University of Reading

Assessing the effects of pressures and their interaction on commercially reared *Apis mellifera* and *Bombus terrestris* colonies and implications for management practices

Ph.D. in Ecology and Agro-Environmental Research

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Declaration of Original Authorship

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Signed:

Abstract

Bees are the most dominant pollinators worldwide, providing many monetary and non-monetary benefits to society. However, wild bee declines and honeybee colony losses are reported in many regions of the world. Several pressures are responsible for these declines, including land use change, pesticides, and diseases, which can occur simultaneously and interact with each other. However, many knowledge gaps on the impacts of multiple pressures on pollinators remain. Here, I focus on managed *Apis mellifera* and *Bombus terrestris* and investigate: (a) the interactive effects of land cover and pesticides on bee health, activity, and crop yield using a large-scale fieldwork; (b) the impact of the new insecticide sulfoxaflor, the pathogen *Crithidia bombi*, and their interaction on bee behaviour and pollination, using a semi-field experiment in flight cages; and (c) beekeepers' perceptions of the Bee Health Card, a tool under development that can help tackle health issues in beehives, using surveys involving 7 European countries. My findings indicate that higher proportions of cropland and lower proportions of woodland in the landscape favour *Bombus terrestris* colony growth in apple orchards, while a higher honeybee activity is linked to higher proportions of woodland. In oilseed rape fields, both *B. terrestris* colony growth and social bee activity are increased by higher fungicide and herbicide pressures. Moreover, I show that sulfoxaflor and *Crithidia bombi*, individually and in combination, do not affect the behaviour or pollination by *Bombus terrestris*. Finally, I observe that beekeepers recognise the opportunity offered by the Bee Health Card, and that the confidence in its effectiveness is key to its adoption. Cost is a barrier when economic incentives are not available, but environmental benefits may help increase the willingness to use the tool in such cases. I conclude that bees face several interacting pressures varying across species, and that the Bee Health Card may be useful in detecting and addressing such pressures, benefitting both wild and managed bees.

Thesis outline

The PoshBee project

This PhD is funded by the EU Horizon 2020 PoshBee project, aiming to assess, monitor, and mitigate pressures responsible of affecting bee health (M. Brown et al., 2021), and the studies presented in my three core chapters are part of it.

'Chapter 2' (*i.e.* 'Responses of *Apis mellifera* and *Bombus terrestris* to pesticides, cropland, and woodland') is the result of my involvement in PoshBee Work Package 1 (WP1), which consisted of a large-scale fieldwork using sentinel colonies of honeybees and bumblebees in 8 European countries: Estonia, Germany, Ireland, Italy, Spain, Sweden, Switzerland, and the UK. Each country had one designated researcher leading the fieldwork, and my role was to lead and carry out such experiment in the UK. Standardised protocols were written by PoshBee researchers and used to perform replicated field experiments in all 8 countries. To write my thesis, I utilised part of the data I collected to show the effect of multiple pressures on bees in the UK. The entirety of data collected during fieldwork in all countries fits into PoshBee deliverables 1.4, 1.5, 1.6, 2.1, 2.2, and 2.3 (available at: https://poshbee.eu).

'Chapter 3' (*i.e.* 'Effects of sulfoxaflor, *Crithidia bombi*, and their interaction on *Bombus terrestris* behaviour and pollination services') is based on the study developed in collaboration with the Royal Holloway University of London in relation to PoshBee Work Package 6 (WP6), which includes both a laboratory and a semi-fieldwork approach. While the Royal Holloway dedicated to laboratory protocols and work, my involvement was related to writing protocols for and performing the semi-field experiment. The Royal Holloway team had the task to standardise the size of bumblebee colonies, screen them for parasites, and inoculate designated colonies with a pathogen. They additionally prepared the treatment solutions, some of which contained an insecticide. Colonies and solutions were then collected and transported to the University of Reading, where my semi-field experiment took place. Afterwards, colonies were frozen and collected by Royal Holloway researchers for further analyses on colony development, fitting into PoshBee deliverable 6.3 (*i.e.* 'Straw, E. A., Cini, E., Gold, H., Garratt, M. P. D., Linguadoca, A., Rockx, J., Senapathi, D., Potts, S. G., Brown, M. J. F., 2021. Manuscript on agrochemical and pathogen effects on bumblebee health at the colony level'. Available at: https://poshbee.eu).

Finally, 'Chapter 4' (*i.e.* 'A survey for beekeepers to investigate perceptions toward a new omics tool for bee health') shows the result of a survey for beekeepers to investigate their perceptions toward the PoshBee Bee Health Card, as part of PoshBee Work Package 10 (WP10). The Bee Health Card is a tool under development as part of PoshBee Work Package 9 (WP9), and it is expected to be one of the main outputs of the PoshBee project (Brown et al., 2021). My role was to prepare the survey questions, which were reviewed by professional beekeepers and the other WP1 leaders before being distributed in the same 8 European countries involved in Work Package 1. Since the Bee Health Card is still being developed and is not ready to

be tested, it was necessary to include in the survey as much information as possible to allow beekeepers to form an opinion on potential benefits and barriers to its use. Therefore, the Centre National de la Recherche Scientifique (CNRS, WP9 lead) provided me with the information available at the time, including expected Bee Health Card functionalities, time window between shipment and results, and approximate cost. Survey results form part of deliverable 10.2, which is due to be published online in 2022 ('Cini, E., Breeze, T. D., Potts, S. G., Senapathi, D., Albrecht, M., Costa, C., De La Rua, P., Klein, A. M., Mänd, M., Raimets, R., Schweiger, O., Stout, J. C., 2022. Report on incentives for, and barriers to, the adoption of PoshBee tools'. *Undergoing internal review*).

Chapter 1

Introduction

1.1. The importance of pollination

Pollination is an ecosystem service key to the reproduction, conservation, and evolution of many flowering plants (Klein et al., 2007; Potts et al., 2016). It is usually mediated by animals or other vectors, such as wind and water (Ollerton, 2017), which transfer pollen from anthers (male parts) to stigmas (female parts) promoting fertilisation and development of seeds and fruits (Cheung & Wu, 2001). Pollination provides a wide range of benefits to humans, both monetary and non-monetary (Potts et al., 2016).

1.1.1. Monetary benefits

Animal pollination is estimated to profit global food crops with an economic value between $235-577 bn per year (Lautenbach, 2012). Worldwide, about 87.5% wild flowering plants and 75% leading food crops benefit to different extents from animal pollination (Klein et al., 2007; Ollerton et al., 2011).

Pollinators may contribute to the productivity of oil crops commonly used for biofuels, such as oilseed rape (Bommarco et al., 2012), physic nut (Negussie et al., 2015), and soybean (Gallai et al., 2009), and help provide construction materials (*e.g.* eucalypts), fibres (*e.g.* cotton), and products for musical instruments (*e.g.* propolis) (IPBES, 2016).

Moreover, they can contribute to enhancing the market value, quality, and shelf-life of many crops (Hanley et al., 2015). For instance, insect pollination has been shown to increase the quality of oilseed rape seeds (*Brassica napus* L.), with higher oil and lower chlorophyll contents (Bommarco et al., 2012), and to increase its yield by 15-50% (Woodcock et al., 2016; Perrot et al., 2018; Catarino et al., 2019). Such contributions to quality and yield are also transferrable to other crops. For instance, insect pollination has been shown to produce heavier, better-shaped, and brighter-coloured strawberries (Klatt et al., 2013; Saridaş et al., 2021), and to benefit the yield of field bean (*Vicia faba* L.) by increasing bean pod set by 60-69% (Garratt et al., 2014b) and plant weight by 40% (Bartomeus et al., 2014). Yield benefits have also been highlighted for apples (*Malus domestica*), which are pollination-dependent and among the most important fruit crops in the world (Pardo & Borges, 2020). Insect pollination has been shown to produce heavier, firmer, and bigger apples, increasing the proportion of class 1 apples, and to highly contribute to apple fruit set and seed set (Garratt et al., 2021). Moreover, it can benefit the market value of different apple varieties, including two of the most important UK varieties, Cox and Gala (Garratt al., 2014a; Garratt et al., 2016), and two popular Irish ones, Jonagored and Dabinett (Burns & Stanley, 2022).

1.1.2. Non-monetary benefits

Non-monetary benefits of pollination include the supply of micronutrients such as some vitamins and minerals, carotenoids, and folate (Eilers et al., 2011). According to Chaplin-Kramer et al. (2014), areas that are highly reliant on pollinator-dependent crops for micronutrient intakes (*i.e.* India, Southeast Asia, central and southern Africa) have three times the risk of experiencing micronutrient deficiencies, which may lead to severe long-term health complications like spina bifida (folate), osteoporosis (calcium), anaemia (iron), and blindness and maternal mortality (vitamin A). Without pollination, the number of people suffering from vitamin A and folate deficiencies is estimated to be 70 and 170 million respectively, with deaths from other preventable malnutrition illnesses increasing by 2.7% per year (Smith et al., 2015).

Among indirect benefits of pollination is the aesthetic importance attributed to biodiversity, which can represent a link between humans and nature (Goldman, 2010). The value of pollinators in terms of heritage is key to preserving pollination services and to pollinator conservation (Hill et al., 2019). Studies have shown that natural environments with a high diversity of plants and grasses are greatly appreciated by the public (Lindemann-Matthies et al., 2010), and that the absence of nature can produce negative effects on human mental health and wellbeing, even in people that do not feel particularly affiliated with it (Grinde & Patil, 2009). The presence of pollinators and flowers they pollinate is perceived as aesthetically pleasant (*e.g.* Soini & Aakkula, 2007; Hanley et al., 2015). For instance, Junge et al. (2015) highlighted how Swiss inhabitants had a high preference for, and found appealing, environments full of flowers, and Breeze et al. (2015) demonstrated that the UK public had a general feeling of care for conservation of bees and services they produce. Moreover, Sumner et al. (2018) showed that the value of bee pollination is understood even by people with no high interest in nature, and that bees are much more appreciated than other insects such as wasps and flies.

Bees have also been historically important parts of different cultures all around the world (Hill et al., 2019), inspiring poetry, literature, art, and becoming symbols of nations and communities (IPBES, 2016). They have also been positively portrayed in films and other art, contributing to increasing the level of engagement with the public (Duffus et al., 2021) and to raising awareness about their importance (Prendergast et al., 2021).

Bee products such as honey and propolis are also widely used to alleviate inflammations and infections, thanks to their vitamin content and antioxidant properties (Kumar & Bhowmik, 2010; Stojko et al., 2021).

1.2. Diversity of pollinators

The term 'pollinators' refers to a wide range of vertebrate and invertebrate animals that visit crops and act as pollen vectors (Ollerton, 2017; Ratto et al., 2018).

Vertebrate pollinators include a few birds, bats, rodents, and reptiles (Ollerton, 2017; Ratto et al., 2018), providing more than 1,000 pollinating species (Ollerton, 2017). The most diverse vertebrate pollinators are

birds (Ollerton, 2017), with more than 900 species involved in pollination, while the major mammal pollinators are bats, pollinating more than 500 plant species globally (Ratto et al., 2018).

Insects constitute the majority of invertebrate pollinators (Ollerton, 2017). When referring to insect pollinators, the four most extensive orders are Lepidoptera, Coleoptera, Hymenoptera, and Diptera (Wardhaugh, 2015; Ollerton, 2017). To date, Lepidoptera are the most diverse with more than 140,000 species (Ollerton, 2017).

Bees (Hymenoptera), comprising more than 20,400 species (Engel et al., 2020), are the most dominant pollinators in most systems worldwide (Ollerton, 2017). Due to its favourable climatic conditions and proximity to Africa and the Middle East, Europe supports around 10% of the world bee diversity; in particular, the Mediterranean basin has an exceptionally high richness of bee species, while such trends tend to decline with higher latitudes and towards the north-east (Nieto et al., 2014).

Solitary bees comprise the majority of bee species, while bees displaying social behaviours are less common (Engel et al., 2020), and constitute only the 6% of bee species (Danforth, 2007). Moreover, while the majority of species around the world are wild, a very small number of bee species are managed (IPBES, 2016). The species responsible of pollinating the majority of crops that rely, at least partially, on animal pollination, are the social species *Apis* spp. and *Bombus* spp. (Wardhaugh, 2015). The western honeybee, *Apis mellifera*, is the most abundant managed pollinator in the world, characterised by its high versatility in pollinating (Klein et al., 2007). To date, it is estimated to visit more than 50% of animal-pollinated crops, although on an individual basis it may not be the most effective pollinator of many crops (IPBES, 2016). Bumblebees, *Bombus* spp., account for about 250 species (P. H. Williams, 1998), including both managed and wild. Together with honeybees, they contribute to the enhancement of yield of many major food crops, such as apples, oilseed rape, sunflower, soybean, and strawberry (Klein et al., 2007). Additionally, bumblebees are also commonly used to pollinate greenhouse crops, such as tomato, thanks to their buzzing-pollinating behaviour and resistance to colder temperatures (Ahmad et al., 2015). In Europe, the main species being bred and commercialised is *Bombus terrestris* (IPBES, 2016).

1.2.1. Importance of bees as pollinators

To measure the effectiveness of bees as pollinators, it is important to consider various aspects, among which their abundance in the environment, their ability to carry pollen, distances that are able to cover, and specific interactions with plants (Rodriguez-Rodriguez et al., 2013; Ollerton et al., 2017).

Although bees are not as diverse as Lepidoptera, they are the most dominant pollinators, and the only ones that completely rely on floral resources during all life stages – from larval to adulthood (Ollerton, 2017) – outweighing any other pollinator in terms of visitation frequency and distances travelled between flowers

(Willmer, 2011). Typically, bees can travel as far as 3-5 km to forage, and have optimal navigation abilities which allow them to safely return to their homes after each trip (Pahl et al., 2011; Osborne et al., 2008a).

Thanks to being hairy, bees can accumulate good quantity of pollen particles that subsequently get moved to their back legs and transported to the nest (Amador et al., 2017). Bees are also capable of using the so-called 'buzzing behaviour', producing vibrations that help pollen collection from flowers characterised by tubular anthers, which do not offer a free and easy access to pollen (Michener, 1962).

Finally, bees and plants are involved in complex mechanisms mediated by specific morphological traits that make some bees particularly effective in pollinating certain types of plants; for example, bee species characterised by tongues of short length are more efficient foragers of short-tubed flowers, while medium- and long-tubed flowers benefit more from long-tongued bees which better exploit their pollen and nectar resources; such mutual mechanism both favours plant reproduction and promotes diverse bee populations (Ranta & Lundberg, 1980; Roof et al., 2020).

1.3. Status and trends of bees

According to the UN, the global human population is expected to rise to ~9 billion by 2050 (UN, 2004). Therefore, to sustain such growth, the demand for bee pollination services and agricultural practices are predicted to increase and intensify, with land areas dedicated to pollination-dependent crops having expanded worldwide (Aizen et al., 2008, 2019), and the degree of dependence on pollination services has been growing (Potts et al., 2016; Aizen et al., 2019).

However, in recent decades, wild bee declines have been recorded globally (*e.g.* IPBES, 2016; Powney et al., 2019), and given the multiple benefits of pollination, this is a worrying perspective. Pollinator trends highly differ according to the species and geographical areas (Ollerton, 2017), with declines mainly documented in the north-west areas of Europe and North America (Potts et al., 2016). The European Red List of Bees reports that ~9% of species are threatened by extinction, ~7% have been declining, and >12% are stable, while <1% are increasing (Nieto et al., 2014). Of particular concern is that 56% of species are data deficient, suggesting that the percentage of threatened species may be higher than estimated (Nieto et al., 2014).

1.3.1. Solitary bees

According to Danforth et al. (2019), solitary bees comprise the majority of threatened species worldwide, and are also the most 'Data Deficient' group, with more than 500 species belonging to Andrenidae and Megachilidae families that cannot be classified. In Europe, the Andrenidae family constitute more than 20% of bee species diversity and hold an important role in pollinating numerous plants, therefore the lack of taxonomic data represents a significant knowledge gap, preventing a clear picture of the status and distribution of many taxa (Nieto et al., 2014). This could be caused by multiple reasons, such as missing information about taxonomy, lack of taxonomic expertise in the southern hemisphere, or difficulty in finding

rare species to sample (Carvalheiro et al., 2013). Moreover, more specific data on distribution and numbers of bee species may be available for some EU nations (*e.g.* Germany, the Netherlands, and the UK), but many others are data deficient (*e.g.* south-east countries in Europe: Greece, Bulgaria, Albania) (Nieto et al., 2014). For instance, Biesmeijer et al. (2006) showed evidence of declines in solitary bee species richness, particularly specialist bees with specific foraging and nesting requirements, in both the Netherlands and the UK. Additionally, through the analysis of past records, Ollerton et al. (2014) reported the extinction of 11 solitary bee species in the UK, though at least 1 is believed to have recently re-established (Sirohi et al., 2015). As a result, several solitary bee species have become important priorities for biodiversity conservation in the UK: *Andrena ferox*, *Andrena tarsata*, *Anthophora retusa*, *Colletes floralis*, *Colletes halophilus*, *Eucera longicornis*, *Lasioglossum angusticeps*, *Nomada armata*, *Nomada errans*, *Osmia inermis*, *Osmia parietina*, *Osmia uncinata*, and *Osmia xanthomelana* (UK BAP, 2007).

1.3.2. *Apis mellifera*

Apis mellifera, the western honeybee, counts to date 31 subspecies, 15 of which can be found in Europe and the Caucasus region (Fontana et al., 2018). Although *A. mellifera* represents the most abundant managed bee (Klein et al., 2007), its presence in the wild in Europe is currently unknown, and is therefore listed as 'Data Deficient' in the European Red List of Bees (Nieto et al., 2014).

In recent decades, several studies have reported cases of honeybee colony losses in Europe (*e.g.* Neumann & Carreck, 2010; Potts et al., 2010b; Chauzat et al., 2013). For instance, surveys conducted in Switzerland between 2003 and 2009 revealed anomalous beehive winter losses in four years, equal to the double or the triple of regular losses (Charrière & Neumann, 2010). During the winter of 2007-2008, 30-40% colony losses in Northern Italy and 10-30% in the centre and south were reported (Mutinelli et al., 2010), accompanied by about 30% losses in England (Aston, 2010) and 13% in Austria (Brodschneider et al., 2010). In the latest COLOSS survey (Prevention of honeybee COlony LOSSes, 2012), Gray et al. (2020) showed an overall rate of winter colony losses in Europe equal to 14.5% between 2018 and 2019, of which about 10% were caused by dead or empty colonies. High mortality rates and health disorders among bee colonies are of notable concern, and can lead to a significant increase in beekeeping expenses, as it is necessary to invest in sanitary practices to avoid colony losses (Breeze et al., 2017; Gray et al., 2019). Such increased costs are thought to be a major factor contributing to long-term declines in honeybee colony numbers across Europe (Potts et al., 2010b).

Despite such evidences of colony declines, the FAO shows an approximately 50% increase in the world stocks of beehives from 1961, and a rise in European stocks from 2010 onwards (FAOSTAT 2022). This is likely caused by globalisation, which is increasing the agricultural demand for pollinators and their services (Potts et al., 2010b). Furthermore, FAO assessments only consider the number of hives estimated in a chosen year, and do not take into consideration records of beehive losses, which may influence the overall results (Potts et al.,

2010b). However, even such a growth may not sufficiently satisfy the demand of pollination services in the future, which in Europe is increasing nearly 5 times faster than the available beehives (Breeze et al., 2014).

1.3.3. *Bombus* spp.

The European Red List of Bees provides a detailed overview of European population trends and status of *Bombus* spp., for which much more is known than other wild bee species. Sixty-eight of the 250 bumblebee species are found in Europe, and among them, 26% are classified as threatened, more than 45% are in decline, in contrast to nearly 30% which are stable, around 13% that are increasing, and 12% for whom data is not sufficient to estimate trends (Nieto et al., 2014). *Bombus terrestris* bees are examples of 'Least Concern' bumblebees, and are defined as some of the most abundant and common bumblebee species in Western Palaearctic, with its domestication contributing to its high widespread (Rasmont et al., 2008).

The UK hold an extensive set of data on the long term distribution of bumblebees, allowing a clearer view of their status and trends than most other European countries (Casey et al., 2015). An important source of information is the Bumblebee Conservation Trust, which set up the monitoring programme 'BeeWalk' to record the presence of bumblebees in the territory (Bumblebee Conservation Trust, 2008). From such monitoring schemes, reports on the abundance of *Bombus* spp. are produced every year, indicating the trends of each detected species, which may show an increase or decrease in numbers (*e.g.* most recent report: Comont et al., 2021).

Two bumblebee species that went extinct in the UK are *Bombus cullumanus* and *Bombus subterraneus* (Ollerton et al., 2014). While the former was last recorded in 1941 (Goulson, 2003), the latter was last observed in 1988, but has since been reintroduced (Ollerton et al., 2014). The UK count 25 known bumblebee species (Goulson, 2005). Among them, 8 are still common and widespread (*i.e. Bombus hortorum*, *Bombus hypnorum*, *Bombus jonellus*, *Bombus lapidarius*, *Bombus lucorum*, *Bombus pascuorum*, *Bombus pratorum*, and *Bombus terrestris,* Comont et al., 2021), and are all listed as 'Least Concern' in the European Red List of Bees (Nieto et al., 2014), while 7 species are conservation-priority: *Bombus distinguendus*, *Bombus humilis*, *Bombus muscorum*, *Bombus ruderarius*, *Bombus ruderatus*, *Bombus subterraneus*, and *Bombus sylvarum* (UK BAP, 2007). *Bombus sylvarum* is one of the rarest and most endangered species in the UK (Goulson et al., 2006; Fitzpatrick et al., 2007), while it is regarded as 'Least Concern' in the European List of Bees.

1.4. Threats to pollinators

Given the importance of bees as providers of pollination, the evidence of wild pollinator declines worldwide (*e.g.* IPBES, 2016; Powney et al., 2019; DEFRA, 2019), coupled with the decline of managed honeybees in Europe (*e.g.* Potts et al., 2010b; Gray et al., 2020), is a cause of rising concerns. To date, there is strong evidence of the presence of multiple individual pressures on pollinators, which may also interact with each other and influence their impact magnitude (*e.g.* González-Varo et al., 2013; Goulson et al., 2015; Siviter et

al., 2021a). Direct drivers of pollinator declines may include: i) land cover, configuration, and management; ii) the use of pesticides; iii) climate change; iv) the spread of parasites, pathogens, and diseases; v) invasive alien species; and vi) genetically modified crops (IPBES, 2016; DEFRA, 2019; Dicks et al., 2021).

1.4.1. Land cover, configuration, and management

The intensification of agriculture to sustain trends of human population growth has inevitably led to losing natural habitats in favour of improved farmland (Potts et al., 2010a), constituting one of the main drivers of pollinator declines worldwide (Dicks et al., 2021). In fact, higher proportions of natural and semi-natural habitats (SNH) in the landscape surrounding agricultural fields have often been linked to a higher bee abundance (*e.g.* Nayak et al., 2015; Bartholomée et al., 2020; Raderschall et al., 2021) and richness (*e.g.* Le Féon et al., 2010; Ricketts et al., 2008; Schurr et al., 2021). For example, Carvalheiro et al. (2010, 2011) showed that the abundance and richness of bees in agricultural fields dropped at 500-1000m distance from SNH, and a field study by Bartholomée et al. (2020) demonstrated that, when the distance between the target orchard and the closest grassland patches increased, the abundance of pollinators decreased. This is explained by the fact that natural and semi-natural areas are characterised by a more diverse and continuous presence of suitable nesting and foraging resources, which may not be offered by cropland all year round (Westphal et al., 2003). With an increasing use of mass flowering crops, species that are less susceptible to landscape variations may be advantaged at the expense of pollinators that are more linked to habitat specificity (Grünewald, 2010; Vanbergen et al., 2013; DEFRA, 2014). For instance, while mass-flowering crops may help colony growth and density of generalist bee species, such as *B. terrestris* and *A. mellifera* (*e.g.* Westphal et al., 2009; DEFRA, 2014; Gervais et al., 2020), specialist pollinators, like many wild bees, are more strictly connected with natural habitats due to their specific dietary and nesting requirements (*e.g.* Rollin et al., 2013; Hanley et al., 2014; Kämper et al., 2016), and may be negatively affected by increasing cropland to the detriment of natural habitat areas (Kline & Joshi, 2020).

Several studies have linked lower bee abundances and richness to higher proportions of cropland in the surrounding landscape (*e.g.* Diekötter et al., 2010; Senapathi et al., 2015; Shaw et al., 2020), showing that land management plays a very important role in pollinator declines (Senapathi et al., 2017). In fact, it can affect the availability of foraging and nesting resources (Kovács-Hostyánszki et al., 2017), and high agricultural inputs may negatively impact pollinators (Bartholomée et al., 2020), threatening their abundance and survival (Dicks et al., 2021).

Past studies have highlighted that fields characterised by organic management and high vegetation diversity have recorded a higher bee abundance and richness as opposed to conventionally-managed fields with lower plant diversity (*e.g.* Kennedy et al., 2013; Lichtenberg et al., 2017). The meta-analysis of Tuck et al. (2014) showed that organic farming may be able to increase species richness by 30% when compared to conventional farming, and Andersson et al. (2014) quantified the positive impact of organic management on

field bean crop yield, with higher numbers of developed pods found on organic farms, and higher numbers of developed beans per pod on organic farms with higher proportions of SNH in the landscape. Moreover, other management practices, such as tillage and the use of fertilisers, may reduce the availability of in-field flowering plants and vegetations, consequently penalising both pollinator abundance and the delivery of their pollination services (Kovács-Hostyánszki et al., 2017; DEFRA, 2019).

In fact, heterogeneous landscapes including hedgerows and flower-rich margins have been shown to support pollinator communities with the provision of foraging and nesting resources (*e.g.* Miñarro & Prida, 2013; Morandin & Kremen, 2013; von Königslöw et al., 2021), with positive implications for crop yield (Castle et al., 2019). Studies have even underlined a positive influence of local floral resources, such as flowering strips, on the growth and reproduction of bumblebee colonies (*e.g.* Herrmann et al., 2017; Adler et al., 2020; Bommarco et al., 2021). For instance, Gardner et al. (2021) showed that ground-nesting bumblebee colonies were larger in size in correspondence with landscapes rich in boundary features, like hedgerows and margins full of flowers. Such landscape components have been observed to be particularly important in intensively-managed fields, as they constitute natural corridors for insect pollinators, improving their movements, the delivery of their pollination services (Van Geert et al., 2010), and contrasting habitat fragmentation, which may isolate bee species leading to more limited populations (DEFRA, 2014).

The global trends toward urbanisation may also have an impact on beneficial insects, although the evidence of such impacts is contrasting; on the one hand, urbanisation may contribute to the decrease of natural habitats (*e.g.* Goulson et al., 2015) and pollinators (*e.g.* Desaegher et al., 2018), and on the other hand it may provide alternative nesting and foraging resources in the form of gardens and other green spaces (DEFRA, 2014; Hall et al., 2016). For instance, in landscapes dominated by agricultural lands, bee abundance and richness have been shown to be higher in closer proximity to gardens (Samnegård et al., 2011) and in urban areas as opposed to farmlands (Baldock et al., 2015), most likely due to the lower pesticide exposure (Samnegård et al., 2011) and to the fact that urban areas may be capable of providing pollinators with a continuity of flowering resources coming from plants flowering at different times of the year (Osborne et al., 2008a; Baldock et al., 2015). Additionally, Osborne et al. (2008a) showed that nests of different bumblebee species had higher densities in urban gardens than in grasslands and woodlands, suggesting that urban areas may offer more diverse and suitable nesting (*e.g.* wall cavities, soil, trees) and foraging resources (native and non-native flowering plants) than those offered by croplands (Osborne et al., 2008a).

1.4.2. The use of pesticides

With the advent of modern agriculture and intensive farming practices, we have witnessed an increasing use of pesticides, including insecticides, fungicides, and herbicides, to counteract pest plants, insects, and plant diseases (Lopez-Uribe et al., 2020). Despite not being their target, bees are often directly or indirectly exposed to pesticides or their metabolites, with consequences on their health and survival (IPBES, 2016).

Such exposure can occur by different routes; (i) ingestion of contaminated water, nectar, or pollen; (ii) a direct contact with the pesticide spray; or (iii) indirect contact with sprayed flowers, leaves, or residues in soil, dust, or water (IPBES, 2016). The exposure to pesticides causes different effects on bee health, which range from lethal to sub-lethal (IPBES, 2016; Havard et al., 2019) and vary according to the pesticide type, exposure levels, and bee species (Siviter et al., 2018b). Additionally, the choice of experimental designs can significantly influence the response of bees to pesticides; if, on the one hand, field experiments are more difficult to control and replicate, and small-size effects of pesticides can sometimes be buffered by the multitude of other variables that can influence bee responses (*e.g.* environment, weather), on the other hand laboratory studies tend to overestimate pesticide exposure, often using a single dose rate instead of multiple doses to mimic the natural pesticide degradation, and concentrations may not always be field-realistic (Carreck & Ratnieks, 2014). Therefore, the choice of pesticide dose and concentration to assess effects on bees are of crucial importance to level the dissimilarities between laboratory and field studies (Carreck & Ratnieks, 2014; Vanbergen, 2021).

While pesticide lethal effects arise with a reduced survival, sub-lethal effects are more difficult to detect, and include changes in foraging performance and behaviour (Siviter et al., 2018b). Moreover, since conventional agriculture resorts to multiple products to target pest insects, plants, and fungal diseases (Lopez-Uribe et al., 2020), interaction between different pesticides may occur, further impairing the health of beneficial pollinators (see section 1.4.7.1.).

1.4.2.1. Insecticides

Insecticides are a category of pesticides utilised to target herbivorous insect pests (Cullen et al., 2019). They comprise different classes, among which neonicotinoids (*e.g.* Lundin et al., 2015), pyrethroids (*e.g.* Mužinić & Željježić, 2018), and sulfoximines (*e.g.* Sparks et al., 2013).

Neonicotinoids are highly versatile insecticides characterised by a long persistence and efficacy at low concentrations, which made them become the most widely used worldwide insecticides since their introduction in the market in the 1990s (Sgolastra et al., 2020). In fact, neonicotinoids are systemic insecticides, meaning they are transported throughout the plant tissues after being absorbed by its roots and leaves, thanks to their high solubility in water (Singla et al., 2020). However, it is well-established that they cause negative effects on bees, including impacts on foraging activity (*e.g.* Gill & Raine, 2014; Stanley et al., 2015a; Siviter et al., 2021b) and learning and memory abilities (*e.g.* Muth et al., 2019; Samuelson et al., 2016; Siviter et al., 2018b). For instance, Muth et al. (2019) observed that imidacloprid-treated bumblebees showed an impaired olfactory learning, accompanied by a lower motivation to forage, and Fischer et al. (2014) linked the exposure of honeybees to imidacloprid, clothianidin, and thiacloprid to a reduced rate of successful returns to the beehives, demonstrating an effect on homing flight abilities. Moreover, Gill & Raine (2014) highlighted that, after a first small impact on pollen collection, the extended exposure to imidacloprid caused

a chronic drop in bumblebee foraging performances, and Stanley et al. (2015a) showed that thiamethoxam affected both bumblebee visitation rate and pollen collection.

Several studies have also observed that the exposure to neonicotinoid insecticides resulted in a lower bumblebee colony fitness, with diminished production of males and queens (*e.g.* Gill et al., 2012; Rundlöf et al., 2015; Wintermantel et al., 2018), and in lower numbers of workers (*e.g.* Gill et al., 2012; Whitehorn et al., 2012; Rundlöf et al., 2015) with a reduced lifespan (Fauser-Misslin et al., 2014). Moreover, neonicotinoids have also been proven to negatively impact the growth of bumblebee colonies, with a lower weight gain compared to untreated ones (*e.g.* Whitehorn et al., 2012; Rundlöf et al., 2015). These findings underline that neonicotinoids may affect worker foraging success (Rundlöf et al., 2015) and colony initiation due to a decrease in queen production (Baron et al., 2014). Furthermore, an effect on the number of workers may consequently cause inadequate brood care (Gill et al., 2012; Rundlöf et al., 2015).

The impact of neonicotinoids on bee health and behaviour can also translate into an effect on crop yield; for example, Hokkanen et al. (2017) showed a decrease in yield linked to higher neonicotinoid usage as seed dressing, and Stanley et al. (2015a) reported a 36% reduction in seed production when apple trees were pollinated by bumblebee colonies exposed to field realistic doses of thiamethoxam.

The several negative effects of neonicotinoid insecticides have driven the European Union to restrict and then ban imidacloprid, clothianidin, and thiamethoxam from the market in 2013 (EU, 2013; Sgolastra et al., 2020). In fact, such neonicotinoids have been proven to be highly toxic to bees at low concentrations, with an acute contact LD_{50} of 0.081, 0.0443, and 0.024 µg/bee respectively (EFSA, 2013a, 2013b, 2013c).

Pyrethroids are another frequently used class of insecticides, they are broad spectrum, and are derived from pyrethrins (Mužinić & Želježić, 2018). Despite their common use, they have drawn much less attention than neonicotinoids, and have therefore been significantly less investigated (Christen & Fent, 2017). However, similarly to neonicotinoids, they have been shown to cause negative effects on non-target insects, including impacts on bee foraging activity (Shires et al., 1984; Decourtye et al., 2004), learning (Decourtye et al., 2004, 2005), and physiology (Bendahou et al., 1999; Christen & Fent, 2017). For instance, the experiment of Christen & Fent (2017) on honeybees showed that cypermethrin affected the expression of *vitellogenin,* a protein able to regulate worker foraging performance and oxidative stress, suggesting implications for bee foraging behaviour and longevity. Moreover, Decourtye et al. (2004) found that deltamethrin significantly increased honeybee worker mortality, contrary to imidacloprid, while a similar impairment of foraging activity was found with both insecticides.

Following the EU ban of the three neonicotinoids, it has become important to find new, safer, and effective alternatives (Siviter et al., 2018a; Azpiazu et al., 2021). The trend is currently heading toward greater use of

sulfoxaflor, the first marketed sulfoximine insecticide, that has a lower toxicity than clothianidin, imidacloprid, and thiamethoxam (acute contact LD_{50}=0.379 µg/bee, EFSA, 2014).

Sulfoxaflor is a systemic insecticide with the additional ability to target some neonicotinoid- and pyrethroid-resistant pests, such as *Myzus persicae* (Zhu et al., 2011; Sparks et al., 2013). Although it was shown to persist in pollen, nectar, and soil for shorter periods of time than neonicotinoids (Siviter & Muth, 2020), it can still be found days after application (maximum tested period: 11 days, EPA, 2019), with consequent risk of bee exposure to the substance (Botías et al., 2015). Studies assessing its effect on non-target insects are still limited (Azpiazu et al., 2021). For example, Siviter et al. (2018a) observed that the chronic exposure of bumblebee colonies to sulfoxaflor significantly reduced the production of workers and impaired colony reproductive success with a 54% drop in the production of males. Even so, no impact on worker foraging behaviour or colony survival was found (Siviter et al., 2018a). Additionally, a sulfoxaflor effect on the number of laid eggs in bumblebee colonies was observed by Siviter et al. (2020a), suggesting that this may be due to a reduction in feeding. Siviter et al. (2019) also found no effect of acute exposure of bumblebees or honeybees to sulfoxaflor in terms of olfactory learning and working memory, however such effects have been shown in previous studies with comparable, field-realistic doses of neonicotinoids (=2.4 ppm, Stanley et al., 2015b; Samuelson et al., 2016). This might suggest a higher safety of sulfoxaflor, but still necessitates further research to be confirmed or disputed (Siviter et al., 2019).

1.4.2.2. Herbicides and fungicides

Herbicides and fungicides are two of the most used classes of pesticides worldwide (EPA, 2017; EUROSTAT, 2019). Although their sales and application loads exceed those of insecticides, their effects on beneficial insects have been significantly less investigated (*e.g.* Cullen et al., 2019; Iwasaki & Hogendoorn, 2021). However, the high fungicide and herbicide residues in pollen collected by bees are a cause of raising concerns (*e.g.* Böhme et al., 2018; Tosi et al., 2018).

While herbicides are utilised to target weeds, their use may reduce the availability of flowering plants that pollinators use as foraging resources, indirectly contributing to their decline (DEFRA, 2014; Cullen et al., 2019). Moreover, they have been found to sometimes cause sub-lethal effects on bees, although the number of studies investigating such aspects are low (*e.g.* Dai et al., 2018; Motta & Moran, 2020; Luo et al., 2021). For instance, Mengoni Goñalons et al. (2018) observed that a chronic exposure to glyphosate reduced honeybee sucrose syrup consumption and gustatory perceptions, suggesting implications for beehive development and health. Additionally, further effects of glyphosate on learning abilities of honeybees was discovered by Hernández et al. (2021) and Luo et al. (2021), who found that exposure to field-realistic dosages caused a decreased ability in retaining memorised olfactory information. This may lead to a compromised foraging efficiency, with honeybees not being able to make optimal foraging decisions based on acquired information and to effectively communicate them to other workers (Hernández et al., 2021; Luo et al., 2021).

This picture is aggravated by the impact of glyphosate on homing flight abilities, for which bees may need more time to successfully return to the hive (Balbuena et al., 2015). Therefore, not only does glyphosate seem to impact bee olfactory information, but also impair the memorisation of environmental information halting bee safe returns to the colony (Balbuena et al., 2015). Glyphosate was also observed to impact the composition of the bee gut microbiota (*e.g.* Dai et al., 2018; Blot et al., 2019; Castelli et al., 2021). For example, Motta et al. (2018) found that glyphosate-treated bees had lower beneficial bacteria colonising their gut, and such findings were confirmed with a later study that used lower glyphosate concentrations than those found in agricultural products (Motta & Moran, 2020).

Fungicides are used to target plant fungal diseases, and can be responsible of several non-lethal effects on bees (Cullen et al., 2019); in fact, since they are often sprayed during crop flowering, bees are highly exposed to them (Iwasaki & Hogendoorn, 2021; Krichilsky et al., 2021). For instance, Degrandi-Hoffman et al. (2015) observed that honeybees ingested less boscalid-contaminated pollen compared to non-contaminated one, also digesting less proteins, suggesting that this fungicide may reduce food palatability (Degrandi-Hoffman et al., 2015). Further studies have demonstrated that diniconazole, fludioxonil (Syromyatnikov et al., 2017), and pyraclostrobin (Nicodemo et al., 2020) can inhibit the respiration of flight muscle mitochondria in *B. terrestris* and *A. mellifera*, which translates into a lower production of ATP impacting the flight activity of foragers (Syromyatnikov et al., 2017; Nicodemo et al., 2020). Hence, fungicides may be able to affect bee foraging activity through the impairment of their flight abilities (Syromyatnikov et al., 2017; Nicodemo et al., 2020). Similarly to herbicides, the exposure to fungicides was also linked to an altered gut microbiota; for instance, Batista et al. (2020) showed that picoxystrobin produced morphological alterations in the midgut, including an increase production of apocrine, whose secretion is higher in response to a damage to the organism (Grella et al., 2019). Such interference with the normal gut microbiota composition may suggest an alteration in the absorption of nutrients in the long run, which may drive the hive to experiencing malnutritional issues (Batista et al., 2020).

Hence, although herbicides and fungicides have not been formulated to specifically target insects (Cullen et al., 2019), the described findings suggest that they may have an impact on the health and behaviour of bees, with potential detrimental consequences.

1.4.3. Climate change

Climate change, including extreme weather conditions, changes in temperatures, and rainfalls, has been reported to significantly impact the distribution of pollinators and their interaction with flowering plants (Belsky & Joshi, 2019; DEFRA, 2019).

With global warming, anomalous seasons and increased temperatures accompanied by early flowering or early pollinator appearances have been occurring, resulting in plant-pollinator mismatches (*e.g.* Bartomeus et al., 2011; Forrest, 2015; Kudo & Ida, 2013). For instance, Kudo & Ida (2013) investigated the mutualism

between *Bombus* spp. and *Corydalis ambigua*, a plant commonly pollinated by bumblebees, and observed that, due to an early beginning of spring, *C. ambigua* flowered earlier than the first bee detection, leading lo less bees pollinating the crop during its flowering period, and consequently impacting the yield with a lower production of seeds.

Such phenological mismatches, where the flowering stage no longer overlaps with pollinator flight periods, may also contribute to pollen limitations, restricting bee dietary resources, with specialist bees being more affected than generalists due to their restricted foraging requirements (Memmott et al., 2007). For instance, through a flight cage experiment, Schenk et al. (2018) showed that the specialist bee *Osmia brevicornis* produced fewer brood cells and showed a decreased survival rate with a mismatch of 3 days only, and was unable to mitigate such negative impacts. However, the generalist *O. cornuta* and *O. bicornis*, although both impacted by the mismatch of 6 and 3 days respectively, were able to adapt through the production of fewer females or nests to stabilise the number of brood cells (Schenk et al., 2018).

Responses to climate change impacts may vary depending on a species tolerance to higher temperatures (*e.g.* Kerr et al., 2015; CaraDonna et al., 2018; Maebe et al., 2021). While Maebe et al. (2021) and Kovac et al. (2014) respectively showed that *B terrestris* and *A. mellifera* thermal tolerance was very high (~50 °C), Martinet et al. (2015) observed that the tolerance of Arctic and Boreo-Alpine bumblebees, and the widespread *B. lucorum,* highly differed; in fact, *B. lucorum* was the only species showing a death rate below 30%, while the others were attested to about 50%. Moreover, with a two-year experiment, CaraDonna et al. (2018) observed that the exposure of *Osmia ribifloris* to temperatures of about 2 °C warmer than the average caused a mean ~27% and ~21% reduction in male and female body mass respectively, accompanied by a mean decrease of 42% and ~51% in male and female body fat, suggesting a lower reproductive fitness and life span (Bosch & Kemp, 2004; CaraDonna et al., 2018). Additionally, an increase in bee mortality was also recorded, reaching over 30% in the first year and 70% during the second year (CaraDonna et al., 2018).

Therefore, global warming may affect bees differently depending on their species, in terms of both mortality and fitness (CaraDonna et al., 2018; Maebe et al., 2021), and specialist bees may be more impacted than generalists by plant-pollinator mismatches (Schenk et al., 2018).

Several studies have also demonstrated that, due to climatic changes, bee populations had to shift towards the poles and to higher elevations (*e.g.* Ploquin et al., 2013; Kerr et al., 2015; Pyke et al., 2016) to keep the same, optimal, average temperatures (Pyke et al., 2016). In particular, a recent study by Soroye et al. (2020) estimated that bumblebee species have been rapidly declining in Europe and North America, and are now shifting from areas with temperatures above their thermal limit to areas that were previously closer to their cold limit, which are now experiencing warmer climates. Since the majority of studies have focussed on future changes in distributions (Urban, 2015), no clear scientific consensus has been yet reached on the extent to which climate change may pose an extinction risk to bees (DEFRA, 2019). However, according to Urban

(2015), the global risk of species extinction is expected to increase and accelerate due to the rising temperatures that have been occurring in the last decades. Therefore, considering that climate change is one of the many stressors which threaten the health of bees, it is widely regarded as a major pressure on pollinators worldwide (DEFRA, 2019; Dicks et al., 2021).

1.4.4. Parasites, pathogens, and diseases

A broad range of diseases, pathogens, and parasites have been found to affect the health of bees at the individual and colony level (IPBES, 2016). Due to the commercialisation of managed bees and beehive products (*e.g.* honey, beeswax), coupled with shifts in bee populations caused by climate change, the transmission of such diseases, pathogens, and parasites once confined to certain areas of the world have been spreading to new areas, favouring an increase in the transmission rate within the same species and between different ones, going as far as to involving wild bee populations (IPBES, 2016; Potts et al., 2016). Examples of a few important parasites, pathogens, and diseases which are found to affect bee colonies are *Varroa destructor*, Deformed Wing Virus (DWV), American and European foulbroods (AFB, EFB), and *Crithidia bombi* (IPBES, 2016).

1.4.4.1. Varroa destructor and DWV

Varroa destructor is the most common ectoparasite in honeybee hives; originating as a parasite of the Asian honeybee *Apis ceranae*, it spread to Europe in the mid-20th century (Le Conte & Navajas, 2008; Navajas, 2010). Female mites feed upon the haemolymph of both larvae and adult bees (Grünewald, 2010), causing a loss in body fat and endangering colony survival (Traynor et al., 2020). *Varroa* can be easily transmitted to nearby beehives through drifting or robbing, which are common phenomena in managed apiaries (Peck & Seeley, 2019), and necessitates regular treatment to be kept under control and prevent colony losses (Grünewald, 2010). *Varroa* mite infections contribute to debilitating the colony, reducing the lifespan of bees (Dainat et al., 2012) and making them more prone to developing other diseases (Le Conte & Navajas, 2008).

Varroa mites can act as vectors for viruses, such as the Deformed Wing Virus (DWV) (de Miranda & Genersch, 2010). While it may not be detrimental per-se, DWV infection levels in association with *V. destructor* may become high, leading to deformities and bloated abdomens (Genersch, 2010) and increased mortality rates (Ryabov et al., 2014), causing even higher colony losses (EU Reference Laboratory, 2011). The DWV has also been proven to be infective for bumblebee colonies, highlighting the fact that honeybee viruses represent a further threat for wild pollinators, without being limited to managed bees, thus contributing to their decline (Fürst et al., 2014). To date, *Varroa* destructor remains one of the main drivers of honeybee colony losses (Steinhauer et al., 2018; Thoms et al., 2019).

1.4.4.2. AFB and EFB

Among the diseases infecting honeybee hives are American and European foulbroods, arising from the bacteria *Paenibacillus larvae* and *Melissococcus plutonius* respectively (Steinhauer et al., 2018). Contrary to

EFB, AFB is responsible of producing spores that can persist for years, and is therefore considered more difficult to handle (Reybroeck et al., 2012). Due to their high contagiousness, the majority of European countries have labelled them as 'notifiable diseases' (*e.g.* Italy, D.P.R., 2006; UK, The Bee Diseases and Pest Control, 2006) and require infected colonies to be destroyed, while others allow the administration of antimicrobials to limit the infection (*e.g.* US and Canada, Reybroeck et al., 2012). However, such medications are not definitive and need to be constantly administered to keep the disease under control and to not risk any further outbreak (Genersch, 2010; Reybroeck et al., 2012).

1.4.4.3. *Crithidia bombi*

Crithidia bombi is a highly prevalent trypanostomatid pathogenic parasite that affects bumblebees (IPBES, 2016; Figueroa et al., 2019). Its infection rate is estimated to reach the 80% (Shykoff & Schmid-hempel, 1991; Gillespie, 2010), and can be transmitted either via faeces or orally (Figueroa et al., 2019). Although it was shown to be relatively benign when circumstances are favourable, it can impact the survival of the colony in stressful environments (*e.g.* adverse weather conditions, nutritional stress, Schaub, 1994; Brown et al., 2000). For instance, the mortality rate of *B. terrestris* colonies infected with *C. bombi* was shown to nearly double when workers were starved (Brown et al., 2000).

Crithidia bombi infections have been linked to many sub-lethal effects on bumblebee health, including the impairment of cognitive abilities and foraging behaviour (Shykoff & Schmid-Hempel, 1991; Otterstatter et al., 2005; Gegear et al., 2005, 2006). For example, Gegear et al. (2005) showed that infected bumblebees took longer to enter flowers and foraging for nectar, and needed double the time to efficiently handle flowers compared to control bees. Moreover, a further study by Otterstatter et al. (2005) observed that higher *C. bombi* infections corresponded to less flower visits per minute, while non-infected bees visited an additional ~12% flowers/minute on average. Such results suggest possible negative implications for bee foraging behaviour and success.

C. bombi was also shown to affect the reproductive fitness of bumblebee colonies (Brown et al., 2003; Yourth et al., 2008; Goulson et al., 2018). For instance, with a laboratory experiment, Brown et al. (2003) found that *Crithidia bombi* infection caused a 9% reduction in *B. terrestris* colony size, which produced less workers, males, and queens, with an overall decrease in fitness of ~40%. In a later study, Yourth et al. (2008) confirmed these findings by highlighting that infected queens produced fewer males, thus impacting colony fitness. Finally, through a one-year fieldwork experiment observing 47 wild bumblebee nests, Goulson et al. (2018) confirmed that *C. bombi* infection may also be transmitted to wild bee populations, observing that wild bumblebee nests with higher infection rates were less likely to produce new queens.

1.4.5. Invasive alien species

Invasive alien species are defined as those introduced to a new environment either intentionally or accidentally, which successfully outperform native species, damaging local ecosystems (Convention on

Biological Diversity, 2010). Therefore, invasive species may include plants, insect pests and predators, and also alien bees (DEFRA, 2014).

1.4.5.1. Alien plants

Although non-native plants have been shown to offer additional nectar and pollen resources to pollinators (Tepedino et al., 2008; Stelzer et al., 2010), their invasion may alter and disrupt pollinator networks, resulting in lower native plant reproduction; in fact, through competition for resources such as water, light, and space (Drossart et al., 2017), or due to their attractiveness to pollinators or high flower abundances, invasive plants may threaten and reduce the presence of native plants (*e.g.* Bezemer et al., 2014; Goodell & Parker, 2017; Gillespie & Elle, 2018), particularly when present at high densities (Dietzsch et al., 2011; Herron-Sweet et al., 2016). As a consequence, such reduction may also affect the abundance of wild bees with a high preference for pollinating native plant species (Moroń et al., 2009; Nienhuis et al., 2009; Goodell & Parker, 2017). For instance, Moroń et al. (2009) observed that alien goldenrod plants in Poland negatively affected the abundance and diversity of both wild bees and native plants, suggesting that conservation measures should be put in place to protect native bee and plant communities from the invasion of alien plant species.

