

ORIGINAL RESEARCH ARTICLE



Investigating the impact of deploying commercial *Bombus terrestris* for crop pollination on pathogen dynamics in wild bumble bees

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Summary

The use of commercial bumble bees for crop pollination has been implicated in the decline of wild bumble bees through the spread of pathogens. This study investigates whether diseases from commercial bumble bees threaten native species in the UK. We sampled bumble bees from ten soft fruit farms: five that deploy commercial *Bombus terrestris* and five that do not. Each farm was visited monthly throughout the summer and workers of *B. terrestris*, *B. pratorum*, *B. pascuorum* and *B. lapidarius* were captured. The faeces of these bees were inspected for the gut microparasites *Crithidia* spp., *Nosema bombi* and *Apicystis bombi*. Prevalence was defined as the proportion of individuals infected and abundance was defined as the number of pathogen cells per volume of bumble bee faeces. The prevalence of *A. bombi* and *N. bombi* was too low to analyse. The prevalence and abundance of *Crithidia* spp. was significantly different among bumble bee species. Overall, the prevalence of *Crithidia* spp. was initially lower on farms deploying commercial bumble bees, possibly due to a dilution effect caused by the high density of imported bees. *Crithidia* spp. prevalence in *Bombus terrestris*, however, rose sharply on commercial farms at the end of the season. One potential explanation is that commercial bumble bees contract the local pathogen, which is then rapidly transmitted among them due to the high bee density. Whilst our data provide no evidence of pathogen spillover to wild species, it would be premature to conclude with certainty that commercial colonies do not represent a disease risk to native bees in the UK and we urge further studies into this phenomenon.

Spanish Summary

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high density of imported bees. *Crithidia* spp. prevalence in *Bombus terrestris*, however, rose sharply on commercial farms at the end of the season. One potential explanation is that commercial bumble bees contract the local pathogen, which is then rapidly transmitted among them due to the high bee density. Whilst our data provide no evidence of pathogen spillover to wild species, it would be premature to conclude with certainty that commercial colonies do not represent a disease risk to native bees in the UK and we urge further studies into this phenomenon.

Keywords: *Bombus*, pathogen spillover, *Crithidia* spp., *Nosema bombi*, *Apicystis bombi*, soft fruit pollination

Introduction

The commercial use of bumble bees as pollinators for agricultural crops has been common practice since the 1980s when techniques for mass rearing bumble bees were developed (Velthuis & van Doorn, 2006). The majority are used for greenhouse tomatoes, but large numbers are also used for the pollination of various cucurbits and soft fruits (Velthuis & van Doorn, 2006; Stanghellini *et al.*, 1997; Stubbs & Drummond, 2001). As bumble bees are highly efficient pollinators, they can provide economic benefits to fruit growers through increased yield (Serrano & Guerra-Sanz, 2006; Lye *et al.*, 2011) but their use does not come without risk (Velthuis & van Doorn, 2006 & references therein). Commercially produced bumble bees pose three main potential threats to native bumble bee fauna; competition for resources (Ings *et al.*, 2006; Inoue *et al.*, 2008; Inoue *et al.*, 2010); hybridisation with native subspecies (Kondo *et al.*, 2009) and finally, the spread of parasites (Colla *et al.*, 2006; Meeus *et al.*, 2011; Arbetman *et al.*, 2013). It is vital to understand the relevance of these threats to bumble bees because populations of many species have been declining over recent decades (Williams & Osborne, 2009; Cameron *et al.*, 2011). These declines have been predominantly attributed to the intensification of agriculture and the associated loss of habitats, on which bumble bees depend (Goulson *et al.*, 2008; Williams & Osborne, 2009).

