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Indirect effects of agricultural pesticide use on parasite prevalence in wild pollinators

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ABSTRACT

Insect pollinators appear to be experiencing worldwide declines, a phenomenon that has been correlated both with exposure to chemical pesticides and disease prevalence. These factors have been found to have strong and often interacting negative effects on multiple pollinator species in laboratory based studies, however their interactions in the field are less clear. To try and understand the link between pesticide use on pollinator communities, and how this might impact on disease transmission, we took two complementary approaches. First, we undertook a series of pollinator surveys to assess the abundance and diversity of pollinator groups across British agricultural field sites subject to varying levels of pesticide use. We then screened the offspring of two taxa of tube nesting solitary bees (*Osmia bicornis* and *Megachile* spp.) for three parasite groups commonly associated with pollinators. We found lower pollinator abundance, group richness and diversity across agricultural sites associated with higher pesticide use. Specifically, there were fewer honey bees, hoverflies, solitary bees and wasps. Surprisingly, we found a lower prevalence of all three parasite groups in *O. bicornis* offspring reared in sites associated with higher pesticide use compared to lower pesticide use. We also found a lower prevalence of *Ascosphaera* but a higher prevalence of Microsporidia in *Megachile* offspring reared in sites associated with higher pesticide use compared to lower pesticide use. Together, our results suggest that agricultural sites associated with higher pesticide use may be affecting pollinators indirectly by disrupting community structure and influencing disease epidemiology and vectoring opportunities. This highlights the importance of understanding the interactions between pesticide use and disease in both managed and wild bee populations for the future mitigation of pollinator declines.

1. Introduction

Animal pollinators provide ecosystem services of environmental, agricultural and economic importance by pollinating an estimated 90% of all plant species, including essential agricultural crops (Kearns et al., 1998). European honey bees (*Apis mellifera*) are often cited as the most valuable agricultural pollinator. However, wild pollinators, such as wild bumblebees (*Bombus* spp), solitary bees, flies, wasps and Lepidoptera appear to pollinate certain (and prevalent) crops such as oilseed rape and orchard fruits more effectively (Velthuis, 2001; Breeze et al., 2011), by for example doubling fruit setting rates compared to the equivalent visitation rate by managed honey bees (Garibaldi et al., 2013). Indeed, wild bees contribute approximately the same value towards crop production as managed bees do (Kleijn et al., 2015). The increasingly evident role of wild insects in crop pollination has led to the suggestion that maintaining both the diversity and abundance of wild pollinators is crucial in meeting the mounting demands on the agricultural industry (Klein et al., 2003; Greenleaf and Kremen, 2006; Hoehn et al., 2008; Winfree et al., 2015). Unfortunately, multiple pollinator taxa are currently experiencing contracting ranges and reductions in species richness (Biesmeijer et al., 2006; Potts et al., 2010). This appears to be the result of a complex interaction between multiple stressors (Goulson et al., 2008; Bacandritsos et al., 2010; Ellis et al., 2010; vanEngelsdorp and Meixner, 2010). Understanding how stressors responsible for pollinator declines interact is therefore a key target both for improving their conservation in the wild and in supporting future global crop production.

A key driver of pollinator decline is believed by many to be the environmental stressors generated via agricultural intensification. For example, habitat fragmentation and landscape homogeneity in large-scale farm systems have been linked to reduced forage and nesting habitats required for wild bees as well as general biodiversity loss (Weibull and Östman, 2003). However, several studies suggest it is the combination of reduced quantity and diversity of flowering plants and

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exposure to high levels agrochemicals that is driving pollinator declines (Nazzi and Pennacchio, 2014; Schmehl et al., 2014; Baude et al., 2016). While significant lethal and sub-lethal effects of certain agrochemicals, such as neonicotinoid insecticides, have been found in laboratory experiments (e.g. Cresswell, 2011; Lundin et al., 2015), there has been less evidence of such detrimental effects on pollinators by field-realistic exposure levels (Rundlöf et al., 2015). Some studies indicate no negative effects (Blacquiere et al., 2012; Nicholls et al., 2017), others indicate inconsistent sub-lethal effects (Woodcock et al., 2017), supporting the idea that prevailing environmental conditions are a key factor determining the lethality of agrochemicals in the field. As of the 1st December 2013, the European Commission initiated a restriction on the application of three major neonicotinoids (imidacloprid, clothianidin and thiamethoxam) on animal-pollinated crops throughout the European Union until there is more conclusive evidence as to whether these pesticides are causing unacceptable pollinator losses (European Commission, 2013). The effect of the memorandum on neonicotinoids is currently under review, but the general consensus remains that farming practises that involve high levels of their use pose a considerable threat to all wild pollinators (Wood and Goulson, 2017). Despite this consensus, the majority of studies on the effects of pesticides on pollinators have focused on honey bees and bumblebees, leaving a gap in knowledge on the effects of agrochemicals on wild pollinators (Blacquiere et al., 2012; Thompson, 2010; FERA, 2013; Lundin et al., 2015; Wood and Goulson, 2017).