Additionally, invasive plants may not always provide bees with the necessary nutritional content (*e.g.* amino acids), potentially affecting their health and growth (Vanbergen et al., 2018). For example, Praz et al. (2008) showed that alien plants offering pollen and nectar with suboptimal nutritive ingredients impaired bee larval growth and survival. This may be caused by poor protein or carbohydrate content, or by the lack of enzymes responsible of digesting cellulose allowing access to nutrients (Praz et al., 2008). Pollen and nectar may also contain secondary compounds which are toxic to bees; for instance, Arnold et al. (2014) observed that *Bombus terrestris* colonies that were fed with pollen containing the compound D-lupanine, found in *Lupinus* crops, produced less and smaller workers than untreated colonies. Given the fact that *Lupinus* crops are usually exploited by many bumblebees for their foraging resources, these findings highlighted the potential impact of alien plants on the diet of pollinators when introduced to new environments (Vanbergen et al., 2018).

1.4.5.2. Alien insect pests and predators

The biological invasions of alien pests and predators may threaten the health and survival of local bee populations (*e.g.* DEFRA, 2019).

The small hive beetle *Aethina tumida* (SHB) is an invasive coleopteran imported from Africa from the 1990s to several countries, including America, Asia, Australia, and parts of southern Europe (Neumann et al., 2016). It represents an example of insect pest, particularly affecting honeybee hives; in fact, SHB is able to exploit the beehive through feeding on pollen, nectar, and bee brood, and can potentially be detrimental for the colony, particularly if further weakened by diseases and parasites (Grünewald, 2010; Sabella et al., 2022). Different treatments against SHB do exist, ranging from insecticides to traps and organic acids, although some

may not represent safe alternatives for bees (Sabella et al., 2022). Thus, a constant beehive monitoring by beekeepers is necessary to prevent or reduce infestations (Sabella et al., 2022), and in some countries, *A. tumida* must be notified to the Government (*e.g.* GOV UK, 2012; EU, 2018), and beehives destroyed (EU, 2021a).

The small hive beetle can also infect bumblebee colonies (*e.g.* Neumann & Elzen, 2004; Neumann et al., 2016; Sharma et al., 2021). In the US, Spiewok & Neumann (2006) observed that managed colonies of *Bombus impatiens* got infested in the field, although some colonies prevented a mass SHB reproduction. Since *A. tumida* is able to reduce successfully reared sexuals and, in severe cases, impact colony survival, it may represent a concrete threat to wild bumblebee colonies, which, contrary to honeybee hives, are not continuously monitored and treated against pests by beekeepers (Spiewok & Neumann, 2006; Roth et al., 2022).

The Asian Hornet (*Vespa velutina*) is an example of an alien predator unintentionally introduced to Europe from Asia in 2004, when it was first recorded in France (DEFRA, 2019). Just like *A. tumida*, *V. velutina* is a notifiable pest in the UK (GOV UK, 2012. Updated: 2021).

The spread of *V. velutina* in Europe and non-native Asian regions is thought to have been driven by its climatic preferences and requirements, and global warming is expected to exacerbate the situation (Laurino et al., 2020). *Apis mellifera* represents an easy prey for Asian hornets, since it is unable to adopt defence mechanisms to protect the hive against them (Couto et al., 2014; Franklin et al., 2017). Moreover, the abundance of bees living in beehives and the odour of beehive products (*e.g.* pollen, nectar) are further contributing to making honeybees attractive targets (Couto et al., 2014). When attacking honeybees, *V. velutina* stays outside the hive, grabbing returning foragers and feeding on them (Laurino et al., 2020). Its disturbance at the hive entrance may lead to the so-called 'foraging paralysis', where bees pause their foraging activity (Monceau et al., 2018; Requier et al., 2019), and to a higher probability of homing flight failures (Monceau et al., 2013; Requier et al., 2019). Such effects may contribute to increasing winter colony mortality, as the beehives will not have collected enough foraging resources to survive (Requier et al., 2019). In addition to honeybees, the Asian hornet may also prey on other wild pollinators depending on their abundance in the environment (Rome et al., 2021), and compete with them for foraging resources (DEFRA, 2019).

1.4.5.3. Alien bees

Generalist managed bees, such as *A. mellifera* or *B. terrestris*, are characterised by high adaptability to different landscapes and crops (Potts et al., 2003), and when introduced to a new environment they can potentially deprive wild, native bees of foraging resources (Vanbergen et al., 2013). Although also solitary species can be managed (*e.g. Osmia bicornis*, IPBES, 2016), social species such as honeybees and bumblebees are managed more frequently, and are likely to reach high colony densities. Thus, they will need to exploit

great quantities of foraging resources, competing with native bee communities and threatening their survival (Russo et al., 2021). For example, Thomson (2004) observed that the vicinity to managed beehives negatively affected pollen collection and reproductive fitness of the native *Bombus occidentalis*, as honeybees deprived them of the necessary pollen quantities to sustain regular larval productions.

Imported, managed bees could also potentially spread new diseases to local bee populations (*e.g.* Meeus et al., 2011; Graystock et al., 2013, 2016). For instance, there is evidence that DWV and *C. bombi* can be transmitted to wild bumblebee population from honeybee hives and managed *B. terrestris* colonies respectively (Fürst et al., 2014; Goulson et al., 2018). Moreover, Graystock et al. (2013) showed that nearly 80% of commercially reared *Bombus terrestris* bees used for their experiment were carrying different parasites, including *C. bombi*, DWV, and *Paenibacillus larvae*, which is particularly dangerous for its transmission of AFB to honeybees (Steinhauer et al., 2018). Therefore, these studies showed a concrete risk of transmitting parasites and diseases not only from managed to wild populations, but also between managed bees (Graystock et al., 2013).

The extreme abundance and dominance of alien species may also lead to over-pollination of crops (Aizen et al., 2020). For example, with a field experiment in raspberry fields, Sáez et al. (2018) showed that, while controlled, lower densities of managed *A. mellifera* and *B. terrestris* colonies increased fruit set, an excessive pollination reduced both fruit set and quality. Moreover, since wild bees have often been shown to outperform honeybees in providing efficient pollination to many crops (*e.g.* Mateos-Fierro et al., 2022), the prevalence of managed over wild bees may have negative consequences on the yield of crops they pollinate.

1.4.6. Genetically modified crops

Genetically modified crops (GM-crops) are included among potential threats to bee health, although there has been very little evidence of their direct effects on non-target insects (IPBES, 2016; DEFRA, 2019). While a few GM-crops are grown in the United States (*e.g.* soybean, cotton, corn, FDA, 2022) only one is currently authorised to be cultivated in the EU (MON810 maize, EFSA, 2021). However, in light of likely future changes in the EU legislation, it is worth investigating any potential negative effect on pollinators (DEFRA, 2019).

The majority of GM-crops have been bred to be insect resistant (IR-crops) or herbicide tolerant (HT-crops), in order to address agricultural issues related to insect and plant pests (DEFRA, 2019).

One common IR modification is represented by the expression of *Bacillus thuringiensis* (Bt) Cry toxins usually targeting lepidoptera (*e.g.* in MON810 maize, Arpaia et al., 2021). Bt-crop pollen may represent a possible direct route of exposure for bees, which may come into contact with Bt-toxins (Arpaia et al., 2021). Several studies have registered no negative effects of Bt-crops on the health and behaviour of bees (*e.g.* Dai et al, 2012, 2016; Geng et al., 2013; Hendriksma et al., 2013), including the latest EFSA report (EFSA, 2021), which confirmed the approval to cultivate MON810 Bt-maize in the EU. However, there is the hypothesis that such

crops may be able to negatively impact bee learning, foraging, and longevity (Ramirez-Romero et al., 2008; Nicodemo et al., 2018). For instance, a recent study by Nicodemo et al. (2018) observed that honeybees feeding on AG8088YG Bt-maize were characterised by a decrease in vitellogenin and lipophorin – essential proteins for bee development – by more than 30%, and by 15% in haemolymph, suggesting a consequent impact on honeybee lifespan. However, in addition to these findings, further evidence is still required before concluding that IR-crops are able to impact the health of non-target insects (DEFRA, 2019). Overall, the less traits bees and target insects share, the lower the risk for bees to be negatively affected by these crops (Potts et al., 2016).

In addition to IR-crops, some negative indirect effects on bees may come from HT-crops, due to their necessity of being regularly treated with herbicides to target unwanted plants (DEFRA, 2019). In fact, changes in herbicide management may lead to the reduction of floral resources, consequently affecting bee foraging activities (Firbank et al., 2003; Arpaia et al., 2021). In this regard, few studies have so far investigated and underlined that herbicide-tolerant crops may be able to affect bee abundance (Hawes et al., 2003; Bohan et al., 2005; Morandin & Winston, 2005). However, since such evidence is still small, it is not yet sufficient to understand the severity and scale of HT-crop effects on bees in the long run (IPBES, 2016).

1.4.7. Threat interactions

In the wild, it is unlikely that bees would be exposed to individual pressures; instead, there are usually multiple threats that have to be faced simultaneously (Goulson et al., 2015). Because of this, the literature has recently started to focus more on the potential interactions between different pressures, although further evidence is still required to better understand interaction mechanisms and their effects on pollinators (*e.g.* Fauser-Misslin et al., 2014; Alburaki et al., 2018; Botías et al., 2020). The summary of effects of individual and combined pressures on pollinators and pollination is presented in Figure 1.4.7 (source: IPBES, 2016).

1.4.7.1. Pesticide-pesticide interactions

Conventional agricultural practices usually need to rely on multiple pesticides to tackle insect and plant pests, and in a recent review including 90 studies, Siviter et al. (2021a) observed that the interaction between pesticides tend to be synergistic, increasing their impact magnitude and, therefore, causing even more serious damages to bees when they are combined than when administered in isolation. The negative effects of a combined exposure to different pesticides have been reported by several studies (*e.g.* Sgolastra et al., 2018; Carnesecchi et al., 2019; Azpiazu et al., 2021). For instance, Gill et al. (2012) found that the simultaneous exposure to the pyrethroid λ-cyhalothrin and the neonicotinoid imidacloprid increased the worker mortality of *B. terrestris* and impacted their foraging behaviour, decreasing the number of foraging trips and the amount of collected pollen. This resulted in an impaired development of brood, whose production decreased by 7% (Gill et al., 2012). Moreover, Sgolastra et al. (2018) observed that the exposure of *O. bicornis* to the neonicotinoid clothianidin and the fungicide propiconazole altered their feeding

behaviour, decreasing the consumption quantity of contaminated syrup. Such combined exposure additionally resulted in an impaired ovary maturation and decreased longevity, and a synergistic effect between the two pesticides was also found on post-exposure survival probability (Sgolastra et al., 2018). However, none of these effects was observed when *O. bicornis* were exposed to clothianidin and propiconazole singularly (Sgolastra et al., 2018). A further example of detrimental effects of combined pesticides was given by Sanchez-Bayo & Goka (2014), who showed that the fungicide propiconazole synergistically interacted with the pyrethroid cyhalothrin and neonicotinoid thiacloprid, highly increasing their toxicity to honeybees. Additionally, Prado et al. (2019) observed that two different pesticide mixtures, usually including fungicides, reduced *A. mellifera* flight activity and their time spent foraging, while one mixture also caused more than 40% decrease in collected pollen, suggesting that such impact on bee behaviour may lead to a lower foraging efficiency.

Past studies have also suggested an effect of pesticide interactions on the growth and reproductive success of bumblebee colonies (*e.g.* Gill et al., 2012; Fauser-Misslin et al., 2014; Rundlöf et al., 2015). For instance, Mallinger et al. (2015) deployed *Bombus impatiens* colonies in apple orchards and found that the exposure to pesticide mixtures, mainly including fungicides and insecticides, reduced the production of workers and males, and also led to producing smaller workers. Moreover, Botías et al. (2020) observed that the simultaneous exposure to the fungicide tebuconazole and the pyrethroid cypermethrin impaired the growth of *B. terrestris* colonies, which were also characterised by a lower number of males and queens. Such findings are in accordance with those of Rundlöf et al. (2015), who demonstrated that the neonicotinoid clothianidin and the pyrethroid β-cyfluthrin used as coating on oilseed rape seeds reduced the weight gain and production of males, queens, and workers of *B. terrestris* colonies. Impairments in the production of males and queens are of particular concern; in fact, they ensure the colony persistence over time, and are therefore an important measure of colony fitness (Goulson, 2010; Bommarco et al., 2021).

1.4.7.2. Pesticide-disease interactions

The increasing use of pesticides in agricultural systems may potentially lead to the susceptibility of bees to parasites, pathogens, and diseases (James & Xu, 2012). Several studies have demonstrated a link between *V. destructor* and the use of pesticides (Di Prisco et al., 2013; Tesovnik et al., 2019; Schwartz et al., 2020). For example, Blanken et al. (2015) observed that honeybee colonies exposed to both the neonicotinoid imidacloprid and *Varroa* mites had shorter mean flight distances than colonies solely exposed to the pesticide, suggesting that such interaction may be able to impact their ability to collect foraging resources and, consequently, halt colony survival. Moreover, Annoscia et al. (2020) demonstrated that neonicotinoids may be able to increase the proliferation of *Varroa mites* in beehives, suggesting a synergistic effect which, in Straub et al. (2019), impacted the survival of winter bees several months after the insecticide exposure. Therefore, pesticides may suppress the immune response of bees, making them more prone to being affected by parasites (Annoscia et al., 2020), and potentially reduce their survival (Straub et al., 2019).

Compared to *Varroa*, the possible interaction between pesticides and the pathogen *C. bombi* has been less thoroughly investigated, and further research is needed to clearly understand any effects from this interaction. While neither Baron et al. (2014) nor Fauser et al. (2017) reported an interaction of pyrethroids or neonicotinoids with *C. Bombi* on *B. terrestris* colonies, Fauser-Misslin et al. (2014) highlighted that the neonicotinoids thiamethoxam and clothianidin significantly interacted to reduce the longevity of the mother queen, although it did not increase the infection per-se. This suggests that such interaction may be able to impair the reproductive fitness of bumblebee colonies, which, according to Whitehorn et al. (2012), may be due to an impact on bee foraging activity.

1.4.7.3. Landscape-disease interactions

In addition to pesticides, few studies have investigated the relation between landscape and bee health disorders so far (*e.g.* Simon-Delso et al., 2014; Leza et al., 2016; Rolke et al., 2016). For instance, Alburaki et al. (2018) observed higher mite loads corresponding to apiaries positioned in agricultural lands, and Leza et al. (2016) showed that beehives with a higher proliferation of mites were linked to a lower proportion of natural habitats in the surrounding landscape. Such findings are in accordance with Simon-Delso et al. (2014), who underlined that the incidence probability of health disorders in *A. mellifera* colonies, including the *Varroa*-vectored DWV, increased with the proportion of cropland and decreased with the proportion of grassland in the landscape surrounding target fields. Higher health issues linked to agricultural landscapes may be due to the fact that such habitats pose a higher risk of pesticide exposure to bees than natural and semi-natural lands; this confirms that pesticides may lead to a reduced immune response in bees, which makes them more susceptible to infections and diseases (Poquet et al., 2016).

1.4.8. Implications for bee health management

Although bee pollination services are able to provide multiple monetary and non-monetary benefits (IPBES, 2016), managing beehives is becoming increasingly challenging, and even unprofitable for small-scale apiaries (Potts et al., 2010b) and amateur beekeepers (Breeze et al., 2017). The high incidence of health issues is pushing beekeepers to investing in sanitary products in order to control the spread of diseases in their beehives and avoid colony losses (Breeze et al., 2017; Gray et al., 2019). Using a survey for beekeepers in the UK, Breeze et al. (2017) estimated that more than 60% of beekeeping expenses were employed for the management of pests and diseases, and that the majority of respondents who provided crop pollination services were either not receiving any payment, or receiving payments that were substantially lower than the pollination service benefits theoretically provided. With the creation of the EU Reference Laboratory (EU Reference Laboratory for honeybee health, 2011), surveillance measures were put in place to directly support European beekeeping and monitor the status of beehive health (EC, 2013). However, monitoring such health issues and reasons behind them through Europe has been proven to be extremely challenging (Chauzat et al., 2013). In fact, the estimated rates of colony losses need to rely on beekeepers' reports, which may differ

in accuracy and representation depending on personal motivations and concerns (Gray et al., 2020). Moreover, while registration of apiaries is mandatory in certain countries (*e.g.* Italy), it is entirely voluntary in others (*e.g.* the UK) (Chauzat et al., 2013), thus the actual number of beehive losses and honeybees could be underestimated (DEFRA, 2014).

Additionally, tracking the spread of infectious diseases in wild bee populations is even more challenging, as they cannot be regularly checked and treated as beekeepers do with their apiaries. Hence, the evidence of a disease spill-over from managed to wild bees (*e.g.* Fürst et al., 2014; Goulson et al., 2018) or between managed bees (Graystock et al., 2013) is of particular concern.

Sustaining healthy bee populations and good beekeeping practices is therefore crucial to halt managed bee declines (Potts et al., 2016; Gray et al., 2019), and promoting methods that help monitor bee health issues may be especially important to ensure the health of both managed and wild bees (Fürst et al., 2014).

The EU has been supporting beekeeping practices through national honeybee health programmes (*e.g.* EU, 2013a; EC, 2019) and surveillance measures (*e.g.* EC, 2013), and through funding research programmes designed to monitor beehive health through new technologies (*e.g.* Chlebo et al., 2020). Examples of widely used technologies are devices able to monitor different colony parameters such as weight, temperature, and humidity, at frequent intervals (Smart et al., 2018; Chlebo et al., 2020). For example, the change in colony weight can estimate the level of food consumption in the hive, swarming events, or forager numbers (Chlebo et al., 2020), and the change in temperature and humidity can detect if the colony is able to regulate its temperature, and is therefore healthy, or if a swarming is happening (Meikle & Holst, 2015). Several are the EU projects which aim to provide new technologies that could help address beehive health issues and sustain optimal beekeeping practices. One of the main projects, which was concluded in 2015, was 'Swarmonitor'; its monitoring tool detects vibration changes within the beehives, indicating possible phenomena such as swarming and decline of beehive health conditions (Swarmonitor, 2017). Among ongoing projects, B-GOOD aims to create a 'Health Status Index' for beehives to facilitate the measurements of bee health status and provide beekeepers with guidance on optimal beekeeping practices (B-GOOD, 2019). Another example is the PoshBee project, which aims to assess, monitor, and mitigate the stressors affecting the health of bees (Brown et al., 2021). Among its objectives, PoshBee is currently developing the Bee Health Card, a monitoring tool that will detect issues in the beehives caused by pesticide exposure, poor nutrition, or diseases, allowing beekeepers to quickly tackle and address such health concerns (Brown et al., 2021).

Despite the existing programmes to support sustainable beekeeping in Europe, no study has yet explored beekeepers' views in regard to adopting such new tools. In fact, beekeepers' expertise on bee health is often underestimated (Donkersley et al., 2020), with so far very few studies addressing the need of directly investigating their knowledge on the adoption of sustainable management practices (El Agrebi et al., 2021).

However, beekeepers represent the most important end users of new tools addressing bee health concerns, and research on their perspectives towards them is an important knowledge gap in the literature.
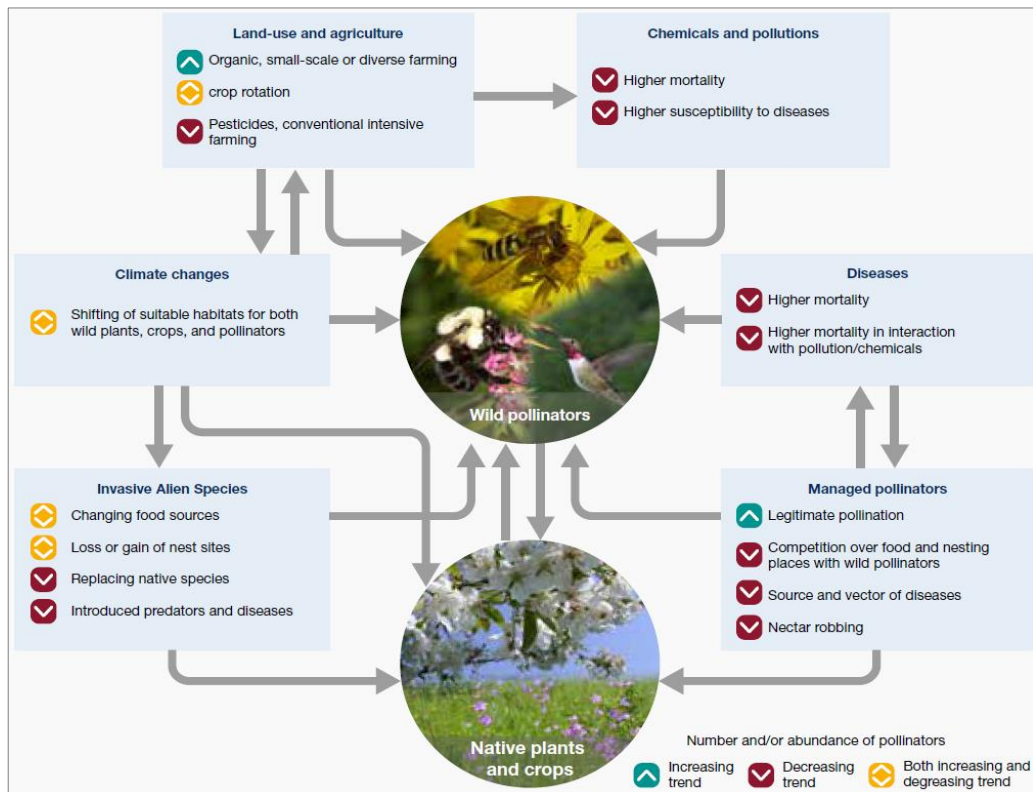
Figure 1.4.7: Summary of the effect of different pressures, alone and combined, on pollinators and their pollination on native plants and crops. Source: IPBES, 2016.

1.5. Thesis aims

With this thesis, I aim to address the following literature knowledge gaps, which were also reviewed in the paragraphs above:

1. **Effects of herbicides and fungicides on bee health and pollination.** So far, the focus of the literature has primarily been on insecticides, but non-insecticidal products have been shown to be capable of impairing bee health, and it is necessary to better understand such mechanisms (see section 1.4.2.).

2. **Interaction effects between different types and classes of pesticides, land cover (related to forage resources), parasites, and pathogens, on bee health and pollination.** Understanding how different pressures may interact between each other and impact beneficial pollinators provides a valuable contribution to the literature, that has recently started to focus more on such interactions (see section 1.4.7.).

3. **Potential impacts of the emerging insecticide sulfoxaflor and its interaction with a common pathogen on bee behaviour and pollination.** Since safer and effective alternative insecticides need to be introduced as substitutes of neonicotinoids, it is important to investigate whether sulfoxaflor, alone or in combination with other stressors, may potentially impact the behaviour of bees and the delivery of

their pollination services, contributing to the limited research that has been done on this matter so far (see section 1.4.2.1. and 1.4.7.).

4. **Beekeepers' perceptions towards a new tool for a quick detection of health issues in their beehives.** To the best of my knowledge, no study has yet addressed beekeepers' interests in regard to new technologies designed to improve the health of their bees, but it is important to take their perceptions into consideration and make sure that such instruments will be widely and effectively used (see section 1.4.8.).

The purposes of each core chapter in this thesis are summarised as follows:

Chapter 2: I investigate how cropland, semi-natural habitats, and pesticide pressures affect (i) the activity of honeybees and bumblebees; (ii) the reproduction and fitness of *Bombus terrestris* colonies; (iii) *Varroa* mite loads in honeybee hives; and (iv) the delivery of pollination services to two important food crops. Pesticide pressures were calculated using two indexes, one including all insecticides, and another including herbicides and fungicides, that were applied in the target fields. I choose to work on two separate crops to detect any difference in the effects of the above stressors, and I base my experimental design on a field-realistic approach through a large-scale experiment in the UK.

Chapter 3: I investigate if the emerging insecticide sulfoxaflor interacts with the common pathogen *Crithidia bombi* on the individual and colony behaviour of *Bombus terrestris* and the delivery of their pollination of an economically important crop. I choose a semi-field experiment in flight cages, using sucrose solutions containing sulfoxaflor in realistic concentrations that mimic its natural degradation over time.

Chapter 4 : Through an online survey, I target beekeepers of 7 European countries, and assess what potential benefits and barriers can encourage or discourage them to use the Bee Health Card, the tool designed by the PoshBee project aiming to help large-scale monitoring of health issues in beehives. In doing so, I consider two different scenarios: one including planned financial incentives, and one excluding them. I further investigate the willingness to accept extra costs related to the Bee Health Card, and the frequency of use in case it was adopted.

The first two chapters, involving large-scale and semi-field studies, focus on two main social bee species: *Apis mellifera* and *Bombus terrestris*. The choice of using honeybees is driven by the fact that they are the most employed pollinators worldwide, characterised by high versatility in pollinating many important food crops (Klein et al., 2007). However, honeybees are the main focus of scientific research, and are also typically used as target species when assessing the safety of pesticides (Siviter et al., 2018b). Thus, to investigate pressure effects related to non-*Apis* bees, I decide to additionally focus on the bumblebee *Bombus terrestris*, largely employed in both greenhouse and outdoor pollination (Wolf & Moritz, 2008) and known for its high adaptability to diverse habitats (Goulson, 2003).

All three core chapters are chained together to point to the final aim of the thesis, which is to investigate what stressors may be tackled to protect bee health, and what measures may be put in place to help address beehive health issues, halting the decline of both wild and managed pollinators (Figure 1.5.1).
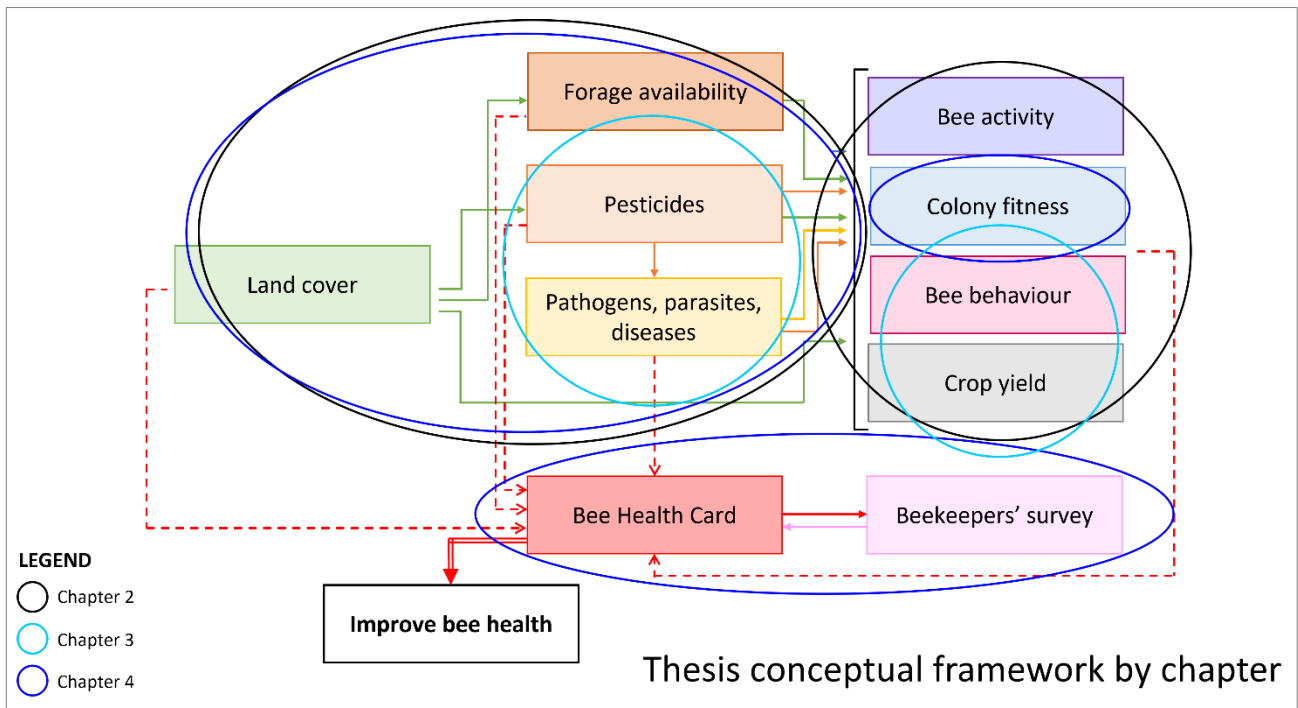
Figure 1.5.1: Thesis conceptual framework. **Chapter 2:** 'Responses of *Apis mellifera* and *Bombus terrestris* to pesticides, cropland, and woodland'. **Chapter 3:** 'Effects of sulfoxaflor, *Crithidia bombi*, and their interaction on *Bombus terrestris* behaviour and pollination services'. **Chapter 4:** 'A survey for beekeepers to investigate perceptions toward a new omics tool for bee health'.

As seen in Figure 1.5.1, this thesis will directly explore not only the impact of land covers, pesticides, and health disorders on bee behaviour, activity, and health, but also beekeepers' expertise and interest in relation to such threats and ways of facing them. In fact, although understanding how different pressures may act and interact between one another is fundamental, it is equally important to find a way to hinder them. Beekeepers hold an extremely important role in this regard, since they are in charge of monitoring managed honeybee apiaries and guaranteeing colony health; unhealthy colonies are capable of transmitting parasites, pathogens, and diseases not only to other apiaries (*e.g.* Peck & Seeley, 2019), but also to wild bee communities (*e.g.* Goulson et al., 2018), and a suboptimal environment with a high use of pesticides could affect both managed and wild bees present in the surrounding landscape (*e.g.* Rundlöf et al., 2015). Therefore, this thesis aims to investigate in parallel (i) how different pressures affect bee health, and (ii) beekeepers' thoughts on the use of a new omics tool to help with an early identification of beehive health issues; thanks to a better understanding of pressures' effects on bees and to an improved monitoring of bee health conditions, it would be possible to improve not only managed, but also wild bee health, and tackle their decline on the long run.

The research studies in this thesis were performed as part of the PoshBee project (Brown et al., 2021), in which my involvement was related to the following tasks:

1. Leading the large-scale fieldwork which took place in the UK (along with other 7 countries, each led by a different researcher) (Work Package 1). Part of the results obtained from this study were used to write Chapter 2.

2. Collaborating with Royal Holloway University of London in designing a semi-field experiment which investigates the potential effects of sulfoxaflor and its interaction with *C. bombi* (Work Package 6). Such analyses were used to write Chapter 3.

3. Designing an anonymous, on-line survey to distribute to beekeepers in 8 countries and investigate their perceptions on the Bee Health Card (Work Package 10). The results were analysed to write Chapter 4.

Chapter 2

Responses of *Apis mellifera* and *Bombus terrestris* to pesticides, cropland, and woodland.

Abstract

Pollination is an important ecosystem service able to enhance the yield and quality of many important food crops. However, evidence of pollinator declines is growing worldwide, driven by multiple stressors which may also interact with each other and influence the overall magnitude of impact. Changes in land cover and the use of pesticides represent two of the main causes of pollinator decline. Using a large-scale field study, we aimed to investigate the impact of landscape and pesticide pressures on managed *Apis mellifera* and *Bombus terrestris* sentinel bees positioned in 8 apple orchards and 8 oilseed rape fields across southern England. Sites were characterised by different proportions of cropland and woodland with a 1 km radius, and each site was attributed two toxicity indexes evaluating (i) the pressures of insecticides, and (ii) the pressures of fungicides and herbicides on bees. We then assessed (a) the activity of social bees through transect surveys, (b) the weight change of bumblebee colonies between the start, middle, and end of flowering and their reproductive fitness, and (c) *Varroa* mite infections in beehives at the end of the season. Our results showed that the growth of *B. terrestris* colonies located in apple orchards was positively influenced by the proportion of cropland and negatively influenced by the proportion of woodland in the landscape, while, surprisingly, the growth in oilseed rape fields was positively influenced by a higher use of fungicides and herbicides. Although previous studies have investigated pesticide effects on the growth of bumblebee colonies, ours is the first large-scale UK field study to take into account a toxicity index which exclusively assesses the pressures of fungicides and herbicides on bees. We recommend caution when addressing the impact of land cover and pesticides on different bee species, particularly specialist pollinators, which may differ in sensitivity to different stressors and may also be threatened by the growing presence of generalist bees competing for foraging resources. Moreover, we stress the importance of expanding the body of research related to fungicides and herbicides, and their impact on the abundance, health, and colony fitness of different bee species.

Contributions

2.1. Introduction

Pollination is an important ecosystem service benefitting about 75% leading food crops worldwide (Klein et al., 2007) and able to enhance their yield and quality (Bartomeus et al., 2014; Garratt et al., 2014a), with a total global value of $235-577 bn per year (Lautenbach et al., 2012).

The most widespread and important pollinators are bees (Potts et al., 2016; Rader et al., 2016). Honeybees (*Apis spp.*) and bumblebees (*Bombus spp.*) are among the most common pollinators to both crops and wild flowers in many part of the world (IPBES, 2016). The western honeybee, *Apis mellifera*, is the most widely used managed pollinator, estimated to visit more than 50% animal-pollinated crops (IPBES, 2016) and characterised by its high versatility (Klein et al., 2007). Bumblebees are among the most important pollinators in Europe and North America (Kleijn et al., 2015) and are therefore being progressively commercialised for their efficient pollination services (IPBES, 2016). Both honeybees and bumblebees contribute to the yield of major food crops, such as apples, oilseed rape, sunflower, soybean, and strawberry (Klein et al., 2007).

However, despite the benefits of pollination, wild bee declines have been recorded in several countries over recent decades (*e.g.* IPBES, 2016; Powney et al., 2019), and managed honeybee declines across Europe (Potts et al., 2010b; Gray et al., 2020), affecting the yield and quality of fruits and seeds (Garratt et al., 2014a; Reilly et al., 2020). The main causes of bee declines in Europe are reported to be changes in land cover configuration and land management, pressures caused by pesticides employed in agricultural lands, and the spread of pests and diseases (Dicks et al., 2021).

In Europe, much of the loss of natural and semi-natural habitats (SNH) has been a result of the expansion and intensification of agriculture (IPBES, 2016). Although mass-flowering crops are able to provide pollinators with foraging resources for short periods, they may not compensate the loss of pollen and nectar offered by natural and semi-natural lands (DEFRA, 2019). Thus, the decrease of such habitats in favour of improved farmlands may result in limited foraging and nesting resources (Potts et al., 2010a; Bretagnolle & Gaba, 2015), thereby negatively impacting bee survival (Smart et al., 2016) and the delivery of their pollination services (IPBES, 2016). The abundance and richness of bees in agricultural fields is positively linked to the nearby presence of SNH, such as grasslands and woodlands (*e.g.* Nayak et al., 2015; Raderschall et al., 2021), which provide 'high-quality' foraging resources (Kennedy et al., 2013). At the same time, bee abundance may be negatively influenced by high distances from SNH and by high areas of cropland in the surrounding landscape (*e.g.* Holzschuh et al., 2016; Bartholomée et al., 2020; Shaw et al., 2020).

Intensive agriculture usually relies heavily upon chemical pesticides, including insecticides, fungicides, and herbicides for sustainable, high yields (Lopez-Uribe et al., 2020). When foraging, bees come into contact with pesticides, which can have lethal and sub-lethal consequences (IPBES, 2016; Havard et al., 2019) that vary depending on the class of pesticides, exposure levels, and bee species (Siviter et al., 2018b).

Insecticides are utilised to target herbivorous pests, however they may have considerable implications for the health of beneficial insects which provide valuable pollination services (Cullen, 2019). Globally, neonicotinoids are the most widely used class of insecticides (Sgolastra et al., 2020a), with well-established evidence of their negative effects on bees, such as reduced reproductive success and colony growth (Gill et al., 2012; Rundlöf et al., 2015; Siviter et al., 2021b, 2021c), foraging activity (*e.g.* Gill & Raine, 2014; Tasman et al., 2020; Siviter et al., 2021b), and memory/learning abilities (*e.g.* Muth et al., 2019; Samuelson et al., 2016; Siviter et al., 2018b). As a result, three neonicotinoids are presently banned from the EU and UK markets (Sgolastra et al., 2020a). Other classes of insecticides and their impact on bees have been significantly less investigated in the literature. For instance, a review by Mužinić & Želježić (2018) highlighted that an effect of pyrethroids has been reported on honeybee foraging activity (Shires et al., 1984; Decourtye et al., 2004), learning ability (Decourtye et al., 2004, 2005), and physiology (Bendahou et al., 1999; Christen & Fent, 2017). Further research is ongoing in regards to sulfoxaflor, a newly emerging insecticide belonging to the class of sulfoximine (Sparks et al., 2013; Brown et al., 2016; Sgolastra et al., 2020), whose chronic exposure has been linked to a lower colony growth and reproductive success of bumblebees (Siviter et al., 2018a, 2020a).

Herbicides (targeting pest plants) and fungicides (targeting fungal diseases) are two of the most utilised pesticides (Krichilsky et al., 2021), outweighing insecticides in terms of sales and application (EPA, 2017; EUROSTAT, 2019). Despite studies investigating the impacts of fungicides and herbicides on managed bee foraging activity (*e.g.* Sprayberry et al., 2013; Christen et al., 2019), current research is mainly confined to insecticides (*e.g.* Feltham et al., 2014; Muth et al., 2019; Siviter et al., 2021b, 2021c). The herbicide glyphosate is the most widely used pesticide worldwide (Benbrook, 2016), and it was shown to affect honeybee learning/memory ability (Mengoni Goñalons & Farina, 2018; Hernández et al., 2021; Luo et al., 2021), larval development (Dai et al., 2018), and gut microbiota composition (*e.g.* Blot et al., 2019; Motta & Moran, 2020; Castelli et al., 2021). Fungicides often sprayed during flowering (Krichilsky et al., 2021) are amongst the most detected residues in pollen collected by bees (McArt et al., 2017). For example, boscalid has been reported to decrease honeybee pollen ingestion (Degrandi-Hoffman et al., 2015), while pyraclostrobin may reduce their longevity (Fisher et al., 2021) and mitochondrial function, which is key to flight activity (Nicodemo et al., 2020). Similarly to herbicides, past studies have also reported an impact of fungicides on the bee gut microbiota (Batista et al., 2020; Carneiro et al., 2020).

Agricultural practices usually rely on multiple agrochemicals to reduce the damage from pests (Tilman et al., 2002), mixing different types of substances which may interact and change their impact magnitude (Mužinić & Želježić, 2018). For instance, Gill et al. (2012) highlighted the impact of two insecticides on bumblebee foraging behaviour and mortality, which led to impaired colony growth. Moreover, Azpiazu et al. (2021) outlined that a fungicide significantly reduced both honeybee and solitary bee survival when in combination with sulfoxaflor, and Botías et al. (2020) showed that the interaction of a pyrethroid insecticide and a

fungicide significantly affected the growth of bumblebee colonies, leading them to produce less males and queens. The production of reproductives (*i.e.* males and queens) is an important measure of colony fitness (Dave Goulson, 2010), as they ensure the persistence of the colony through time (Bommarco et al., 2021). Therefore, it is important to tackle and limit any threat impact on the production of males and queens in the colony (Bommarco et al., 2021). A potential link between pesticide usage and land cover has also been suggested, with Gervais et al. (2020) showing that the proportion of high-intensity cropland in the landscape affected the weight of bumblebee colonies and their longevity, which were higher with a higher proportion of low-intensity or flower-rich areas.

The use of pesticides may also influence bee immune systems, increasing their susceptibility to pathogens and diseases (James & Xu, 2012), which are a significant pressure on European honeybee populations in particular (Dicks et al., 2021). *Varroa destructor* is the most common ectoparasite in honeybee hives, contributing to colony debilitation and making bees more prone to developing infections (Le Conte & Navajas, 2008). *Varroa* mites can also act as vectors for the transmission of viruses, such as Deformed Wing Virus (DWV) (de Miranda & Genersch, 2010), therefore regular treatments with organic or chemical miticides are necessary to reduce colony losses (Grünewald, 2010). Neonicotinoids have been reported to favour *Varroa destructor* infestations reducing the immune response of bees (*e.g.* Di Prisco et al., 2013; Tesovnik et al., 2019; Annoscia et al., 2020), suggesting that pesticides and parasites may act in synergy to negatively affect the health of bee colonies (Annoscia et al., 2020). Other studies have also linked a higher mite load to a lower availability of natural flowering resources in the landscape (Leza et al., 2016) and to agricultural lands, which are at higher risk of pesticide exposure (Alburaki et al., 2018).

To date, few studies have explored the effects of combinations of different pesticide classes on bees (*e.g.* Park et al., 2015; Yasrebi-de Kom et al., 2019), with most of the literature mainly focussing on insecticides only, while fungicide and herbicide effects are much less often investigated (see Iwasaki & Hogendoorn, 2021). However, due to increased agricultural practices, the potential impact of different pesticide mixtures is becoming a cause of concern (*e.g.* Tosi et al., 2018).

Using a large-scale fieldwork experiment in sixteen sites across south England, we investigated how pesticide pressures (including not only insecticides, but also fungicides and herbicides) and land cover (including semi-natural habitats and cropland) may interact to affect the health and activity of social bees, *i.e.* honeybees and bumblebees, and the delivery of their pollination services, addressing the following research questions:

a. Is honeybee and bumblebee activity influenced by pesticide pressures and the proportion of semi-natural habitat and cropland in the surrounding 1 km landscape?

b. Are bumblebee colony weight and reproductive fitness influenced by pesticide pressures and the proportion of semi-natural habitat and cropland in the surrounding 1 km landscape?

c. Is the proliferation of *Varroa destructor* mites in honeybee hives impacted by pesticide applications and the proportion of semi-natural habitat and cropland in the surrounding 1 km landscape?

d. Is the yield of crops influenced by pesticide applications and the surrounding 1 km landscape?

The fieldwork experiment took place between April and June 2019 as part of the EU Horizon 2020 PoshBee project (Brown et al., 2021), following standardised protocols written by expert scientists involved in the programme (see Appendix 2.1).

2.2. Methodology

2.2.1. Site selection

Twenty-four candidate sites were identified in Southern England – 12 oilseed rape fields (OSR) and 12 apple orchards (APP) – and coordinates of each site centre point were sent to UFZ (Helmholtz Centre for Environmental Research, Germany), which calculated the approximate proportion of cropland within 5 km radius using *ArcGIS* v10 on the basis of the Map of European Ecosystem Types (www.eea.europa.eu). Such proportions were used as a proxy to describe pesticide exposure as a first indication to help with site selection procedures; the 8 OSR and 8 APP sites that were spread more evenly across the agrochemical use gradient were selected as target sites, with sites in the lower gradient spectrum being organic and/or surrounded by less croplands and more semi-natural habitats, and sites in the higher spectrum being characterised by conventional management and mostly surrounded by arable lands and orchards (Appendix 2.1, WP1.1.1). Eventually, 8 oilseed rape fields (OSR) of mixed varieties (*Brassica napus spp.*) and 8 apple orchards (APP) of *Gala* variety (Figure 2.2.1) were selected, with a distance of at least 3 km between each site to promote statistical independence of data and allow researchers to easily travel among sites even on the same day (Hodge et al., *in review*). While two APP sites were organic, only conventionally managed oilseed rape fields were used due to the absence of organic sites in England (Appendix 2.1, WP1.1.1).

2.2.1.1. Landscape gradient

Using Google Earth (Yu & Gong, 2011), different EUNIS habitat codes (EEA, 2016) were attributed to the land adjacent to each field boundary to generate basic landscape data (Appendix 2.1, WP1.3.1). Afterwards, a detailed characterisation of the landscape surrounding target sites was drawn by UFZ classifying the adjacent lands in 1 km radius around the targeted fields using *ArcGIS* Pro 2.4.1 based on a combination of 0.5m resolution imagery from DigitalGlobe (www.maxar.com) and 2.5m resolution imagery from SPOT (www.earth.esa.int) provided by World Imagery (Scott & Janikas, 2010). The final landscape dataset included land cover features in a 1:2500 scale, among which the total class area of arable and orchard lands, grassland, and woodland (Appendix 2.1, WP1.3.2). In order to make direct comparisons among sites, the total class area of each land type was divided by the sum of total class areas of all land types, obtaining the corresponding proportions for each site. Although details on the proportion of grassland were successfully obtained (Appendix 2.1), no distinction could be made between intensively and non-intensively managed grassland in

the original dataset[1]. This distinction is crucial, since grassland intensification practices, such as defoliation and high use of fertilisers, can reduce the availability of foraging and nesting resources for pollinators, whose abundance is linked to flower-rich and low-input grassland (Carvell, 2002; Potts et al., 2009). Therefore, it was decided to only use woodland to represent semi-natural habitats, and arable and orchard lands were merged into a single proportion of cropland to represent the areas employed as agricultural lands in the analysis.
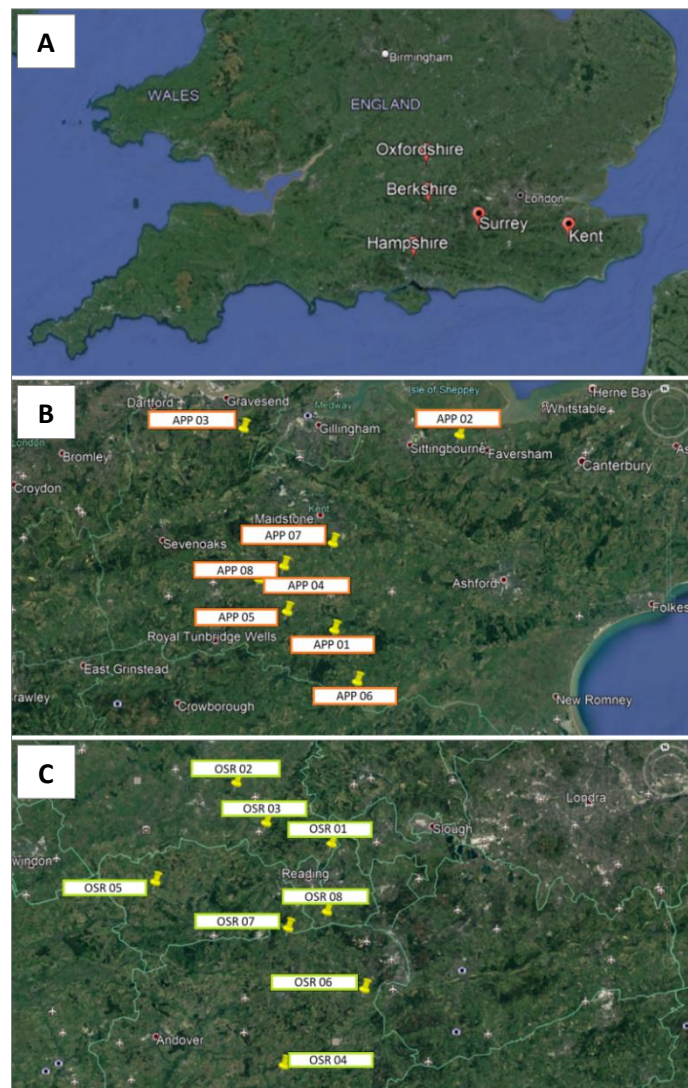
Figure 2.2.1: (A) map of southern England, (B) APP sites in Kent, (C) OSR sites in Berkshire (OSR 05, 08), Hampshire (OSR 04, 07), Oxfordshire (OSR 01-03), and Surrey (OSR 06)

2.2.1.2. Intensity gradient

In order to describe the exposure of pollinators to pesticides, UFZ attributed an Intensity Gradient Index (IGI) to each site ranging from 1 to 8 (lowest to highest), using as a proxy the proportion of cropland in 1 km radius obtained through *ArcGIS* Pro 2.4.1 as described in section 2.2.1.1. (Table 2.1).

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[1] The proportion of total grassland in the surrounding landscapes (1 km radius) ranged from 0.21 to 0.39 in APP sites and from 0.10 to 0.34 in OSR sites. See Appendix 2.1 for each site proportion.