Recent work from North America suggests that diseases from commercial bumble bees may pose a significant additional threat to native species (Winter *et al.*, 2006; Colla *et al.*, 2006; Cameron *et al.*, 2011; Szabo *et al.*, 2012) and this threat can take two forms. Firstly, the use of commercial bumble bees, frequently imported from foreign countries, could introduce a novel pathogen or pathogen genotype, which is virulent in wild populations (Goka *et al.*, 2000; Goka *et al.*, 2006). Secondly, if the unusually high densities of bumble bees associated with commercial use elevate disease prevalence, pathogens may spill over to cause increased infection rates in wild bumble bee populations (Otterstatter & Thomson, 2008; Szabo *et al.*, 2012; Murray *et al.*, 2013). It may be possible for such pathogen spillover to occur even if the commercial bees arrive uninfected if they contract and amplify local pathogens. The potential exists for both processes to occur when commercial bumble bees are deployed because they regularly forage on wild flowers adjacent to the crop (Morandin *et al.*, 2001; Whittington *et al.*, 2004). Transmission of parasites can then occur when infected and uninfected individuals forage on the same flower (Durrer & Schmid-Hempel, 1994). Infection with intestinal parasites such as *Crithidia* spp. and *Nosema bombi* can substantially

reduce the fitness of individual bumble bees and the reproductive output of colonies (Brown *et al.*, 2003; Otti & Schmid-Hempel, 2008).

The introduction of novel pathogens can potentially have severe consequences. In North America, the accidental introduction of the gut parasite *Nosema bombi* with commercial bumble bees is thought by many (e.g., Thorp, 2005; Thorp & Shepherd, 2005; Winter *et al.*, 2006) to be responsible for the dramatic decline of four species of native bumble bees since the 1990s (Cameron *et al.*, 2011), although direct evidence is lacking (Brown, 2011). In South America, the native *Bombus dahlbomii* has disappeared from all areas invaded by the rapidly spreading European *Bombus terrestris*, possibly due to one or more non-native pathogens carried by the invading species (Arbetman *et al.*, 2012). Within Europe, this may be considered less of a threat because the source and destination locations of commercial bees contain the same parasite species. The introduction of novel pathogen strains, however, remains a risk. For example, the gut trypanosome *Crithidia bombi* is known to consist of a large number of different strains (Schmid-Hempel & Reber Funk, 2004). Higher mortality has been found when bumble bees are infected with a *Crithidia bombi* strain from a distant location compared to infection from a local source (Imhoof & Schmid-Hempel, 1998). Thus, the importation of bumble bees from abroad could potentially introduce novel parasite strains to which the local populations are more susceptible.

Pathogen spillover occurs when a heavily infested host reservoir population transmits a pathogen to a nearby susceptible population (Daszak *et al.*, 2000). In the case of the commercial use of bumble bees, the reservoir population consists of the imported colonies and the susceptible population is the local natural bumble bee fauna. The pathogen may already exist within the susceptible population but spillover occurs if the commercial bees maintain higher parasite loads, which is likely due to the high densities of commercial colonies within greenhouses or polytunnels (tunnels made of polyethylene for crop propagation). Pathogen spillover from commercial to wild bees has been shown to occur in Canada. The prevalence of parasites was compared between sites close to greenhouses using commercial bumble bees and sites over 50km from any commercial greenhouse. It was found that *Crithidia bombi* was present at significantly higher prevalence at the sites near greenhouses. Additionally, bees foraging closest to the greenhouse had more intense infections (Colla *et al.*, 2006; Otterstatter & Thomson, 2008). More recently, Murray *et al.* (2013) have provided evidence of pathogen spillover in Ireland; again the prevalence of *C. bombi* was significantly higher closer to greenhouses and the probability of infection declined with increasing distance from

the greenhouses. It should be noted that pathogen spillover can occur even if the commercial bees are free of disease in the factory; high densities of bumble bees in greenhouses provide suitable conditions for rapid spread of any pathogen with which they come into contact.

Apart from Murray *et al.* (2013), no other research into the potential threat of pathogens and parasites from commercial bumble bees has been published in Europe and this paper aims to investigate whether such a threat exists in the UK. We focus on the use of commercial bumble bees for the pollination of soft fruit where nest boxes are placed in open-ended polytunnels and open-field situations. The spread of pathogens to wild bumble bees is of particular concern in such situations because there is no containment of the commercial bees. We investigate this using soft fruit farms in Scotland as a study system, where there is undoubtedly the potential for commercial bumble bees to pose a threat because approximately 60,000 *Bombus terrestris* nests are currently imported from mainland Europe to the UK each year (Goulson, 2010). We compare the prevalence and abundance of pathogens in bumble bees on farms that do deploy commercial bumble bees and on farms that do not. If commercial bumble bees amplify pathogen prevalence, we would predict infections to be more common among foraging bumble bees on the farms where they are deployed.