Several studies have also correlated pollinator declines with the spread of pathogens and parasites (Goka et al., 2001; Otterstatter and Thomson, 2008; Meeus et al., 2011; Arbetman et al., 2012; Szabo et al., 2012). Again, the focus of research has largely centred on honey bees, and to a lesser extent bumblebee species. However, honey bees are generalist pollinators, which share their foraging sites with wild pollinators (Hudewenz and Klein, 2015). They are host to more than 70 different parasites (Morse and Flottum, 1997), and provide a significant reservoir of disease and potential for inter-species transmission, for example though shared flower patches (Graystock et al., 2015a). Indeed, several non-*Apis* UK pollinator species have been associated with a multitude of 'traditional' honey bee parasites (Evison et al., 2012; Fürst et al., 2014; Tehel et al., 2016; Villalobos 2016). Disease associations between honey bees and bumblebees (Fürst et al., 2014), and parasite spillover between commercially reared and wild pollinators (Graystock et al., 2013; Tehel et al., 2016) together suggest that inter-species transmission and/or novel vectoring routes are exacerbating the effects of disease driven pollinator decline. For example, co-infection in bumblebees by their neogregarine parasite *Apicystis bombi* and deformed wing virus (DWV), which is usually associated with honey bees, were shown to severely increase mortality (Graystock et al., 2015b). Damaging epidemics resulting from parasites switching between honey bee species, such as *Varroa destructor* (Mondet et al., 2014; Wilfert et al., 2016) and *Nosema ceranae* (Natsopoulou et al., 2015), are well documented and have taught us a great deal about emerging infectious diseases (EIDs) of honey bees, but their interactions with non-*Apis* species requires much more investigation.

The way in which parasites and pesticides interact may be a key reason for the contrasting results of studies investigating the effect of pesticides on pollinator health (e.g. Woodcock et al., 2017). Laboratory studies consistently suggest that exposure to pesticides increases the susceptibility of honey bees to disease, increasing mortality (e.g. Vidau et al., 2011; Wood and Goulson 2017), as well as causing harmful sub-lethal effects such as a reduced ability to sterilize colony and brood food (e.g. Alaux et al., 2010). There have also been reports of some insecticides, such as the carbamate Carbofuran, and the organophosphate Dimethoate, reducing the peak larval weights of honey bee larvae (Davis et al., 1988), which may have knock on effects in terms of immunocompromisation of adult honey bees (Yearsley et al., 2004). When adult workers of social species of bee are immunocompromised through exposure to pesticides, an increased susceptibility to disease, particularly to those that are commonly spread through shared foraging patches (Pettis et al., 2012, 2013; Wu et al., 2012), is likely to exaccerbate its spread. For example, long range generalist foraging habits of honey bees, and high levels of intra-colony transmission predispose social species like these as superspreaders of disease, particularly if those hosts are already infected with other parasites (Vidau et al., 2011). Consequently, synergistic interactions between emerging infectious diseases (Natsopoulou et al., 2015) and pesticide exposure (e.g. Doublet et al., 2015) are likely to have serious consequences for wild pollinators such as solitary bees, but there is a dearth of information on how these factors might interact in wild populations.

Based on this information, here we aimed to start to disentangle the mechanisms underlying the documented pollinator declines by assessing, first, how differing levels of agricultural pesticide use impacts on the abundance, diversity and reproductive success of populations of British pollinators, and second, how this might influence the prevalence of parasites across wild bees in the same populations. We assessed the effect of level of pesticide use on wild pollinators using field surveys to measure general pollinator abundance, group richness and diversity. As an additional measure to the flying pollinator activity, we also measured the reproductive success of tube-nesting pollinator species, and the larval weight of their offspring (as an indicator of stable development and the production of healthy adults; Bosch and Vicens, 2002). Collecting tube-nesting pollinators as a method of assessing pollinator biodiversity is useful because they provide a small, interacting and reproducing community within the wider pollinator community (Tscharntke et al., 1998), and provide a more robust assessment of the local pollinator community than flying insect surveys alone can. We then measured the prevalence of three parasites previously associated with pollinators (Evison et al., 2012) across the same landscape, using tube-nesting solitary bees of the genus *Megachile* as a consistent way to sample the environment. These bees share a similar ecological niche to honey bees, as generalist pollinators (Hudewenz and Klein, 2015), so are a useful tool for detecting inter-species disease transmission across pollinator communities. Considering the potential impact of parasites on pollinator health, a deeper understanding of how pesticide use influences their prevalence in wild pollinators is invaluable.

2. Materials and methods

2.1. Field site selection and method overview

Twenty-three agricultural sites across Cambridgeshire and East Anglia were used in the study (Fig. 1), which was performed during 2012. This set of sites were selected from a larger database of field sites (Fig. S1) originally identified by the IPI AgriLand project (Linking agriculture and land use change to pollinator populations, BB/I000364/1; Supplementary material Section S4; Gillespie et al., 2017). The farms in this database are a randomised selection of farms that were chosen to encompass variation in four specific variables thought to be important in driving pollinator declines, yet were otherwise comparable (Gillespie et al., 2017). These variables were pesticide use, habitat diversity, floral resource availability, and managed honey bee colony density (see Gillespie et al., 2017 and Supplementary materials, Section S3 for specific details on how these were calculated). From the farms in the Cambridgeshire and East Anglia regions of this database, we selected the 23 sites used in this study from conventional farms only, based on their pesticide use figure. Pesticide use was estimated based on information from the UK Pesticide Survey, and was calculated by multiplying areas of different crop cover by recommended insecticide application, weighted by toxicity to honey bees (Supplementary materials, Section S4.1). We chose sites that differed in extremes of their pesticide use, and categorised 13 sites as high and 10 as low pesticide use, based on whether their estimated pesticide application levels fell above or below the mean pesticide use estimation figure (detailed in Tables S1.1.1 and S1.2). We used a series of survey protocols to assess