2.2.1.2.1. Pesticide Pressure Indexes

Since collecting information on whether the cropland areas in 1 km radius were intensively or non-intensively managed was not feasible, a measure to describe pollinator exposure to pesticides was adopted. A survey for the growers of apple and oilseed rape sites was designed to collect information on plant protection products used on the PoshBee sites (Appendix 2.1, WP1.3.5). Growers were asked to provide the name and application rate of every product they applied since the previous harvest, including insecticides, herbicides, and fungicides. To make direct comparisons among sites, two different Pesticide Pressure Indexes (PPI) were calculated, one for insecticides (Insecticide Pressure Index = IPI) and another for herbicides and fungicides (Other Pesticides Pressure Index = OPPI), modifying the approach of Yasrebi-de Kom et al. (2019) and using the following formula:

$$PPI = \Sigma \, [A \cdot (PPP_{AR} \cdot AI_c) \, / \, LD_{50}]$$

Here, A is the area of the PoshBee site (ha), PPP_{AR} is the application rate of the plant protection product which multiplied by the concentration of the active ingredient (AI_c) resulting in the application rate of the active ingredient (AR_{AI}), and LD_{50} is the honeybee acute contact toxicity of the AI (median lethal dose per bee, µg/bee). AI_c and LD_{50} were obtained from published databases (EFSA: https://european-union.europa.eu; ECHA: https://echa.europa.eu; EPA: https://www.epa.gov/; Agrobase: https://agrobaseapp.com), literature sources (Dinter et al., 2010; Pettis et al., 2013; Sanchez-Bayo & Goka, 2016), and product labels. If no information was available from any of the listed sources, the ingredient was not included in the analysis (Yasrebi-de Kom et al., 2019). Table 2.2.1 shows site IDs paired with their location, management, proportion of cropland and woodland, and Pesticide Pressure Indexes that were used in the analysis.

Table 2.2.1: Landscape and pesticide data of APP and OSR sites, with IGIs ranging from lowest (1) to highest (8) obtained using IPI and OPPI as proxies. Such IGIs classify APP and OSR sites differently than the IGI attributed to each site using the proportion of cropland as proxy. Higher IPI and OPPI: higher pesticide pressures on pollinators. '0': no insecticides (IPI)/other pesticides (OPPI) sprayed in the field. 'NA': no response to survey question. See Appendix 2.1 for further data.

Site ID	Location	Management	Prop. cropland	Prop. woodland	IPI	OPPI	IGI from prop. cropland	IGI from IPI	IGI from OPPI
APP 01	Kent	Organic	0.26	0.44	116618	9442	2	7	6
APP 02	Kent	Organic	0.66	0.06	3459049	66927	8	8	8
APP 03	Kent	Conventional	0.53	0.14	74	595	7	3	4
APP 04	Kent	Conventional	0.47	0.16	6691	1563	6	6	5
APP 05	Kent	Conventional	0.38	0.28	3824	49552	4	5	7
APP 06	Kent	Conventional	0.27	0.33	1959	510	3	4	3
APP 07	Kent	Conventional	0.26	0.35	56	0	1	2	1
APP 08	Kent	Conventional	0.40	0.16	11	355	5	1	2
OSR 01	Oxfordshire	Conventional	0.49	0.18	1776	177	4	4	2
OSR 02	Oxfordshire	Conventional	0.47	0.17	25268	144	3	7	1
OSR 03	Oxfordshire	Conventional	0.75	0.09	5238	350	7	5	4
OSR 04	Hampshire	Conventional	0.70	0.13	896	245	6	3	3
OSR 05	Berkshire	Conventional	0.66	0.09	NA	NA	5	NA	NA
OSR 06	Surrey	Conventional	0.80	0.08	0	529	8	1	5
OSR 07	Hampshire	Conventional	0.38	0.22	0	673	1	1	6
OSR 08	Berkshire	Conventional	0.43	0.17	17511	1341	2	6	7

2.2.2. Sentinel bees

Three honeybee hives and three bumblebee colonies were employed as sentinel bees in each selected site, where they were placed right before the beginning of the flowering season[2] (Appendix 2.1, WP1.2.4). The location of sentinel beehives was used as the centre point of the 1 km radius circular area (Appendix 2.1, WP1.1.1).

2.2.2.1. Honeybees

A total of 30 *Apis mellifera spp.* hives were ordered from Denrosa Apiaries (Scotland) and transported to Sonning Farm (Reading) in February 2019. Honeybees were fed with proteins and sucrose water, and the 24 strongest hives were selected as sentinel hives for the 8 oilseed rape fields. The remaining 24 hives for the 8 apple orchards were hired from local beekeepers, which took care of them for the whole duration of the experiment (Appendix 2.1, WP1.2.1).

2.2.2.2. Bumblebees

Forty-eight colonies of *Bombus terrestris audax* were provided by Agralan with a standardised number of 80 workers (Appendix 2.1, WP1.2.3). All colonies were kept at the University of Reading with *ad libitum* access to glucose reservoirs before being placed in the fields, where they were provided with a thin layer of cotton wool to help build their nests (Carnell et al., 2020) and with waterproof covers to protect them from adverse weather. Set-up of sentinel bees in APP and OSR sites is shown in Figure 2.2.2.
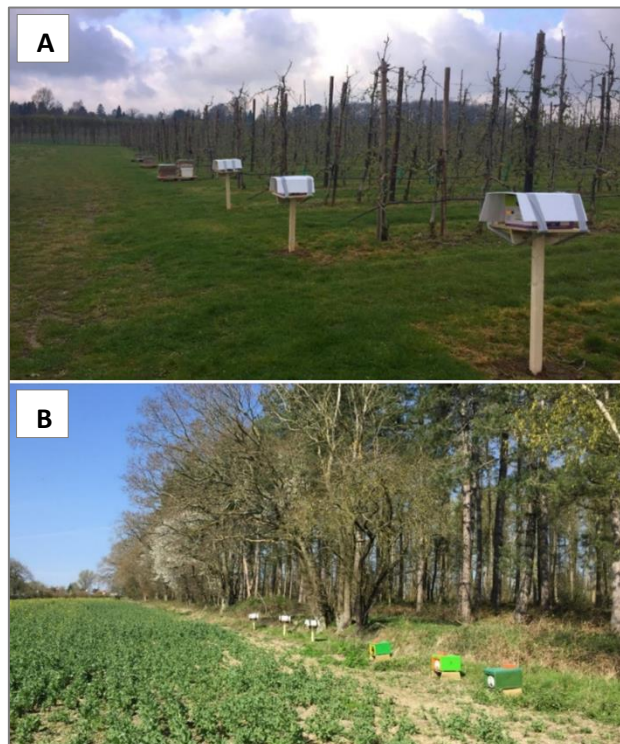

Figure 2.2.2: Example of sentinel beehive and bumblebee colony set-up in APP (A) and OSR (B) sites.

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[2] While the PoshBee project originally planned to employ three *Osmia bicornis* aggregations as additional sentinel bees in each site, the cold spring weather in the UK did not allow the cocoons to open in time, therefore *O. bicornis* could not be included in our fieldwork experiment.

2.2.3. Bumblebee colony performance

After being weighed prior to being deployed to apple orchards and oilseed rape fields, bumblebee colonies were weighed a second time at peak flowering and a third time after being terminated at the end of the season, in order to assess their growth. Colonies were then opened to separate workers, males, and queens to assess the sex ratio and reproductive fitness (Appendix 2.1, WP1.5.9).

2.2.4. Social bee activity

Pollinator transect surveys were carried out in each site in three time points (at the start, middle, and end of flowering) to evaluate the activity of pollinators along four transects – two on the boundaries and two within crops. The surveyor walked along a 50m long, 2m wide strip for five minutes and recorded all the insect pollinators in their sight, including those pollinating on flowers (Appendix 2.1, WP1.3.4). Since our experiment focussed on social bees, we aimed to investigate honeybees (*Apis mellifera*) and bumblebees (*Bombus spp.*) and how their presence may be influenced by pesticides and landscape. The boundary where the sentinel hives and colonies were located was not used as a transect, and the same person performed all pollinator surveys to avoid any surveyor effect.

2.2.5. Beehive health checks

After the flowering stage, hives were checked for *Varroa destructor* infestations through yellow sticky traps that were placed onto the hive bottom board and left there for a minimum of 3 days (Appendix 2.1, WP1.5.2). Traps were then collected and debris were examined, counting *Varroa* mites visible on the surface. While in APP sites beekeepers took care of their own hives, in OSR sites we utilised 'PLA Biodegradable Yellow Sticky Traps', polylactic acid traps produced by the brand 'Plai'.

2.2.6. Crop yield

After the harvest and through the same survey that allowed us to collect information on plant protection products, growers were requested to indicate the total yield of the year in the orchards and oilseed rape fields where the study took place, expressed in tonnes. Growers were additionally asked about the total percentage of class one apples (Appendix 2.1, WP1.3.5).

2.2.7. Statistical analysis

Data collected from apple orchards and oilseed rape fields were analysed separately. Landscape and pesticide pressure variables were first checked for collinearity using the Pearson Product-Moment test to avoid multicollinearity issues, and mixed-effect models were built in Genstat v21 (Goedhart & Thissen, 2021) to assess the impact of cropland, woodland, IPI, and OPPI on the activity and health of honeybees and bumblebees. Variables that were significantly correlated, *i.e.* correlation coefficients ≥0.30 and p<0.05 (Ratner, 2009), were not used within the same model, thus multiple models were built to test impacts of the different explanatory variables (see Appendix 2.2 for correlation matrixes and global models). Linear Mixed

Models (LMMs) were used for response variables with normal distributions, whilst Generalized Linear Mixed Models (GLMMs) were employed to analyse count data. Count data were first tested for Poisson distribution with the goodness-of-fit Chi-Square test for observed versus expected counts, and if they did not follow a Poisson distribution (p<0.05) they were analysed using a Quasi-Poisson distribution accounting for under- or over-dispersion (dispersion parameter allowed to be ≠ 1). Final models were selected based on AICc and ΔAICc scores, where ΔAICc is the AICc difference between the candidate model and the lowest AICc score of the tested models, and models within a ΔAICc of 2 from the lowest AICc model were reported (Burnham & Anderson, 2004; Snipes & Taylor, 2014). Each final model was assigned a code as a unique identifier (M1-M77, full set of models in Appendix 2.3).

2.2.7.1. Bumblebee colony performance

2.2.7.1.1. Bumblebee colony weight change

To investigate the weight variation of bumblebee colonies between the start (period 1) and the middle of the flowering season (period 2), and between the middle and the end (period 3), 'weight of period 1' was subtracted from 'weight of period 2' (=Δweight 1-2), and 'weight of period 2' was subtracted from 'weight of period 3' (=Δweight 2-3). 'Δweight 1-2' and 'Δweight 2-3' were then analysed using LMMs including the proportion of cropland and woodland and the two PPIs as fixed terms, and 'site and colony ID' as random term. Contrary to the two APP Pesticide Pressure Indexes, which resulted to be significantly correlated between each other (coeff≥0.30, p<0.05), the OSR IPI and OPPI did not show any sign of collinearity, therefore their interaction effect was incorporated as an additional fixed term in all OSR analyses, including those described in the paragraphs below.

2.2.7.1.2. Bumblebee colony reproductive fitness

To assess the reproductive fitness of *Bombus terrestris* colonies, colonies were terminated by being frozen at -20°C at the end of the fieldwork season. They were later opened and workers, males, natal queen, and new queens were separated, calculating the percentage of workers and reproductives (*i.e.* males and new queens combined) that were produced by the colony, plus the percentage of males and new queens among the reproductives. Percentages were then analysed using LMMs including the proportion of cropland and woodland and the two PPIs as fixed terms, while 'site and colony ID' was added as random term.

2.2.7.2. Social bee activity

The number of honeybees and bumblebees surveyed during pollinator transect surveys were averaged across the three sampling periods and analysed using LMMs. Additionally, to investigate any potential pressure impact on bee activity after a long period in the fields, data on number of honeybees and bumblebees surveyed at the end of the flowering period was analysed using GLMMs with a Poisson distribution. Time, temperature, transect, proportion of cropland and woodland, and the two PPIs were used as fixed terms, and 'site ID' as random term.

2.2.7.3. Varroa mites

GLMMs with a Quasi-Poisson distribution were built to analyse the number of *V. destructor* mites on the yellow sticky traps in both APP and OSR sites, adding the proportion of cropland, the proportion of woodland, IPI, and OPPI as fixed terms, while 'site and hive ID' was used as random term.

2.2.7.4. Yield and percentage of class 1 apples

The yield of apple orchards and oilseed rape fields, expressed as tonnes on hectares (t/ha), was analysed with LMMs using the proportion of cropland and woodland and the two PPIs as fixed terms, and 'site ID' as random term. A further statistical analysis with LMMs including the same random and fixed terms was conducted on apple orchard data using 'percentage of class 1 apples' as response variable[3].

2.3. Results

2.3.1. Bumblebee colony performance

2.3.1.1. Bumblebee colony weight change

Between the start and the middle of flowering, when new workers are produced (Whitehorn et al., 2012), APP and OSR colonies averagely gained weight with 0.08 kg (±0.093 SD) and 0.23 kg (±0.15 SD) respectively. Between the middle and the end of flowering, APP and OSR colonies averagely experienced a weight decline with 0.10 kg (±0.09 SD) and 0.20 kg (±0.15 SD) respectively, in correspondence with the production of new queens and males (Whitehorn et al., 2012).

LMMs investigating the weight change of bumblebee colonies between the start and the middle of flowering in APP sites found no effect of IPI or OPPI, which were excluded from final models (Appendix 2.3). However, significant effects of the proportion of cropland and woodland were shown, with a higher weight gain corresponding to higher proportions of cropland and lower proportions of woodland (Table 2.3.1, Figure 2.3.1). The weight change between the middle and end of flowering was not affected by any variable (M4-6, Appendix 2.3).

Contrary to apple sites, a statistically significant effect of herbicides and fungicides was found on the weight change of bumblebee colonies in OSR sites between the start and the middle flowering, with a higher weight gain corresponding to higher OPPI (Table 2.3.1, Figure 2.3.2,). No impact of IPI, proportion of cropland, or proportion of woodland was found, and such variables were not included in final models (Appendix 2.3). No variable resulted to be significant in the models investigating the weight change between the middle and end of flowering (M8-9, Appendix 2.3).

Table 2.3.1: Final LMMs on *B. terrestris* colony weight change between start and middle flowering (period 1 and 2). ΔAICc of 0: lowest AICc model. ΔAICc GM: ΔAICc with global models. Significant p-values (<0.05) are highlighted in bold. 'M3': single parameter model due to multicollinearity (no AICc is shown). See Appendix 2.3 for AICc selection and models with only non-significant parameters.

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[3] Growers of OSR sites were additionally asked about the percentage of oil content in oilseeds, but due to the lack of responses it was not possible to include it in the analysis.

Response variable	Model ID	Random term	Fixed terms	Estimate	SE	F	ndf, ddf	p-value	AICc	ΔAICc	ΔAICc GM	R²
APP Δweight 1-2	M2	Site and colony	Cropland	0.3607	0.1306	7.63	1, 19	**0.012**	-	-	-	28.66
	M3	Site and colony	Woodland	-0.4407	0.1326	11.05	1, 19	**0.004**	-39.89	0	19.10	36.77
OSR Δweight 1-2	M7	Site and colony	OPPI	0.0002	0.0001	9.88	1, 19	**0.005**	3.12	0	35.58 37.77	34.20
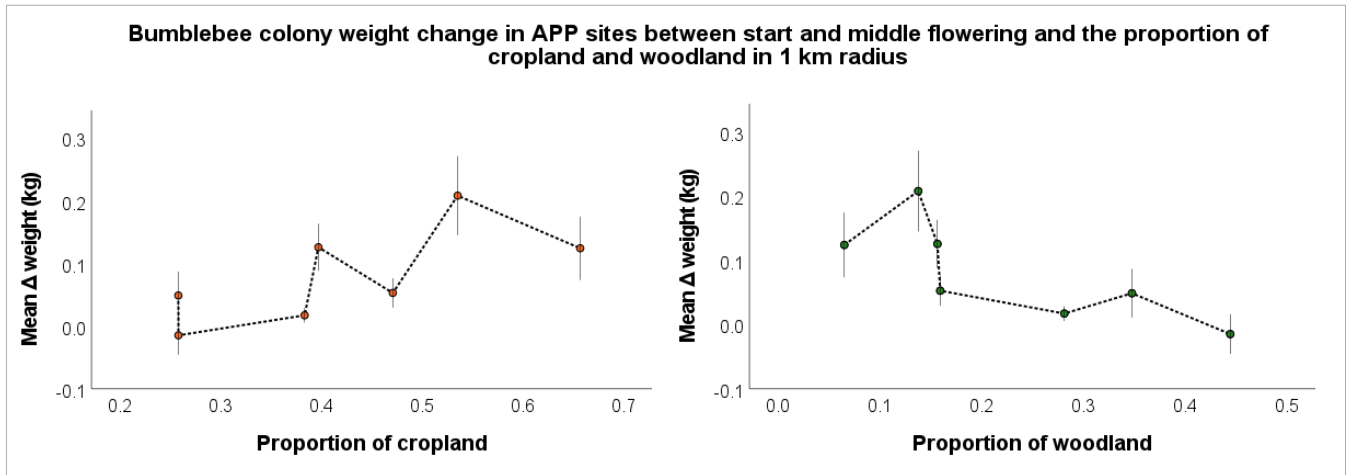
Figure 2.3.1: Between the start and middle of flowering, bumblebee colonies in APP sites gained more weight with higher proportions of cropland and lower proportions of woodland in the landscape (Table 2.3.1). Error bars: ± 1 SE from the mean.
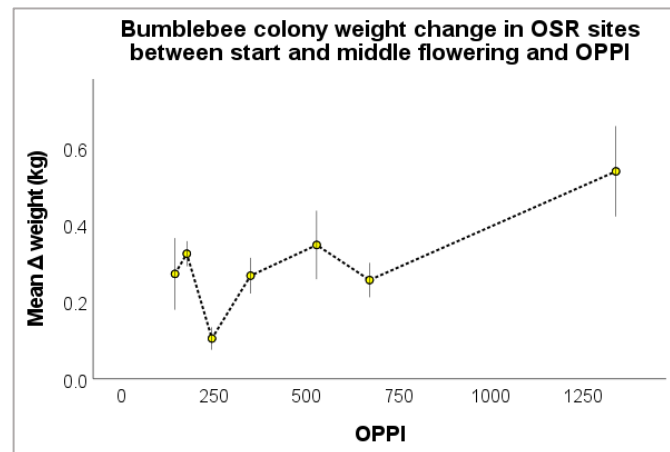
Figure 2.3.2: Between the start and middle of flowering, bumblebee colonies in OSR sites gained more weight with higher OPPI (Table 2.3.1). Error bars: ± 1 SE from the mean.

2.3.1.2. Bumblebee colony reproductive fitness

LMMs investigating the reproductive fitness of bumblebee colonies in APP and OSR sites did not underline any significant effect of IPI, OPPI, proportion of cropland, or woodland on the percentage of workers, reproductives, and males and queens among reproductives (M10-29, Appendix 2.3).

2.3.2. Social bee activity

2.3.2.1. Activity averaged across three sampling periods

LMMs investigating bee activity across three sampling periods did not show any influence of IPI, OPPI, or proportion of cropland and woodland in APP or OSR sites (M30-38, Appendix 2.3), while bumblebees in APP

sites and honeybees in OSR sites were each found to be significantly more active within crops than on boundaries (APP bumblebees: range F=5.44-6.04, range ndf, ddf=1, 8.1-8.4, range p=0.038-0.044, range R^2=27.97-45.02; OSR honeybees: range F=5.33-5.77, range ndf, ddf=1, 12-13, range p=0.032-0.040, range R^2=43.92-44.09) (M34-38 and M48-50, Appendix 2.3).

2.3.2.2. Activity at the end of flowering

GLMMs on social bee activity at the end of flowering showed that, in APP sites, honeybees were more active with higher proportions of woodland in 1 km radius (Table 2.3.2, Figure 2.3.3). No effect of proportion of cropland, IPI, or OPPI was found (M39-44, Appendix 2.3).

In OSR sites, both honeybees and bumblebees were more active in sites with higher OPPI (Table 2.3.2, Figure 2.3.4). Additionally, honeybees were also more active within crops than on boundaries (χ^2=4.53, df=1, p=0.033, range R^2=38.61-45.69), and both honeybees and bumblebees were more active at earlier times of day (honeybees: range χ^2=6.19-6.24, df=1, range p=0.012-0.013, range R^2=38.61-45.69; bumblebees: χ^2=5.50, df=1, p=0.017, R^2=48.21). No effect of IPI, proportion of cropland, or woodland was observed (M56-63, Appendix 2.3).

Table 2.3.2: Final GLMMs on honeybee and bumblebee activity at the end of flowering with ΔAICc ≤ 2. ΔAICc of 0: lowest AICc model. ΔAICc GM: ΔAICc with global models. Significant p-values (<0.05) are highlighted in bold. See Appendix 2.3 for AICc selection.

Response variable	Model ID	Random term	Fixed terms	Estimate	SE	X^2	df	p-value	AICc	ΔAICc	ΔAICc GM	R^2
APP honeybees	M41	Site	Woodland	3.9420	0.2000	5.45	1	**0.020**	39.04	0	30.13	46.77
OSR honeybees	M56	Site	OPPI	0.0007	0.0003	4.66	1	**0.031**	48.12	0	46.33	22.95
	M57	Site	Crop transect	0.5021	0.2641	3.61	1	0.057	49.74	1.62	44.71	27.84
			OPPI	0.0007	0.0003	4.66	1	**0.031**				
	M58	Site	Crop transect	0.5021	0.2642	3.61	1	0.057	49.99	1.87	44.46	34.62
			OPPI	0.0007	0.0003	3.92	1	**0.048**				
			Woodland	-2.5380	3.0065	0.71	1	0.399				
OSR bumblebees	M61	Site	OPPI	0.0008	0.0004	4.29	1	**0.038**	49.27	0	50.74	18.26
	M62	Site	Crop transect	0.5596	0.3619	2.39	1	0.122	51.15	1.88	48.86	37.28
			OPPI	0.0008	0.0004	4.29	1	**0.038**				
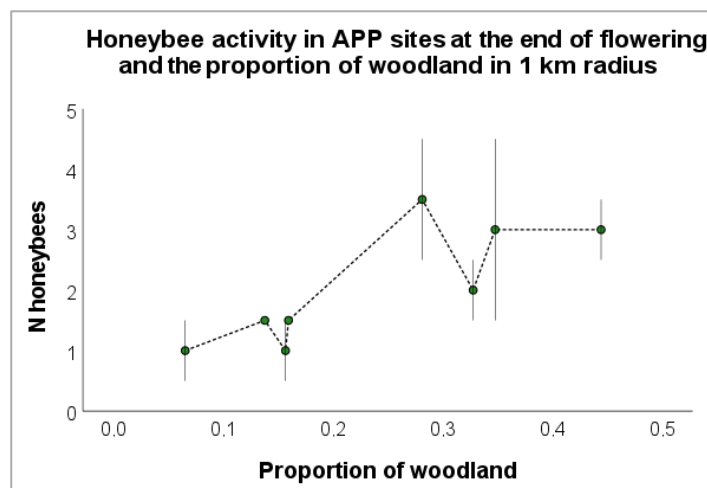
Figure 2.3.3: Higher proportions of woodland increased honeybee activity in APP sites at the end of flowering (Table 2.3.2). Error bars: ± 1 SE from the mean.
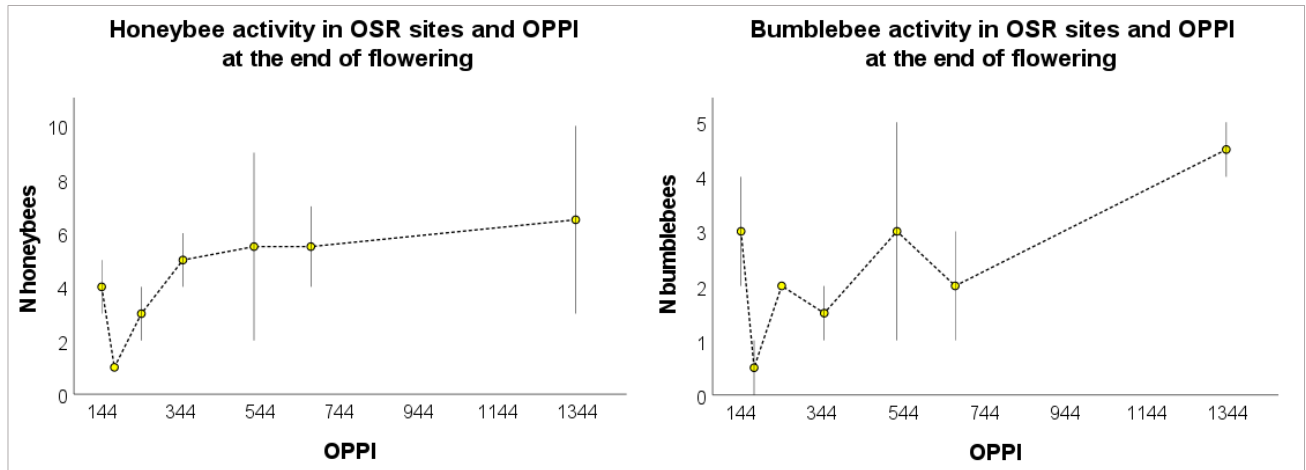
Figure 2.3.4: Higher OPPI increased honeybee and bumblebee activity in OSR sites at the end of flowering (Table 2.3.2). Error bars: ± 1 SE from the mean.

2.3.3. *Varroa destructor* mites in honeybee hives

GLMMs showed no significant effect of IPI, OPPI, the proportion of cropland, or the proportion of woodland on *Varroa* mite counts in APP or OSR beehives (M64-69, Appendix 2.3).

2.3.4. Crop yield and percentage of class 1 apples

There was no effect of IPI, OPPI, the proportion of cropland, or the proportion of woodland on the yield of apple orchards or percentage of class 1 apples (M70-77, Appendix 2.3). Similarly, no effect of any of the variables was shown on oilseed rape yield (M78-79, Appendix 2.3).

2.4. Discussion

Using a large-scale fieldwork experiment across eight apple orchards and eight oilseed rape fields in Southern England, we assessed the effect of land cover and the full range of insecticide, herbicide, and fungicide pressures on *Apis mellifera* and *Bombus terrestris* bees.

2.4.1. Effect of surrounding landscape and pesticide pressures on bumblebee colony growth and reproductive fitness

While neither landscape nor pesticide use were shown to influence bumblebee colony weight variation between the middle and the end of flowering, our study highlighted significant results between the start and the middle of the flowering season, which differed between apple and OSR sites.

In apple orchards, we observed that the weight gain of *Bombus terrestris* colonies was higher in landscapes with higher proportions of cropland. This is in line with previous studies, which observed a higher growth rate of bumblebee colonies in landscapes characterised by larger amounts of flowering crops (*e.g.* Herrmann et al., 2007; Westphal et al., 2009; Gervais et al., 2020). However, we also found that the colony weight gain was lower with higher proportions of woodland in the landscape. Although Kämper et al. (2016) found a similar outcome, such result contrasts with past studies highlighting the importance of semi-natural habitats

in providing high-quality foraging resources and promoting bumblebee colony growth (Spiesman et al., 2017; Proesmans et al., 2019). Our findings may be explained by the fact that generalist pollinators, such as our *Bombus terrestris* bees, are able to exploit a wide range of foraging resources, and may prefer to forage on flowering crops to collect pollen and nectar (Westphal et al., 2009; Kämper et al., 2016). On the contrary, specialist bees may rely to a greater extent on semi-natural habitats due to more restricted dietary choices (*e.g.* Rollin et al., 2013; Hanley et al., 2014; Kämper et al., 2016). Therefore, higher proportions of woodland in the surrounding landscape may have reduced *Bombus terrestris* colony growth (Kämper et al., 2016), while higher proportions of cropland may have driven to a higher growth rate (Westphal et al., 2009; Gervais et al., 2020). This outcome is concerning if we consider the impact on wild bee species; in fact, while expanding the areas dedicated to mass-flowering crops to the detriment of semi-natural habitats may potentially increase the abundance of generalist pollinators, it will not benefit wild, specialist bees, whose survival will be threatened by the spill-over of alien species into the landscape, resulting in a higher competition for foraging resources (Diekötter et al., 2010). Therefore, despite our findings, caution is advised in relying on mass-flowering crops to enhance the growth of *Bombus terrestris* bees, and we stress the importance of preserving semi-natural habitats to support wild bee populations, which provide key pollination services to many important food crops (*e.g.* Holzschuh et al., 2012; Kennedy et al., 2013; Pfister et al., 2018), and have often been shown to be more effective pollinators than honeybees (*e.g.* Garibaldi et al., 2014; Garratt et al., 2016; Eeraerts et al., 2019).

Surprisingly, in OSR sites, the weight gain of *Bombus terrestris* colonies was positively influenced by fungicides and herbicides (OPPI). To the best of our knowledge, this is the first study to underline such an effect on the growth of bumblebee colonies, and we can only speculate about the reasons leading to this result. We hypothesise that herbicides and fungicides were safely applied by farmers, for instance avoiding applications during flowering and preventing a direct exposure of bumblebees to spraying (Biddinger & Rajotte, 2015; IPBES, 2016). Moreover, considering that, between the start and the middle of the flowering season, bumblebee colony weight is expected to increase due to the production of workers and their foraging activity (Rundlöf et al., 2015), herbicides and fungicides may have successfully targeted fungal diseases and pest plants (Cullen et al., 2019), improving crop health, from which bumblebees may have benefitted by actively foraging and increasing their colony weight. This view is partially supported by Muratet & Fontaine (2015), who, despite finding a negative effect of herbicides, observed that fungicides increased the abundance of bumblebee and butterfly species in privately-managed gardens, explaining that it was likely due to the fact that fungicides may have promoted healthier plants, which in turn produced better nectar and pollen resources for pollinators (Muratet & Fontaine, 2015).

However, pesticide risk assessments mainly rely on data from *Apis mellifera* to produce information on toxicity (Sgolastra et al., 2019), therefore our PPI describing herbicide, fungicide, and insecticide pressures are based on *A. mellifera* LD$_{50}$ values. This process assumes that the toxicity measured on honeybees may

successfully represent other bee species too, including bumblebees and solitary bees (Sgolastra et al., 2019). Nonetheless, bee species differ in both sensitivity and exposure routes towards pesticides (Sgolastra et al., 2019, 2020). For instance, soil represents a key exposure route for ground-nesting bees, but not for *A. mellifera* or *B. terrestris* (Sgolastra et al., 2019). Moreover, social bees substantially differ from solitary bees in life history and resilience, and may react differently to environmental stressors (Straub et al., 2015). For example, past studies have already underlined different sensitivities to fungicides between *Apis mellifera* and solitary mason bees, showing the necessity of differentiating LD_{50} information depending on bee species (Ladurner et al., 2005; Biddinger et al., 2013). Therefore, although our results underlined no negative impact of insecticides and a positive influence of herbicides and fungicides on *Bombus terrestris* colony growth, there is the strong need to assess pesticide toxicity on *non-Apis* bees to investigate whether other bee species may be impacted differently (Arena & Sgolastra, 2014).

The influence of land cover and pesticide pressures on bumblebee colony growth does not appear to be consistent between apple and oilseed rape sites, with landscape affecting the growth of APP colonies only, and fungicides and herbicides influencing OSR ones. The two crops are in fact very different between each other, with oilseed rape being part of an annual rotation system and the other being a perennial orchard, and have also distinct landscapes in the surrounding areas. The effects we observed may be the product of different interacting factors, many of which could not be included in the present study. For instance, as semi-natural habitats may differ in floral resources they offer to pollinators (Bukovinszky et al., 2017), the habitats around oilseed rape sites might have contributed to offering better or more abundant foraging resources than those surrounding orchards, leading to having no land cover effect on colony growth. The fact that low-input grasslands had to be excluded from the proportion of SNH may have influenced our results, since several studies have shown that grasslands offer important foraging resources to pollinators (*e.g.* Öckinger & Smith, 2007; Kennedy et al., 2013; Bartholomée et al., 2020). Or else, the range of woodland cover around OSR might have been too small to produce any visible effect on sentinel colonies (mean proportion of woodland cover in 1 km radius around OSR fields = 0.14±0.05 SD, against 0.24±0.13 SD of APP sites) (Bukovinszky et al., 2017). Moreover, we may hypothesise that bumblebees were more willing to forage from the local, closest oilseed rape flowers instead of travelling to the nearby fields or SNH to collect foraging resources, explaining the detection of the solely effect of OPPI on their growth, and no effect of the proportion of cropland. In turn, since bumblebees are able to fly long distances to collect pollen and nectar from suitable resources (Osborne et al., 2008b), colonies placed in apple orchards may have preferred to forage from other agricultural lands in the surroundings, resulting in a higher weight gain in correspondence with higher proportions of cropland, but no OPPI effect, as the toxicity index was calculated based on pesticide products applied in the field only. Alternatively, pesticide pressures in apple orchards might have been below the threshold that would have resulted in a validated impact on colony growth (Milano et al., 2019).

We also underlined no significant effect of land cover on the percentage of workers, males, and queens in APP or OSR bumblebee colonies. Our results may be comparable to those of Westphal et al. (2009), who measured colony reproductive success of *B. terrestris* in two different landscapes finding out that, although colonies placed in areas with higher oilseed rape flower sources gained more weight than those positioned in areas with a lower amount of oilseed rape, there was no significant impact on their reproduction success. Moreover, Milano et al. (2019) found no significant effect of natural habitats or agricultural land covers on the number of workers (both adults and cells), male cells, or queen cells of *Bombus impatiens*. However, past studies have observed that floral resources are necessary to support the production of workers (Herrmann et al., 2017; Adler et al., 2020; Gardner et al., 2021) and reproductives (Williams et al., 2012; Klatt et al., 2020; Bommarco et al., 2021). In fact, queens need to rely on foraging resources throughout the whole season (Westphal et al., 2009), and the lack of such resources may lead to a lower colony fitness (Rundlöf et al., 2014). Moreover, previous studies have also found a positive influence of flower-rich areas on bumblebee colony growth (*e.g.* Crone & Williams, 2016; Bukovinszky et al., 2017; Spiesman et al., 2017). Hence, we suggest that integrating the proportion of semi-natural habitats with a landscape floral-abundance index, and including low input grasslands, may give further insight into the influence of land cover on bumblebee colony reproductive fitness and growth.

Additionally, no significant relationship between pesticide pressures and the percentage of workers or reproductives was found either in APP or OSR sites. The evidence from the literature on this topic is mixed; while some studies have found no impact on the number of workers (Whitehorn et al., 2012; Baron et al., 2014; Wintermantel et al., 2018), males (Whitehorn et al., 2012; Baron et al., 2014), or queens present in the colony (Mallinger et al., 2015), others have observed a significant pesticide effect on colony reproductive fitness (*e.g.* Gill et al., 2012; Rundlöf et al., 2015; Wintermantel et al., 2018), suggesting that pesticides may impair worker foraging efficacy (Rundlöf et al., 2015), reduce colony initiation by queens (Baron et al., 2017), or cause an inadequate brood care, provided by fewer workers (Gill et al., 2012; Rundlöf et al., 2015). Previous research have also shown that bumblebee colonies exposed to pesticides produced significantly smaller workers, even when the production of workers, males (Baron et al., 2014), or queens (Mallinger et al., 2015) was not affected. Therefore, although we did not find pesticides directly impacted the proportion of workers and reproductives in the colonies, investigating how insecticides, herbicides, and fungicides affect the size of worker bumblebees may benefit further studies directed towards pesticide pressures on bee reproductive fitness.

It is also worth highlighting that the majority of the literature investigating the impact of pesticides on the growth and reproduction of bumblebee colonies mainly focussed on insecticides (*e.g.* Whitehorn et al., 2012; Rundlöf et al., 2015; Wintermantel et al., 2018). Very few took fungicides into account (Mallinger et al., 2015; Botías et al., 2020), and to the best of our knowledge, none included herbicide effects. In contrast, ours is the first large-scale field study to calculate a toxicity index that includes fungicides and herbicides.

Overall, our results underline that a high proportion of cropland (including mass-flowering crops) in the surrounding landscape may promote a higher colony growth of *Bombus terrestris* bees thanks to their high adaptability to different landscapes and their ability to exploit different foraging resources (C. Westphal et al., 2009), however this may pose a high threat to the survival of wild, specialist bee populations (Diekötter et al., 2010b). We also hypothesise that, under certain conditions, herbicides and fungicides may be safely employed to target pest plants and fungal diseases to improve crop health and, in return, promote the growth of *Bombus terrestris* colonies. However, caution is required when addressing the impact of pesticides on other bee species, particularly specialist pollinators, that may have different sensitivities to insecticides, herbicides, or fungicides, and may also be threatened by the growing presence of generalist bees competing for foraging resources (Diekötter et al., 2010b). More evidence is also required on herbicide and fungicide effects to properly understand their impact on the health, growth, and reproduction of bees, as the literature mainly focus on the impact of insecticides. Finally, we suggest to further investigate how semi-natural habitats, croplands, and pesticides may affect the colony growth and fitness of specialist bumblebees, without limiting to generalists, which may react differently to environmental stressors.

2.4.2. Effect of surrounding landscape and pesticide pressures on social bee activity

Although no effect of land cover was observed on the activity of social bees in APP or OSR sites using averaged data collected at three points in time, our study indicated that the proportion of woodland positively influenced the activity of honeybees in apple orchards at the end of the flowering period, presumably because a longer exposure to the surrounding habitats was able to produce a visible impact. The positive influence of semi-natural habitats on bee activity is in line with several previous studies (*e.g.* Le Féon et al., 2010; Nayak et al., 2015; Raderschall et al., 2021), as SNH provide a more diverse and continuous presence of suitable nesting and foraging resources which may not be offered by landscapes dominated by cropland areas all year round (Westphal et al., 2003).

However, no effect of proportion of SNH was shown on bumblebees surveyed in apple orchards, nor on either honeybees or bumblebees in oilseed rape fields, and no effect of proportion of cropland was observed in APP or OSR sites. This is not only in contrast with studies linking a higher bee activity to higher proportions of SNH, as mentioned above, but also with research showing a lower bee activity associated with more cropland areas in the landscape, where floral resources are less diverse and pesticide pressures are higher (Holzschuh et al., 2016; Bartholomée et al., 2020; Shaw et al., 2020). Nonetheless, these studies have been mainly focussed on wild bee communities, while managed bee activity was much less investigated (*e.g.* Carvalheiro et al., 2010; Le Féon et al., 2010; Kovács-Hostyánszki et al., 2011), and we cannot exclude that a landscape effect would have been found on local bee species in our sites, particularly if considering that a land cover effect was visible on the activity of the generalist honeybee in apple orchards. In fact, while generalist pollinators are able to exploit a wide range of foraging resources (Potts et al., 2003) and are highly adaptable

to different landscapes, some wild, specialist bees tend to be more affected by the presence of natural and semi-natural habitats, as they have more limited foraging abilities and specific nesting requirements (DEFRA, 2014). Moreover, although pollinator transect surveys were looking at *Bombus* spp., it is likely that the generalist *B. terrestris* was the dominant species due to the presence of our sentinel colonies, and a distinction between bumblebee species could have produced different results.

The lack of a landscape effect may be also due to the fact that, contrary to several past studies (*e.g.* Nayak et al., 2015; Bartholomée et al., 2020; Raderschall et al., 2021), we were unable to include the proportion of low-input grassland in the semi-natural habitat range, as we could not distinguish between high- and low-input grassland management based solely on landscape cover maps. Additionally, including the distance from semi-natural areas as a variable together with the proportion of cropland and SNH might give further insights into the influence of landscape on bee activity. In fact, other studies have shown declines in social bees within crop fields with increasing distance to SNH (Carvalheiro et al., 2010, 2011; Bartholomée et al., 2020).

When it comes to pesticide pressures, the use of herbicides and fungicides was observed to increase both honeybee and bumblebee activity in oilseed rape fields at the end of the flowering period. This appears to be somewhat consistent with findings related to the positive influence of OPPI on the growth of *B. terrestris* colonies, suggesting again that such products may have been not only safely applied by farmers (Biddinger & Rajotte, 2015), but also able to successfully target fungal diseases and pest weeds, making plants healthier and more appealing to social bees (Muratet & Fontaine, 2015). Moreover, as stated before, Pesticide Pressure Indexes were based on *Apis mellifera* LD_{50} values, however pesticide toxicity may vary depending on bee species, life history, and resilience (Sgolastra et al., 2019, 2020); PPI values could be different if calculated using LD_{50} values based on other bee species, *e.g.* solitary bees, which are worth being further researched and their sensitivity to pesticides investigated.

No additional pesticide effect was found using data collected at the end of flowering in APP or OSR sites, although an impact on bee activity could have been expected after a longer exposure of bees to pesticides, and similar to land cover effects, no IPI or OPPI impact was observed when data on social bee activity from the start, middle, and end of flowering was combined.

Such results are in contrast with multiple studies underlining how a reduced use of pesticides correlated with a higher pollinator activity, although the majority of them focussed on wild bees (*e.g.* Marini et al., 2012; Kennedy et al., 2013; Le Provost et al., 2021), or on just one, or a small number, of active ingredients (*e.g.* Dubey et al., 2020; Hatfield et al., 2021; Main et al., 2021). The lack of a pesticide effect on social bees could be explained by the fact that managed bees are usually employed in agricultural fields during crop bloom season, without being exposed to pesticides for longer periods of time (Park et al., 2015). However, since we did not investigate bee foraging behaviour, we are unable to verify whether the exposure to pesticides did have an impact on the visitation rates of social bees on flowers along the transects, even when not impacting

their activity. In fact, several past studies have shown that both insecticides (*e.g.* Gill & Raine, 2014; Christen & Fent, 2017; Siviter et al., 2021b) and fungicides and herbicides (*e.g.* Syromyatnikov et al., 2017; Christen et al., 2019; Macri et al., 2021) may be capable of affecting the foraging activity of bees, including bumblebees and honeybees. We hypothesise that integrating transect surveys with further observations, such as the number of flowers visited by surveyed bees and flowers present along the transects (*e.g.* in Carvalheiro et al., 2010), may produce a more balanced dataset among different sites, and provide further information on how bee visitation rates may be impacted by pesticide pressures in the landscape.

To conclude, it is worth considering that even though our pollinator transect surveys were not species-specific, but rather taxa-specific, it is likely that the placement of the sentinel hives and colonies in each site gave a standard density of such pollinators in the fields, and that *Apis mellifera* and *Bombus terrestris* were the dominant species in every sites, with implications for pollinator transect survey analyses related to both landscape and pesticide effects. This aspect should be considered when interpreting our findings on a broader scale.

In light of such results, the importance of preserving natural and semi-natural habitat to provide bees with suitable, high-quality foraging and nesting sources should be further underlined (*e.g.* Potts et al., 2009; Bartholomée et al., 2020). Moreover, since some bee species may be more sensitive than others to pesticides (Arena & Sgolastra, 2014), it is appropriate to keep pesticide pressures under control to safeguard beneficial insects and pollination services they provide.

2.4.3. Effect of surrounding landscape and pesticide pressures on the proliferation of *Varroa* mites in honeybee hives

In our experiment, we found that landscape cover did not affect the proliferation of *Varroa destructor* in honeybee hives in either APP or OSR sites. Our results seem to be in accordance with Dolezal et al. (2016), who found no impact of agricultural land or non-cultivated land, including grassland and woodland, on *Varroa* mite loads in beehives. However, this is in contrast with other studies, where higher *Varroa* mite loads have been reported in agricultural lands (Alburaki et al., 2018) and landscapes with a lower proportion of natural habitats (Leza et al., 2016). This may be due to the fact that agricultural lands pose a higher risk of pesticide exposure to bees than natural and semi-natural habitats, causing a reduced immune response and making bees more prone to developing infections and diseases (Poquet et al., 2016).

Additionally, we found no impact of insecticides, fungicides, or herbicides on the proliferation of *Varroa* mites in APP and OSR sites, in line with the findings of Rolke et al. (2016) on neonicotinoids effect on *Varroa* mite loads. On the contrary, other studies registered an increase in *Varroa* infestations in beehives feeding on crops exposed to neonicotinoids (*e.g.* Di Prisco et al., 2013; Alburaki et al., 2015; Annoscia et al., 2020), and despite not being thoroughly addressed by the literature, fungicides were shown to be the main cause of beehive disorders, including DWV vectored by *Varroa* mites (Simon-Delso et al., 2014), and to double the risk

of infection of *Nosema ceranae* in honeybees (Pettis et al., 2013). Such results suggest that pesticides may suppress bee immune systems, enhancing parasitic infections (Annoscia et al., 2020). However, there is a substantial literature gap regarding the impact of herbicides and fungicides on parasite proliferation, and no study has yet investigated the impact of pesticide mixtures on *Varroa* mite loads in beehives.

It is also worth considering that synergistic effects between pesticides and parasites may happen for specific combinations and not for others; for instance, the neonicotinoids clothianidin and imidacloprid were both shown to reduce the honeybee immune response to viruses, impacting their defence and consequently favouring the infection, but this was not observed for the insecticide chlorpyriphos (Di Prisco et al., 2013). Hence, the various effects of different pesticide combinations might further explain why we did not observe any effect on parasitic infestations.

Finally, beehives in apple orchards were subject to *Varroa* treatments, which were mostly performed between August and September of the year prior to the experiment by beekeepers that owned these hives. Leza et al. (2016) found that anti-*Varroa* treatments significantly lowered the number of *Varroa* mites in beehives, particularly when they were carried out in the second half of the year. Thus, treatments performed on apple beehives might have buffered the effect of pesticide or landscape on parasite loads. Moreover, beekeepers may have utilised different active ingredients to treat *Varroa* mites, some of which may be more efficient than others. For example, organic treatments have been previously found to outperform synthetic ones (Leza et al., 2015, 2016).

Therefore, despite not finding any pesticide effect on *Varroa* mite loads, we hypothesise that investigating the relation between (i) pesticides and viruses vectored by *Varroa*, or other common infections in honeybee hives, and between (ii) different specific pesticide-*Varroa* combinations, may produce further results contributing to shed light on the way insecticides, fungicides, and herbicides may interact between each other and impact bee health disorders. Moreover, including anti-*Varroa* treatments in future analyses may provide further insight into the effect of pesticides and landscape on parasite loads. Finally, although our study contributes to filling a key knowledge gap, more research is needed to address the impact of different pesticides and mixtures on the proliferation of parasites in beehives.

2.4.4. Effect of surrounding landscape and pesticide pressures on pollination services

With our field study, we show that pesticides and land cover did not have any influence on the yield of apple orchards and oilseed rape fields, or on the proportion of class 1 apples. Since we did not find an effect of pesticides or land cover on the abundance of social bees, not finding any repercussion on crop yield may be in line with the expectancies. In fact, pollinator deficits have been found to affect crop yield (*e.g.* Bartomeus et al., 2014; Potts et al., 2016) due to lower flower visitations (Öckinger & Smith, 2007), and higher yields have been linked to a higher bee abundance (*e.g.* Hokkanen et al., 2017; Perrot et al., 2018; Catarino et al., 2019a). However, our results are in contrast with previous studies observing that higher proportions of arable

land in the surrounding landscape negatively affected the yield of insect-pollinated crops (Hokkanen et al., 2017), and that pesticide pressures on insect pollinators did affect the delivery of their pollination services, leading to a reduced crop yield (*e.g.* Stanley et al., 2015a; Hokkanen et al., 2017). Although our study did not underline any pesticide or landscape effect on the delivery of pollination services, a direct impact of such threats could still be possible; in fact, both apple and oilseed rape crops are pollinated by a high range of bee species (Hutchinson et al., 2021), which may have been enough to buffer such negative effects, ensuring no pollination deficit in our target sites.

Moreover, possible indirect effects of landscape or pesticides on crop yield should also be considered. For instance, Catarino et al. (2019a) showed that, although no direct effect of pesticides on crop yield was found, the interaction between pollinator abundance and pesticides did produce an effect on the yield, which resulted to be higher in correspondence to a higher abundance of pollinators and a lower use of pesticides. Another field study demonstrated that allowing the co-existence of ruderal plants and field crops by reducing herbicide applications may promote the diversity and abundance of insect pollinators and, as a result, optimise crop yields (Carvalheiro et al., 2011). Additionally, it has to be considered that the distance of our selected sites from woodland areas was not a measured variable included in our study, but Carvalheiro et al. (2011) did register lower crop yields corresponding to a higher distance from natural habitats.

Therefore, we suggest that investigating the interaction effect between bee abundance, land cover, and pesticide pressures, and including the field distance from natural and semi-natural areas may give further insight into how threats of different nature may interact among one-another and impact the delivery of pollination services. Moreover, we propose to measure the delivery of pollination services through pollinator-exclusion experiments (*e.g.* see Garratt et al., 2014) to assess the contribution of pollinators to the yield of crops and detect any pollination deficit in the field.

2.4.5. Limitations and further research implications

Our study is the first large-scale UK field experiment to utilise two different pesticide pressure indexes (PPI), one of which exclusively including fungicides and herbicides used in the fields. While several studies classified their selected sites based on management practices (*i.e.* organic or conventional, *e.g.* Andersson et al., 2014; Tuck et al., 2014; Lichtenberg et al., 2017), we utilised PPI to better represent pesticide use across our sites.

The PPI showed that the two organic apple orchards had indeed higher indexes than other conventional ones, similar to what is described by Mallinger et al. (2015). In fact, if organic pesticides are applied numerous times or at high rates, organic management is not necessarily going to be less impactful on beneficial insects (Mallinger et al., 2015). For example, the use of sulphur as fungicide and spinosad as organic insecticide in both organic orchards significantly increased their OPPI and IPI respectively. In fact, despite having a relatively low toxicity (acute contact LD_{50}=100 µg/bee, EFSA, 2008), sulphur was applied at high application rates and frequency in the fields, while spinosad was applied only once but holds a considerably high LD_{50}

(acute contact LD_{50}=0.0036 µg/bee, ECHA, 2010). Thus, the choice of using pesticide pressure indexes was proven to be an accurate method to describe the pressure of insecticides, herbicides, and fungicides on pollinators.