Materials and methods

Ten soft fruit farms in East and Central Scotland were selected for this study (Table 1). The farms were comparable because all grew raspberries. Some of the farms also grew a selection of other soft fruit, including strawberries. Five farms deployed commercially reared *B. terrestris* to aid pollination (hereafter referred to as “commercial farms”) and five did not (“wild farms”). Commercial bumble bees originated from the suppliers Koppert and Biobest. Only one farm (SCRI) bought in the native subspecies *Bombus terrestris audax*, whilst the other commercial farms bought *B. t. terrestris*. Wild farms were located at least 4 km from a farm that used commercial bumble bees to minimise the presence of any foraging commercial bees. The foraging range of bumble bees is difficult to measure and estimates vary, but most studies agree that *B. terrestris* rarely forage more than 1.5 km from their nest (Darvill *et al.*, 2004; Knight *et al.*, 2005; Osborne *et al.*, 2008; Wolf & Moritz, 2008). Sampling took place at each farm for one day each month in May, June, July and August in 2010. Farms were visited over several days, approximately alternating between commercial and wild treatments. We ensured that there was no bias between treatments in the order the farms were visited. Worker bumble bees of the species *B. terrestris*, *B. pascuorum*, *B. pratorum* and *B. lapidarius* were collected using sweep nets. Bees were collected either directly from the raspberry or strawberry crop or from wildflowers growing within 10 metres of the crop. No attempt was made to genetically distinguish between the morphologically similar *B. terrestris*, *B. lucorum*, *B. magnus* and

Table 1. Information on the ten farms from which samples were collected.

Farm name	Longitude	Latitude	Farm type	ha soft fruit	No. nest boxes imported per year
Allanhill	2°46.8' W	56°19.2' N	Commercial	45	300
Blacketside	2°59.2' W	56°12.7' N	Commercial	40	200
Broadslap	3°36.5' W	56°19.7' N	Commercial	8	6
SCRI	3°04.2' W	56°27.4' N	Commercial	18.5	6
Seaton	2°33.1' W	56°34.2' N	Commercial	40	350
Briarlands	4°02.6' W	56°10.1' N	Wild	0.5	-
Kinreich	2°55.2' W	56°35.3' N	Wild	6	-
Mill of Montague	3°19.2' W	56°26.2' N	Wild	6	-
Milton of Ruthven	3°09.6' W	56°38.5' N	Wild	40	-
Newmills	3°18.0' W	56°30.4' N	Wild	6	-

B. cryptarum due to financial constraints and this species group is referred to as simply *B. terrestris*. On the commercial farms this group includes the commercial bumble bees. Commercial bumble bees were also sampled directly from their colonies on one day in May and June. At this time the nest boxes had been open and the bees foraging for varying periods of time; it was not possible to sample from nest boxes immediately on arrival from the suppliers. Bees were held individually in clear sampling tubes with ventilation holes in the lids and were left until they had defecated. The faeces were collected into microcapillary tubes, which were then sealed at each end and stored in a chilled box. Bees were released at the end of the sampling period unharmed; lethal sampling was avoided because removing the pollinators from the crops was unacceptable to the farmers. The faeces were later inspected at x400 magnification to detect the presence of *Crithidia spp.*, *Nosema bombi* and *Apicystis bombi*. No attempt was made to distinguish between *Crithidia bombi* and the newly discovered *C. expoeki* (Schmid-Hempel & Tognazzo, 2010). If present, the intensity of infection was recorded using a haemocytometer: the number of cells in a 0.1µl grid was counted.

Statistical analysis

Data were analysed in R, version 2.12.0 (2010 The R Foundation for Statistical Computing). Chi-squared tests established whether differences existed between the proportion of infected bees in different species. Binomial generalised linear mixed effect models were used to analyse determinants of *Crithidia spp.* prevalence and each bumble bee species was analysed first together and then separately. The residuals were tested for autocorrelation using the Durbin-Watson statistic but this was not detected. *Crithidia spp.* abundance (the number of *Crithidia spp.* cells per 0.1µl faeces, including uninfected bees) was analysed in a