To calculate the PPI, we modified the approach of Yasrebi-de Kom et al. (2019) using not only the active ingredient application rates and LD_{50}, but also the area of application of the product ($PPI=\Sigma(A \cdot AR_{AI}/LD_{50})$). This allowed us to take into account potential differences in dimensions among sites. However, pesticide formulations do not only contain active ingredients, but also co-formulants, and are often applied together with adjuvants, whose functions range from emulsifiers, to solvents, to surfactants, and facilitate the action of the active ingredient (Straw et al., 2022). Such 'inactive' ingredients are not regulated by any toxicity testing (EU, 2021b), although they have been demonstrated to be potentially dangerous to bees (*e.g.* Mullin et al., 2010; Ciarlo et al., 2012; Mullin et al., 2015, 2016), and could even interact with active ingredients to produce synergistic effects on the health of beneficial insects (Park et al., 2015; Straw et al., 2022). On the contrary, active ingredients are indeed regulated (EFSA, 2013d). However, as pointed out earlier, current pesticide risk assessments mainly focus on *Apis mellifera*, and sub-lethal effects may vary among species (Siviter et al., 2018b). Thus, toxicity data used to calculate PPI may not be representative of all bees, and land cover and pesticide pressures may impact wild, solitary bees differently than social bees. A recent case-study on Great Britain fields showed that solitary bee species were dominant pollinators in both apple orchards and oilseed rape fields, particularly *Andrena* and *Lasioglossum* species (Hutchinson et al., 2021). Therefore, we suggest that future research should investigate pollinator abundance at the species level to shed light on the impact of landscape and pesticide usage on different bee species. Moreover, utilising not only generalist pollinators, but also specialists, may help address competition issues between the two, and further investigate the extent to which land cover and pesticide pressures impact different bee species. Finally, integrating additional species into pesticide risk assessments and accounting for the co-occurrence of multiple compounds could help safeguard non-*Apis* bees, and ensure the safety of both active and 'inactive' ingredients.

Bee responses to different stressors are highly influenced by many variables, some of which could not be included in the present study. For instance, calculating pesticide pressure indexes of agricultural lands in 1 km radius was not feasible. However, distinguishing between high- and low-intensity surrounding areas may provide further insight into the impact of land cover and usage of pesticides on bee health and abundance. In the same way, distinguishing between intensively- and non-intensively managed grassland, and classifying the surrounding land cover based on the richness in flowering resources, would have contributed to better describing the proportion and characteristics of semi-natural habitats in our study. In fact, previous studies have shown that low input grasslands are one of the most important source of floral resources for a wide range of pollinators, including both wild and managed bees (e.g. Öckinger & Smith, 2007; Kennedy et al., 2013; Proesmans et al., 2019).

Our large-scale fieldwork provided valuable evidence on the impact of both landscape and pesticide pressures on social bees and the delivery of pollination services in a short-time period. However, ecology long-term impact studies are fewer and more challenging to conduct (*e.g.* Senapathi et al., 2015; Hokkanen et al., 2017; Gardner et al., 2021), making it difficult to properly understand the real impact of pesticide usage and land cover on the long run. In this regard, pollinator monitoring schemes may be proven to be useful to monitor and tackle the overall decline of wild pollinators and its main causes in Europe, such as the UK PoMS and the EU Pollinator Monitoring Schemes (UKpoms.org.uk; EC, 2021).

2.5. Conclusions

The results of our large-scale fieldwork experiment in 16 sites across England, utilising *Apis mellifera* and *Bombus terrestris* as sentinel bees, may be summarised as follows:

a. **The proportion of cropland and woodland in 1 km radius significantly influenced the weight gain of *Bombus terrestris* colonies in apple orchards between the start and the middle of flowering.** A higher proportion of cropland and a lower proportion of woodland corresponded to a higher weight gain.

b. **Although no effect of insecticides was shown, fungicides and herbicides increased the weight gain of *Bombus terrestris* colonies in oilseed rape fields** between the start and the middle of flowering.

c. **No effect of land cover or pesticides on the percentage of workers and reproductives was shown in *B. terrestris* colonies** in apple orchards or oilseed rape fields.

d. **A positive effect of woodland was observed on the activity of *Apis mellifera* in apple orchards**, with higher proportions of SNH corresponding to a higher activity.

e. **Fungicides and herbicides increased the activity of bumblebees in oilseed rape fields**, consistent with findings on *B. terrestris* colony growth in the same sites.

f. **No effect of land cover or pesticides on the delivery of pollination services was observed,** either in terms of crop yield or percentage of class 1 apples, in apple orchards or oilseed rape fields.

In view of our results, we are able to conclude that landscape characteristics and pesticide pressures can influence the growth of *Bombus terrestris* colonies and the activity of social bees, although these effects were not consistent between the two crops. This suggests that multiple stressors, including pesticides and landscape characteristics, may interact between one-another and affect bees in many different ways, and underlines the importance of directing future research towards the impact of fungicides and herbicides on the health of wild, specialist pollinators whose survival may be threatened by high abundances of generalist bees.

Chapter 3

Effects of sulfoxaflor, *Crithidia bombi,* and their interaction on *Bombus terrestris* behaviour and pollination services.

Abstract

Bees are important contributors to biodiversity and ecosystem services, such as the pollination of many food crops and wild flowering plants. However, there is growing evidence of their decline at a national and global level, driven by different stressors which may also interact synergistically. Two of the main drivers of pollinator declines are the exposure to pesticide and parasites. Here, using a semi-field experiment, we investigated the effect of the novel insecticide sulfoxaflor and the common gut parasite *Crithidia bombi*, individually and in combination, on the individual and colony behaviour of *Bombus terrestris* and the delivery of their pollination services to field bean (*Vicia faba*). We found no evidence of an effect of sulfoxaflor or *C. bombi*, alone or in combination, on the behaviour or pollination of bumblebees, indicating that sulfoxaflor may potentially represent a safer alternative to neonicotinoid insecticides. However, further research is still required to confirm our results, as sub-lethal effects of pesticides may vary depending on bee species and exposure levels.

Contributions

3.1. Introduction

Pollination is a key ecosystem service benefitting about 75% of the leading food crops worldwide (Klein et al., 2007). In particular, animal pollination is estimated to profit global food crops with an economic value between \$235-577 bn per year (Lautenbach, 2012), enhancing their yield and quality (Bartomeus et al., 2014; Garibaldi et al., 2014; Garratt et al., 2014a).

Bumblebees (*Bombus spp.*) are among the most important pollinators in Europe and North America (Kleijn et al., 2015), responsible for pollinating many wild flowers and crops (Polce et al., 2018). Bumblebees have been shown to contribute to the yield of many important crops, including oilseed rape (Bommarco et al., 2012), apples (M. P.D. Garratt et al., 2016), and field beans (Bishop et al., 2016), and they are also commonly used to pollinate greenhouse crops (*e.g.* tomato), for which they are more efficient pollinators than *Apis* bees thanks to their buzz pollination behaviour and ability to remain active at cooler temperatures (Ahmad et al., 2015).

However, despite these benefits, there is well-documented evidence of a pollinator decline worldwide (*e.g.* IPBES, 2016; DEFRA, 2019; Dicks et al., 2021) which can impact the yield of crops and the quality of their fruits and seeds (Klatt et al., 2013; Reilly et al., 2020). In Europe, the most important drivers of such declines are changes in land cover, land configuration and management, and the impact of pesticides (Dicks et al., 2021). Pesticide exposure is one of the most investigated threats to bee health (Havard et al., 2019). Intensive farming practices usually necessitate a reliance on plant protection products to which bees are increasingly exposed to (Lopez-Uribe et al., 2020), and such exposure may threaten their health leading to both lethal and sub-lethal effects (IPBES, 2016; Havard et al., 2019).

Since their introduction in the market, as a result of their long persistence and efficacy at low concentrations, neonicotinoids have become the most widely used class of insecticides worldwide (Sgolastra et al., 2020a). Due to growing scientific evidence of the impact of field-realistic doses of neonicotinoids on the health of pollinators, the use of clothianidin, imidacloprid, and thiamethoxam have been banned in the EU (IPBES, 2016; Sgolastra et al., 2020a). Several studies have linked the use of neonicotinoids to lethal and sub-lethal effects at the individual and colony level, including impacts on foraging behaviour (*e.g.* Gill & Raine, 2014; Tasman et al., 2020; Siviter et al., 2021b), memory and/or learning abilities (*e.g.* Muth et al., 2019; Samuelson et al., 2016; Siviter et al., 2018b), worker production (Gill et al., 2012; Gill & Raine, 2014; Whitehorn et al., 2012), reproductive success (*e.g.* Whitehorn et al., 2012; Rundlöf et al., 2015; Siviter et al., 2021c), and colony growth (*e.g.* Gill et al., 2012; Rundlöf et al., 2015; Siviter et al., 2021b). As such, particularly following the EU ban, investigating the impact of other classes of insecticides on bee health is key to finding safer and effective alternatives for crop pest management (Siviter et al., 2018a; Azpiazu et al., 2021).

Sulfoxaflor is the first marketed insecticide belonging to the class of sulfoximine and is currently emerging as a potential substitute for neonicotinoids (Sparks et al., 2013; Brown et al., 2016; Sgolastra et al., 2020). According to the European Food Safety Authority (EFSA), sulfoxaflor acute contact LD_{50} amounts to 0.379 µg/bee (EFSA, 2014), indicating a lower toxicity than clothianidin, imidacloprid, and thiamethoxam, for which the value is estimated to be 0.0443, 0.081, and 0.024 µg/bee respectively (EFSA, 2013a, 2013b, 2013c). Sulfoxaflor has been proven to be able to target some neonicotinoid-resistant pests (Zhu et al., 2011), and to have a shorter persistence than neonicotinoids in pollen, nectar and soil (Siviter & Muth, 2020). However, as a systemic insecticide, its residues can still persist for days (maximum tested period: 11 days. Source: EPA, 2019), and bees could still be exposed to it during foraging (Botías et al., 2015). Chronic exposure to sulfoxaflor has been linked to a lower worker production and reproductive success of bumblebee colonies similar to those caused by neonicotinoids (Siviter et al., 2018a), and to a lower production of eggs potentially driven by a reduction in feeding (Siviter et al., 2020a). However, no effect of chronic exposure to sulfoxaflor was found on bumblebee foraging performance (Siviter et al., 2018a), and no impact of acute sulfoxaflor exposure on bee learning and behaviour was observed (Siviter et al., 2019), contrary to neonicotinoids at comparable dosages (Stanley et al., 2015b; Samuelson et al., 2016). Studies assessing sulfoxaflor effects on bees are limited, and further evidence is still required before it can be effectively considered as a safe replacement for neonicotinoids (DEFRA, 2019; Siviter et al., 2019; Azpiazu et al., 2021).

Exploring potential interactions between sulfoxaflor and other common stressors is also essential, as they may act synergistically when combined (Azpiazu et al., 2021). For instance, the simultaneous exposure of bumblebee larvae to sulfoxaflor and the parasite *Nosema bombi* was shown to increase their mortality, while the exposure to both stressors in isolation did not lead to a higher death rate (Siviter et al., 2020b). Moreover, Azpiazu et al. (2021) showed that the interaction between sulfoxaflor and the fungicide fluxapyroxad did not decrease *Bombus terrestris* survival, but it did affect the survival of other bee species.

The spread of parasites and diseases represents another driver of pollinator decline (Dicks et al., 2021), and is linked to the commercialisation, movement, and trade of managed bees and beehive products (Dormann et al., 2008; Graystock et al., 2014). In particular, *Crithidia bombi* is a highly prevalent gut parasite which can be transmitted via faeces or orally (Figueroa et al., 2019), with an infection rate that could be up to 80% (Gillespie, 2010). Despite being relatively benign in favourable circumstances, this parasite was shown to negatively impact colony survival under stress conditions (Brown et al., 2000). Moreover, *C. bombi* infection is thought to be responsible for sub-lethal effects on bumblebees, such as a lower colony reproduction and fitness (Brown et al., 2003; Yourth et al., 2008; Goulson et al., 2018), impaired cognitive abilities (Gegear et al., 2006) and foraging behaviour (Shykoff & Schmid-Hempel, 1991; Otterstatter et al., 2005; Gegear et al., 2005, 2006). Although existing research has investigated the interaction effect of *C. bombi* with some common insecticides (Baron et al., 2014; Fauser-Misslin et al., 2014; Fauser et al., 2017), no study has yet analysed its effect in combination with the newly emerged insecticide sulfoxaflor.

Our study aimed to address gaps in the literature on sulfoxaflor and its potential interactive effect with *Crithidia bombi* on bee health and crop pollination using a semi-field experiment performed in outdoor flight-cages with commercially reared bumblebee colonies (*Bombus terrestris audax*) and field bean plants (*Vicia faba*). Field bean is an extensively grown crop in Europe, mostly due to its high protein, carbohydrate, mineral, and B-vitamin content (Crépon et al., 2010), and to its capacity of maintaining soil fertility thanks to biological N-fixation and solubilisation of phosphorus (Rashid et al., 2016). Moreover, bumblebees have been shown to effectively contribute to its yield in terms of both pod set (Garratt et al., 2014b) and plant weight (Bartomeus et al., 2014). Through the experiment, we investigated the impact of these two stressors and their interaction on: (i) the foraging behaviour of bumblebees at the individual and colony level, and (ii) the yield of field bean plants to which they were exposed.

The study was adapted from the work of Stanley et al. (2015a), which showed how field-realistic dosages of a neonicotinoid insecticide can affect the ability of bumblebees to pollinate apple crops influencing their visitation rates, pollen collection, and yield. Therefore, we aim to address the following questions:

a. Does the exposure to sulfoxaflor at field-realistic levels, and the infection with *C. bombi*, affect the behaviour of bumblebees at the colony and individual level?

b. Do sulfoxaflor and *C. bombi* interact in any way impairing the behaviour of bumblebees?

c. Are pollination services provided by bumblebees affected by exposure to sulfoxaflor, inoculation with *C. bombi*, or their interaction?

3.2. Methodology

3.2.1. Experimental design

A semi-field experiment was conducted at the University of Reading between May and June 2021 for a period of 7 weeks. A total of 9 experimental blocks were used, each including 8 colonies except the first block, which comprised four colonies as per experiment logistics (*i.e.* total number of colonies and flight cages was 68 and 8 respectively, therefore it was not possible to process more than 8 colonies at a time). A final number of 36 colonies in five experimental blocks was used.

The main experiment was preceded by a pilot season of four weeks with 50 field bean (FB) plants and four bumblebee colonies between April and May 2021 to finalise the experimental design.

3.2.1.1. Preparation of bumblebee colonies

3.2.1.1.1. Crithidia bombi inoculation

Bombus terrestris audax Biobest colonies were supplied by Agralan Ltd. (www.agralan.co.uk) and prepared at the Royal Holloway University of London. Each experimental block was screened for parasites and culled down to 20 workers per colony plus the queen. Colonies were then weighed and allocated to a specific treatment group by weight ranking which was rotated for each block. With the exception of the first

experimental block – which was made of four colonies, *i.e.* one per treatment – two colonies per block were allocated to one of the following treatment groups: 'control', 'sulfoxaflor', '*Crithidia*', '*Crithidia**sulfoxaflor'. The whole experiment was blind, meaning that observers were unaware of which solutions contained sulfoxaflor and which contained distilled water only, and which colonies were infected and which were not. The blind was only broken at the end of the trial.

Colonies assigned to '*Crithidia*' or '*Crithidia**sulfoxaflor' groups were inoculated with *Crithidia bombi* (dose: 25,000 cells per bee), and the whole block was left to develop for one week with access to reservoirs filled with glucose solutions, allowing the infection of designated colonies to establish. A further screen was then conducted to discard potential '*Crithidia*' and '*Crithidia**sulfoxaflor' colonies with infection <25%, or any 'control' and 'sulfoxaflor' colony that got accidentally infected (see Appendix 3.1 for protocols). The block was then transported to the University of Reading (Crops and Environment Laboratory, Reading, UK) where it was stored in a well-ventilated room with controlled temperature (24-26 °C) and humidity (50±20%) to rest for 24 hours, during which time bees had access to glucose reservoirs.

3.2.1.2. Pesticide treatment

After the 24-hour rest period, reservoirs were closed and colony boxes were covered with thick layers of cotton wool to protect them from the cold. Each colony was then placed in a flight cage with designated FB plants.

After one day of acclimatisation (day 0), behavioural observations were carried out during the next three days (day 1, 2 and 3). While in cages, colonies were supplied with *ad libitum* 30% w/w sucrose solutions every 24 hours; solutions for 'control' and '*Crithidia*' groups contained distilled water only, while solutions prepared for 'sulfoxaflor' and '*Crithidia**sulfoxaflor' groups contained sulfoxaflor with realistic concentrations that mimicked the natural degradation of the insecticide over time after spray applications: day 0 = 0.161 mg/kg, day 1 = 0.047 mg/kg, day 2 = 0.014 mg/kg, day 3 = 0.004 mg/kg (Linguadoca et al., 2021). This time-decaying, realistic exposure regime was modelled by Linguadoca et al. (2021), who re-analysed EFSA sulfoxaflor residue dataset published in 2019 (EFSA, 2019).

Pesticide solutions were prepared at Royal Holloway and frozen in individual falcon tubes (10 mL) before being sent to Reading, where they were defrosted and mixed with 390 g sucrose solutions right before feeding time. Each morning colonies were fed *ad libitum* solutions through gravity feeders attached at the base of the box and refilled every 24 hours with solutions matching the appropriate treatment day and concentration (day 0, 1, 2, and 3) (see Appendix 3.1 for protocols). Exposure took place exclusively while bees were in the cages.

3.2.1.3. Flight cages

Eight outdoor flight cages (each 4.2 x 2.1 x 4.2 m) were equipped with a stand to keep bumblebee colonies raised from the ground and a shelter to protect them from adverse weather. Each cage was randomly assigned 1 bumblebee colony for each study block, where they were left for the four-day experimental period including overnight. A cage rotation system was in place so that by the end of the trial every treatment had been allocated to all cages at least once (see Appendix 3.1 for protocols).

3.2.1.4. Field bean plants

Field bean plants were used to assess the impact of treatments on bumblebee foraging behaviour, as they are economically important insect-pollinated crops for which bumblebees are effective pollinators (Garratt et al., 2014b). Bean plants of the 'Fuego' variety were grown in 3L pots containing 'John Innes n° 2' compost and thinned down to 1 plant per pot when they reached an adequate size.

Three hundred and twenty FB plants were grown in two temporal cohorts to ensure plants at the appropriate flowering stage were used for the experiments. Plants were grown in a glasshouse and moved to pollinator-free flight cages when in flower, and test plants were selected which had enough fresh flowers for each day of bee visit monitoring.

3.2.1.5. Behavioural observations

Observations of bee behaviours were based on the work of Stanley et al. (2015a). One colony and field bean plants were placed in each of the 8 flight cages per study block for four days. On day 0, bees were left to acclimatise to cages for 6 hours with two FB plants, after which colonies were closed. On observation day 1, 2 and 3, three FB plants were moved into the flight cages each day for colony observations and two for individual observations (Figures 3.2.1-3.2.2). All flowers on each FB plant were also counted to allow calculation of colony visitation rates (visits per flower per minute). Observers were assigned 1 cage each following a rotation scheme that allowed each of them to cover two-four cages per day, so that by the end of the day all cages would have been observed. Observers always started their observations from a different cage to further minimise observer effects. However, due to time constraints, it was often necessary for one observer to assess additional treatments to those on their individual rotation scheme, and adverse weather conditions such as cold temperatures, rainfalls, and wind did not allow all colonies to be observed every day.

3.2.1.5.1. Colony observations

Colony activity was measured by filming and later scoring the number of bees leaving and returning to the colony using the event-logging software 'BORIS' (Friard & Gamba, 2016. See Appendix 3.1 for 'Plant exposure' protocol and ethogram codes). The observer opened the colony entrance and allowed 10 minutes of acclimatisation starting from the moment the first bee left the colony, then turned on the camera to record the entrance of the colony. The number of visits made by bees to three FB plants was also recorded for five minutes per plant to calculate visitation rates. Plants were placed in a randomised order and observations started from the right to the left. When observations had ended, all bees were returned to colonies and

plants were moved out of the cages into a pollinator-free cage. This allowed all plants in different cages to be exposed to colonies for the same amount of time. The same three plants were exposed to the same colony throughout the three days of observations. The length of exposure was agreed upon to avoid over-pollination, which is defined as extreme pollination, potentially capable of damaging flowers and, consequently, limiting crop production (Sàez et al., 2014). During the pilot experiment, we observed that, in a typical warm, sunny day, a plant could require an average of approximately 100 minutes to have all its flowers visited once, depending on the number of flowers and bee visits (see Appendix 3.1); considering that (i) the same three plants would have been exposed to bees for three days, (ii) bees would have needed 10 minutes of acclimatisation before observations, (iii) flower numbers could be highly variable, and (iv) observers would have required sufficient time to process 8 cages in a day, we opted for a standardised exposure of 75 minutes over three days (*i.e.* 25 minutes per day).

3.2.1.5.2. Individual observations

Two FB plants were assigned to one colony for individual observations throughout the three-day observation period. The observer allowed one bee out of the colony at a time and recorded its behaviour for a maximum of 15 minutes starting from the moment it left the colony. Recorded behaviours included latency (time taken to visit the first flower), overall duration of foraging trip, time spent on flowers (average time of flower visits per bee), time between one flower visit and the next (average time per bee), foraging rate (number of flowers visited divided by foraging trip duration), and if pollen was collected or not. Observations of individual bees were carried out with the help of the event-logging software 'BORIS' (Friard & Gamba, 2016. See Appendix 3.1 for 'Plant exposure' protocol and ethogram codes). If the bee did not start foraging within 10 minutes, it was captured in a falcon tube and another bee was allowed out of the colony. If the bee attempted to return to the colony before 15 minutes had elapsed (*i.e.* landing on the entrance), it was assumed the foraging trip had ended. At the end of the trip, the bee was captured in a falcon tube and returned to the colony after all individual observations had been completed. Although the aim was to observe three bees per colony, this was not always possible due to suboptimal weather conditions, which sometimes forced us to observe one-two bees per colony (see Appendix 3.2).

3.2.1.6. Pollination services

To assess the level of pollination delivered by each colony, the three plants used for colony-level observations were also employed as phytometer plants. One stem of each phytometer plant was marked with cable ties above and below two floral nodes which were in flower (*i.e.* nodes with freshest and most receptive flowers) and flowers between cable ties were counted. After carrying out colony observations, the plants were removed and transferred to an insect-free flight cage where they continued to grow and ripen for two months. At harvest, the number of pods per node between cable ties and node location was recorded, and pods were then dried in the oven for 48h at 80 °C, after which pod weight, number of pods per node, beans

per pod, and weight of individual beans was recorded (see 'Plant yield measurements' protocol in Appendix 3.1).

Figure 3.2.1: Arrangements in one of the cages for colony-level observations.
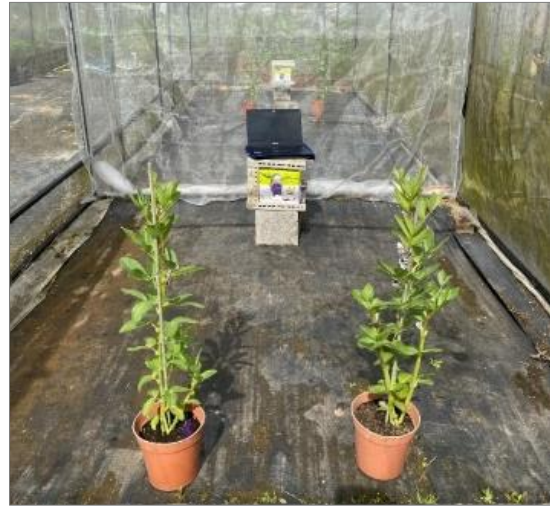
Figure 3.2.2: Arrangements in one of the cages for individual-level observations.

3.2.1.7. Colony development

Following the final day of observations, colonies were returned to controlled temperature rooms (24-26°C) where they remained for 6 more weeks. During this time, colonies were fed 1 tablespoon of pollen through the lid once a week, and had *ad libitum* access to their glucose reservoirs, which were topped up with 50% w/w sugar when required. After 6 weeks, colonies were frozen at -20°C and later collected by Royal Holloway to assess whether any treatment affected their development (see Appendix 3.1 for 'Colony development requirements' protocol).

3.2.2. Statistical analysis

A total of 88 colony observations, 149 individuals, and 106 plants were analysed (Table 3.2.1). Due to adverse weather conditions, the first three experimental blocks and the last one were excluded from the analysis, together with two colonies that were accidentally infected with *Crithidia bombi* and two others that were supplied with incorrect treatment solutions. Moreover, 21 files with data on number of bees leaving and re-entering colonies collected throughout the whole trail period were lost because of technical issues of one of the laptops, thus they could not be included in the analysis investigating such response variables (n=67). As individual observations were not possible for all treatments in block 1, only blocks 2 to 5 were included in the individual observation analysis. Finally, one of the phytometer plants was discarded as it was dead. The number of replicates per colony for both individuals and plants are presented in Table 3.2.2 (see Appendix 3.2 for further data summary on colony and individual observations).

Data on treatment and observation day were checked for correlations using Pearson Product-Moment test to avoid multicollinearity issues (see Appendix 3.2), and mixed-effect models were built in Genstat 21 (Goedhart & Thissen, 2021) to assess the impact of treatments on bee behaviour and plant yield (see

Appendix 3.2 for global models). Models with the lowest AICc value and ΔAICc ≤ 2 were selected, where ΔAICc is the difference between the AICc of the candidate model and the lowest AICc (Burnham & Anderson, 2004; Symonds & Moussalli, 2011). Fisher's protected LSD post-hoc tests were planned in case observation day or treatment would have been significant.

Table 3.2.1: Details on data included in statistical analyses of individuals, colonies, and plant yield. See Appendix 3.2 for numbers of observations divided by experimental block, observation day, and colony.

Treatment	N colony observations (visitation rate)	N colony observations (bees leaving/returning)	N individuals	N plants
Control	23	19	42	26
Crithidia	23	17	43	26
Sulfoxaflor	22	16	31	27
Crithidia*sulfoxaflor	20	15	33	27
Total	**88**	**67**	**149**	**106**

Table 3.2.2: Number of replicates of individual bees ('Indiv'), and plants per colony over the three-day period (two colonies of the same treatment per block). As explained above (see 3.2.2), no block 1 individuals were included in the analysis. See Appendix 3.2 for individuals that were observed each of the three days.

Treatment colony	Block 1 Plants	Block 2 Indiv	Plants	Block 3 Indiv	Plants	Block 4 Indiv	Plants	Block 5 Indiv	Plants
Control C1	2	3	3	6	3	9	3	8	3
Control C2	3	7	3	0	0	6	3	3	3
Crithidia C1	3	7	3	4	3	9	3	4	3
Crithidia C2	3	8	3	3	2	0	0	8	3
Sulfoxaflor C1	3	5	3	6	3	7	3	1	3
Sulfoxaflor C2	3	6	3	0	3	2	0	4	3
Crithidia*sulfoxaflor C1	3	8	3	6	3	3	3	6	3
Crithidia*sulfoxaflor C2	3	1	3	0	0	0	3	9	3
Total	**23**	**45**	**24**	**25**	**17**	**36**	**18**	**43**	**24**

3.2.2.1. Individual and colony observations

Response variables for individual observations included latency, duration of foraging trip, duration of flower visits, time between visits, foraging rate, and pollen collection, while visitation rate and number of bees leaving and entering colonies were dependent variables for colony-level assessments. Treatment, observation day, and interaction between the two were included as fixed terms, while 'experimental block and colony ID' and 'observer' were included as random factors to account for potential variation in observations. Data were analysed using either Linear Mixed Models (LMMs) for normal distributions, or Generalized Linear Mixed Models (GLMMs) for count and binary data. Count data were first tested with the goodness-of-fit Chi-Square test for observed versus expected counts (see Appendix 3.2), and data appearing not to follow a Poisson distribution (p<0.05) were analysed using a Quasi-Poisson distribution to account for under- or over-dispersion (dispersion parameter allowed to be ≠ 1).

In addition, a further separate analysis was conducted to test for the effect of prevalence of *Crithidia bombi* infection. For this specific analysis, both 'control' and 'sulfoxaflor' colonies were excluded, and only comparisons between '*Crithidia*' and '*Crithidia**sulfoxaflor' colonies were included (n individuals=76, n colony observations for visitation rate=43, n colony observations for number of leaves and returns=32). The

same response variables, random factors, and fixed factors were used, except 'treatment' was the percentage of *Crithidia* infection.

3.2.2.2. Pollination services

Linear Mixed Models were used to test treatment effects on mean number of pods per node, beans per pod, and pod and bean weight between the cable ties. Treatment and location of first node were included as fixed terms, while 'plant' nested within 'experimental block and colony ID' were used as random factors. 'First node location' was treated as a categorical variable including early (1 to 5), middle (6 to 10), and late flowering nodes (11 to 16).

A further analysis was performed with plants exposed to *Crithidia*-infected colonies only, where 'treatment' was the percentage of *Crithidia bombi* infection. In this case, as we aimed to investigate yield data and not changes in bee behaviours, plants exposed to control colonies were also included in the analysis (n=80).

3.3. Results

3.3.1. Colony observations

The analyses including all treatment groups and *Crithidia*-infected colonies only did not show any significant effect of treatment on the visitation rate or number of bees leaving and returning to colonies (Tables 3.3.1-3.3.2.). A significant effect of observation day on visitation rate was observed in both analyses, with the lowest rate on day 1 (Table 3.3.1, Figure 3.3.1). Moreover, observation day significantly influenced the number of bees leaving the colony in the analysis including all treatments, with fewer bees leaving on day 1 compared to day 3 (Table 3.3.2, Figure 3.3.2).

Table 3.3.1: Final LMMs investigating bee visitation rates. ΔAICc = 0 is the lowest AICc model. ΔAICc GM = ΔAICc with the global model. See Appendix 3.3 for model selection, estimates, SE, and predicted means.

Visitation rate	Fixed terms	Random terms	F	ndf, ddf	p-value	AICc	ΔAICc GM	ΔAICc	R^2
All treatments	Observation day	Block and colony	9.43	2, 53.9	**<0.001**	-294.06	43.56	0	18.15
% *Crithidia* infection	Observation day	Block and colony	8.39	2, 25.5	**0.002**	-144.75	43.50	0	29.56

Table 3.3.2: Final GLMMs on number of bees leaving and returning to colony. ΔAICc = 0 is the lowest AICc model. ΔAICc GM = ΔAICc with the global model. See Appendix 3.3 for model selection, estimates, SE, and predicted means. No AICc is shown for models with all non-significant terms since no selection criteria was applied.

N bees leaving	Random terms	Fixed terms	χ^2	df	p-value	AICc	ΔAICc GM	ΔAICc	R^2
All treatments	Block and colony + observer	Observation day	8.33	2	**0.016**	118.95	24.01	0	4.23
% *Crithidia* infection	Block and colony + observer	Observation day	2.93	2	0.231	-	-	-	10.81
		Treatment	0.30	1	0.587				
		Interaction	0.00	2	0.998				
N bees returning	**Random terms**	**Fixed terms**	χ^2	**df**	**p-value**	**AICc**	**ΔAICc GM**	**ΔAICc**	R^2
All treatments	Block and colony + observer	Observation day	1.36	2	0.507	-	-	-	12.82
		Treatment	1.51	3	0.680				
		Interaction	2.28	6	0.892				

|									
***% Crithidia* infection**	Block and colony + observer	Observation day	0.19	2	0.908	-	-	-	26.56
		Treatment	0.56	1	0.455				
		Interaction	5.20	2	0.074				
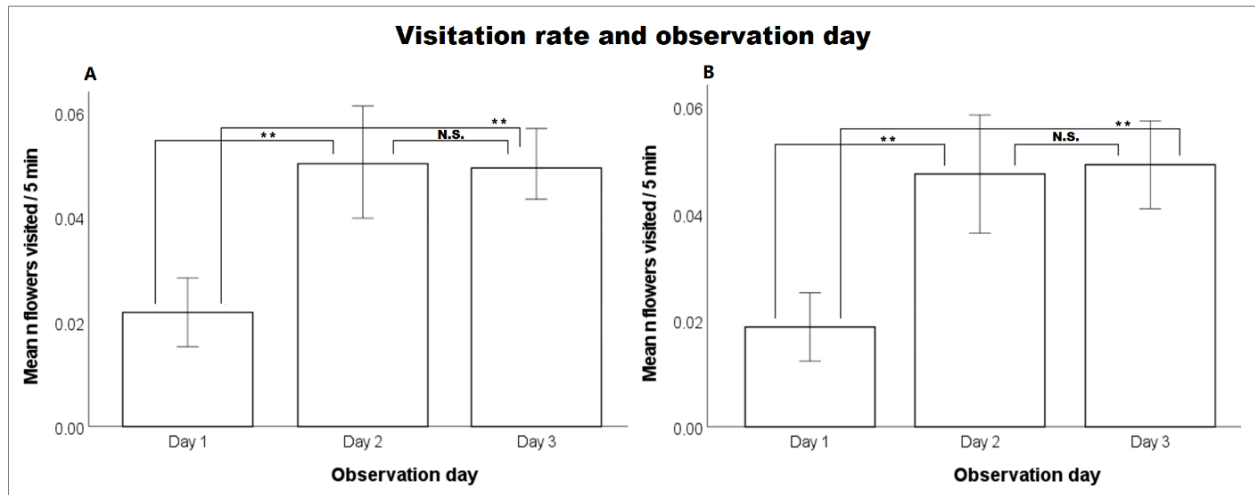
Figure 3.3.1: Significant effect of observation day on visitation rates of all treatment groups (A) and *Crithidia*-infected colonies only (B), **p<0.01, N.S. not significant. See Appendix 3.2 for post-hoc tests. Error bars: ± 1 SE from the mean.
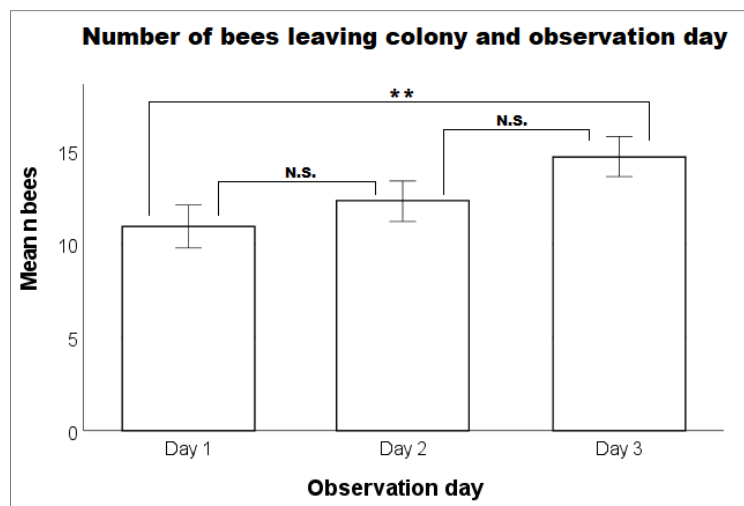

Figure 3.3.2: Significant effect of observation day on number of bees that left colonies (analysis on all treatment groups), **p<0.01, N.S. not significant. See Appendix 3.3 for post-hoc test. Error bars: ± 1 SE from the mean.

3.3.2. Individual observations

The analysis including all treatment colonies found no significant effect of treatment on foraging rate, duration of foraging trip, latency, average time between visits, average duration of flower visits, and pollen collection (Table 3.3.3, Figure 3.3.3). No significant effect of observation day or interaction between observation day and treatment was detected, however the model analysing the effect of observation day-treatment interaction on pollen collection returned a p-value of 0.051, which is near significance (Table 3.3.3). The non-significant result was however confirmed by Fisher's LSD post-hoc test, which returned a non-significant variance ratio for the interaction term (Appendix 3.3).

Similarly, analyses including only *Crithidia*-infected colonies did not show any significant effect of the percentage of *Crithidia bombi* infections on foraging rate, pollen collection, duration of foraging trip, latency,

average time between visits, or average duration of flower visit, and no effect of observation day or interaction with treatment was found on any response variable (Table 3.3.3, Appendix 3.3).

Table 3.3.3: Models investigating individual-colony behaviours of (1) all treatment colonies and (2) *Crithidia*-infected colonies, showing no significant terms. See Appendix 3.3 for estimates, SE, and predicted means.

| Model variables			(1) All treatments			(2) % *Crithidia* infection only		
Response variable	Random terms	Fixed terms	F	ndf, ddf	p-value	F	ndf, ddf	p-value
Foraging rate	Block and colony Observer	Observation day Treatment Interaction	0.49 0.51 1.74	2, 129.6 3, 21.5 102.7	0.614 0.677 0.119	0.38 0.70 0.03	2, 69.1 1, 68.9 2, 69.7	0.685 0.405 0.972
		R^2	R^2	8.69		R^2	2.13	
Duration of foraging trip	Block and colony Observer	Observation day Treatment Interaction	0.56 0.81 1.66	2, 124.2 3, 20.2 6, 109.9	0.575 0.504 0.138	1.65 0.42 0.11	2, 64.7 1, 12.7 2, 67.0	0.199 0.528 0.989
		R^2	R^2	8.99		R^2	5.33	
Latency	Block and colony Observer	Observation day Treatment Interaction	0.61 1.11 0.63	2, 135.0 3, 136.3 6, 111.9	0.545 0.346 0.707	0.30 0.01 0.46	2, 69.7 1, 69.9 2, 69.6	0.744 0.918 0.631
		R^2	R^2	5.74		R^2	2.14	
Time between visits	Block and colony Observer	Observation day Treatment Interaction	0.18 0.74 0.35	2, 135.1 3, 136.3 6, 111.8	0.836 0.527 0.906	0.61 1.29 0.80	2, 66.6 1, 15.2 2, 68.4	0.546 0.273 0.452
		R^2	R^2	3.34		R^2	5.56	
Duration of flower visits	Block and colony Observer	Observation day Treatment Interaction	0.53 0.86 0.77	2, 131.0 3, 20.6 6, 90.3	0.588 0.576 0.596	0.35 1.05 0.06	2, 69.4 1, 69.4 2, 69.9	0.704 0.308 0.943
		R^2	R^2	5.36		R^2	2.61	
Pollen collection	Block and colony Observer	Observation day Treatment Interaction	0.73 0.89 2.35	2, 124.9 3, 19.7 6, 35.6	0.484 0.463 0.051	0.71 0.82 0.57	2, 64.9 1, 13.3 2, 68.0	0.494 0.382 0.571
		R^2	R^2	19.95		R^2	8.25	

Figure 3.3.3: There was no significant effect of treatment on foraging rate (A), duration of foraging trip (B), latency (C), time between visits (D), duration of flower visits (E), or pollen collection (F). Error bars: ± 1 SE from the mean.

3.3.3. Pollination services

Final models of plant yield measurements including all treatment groups and *Crithidia*-infected colonies did not show any significant effect of treatment or location of first node on average number of beans, average number of pods, average pod weight, or average bean weight (Table 3.3.4, Figure 3.3.4, Appendix 3.3).

<u>Table 3.3.4</u>: LMMs investigating the yield of plants exposed to (1) all treatment colonies, and (2) *Crithidia*-infected colonies only, showing no significant terms. See Appendix 3.3 for estimates, SE, and predicted means.

| Model variables			(1) All treatments			(2) % *Crithidia* infection only		
Response variable	Random terms	Fixed terms	F	ndf, ddf	p-value	F	ndf, ddf	p-value
Average n. beans	Plant nested within block and colony	Treatment 1st node location	1.94	3, 27.0	0.147	0.18	1, 24.8	0.673
			0.10	2, 68.3	0.907	0.50	2, 63.9	0.610
			R^2	8.01		R^2	1.78	
Average n. pods	Plant nested within block and colony	Treatment 1st node location	1.63	3, 31.6	0.203	1.99	1, 28.6	0.170
			0.19	2, 92.4	0.831	1.04	2, 85.2	0.357
			R^2	4.99		R^2	4.47	
Average pod weight	Plant nested within block and colony	Treatment 1st node location	0.33	3, 24.9	0.803	0.00	1, 65.0	0.970
			0.51	2, 67.7	0.604	0.02	2, 65.0	0.981
			R^2	2.83		R^2	0.06	
Average bean weight	Plant nested within block and colony	Treatment 1st node location	0.43	3, 24.7	0.734	0.23	1, 21.7	0.637
			0.88	2, 63.5	0.421	0.28	2, 63.5	0.760
			R^2	4.22		R^2	1.19	
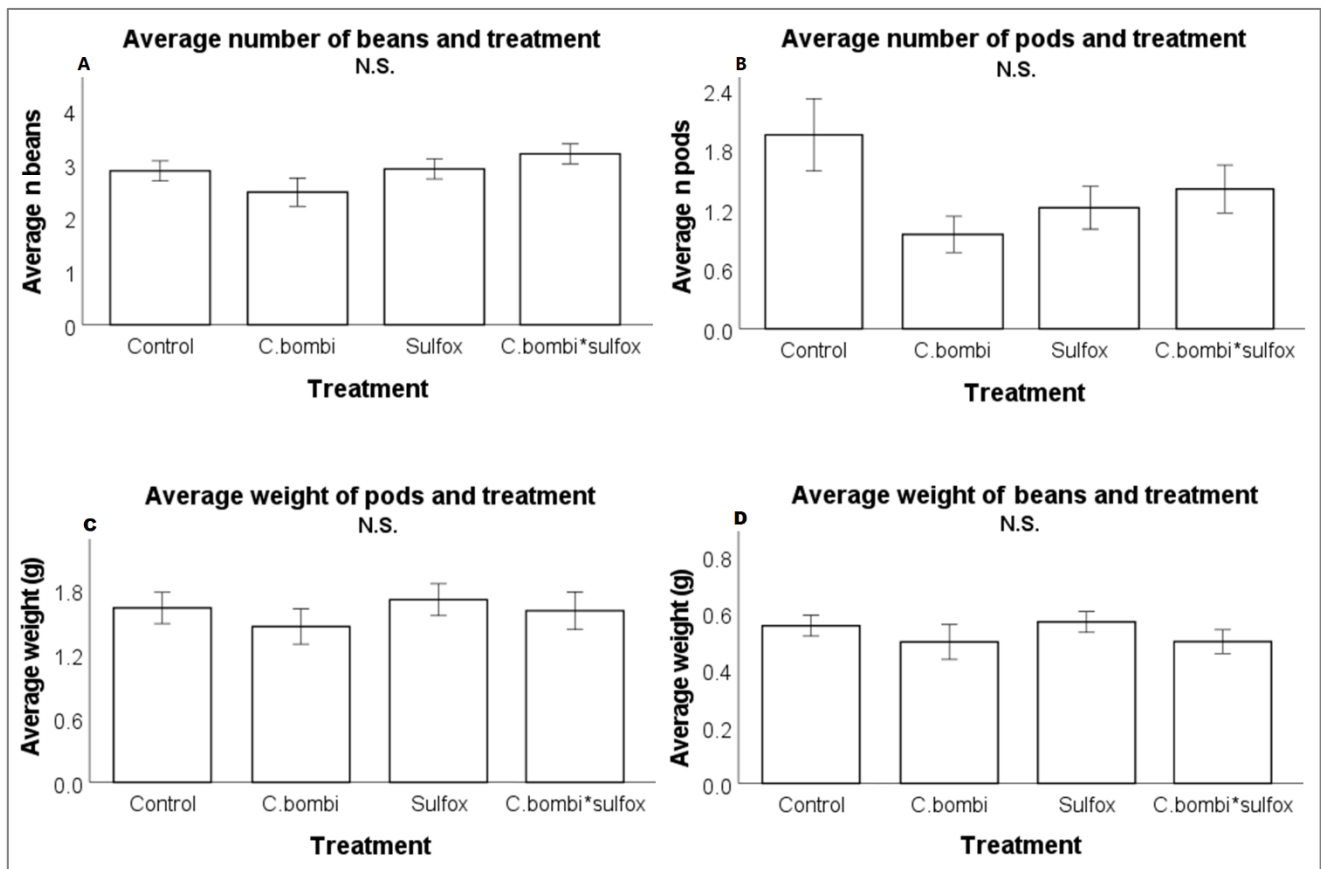
<u>Figure 3.3.4</u>: There was no significant effect of treatment on average number of beans (A), average number of pods (B), average pod weight (C) or average bean weight (D). Error bars: ± 1 SE from the mean.

3.4. Discussion

Using a semi-field study in outdoor flight cages, we assessed the impact of sulfoxaflor and *Crithidia bombi*, both individually and in combination, on the behaviour of *Bombus terrestris* colonies and their pollination of field bean (*Vicia faba*) plants.

3.4.1. Impact of sulfoxaflor and *Crithidia bombi* on bee behaviour and pollination services

Whilst the previous work of Stanley et al. (2015a) found that the neonicotinoid thiamethoxam affected the visitation rate and pollen collection of bumblebees on apple, our experiment showed that bumblebees were not impacted by sulfoxaflor at field-realistic levels of exposure, with no significant differences in colony or individual behaviours between treatment and control groups. No effect of *Crithidia bombi* on bee behaviour was observed either, contrary to previous studies which showed that this parasite can affect foraging behaviours in terms of visitation rate and time spent on flowers (Gegear et al., 2005; Otterstatter et al., 2005). This study is the first to investigate the interaction between sulfoxaflor and *C. bombi*, and shows that they do not to impair bee behaviour at the individual or colony level under the conditions of our experiment. Neonicotinoid exposure has been linked to a reduction in visitation rate and pollen collection (Feltham et al., 2014; Gill et al., 2012; Stanley et al., 2015a; Whitehorn et al., 2017), which translates into less efficient foraging behaviour. In fact, neonicotinoid impacts on bumblebee colony behaviour were shown to cause a lower production of seeds, with repercussions on apple yield and fruit set (Stanley et al., 2015a). However, since no significant differences in bee behaviour were observed among treatments, our study reported no effect of sulfoxaflor, *Crithidia bombi*, or their combination on the yield of field beans in terms of pod set, bean set, pod weight and bean weight, indicating that sulfoxaflor might be a less harmful alternative to neonicotinoids. This view is supported by past research showing no impact of acute sulfoxaflor exposure on working memory and behaviour of bumblebees (Siviter et al., 2019), while such effects were observed for the neonicotinoid thiamethoxam at comparable dosages (Stanley et al., 2015b).

The field-realistic exposure regime we used for the experiment was based on the strawberry exposure scenario proposed by Linguadoca et al. (2021), who re-analysed EFSA sulfoxaflor residue dataset published in 2019 (EFSA, 2019) and modelled a time-decaying, realistic exposure mechanism of sulfoxaflor in nectar, which estimated its residues after spray applications. While EU countries are encouraged to stop any sulfoxaflor applications 5 days before flowering, nearby non-target crops, that are already in flower could be reached by the spray drift directed to target crops that are not yet flowering, potentially exposing pollinating insects to higher doses of sulfoxaflor than expected. Moreover, the direct spray of sulfoxaflor on flowering plants is allowed in other non-EU countries, including USA, Australia, New Zealand, and South Africa (Siviter et al., 2021a), while sulfoxaflor use has not yet been approved in the UK, although pesticide authorisations made under the EU pesticide regimes still applies after Brexit (HSE, 2021). Although the absence of mitigation measures made our exposure regime a worse-case scenario, it has to be noted that the experiment consisted

of providing bumblebees with spiked sugar solutions only, without contaminated pollen. Since residues are often higher in pollen than in nectar (EFSA, 2019; Linguadoca et al., 2021), repeating the experiment exposing bees to both contaminated pollen and nectar might lead to different outcomes, that are worth being investigated.

Sub-lethal effects caused by pesticides may also vary depending on exposure levels (Siviter et al., 2018b). Stanley et al. (2015a) chose to expose *B. terrestris* colonies to 0.0024 mg/kg and 0.01 mg/kg thiamethoxam solutions over a period of 13 days. However, only colonies exposed to the higher concentration showed a treatment effect on their behaviour and pollination, though both doses are considered field realistic. This indicates that different dosages can produce distinct effects on bees. For our experiment with sulfoxaflor, we followed the time-decaying, realistic exposure regime modelled by Linguadoca et al. (2021) which consisted of a much shorter exposure, starting with a concentration of 0.161 mg/kg on day 0 that quickly dropped to 0.004 mg/kg on day 3. Although the differences with Stanley et al. (2015a) in exposure regimes are due to the fact that we used a different insecticide, it is however possible that a longer exposure mechanism with lower concentrations might be more toxic to bees than a shorter exposure at higher dosages (Medrzycki et al., 2013). Therefore, this should be considered when drawing conclusions regarding the safety of sulfoxaflor compared to neonicotinoid insecticides, and should be further investigated.