Bayesian framework using the MCMCglmm package in R (Hadfield, 2010). Generalised linear mixed models with a zero-inflated poisson distribution were used and non-informative priors were set in all analyses. Prior sensitivity analysis was carried out and the final models are robust to variation in the values of priors. Model convergence was confirmed using Geweke's diagnostic (Geweke, 1992) and visual examination of the model output. Parameter estimates reported are means from the posterior distribution with 95% lower and upper credible intervals (CI). A binomial generalised linear mixed effect model was used to investigate the difference in prevalence of *Nosema bombi* between the treatments. Prevalence of infection was too low to allow bumble bee species to be analysed separately for this parasite. In all the mixed effect models, sampling month (entered as a covariate 1, 2, 3 or 4), treatment (presence or absence of commercial bumble bees), bumble bee species and farm size (hectares of soft fruit) were entered as fixed effects and the individual farms were entered as a random effect. Means are recorded \pm their standard errors throughout.

Results

A total of 946 worker bumble bees was collected from the ten farms and screened for pathogens over the four month sampling period. Additionally, 103 commercial bumble bee workers were collected directly from their nest boxes in May and June. All three parasite species were detected and the overall prevalence in the bees collected foraging were: *Crithidia spp.* 39.22%; *Nosema bombi* 2.01% and *Apicystis bombi* 0.74%. The number of bees infected with *A. bombi* was too small to allow further analyses on this parasite.

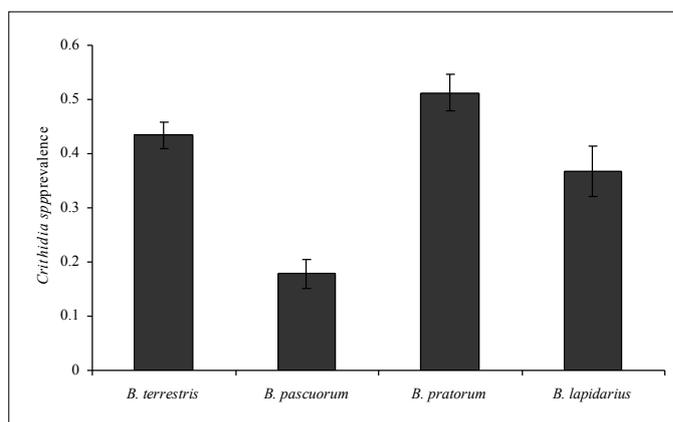


Fig. 1. Prevalence of *Crithidia spp.* in the four species. The proportion of bees infected was significantly different among the species ($\chi^2 = 53.09$, $df = 3$, $p < 0.001$). Bars represent the mean prevalence and their standard errors.

Crithidia bombi prevalence

The proportion of bees infected differed significantly across the different species, being highest in *B. pratorum* and lowest in *B. pascuorum* ($\chi^2 = 53.09$, $df = 3$, $p < 0.001$, Fig. 1, Table 2). The prevalence of *Crithidia spp.* infection in commercial bumble bees collected directly from their nestbox in May and June was $35.92 \pm 4.75\%$, which is similar to the prevalence in *B. terrestris* collected from commercial farms ($28.57 \pm 5.66\%$; $\chi^2 = 1.09$, $df = 1$, $p = 0.297$) and wild farms ($47.73 \pm 5.36\%$; $\chi^2 = 2.73$, $df = 1$, $p = 0.099$) in May and June. There was a significant three-way interaction between species, sampling month and farm type ($\chi^2 = 124.08$, $df = 15$, $p < 0.001$). This indicates that the change in *Crithidia spp.* prevalence across the sampling period in the two farm types was different among bumble bee species and for this reason we present separate analyses for each species (table 3). Farm size did not significantly influence *Crithidia spp.*

Table 2. Mean prevalence of *Crithidia spp.* infection for the four bumble bee species in the two farms types across the sampling period.

	<i>B. terrestris</i>						<i>B. pascuorum</i>						<i>B. pratorum</i>						<i>B. lapidarius</i>					
	Commercial			Wild			Commercial			Wild			Commercial			Wild			Commercial			Wild		
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
May	0.250	0.25	4	0.500	0.29	4	0.000	-	1	0.400	0.25	5	0.417	0.15	12	0.345	0.09	29	0.333	0.33	3	1.000	-	1
June	0.283	0.06	60	0.476	0.05	84	0.069	0.05	29	0.300	0.15	10	0.257	0.08	35	0.486	0.09	35	0.423	0.10	26	0.191	0.09	21
July	0.307	0.05	75	0.417	0.06	72	0.154	0.06	39	0.143	0.07	28	0.400	0.08	40	0.824	0.07	34	0.316	0.11	19	0.400	0.25	5
Aug	0.712	0.06	66	0.406	0.06	64	0.243	0.07	37	0.191	0.06	42	0.792	0.08	24	0.875	0.13	8	0.412	0.12	17	0.471	0.13	17

Table 3. Parameter estimates and 95% CIs from the generalised linear mixed effect models for *Crithidia bombi* prevalence. The parameter estimates for *C. bombi* prevalence shown here are with reference to the commercial farm type and are on the logit scale.

	<i>B. terrestris</i>			<i>B. pratorum</i>			<i>B. pascuorum</i>			<i>B. lapidarius</i>		
	Parameter estimate	Lower 95% CI	Upper 95% CI	Parameter estimate	Lower 95% CI	Upper 95% CI	Parameter estimate	Lower 95% CI	Upper 95% CI	Parameter estimate	Lower 95% CI	Upper 95% CI
Intercept	-3.083	-4.304	-1.861	-2.459	-3.855	-1.064	-3.875	-6.373	-1.376	-0.311	-2.160	1.539
Treatment	3.268	1.717	4.820	0.340	-1.552	2.231	2.960	-0.709	6.629	-2.396	-5.360	0.569
Month	0.918	0.539	1.297	0.803	0.336	1.269	0.693	-0.035	1.421	-0.052	-0.676	0.572
Treatment*Month	-1.075	-1.567	-0.582	0.347	-0.376	1.070	-0.857	-1.927	0.214	0.710	-0.239	1.660

prevalence ($\chi^2 = 0.258$, $df = 1$, $p = 0.611$) and has been excluded from the following analyses of individual bumble bee species.

Bombus terrestris

Averaging across the whole season, the proportion of *Bombus (s.s)* spp. infected with *Crithidia* spp. was significantly higher on the wild farms compared to the commercial farms ($\chi^2 = 17.95$, $df = 1$, $p < 0.001$). There was also a significant interaction between the farm type and the sampling month ($\chi^2 = 19.07$, $df = 1$, $p < 0.001$): month significantly predicted *Crithidia* spp. prevalence on commercial farms due to the marked increase in August, whilst prevalence on wild farms did not change significantly over time (Fig. 2).

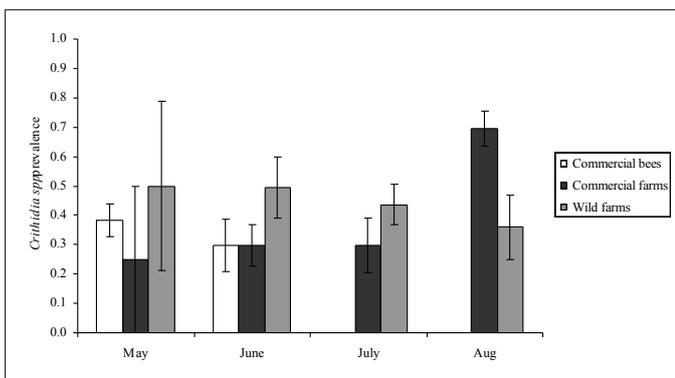


Fig. 2. Prevalence of *Crithidia* spp. in *B. terrestris* over the sampling period in the two farm types. Prevalence on commercial farms was significantly affected by month due to the marked increase in August ($Z = 4.75$, $p < 0.001$). No significant change in prevalence occurred on wild farms ($Z = 0.976$, $p = 0.329$). There was no difference in the prevalence of *Crithidia* spp. in commercial bees collected from nest boxes and in foraging *B. terrestris* collected on commercial farms ($\chi^2 = 1.09$, $df = 1$, $p = 0.297$). Bars represent the mean prevalence and their standard errors.