An additional difference with the experiment of Stanley et al. (2015a) is that our behavioural observations on bumblebees were carried out while they had *ad libitum* access to the spiked solutions, and not after the exposure period was over. This choice was driven by the fact that sulfoxaflor concentrations would have quickly decreased (Linguadoca et al., 2021), with the risk of not being able to observe any pesticide effect on bees. However, having *ad libitum* access to gravity feeders might have discouraged workers to exit the colony and start foraging, particularly when weather conditions were not ideal. Although this may not have impacted the delivery of pollination services, the experiment could be repeated in the fields and with no access to feeders during foraging. In fact, a fully field-realistic experiment may offer new insights into the effect of sulfoxaflor and its interaction with *Crithidia bombi* on bee behaviour and pollination.

3.4.2. Future research implications

Overall, the lack of a treatment effect in our semi-field experiment indicates that sulfoxaflor may be less harmful to *Bombus terrestris* than neonicotinoid insecticides. Its potentially higher safety, coupled with its ability to target neonicotinoid-resistant pests (Zhu et al., 2011), might potentially make sulfoxaflor a better and safer alternative to neonicotinoids.

The only statistically significant effect found in our study was that of the observation day on colony behaviour of bumblebees. In fact, observation day 1 showed a significantly lower visitation rate and number of bees leaving the colony compared to observation day 2 and 3. This may be explained by the fact that bees needed a longer acclimatisation time to get used to the flight cages than 6 hours; the more time passed, the more

bees may have become comfortable in exiting the colony and starting foraging. Moreover, better weather conditions would have allowed us to collect more data and thus to have more replications which would have benefitted the statistical power of our experiment, particularly in regard to individual-level observations. In fact, the interaction of treatment and observation day on pollen collection that was close to significance (p=0.051) may be an artefact derived from difficulties in producing a balanced dataset of individual observations. However, research has previously underlined that neonicotinoid insecticides are capable of affecting bee pollen collection; for example, Stanley et al. (2015a) observed that *Bombus terrestris* colonies exposed to field-realistic dosages of thiamethoxam had fewer bees collecting pollen than control colonies, similar to Feltham et al. (2014), who showed that imidacloprid-treated bees returned with pollen less often than non-treated bees, and when they did, pollen collected by hour was significantly less than control colonies. This suggests that neonicotinoids may produce behavioural changes in colony activity, and further research is needed to make sure that sulfoxaflor is not likely to cause such changes.

It is also worth underlining that, during the experiment, *B. terrestris* nectar robbing on plants has sometimes been observed. Nectar robbing is a type of behaviour in which bees create holes at the base of flowers, or use holes created by others, to forage for nectar without entering corollas themselves (Inouye, 1980). Possible reasons leading to such behaviour include a less effort required by bees, and a higher nectar reward than that obtained by legitimate visitations (Dedej & Delaplane, 2005). Nectar robbing was not observed at the immediate beginning of the experiment, but noticed later in the trial period, and this could be explained by the fact that bees may learn such procedure with time, and that nectar robbing appears to be socially transmitted to other bees exposed to robbed flowers (Leadbeater & Chittka, 2008). However, it would be worth investigating if sulfoxaflor is capable of playing a role in influencing such behaviour. In fact, although an indirect pollination could still take place by moving pollen from flower anthers to stigmas, pollen transfer by nectar robbing is reduced, and holes created by biting flowers may be used by other pollinators to collect nectar instead of entering the corolla to forage, with implications for pollination efficiency and plant yield (Kendall & Smith, 1975; Sàez et al., 2017). Moreover, nectar robbing performed by introduced, managed bees may reduce flower visits by native, wild bees, interfering with native plant-pollinator mutualism and contributing to wild bee declines (Dohzono et al., 2008). Hence, it is suggested to explore whether the exposure to different dosages of sulfoxaflor could contribute to increasing such behaviour.

Even though we produced valuable results on the absence of a sulfoxaflor effect on *B. terrestris* when alone or in combination with *C. bombi*, it is necessary to also consider its impact when combined with other agrochemicals. In conventional agriculture it is common practice to rely on several agrochemicals for pest control and increasing crop yield (Tilman et al., 2002), and it is therefore expected that sulfoxaflor would be applied with other insecticides, fungicides, herbicides, and fertilisers in the fields. A recent meta-analysis of studies where bees were exposed to multiple stressors revealed that, overall, the interaction effect between different agrochemicals at field-realistic levels tend to be synergistic, with detrimental effects on bees (Siviter

et al., 2021a). So far, few studies have addressed interaction effects of sulfoxaflor with other agrochemicals; for example, sulfoxaflor did not impair the activity or development of *A. mellifera* when combined with the fungicide azoxystrobin (Tamburini et al., 2021), but it did decrease the survival of both *A. mellifera* and *O. bicornis* when in conjunction with the fungicide fluxapyroxad (Azpiazu et al., 2021). Hence, further investigation on sulfoxaflor mode of interaction in a multi-agrochemical scenario and on different bee species would be helpful in assessing its safety.

The presence of sub-lethal effects may vary among bee species (Siviter et al., 2018b). For instance, Azpiazu et al. (2021) showed that the interaction between sulfoxaflor and the fungicide fluxapyroxad significantly decreased both *Osmia bicornis* and *Apis mellifera* survival, yet no effect was observed on *Bombus terrestris*, implying that the occurrence of interaction effects may also change depending on the species. Bee species have been shown to differ in sensitivity and exposure routes to pesticides (Sgolastra et al., 2019, 2020). For example, the exposure to pesticide residues in soil represents a relevant route for ground-nesting bees, but not for *A. mellifera* or *B. terrestris* (Sgolastra et al., 2019). Therefore, social bees such as *Apis* and *Bombus* may not always be representative of all other species (Siviter et al., 2021c), yet the majority of field studies have been focussing on honeybees, and studies on non-*Apis* bees were predominantly conducted on *Bombus* (Siviter et al., 2021c). According to Boff et al. (2021), *Osmia bicornis* exposed to field-realistic doses of sulfoxaflor showed signs of changes in foraging behaviour, including the number of flower visits and flight performance. Therefore, investigating whether other bee species may have a different sensitivity to sulfoxaflor at field-realistic dosages could contribute to filling the knowledge gap on sulfoxaflor as a potential neonicotinoid substitute. Currently, pesticide risk assessments tend to focus on *Apis mellifera*, and consequently, potential impacts of pesticides on other bee species are often not considered. Moreover, although it is very common practice in agriculture, assessments are rarely performed on pesticide formulations, and usually only on single compounds (Cedergreen, 2014). Integrating different bee species into risk assessments and accounting for the co-occurrence of multiple compounds could help safeguard non-*Apis* bees and ensure the safety of new pesticides, such as sulfoxaflor, on multiple species before they are authorised (Cedergreen, 2014; Sgolastra et al., 2020a).

While risk assessments could be improved and could support evaluating the impact of pesticides even on non-*Apis* bees, including wild bees, understanding the incidence of diseases and parasites is much more challenging. In fact, while honeybee hives and commercial bumblebee colonies can be more easily monitored, much less is known about the extent of parasite loads on wild bees. Past research has successfully addressed the incidence of *Crithidia bombi* in wild bumblebee populations, indicating it to be up to 80% (Shykoff & Schmid-Hempel, 1991; Gillespie, 2010). However, rates may vary depending on bee biology, with some species being more susceptible than others to parasites and diseases and, consequently, leading to a decline (Gillespie, 2010). In this regard, pollinator monitoring schemes such as the UK Pollinator Monitoring

Scheme and the EU Pollinator Monitoring Schemes can be particularly useful in understanding the decline of wild pollinators in Europe and the major causes of such declines (https://ukpoms.org.uk/; Potts et al., 2021).

3.5. Conclusions

The results of our semi-field experiment with bumblebees foraging on field bean plants may be summarised as follows:

a. **Field-realistic concentrations of sulfoxaflor did not affect the behaviour of managed *Bombus terrestris*** at the individual or colony level.

b. **The inoculation with *Crithidia bombi* at an infection rate above 25% did not affect the behaviour of *Bombus terrestris*** at the individual or colony level.

c. **There was no interaction effect of sulfoxaflor and *Crithidia bombi* on the individual or colony behaviour of *Bombus terrestris***, indicating that these stressors may not increase their impact magnitude on bumblebees.

d. Since no treatment effect was shown on *Bombus terrestris* behaviour, **no effect on the delivery of pollination services on field bean plants was observed for any of the treatments or their interaction.**

In light of such results, we can conclude that sulfoxaflor might represent a potentially effective alternative to neonicotinoid insecticides due to its apparently higher safety and ability to overcome pest-resistance issues. However, considering that sub-lethal pesticide effects may differ depending on bee species or exposure levels, further research is required to assess its safety when alone or combined with other stressors.

Chapter 4

A survey for beekeepers to investigate perceptions toward a new omics tool for bee health.

Abstract

Pollination is a crucial service in crop agriculture, to which both wild and managed bees, including *Apis mellifera*, contribute. Despite their role in crop production, honeybee colony losses in Europe have recently doubled due to multifactorial threats to their health. Such health issues can result in substantial costs for many beekeepers, who need to constantly manage the spread of diseases, pests, and pathogens in the beehives to avoid colony losses. Therefore, ensuring bee health is critical in maintaining honeybee populations and supporting beekeeping practices, however research into perceptions and attitudes of beekeepers in Europe is very limited. Our study is the first to investigate beekeepers' willingness to adopt an omics tool (here, the PoshBee 'Bee Health Card'), that has the potential to rapidly assess bee health. Through an on-line survey for beekeepers in seven European countries, we showed that beekeepers recognise the potential for the new tool to improve colony health, with typically moderate confidence levels in its effectiveness, and confidence may be increased if the tool is easy to use and not too time consuming. Moreover, planning well targeted economic incentives such as subsidises is necessary to prevent the cost from being a barrier to the use of the health card, and to increase its use frequency. Finally, environmentally friendly benefits, such as pollinator and environment protection, may influence beekeepers when deciding whether or not to use the tool. With the Bee Health Card, we estimate that there might be a reduction of colony winter losses of 28.96% considering a hypothetical 75% effectiveness of the tool and 95% probability of using it at least once a year with high confidence in its effectiveness.

Contributions

I created, distributed, and advertised the survey, which was peer reviewed and later advertised by PoshBee experts including Prof. Marika Mand, Dr. Risto Raimets (Estonia), Prof. Alexandra-Maria Klein (Germany), Dr. Oliver Schweiger (Germany), Prof. Jane Stout (Ireland), Dr. Cecilia Costa (Italy), Prof. Pilar De La Rua (Spain), Dr. Matthias Albrecht, Dr. Anina Knauer (SWI), Prof. Simon Potts (UK), Dr. Deepa Senapathi (UK), Dr. Tom Breeze (UK), and Matt Allan (UK), while Dr. Philippe Bulet and Dr. Dalel Askri (France) provided information related to the Bee Health Card. Additionally, I created the advertisements on social media platforms and, eventually, performed data collection, data selection, and data analysis.

4.1. Introduction

Pollination represents a key ecosystem service for crop production, benefitting about 75% leading food crop types worldwide (Klein et al., 2007). Animal pollination in particular is estimated to provide global food crops with benefits with an economic value between $235-577 bn per year (Lautenbach et al., 2012).

Bees are the most widespread pollinators in the world (Simon G. Potts et al., 2016; Rader et al., 2016), 2% of which are estimated to pollinate about 80% crops (Kleijn et al., 2015). Since land areas dedicated to pollination-dependent crops have been increasing, so has the reliance on pollination services (Aizen et al., 2019).

While most insect pollinators are wild, a minority of species are managed (IPBES, 2016). Wild bees may be more efficient and better contribute to crop pollination than managed bees (Garibaldi et al., 2013), however they may be more prone to being affected by several pressures, such as the loss of natural habitats and change in habitat configuration and composition (Winfree et al., 2010; DEFRA, 2014). Moreover, unlike managed honeybees, wild bees are not actively monitored and taken care of by beekeepers. As such, there is no intervention to control pests and diseases within colonies, making them more challenging to keep under control (Spiewok & Neumann, 2006; Roth et al., 2022). Therefore, also thanks to their ability to rapidly adapt to new landscapes and foraging resources, managed bees including *Apis mellifera* are often employed in many commercial crop systems (DEFRA, 2014).

Honeybees are the most widely used managed pollinators, estimated to visit more than 50% of animal-pollinated crops (IPBES, 2016). However, in the last decades, several European studies have reported high incidence of honeybee colony losses (Neumann & Carreck, 2010; Gray et al., 2019), together with an overall decline of colonies in Europe (Potts et al., 2010b). As such, beehive supplies may not sufficiently satisfy the demand for honeybee pollination services, which is rising at a faster pace (Tom D. Breeze et al., 2014).

There is strong evidence of multiple anthropogenic stressors negatively affecting bee health. In Europe, the most important drivers of pollinator decline are thought to be changes in land cover and configuration, land management, and the impact of pesticides (Dicks et al., 2021). The planting of mass-flowering crops at the expense of semi-natural habitats is depriving wild pollinators of nesting and foraging resources, and agricultural intensification is inevitably leading to the loss of natural habitats in favour of improved farmlands, impacting the survival of both wild and managed bees (Smart et al., 2016). Moreover, with intensive farming practices, bees are increasingly exposed to pesticides that may have sub-lethal effects on their health (IPBES, 2016; Havard et al., 2019). Commercialisation of managed bees and beehive products are also increasing the risk of disease and pathogen spill-over, such as the spread of *Varroa destructor*, and associated viruses, for which honeybee hives necessitate regular treatments (Grünewald, 2010) that can weaken colonies

(Donkersley et al., 2020). Shifts in climate may also influence bee and pathogen distributions worldwide, leading to further spreads of diseases (Dormann et al., 2008; IPBES, 2016).

Health issues arising among honeybee populations are therefore a notable concern, with many countries in Europe reporting colony high rates of health disorders (Chauzat et al., 2013; Gray et al., 2020). Such health issues can lead to significant expenses for many beekeepers, often forcing them to adopt sanitary practices to manage the spread of diseases, pests and pathogens in the beehives and avoid colony losses (Breeze et al., 2017; Gray et al., 2019). Such increased costs are thought to be a major factor driving long-term declines in honeybee colony numbers across Europe (Potts et al., 2010b), and are seldom compensated for through pollination activities. For example, using an on-line survey for UK beekeepers, Breeze et al. (2017) found that most respondents who provide pollination services by renting or lending their beehives are not paid, and those who are often receive lower payments than their costs, resulting in a net loss, with payments usually lower than the benefits provided to the crops. Moreover, Breeze et al. (2019) showed that some beekeepers are often reluctant to place their hives by certain crops due to perceived pesticide pressures, even if the crop would otherwise be attractive as a source of nectar.

Supporting healthy beekeeping practices is therefore critical to help improve bee health (Potts et al., 2016; Gray et al., 2019). The EU directly support beekeeping through various national honeybee health programmes (*e.g.* Apiculture programmes, EU, 2013a; EC, 2019) and surveillance measures such as the creation of the EU Reference Laboratory (EC, 2013), and also indirectly through agri-environment schemes (AES) for rural areas growth (Donkersley et al., 2020; EC, 2017). However, monitoring bee health issues and their causes throughout Europe is extremely challenging (Chauzat et al., 2013), and good estimates of colony loss rates still depends on how accurate and representative beekeeper reports are, which may itself vary depending on personal motivations and concerns (Gray et al., 2020).

Moreover, there are different legislations regulating beekeeping activities between European countries with registration of apiaries with a central authority ranging from mandatory for all beekeepers (*e.g.* Italy), only those who sell honey (*e.g.* Ireland), or entirely voluntary (*e.g.* UK) (Chauzat et al., 2013). Further differences in legislations exist in notifiable diseases lists among European countries (Chauzat et al., 2013).

While the EU does support many research programmes to improve bee health, some of which involve new technologies to monitor the health of beehives (*e.g.* 'SmartBees' and 'Swarmonitor', Chlebo et al., 2020), it is nevertheless necessary to investigate whether adopting novel farming technologies represent barriers or opportunities for different users. For instance, Vecchio et al. (2020) looked at factors that may affect the adoption of precision farming tools (PFTs) among selected Italian farmers, finding that the perceived complexity of such tools may represent a barrier to their use. Therefore, it is crucial to understand the barriers and incentives to the implementation of new technologies for beekeepers and what benefits may be able to oppose them.

Research into perceptions and attitudes of beekeepers in Europe is limited to few studies (*e.g.* Carreck et al., 1997, Breeze et al., 2017, 2019; Gray et al., 2019), and to date no study has looked at the willingness of European beekeepers to adopt new technologies. Here we present the results of a survey circulated in 8 European countries to investigate beekeepers' perceptions on the Bee Health Card (BHC), a tool under development by the PoshBee project (Brown et al., 2021) which represents a significant advancement in assessing a range of stressors (pesticides, pathogens, and malnutrition) from a small sample of bees and hive products. In order to promote the wide uptake of such tool among beekeepers, we investigate possible barriers and benefits to its adoption. We then proceed to explore the willingness to adopt such a tool with associated extra costs linked to it, and the frequency of use, considering a scenario both with and without planned economic incentives.

With this survey, we aim to address the following research questions:

a. What factors could incentivise beekeepers to use the BHC tool, accept extra costs linked to it, or use it more frequently?
b. What factors could form potential barriers to beekeepers using the BHC tool, accepting extra costs linked to it, or using it frequently?
c. Are beekeepers confident in the effectiveness of the BHC, and how important is their level of confidence when it comes to using the tool, accepting extra costs, or deciding how frequently to use it?

4.2. Methodology

4.2.1. Survey for beekeepers

An online survey addressed to beekeepers was built using the software 'Qualtrics' (Qualtrics, 2005). The purpose of the survey was to investigate what incentives and barriers could encourage or discourage beekeepers to adopting the new PoshBee tool, so as to understand how to better support its wide uptake.

At the time the survey was developed, the BHC had not been field tested yet, and no statistics on its effectiveness were available. Therefore, in order to provide respondents with the necessary information, an infographic was created to communicate what the health card tool would do and how it would be used (Figure 4.2.1.). This was translated into each of the survey languages (Table 4.2.1).

The survey included six sections made of 19 closed questions, in order to make the survey more accessible and thereby encourage responses, and to facilitate the interpretation of answers given. In the first section, beekeepers were asked a series of questions, based on prior work by Breeze et al. (2017, 2019), about their experience and reasons for practicing beekeeping and whether they engaged in frequent, infrequent, or no communication with growers. Questions were framed to be as neutral, specific, and inclusive as possible. The next section was dedicated to investigating the sources of information on the health of beehives and the interest and perceptions of beekeepers in regard to bee decline and health. The final two sections were

centred on the benefits and barriers to the use of the Bee Health Card, the willingness to adopt it with or without associated costs, and the frequency of use, considering a scenario with planned economic incentives (such as subsidies, grants, certified products…) and without. The survey terminated with one optional open question, aiming to further explore which aspects of the Bee Health Card respondents were more enthusiastic or interested about.
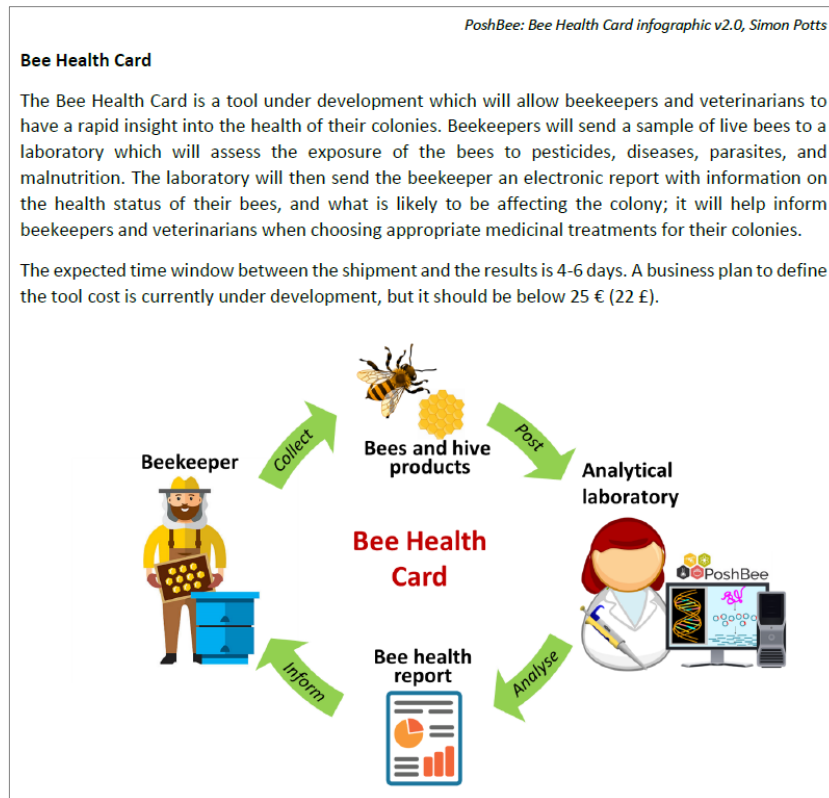
Figure 4.2.1: The Bee Health Card infographic shown in the survey. Beekeepers collect beehive products or a sample of their bees to send to an analytical laboratory, which processes the samples and produce a report with information on beehive health to send back to beekeepers, who will be able to make informed decisions to safeguard their bees.

Before being circulated, the survey was peer reviewed by experts from each of the 8 target European countries and the experienced beekeepers that were part of each team. These experts were asked to suggest any additional answer to include in closed questions, giving any further opinion on whether a question was useful to include or not, and ensuring all questions were clear. Moreover, BIOP (BioPark Archamps) and CNRS (Centre National de la Recherche Scientifique) researchers, who are leading the production of the Bee Health Card, made sure that the tool description presented in the survey was easily comprehended and included all important elements that were provided. Ultimately, the final version of the survey (Appendix 4.1) was translated by study leaders into each language and distributed in the 8 countries (Table 4.2.1). The survey was advertised through the PoshBee social media channels (Twitter and Facebook) and website, and was promoted through various beekeeping associations (official Facebook pages, Twitter accounts, webpages) and magazines (Appendix 4.1). The anonymity of participants was guaranteed by identification through a unique ID.

'Display Logic' functions were used to show selected questions to respondents based on the answers that were previously given. For example, if respondents were not interested in the BHC, they were not shown subsequent questions regarding the frequency of use.

Table 4.2.1: Countries and languages of distribution.

Country	Survey language
Estonia	Estonian
Germany	German
Ireland	English
Italy	Italian
Spain	Spanish
Sweden	Swedish
Switzerland	German
United Kingdom	English

The survey remained online for a period of 6 months, from July 31st, 2020, until February 2nd, 2021. The target was a minimum of 30 responses from each country, and this resulted in a final dataset from 7 countries (Table 4.3.1). Before being published, the survey was approved by the University of Reading Ethics Committee and participants expressed their consent prior to submitting their answers.

4.2.2. Statistical analysis

4.2.2.1. Multiple Correspondence Analysis

Data derived from Qualtrics was organised in Microsoft Excel 2019 (Divisi et al., 2017), and correlations among all survey responses were explored using Kendall Rank Correlation Analysis in IBM SPSS Statistics 27.0.1 (Okagbue et al., 2021) (see Appendix 4.2). Given the very high number of correlations, two Multiple Correspondence Analyses (MCA) were conducted in Minitab 19 (Okagbue et al., 2021) to identify groups of variables that could be clustered for use in further analyses. The first MCA (MCA 1) was performed with variables related to the willingness to use the tool and accept extra costs with and without planned incentives, and the second MCA (MCA 2) was conducted with variables related to the frequency of use of the tool with and without planned incentives. List of variables used in MCAs and correspective codes can be found in Table 4.2.2.

To reduce the number of categories shown on the MCA maps and avoid very polarised results (*e.g.* very few people strongly agreeing with a statement, but many agreeing with it), 'strongly agree' and 'agree' answers were grouped together as well as 'strongly disagree' and 'disagree' ones. Also, 'extremely confident' and 'very confident' beekeepers were grouped together, such as 'moderately confident' and 'slightly confident' ones. We then proceeded to build clusters based on the proximity of variables on the graph that belonged to the same group (*i.e.* benefits or barriers).

Table 4.2.2: Variables, their codes, and colours used in Multiple Correspondence Analyses.

Survey question	Variable	Code on MCA map	MCA
Country where respondent practices beekeeping	Estonia	**est**	Both
	Germany	**ger**	
	Ireland	**ire**	

	Italy	**ita**	
	Spain	**spa**	
	Switzerland	**swi**	
	United kingdom	**uk**	
Benefits of the use of the Bee Health Card	Increased bee health	**bh**	Both
	Pollinator protection	**pp**	
	Environment protection	**ep**	
	Toll is quick and easy to use	**qe**	
	Enhanced crop production	**cp**	
	Lower treatment cost	**tc**	
	Higher productivity	**p**	
	Better communication with growers	**g**	
Barriers to the use of the Bee Health Card	Tool effectiveness	**e**	Both
	Tool cost	**c**	
	Tool is time consuming	**t**	
	Tool is difficult to use	**d**	
	Tool is not important to be used	**i**	
	Lack of communication with growers	**g**	
Confidence level in the effectiveness of the BHC	Extremely/very confident	**evc**	Both
	Moderately/slightly confident	**msc**	
	Not confident	**nc**	
Willingness to use the tool and accept extra costs with and without incentives	Use with no incentives and no extra costs	**ninc**	MCA 1
	Use with incentives and no extra costs	**inc**	
	Use with incentives and extra costs	**ic**	
	Use with no incentives and extra costs	**nic**	
	Use with incentives	**i**	
	Use with no incentives	**ni**	
Frequency of use of the tool with and without incentives	Regular to irregular use with incentives	**iri**	MCA 2
	Regular to irregular use without incentives	**niri**	
	Limited to no use with incentives	**iln**	
	Limited to no use without incentives	**niln**	

4.2.2.2. Binary logistic regression

Six Binary Logistic Regression analyses were performed in Minitab 19 to investigate the followings:

a. The willingness to use the PoshBee tool with incentives

b. The willingness to use it without incentives

c. The willingness to accept its extra costs with incentives

d. The willingness to accept its extra costs without incentives

e. The frequency of use of the PoshBee tool with incentives

f. The frequency of use without incentives.

For each of the six response variables, the final set of variables and clusters obtained from the MCAs and listed in Table 4.3.4 ('3.4 Multiple Correspondence Analysis') were used as explanatory variables. Due to low frequencies of some responses (*i.e.* 'never' = 4 answers (0.7%) in case of incentives and 8 answers (1.9%) without incentives, and 'regular use' = 52 answers (12.6%) without incentives), we chose to merge the frequency of use into two categories: (1) 'more frequent use', including respondents who would use the tool somewhat frequently, either a more regular monthly use or more irregularly but always a few times during the year, and (2) 'limited to no use', comprising beekeepers that would either use the tool just with a reasonable suspicion, or never use it. To perform the regressions, a score of '0' was attributed to each

'disagree', '1' to each 'neutral', and '2' to each 'agree' answer. An average score of '0', '1', or '2' was attributed to each cluster corresponding to an overall tendency to disagree, having neutral views, or agree with the variables grouped in the cluster, rounding decimals to the nearest whole number to facilitate the result discussion. Finally, response variables (binary data) were expressed as '0' or '1'.

After creating the global models, terms with a Variance Inflation Factor (VIF) equal or higher than 5.0 were removed to avoid multicollinearity issues (Gareth et al., 2013) (see Appendix 4.4). We then proceeded to remove terms with the highest *p*-value until only significant terms were left in the model. Final models were selected based on the Schwarz's Bayesian Information Criterion (BIC) (Simon-grifé et al., 2013; Nikolaus et al., 2019; Farwell et al., 2020), reporting models with the lowest BIC and ΔBIC≤2 from the lowest BIC model (Neath & Cavanaugh, 2012).

4.3. Results

4.3.1. Sample description

The usable response rates across the survey network varied substantially, with UK and Irish beekeepers comprising more than 50% of all responses (Table 4.3.1). This was most likely due to the nature of the survey advertisement, which was highly distributed on social media by WP1 researchers with an often predominant English-speaking public. Advertisements were also frequently made in English (see Appendix 4.1), reaching a higher proportion of English-speaking beekeepers and, presumably, increasing the response rate of both Ireland and the UK. Additionally, the UK is the sixth leading country in the world for Twitter usage (Statista.com, 2022); since Twitter was one of the main channels used to advertise the survey, this could have influenced its response rate. However, with the exception of Sweden, response rates were always above the minimum threshold of 30 required to be included in the analysis.

Table 4.3.1: Final usable response rate by country (progress ≥ 97%). Seven out of 8 countries reached the minimum target of 30 responses and were therefore included in further analyses.

Country	Code	N respondents	% respondents
Ireland	IRE	115	24.1%
Sweden	SWE	3	0.6%
United Kingdom	UK	136	28.5%
Spain	SPA	40	8.4%
Italy	ITA	66	13.8%
Germany	GER	33	6.9%
Switzerland	SWI	52	10.9%
Estonia	EST	32	6.7%
Total: 477			
Final total: 474			

Across the sample of respondents, the majority (74%) were hobbyist beekeepers, while only 24% were professionals. This may be due to the fact that, in many countries, respondents were mostly recruited from national associations with a hobby focus (Appendix 4.1, 4.3). The overall average of beehives kept per year in the last 3 years varied among countries, with most Italian respondents having the highest average (50 per year) and Ireland and the UK being accounted for the lowest (3 per year) (Appendix 4.3). This may be

explained by the fact that respondents from the UK and Ireland were largely hobbyists, while Italian beekeepers are mainly professionals (Appendix 4.3).

Among the reasons to practice beekeeping, more than 77% stated 'personal hobby', followed by nearly 45% that sell honey and other beehive products, of which this was more than 70% from Estonia and 67% from Italy. Italian respondents mainly practiced beekeeping to sell beehive products rather than as hobby, with percentages of nearly 70% and less than 50% respectively (Figure 4.3.1). Among those who selected 'others', the most popular driver for being a beekeeper was the fascination for bees or nature followed by self-learning purposes with 27.69% and 15.38% respectively (Appendix 4.3).
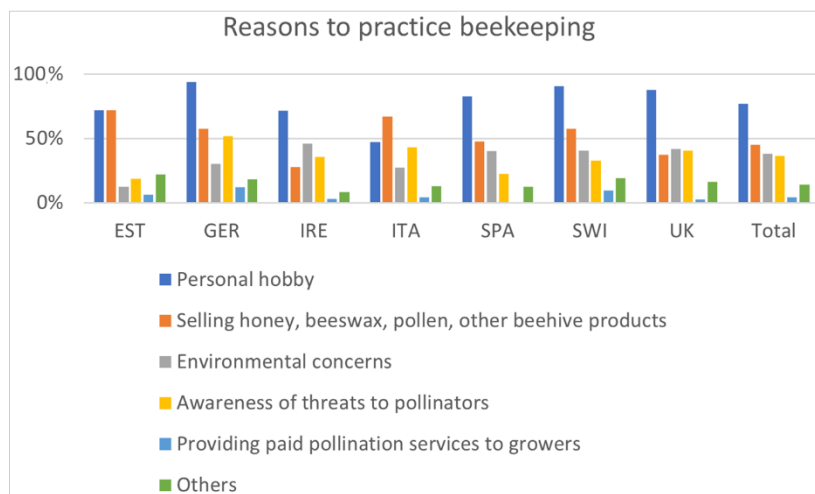

Figure 4.3.1: Reasons to practice beekeeping according to respondents from all countries.

4.3.2. Beekeepers' knowledge exchange

There were notable differences in the rate at which beekeepers communicate with growers. Overall, more than 40% respondents never communicate with growers, particularly in the UK and Ireland (>60%) and Germany (>40%). By contrast, more than 25% total respondents do communicate with growers more than twice a year – specifically, more than 50% in Switzerland, 47% in Spain and about 40% in Italy, in contrast with only 17% in Ireland and 12% in the UK. Finally, about 40% Estonian beekeepers engage communication with growers once or twice a year, with more than 20% reporting a more frequent communication (Figure 4.3.2).

Across all 7 countries beekeeping associations (BKA) were consistently the most important sources of information, with nearly 80% respondents reporting them as 'extremely' or 'very important' sources; only 1.9% think they are 'not important' sources (Figure 4.3.3). This was very consistent across countries (Appendix 4.3).

Other very important sources of beehive health information are 'other beekeepers' ('extremely' and 'very important': 32.7% and 41.1% respectively across countries) and 'training in person' ('extremely' and 'very important': 33.5% and 39.2% respectively across countries). The former is particularly relevant in Ireland and Switzerland, while the latter is more important in Italy, Switzerland, and Spain (Appendix 4.3). NGOs and

TV/Radio are regarded as the least important sources, with more than 30% respondents labelling them as 'not at all important' (Figure 4.3.3).
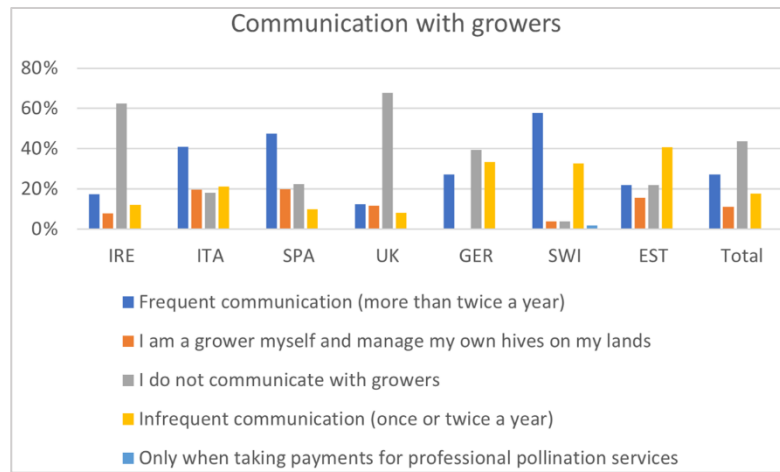

Figure 4.3.2: Percentage of respondents who communicate with growers at different regularities.
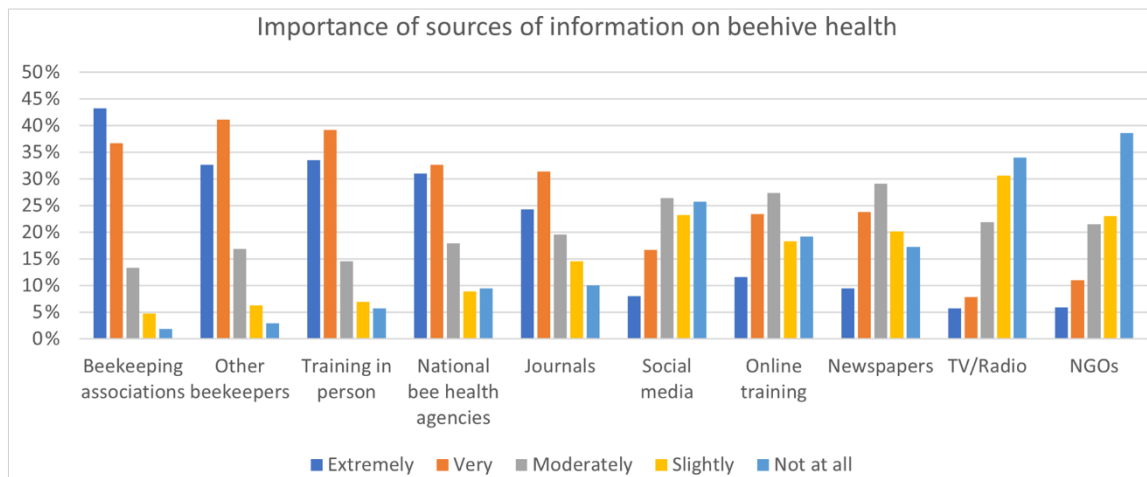
Figure 4.3.3: Importance of each source of information to all sampled beekeepers.

4.3.3. Bee decline

The loss of semi-natural habitats and the use of agrochemicals were the perceived causes of bee decline most beekeepers agreed with, chosen by more than 80% beekeepers. Similarly, more than 75% respondents felt that diseases and parasites were important drivers of bee declines. Climate change was also perceived as a threat to bee populations by more than half of respondents. The least supported reason was 'competition between wild and managed pollinators', with more than 40% respondents expressing disagreement with the statement. While this finding may be expected, given the focus of the survey on professional and hobbyist beekeepers, it does indicate that about 20% respondents believe competition is a significant driver of declines (Figure 4.3.4, Appendix 4.3).

More than 80% participants agreed with all measures to reduce bee decline proposed by the survey. In particular, nearly 95% agreed with the importance of preserving natural habitats and flower areas, followed by 'monitoring diseases and parasites' with more than 92%, and 'monitoring agrochemicals' with 91.53%.

(Figure 4.3.5). Very few beekeepers disagreed with the listed measures, with no notable differences among countries except for nearly 22% of German beekeepers who disagreed with the importance of monitoring diseases in beehives, against 0% from the other 6 countries (Appendix 4.3).
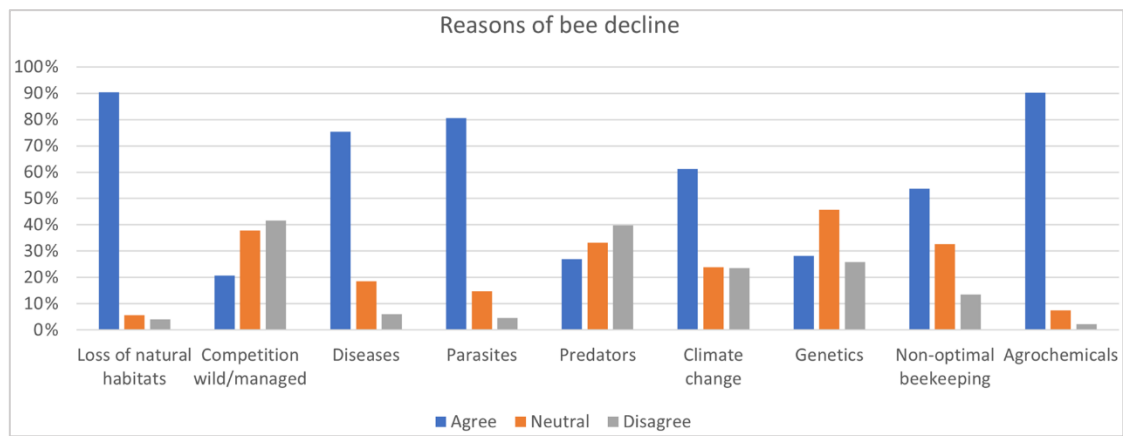

Figure 4.3.4: Proportion of respondents agreeing/disagreeing with proposed reasons for bee decline.
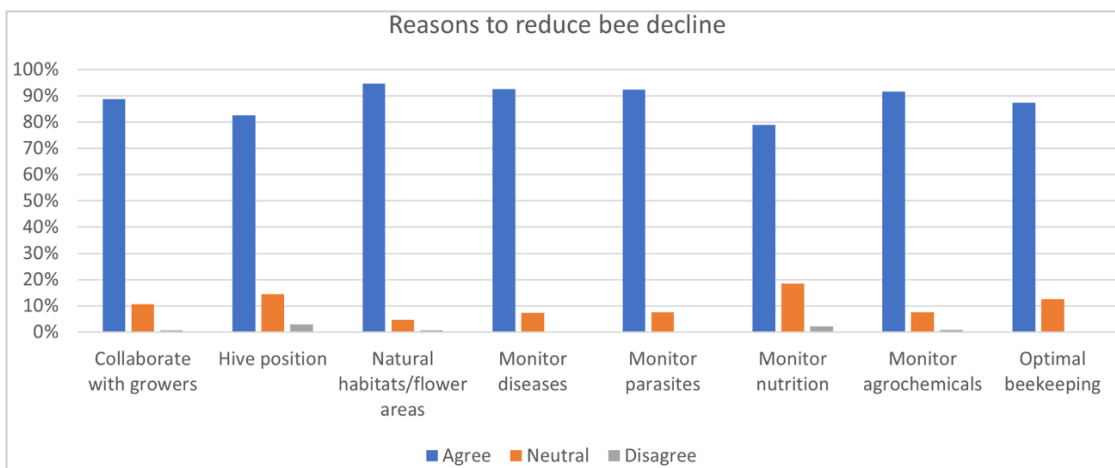

Figure 4.3.5: Proportion of respondents agreeing/disagreeing with proposed measures to reduce bee decline.

The majority of participants agreed with all listed reasons to protect bees. However, pollinators conservation was by far the most widely supported with nearly 96% of respondents agreeing with the statement, while food security and maintaining crop varieties came second and third with about 82% each. Economic motives, such as pollination contracts, were thought to be a reason to protect bees by more than 60% respondents, though there is substantial variation in the agreement across countries. Most notably, less than 50% respondents in Switzerland and Germany agreed that these were major reasons to protect bees (Appendix 4.3). Respondents were much less concerned about public perceptions or legal reasons to protect bees, indicating a lean towards ecological, food security and economic arguments for preserving bee health over social dimensions. No major disagreement with the listed reasons was shown, but it is worth mentioning that nearly 14% participants disagreed with 'legal motives' and 'public perception' as measures to protect bee health (Figure 4.3.6).
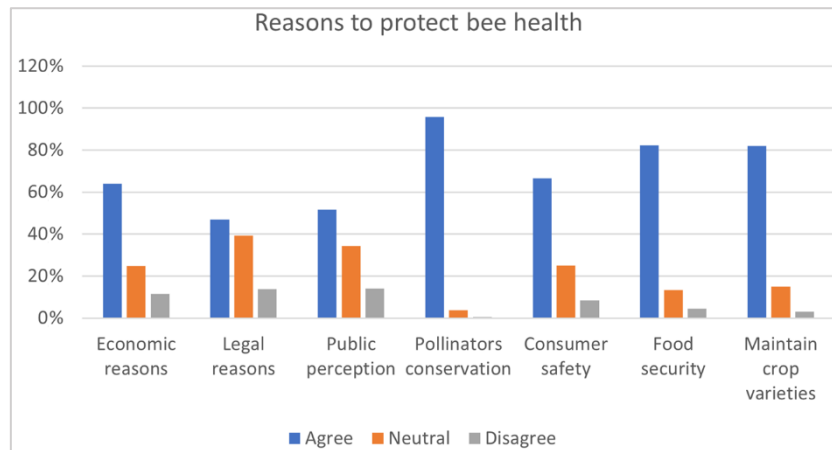
Figure 4.3.6: Proportion of respondents agreeing/disagreeing with reasons to protect bee health.

4.3.4. Bee health

The frequency of health checks performed by beekeepers on beehives varied between the different stressors. Beekeepers from all 7 countries mainly performed checks for diseases and nutrition either weekly or fortnightly, while checks for chemicals were mostly carried out only when there was a reasonable suspicion. Parasite checks were usually conducted either monthly or more than once a year (Figure 4.3.7). Such results varied between countries, with UK and Italy generally checking for pressures more regularly than others (Appendix 4.3). It is worth considering that the frequency of performing health checks may also depend on national policies; for example, despite not being listed as notifiable diseases under EU legislation (EU, 2016), both Italy and the UK consider EFB as notifiable (D.P.R., 2006; Statutory Instruments, 2006), with Italy also adding Nosemosis caused by *Nosema Apis* (D.P.R., 2006). Thus, the presence of more notifiable diseases in national regulations may drive beekeepers to perform more regular health checks.
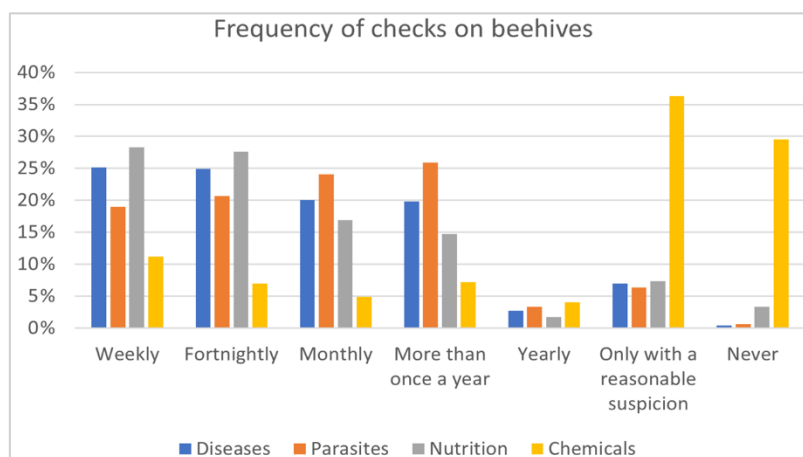

Figure 4.3.7: Proportion of total beekeepers who check their hives for different pressures at each regularity.

'Improving bee health' is the most widely perceived benefit of using the Bee Health Card, followed by 'pollinators protection'. Throughout the sample, there was a high degree of neutral opinion on the prospective benefits of the tool indicating strong respondent uncertainty, given that the tools effectiveness has yet to be demonstrated to them. Agreement was weakest for increased crop pollination, which was thought to be a benefit by just over 30% of the sample. This could be an artefact of the prevalence of amateur

beekeepers who may not provide pollination services. Although few respondents disagreed with any of the potential benefits, more than 20% respondents disagreed with the suggestion that using the Bee Health Card could reduce treatment costs for the beehives (Figure 4.3.8), which is in congruence with the agreed concern of about 65% participants that cost could be a potential barrier to the use of the tool. These trends are broadly held at a national level, although beekeepers in Spain and Italy were more likely to agree with the proposed benefits than those from other countries (Appendix 4.3).
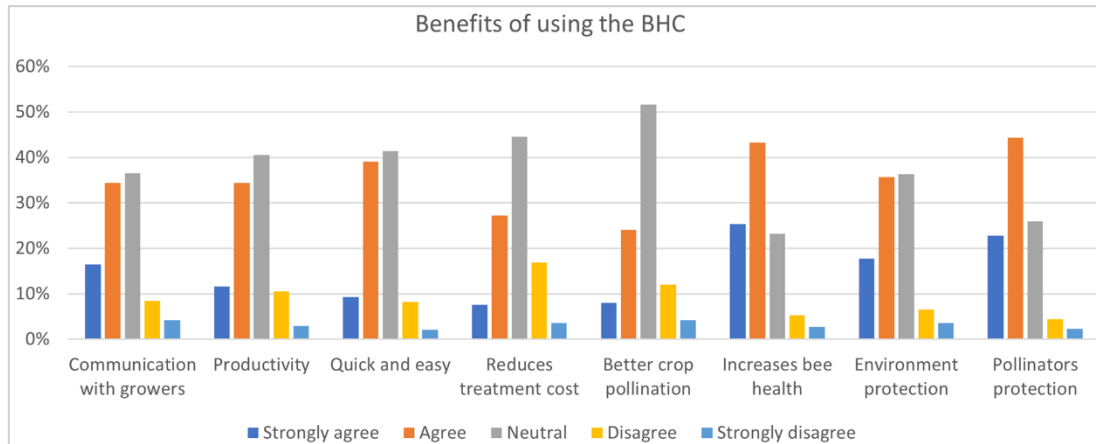

Figure 4.3.8: Percentages of total respondents agreeing/disagreeing with factors being benefits of using the PoshBee health card tool.

In terms of barriers to using the proposed PoshBee health card, nearly 65% respondents agreed with 'cost' being a potential obstacle, followed by 'lack of communication with growers' with about 61% (Figure 4.3.9). Specifically, 'cost' represents a particularly strong barrier for beekeepers from the UK, Ireland, Estonia, and Switzerland (Appendix 4.3), while 'lack of communication with growers' was selected the most by beekeepers from Italy and Spain. However, only 10% participants strongly agreed with 'effectiveness' being a barrier, which is in line with responses obtained to the question investigating the confidence in the effectiveness of the tool. The barriers respondents either disagreed or strongly disagreed the most with are the difficulty of the tool and the lack of importance in using it, with more than 40% respectively. Therefore, the majority of beekeepers are aware of the potential role of the tool in helping improve bee health and are not concerned that it would turn out to be difficult to use (Figure 4.3.9).
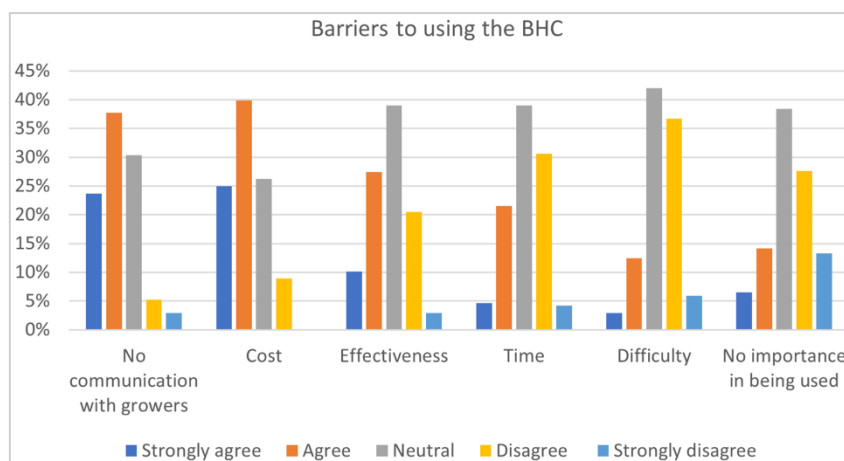

Figure 4.3.9: Percentages of total respondents agreeing/disagreeing with factors being barriers to using the PoshBee health card tool.