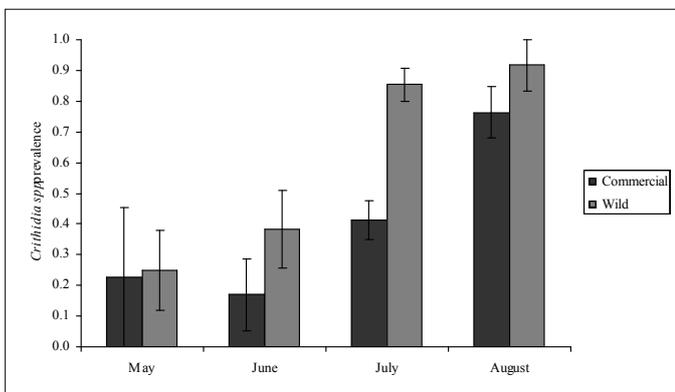


Fig. 3. Prevalence of *Crithidia* spp. in *B. pratorum* over the sampling period in the two farm types. Prevalence was higher in wild farms ($p = 0.012$) and significantly increased over time ($p < 0.001$). Bars represent the mean prevalence and their standard errors.

Bombus pratorum

The prevalence of *Crithidia* spp. was significantly higher on wild farms than on commercial farms ($\chi^2 = 6.33$, $df = 1$, $p = 0.012$, Fig. 3) and also significantly increased over the sampling period ($\chi^2 = 30.27$, $df = 1$, $p < 0.001$). There was no interaction between the farm type and month ($\chi^2 = 0.887$, $df = 1$, $p = 0.346$), indicating that this increase occurred at a similar rate on both farm types.

Table 4. MCMCglmm output for *Crithidia* spp. abundance. The parameter estimates shown here are with reference to *B. terrestris* and the commercial farm type and are on the log scale. The MCMC procedure for this model has a burn-in period of 5000, a total of 505,000 iterations and a thinning interval of 500. P values < 0.05 are written in bold.

	Parameter estimate	Lower 95% CI	Upper 95% CI	P value
<i>Crithidia</i> spp. abundance	4.770	3.001	6.562	<0.001
Species				
<i>B. lapidarius</i>	1.094	0.121	1.903	0.022
<i>B. pascuorum</i>	-3.025	-4.321	-1.741	<0.001
<i>B. pratorum</i>	-1.040	-1.629	-0.416	<0.001
Month	-0.005	-0.487	0.445	0.980
Treatment	1.697	-0.459	4.129	0.110
Month*Treatment	-0.609	-1.258	0.025	0.064
Farm size	-0.018	-0.056	0.034	0.312

Bombus pascuorum and Bombus lapidarius

Similar results were obtained for both species and because so few workers were collected in May, this month was excluded from the analysis of both. The prevalence of *Crithidia* spp. in *B. pascuorum* and *B. lapidarius* was not significantly different in each farm type ($\chi^2 = 0.038$, $df = 1$, $p = 0.847$ and $\chi^2 = 0.473$, $df = 1$, $p = 0.492$ respectively) and did not significantly change over time ($\chi^2 = 1.52$, $df = 1$, $p = 0.217$ and $\chi^2 = 1.26$, $df = 1$, $p = 0.262$ respectively). Temporal patterns were similar on commercial and wild farms: there was no significant interaction between month and farm type ($\chi^2 = 2.46$, $df = 1$, $p = 0.117$ and $\chi^2 = 2.20$, $df = 1$, $p = 0.138$).

Crithidia spp. abundance

Considering the load of infection in each individual bee, *Crithidia* spp. abundance for all bumble bee species did not differ significantly between the two farm types and did not change significantly over time. Additionally, there was no interaction between these two variables. There was also no significant effect of farm size (Table 4). The abundance was, however, significantly different among the four bumble bee species (Fig. 4).

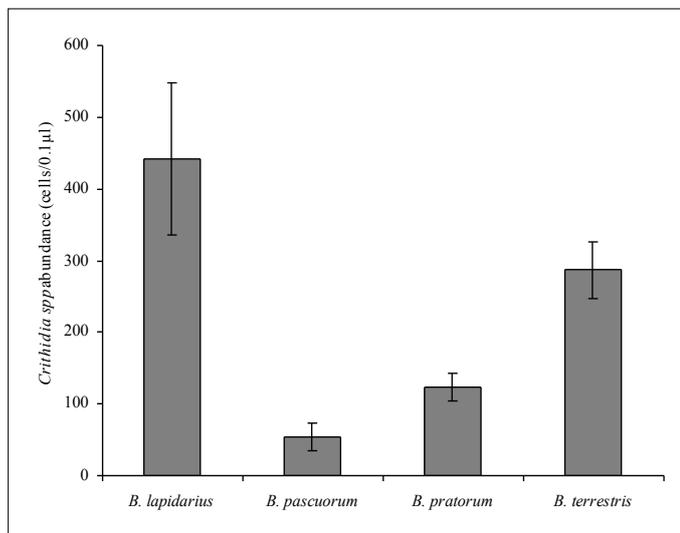


Fig. 4. Mean *Crithidia* spp. abundance for the four bumble bee species. All species comparisons were significant: *B. lapidarius* had a significantly greater mean load than all the other species (*B. pascuorum*: $p < 0.001$; *B. pratorum*: $p < 0.001$; *B. terrestris*: $p = 0.022$). *B. terrestris* had a significantly greater mean load than *B. pascuorum* and *B. pratorum* ($p < 0.001$ and $p < 0.001$ respectively). *B. pratorum* had a significantly higher mean load than *B. pascuorum* ($p = 0.004$). Bars represent the mean abundance and their standard errors.

Nosema bombi

When all bumble bee species were pooled, a greater proportion was infected with *N. bombi* on commercial farms ($2.95 \pm 0.97\%$) compared to wild farms ($1.15 \pm 0.54\%$). This difference was not, however, significant ($\chi^2 = 3.09$, $df = 1$, $p = 0.079$). Farm size and sampling month also did not affect infection with *N. bombi* ($\chi^2 = 1.197$, $df = 1$, $p = 0.274$ and $\chi^2 = 0.795$, $df = 1$, $p = 0.372$ respectively). Due to the small number of bees infected ($n = 19$) it was not possible to analyse species separately. This comparison could be confounded, however, by an uneven distribution of species infected in the two farm types: only *B. terrestris* were found to be infected on wild farms whilst a few individuals of all four bumble bee species were infected on commercial farms. Additionally, two of 103 commercial bumble bees collected directly from their nest boxes were infected with *N. bombi*.

Discussion

The decline of insect pollinators is of universal concern due to the ecological and economic benefits they provide. The global trade in commercial bumble bees may have contributed to this decline, partially through the spread of pathogens and parasites (Colla *et al.*, 2006; Brown, 2011; Cameron *et al.*, 2011). However, the impact of commercial pollination practices is likely to differ depending on location and ecological circumstances. This paper offers an insight into the potential

impacts of commercial bumble bees on parasite dynamics in European bumble bee populations, using Scottish farms as a study system.

No evidence for the spread of pathogens from commercial bees to other bumble bee species was found: parasitic infection in wild bumble bee species was no higher at commercial farms compared to wild farms (and was lower in one wild bumble bee species). This contrasts markedly with the situation in Canada and Ireland, where commercial bumble bees used in greenhouses acted as a source of infection to wild bumble bees in the surrounding area (Colla *et al.*, 2006; Otterstatter & Thomson, 2008; Murray *et al.*, 2013). Overall, we found a lower prevalence of *Crithidia* spp. in *B. terrestris* on commercial farms compared to wild farms, particularly early in the season. This could be a dilution effect caused by the new arrival of large numbers of predominantly uninfected commercial bumble bees. Our study did not investigate whether parasites were present in commercial nest boxes when they arrived from the suppliers; hence we cannot discern whether the infections observed in commercial bees were contracted largely or exclusively whilst bees were foraging on farms following deployment. Previous studies, however, have found commercial bees to arrive from the supplier infected with parasites in Japan, North America and Ireland (Goka *et al.*, 2000; Colla *et al.*, 2006 and references therein; Murray *et al.*, 2013).

Interestingly, the prevalence of *Crithidia* spp. increased through the season in *B. terrestris* on commercial farms, whilst it remained approximately constant on wild farms. This was driven by a marked increase in infection rate at the end of the season in August. Although both wild and commercial *B. terrestris* (and also *B. lucorum*, *B. magnus* and *B. cryptarum*) would have been sampled on commercial farms, the majority are likely to have been commercial bees due to the close proximity of their nest boxes. One possible explanation for this pattern is that the commercial bumble bees contract *Crithidia* spp. from local bees and the elevated bumble bee density on commercial farms causes a high rate of transmission, resulting in an increase in the prevalence of this parasite. Additionally, commercial bumble bees may have higher susceptibility to *Crithidia* spp. than local bumble bees. Genetic variation exists in *B. terrestris* for *Crithidia* spp. susceptibility (Wilfert *et al.*, 2007), although recent studies suggest that the bee's gut flora has a more important role in determining susceptibility (Koch & Schmid-Hempel, 2012). Consequently, it is possible that commercial *B. terrestris* and their gut flora, which originate from mainland Europe, could be poorly adapted to defend against local *Crithidia* spp. genotypes. This effect may be intensified because commercial *B. terrestris* have undergone selection in a factory environment for several generations, which might have altered immune investment, as well as being fed honey bee pollen and artificial nectar, which is likely to have altered their gut flora.