Despite the Bee Health Card being currently under development and its effectiveness still needing to be fully demonstrated, more than 30% German and nearly 38% Italian respondents stated to be extremely confident the tool would be effective, which may represent a promising insight. Additionally, beekeepers that are 'not at all confident' are generally few, ranging from 12.5% in the UK to 3.13% in Estonia (Figure 4.3.10).
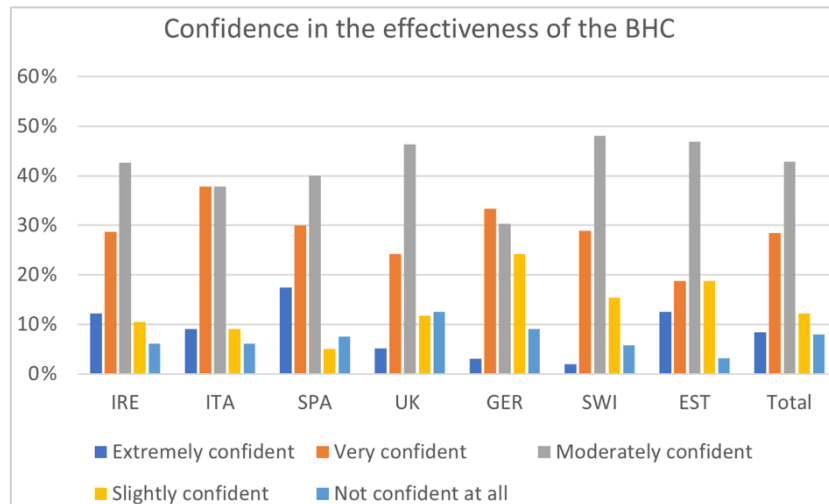

Figure 4.3.10: Level of confidence of total respondents in the effectiveness of the PoshBee health card tool.

The presence of economic incentives did not affect beekeepers' decision of whether or not to use the tool, however it does become relevant when deciding the frequency of such use. Despite the limited available description of the tool and its outputs, about the half of respondents in Ireland, UK, Spain, Italy, and Germany would use it even with extra costs, while more than half of Estonian beekeepers would use it only without extra costs. However, in terms of using the PoshBee health card with or without incentives, results among countries do not vary much (Table 4.3.2).

Table 4.3.2: Percentage of beekeepers who would use the PoshBee health card with and without economic incentives. The higher percentages for each country are highlighted in bold.

Use with incentives	IRE	UK	SPA	ITA	GER	SWI	EST	Total
Yes - even with extra costs	**49.57%**	**47.79%**	**55.00%**	**54.55%**	**48.48%**	**40.38%**	40.63%	**48.52%**
Yes - only if there were no extra costs to me	43.48%	42.65%	35.00%	39.39%	24.24%	**40.38%**	**56.25%**	41.14%
No	6.96%	9.56%	10.00%	6.06%	27.27%	19.23%	3.13%	10.34%
Use without incentives	**IRE**	**UK**	**SPA**	**ITA**	**GER**	**SWI**	**EST**	**Total**
Yes - even with extra costs	**49.57%**	**46.32%**	**55.00%**	**45.45%**	**45.45%**	**46.15%**	34.38%	**46.84%**
Yes - only if there were no extra costs to me	40.87%	42.65%	35.00%	42.42%	21.21%	38.46%	**56.25%**	40.51%
No	9.57%	11.03%	10.00%	12.12%	33.33%	15.38%	9.38%	12.66%

Things change if we look at the frequency of use in a year: with no incentives, participants who would use it only with a reasonable suspicion increase by approximately 15 percentage points, while there is a significant decrease among those who would use it more frequently; in particular, beekeepers who would opt for a regular use drop from 24.11% to 12.62% if incentives are not expected. For instance, nearly 20% of UK beekeepers would use the Bee Health Card regularly with economic incentives, but this drops to 7.50% with no incentives. Again, more than 40% beekeepers from Italy would use the tool regularly with incentives, but this becomes less than 16% with no planned incentives (Table 4.3.3).

Table 4.3.3: Proportion of beekeepers who would use the PoshBee health card at different regularity with/without economic incentives. Higher percentages in each country are highlighted in bold.

Use frequency of the BHC		IRE	UK	SPA	ITA	GER	SWI	EST	Total
With incentives	Regularly	27.10%	19.67%	13.89%	40.98%	20.83%	23.81%	12.90%	24.11%
	Irregularly	**50.47%**	**51.64%**	**69.44%**	40.98%	**54.17%**	**45.24%**	41.94%	**50.12%**
	Suspicion only	22.43%	27.87%	13.89%	18.03%	25.00%	30.95%	**41.94%**	25.06%
	Never	0.00%	0.82%	2.78%	0.00%	0.00%	0.00%	3.23%	0.71%
Without incentives	Regularly	15.38%	7.50%	11.11%	15.79%	13.64%	18.18%	10.34%	12.62%
	Irregularly	**45.19%**	**50.83%**	**50.00%**	**47.37%**	**54.55%**	31.82%	27.59%	**45.39%**
	Suspicion only	37.50%	40.00%	33.33%	35.09%	31.82%	**50.00%**	**58.62%**	40.05%
	Never	1.92%	1.67%	5.56%	1.75%	0.00%	0.00%	3.45%	1.94%

Finally, many beekeepers' responses to the open question stated it would be useful to detect *Varroa destructor* and diseases linked to it (25.91%), foulbrood diseases (23.64%), and the presence of pesticide residues in the beehives (21.36%) (Appendix 4.3).

4.3.5. Multiple Correspondence Analyses

4.3.5.1. Willingness to use the PoshBee tool with and without extra costs

The first Multiple Correspondence Analysis (MCA 1) conducted on variables related to the willingness to adopt the tool with and without extra costs, in a scenario with and without planned economic incentives, allowed us to cluster barriers and benefits based on their proximity on the map to use in the next statistical analyses (Table 4.3.4, Figure 4.3.11). The map shows that, while 'Cluster 2a' is clearly grouped the same way for agreement, disagreement, and neutral answers, this is not the case for the six 'BHC benefit' variables grouped in Q4. In fact, despite their 'agrees' cluster all together in Q4, their 'disagrees' are clustered into two separate groups in opposite quadrants (Q1 and Q3). Similarly, their 'neutrals' are grouped into the same two groups in quadrant 2 and 3. Therefore, to account for potential differences between such benefits, two different clusters were built based on the way the 'disagrees' and 'neutrals' grouped on the map, forming 'Cluster 1a' and 'Cluster 1b'.

Table 4.3.4: Final set of clusters and variables obtained after the two MCAs to use as predictors in further analyses.

Code	Variables	Classification
Cluster 1a (cp + bh + g)	Improved crop pollination + improved bee health + better communication with growers	**Benefits of the tool**
Cluster 1b (qe + ep + pp)	Tool quick and easy to use + environment protection + pollinators protection	**Benefits of the tool**
Cluster 2a (i + e + d + t)	Tool is not important + not effective + difficult + time-consuming	**Barriers to the tool**
p	Higher productivity	**Benefits of the tool**
tc	Lower treatment costs	**Benefits of the tool**
c	Tool cost	**Barriers to the tool**
g	Lack of communication with growers	**Barriers to the tool**
evc, msc, nc	Confidence in the effectiveness of the tool	**Effectiveness of the tool**

## Use of the Bee Health Card MCA - variable categories
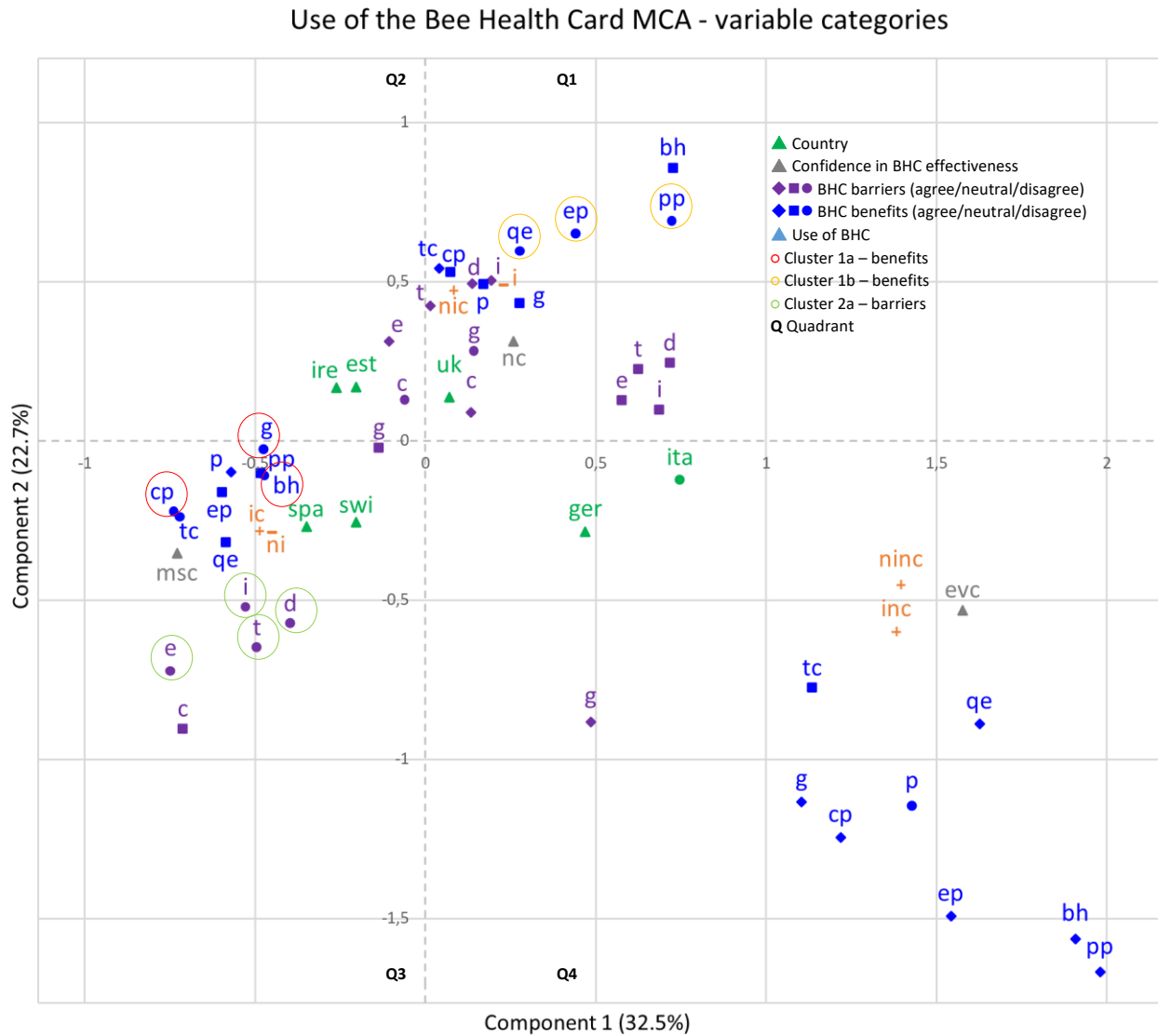
<u>Figure 4.3.11:</u> MCA factor map 1 showing variables to utilise as predictors in further statistical analysis investigating the willingness to use the tool and accept extra costs, with or without planned incentives. Components 1 and 2 account for 32.5% and 22.7% of variation in the data respectively.

4.3.5.2. Frequency of use of the PoshBee tool

The second Multiple Correspondence Analysis (MCA 2) conducted on variables related to the frequency of use of the tool in a scenario with and without planned economic incentives (n=423 and n=412 respectively), showed that no new cluster was formed, and variables on the map grouped the same way as in MCA 1 (Figure 4.3.12).
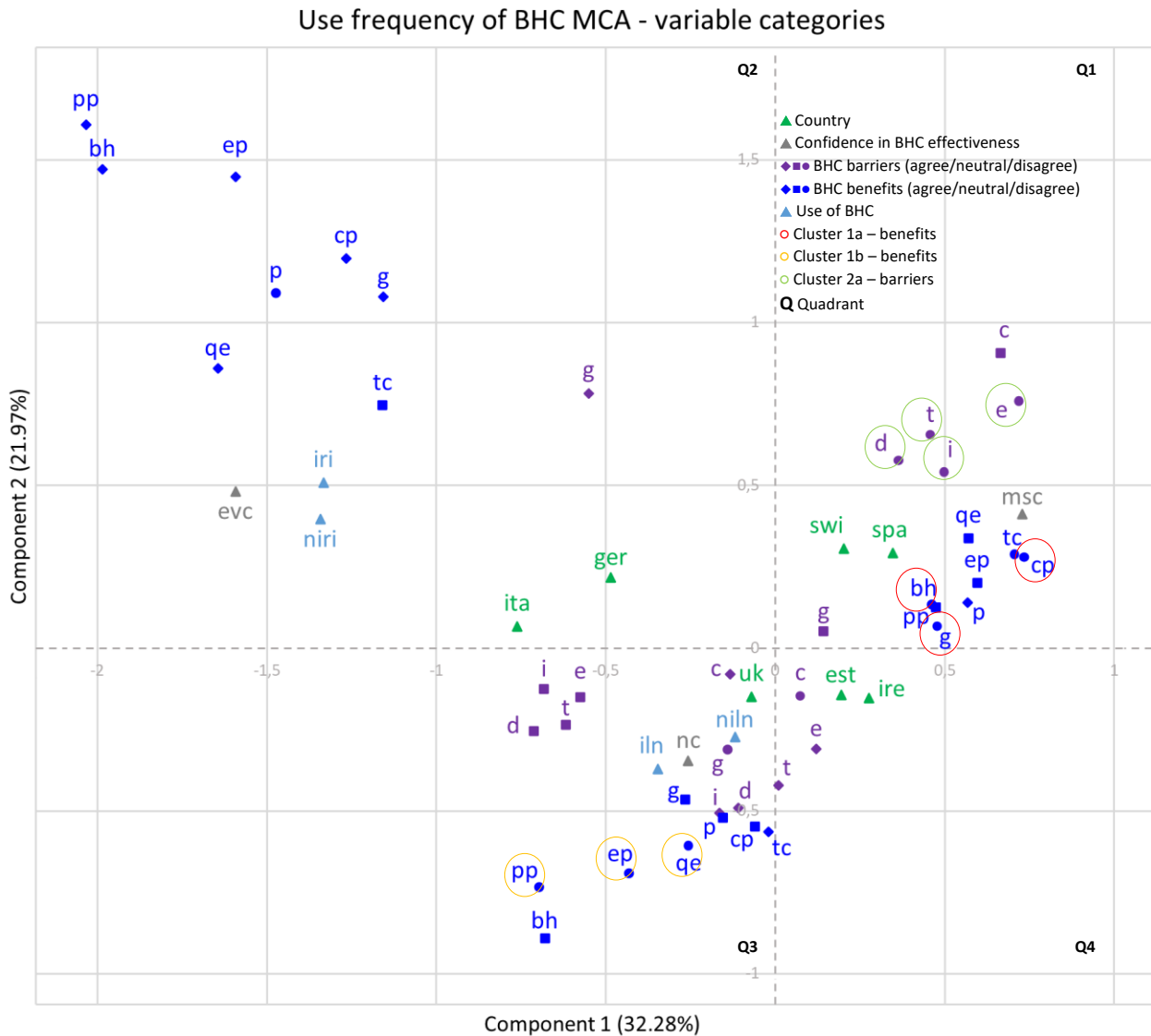
Figure 4.3.12: MCA factor map 2 showing variables to utilise as predictors in further statistical analysis investigating the frequency of use of the tool with or without planned incentives. Components 1 and 2 account for 32.28% and 21.97% of variation in the data respectively. Variables are grouped in the same way as in MCA 1, with 'Cluster 1a' and 'Cluster 2a' being in the opposite quadrant of 'Cluster 1b'.

4.3.6. Binary logistic regressions

4.3.6.1. Willingness to use the PoshBee tool

4.3.6.1.1. Scenario with economic incentives

With planned economic incentives, a higher level of confidence in the effectiveness of the tool is linked to a higher willingness to use it, while tending to agree with the variables grouped in 'Cluster2a' (*i.e.* tool being potentially time-consuming, difficult to use, not effective, and not important) indicates a lower probability of use (Table 4.3.5, Figures 4.3.13-14). Additionally, regression analysis shows that beekeepers inclined to perceive 'increase in productivity' as a benefit of the BHC have a higher probability of using it (Table 4.3.5, Figure 4.3.15).

Table 4.3.5: Final model investigating the willingness to use the BHC with economic incentives. Goodness-of-fit > 0.05 indicates no evidence of a lack of model fit. Significant p-values (>0.05) are highlighted in bold. See Appendix 4.4 for model selection and table of coefficients.

Willingness to use the PoshBee tool with economic incentives			
Terms	**χ²**	**df**	**p-value**
Confidence level in effectiveness	19.72	2	**<0.001**
Productivity as benefit	11.79	2	**0.003**
Time, effectiveness, difficulty, and importance as barriers	11.26	2	**0.004**
Goodness-of-fit	**χ²**	**df**	**p-value**
Hosmer-Lemeshow test	5.64	5	0.343
Model summary	**R²**	**BIC**	**BIC global model**
	22.21	288.28	325.38
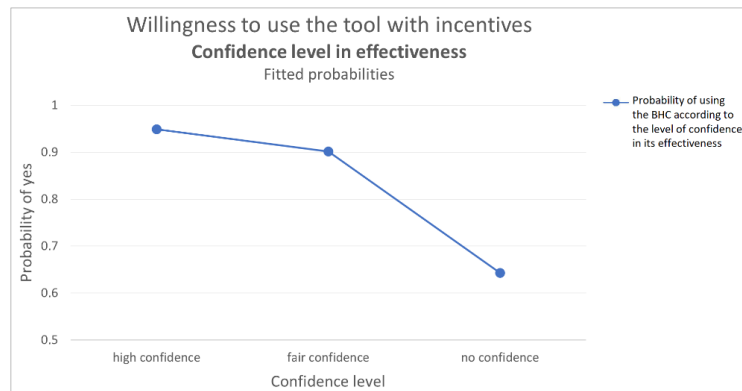
Figure 4.3.13: The probability of using the BHC is 0.95 when the confidence is high, and it drops to 0.64 with no confidence.
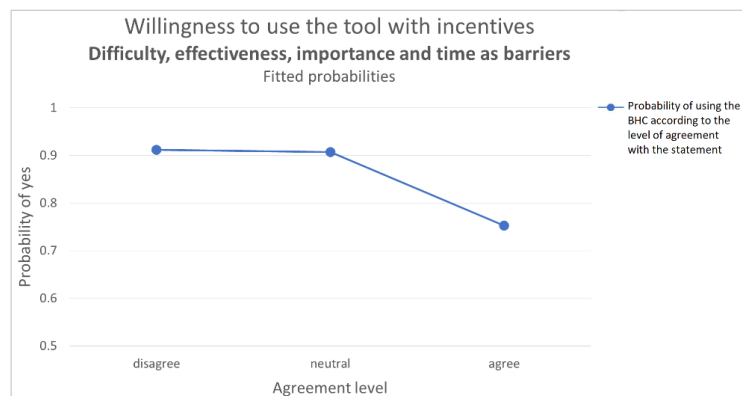
Figure 4.3.14: The probability of using the BHC is 0.91 when respondents disagree with 'Cluster 2a' barriers, while the probability drops to 0.75 when they agree with them.
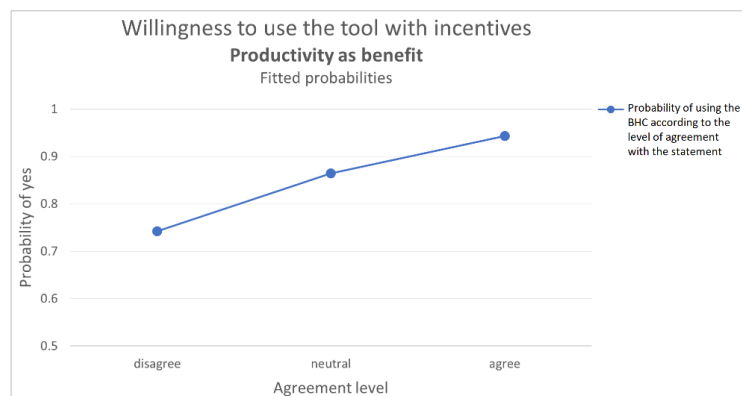
Figure 4.3.15: The probability of using the BHC is 0.74 when respondents do not recognise 'increase in productivity' as a potential benefit, and it increases to 0.94 when they do.

4.3.6.1.2. Scenario without economic incentives

In the scenario without planned economic incentives, regression analysis shows that to a higher level of confidence in the effectiveness of the BHC corresponds a higher probability of use, and that tending to agree with the barriers of 'Cluster 2a' (tool being 'time-consuming', 'difficult to use', 'not effective', and 'not important') indicates a lower probability of use (Table 4.3.6, Figures 4.3.16-17). Moreover, being prone to agree with benefits grouped in 'Cluster 1b' ('pollinator protection', 'environment protection', and tool being 'easy to use') increases the probability of using the PoshBee health card (Table 4.3.6, Figure 4.3.18).

Table 4.3.6: Final model investigating the willingness to use the BHC without economic incentives. Goodness-of-fit > 0.05 indicates no evidence of a lack of model fit. Significant p-values (>0.05) are highlighted in bold. See Appendix 4.4 for model selection and table of coefficients.

| Willingness to use the PoshBee tool without economic incentives			
Terms	χ^2	**df**	**p-value**
Confidence level in effectiveness	18.46	2	**<0.001**
Time, effectiveness, difficulty, and importance as barriers	7.13	2	**0.028**
Pollinator protection, environment protection, and easy to use as benefits	15.95	2	**<0.001**
Goodness-of-fit	χ^2	**df**	**p-value**
Hosmer-Lemeshow test	0.59	2	0.965
Model summary	R^2	**BIC**	**BIC global model**
	24.80	313.92	373.76
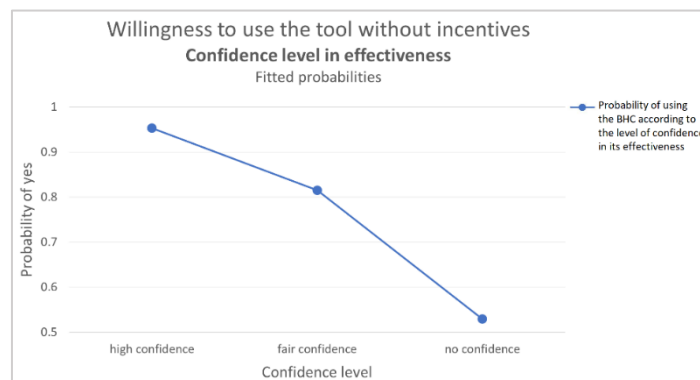
Figure 4.3.16: The probability of using the BHC is 0.95 when the confidence is high, and it decreases to 0.53 with no confidence.
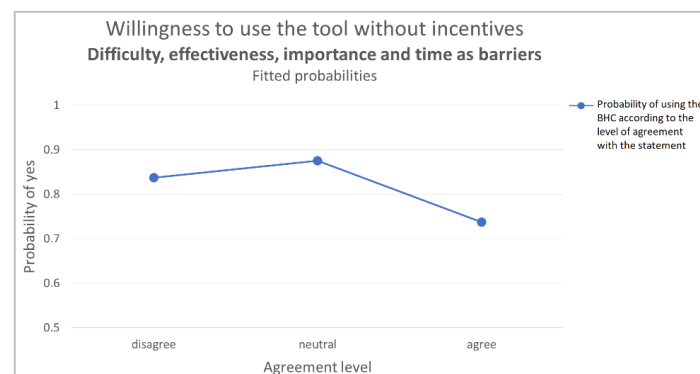

Figure 4.3.17: The probability of using the BHC is 0.84 when respondents disagree with 'Cluster 2a' barriers, while it drops to 0.74 when they agree with them.
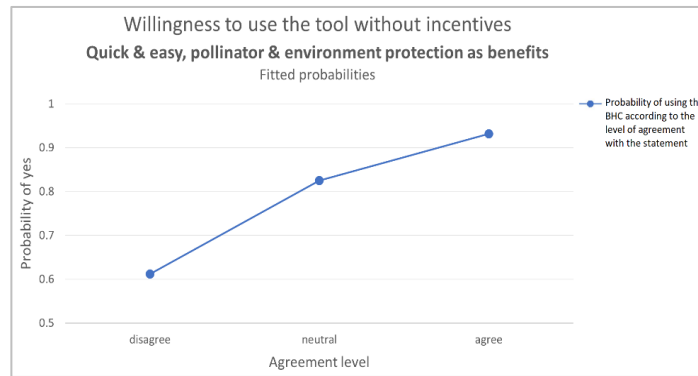
Figure 4.3.18: The probability of using the tool is 0.93 when respondents agree with 'Cluster 1b' benefits, while it drops to 0.61 when they disagree with them.

4.3.6.2. Willingness to accept extra costs linked to the PoshBee tool

4.3.6.2.1. Scenario with economic incentives

In the scenario with planned economic incentives, the probability of accepting extra costs significantly increases with the perceived level of confidence in the effectiveness of the BHC (Table 4.3.7, Figure 4.3.19), while it appears to decrease for respondents that tend to perceive the tool as being time-consuming, difficult to use, not effective, and not important ('Cluster 2a') (Table 4.3.7, Figures 4.3.19-20).

Table 4.3.7: Final model investigating the willingness to accept BHC extra costs with economic incentives. Goodness-of-fit > 0.05 indicates no evidence of a lack of model fit. Significant p-values (>0.05) are highlighted in bold. See Appendix 4.4 for model selection and table of coefficients.

| Willingness to accept extra costs related to the PoshBee tool with economic incentives			
Terms	**χ²**	**df**	**p-value**
Confidence level in effectiveness	19.47	2	**<0.001**
Time, effectiveness, difficulty, and importance as barriers	25.81	2	**<0.001**
Goodness-of-fit	**χ²**	**df**	**p-value**
Hosmer-Lemeshow test	1.37	2	0.503
Model summary	**R²**	**BIC**	**BIC global model**
	10.99	615.39	676.64
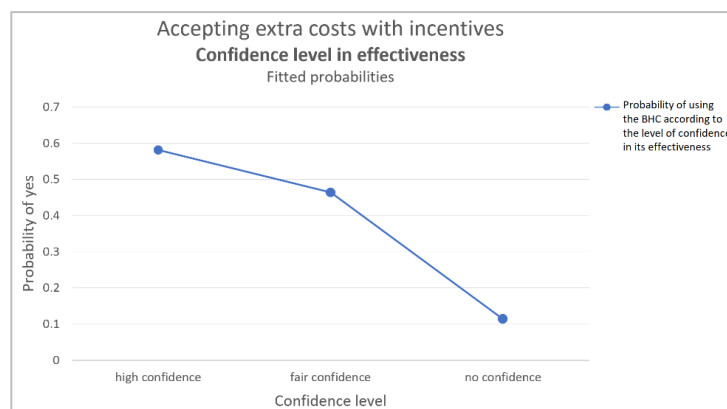
Figure 4.3.19: The probability of accepting BHC extra costs is 0.58 when the confidence is high, but it drops to 0.11 with no confidence.
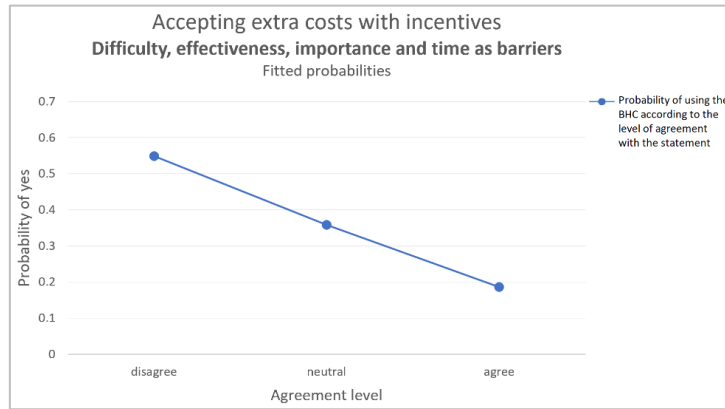
Figure 4.3.20: The probability of accepting BHC extra costs is 0.55 when respondents disagree with 'Cluster 2a' barriers, and it drops to 0.19 when they agree with them.

4.3.6.2.2. Scenario without economic incentives

When economic incentives are not planned, the level of confidence in the effectiveness of the PoshBee health card and 'Cluster 2a' including barriers related to time, difficulty, efficacy, and importance of the tool are also statistically significant in driving beekeepers' acceptance of extra costs related to the BHC (Table 4.3.8, Figures 4.3.21-22). However, contrary to the scenario including incentives, beekeepers that recognise 'cost' as a potential barrier to the use of the BHC are less likely to accept extra costs of the tool if economic incentives are not planned (Table 4.3.8, Figure 4.3.23).

Table 4.3.8: Final model investigating the willingness to accept BHC extra costs without economic incentives. Goodness-of-fit > 0.05 indicates no evidence of a lack of model fit. Significant p-values (>0.05) are highlighted in bold. See Appendix 4.4 for model selection and table of coefficients.

| Willingness to accept extra costs related to the PoshBee tool without economic incentives			
Terms	χ^2	**df**	**p-value**
Confidence level in effectiveness	15.11	2	**0.001**
Cost as barrier	8.37	2	**0.015**
Time, effectiveness, difficulty, and importance as barriers	14.03	2	**0.001**
Goodness-of-fit	χ^2	**df**	**p-value**
Hosmer-Lemeshow test	3.39	5	0.640
Model summary	R^2	**BIC**	**BIC global model**
	10.33	630.62	678.82
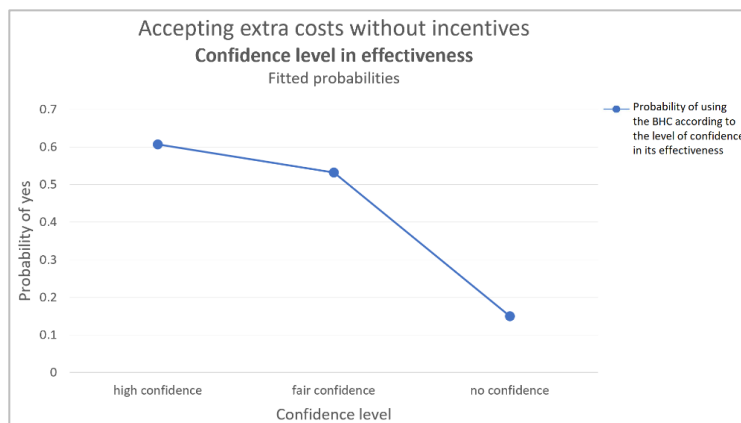
Figure 4.3.21: The probability of accepting BHC extra costs is 0.60 when the confidence is high, and it drops to 0.15 with no confidence. See Table 4.3.5 and Appendix 4.4 for further model details.
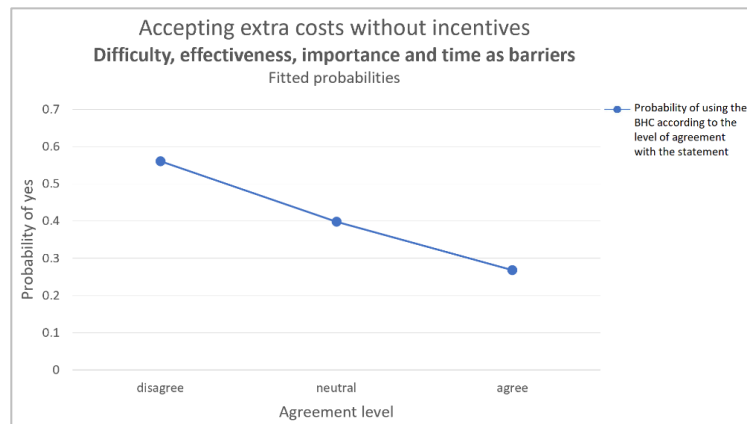
Figure 4.3.22: The probability of accepting BHC extra costs is 0.56 when respondents disagree with 'Cluster 2a' barriers, and drops to 0.27 when they agree.
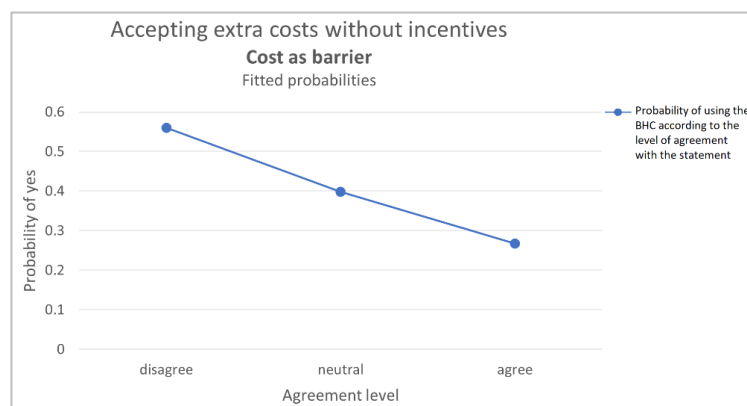
Figure 4.3.23: Respondents agreeing with 'cost' being a barrier have a 0.30 probability of accepting BHC extra costs, but it rises to 0.54 when they disagree.

4.3.6.3. Frequency of use of the PoshBee tool

4.3.6.3.1. Scenario with economic incentives

With planned economic incentives, the level of confidence in the effectiveness of the Bee Health Card is found to be statistically significant, with a higher confidence level corresponding to a higher probability of using the PoshBee tool more frequently (Table 4.3.9, Figure 4.3.24). Additionally, respondents considering 'cost' as a potential barrier to the use of the BHC are also linked to a lower probability of using it more frequently (Table 4.3.9, Figure 4.3.25).

Table 4.3.9: Final model investigating the BHC use frequency with economic incentives. Goodness-of-fit > 0.05 indicates no evidence of a lack of model fit. Significant p-values (>0.05) are highlighted in bold. See Appendix 4.4 for model selection and table of coefficients.

Frequency of use of the PoshBee tool with economic incentives			
Terms	**χ²**	**df**	**p-value**
Confidence level in effectiveness	20.81	2	**<0.001**
Cost as barrier	6.53	2	**0.038**
Goodness-of-fit	**χ²**	**df**	**p-value**
Hosmer-Lemeshow test	2.52	2	0.283
Model summary	**R²**	**BIC**	**BIC global model**
	6.60	481.11	537.33
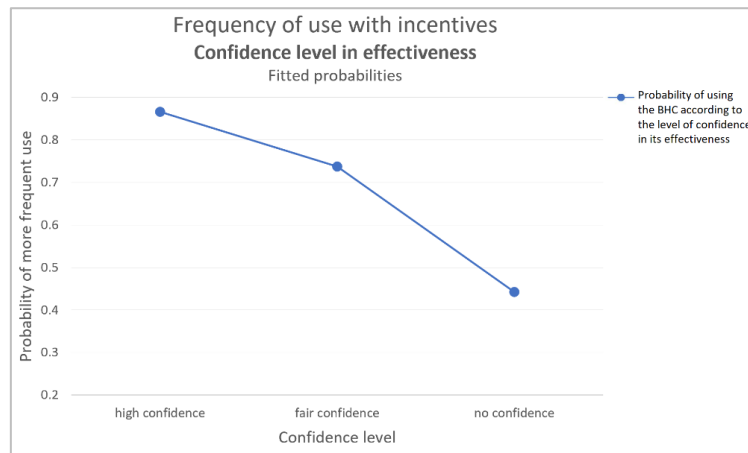
Figure 4.3.24: The probability a more frequent BHC use is 0.87 when confidence is high, while it drops to 0.44 with no confidence.
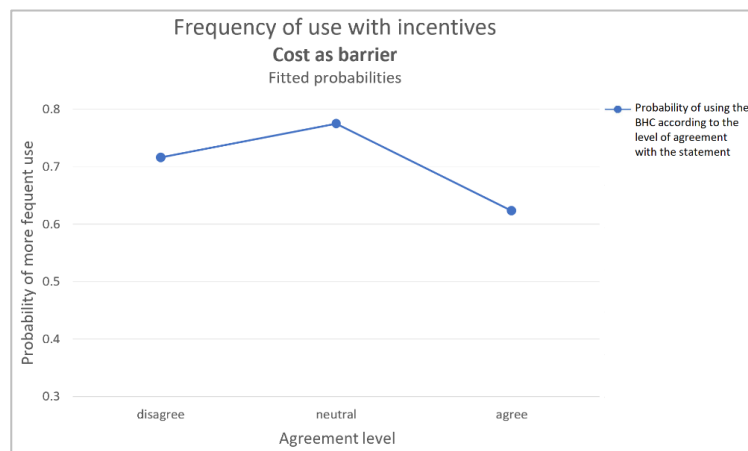
Figure 4.3.25: Respondents agreeing with 'cost' being a barrier have a 0.72 probability of using the BHC more frequently, and it rises to 0.62 when they disagree with it.

4.3.6.3.2. Scenario without economic incentives

When economic incentives are not planned, both 'confidence in the effectiveness of the BHC' and 'cost' have a statistically significant effect on the frequency of use, with a higher probability of a more frequent use when there are higher confidence levels (Table 4.3.10, Figure 4.3.26) and when respondents tend to disagree with 'cost' being a barrier (Table 4.3.9, Figure 4.3.27).

4.3.10: Final model investigating the BHC use frequency without economic incentives. Goodness-of-fit > 0.05 indicates no evidence of a lack of model fit. Significant p-values (>0.05) are highlighted in bold. See Appendix 4.4 for model selection and table of coefficients.

| Frequency of use of the PoshBee tool without economic incentives			
Terms	**χ²**	**df**	**p-value**
Confidence level in effectiveness	23.42	2	**<0.001**
Cost as barrier	13.54	2	**0.001**
Goodness-of-fit	**χ²**	**df**	**p-value**
Hosmer-Lemeshow test	1.01	3	0.798
Model summary	**R²**	**BIC**	**BIC global model**
	7.92	546.23	609.53
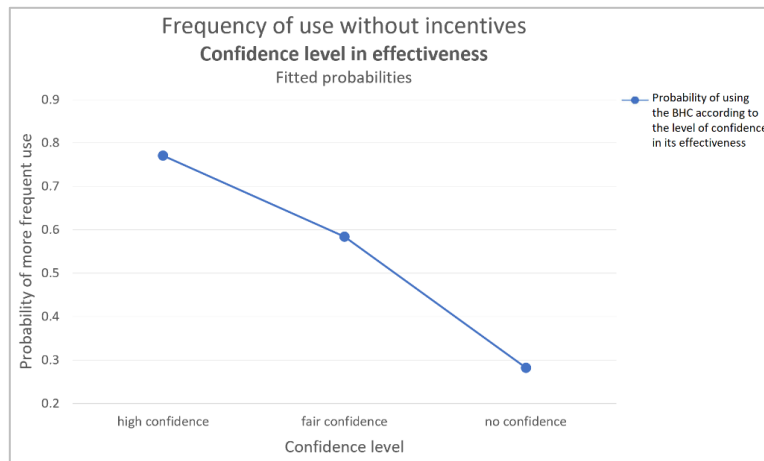
Figure 4.3.26: The probability a more frequent BHC use is 0.77 when confidence is high, and it drops to 0.28 with no confidence.
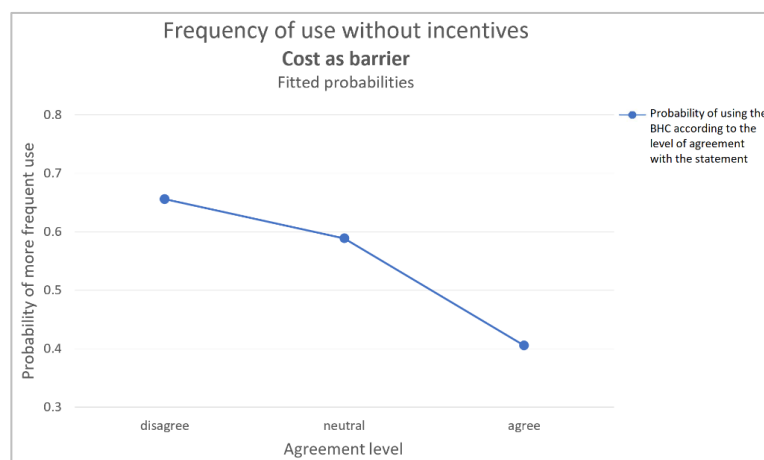

Figure 4.3.27: Respondents agreeing with 'cost' being a barrier have a 0.66 probability of using the BHC more frequently, and it rises to 0.40 when they disagree.

4.4. Discussion

In this survey, we explored key sources of beekeeper information around bee health and developed the first investigation into beekeepers' willingness to adopt novel bee health technology in the PoshBee Health Card, a tool currently under development which will provide accessible and rapid evaluation of the health of honeybee hives. The study findings allow us to understand what barriers to tackle and what perceived benefits may encourage adoption, not only to maximise the willingness to use the tool, but also to ensure its wide and frequent applications by beekeepers.

4.4.1. Beekeepers' perceived barriers and benefits towards the PoshBee tool

Research into beekeepers' interests and attitudes is limited (*e.g.* Breeze et al., 2017, 2019; Bieńkowska et al., 2020), and their knowledge and experience of bee health is sometimes underestimated (Donkersley et al., 2020). Very few studies address the need to directly investigate the impact of beekeepers' knowledge on management practices (El Agrebi et al., 2021), and despite being the most important end-users, no study has to date investigated beekeepers' perceptions in regard to the adoption of new technologies that may help

improve the health of their beehives. However, there are numerous studies investigating farmers' interests in adopting new technologies, such as precision agriculture technologies (PATs), and our survey results will be discussed looking at parallelisms between farmers' and beekeepers' perceptions.

The literature shows that farmers may be held back from using new technologies if they are perceived as difficult to use, while they are more eager to adopt them if regarded as easy to use and not time-consuming (*e.g.* Reichardt & Jürgens, 2009; Aubert et al., 2012; Vecchio et al., 2020). This is in line with our results, where 'difficulty' and 'time-consuming' factors were shown to negatively influence beekeepers' willingness to adopt the PoshBee tool and accept any related extra costs, while they would be more inclined to adopt the tool if it was quick and easy to use. In fact, technologies that are easy to use and not time-consuming do not usually require any specific additional knowledge, which may be an obstacle for some users (Vecchio et al., 2020). Moreover, perceiving new technologies as helpful and functional may encourage users to employ them (Davis, 1989; Aubert et al., 2012). This is also highlighted by our survey, where beekeepers with a high confidence in the effectiveness of the Bee Health Card are also more likely to be willing to use it, accept possible extra costs, and adopt it more frequently than beekeepers with fair or no confidence in its effectiveness. In this regard, increasing the perceived effectiveness will be easier when the PoshBee card will be fully developed and not just a hypothetical concept, as beekeepers will either have the opportunity to test it or at least have access to more practical information on its functions and characteristics; in fact, demonstrating how new technologies work does encourage their implementation (Barnes et al., 2019).

One of the most important barriers for beekeepers, underlined by more than half of respondents, is the potential cost of the PoshBee tool. This finding is in line with literature on farmers' perceptions, and highlights how high costs and uncertainties regarding economic returns are key factors in user's hesitation to adopt new technologies (*e.g.* Cullen et al., 2013; Barnes et al., 2019; Vecchio et al., 2020). In our study, cost becomes a significant barrier only in the scenario with no economic incentives, where it is associated with a lower frequency of use and probability of accepting extra costs linked to the BHC. Such an outcome is expected; in fact, beekeepers are constantly dealing with treatments to keep their beehives healthy (*e.g.* regular treatments to combat *Varroa destructor* mites, Steinhauer et al., 2018), the cost of which have increased to the point of becoming unprofitable for some owners of small-scale apiaries (Potts et al., 2010b). Thus, if a new tool does not entail the need of further investments in time and economic resources, it may be considered a good way of easing monetary pressures on beekeepers, particularly professionals (Rucker et al., 2012; Breeze et al., 2017; Gray et al., 2019). From this perspective, even if our results highlight that economic incentives are predominantly relevant only to maximise the use frequency of the tool, and not the willingness to use it, it is worth considering to offer them.

A recent survey highlighted that 'farmers' willingness to pay for pollination services' and 'subsidies' were among the most common suggestions made by beekeepers to support beekeeping services and crop

pollination (Tom D. Breeze et al., 2019). Such routes may be explored to support a widespread adoption of the Bee Health Card. Farmers need to sustain a multitude of important decisions when it comes to farming operations, and despite the large availability of precision tools which can help enhance both sustainable farming and farming efficiency, the adoption of such technologies is low (Aubert et al., 2012). Breeze et al. (2019) showed that half of surveyed farmers believed their crops were subject to pollination deficits, negatively impacting the yield. However, only a third answered that they actively hire beehives to promote pollination services, with 'cost' and 'lack of experience' as important barriers to this decision (Breeze et al., 2019). Considering that past studies highlighted the need of increasing farmers' knowledge in order to raise the chances of implementing new technologies (*e.g.* Kitchen et al., 2002; Aubert et al., 2012; Gürer & Akyol, 2018) or improve bee conservation (Tarakini et al., 2020), we speculate that targeting farmers' knowledge of pollination service importance may increase their chances of being willing to hire healthy hives, monitored through the Bee Health Card, thanks to which bee health concerns may be addressed quickly. Targeting farmers' knowledge may also help address beekeepers' concerns related to the lack of communications with them, which is regarded as a potential barrier to the use of the BHC by 60% respondents. Therefore, increasing farmers' awareness of protecting bee health may favour the cooperation with beekeepers and knowledge exchange related to issues affecting beehives, which would need to be tackled to prevent an impact on crop yield. Although investigating farmers' willingness to pay for such new technology would contribute to our research with new, useful insights, we anticipate that exclusively relying on this route to support the new PoshBee tool may be impractical.

Government subsidies may also represent a fruitful measure to support the Bee Health Card. Overall, the European Union provide support for beekeeping-related issues through national programmes that, between 2020-2022, amount to 40 million € per year (Commission Implementing Decision EU, 2019; EC, 2019). For instance, Majewski et al (2017) reported that, in 2019, EU contributions ranged from a minimum of 2.32 €/beehive in Denmark to a maximum of 5.3 €/beehive in Malta. Other examples of government supports which may also benefit beekeepers are the Rural Development Programmes (RDPs), EU-implemented funding programmes whose aims include, among others, supporting innovative technologies, agricultural innovations, and national quality schemes (EU, 2013b; Novelli et al., 2021). Therefore, we hypothesise that expanding funding opportunities to subsidises directed to the implementation of the PoshBee health card, at least initially, would allow a rapid evaluation of the beehives, helping beekeepers to quickly tackle and address potential health issues, and supporting a successful delivery of pollination services with profitable yields. Such profitable yields might also make growers more prompted to hire hives from beekeepers who use the PoshBee health card. To validate this consideration, when the tool will be finalised and ready to use, it would be worth investigating what factors may potentially favour or discourage growers' reliance on beehives monitored by the BHC.

If, however, economic incentives cannot be included, our results indicate that increasing the perception of wider, environmentally conscious benefits, such as pollinator and environment protection, may make beekeepers more willing to use the PoshBee tool. The importance of stressing environmental benefits is also underlined by Cullen et al. (2013) in regard to the adoption of new technologies by agricultural businesses. Safeguarding pollinators and the environment may also benefit the health of all bees, allowing beekeepers to deal with less health issues in their beehives, with consequently lower monetary expenses.

In addition to the safeguard of pollinators and environment, good beekeeping practices are also key to dealing with bee health concerns, as the lack of expertise of some beekeepers and poor beekeeping practices are one of the main causes of beehive losses in Europe (Jacques et al., 2017; Havard et al.,2019). With poor beekeeping practices, the risk of accentuating beehive hazards is high, while being educated on the matter may significantly contribute to overcoming beehive health issues (Steinhauer et al., 2018). Respondents generally agree with this, as they recognise that sub-optimal beekeeping practices may have a role in the decline of bees, while optimal methods may help counteract such declines. Given the importance of beekeepers' education, relying on well-grounded sources of information is key to building a strong knowledge background and being able to address health concerns in the beehives. This is consistent with studies on farmers' perceptions towards using new technologies, which report that farmers who hold higher educational backgrounds or are more dedicated to acquiring knowledge from external sources are also more likely to adopt new technologies (Aubert et al., 2012; Barnes et al., 2019; Vecchio et al., 2020). In this regard, our study shows that the majority of respondents consider beekeeping associations as highly important sources of information on bee health, indicating that a more direct collaboration with them is key to exchanging and disseminating knowledge, allowing beekeepers to build stronger expertise to address health issues correctly and efficiently in their beehives. The need to deal with such health concerns is reflected by respondents' perceived role of habitat loss, use of agrochemicals, and presence of parasites and diseases as the main causes of bee decline, in line with the most important and well documented threats to the health of bees highlighted in the literature (IPBES, 2016; Havard et al., 2019; Dicks et al., 2021).

4.4.2. Implications of adopting the PoshBee tool

The importance of addressing honeybee health concerns is reflected by responses to our survey, where beekeepers listed *Varroa* mites and related diseases, foulbrood diseases, and pesticide residues as important issues to be detected by the PoshBee tool. This is in accordance with other past studies, which show that beekeepers are constantly challenged by the necessity of restricting the spread of diseases and parasites in their beehives and dealing with pesticide pressures (Breeze et al., 2017, 2019).

Using the Bee Health Card, beekeepers could detect pesticide residues which may cause health issues in their beehives, and address such issues through cooperating with farmers and reducing the risk of pesticide effects on pollinators (IPBES, 2016). For instance, farmers should always let beekeepers know when they plan to

apply pesticides, so that hives may be closed and bees may not be directly exposed to spraying (Hooven et al., 2013). Moreover, farmers should avoid spraying crops during blooming, or using pesticides with high bee toxicity (Biddinger & Rajotte, 2015; IPBES, 2016). This also applies to organic fields, as some organic insecticides may hold high toxicity levels (*e.g.* Spinosad, acute contact LD_{50}=0.0036 µg/honeybee, ECHA, 2010) (Mallinger et al., 2015). The adoption of Integrated Pest Management measures (IPM) may also help reduce pesticide use and pressures on beneficial pollinators (DEFRA, 2019). Although some studies have reported that plant protection products may help increase crop yield (*e.g.* Ijaz et al., 2015; Popp et al., 2013; Sutter et al., 2018), others have highlighted that pesticide pressures on pollinators can negatively affect the yield of the crops they pollinate (*e.g.* Stanley et al., 2015a; Hokkanen et al., 2017), and there are examples where reducing to some extent the use of pesticides did not affect the productivity or profitability of farmlands (*e.g.* Lechenet et al., 2017; Cui et al., 2018; Catarino et al., 2019b). Moreover, crop yield may also be impacted by pollinator deficits (*e.g.* Bartomeus et al., 2014; Potts et al., 2016), which have also been linked to a higher use of pesticides (*e.g.* Le Féon et al., 2010; Kennedy et al., 2013; Le Provost et al., 2021). Limiting pesticide inputs also contributes to lower pressures on wild pollinators, whose communities play a key role in pollinating important crops (Woodcock et al., 2013; Hutchinson et al., 2021), but are highly impacted by intensive agricultural practices (*e.g.* Rundlöf et al., 2015). For example, if the BHC detected a sub-optimal environment characterised by high pesticide residues, local wild bees would also be affected, and reducing pesticide usage would benefit not only managed beehives, but also wild bee populations. Thus, monitoring pesticide issues through the Bee Health Card could encourage the adoption of lower input management practices, benefitting both beekeeping and farming activities in lowering pressures on bees and, consequently, their pollination services.

In addition to pesticide issues, a quick BHC detection of parasites and pathogens in beehives could greatly facilitate response to such threats. For instance, when American or European foulbroods are detected, many countries necessitate to notify them to the government (e.g. Italy, D.P.R., 2006; UK, The Bee Diseases and Pest Control, 2006), and the application of specific anti-microbial agents is required to prevent the spread of such diseases and reduce consequent economic losses (Genersch, 2010; Reybroeck et al., 2012). If antibiotic treatments are not permitted (*e.g.* see EU, 2009), the hives will need to be destroyed (Genersch, 2010; Reybroeck et al., 2012). Contrary to foulbrood diseases, if promptly detected, *Varroa mite* infections are usually easier to deal with, and necessitate the periodical administration of anti-*Varroa* treatments to keep the proliferation under control (Hernandez et al., 2022). However, there is also emerging evidence that acaricide residues can be toxic to bees (Premrov Bajuk et al., 2017; Ostiguy et al., 2019; Kast et al., 2020). If acaricides are detected as potential threats to the health of beehives, beekeepers will be able to quickly tackle the issue, following low-input procedures and reducing their usage (Noël et al., 2020) and favouring organic-based treatments, which have also been shown to be more effective than synthetic ones (Leza et al., 2015, 2016). Tackling health issues in beehives in time would also prevent the spread of diseases from

managed to wild bees, which have been recorded in several studies (e.g. Graystock et al., 2013; Fürst et al., 2014; Steinhauer et al., 2018).

The most critical period for beehives in Europe is winter, when mortality is a widespread issue (EU Reference Laboratory, 2011; Popovska Stojanov et al., 2021). For instance, Chauzat et al. (2016) found that, during Winter 2012-2013, Varroosis, Nosemosis, and American Foulbrood (AFB) diseases significantly reduced honeybee colony health and survival. According to the latest COLOSS questionnaire (International surveillance network for honeybee colony losses – Prevention of colony losses), the overall rate of winter colony losses in Europe between 2018-2019 amounted to 14.5% (Gray et al., 2020), of which ~10% are caused by dead or empty colonies, ~4% from queens problems, and >1% from natural disasters. An effective and widespread use of the Bee Health Card may be able to lower such winter losses, and assuming that our sample of beekeepers is representative, we can predict how much this reduction would be. Considering (a) 14.5% colony winter losses in Europe between 2018-2019, (b) a hypothetical 75% effectiveness of the tool, and (c) 95% probability of using the tool at least once per year with high confidence in its effectiveness as shown by the binary logistic regression (Figure 4.3.13), in a best case scenario we could expect an overall 10.3% colony winter losses[4], for a reduction of 28.96%[5]. Therefore, the widespread adoption of the health card may represent a significant possible mechanism to reducing winter colony losses in Europe.

4.4.3. Limitations and further work

Although this study presents many valuable results, the most important limitation is undoubtedly that the PoshBee tool is still an instrument under development, and therefore could not be used and directly appraised by respondents. When the card is ready, a further comprehensive survey involving a more representative and numerous samples of beekeepers should be circulated to look at the way respondents perceive the Bee Health Card after giving them the opportunity to use it for a certain period of time.

The study was also limited by the use of social media dissemination. Using social media was necessary to distribute the survey, but it led to substantive differences in the distribution among partner countries and, consequently, in reaching the target of beekeepers. Ideally, a professional sample would have been more representative and therefore more suitable to be used for the research, however such a sample is likely to be very costly and many market research agencies do not have access to the contact details of niche demographics like beekeepers. Furthermore, recruited respondents may not have been representative of all national beekeepers, as (i) they are likely to have an interest in bee health tools and/or bee declines, which would influence their responses, and (ii) in many of the countries involved, beekeepers were mostly recruited from national associations with a hobby focus (*e.g.* BBKA – UK; FIBKA – Ireland, Appendix 4.1).

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[4] 0.145*(0.75*0.95) = 10.3% new expected colony winter loss
[5] (10.3-14.5)/14.5= -28.96% reduction

As we have focussed on investigating which benefits and barriers may be significant to the use of the Bee Health Card, further studies may focus on understanding how to increase the perception of its effectiveness, and whether it is more efficacious to decrease the perception of barriers such as cost, time, and difficulty, or to increase the perception of benefits such as environmentally conscious ones.

Ultimately, our findings highlight the important role of beekeeping associations in knowledge dissemination, thus further analyses may be effective in exploring the relations to different sources of information on the health of beehives, and the way beekeepers may change their view on the PoshBee health card with the engagement of beekeeping associations, training courses in person, and other important sources. One suitable approach to explore such behaviours may be the Social Network Analysis, providing the collection of enough data from a larger representative sample of beekeepers (Makagon et al., 2012).

4.5. Conclusions

The survey key findings and recommendations may be summarised as follows:

a. **Beekeeping associations should be the main points of contact for disseminating knowledge.** The survey outcome highlighted the importance given to often small, independently run organisations in providing beekeepers with information on beehive health, as such they should be the primary focus of efforts to maximise knowledge exchange. Training workshops as support for a strong educational background should also be encouraged.

b. **Beekeepers' perceptions of drivers of bee decline, and measures to reduce it, are in line with the scientific consensus.** Beekeepers recognised a number of factors as the main threats to overall bee health, most notably the loss of natural habitats and agrochemical use.

c. **Beekeepers recognise the potential for the BHC to improve colony health, with moderate confidence level in its effectiveness.** Such confidence is crucial for the willingness to use the tool, and it becomes even more important to maximise its use frequency.

d. **Wider environmental benefits may influence beekeepers' willingness to use the tool.** Safeguarding pollinators and the environment may also lead to less health concerns regarding the hives, and less monetary expenses from beekeepers to deal with them.

e. **The BHC needs to be easy to use and highly effective.** It is necessary to increase beekeepers' confidence in the effectiveness of the tool and to underline it does not require any specific knowledge to be used. Practical demonstrations and testing of prototypes by beekeepers would help address this.

f. **Well targeted economic incentives should be planned to establish frequent use, and may be particularly useful if extra costs are involved.** This would also prevent cost from being a very influential barrier to the use of the tool. Additionally, with economic incentives, an increase in productivity may be one of the benefits capable of contrasting the obstacles to using the PoshBee health card.

Chapter 5

Discussion and conclusions

5.1. Result summary

Bees represent the most dominant pollinators in the world, providing the human population with many monetary and non-monetary benefits (IPBES, 2016). For this reason, reports of bee declines in the last decades (*e.g.* Powney et al., 2019), coupled with high rates of honeybee colony losses in Europe (*e.g.* Gray et al., 2020), are a cause of rising concern. Bee health is threatened by numerous pressures which may also occur simultaneously and interact synergistically (Goulson et al., 2015). Understanding how to sustain healthy bee populations of both managed and wild bees and optimal beekeeping practices is key to tackling and reversing such declines (Potts et al., 2016).

This thesis aimed to address some important knowledge gaps in the literature, among which the effects on bee health, behaviour, and pollination of: (i) multiple pressures and their interactions, including insecticides, herbicides, fungicides, land covers, and diseases; and (ii) a new insecticide that may be used as an alternative to neonicotinoids, and its potential interaction with a common bumblebee pathogen. Moreover, I investigated beekeepers' perceptions regarding the adoption of a new tool that could help improve bee health and counteract colony losses.

In order to address the multiple challenges of safeguarding bee health and halting their decline, I have utilised a combination of natural and social science approaches from multiple perspectives. The effect of land cover, pesticides, and diseases were investigated using both a large-scale and a small-scale study. The large-scale study (Chapter 2) was performed using a field-realistic scenario; field experiments are more difficult to control, but do not overestimate or underestimate pesticide exposure and allow a more accurate representation of real pressures affecting bee health than a laboratory approach. The semi-field experiment (Chapter 3) was performed using flight cages, which provide a more controlled environment and an easier replication design compared to a complex field experiment, and also lower the risk for small-size effects to be buffered by environmental variables (*e.g.* landscape effects). Additionally, the choice of using multiple doses of insecticide instead of a single dose was made to mimic its natural degradation over time, enabling the adoption of a more field-realistic approach. Finally, I also used a social science study (Chapter 4) investigating beekeepers' opinions on a new omics tool, offering a completely different approach to the first two experiments, and focussing on practical actions to put in place to safeguard bee health.

As shown in Chapter 1 (section 1.5), all three core chapters are linked together to explore not only stressor interactions and impacts on bees, but also measures to help large-scale monitoring of bee health issues, consequently benefitting both managed and wild bee populations (Figure 1.5.1).
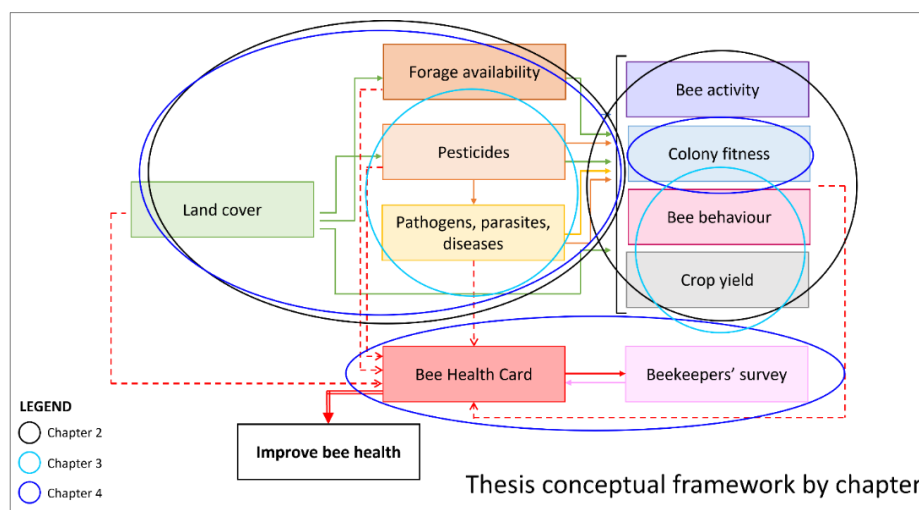
Figure 1.5.1: Thesis conceptual framework. See Chapter 1, section 1.5.

My findings on pesticide pressures on bees highlighted that effects may be different depending on pesticide combinations, class, and dosages. In fact, mixtures of fungicides and herbicides used in oilseed rape fields were able to interact between each other, surprisingly increasing the activity of honeybees and bumblebees, and positively affecting the growth of *B. terrestris* colonies. However, no effect of insecticide mixtures on bee activity, health, or colony fitness in such sites was found, and it was later highlighted by the semi-field study that even the emerging insecticide sulfoxaflor did not affect the behaviour of *B. terrestris* bees or their pollination on field bean plants. At the same time, I also found that no pesticide mixture affected the proliferation of *Varroa destructor* in honeybee hives in target apple or oilseed rape sites, and, similarly, that sulfoxaflor did not interact with the pathogen *C. bombi* in affecting *B. terrestris* behaviour or pollination of field beans.

When investigating land cover effects on bees, I observed that different types of land covers can have very polarised impacts on different bee species. In fact, while the growth of *B. terrestris* colonies in apple sites was higher in correspondence with more croplands and less woodlands in the surroundings, semi-natural habitats were found to increase honeybee activity in the very same sites. Moreover, similar to pesticides, land covers were not observed to interact with *Varroa destructor* in any crop sites.

Such results proved the complexity of stressor interactions, but a good understanding of their issues and well-structured response measures are necessary to counteract detrimental effects on bees. In this regard, beekeepers may hold a fundamental role not only in ensuring beehive health, but also in contributing to protecting wild bee populations, securing safe habitats in terms of nutrition and pesticides, and lowering the risk of disease transmission from managed to wild bees. The investigation of beekeepers' opinions on the use of the Bee Health Card provided important perspectives to consider in relation to favouring the wide use of such tool in the future; with a promising fair level of confidence in its effectiveness, the Bee Health Card may be successfully used to target beehive health issues, and planning economic incentives may also be important to ensure a more frequent use, especially with extra costs involved, so as to reduce the high

expenses beekeepers need to sustain to prevent beehive health disorders or losses. Maximising the confidence in the tool effectiveness may also guarantee a higher willingness to use it, higher use frequency, and higher probability of accepting its extra costs. To favour a correct and useful uptake of the Bee Health Card, scientists should also work in close contact with beekeeping associations, since they were revealed to be the main source of information on beehive health that beekeepers relied on.

Hence, with three chapters using three different methodologies, this thesis showed how investigating the mode of actions and interactions of pesticides, land cover, and diseases on the health of bees, and using a new tool able to quickly detect issues within beehives, may allow a better management of commercial colonies and environment control, also benefitting wild bee populations in the surrounding habitats.

5.2. Implications for pesticide approval and use

5.2.1. Active ingredient toxicity

One of the major implications outlined in this thesis is the way pesticides are reviewed for safety and approval (sections 2.4.1, 2.4.2., 3.4.2). Current pesticide risk assessments attribute an LD_{50} index to the active ingredient, related to the dose expected to cause mortality in 50% of tested bees (ECHA, 2016). Such assessments tend to rely on *Apis mellifera*, assuming that it is representative of all other bee species (Sgolastra et al., 2019). However, species sensitivity towards pesticides is characterised by high variability (Sgolastra et al., 2020).

For instance, while honeybees process and store nectar and pollen for a long time before feeding their larvae, solitary bee larvae directly feed on unprocessed, recently collected food, but take longer to consume it (Sgolastra et al., 2019). Hence, pesticide residues may be more or less degraded, or more or less diluted, depending on bee foraging behaviour (Sgolastra et al., 2019). Moreover, the route through which bees are exposed to pesticides differ depending on nesting behaviours; soil may represent an important route of pesticide exposure for certain ground-nesting bee species, and not for others, like *A. mellifera* and *B. terrestris* (Sgolastra et al., 2019). Additionally, bees adopt different behaviours in response to environmental pressures that influence their ability to endure them (Straub et al., 2015), with social species being considered less vulnerable than solitary ones, since they are likely to put in place mechanisms to protect their colonies (Sgolastra et al., 2017), for example through a more frequent queen replacement (Sandrock et al., 2014) or a lower production of new queens (Whitehorn et al., 2012).

Species dissimilarities are crucial to acknowledge when approving the use and safety of pesticides (Arena & Sgolastra, 2014). For example, through a laboratory experiment, Ladurner et al. (2005) showed that the fungicide captan, even at high doses, did not affect the health and behaviour of *A. mellifera*, but it had a significant impact on the solitary bee *Osmia lignaria*, reducing its survival and causing several behavioural changes, such as inactivity and regurgitation of the administered pesticide solution. Moreover, the fungicide

fenbuconazole was found to hold minimal toxicity to *A. mellifera* and to *Osmia cornifrons* (Biddinger et al., 2013), but Boff et al. (2022) observed that it affected the mating success of *Osmia cornuta* males, suggesting an alteration of chemical signals that females use to assess male fitness. Additionally, Abraham et al. (2018) observed that a glyphosate-based herbicide caused a higher mortality in *Hypotrigona ruspolii*, a stingless bee, than in *Apis mellifera*, while Heard et al. (2017) found that 2,4-dichlorophenoxyacetic acid, a systemic herbicide, was more toxic to *Apis mellifera* and *Bombus terrestris* than to *Osmia bicornis*, a trap nesting solitary bee. Hence, even though my large-scale fieldwork showed that higher herbicide and fungicide pressures in oilseed rape fields favoured a higher growth of *B. terrestris* colonies and a higher activity of both honeybees and bumblebees, caution is required when addressing such effects on other bees, as it cannot be excluded that they may be impacted in different ways.

Similarly, insecticides also affect bee species differently; for instance, Rundlöf et al. (2015) found that the use of a neonicotinoid and pyrethroid did not have any impact on honeybee colony strength (*i.e.* number of adult bees), but it did negatively affect the weight gain, reproductive fitness, and colony strength of *B. terrestris*, the density of wild bumblebees and solitary bees, and the nesting of the solitary bee *O. bicornis*. My semi-field experiment investigated the impact of field-realistic concentrations of sulfoxaflor on *B. terrestris* only, and underlines no effect on foraging behaviour, consistent with past research on bumblebees (Siviter et al., 2019) (section 3.4.1.). However, a previous study by Boff et al. (2021) highlighted that field-realistic doses of sulfoxaflor did affect the behaviour of *O. bicornis*, including a reduced foraging activity, visitation rate, and flight performance. Thus, although it can be concluded that, under the conditions of my experiment, sulfoxaflor might potentially be a safer alternative to neonicotinoids for *Bombus terrestris*, it is necessary to conduct more research on other bee species, such as wild bumblebees and solitary bees, to confirm such result.

Moreover, my study showed that sulfoxaflor did not interact with the pathogen *C. bombi* and reported no effect on the behaviour of *B. terrestris* (section 3.4.1.). However, no other study has yet investigated such interaction, and further research is needed to investigate the possible synergy between sulfoxaflor and *C. bombi* on different bumblebee species before corroborating my result.

Hence, more data on non-*Apis* bees is needed to address differences between species when it comes to pesticide exposure, and to make sure that a pesticide considered safe for certain species, such as honeybees or commercial bumblebees, is also proven to be safe for others, such as solitary bees or wild bees.

5.2.2. Pesticide formulations

In addition to mainly relying on honeybees, it is of notable concern that pesticide risk assessments are often performed on single active ingredients instead of formulations (sections 2.4.5., 3.4.2.) (Cedergreen, 2014). Pesticides employed in agriculture nearly always involve co-formulants and adjuvants, and although such ingredients are often assumed 'inert', their safety to bees has not been fully tested (Straw et al., 2022). On

the contrary, the literature suggests that 'inert' ingredients may even be more toxic than active ingredients themselves (Mullin, 2015), and that they can cause both lethal and sub-lethal effects on bees; for instance, organosilicon adjuvants were shown to impair honeybee learning capacity (Ciarlo et al., 2012), alcohol ethoxylates reduced bumblebee colony weight change and sucrose consumption (Straw & Brown, 2021), and a non-ionic adjuvant was observed to affect solitary bee nest recognition abilities (Artz & Pitts-Singer, 2015). 'Inert' ingredients have also been shown to interact with pesticides, increasing their toxicity to bees; for example, Wernecke et al. (2022) showed that, when applied alone, neither surfactant adjuvants nor different insecticide formulations affected the survival of honeybees. However, when applied in combination, adjuvants and insecticides interacted and significantly increased bee mortality (Wernecke et al., 2022).

Despite such evidence, testing of 'inert' ingredients is not currently included in any toxicity testing regulation (EU, 2021b), contrary to active ingredients (EFSA, 2013d). Information on 'inert' ingredient toxicity, or their interaction with pesticides, would have highly benefitted my large-scale field study design and analysis. The availability of LD_{50} information on such ingredients would have allowed us to include them in pesticide pressure index calculations, and to investigate any synergistic effect with other pesticides or active substances on the activity, fitness, and growth of bee colonies, and on the delivery of pollination services. Therefore, introducing regulatory testing on entire formulations instead of only active ingredients is needed to assess pesticide safety with higher accuracy and to better tackle lethal and sub-lethal effects on bees (Cedergreen, 2014; Straw et al., 2022).

5.2.3. Organic and conventional farming systems

When it comes to pesticide use implications, an important distinction needs to be made between low-pressure and organically managed systems. In fact, although organic farming in most cases is likely to be less impactful on beneficial insects than conventional farming (*e.g.* Holzschuh et al., 2008; Tuck et al., 2014; Lichtenberg et al., 2017), organic management does not always equal lower-input systems (*e.g.* Bahlai et al., 2010; Mallinger et al., 2015). An example is given by my large-scale field study, where the organic apple orchards had among the highest Pesticide Pressure Indexes of all apple sites (section 2.4.5.).

The increase in insecticide indexes of the two organic sites was primarily driven by the relatively high acute contact toxicity of the organic insecticide spinosad (LD_{50} = 0.0036 µg/bee, ECHA, 2010). Although this may appear unexpected, organic pesticides are not always the least toxic alternatives; for instance, spinosad is globally used on more than 150 crops (Miles et al., 2011), but its acute contact toxicity overcomes that of clothianidin (LD_{50} = 0.0443 µg/bee), imidacloprid (LD_{50} = 0.081 µg/bee), and thiamethoxam (LD_{50} = 0.024 µg/bee), the three neonicotinoids that were banned from the EU market (EFSA, 2013a, 2013b, 2013c). Several knowledge gaps on spinosad effects on bees still need to be investigated, including further information on risks to bumblebees, risks of chronic exposure and its potential sub-lethal effects on honeybees, and data on solitary bees (EFSA, 2018).

In contrast, the increase of herbicide and fungicide pressure indexes in the two organic orchards was caused by the high number of applications of the fungicides potassium bicarbonate (LD_{50} = 24 µg/bee, EFSA, 2012) and sulphur (LD_{50} = 100 µg/bee, EFSA, 2008). In fact, despite having a low toxicity, some organic pesticides need to be applied multiple times in order to be effective; high rates and frequency of application may increase pesticide exposure levels, and, consequently, their toxicity to bees (*e.g.* Medrzycki et al., 2013; Mallinger et al., 2015).

Thus, the approach of calculating pesticide indexes to understand the level of pressures on bees can be a more accurate method than classifying target sites based purely on whether they have conventional or organic management systems, and this is backed up by past studies in the literature (Mallinger et al., 2015; Park et al., 2015; Yasrebi-de Kom et al., 2019). Such an approach allowed me to account for both toxicity and application rates, and to highlight that organic pesticides, if applied at high rates or frequency, are capable of increasing pesticide pressures on pollinators despite holding low toxicity scores (Bahlai et al., 2010; Mallinger et al., 2015).

In order to reduce pesticide inputs, not only is it necessary to choose pesticides with low toxicity, but also to consider the rate and frequency of applications that need to be implemented. Hence, when pesticide application schemes are discussed and prepared, it is important not to assume that organic products have a lower environmental impact than synthetic ones, and instead explore all possible exposure routes taking into account both toxicity and application rates.

5.2.4. Cooperation between beekeepers and growers to reduce pesticide use

Beekeepers involved in my survey were aware of the pressures leading to bee declines (section 4.4.2.). Particularly, they expressed the need to deal with the increasing use of pesticides caused by agricultural land expansion and intensification, with 90% of beekeepers agreeing with the statement that pesticides are a cause of concern for bee health and decline. The presence of pesticide residues in beehives was also listed by ~21% of respondents among the issues that should be detected by the PoshBee Bee Health Card, which was among the most common answers (section 4.4.2.).

My results are in line with the major pressures on bees highlighted in the literature (*e.g.* IPBES, 2016; Havard et al., 2019; Dicks et al., 2021), and with past surveys revealing beekeepers' concerns and changes in behaviour over pesticide exposure (Breeze et al., 2019). Considering that pesticides can make beehives more vulnerable and prone to infections and diseases (Pettis et al., 2013), beekeepers are forced to invest in sanitary practices to avoid colony losses (Steinhauer et al., 2018). Thus, the use of pesticides in agricultural lands is of particular interest for beekeeping.

However, beekeepers' concerns related to pesticides are not easily addressed. Reducing pesticide use to unburden pressures on bee health is difficult without a good communication and cooperation with growers;

more than 60% of respondents stated that communication is poor or inexistent, and that it was one of the perceived, major barriers to adopting the Bee Health Card (section 4.4.1.). Thus, the lack of constructive dialogues between growers and beekeepers should be considered a priority issue to address, as a potential way to reduce pesticide pressures on bees.

If, on the one hand, the use of pesticides to target pest insects and plants is necessary to help improve crop yield (*e.g.* Popp et al., 2013), on the other hand such products have been shown to negatively affect bees and, consequently, may affect the yield of insect-pollinated crops (*e.g.* Stanley et al., 2015a). Instead, farm profitability may increase if the use of pesticides is limited to some extent (*e.g.* Catarino et al., 2019b). In this regard, cooperation between beekeepers and growers could be used to modify changes to pesticide application programmes by reducing the risk of such products, without compromising crop yield.

In order to both reach safer environmental conditions for bees and maintain farm profits, growers should always update beekeepers with precise dates when they are planning to conduct any spraying and with active ingredients that are being used (Hooven et al., 2013), which should ideally not happen during the flowering period (Biddinger & Rajotte, 2015). This way, beekeepers will be able to close the hive entrances and bees will not be allowed outside, potentially minimising the risk of a direct exposure to pesticides (Hooven et al., 2013).

5.3. Implications for biodiversity conservation

5.3.1. Landscape pressures

Above, it was outlined how pesticides may affect bees to different extents, and how the exposure to such substances should be regulated by testing the entire formulation on multiple bee species (*e.g.* Siviter et al., 2018b; Sgolastra et al., 2020) (sections 2.4.1., 2.4.2., 2.4.5., 3.4.2.). Together with pesticide use, different land covers can influence bee species differently (*e.g.* Diekötter et al., 2010), with implications for land management and bee conservation.

The large-scale study showed that, during their growth phase, *Bombus terrestris* colonies in apple orchards tended to gain more weight when the proportion of cropland in the surrounding landscape was higher, and the proportion of woodland was lower (section 2.4.1.). Although this may seem contradictory at first, given the importance of natural and semi-natural habitats for bee populations (*e.g.* Proesmans et al., 2019; Raderschall et al., 2021), it should be considered that *B. terrestris* are generalist bees with the ability to exploit a wide range of foraging resources, for which mass-flowering crops can provide short bursts of copious nectar and pollen (*e.g.* Westphal et al., 2009). By contrast, specialist bees have much more restricted feeding and nesting requirements, and are not as versatile as generalist species; they tend to be more strongly associated with natural and semi-natural habitats, as they cannot exploit the same wide range of agricultural habitats and flowers as generalist pollinators (*e.g.* Kämper et al., 2016; Kline & Joshi, 2020).

The expansion of agricultural areas, however, is threatening the conservation of natural and semi-natural habitats, and, consequently, many bee species that rely on them for their floral and nesting sources (Kline & Joshi, 2020). The spread of generalist bees to new environments can establish competition mechanisms with wild, native bees, potentially depriving them of foraging resources (*e.g.* Russo et al., 2021), and in some cases can drive to disease spill-overs from managed to wild bee communities (*e.g.* Graystock et al., 2016). The spread of managed bees and their competition with wild bees is a current hotspot of debate; with a systematic literature review, Mallinger et al. (2017) concluded that there is enough evidence to support that such competition is negatively affecting wild bee populations, even though more evidence is required to establish that it is leading to wild bee declines.

Nevertheless, more than 40% of beekeepers who participated to my survey did not believe that the competition between managed and wild bees is contributing to the decline of bee populations (section 4.3.3.). This aligns with the fact that beekeeping practices are often assumed to be sustainable, and they are consequently encouraged even in national parks and other protected areas (*e.g.* Côte Bleue coastal area in France, Parco Nazionale dell'Aspromonte in Italy, Schweizerische Nationalpark in Switzerland) (Torné-Noguera et al., 2016; Henry & Rodet, 2018). Thus, my findings represent a significant concern for biodiversity conservation. In fact, wild bees strongly contribute to the yield of several food crops (*e.g.* Eeraerts et al., 2019), and often deliver even more effective pollination services than the highly versatile honeybee (*e.g.* Garratt et al., 2016). However, the increase of cropland areas at the expense of natural and semi-natural lands, coupled with the increasing dominance of managed bees in some areas, has the potential to negatively impact wild bee health and survival (*e.g.* Aizen et al., 2020; Kline & Joshi, 2020); the lack of beekeepers' acknowledgement of such impact, highlighted by my survey, may exacerbate the issue.

In light of such results, in order to limit the rapid spread of generalist pollinators and protect wild bee communities, natural and semi-natural habitats should be preserved, and high reliance on mass-flowering crops should be reduced (*e.g.* Aizen et al., 2020). Moreover, it is important to increase beekeepers' knowledge of how high abundances of managed bees can affect the survival of local, wild bee populations (section 4.4.1.).

Although beekeepers are reluctant to acknowledge this issue, my survey pointed out that they often rely on beekeeping associations as important sources of information on beehive health (section 4.4.1.). Scientists could harness such reliance and work in close contact with beekeeping associations, to increase knowledge exchange and enable beekeepers to build stronger expertise not only for honeybees, but also for the health of other bee species and how they may be harmed by an excessive density of managed hives. Stronger background knowledge also has the potential of increasing the use of the Bee Health Card, particularly among environmentally conscious beekeepers. Furthermore, research previously surveying farmers showed that higher educational backgrounds or dedication to acquiring knowledge is linked to higher probabilities of

adopting new technologies (Aubert et al., 2012; Barnes et al., 2019; Vecchio et al., 2020), and such findings could be similar for beekeepers. Therefore, leveraging on beekeepers' trust for beekeeping associations may both enable a high exchange and dissemination of knowledge, and favour the use of the Bee Health Card tool to address bee health issues.

5.3.2. Monitoring bee health issues

In addition to concerns related to pesticide exposure, it was clear from the beekeepers' survey that the loss of natural habitats and the spread of diseases were recognised as further pressures threatening the health of bees, in accordance with the evidence in the literature (*e.g.* Havard et al., 2019; Dicks et al., 2021) (section 4.4.2.).

The Bee Health Card may play a crucial role in monitoring such pressures. In particular, in a scenario without economic incentives, environmentally conscious beekeepers, who considered environmental and pollinator protection as potential benefits of the tool, were shown to have higher probabilities of using the Bee Health Card compared to beekeepers that disregarded such benefits (section 4.4.1.). Widespread use of the Bee Health Card could not only potentially reduce beehive losses, but also help monitor pressures on bee populations in the surrounding landscape. In this respect, large-scale pollinator monitoring initiatives, like the EU Pollinator Monitoring Scheme (EU-PoMS), could also be particularly useful in helping detect changes in the status of wild bees and address the major causes linked to their decline (Potts et al., 2021).

Monitoring the health of wild bee communities is challenging, as they are not supported by beekeepers' care. The Bee Health Card may help identify and quantify the nature of the pressures impacting the health of beehives, and as a consequence, this would enable to gather information on the environment and its sustainability for wild bees in the surrounding landscape.

Therefore, the Bee Health Card could keep beehives under control and, at the same time, help protect the wider environment and pollinators by identifying areas with high pesticide loads or poor nutritional resources, and tackling the spread of diseases. Beekeepers will be able to promptly intervene, allowing both managed and wild bee populations to benefit from the use of the tool. For example, if the tool detects pesticide residues in beehives, it indicates that wild bees foraging in the same areas may also potentially be exposed. Considering that exposure routes differ depending on several factors, such as feeding and nesting behaviours, wild bee species may even be more impacted than honeybees by pesticide use (Sgolastra et al., 2019). Furthermore, beehives that are weakened due to pesticides have higher risks of developing diseases (*e.g.* Pettis et al., 2013), which can even be transmitted to wild bee populations (*e.g.* Fürst et al., 2014; Goulson et al., 2018). By enabling beekeepers to tackle pests and diseases more quickly, the Bee Health Card could support wider pollinator health through reducing their spill-over to wild bees.

5.4. Implications for future research

Overall, my studies contributed to filling some important knowledge gaps on how different pressures may act and interact to affect the health of bees. However, many other questions remain, and here I present some suggestions that may be considered for future research.

5.4.1. Target species

The focus of this thesis was primarily on two social bees, *A. mellifera* and *B. terrestris*. *A. mellifera* is often used as a model species to assess pesticide effects on bees, and studies investigating non-*Apis* bees are mostly based on bumblebees (Siviter et al., 2021c). However, the great majority of bee species are solitary, and only 6% show social behaviours (Engel et al., 2020). Therefore, neither honeybees nor bumblebees are necessarily representative of most bee species (Siviter et al., 2021c). Particularly, research assessing the impact of land cover and pesticides on bumblebee behaviour and colony growth and fitness generally focusses on *B. terrestris* (Europe) or *B. impatiens* (North America) (*e.g.* Rundlöf et al., 2015; Milano et al., 2019; Siviter et al., 2019), which are both widespread, generalist pollinators. Therefore, I suggest exploring pressures on wild, specialist bees, including non-generalist bumblebees and solitary bees, contributing to filling further knowledge gaps in the literature.

5.4.2. Pesticide pressures

Using Pesticide Pressure Indexes for my large-scale field study was a novel approach to estimate the effect of a combination of different active ingredients on bees. However, I did not have the opportunity to calculate indexes for croplands surrounding target fields, which would have provided further information on the level of exposure that sentinel bees were subject to. It would therefore be useful to consider retrieving such data and generate a second index, including pesticide pressures of all croplands in a short radius. This information could also be fed into models such as the 'BEEHAVE', simulating the health status of bee colonies in mapped landscapes (Becher et al., 2014, 2018), to obtain an even more accurate prediction of the effect of different pressures on bee health.

In contrast to the large-scale fieldwork, the design of the flight-cage experiment was based on only one insecticide, and how it affected bumblebees. Although I found no evidence of a sulfoxaflor impact on bee health, in accordance with Siviter et al. (2019), no interaction with other pesticide products was investigated. Although very few studies have so far covered the effects of sulfoxaflor on bee health (*e.g.* Siviter et al., 2019; Azpiazu et al., 2021; Tamburini et al., 2021), one demonstrated that sulfoxaflor interacted with the fungicide fluxapyroxad, increasing the mortality of both *A. mellifera* and *O. bicornis* (Azpiazu et al., 2021). Thus, I suggest further work on the synergistic interactions with other products, considering a typical farmland, multi-agrochemical scenario in which pesticides are used in combination (Siviter et al., 2021a).

In April 2022, the EU announced that sulfoxaflor will be banned for outdoor use due to the inability to demonstrate its safety for bumblebees and solitary bees, and its use will be restricted to greenhouses only (EU, 2022). However, my study has contributed to showing that field-realistic doses of sulfoxaflor did not

affect the behaviour and pollination of *B. terrestris* in flight cages, and further research is needed to assess its potential effects particularly on solitary bees and other bumblebee species.

My study was also the first to assess the interaction between sulfoxaflor and the pathogen *C. bombi*, showing no significant impact of such combination. However, as synergistic effects between diseases and pesticides may be very specific (*e.g.* Di Prisco et al., 2013), I cannot exclude possible interactions between *C. bombi* and pesticide formulations with sulfoxaflor as an active ingredient. Hence, it would be useful to investigate other pesticide-pathogen combinations involving entire formulations, as it is expected that sulfoxaflor will be applied together with other co-formulants and pesticides in the fields (Siviter et al., 2021a).

5.4.3. Semi-natural habitats

In the large-scale study, woodlands surrounding target fields were utilised to represent the proportion of semi-natural habitats in the landscape, while grassland had to be excluded as it was not possible to separate high-input (*e.g.* intensive pasture) from low-input lands (chalk grassland) using landscape maps. Such distinction is necessary, since high-input grassland utilises intensification practices, like a high use of fertilisers and defoliation, that may reduce floral resources and nesting habitats (*e.g.* Potts et al., 2009), and should not be considered part of semi-natural habitats.

Therefore, in addition to woodland, future research should account for non-intensively managed grassland in defining the proportion of semi-natural habitats, as it provides high-quality foraging and nesting resources to bees (*e.g.* Potts et al., 2009; Bartholomée et al., 2020).

5.4.4. Pollinator abundance and richness

My study used pollinator transect surveys to investigate how pesticides and land cover affected the abundance of honeybees and bumblebees. In addition to assessing taxa abundance, future research should also focus on species richness, so as to account for potential differences between species belonging to the same taxa (*e.g.* Le Féon et al., 2010; Kennedy et al., 2013; Park et al., 2015).

I also suggest integrating additional information on flower abundance along the transects, coupled with bee visitation rates, to produce a more balanced dataset; in fact, floral resource availability may influence bee abundance and visitation, and it is therefore an important information to capture (*e.g.* Lopezaraiza-Mikel et al., 2007; Carvalheiro et al., 2010). For example, Lopezaraiza-Mikel et al. (2007) registered bee visitations made to floral units, and this could be a valid approach to standardise floral abundance across surveyed areas.

Further data on the distance between the target site and natural and semi-natural areas could also be included, without limiting the information to the proportion of such areas in the surroundings; in fact, the distance from natural and semi-natural habitats can be capable of affecting bee abundance and richness, and could therefore be an important variable to measure (*e.g.* Bartholomée et al., 2020).

5.4.5. Parasite loads

When investigating the influence of different pressures on *Varroa* mite loads, it would be important to consider if and when beehives were previously treated for *Varroa* and what treatments were applied. In fact, the use of such products might be capable to buffer pesticide or land cover effects on beehive diseases; anti-*Varroa* treatments were found to be particularly efficacious when applied in the second half of the year, and less efficacious when applied earlier (Leza et al., 2016). Moreover, the type of treatment should be considered; for instance, organic products have been observed to be more effective than synthetic ones (Leza et al., 2015).

Additionally, synergistic effects between parasites and pesticides may recur for specific combinations, but not be observed for others (*e.g.* Di Prisco et al., 2013). Investigating the impact on *Varroa* mites of two comprehensive pesticide indexes may buffer interaction effects between specific insecticides, herbicides, or fungicides; I therefore suggest to explore interactions between *Varroa destructor* and specific substances, so as to further inform on possible immune responses of bees to certain pesticide combinations.

5.4.6. The Bee Health Card as demonstrable output

As previously described, the Bee Health Card may be useful in detecting optimal and sub-optimal environments, benefitting both managed and wild bee communities. My analyses underline that maximising the confidence level in the Bee Health Card effectiveness is key to guaranteeing its widespread and frequent use. Although survey respondents demonstrated an overall fair confidence level in its effectiveness, the Bee Health Card is currently still under development (as of May 2022), and beekeepers did not have the opportunity to test it. However, perceiving new technologies as helpful and functional may encourage users to adopt them (*e.g.* Aubert et al., 2012). It is thus suggested to conduct a new investigation when a tool prototype is ready, to offer new insights into beekeepers' perceptions of its effectiveness. A demonstrable output would enable beekeepers to fully understand its benefits and barriers, to detect particular aspects that could be refined, and to provide more practical advices on how to improve its functionalities. Furthermore, directly testing the Bee Health Card would give beekeepers better understanding of what costs are to be expected; in fact, the uncertainty on economic returns can be responsible for the hesitancy of investing in new technologies (*e.g.* Vecchio et al., 2020), and addressing such concerns will enable final users to understand whether this new tool will entail the need of further investments in both time and money, and whether it will be worth it.

Given the trust beekeepers place in beekeeping associations, such associations should be used as points of contact by scientists to distribute Bee Health Card prototypes and enable beekeepers to directly experiment its functionalities. Beekeeping associations could play a key role in strengthening beekeepers' background knowledge on the importance of the Bee Health Card, not only for managed honeybees, but also for the surrounding environment and wild bee communities populating it. Such associations could report to

scientists collective thoughts on what are the advantages and difficulties experienced after a certain period of testing the prototype, and this information should be used by PoshBee to refine and finalise the tool. This would also enable researchers to compare beekeepers' perspectives before and after the engagement of beekeeping associations, and further understand the role they play in increasing knowledge exchange and favouring the use of new technologies (*e.g.* see Caffaro et al., 2020).

5.4.7. Survey sample

Due to the limited availability of financial resources, the survey sample of beekeepers had to be recruited through social media channels, leading to differences in survey distributions among countries; in fact, the sample was mostly made of beekeepers recruited from national associations with a hobby focus (*e.g.* BBKA in UK, FBKA in Ireland, see Appendix 4.1), who were likely to be biased due to their interest in bee health and biodiversity conservation. Italy was the only country which counted way more professional than hobbyist respondents, due to the fact that they were recruited through UNAAPI, the national beekeeping union incorporating three different professional beekeeping associations (see www.unaapi.it and Appendix 4.3). As such, recruited beekeepers may not be fully representative of all national beekeepers. In fact, getting a truly representative sample of beekeepers in each country is challenging, particularly where apiary registration is entirely voluntary (*e.g.* UK), or extended only to certain categories of beekeepers (*e.g.* those who sell honey in Ireland) (Chauzat et al., 2013).

Further research should also target associations with a specific focus on professional beekeepers, who could offer new insights into the practicalities of a broad use of the Bee Health Card. For example, member associations of the European Professional Beekeepers Association (EPBA) could represent a good target to ensure both a wide survey distribution and a professional perspective towards the use of a new technology (http://www.professional-beekeepers.eu/).

When the Bee Health Card is finalised and ready to be tested, it is therefore suggested to distribute another survey to a balanced dataset of (i) hobbyist and (ii) professional beekeepers to investigate both their perceptions toward the tool, and what potential issues and concerns need to be addressed in relation to the type of activity they engage with. A further survey could also be distributed to growers of insect-pollinated crops to explore the factors that may encourage or discourage the use of the tool, and to investigate their interests in hiring Bee Health Card monitored beehives. Knowing both beekeepers' and growers' perspectives would help identify what factors to tackle in order to maximise the willingness to use the tool, its frequency of use, and its widespread adoption.

5.5. Concluding remarks

The main thesis discussion points and recommendations are summarised as follows:

a. **Pesticide risk assessments should be conducted on multiple bee species and on the entire product formulation**. Pesticide safety needs to be assessed on species other than *A. mellifera*, and it is crucial to provide toxicity information on both active and 'inert' ingredients to detect any possible synergistic interaction between them.

b. **'Organic' does not always equal 'low-input'**. Products with low toxicity may have high pressures on bees due to their high rates of applications, and organic pesticides are not always less toxic than synthetic ones. Informed choices need to be made when discussing pesticide application schemes, considering both the aspect of low-toxicity and the number of required applications and rates to make the product effective.

c. **Good communication between beekeepers and growers is essential for lowering pesticide pressures on bees and maintaining high farm profits.** Pesticide management decisions should be discussed and agreed upon to minimise any exposure risk and, at the same time, guarantee an efficient delivery of pollination services.

d. **Landscape cover can affect bee species differently, with potential implications for biodiversity conservation.** While generalist pollinators are able to successfully exploit mass-flowering crops, specialist bees have restricted dietary and nesting requirements that are better supported and met by natural and semi-natural lands. Preserving such habitats is needed to avoid detrimental impacts on wild bee communities.

e. **Both managed and wild bee populations may benefit from the Bee Health Card.** It may represent a useful instrument to identify areas with high pesticide loads or poor nutritional sources, and to tackle the spread of diseases, which may be transmitted not only between managed colonies, but also from managed to wild bees.

f. **Beekeepers recognise the benefits of the Bee Health Card for pollinators and the environment.** Environmentally conscious beekeepers may be more willing to adopt such tool when economic incentives are not available, giving a consistent contribution to preventing beehive losses and preserving healthy bee communities in the surrounding landscape.

In conclusion, bees face several pressures including land use change, pesticides, and diseases that may act individually and in combination to affect their health, behaviour, and pollination. Modes of interactions are multiple, and may affect bee species differently. This thesis investigated some of the most common pressures on two managed bee species, and provided valuable information on how they could be prevented and controlled through the use of an innovative tool under development. Future research should be directed towards the impact of such pressures on additional bee species, and a specific focus should be given on the Bee Health Card to explore its functions when it will be ready to use.

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Appendices

Chapter 2

Appendix 2.1

This appendix includes the following parts:

- Part A: List of PoshBee protocols.

- Part B: UK EUNIS habitat codes.

- Part C: Growers' survey to collect information on pesticide pressures and yield.

- Part D: Summary data on APP and OSR sites.

Part A: list of protocols used for the PoshBee experiment

Protocol code	Protocol title
WP1.1.1	Field site selection
WP1.1.2	Site labelling scheme
WP1.1.3	Basic site data
WP1.1.4	Data management plan
WP1.2.1	Guidance for preparation of Apis mellifera colonies for 2019 field site network
WP1.2.2	Sourcing Osmia pupae and nests
WP1.2.3	Obtaining Bombus colonies and nests
WP1.2.4	Hive, nest, colony installation on site
WP1.3.1	Basic site landscape data
WP1.3.2	Complex landscape data
WP1.3.3	Floral survey of target field boundaries
WP1.3.4	Surveys of wild and managed pollinator insects
WP1.3.5	Grower survey
WP1.4.1	Water from puddles
WP1.4.2	Collecting pollen from target crop and from foraging bees
WP1.4.3	Collecting nectar from stomachs of *Apis* and *Bombus* for WP2
WP1.4.4	Collecting bee samples for WP2
WP1.4.5	Collecting beebread / stored pollen samples for WP2
WP1.4.6	Collecting bee wax (*Apis* & *Bombus*)
WP1.4.7	Collecting Royal Jelly for WP2 (*Apis*)
WP1.5.1	Colony strength evaluations
WP1.5.2	*Varroa* mite infestation
WP1.5.3	Small Hive Beetle infestation
WP1.5.4	Presence of *Vespa velutina*
WP1.5.5	Chalkbrood
WP1.5.6	American Foulbrood
WP1.5.7	European Foulbrood
WP1.5.8	Assessment of the presence of Deformed Wing Virus (DWV)
WP1.5.9	Assessment of *Bombus terrestris* colony performance and natural enemies
WP1.5.10	Assessment of *Osmia bicornis* performance and exposure to natural enemies
WP1.6.1	Field collection of bee haemolymph for WP9
WP1.6.2	Collecting bee samples for wing asymmetry, fat bodies, gut microbiota

Part B: EUNIS habitat codes

Habitat level	EUNIS habitat code	EUNIS habitat name
1	C	Inland surface waters
1	D	Mires, bogs, fens
1	E	Grasslands, lands dominated by forbs, mosses, or lichens
1	F	Heathland, scrub, tundra
1	G	Woodland, forest, other wooded land
1	H	Inland unvegetated/sparsely vegetated habitats
1	I	Regularly or recently cultivated agricultural, horticultural, domestic habitats
1	J	Constructed, industrial, other artificial habitats
2	E1	Dry grasslands
2	E2	Mesic grasslands
2	E3	Seasonally wet and wet grasslands
2	E4	Alpine and subalpine grasslands
2	E5	Woodland fringes, clearings, tall forb stands
2	E6	Inland salt steppes
2	E7	Sparsely wooded grasslands
2	F1	Tundra
2	F2	Arctic, alpine, subalpine scrub
2	F3	Temperate, Mediterranean-montane scrub
2	F4	Temperate shrub heathland
2	F5	Maquis, arborescent matorral, thermo-Mediterranean brushes
2	F6	Garrigue
2	F7	Spiny Mediterranean heaths (phrygana, hedgehog-heaths, related coastal cliff vegetation)
2	F8	Thermo-Atlantic xerophytic scrub
2	F9	Riverine, fen scrubs
2	FA	Hedgerows
2	FB	Shrub plantations
2	G1	Broadleaved deciduous woodland
2	G2	Broadleaved evergreen woodland
2	G3	Coniferous woodland
2	G4	Mixed deciduous, coniferous woodland
2	G5	Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland, coppice
2	I1	Arable land, market gardens
2	I2	Cultivated areas of gardens, parks
2	J1	Buildings of cities, towns, villages
2	J2	Low density buildings
2	J3	Extractive industrial sites
2	J4	Transport networks, other constructed hard-surfaced areas
2	J5	Highly artificial man-made waters, associated structures
2	J6	Waste deposits

Habitat codes assigned to sites

Habitat codes assigned to APP and OSR sites are presented below.

Further legend
NB= no boundary; BB= bare boundary; AP= apples; OSR= oilseed rape; I1.1= cereals/arable crops (excl. OSR); I1.2= horticulture (excl. apples); SN= semi-natural habitat/meadows.