The significantly higher prevalence of *Crithidia* spp. on commercial farms by the end of the season does suggest that pathogen spillover is a threat because there is a possibility that wild bumble bees, including

newly emerged queens, may become infected by contact with commercial bees. Such infection of queens would cause fitness losses because *Crithidia* spp. is known to substantially reduce their colony founding success (Brown *et al.*, 2003). However, recent research suggests that queens may be more resistant to *Crithidia* spp. than workers, which would lessen the impact of any epidemic (Ulrich *et al.*, 2011). Further research into the rates of interspecific transmission by the strains of *Crithidia* spp. infecting wild and commercial bumble bees would be required to assess the risks of these late-season epidemics spreading to other species in the surrounding areas.

The overall mean prevalence of *Crithidia* spp. was similar to that in central Europe and was also significantly different among bumble bee species (Shykoff & Schmid-Hempel, 1991). *Bombus pratorum* suffered from the highest rate of infection, particularly at the end of the sampling period. This species emerges early from hibernation in the spring throughout the UK and nests can produce reproductives as early as April (Goulson, 2010). Therefore, individuals still on the wing by the end of the summer are highly likely to be infected because they would have had a long period of exposure to *Crithidia* spp. The intensity of infection with *Crithidia* spp. also varied significantly across bumble bee species but interestingly shows a different pattern to the prevalence of infection. *B. lapidarius* was found to suffer from considerably higher parasite loads than all three other bumble bee species and *B. terrestris* had significantly higher loads than *B. pascuorum* and *B. pratorum*. The reasons behind these differences remain unknown but it may relate to inter-specific differences in host genetics and parasite defence, environmental factors or parasite virulence (Ruiz-González *et al.*, 2012).

The proportion of bees infected with *Nosema bombi* was too low in this study to allow an in-depth analysis. To obtain a good picture of the infection dynamics of this parasite species, results from more than one season would be required because the prevalence of *N. bombi* is known to vary spatially, temporally and across species by substantial amounts (Larsson, 2007). *Nosema bombi* appears to be a rare pathogen in this habitat and consequently may only have a small impact on the bumble bee populations in the area. Our dataset is too small to make any conclusions but it is interesting to note that the prevalence of this parasite was higher on the commercial farms, although this difference was not significant. Previous authors have thought that the presence of commercial bumble bees can possibly amplify the prevalence of *N. bombi* (Colla *et al.*, 2006; Otterstatter & Thomson, 2008). This is potentially concerning because bumble bees infected with *N. bombi* have substantially reduced fitness (Otti & Schmid-Hempel, 2008; Rutrecht & Brown, 2009).

This study assesses one aspect of the risks associated with the use of commercial bumble bees for pollination services. Our data suggest that the high density of bees on commercial farm amplifies the prevalence of *Crithidia* spp. by the end of the season. This high prevalence has the potential to spill back over to local wild bumble bees but we find no evidence that this threat is being realised; our

data suggest that this late-season epidemic may remain within the commercial bees. More research over a larger temporal and spatial scale is needed, however, before any conclusive generalisations on the disease risks posed by commercial bees can be made. Any future research should also use genetic methods to differentiate between commercial *B. terrestris* and wild *B. terrestris* on commercial farms. Further research is also needed into the other detrimental ecological consequences associated with commercial bumble bees, such as hybridisation with native subspecies and competition for resources (Goulson, 2003). Due to the uncertainties surrounding these potential costs, it would be preferable to develop viable alternatives where possible and thus reduce the need for commercial bumble bees. For example, sowing wild flower mixes can boost natural pollinator populations (Carvell *et al.*, 2007), which in turn may benefit outdoor soft fruit pollination.

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