APP sites

Site	Boundary 1	Boundary 2	Boundary 3	Boundary 4	Adjacent field 1	Adjacent field 2	Adjacent field 3	Adjacent field 4
APP 1	FA	FA	NB	FA	J2	AP	AP	J2
APP 2	NB	BB/FA	BB/FA	BB	J2	AP	E2	J2
APP 3	FA	G5	FA	G5	AP	AP	AP	AP
APP 4	FA	FA	FA	FA	SN	I1.2	AP	E2
APP 5	FA	FA	FA	FA	E2/SN	AP	AP	AP

APP 6	G5	FA	FA	FA	AP	E2	E2	AP
APP 7	FA	FA	FA	FA	G4	AP	AP	G4/E2
APP 8	NB/FA	FA	FA	NB	SN	E2	E2	AP

OSR sites

Site	Boundary 1	Boundary 2	Boundary 3	Boundary 4	Adjacent field 1	Adjacent field 2	Adjacent field 3	Adjacent field 4
OSR 1	NB	FA	BB	E5	I1.1	J2	I1.1	E2
OSR 2	G5	FA	FA	G5/BB	I1.1	OSR	SN	OSR/E2
OSR 3	NB	NB	FA	FA	I1.1	I1.1	J2	I1.1
OSR 4	FA	FA	FA	FA	OSR	I1.1	I1.1	E2/SN
OSR 5	FA	FA	NB	BB	I1.1	J2	I1.1	I1.1
OSR 6	FA	NB	E2	FA/E2	I1.1	I1.1	I1.1	I1.1
OSR 7	E5	FA	NB	E5	G4	OSR	I1.1	G4
OSR 8	FA	FA/BB	FA	FA	J2	J2	E2	J2

Part C: Growers' survey

The surveys addressed to APP and OSR growers are presented below. Questions relevant to the chapter, that were therefore used for statistical analyses, are highlighted in bold.

APP growers

Question title	Full question
Farm size hectares	Approximately how large are the following? (in hectares) - Your total farming operation
Area of apples	Approximately how large are the following? (in hectares) - The area of apples you are growing this year
Area of PoshBee field	**Approximately how large are the following? (in hectares) - The area of the field where the PoshBee survey took place**
Main crop variety	Which maincrop variety(s) of apple did you grow in the orchard where the PoshBee experiment took place?
Polliniser variety	Which polliniser variety(s) of apple did you grow in the orchard where the PoshBee experiment took place?
Age of orchard	How old (in years) is the orchard where the PoshBee Experiment took place?
Growth regulators	Which (if any) chemical growth regulators (auxins etc.) did you apply to the orchard where the PoshBee experiment took place?
Scheme involvement	**Are you involved in any of the following? (please select all that apply)**
Years of organic farming	Including this year, how many years have you been practicing organic farming in the orchard where the PoshBee experiment took place?
Biological control	This year, did you use any of the following biological control strategies in the orchard where the PoshBee experiment took place? Please tick all that apply
Plant Protection Products	**Since your last harvest, which plant protection products (including herbicides, insecticides, fungicides, soap, copper etc.) did you apply to the orchard where the PoshBee experiment will take place? For each product, please indicate when you apply the product and at what rate are they applied (l/ha).**
Tank Mix	Which, if any, of these plant protection products did you apply to the orchard where the PoshBee experiment took place using a tank mix? Please tick all that apply.
Representative	Finally, is the management in the field where the PoshBee experiment took place representative of how you manage your other apple orchards?
Difference	Please use this space to describe how it is different from how you manage your other orchards (different plant protection products etc.)
Apple Yield	**This year, what was the total yield of the orchard where the PoshBee study took place?**
% Class 1 apples	**This year, what was the total percentage of class one apples in the orchard where the PoshBee study took place?**
Deficits	Do you feel that the yield of the orchard where the PoshBee study took place was lower than it could have been because it did not have enough pollinators?
Level of deficit	How much lower do you think your yields were because of a lack of pollination in the field where the PoshBee survey took place? You may answer in either tonnes/ha or as a percentage of the total yield.

OSR growers

Question title	Full question

Farm Size Hectares	Approximately how large are the following? (in hectares) - Your total farming operation
Area of OSR	Approximately how large are the following? (in hectares) - The area of oilseed rape you are growing this year
Area of PoshBee Field	**Approximately how large are the following? (in hectares) - The area of the field where the PoshBee survey took place**
OSR Variety	Which variety(s) of oilseed rape did you grow in the field where the PoshBee experiment took place?
Rotation 2014	In the field where the PoshBee experiment took place, which crops did you grow in the following years? - 2014
Rotation 2015	In the field where the PoshBee experiment took place, which crops did you grow in the following years? - 2015
Rotation 2016	In the field where the PoshBee experiment took place, which crops did you grow in the following years? - 2016
Rotation 2017	In the field where the PoshBee experiment took place, which crops did you grow in the following years? - 2017
Rotation 2018	In the field where the PoshBee experiment took place, which crops did you grow in the following years? - 2018
Seeding strategy	This year, which of the following describes the seeding strategy did you use in the field where the PoshBee experiment took place?
Seed treatments	Which (if any) plant protection products were these seeds treated with?
Certification	**Are you involved in any of the following? (please select all that apply)**
Years of organic farming	Including this year, how many years have you been practicing organic farming in the field where the PoshBee experiment took place?
Biological control strategies	This year, did you use any of the following biological control strategies in the field where the PoshBee experiment took place? Please tick all that apply
Plant Protection Products	**Following seeding this year, which plant protection products (including herbicides, insecticides, fungicides, soap, copper etc.) did you apply to the field where the PoshBee experiment will take place? For each product, please indicate when you apply the product and at what rate are they applied (l/ha)**
Tank Mix	Which, if any, of these plant protection products did you apply to the field where the PoshBee experiment took place using a tank mix? Please tick all that apply.
Representative	Finally, is the management in the field where the PoshBee experiment took place representative of how you manage your other oilseed fields?
Difference	Please use this space to describe how it is different from how you manage your other fields (different plant protection products, seeding strategies etc.)
Oilseed rape Yield	**This year, what was the total yield of the field where the PoshBee study took place?**
Oil content	**This year, what was the total percentage oil content of the oilseed in the field where the PoshBee study took place?**
Deficits	Do you feel that the yield of the field where the PoshBee study took place was lower than it could have been because it did not have enough pollinators?
Level of deficit	How much lower do you think your yields were because of a lack of pollination in the field where the PoshBee survey took place? You may answer in either tonnes/ha or as a percentage of the total yield.

Part D: Summary data on APP and OSR sites

The proportion of grassland in 1 km radius, including intensively and non-intensively managed, is displayed below.

Site ID	Proportion of grassland	Site ID	Proportion of grassland
APP 01	0.25	OSR 01	0.25
APP 02	0.21	OSR 02	0.29
APP 03	0.24	OSR 03	0.13
APP 04	0.24	OSR 04	0.13
APP 05	0.27	OSR 05	0.19
APP 06	0.34	OSR 06	0.10
APP 07	0.36	OSR 07	0.34
APP 08	0.39	OSR 08	0.30

Appendix 2.2

This appendix is divided into 2 parts:

- Part A: Global models.
- Part B: Pearson product-moment test for correlations.
- Part C: Goodness-of-fit Chi-square test for Poisson distributions.

Part A: Global models

Global models to investigate response variables in APP and OSR sites are presented, together with model type and distribution used. Given the high number of correlations between variables, multiple models have been built.

APP sites

Response variable	Random term	Fixed terms	Model	Data distribution
Δweight 1-2 and Δweight 2-3	Site and colony	Model1: IPI Model2: OPPI + woodland Model3: cropland	LMM	Normal
% workers, reproductives, males and new queens in reproductives	Site and colony	Model1: IPI Model2: OPPI + woodland Model3: cropland	LMM	Normal
Social bee activity averaged across three time points	Site	Model1: transect + temp + time + IPI Model2: transect + woodland + OPPI Model3: transect + time + woodland Model4: transect + temp + cropland	LMM	Normal
Social bee activity after flowering	Site	Model1: transect + temp + time + IPI Model2: transect + temp + time + cropland Model3: transect + temp + time + OPPI + cropland	GLMM	Poisson
Varroa mites	Site and hive	Model1: IPI Model2: OPPI Model3: cropland Model4: woodland	GLMM	Quasi-Poisson
Yield and % class 1 apples	Site	Model1: IPI + cropland Model2: OPPI + cropland Model3: IPI + woodland Model4: OPPI + woodland	LMM	Normal

OSR sites

Response variable	Random term	Fixed terms	Model	Data distribution
Δweight 1-2 and Δweight 2-3	Site and colony	Model1: IPI + OPPI + IPI.OPPI + cropland Model2: IPI + OPPI + IPI.OPPI + woodland	LMM	Normal
% workers, reproductives, males and new queens in reproductives	Site and colony	Model1: IPI + OPPI + IPI.OPPI + cropland Model2: IPI + OPPI + IPI.OPPI + woodland	LMM	Normal
Social bee activity averaged across three time points	Site	Model1: transect + IPI + OPPI + IPI.OPPI + cropland Model2: transect + IPI + OPPI + IPI.OPPI + woodland Model3: transect + time + cropland Model4: transect + time + woodland	LMM	Normal
Social bee activity at the end of flowering	Site	Model1: transect + temp + time + IPI + cropland Model2: transect + temp + time + IPI + woodland Model3: transect + IPI + OPPI + IPI.OPPI + cropland Model4: transect + IPI + OPPI + IPI.OPPI + woodland	GLMM	Poisson
Varroa mites	Site and colony	Model1: IPI + OPPI + IPI.OPPI + cropland Model2: IPI + OPPI + IPI.OPPI + woodland	GLMM	Quasi-Poisson
Yield	Site	Model1: IPI + OPPI + IPI.OPPI + cropland	LMM	Normal

	Model2: IPI + OPPI + IPI.OPPI + woodland			

Part B: Correlation matrixes

Pearson product-moment tests for correlations between all variables in each analysis are shown below. Moderate and strong significant correlations (correlation coefficient≥0.30 and p<0.05) are highlighted in bold.

APP sites

Bumblebee colony weight and fitness		IPI	OPPI	Cropland	Woodland
IPI	Correl. coeff.	-	0.7674	0.6989	-0.5115
	p-value	-	**<0.001**	**<0.001**	**0.0178**
OPPI	Correl. coeff.	0.7674	-	0.5063	-0.2815
	p-value	**<0.001**	-	**0.0192**	0.2164
Cropland	Correl. coeff.	0.6989	0.5063	-	-0.9161
	p-value	**<0.001**	**0.0192**	-	**<0.001**
Woodland	Correl. coeff.	-0.5115	-0.2815	-0.9161	-
	p-value	**0.0178**	0.2164	**<0.001**	-

Social bee activity averaged across three time points		IPI	OPPI	Cropland	Woodland	Transect	Temperature	Time
IPI	Correl. coeff.	-	0.7724	0.6974	-0.5271	0.0000	0.1733	0.4185
	p-value	-	**<0.001**	**0.0027**	**0.0359**	1.0000	0.5210	0.1067
OPPI	Correl. coeff.	0.7724	-	0.5445	-0.3280	0.0000	0.2135	0.5359
	p-value	**<0.001**	-	**0.0292**	0.2149	1.0000	0.4273	**0.0324**
Cropland	Correl. coeff.	0.6974	0.5445	-	-0.9202	0.0000	-0.2533	0.4694
	p-value	**0.0027**	**0.0292**	-	**<0.001**	1.0000	0.3438	0.0666
Woodland	Correl. coeff.	-0.5271	-0.3280	-0.9202	-	0.0000	0.5035	-0.3741
	p-value	**0.0359**	0.2149	**<0.001**	-	1.0000	**0.0468**	0.1535
Transect	Correl. coeff.	0.0000	0.0000	0.0000	0.0000	-	0.0000	0.0000
	p-value	1.0000	1.0000	1.0000	1.0000	-	1.0000	1.0000
Temperature	Correl. coeff.	0.1733	0.2135	-0.2533	0.5035	0.0000	-	-0.2945
	p-value	0.5210	0.4273	0.3438	**0.0468**	1.0000	-	0.2682
Time	Correl. coeff.	0.4185	0.5359	0.4694	-0.3741	0.0000	-0.2945	-
	p-value	0.1067	**0.0324**	0.0666	0.1535	1.0000	0.2682	-

Social bee activity at the end of flowering		IPI	OPPI	Cropland	Woodland	Transect	Temperature	Time
IPI	Correl. coeff.	-	0.7724	0.6974	-0.5271	0.0000	0.2267	-0.1982
	p-value	-	**<0.001**	**0.0027**	**0.0359**	1.0000	0.3984	0.4617
OPPI	Correl. coeff.	0.7724	-	0.5445	-0.3280	0.0000	0.0830	0.0426
	p-value	**<0.001**	-	**0.0292**	0.2149	1.0000	0.7600	0.8754
Cropland	Correl. coeff.	0.6974	0.5445	-	-0.9202	0.0000	0.4735	-0.2657
	p-value	**0.0027**	**0.0292**	-	**<0.001**	1.0000	0.0640	0.3199
Woodland	Correl. coeff.	-0.5271	-0.3280	-0.9202	-	0.0000	-0.2053	0.4614
	p-value	**0.0359**	0.2149	**<0.001**	-	1.0000	0.4456	0.3199
Transect	Correl. coeff.	0.0000	0.0000	0.0000	0.0000	-	0.0000	0.0000
	p-value	1.0000	1.0000	1.0000	1.0000	-	1.0000	1.0000
Temperature	Correl. coeff.	0.2267	0.0830	0.4735	-0.2053	0.0000	-	0.3371
	p-value	0.3984	0.7600	0.0640	0.4456	1.0000	-	0.2017
Time	Correl. coeff.	-0.1982	0.0426	-0.2657	0.4614	0.0000	0.3371	-
	p-value	0.4617	0.8754	0.3199	0.3199	1.0000	0.2017	-

***Varroa* mites**		IPI	OPPI	Cropland	Woodland
IPI	Correl. coeff.	-	0.9998	0.7136	-0.5814
	p-value	-	**<0.001**	**<0.001**	**0.0114**
OPPI	Correl. coeff.	0.9998	-	0.7210	-0.5893
	p-value	**<0.001**	-	**<0.001**	**0.0101**

Cropland	Correl. coeff.	0.7136	0.7210	-	-0.9490
	p-value	**<0.001**	**<0.001**	-	**<0.001**
Woodland	Correl. coeff.	-0.5814	-0.5893	-0.9490	-
	p-value	**0.0114**	**0.0101**	**<0.001**	-

Yield and class 1 apples		IPI	OPPI	Cropland	Woodland
IPI	Correl. coeff.	-	0.7724	0.6974	-0.5271
	p-value	-	0.0247	0.0545	0.1795
OPPI	Correl. coeff.	0.7724	-	0.5445	-0.3280
	p-value	0.0247	-	0.1629	0.4277
Cropland	Correl. coeff.	0.6974	0.5445	-	-0.9203
	p-value	0.0545	0.1629	-	**0.0012**
Woodland	Correl. coeff.	-0.5271	-0.3280	-0.9203	-
	p-value	0.1795	0.4277	**0.0012**	-

OSR sites

Bumblebee colony weight and fitness		IPI	OPPI	Cropland	Woodland
IPI	Correl. coeff.	-	0.1756	-0.4226	0.1931
	p-value	-	0.4463	0.0563	0.4018
OPPI	Correl. coeff.	0.1756	-	-0.3244	0.1544
	p-value	0.4463	-	0.1514	0.5041
Cropland	Correl. coeff.	-0.4226	-0.3244	-	-0.9525
	p-value	0.0563	0.1514	-	**<0.001**
Woodland	Correl. coeff.	0.1931	0.1544	-0.9525	-
	p-value	0.4018	0.5041	**<0.001**	-

Social bee activity averaged across three time points		IPI	OPPI	Cropland	Woodland	Transect	Temperature	Time
IPI	Correl. coeff.	-	0.1756	-0.4226	0.1931	0.0000	0.0982	-0.6170
	p-value	-	0.5481	0.1322	0.5085	1.0000	0.7383	**0.0188**
OPPI	Correl. coeff.	0.1756	-	-0.3244	0.1544	0.0000	-0.7056	-0.7409
	p-value	0.5481	-	0.2579	0.5983	1.0000	**0.0048**	**0.0024**
Cropland	Correl. coeff.	-0.4226	-0.3244	-	-0.9295	0.0000	0.6228	0.2748
	p-value	0.1322	0.2579	-	**<0.001**	1.0000	**0.0131**	0.3215
Woodland	Correl. coeff.	0.1931	0.1544	-0.9295	-	0.0000	-0.4632	0.0327
	p-value	0.5085	0.5983	**<0.001**	-	1.0000	0.0821	0.9080
Transect	Correl. coeff.	0.0000	0.0000	0.0000	0.0000	-	0.0000	0.0000
	p-value	1.0000	1.0000	1.0000	1.0000	-	1.0000	1.0000
Temperature	Correl. coeff.	0.0982	-0.7056	0.6228	-0.4632	0.0000	-	0.6430
	p-value	0.7383	**0.0048**	**0.0131**	0.0821	1.0000	-	**0.0097**
Time	Correl. coeff.	-0.6170	-0.7409	0.2748	0.0327	0.0000	0.6430	-
	p-value	**0.0188**	**0.0024**	0.3215	0.9080	1.0000	**0.0097**	-

Social bee activity at the end of flowering		IPI	OPPI	Cropland	Woodland	Transect	Temperature	Time
IPI	Correl. coeff.	-	0.1756	-0.4226	0.1931	0.0000	0.2108	-0.4526
	p-value	-	0.5481	0.1322	0.5085	1.0000	0.4695	0.1042
OPPI	Correl. coeff.	0.1756	-	-0.3244	0.1544	0.0000	-0.6692	-0.7861
	p-value	0.5481	-	0.1322	0.5983	1.0000	**0.0089**	**<0.001**
Cropland	Correl. coeff.	-0.4226	-0.3244	-	-0.9305	0.0000	0.0232	0.2497
	p-value	0.1322	0.1322	-	**<0.001**	1.0000	0.9320	0.3510
Woodland	Correl. coeff.	0.1931	0.1544	-0.9305	-	0.0000	0.1489	0.0139
	p-value	0.5085	0.5983	**<0.001**	-	1.0000	0.5821	0.9592
Transect	Correl. coeff.	0.0000	0.0000	0.0000	0.0000	-	0.0000	0.0000
	p-value	1.0000	1.0000	1.0000	1.0000	-	1.0000	1.0000
Temperature	Correl. coeff.	0.2108	-0.6692	0.0232	0.1489	0.0000	-	0.6015
	p-value	0.4695	**0.0089**	0.9320	0.5821	1.0000	-	**0.0137**
Time	Correl. coeff.	-0.4526	-0.7861	0.2497	0.0139	0.0000	0.6015	-

p-value	0.1042	**<0.001**	0.3510	0.9592	1.0000	**0.0137**	-

Varroa mites		IPI	OPPI	Cropland	Woodland
IPI	*Correl. coeff.*	-	0.1794	-0.2883	-0.1765
	p-value	-	0.5223	0.2974	0.5291
OPPI	*Correl. coeff.*	0.1794	-	-0.4516	0.1703
	p-value	0.5223	-	0.0910	0.5440
Cropland	*Correl. coeff.*	-0.2883	-0.4516	-	-0.8555
	p-value	0.2974	0.0910	-	**<0.001**
Woodland	*Correl. coeff.*	-0.1765	0.1703	-0.8555	-
	p-value	0.5291	0.5440	**<0.001**	-

Yield		IPI	OPPI	Cropland	Woodland
IPI	*Correl. coeff.*	-	0.1756	-0.4226	0.1931
	p-value	-	0.7064	0.3448	0.6783
OPPI	*Correl. coeff.*	0.1756	-	-0.3244	0.1544
	p-value	0.7064	-	0.4779	0.7411
Cropland	*Correl. coeff.*	-0.4226	-0.3244	-	-0.9525
	p-value	0.3448	0.4779	-	**<0.001**
Woodland	*Correl. coeff.*	0.1931	0.1544	-0.9525	-
	p-value	0.6783	0.7411	**<0.001**	-

Part C: Goodness-of-fit tests for Poisson distribution

Below are presented the goodness-of-fit Chi-square tests for observed vs. expected counts (method: Maximum Likelihood) used to assess the distribution of count data. P-values < 0.05 are highlighted in bold and indicate that data do not follow a Poisson distribution, therefore a Quasi-Poisson distribution was adopted in corresponding GLMMs.

APP sites

Count data	r	N observed	N expected	Likelihood Chi-square	df	*p*-value
Honeybee activity at the end of flowering	0-2	10	8.33	0.71	1	0.400
	2-4+	6	7.67			
Bumblebee activity at the end of flowering	0-2	5	4.53	0.18	2	0.912
	2-4+	8	7.38			
Varroa destructor mites	0	6	0.31	29.98	1	**<0.001**
	1-5	4	10.79			

OSR sites

Count data	r	N observed	N expected	Likelihood Chi-square	df	*p*-value
Honeybee activity at the end of flowering	0-5	10	8.78	0.38	1	0.537
	5+	6	7.22			
Bumblebee activity at the end of flowering	0-2	6	5.24	0.16	1	0.690
	2-3+	10	10.75			
Varroa destructor mites	0-5	5	1.94	4.22	1	**0.040**
	5-8	4	5.85			
	8+	6	7.21			

Appendix 2.3

This appendix includes the following parts:

- Part A: Model selection using AICc and ΔAICc.

- Part B: List of selected models with ID codes.

- Part C: Table of effects of final models.

- Part D: Additional figures showing significant effects of temperature and transect not included in the chapter.

Part A: Model selection

Model selection tables below show candidate models for each analysis with significant parameters, from the lowest to the highest AIC. Models in bold represent the final, selected models with the lowest AICc and ΔAICc≤2, where ΔAICc is given by the difference between the candidate model and the model with the lowest AICc. AICc and ΔAICc values are not given for global models with a single parameter or no significant terms since no selection criterion was applied.

APP

Δweight 1-2. 'Site and colony' was used as random term. Same models were used for Δweight 2-3 (no significant terms).			
Global models	**Fixed effects**	**AICc**	**ΔAICc**
Model 1: IPI	**IPI**	-	-
Model 2: OPPI + woodland	**Woodland**	**-34.89**	**0**
	OPPI + woodland	-20.82	14.07
Model 3: cropland	**Cropland**	-	-

Bumblebee activity averaged across three time points. 'Site' was used as random term. Same models were used for honeybee activity (no significant terms).			
Global models	**Fixed effects**	**AICc**	**ΔAICc**
Model 1: transect + temp + time + IPI	**Transect + time**	**56.08**	**0**
	Transect	58.45	2.37
	Transect + time + IPI	75.58	19.05
	Transect + temp + time + IPI	84.47	28.39
Model 2: transect + woodland + OPPI	**Transect + woodland**	**56.22**	**0**
	Transect	58.45	2.23
	Transect + woodland + OPPI	68.57	12.35
Model 3: transect + time + woodland	**Transect + time + woodland**	**55.91**	**0**
	Transect + woodland	**56.22**	**0.31**
	Transect	58.45	2.54
Model 4: transect + temp + cropland	**Transect + cropland**	**57.60**	**0**
	Transect	**58.45**	**0.85**
	Transect + temp + cropland	65.32	7.72

Honeybee activity at the end of flowering. 'Site' was used as random term. Same models were used for bumblebee activity (no significant terms).			
Global models	**Fixed effects**	**AICc**	**ΔAICc**
Model 1: transect + temp + time + OPPI + woodland	**Woodland**	**39.04**	**0**
	OPPI + woodland	59.12	20.08
	Transect + OPPI + woodland	62.29	23.25
	Transect + time + OPPI + woodland	62.98	23.94
	Transect + temp + time + OPPI + woodland	69.17	30.13

Model 2: transect + temp + time + IPI	Transect + temp + time + IPI	-	-
Model 3: transect + temp + time + cropland	Transect + temp + time + cropland		

OSR

Δweight 1-2. 'Site and colony' was used as random term. Same models were used for Δweight 2-3 (no significant terms).			
Global models	**Fixed effects**	**AICc**	**ΔAICc**
Model 1: IPI + OPPI + IPI.OPPI + cropland	**OPPI**	**3.12**	**0**
	IPI + OPPI	14.17	11.05
	IPI + OPPI + IPI.OPPI	38.87	35.75
	IPI + OPPI + IPI.OPPI + woodland	41.82	38.70
Model 2: IPI + OPPI + IPI.OPPI + woodland	**OPPI**	**3.12**	**0**
	IPI + OPPI	14.17	11.05
	IPI + OPPI + IPI.OPPI	38.87	35.75
	IPI + OPPI + IPI.OPPI + cropland	44.01	40.89

Honeybee activity averaged across three time points. 'Site and colony' was used as random term. Same models were used for bumblebee activity (no significant terms).			
Global models	**Fixed effects**	**AICc**	**ΔAICc**
Model 1: transect + time + woodland	**Transect + time + woodland**	**63.54**	**0**
	Transect + time	**64.03**	**0.49**
	Transect	68.41	4.87
Model 2: transect + time + cropland	**Transect + time**	**64.03**	**0**
	Transect + time + cropland	**65.62**	**1.59**
	Transect	68.41	4.38
Model 3: transect + temp + IPI + woodland	Transect + temp + IPI + woodland	-	-
Model 4: transect + IPI + OPPI + IPI.OPPI + cropland	Transect + IPI + OPPI + IPI.OPPI + cropland	-	-
Model 5: transect + IPI + OPPI + IPI.OPPI + woodland	Transect + IPI + OPPI + IPI.OPPI + woodland	-	-

Honeybee activity at the end of flowering. 'Site' was used as random term.			
Global models	**Fixed effects**	**AICc**	**ΔAICc**
Model 1: transect + IPI + OPPI + IPI.OPPI + woodland	**OPPI**	**48.12**	**0**
	Transect + OPPI	**49.74**	**1.62**
	Transect + OPPI + woodland	**49.99**	**1.87**
	Transect + IPI + OPPI + IPI.OPPI + woodland	94.45	46.33
Model 2: transect + IPI + OPPI + IPI.OPPI + cropland	**OPPI**	**48.12**	**0**
	Transect + OPPI	**49.74**	**1.62**
	Transect + OPPI + cropland	52.25	4.13
	Transect + IPI + OPPI + IPI.OPPI + cropland	98.23	50.18
Model 3: transect + temp + time + IPI + cropland	**Transect + time**	**32.19**	**0**
	Transect + temp + time	40.87	8.68
	Transect + temp + time + cropland	43.34	11.15
	Transect + temp + time + IPI + cropland	59.84	27.65
Model 4: transect + temp + time + IPI + woodland	**Transect + time**	**33.94**	**0**
	Transect + time + woodland	**34.03**	**0.09**
	Transect + time + IPI + woodland	47.53	13.59
	Transect + temp + time + IPI + woodland	58.12	24.18

Bumblebee activity at the end of flowering. 'Site' was used as random term.			
Global models	**Fixed effects**	**AICc**	**ΔAICc**
Model 1:	**OPPI**	**49.27**	**0**
	Transect + OPPI	**51.15**	**1.88**

transect + IPI + OPPI + IPI.OPPI + woodland	Transect + OPPI + woodland	51.52	2.25
	Transect + IPI + OPPI + IPI.OPPI + woodland	97.20	47.93
Model 2: transect + IPI + OPPI + IPI.OPPI + cropland	**OPPI**	**49.27**	**0**
	Transect + OPPI	**51.15**	**1.88**
	Transect + OPPI + cropland	53.95	4.68
	Transect + IPI + OPPI + IPI.OPPI + cropland	100.01	50.74
Model 3: transect + temp + time + IPI + cropland	**Temp + time**	**39.59**	**0**
	Transect + temp + time	42.99	3.40
	Transect + temp + time + cropland	44.72	5.13
	Transect + temp + time + IPI + cropland	61.31	21.72
Model 4: transect + temp + time + IPI + woodland	**Temp + time**	**39.59**	**0**
	Transect + temp + time	42.99	3.40
	Transect + temp + time + woodland	43.82	4.24
	Transect + temp + time + IPI + woodland	59.31	19.72

Part B: List of final models with ID codes

Final models with corresponding ID codes are shown below. 'NS' = models with only non-significant terms,

'SP' = single parameter models, both of which did not undergo the AICc selection procedure.

Weight change of bumblebee colonies (M1-M9)

Three APP colonies collapsed during the season, therefore they were excluded from the analysis.

APP: Δweight 1-2	
Model ID	**Fixed effects**
M1 (SP, NS)	IPI
M2	Cropland
M3 (SP)	Woodland

APP: Δweight 2-3	
Model ID	**Fixed effects**
M4 (SP, NS)	IPI
M5 (NS)	OPPI + woodland
M6 (SP, NS)	Cropland

OSR: Δweight 1-2	
Model ID	**Fixed effects**
M7	OPPI

OSR: Δweight 2-3	
Model ID	**Fixed effects**
M8 (NS)	IPI + OPPI + IPI.OPPI + cropland
M9 (NS)	IPI + OPPI + IPI.OPPI + woodland

Bumblebee colony fitness (M10-M29)

The 3 collapsed APP colonies were not included in the analysis.

APP: % workers	
Model ID	**Fixed effects**
M10 (SP, NS)	IPI
M11 (NS)	OPPI + woodland
M12 (SP, NS)	cropland

APP: % reproductives (pooled males and new queens)	
Model ID	**Fixed effects**
M13 (SP, NS)	IPI
M14 (NS)	OPPI + woodland
M15 (SP)	cropland

APP: % males in reproductives	
Model ID	**Fixed effects**
M16 (SP, NS)	IPI
M17 (NS)	OPPI + woodland

M18 (SP, NS)	cropland
APP: % new queens in reproductives	
Model ID	**Fixed effects**
M19 (SP, NS)	IPI
M20 (NS)	OPPI + woodland
M21 (SP, NS)	cropland
OSR: % workers	
Model ID	**Fixed effects**
M22 (NS)	IPI + OPPI + IPI.OPPI + cropland
M23 (NS)	IPI + OPPI + IPI.OPPI + woodland
OSR: % reproductives (pooled males and new queens)	
Model ID	**Fixed effects**
M24 (NS)	IPI + OPPI + IPI.OPPI + cropland
M25 (NS)	IPI + OPPI + IPI.OPPI + woodland
OSR: % males in reproductives	
Model ID	**Fixed effects**
M26 (NS)	IPI + OPPI + IPI.OPPI + cropland
M27 (NS)	IPI + OPPI + IPI.OPPI + woodland
OSR: % new queens in reproductives	
Model ID	**Fixed effects**
M28 (NS)	IPI + OPPI + IPI.OPPI + cropland
M29 (NS)	IPI + OPPI + IPI.OPPI + woodland

Social bee activity (M30-M63)

APP: Honeybee activity averaged across three time points	
Model ID	**Fixed effects**
M30 (NS)	Transect + temp + time + IPI
M31 (NS)	Transect + OPPI + woodland
M32 (NS)	Transect + temp + cropland
M33 (NS)	Transect + time + woodland
APP: Bumblebee activity averaged across three time points	
Model ID	**Fixed effects**
M34	Transect
M35	Transect + time
M36	Transect + cropland
M37	Transect + woodland
M38	Transect + time + woodland
APP: Honeybee activity at the end of flowering	
M39 (NS)	Transect + temp + time + IPI
M40 (NS)	Transect + temp + time + cropland
M41	Woodland
APP: Bumblebee activity at the end of flowering	
M42 (NS)	Transect + temp + time + IPI
M43 (NS)	Transect + temp + time + cropland
M44 (NS)	Transect + temp + time + OPPI + woodland
OSR: Honeybee activity averaged across three time points	
Model ID	**Fixed effects**
M45 (NS)	Transect + IPI + OPPI + IPI.OPPI + cropland
M46 (NS)	Transect + IPI + OPPI + IPI.OPPI + woodland
M47 (NS)	Transect + temp + IPI + woodland
M48	Transect + time
M49	Transect + time + cropland
M50	Transect + time + woodland
OSR: Bumblebee activity averaged across three time points	
Model ID	**Fixed effects**
M51 (NS)	Transect + IPI + OPPI + IPI.OPPI + cropland
M52 (NS)	Transect + IPI + OPPI + IPI.OPPI + woodland
M53 (NS)	Transect + temp + IPI + woodland
M54 (NS)	Transect + time + cropland
M55 (NS)	Transect + time + woodland

OSR: Honeybee activity at the end of flowering	
M56	OPPI
M57	Transect + OPPI
M58	Transect + OPPI + woodland
M59	Transect + time
M60	Transect + time + woodland
OSR: Bumblebee activity at the end of flowering	
M61	OPPI
M62	Transect + OPPI
M63	Temp + time

Varroa mite counts (M62-M67)

Due to the lack of information on *Varroa* mite counts – which were not reported by the beekeeper – 6 APP beehives were excluded from the analysis.

APP: Varroa in beehives	
Model ID	**Fixed effects**
M64 (SP, NS)	IPI
M65 (SP, NS)	OPPI
M66 (SP, NS)	Cropland
M67 (SP, NS)	woodland
OSR: Varroa in beehives	
Model ID	**Fixed effects**
M68 (NS)	IPI + OPPI + IPI.OPPI + cropland
M69 (NS)	IPI + OPPI + IPI.OPPI + woodland

Yield and % class 1 apples (M70-M79)

One of the OSR sites had to be excluded from the analysis since no data was provided by the grower.

APP: Yield	
Model ID	**Fixed effects**
M70 (NS)	IPI + cropland
M71 (NS)	IPI + woodland
M72 (NS)	OPPI + cropland
M73 (NS)	OPPI + woodland
APP: % class 1 apples	
Model ID	**Fixed effects**
M74 (NS)	IPI + cropland
M75 (NS)	IPI + woodland
M76 (NS)	OPPI + cropland
M77 (NS)	OPPI + woodland
OSR: yield	
Model ID	**Fixed effects**
M78 (NS)	IPI + OPPI + IPI.OPPI + cropland
M79 (NS)	IPI + OPPI + IPI.OPPI + woodland

Part C: Table of results of final models

Table of effects of final models including estimates, SE, χ^2 values (count data), F values (normal data), df, p-values, and R^2. Significant terms ($p<0.05$) are highlighted in bold.

APP: weight change of bumblebee colonies

Response variable	Model ID	Fixed effects	Estimate	SE	F	ndf, ddf	p-value	R^2
Δweight 1-2	M1	IPI	0.000014	0.000017	0.67	1, 19	0.424	3.39
	M2	**Cropland**	**0.360700**	**0.130570**	**7.63**	**1, 19**	**0.012**	28.66
	M3	**Woodland**	**-0.440700**	**0.132570**	**11.05**	**1, 19**	**0.004**	36.77

Δweight 2-3	M4	IPI	-0.000018	0.000015	1.36	1, 19	0.258	6.67
	M5	OPPI	-0.000064	0.000769	0.17	1, 18	0.683	7.33
		Woodland	0.178300	0.160070	1.25	1, 18	0.278	
	M6	Cropland	-0.136500	0.140300	0.95	1, 19	0.343	4.74

OSR: weight change of bumblebee colonies

Response variable	Model ID	Fixed effects	Estimate	SE	F	ndf, ddf	p-value	R²
Δweight 1-2	M7	**OPPI**	**0.0002375**	**0.0000756**	**9.88**	**1, 19**	**0.005**	34.20
Δweight 2-3	M8	IPI	-0.0028710	0.0025304	0.17	1, 16	0.684	2.87
		OPPI	-0.0000073	0.0000896	0.01	1, 16	0.929	
		IPI.OPPI	0.0000022	0.0000081	0.12	1, 16	0.729	
		Cropland	0.0621600	0.1511030	0.17	1, 16	0.686	
	M9	IPI	-0.0005894	0.0024229	0.17	1, 16	0.685	1.93
		OPPI	-0.0000164	0.0000877	0.01	1, 16	0.929	
		IPI.OPPI	0.0000026	0.0000081	0.12	1, 16	0.730	
		Woodland	-0.0516500	0.0000081	0.01	1, 16	0.908	

APP: bumblebee colony fitness

Response variable	Model ID	Fixed effects	Estimate	SE	F	ndf, ddf	p-value	R²
% workers	M10	IPI	-0.000171	0.000358	0.23	1, 19	0.638	1.188
	M11	OPPI	0.000170	0.000175	0.76	1, 18	0.395	5.195
		Woodland	17.350000	36.306000	0.23	1, 18	0.638	
	M12	Cropland	0.617900	32.288590	0.00	1, 19	0.985	0.002
% reproductives	M13	IPI	0.000163	0.000360	0.21	1, 19	0.655	1.075
	M14	OPPI	-0.000173	0.000175	0.77	1, 18	0.392	5.387
		Woodland	-18.470000	36.447000	0.26	1, 18	0.618	
	M15	Cropland	-0.390900	32.447230	0.00	1, 19	0.991	0.001
% males in reproductives	M16	IPI	0.000169	0.000271	0.39	1, 19	0.541	2.000
	M17	OPPI	0.000010	0.000123	0.43	1, 18	0.519	19.130
		Woodland	-49.970000	25.545000	3.83	1, 18	0.066	
	M18	Cropland	34.070000	23.324000	2.13	1, 19	0.160	10.090
% new queens in reproductives	M19	IPI	-0.000169	0.000271	0.39	1, 19	0.541	2.000
	M20	OPPI	-0.000010	0.000122	0.43	1, 18	0.519	19.130
		Woodland	49.970000	25.545000	3.83	1, 18	0.066	
	M21	Cropland	-34.070000	23.324000	2.13	1, 19	0.160	10.090

OSR: bumblebee colony fitness

Response variable	Model ID	Fixed effects	Estimate	SE	F	ndf, ddf	p-value	R²
% workers	M22	IPI	-0.000342	0.000575	0.00	1, 16	0.972	9.78
		OPPI	0.017570	0.020343	0.00	1, 16	0.989	
		IPI.OPPI	-0.000002	0.000009	1.64	1, 16	0.219	
		Cropland	-10.660000	34.313000	0.10	1, 16	0.760	
	M23	IPI	-0.000317	0.000543	0.00	1, 16	0.972	11.26
		OPPI	0.016970	0.019656	0.00	1, 16	0.989	
		IPI.OPPI	-0.000002	0.000009	1.66	1, 16	0.215	
		Woodland	59.400000	98.307000	0.37	1, 16	0.554	
% reproductives	M24	IPI	0.000330	0.000607	0.00	1, 16	0.964	8.48
		OPPI	-0.016350	0.021486	0.00	1, 16	0.947	
		IPI.OPPI	0.000002	0.000002	1.41	1, 16	0.253	
		Cropland	9.513000	36.241300	0.07	1, 16	0.796	
	M25	IPI	0.000311	0.000574	0.00	1, 16	0.963	9.97
		OPPI	-0.015510	0.020762	0.00	1, 16	0.947	
		IPI.OPPI	0.000002	0.000002	1.43	1, 16	0.249	
		Woodland	-60.080000	103.838000	0.33	1, 16	0.571	
	M26	IPI	-0.005327	0.049279	0.00	1, 16	0.949	1.11

Response variable	Model ID	Fixed effects	Estimate	SE	F	ndf, ddf	p-value	R²
% males in reproductives		OPPI	0.006172	0.017446	0.11	1, 16	0.934	
		IPI.OPPI	-0.000063	0.000157	0.17	1, 16	0.687	
		Cropland	-0.180200	29.426960	0.00	1, 16	0.995	
	M27	IPI	-0.003955	0.046836	0.00	1, 16	0.949	1.63
		OPPI	0.007257	0.016953	0.01	1, 16	0.933	
		IPI.OPPI	-0.000071	0.000157	0.17	1, 16	0.686	
		Woodland	-24.630000	84.785000	0.08	1, 16	0.775	
% new queens in reproductives	M28	IPI	0.005327	0.049279	0.00	1, 16	0.949	1.11
		OPPI	-0.006172	0.017446	0.11	1, 16	0.934	
		IPI.OPPI	0.000063	0.000157	0.17	1, 16	0.687	
		Cropland	0.180200	29.426960	0.00	1, 16	0.995	
	M29	IPI	0.003955	0.046836	0.00	1, 16	0.949	1.63
		OPPI	-0.007257	0.016953	0.01	1, 16	0.933	
		IPI.OPPI	0.000071	0.000157	0.17	1, 16	0.686	
		Woodland	24.630000	84.785000	0.08	1, 16	0.775	

APP: social bee activity

Response variable	Model ID	Fixed effects	Estimate	SE	F	ndf, ddf	p-value	R²
Honeybee activity averaged across three time points	M30	Crop transect	1.0288000	0.9356000	1.35	1, 7.8	0.280	21.07
		Temp	0.2384000	0.2419400	0.32	1, 3.9	0.603	
		Time	11.440000	12.661000	0.20	1, 3.9	0.678	
		IPI	-0.0000676	0.0000655	1.07	1, 3.9	0.362	
	M31	Crop transect	0.9858000	0.8770000	1.07	1, 12	0.320	41.88
		OPPI	0.0000335	0.0000184	4.27	1, 12	0.094	
		Woodland	9.6480000	0.8770000	3.30	1, 12	0.061	
	M32	Crop transect	1.1416000	0.9311000	1.28	1, 7.8	0.292	20.81
		Temp	0.0588700	0.2034000	0.39	1, 5	0.558	
		Cropland	-5.4060000	4.4384000	1.48	1, 5	0.277	
	M33	Crop transect	1.0716000	0.9136000	1.10	1, 8.4	0.324	32.46
		Time	9.5970000	8.5941000	0.12	1, 4.9	0.741	
		Woodland	9.2230000	4.3245000	4.55	1, 4.9	0.087	
Bumblebee activity averaged across three time points	M34	**Crop transect**	**1.1897000**	**0.5103000**	**5.44**	**1, 8.1**	**0.048**	27.97
	M35	Crop transect	1.1693000	0.5082000	5.62	1, 8.2	0.044	36.16
		Time	6.4620000	4.8901000	1.75	1, 5.9	0.235	
	M36	**Crop transect**	**1.1321000**	**0.5094000**	**5.64**	**1, 8.1**	**0.044**	36.51
		Cropland	2.9810000	2.2024000				
	M37	**Crop transect**	**1.1543000**	**0.5040000**	**6.04**	**1, 8.4**	**0.038**	42.32
		Woodland	-4.1560000	2.2220000		1, 6	0.111	
	M38	**Crop transect**	**1.1383000**	**0.5054000**	**5.90**	**1, 8.3**	**0.040**	45.02
		Time	3.9170000	4.9118000	2.00	1, 4.9	0.218	
		Woodland	-3.4260000	2.4713000	1.92	1, 4.9	0.225	

Response variable	Model ID	Fixed effects	Estimate	SE	X²	df	p-value	R²
Honeybee activity at the end of flowering	M39	Crop transect	0.2412000	0.4029000	0.36	1	0.549	37.86
		Temp	-0.1085000	0.3065500	0.06	1	0.807	
		Time	2.2130000	2.4215000	1.27	1	0.259	
		IPI	-0.0002795	0.0003213	0.76	1	0.384	
	M40	Crop transect	0.2412000	0.4029000	0.36	1	0.549	46.00
		Temp	-0.1040000	0.3537400	0.05	1	0.824	
		Time	0.9975000	2.4587800	1.35	1	0.244	
		Cropland	-3.4980000	2.2803000	2.35	1	0.125	
	M41	**Woodland**	**3.9420000**	**1.6887000**	**5.45**	**1**	**0.020**	39.04
Bumblebee activity at the end of flowering	M42	Crop transect	0.4055000	0.3227000	1.58	1	0.209	40.43
		Temp	0.4503000	0.6872300	0.01	1	0.916	
		Time	-7.6530000	6.2373000	1.38	1	0.240	
		IPI	-0.0001720	0.0004877	0.12	1	0.724	
	M43	Crop transect	0.4055000	0.3227000	1.58	1	0.209	49.19
		Temp	-0.0929100	0.7813660	0.02	1	0.895	
		Time	-4.2610000	6.1859000	1.39	1	0.238	
		Cropland	4.5600000	4.9930000	0.83	1	0.361	
	M44	Crop transect	0.4055000	0.3227000	1.58	1	0.209	53.03

			Estimate	SE	F	ndf, ddf	p-value	R²
		Temp	0.0111000	0.8672850	0.01	1	0.903	
		Time	-3.7120000	8.4465000	1.20	1	0.273	
		OPPI	0.0000905	0.0026214	0.00	1	0.972	
		Woodland	-6.2950000	7.4765000	0.88	1	0.347	

OSR: social bee abundance

Response variable	Model ID	Fixed effects	Estimate	SE	F	ndf, ddf	p-value	R²
Honeybee activity averaged across three time points	M45	Crop transect	1.6214000	0.7744000	4.38	1, 8	0.070	61.95
		IPI	0.0004167	0.0048095	0.65	1, 8	0.442	
		OPPI	0.0047180	0.0017026	3.88	1, 8	0.084	
		IPI.OPPI	-0.0000303	0.0000153	3.90	1, 8	0.084	
		Cropland	2.3550000	2.8720000	0.21	1, 8	0.662	
	M46	Crop transect	1.6214000	0.7808000	4.31	1, 8	0.071	61.32
		IPI	-0.0005142	0.0046210	0.86	1, 8	0.380	
		OPPI	0.0045500	0.0016726	3.72	1, 8	0.090	
		IPI.OPPI	-0.0000030	0.0000155	3.78	1, 8	0.088	
		Woodland	-6.0930000	8.3652000	0.00	1, 8	0.071	
	M47	Crop transect	1.6214000	0.8684000	3.49	1, 6	0.111	33.94
		Temp	-0.4587000	0.5340900	0.24	1, 3	0.658	
		IPI	0.0056570	0.0065735	0.74	1, 3	0.453	
		Woodland	-10.9700000	16.4950000	0.16	1, 3	0.719	
	M48	**Crop transect**	**1.7100000**	**0.7117000**	**5.77**	**1, 13**	**0.032**	64.03
		Time	-8.9970000	4.2858000	4.41	1, 13	0.056	
	M49	**Crop transect**	**1.7100000**	**0.7396000**	**5.35**	**1, 12**	**0.039**	44.09
		Time	-9.2510000	4.6475000	4.08	1, 12	0.066	
		Cropland	0.4930000	2.5772900	0.04	1, 12	0.851	
	M50	**Crop transect**	**1.7100000**	**0.7407000**	**5.33**	**1, 12**	**0.040**	63.54
		Time	-8.9940000	4.4613000	4.07	1, 12	0.067	
		Woodland	-0.2486000	7.4374000	0.00	1, 12	0.974	
Bumblebee activity averaged across three time points	M51	Crop transect	0.1900000	0.3635000	0.27	1, 6	0.620	29.43
		IPI	0.0030610	0.0023510	1.99	1, 2	0.294	
		OPPI	-0.0001766	0.0008323	0.40	1, 2	0.594	
		IPI.OPPI	-0.0000017	-0.0000075	0.05	1, 2	0.842	
		Cropland	-0.3476000	1.4039100	0.62	1, 2	0.512	
	M52	Crop transect	0.1900000	0.3635000	0.27	1, 6	0.620	28.53
		IPI	0.0032330	0.0022725	2.41	1, 2	0.260	
		OPPI	-0.0001237	0.0008225	0.33	1, 2	0.626	
		IPI.OPPI	-0.0000019	0.0000076	0.06	1, 2	0.823	
		Woodland	0.2422000	4.1137400	0.12	1, 2	0.766	
	M53	Crop transect	0.1900000	0.3482500	0.30	1, 9	0.599	29.78
		Temp	0.1078000	0.1619900	0.40	1, 9	0.545	
		IPI	0.0028600	0.0019937	2.06	1, 9	0.185	
		Woodland	2.4170000	5.0029000	1.06	1, 9	0.329	
	M54	Transect	0.2912000	0.3307000	0.78	1, 7	0.408	21.32
		Time	-3.7460000	2.4497000	2.47	1, 5	0.177	
		Cropland	0.1091000	1.3584900	0.01	1, 5	0.939	
	M55	Transect	0.2912000	0.3307000	0.78	1, 7	0.408	22.65
		Time	-3.6710000	2.3081000	2.56	1, 5	0.171	
		Woodland	-1.6390000	0.1916000	0.18	1, 5	0.688	

Response variable	Model ID	Fixed effects	Estimate	SE	X²	df	p-value	R²
Honeybee activity at the end of flowering	M56	**OPPI**	**0.0006622**	**0.0003068**	**4.66**	**1**	**0.031**	22.95
	M57	Transect	0.5021000	0.2641000	3.61	1	0.057	27.84
		OPPI	**0.0006622**	**0.0003068**	**4.66**	**1**	**0.031**	
	M58	Transect	0.5021000	0.2642000	3.61	1	0.057	34.62
		OPPI	**0.0007393**	**0.0003486**	**3.92**	**1**	**0.048**	
		Woodland	-2.5380000	3.0065000	0.71	1	0.399	
	M59	**Transect**	**0.5261000**	**0.2472000**	**4.52**	**1**	**0.033**	38.61
		Time	**-2.5480000**	**1.0200000**	**6.24**	**1**	**0.012**	
	M60	**Transect**	**0.5261000**	**0.2474000**	**4.52**	**1**	**0.033**	45.69
		Time	**-2.5760000**	**1.0263000**	**6.19**	**1**	**0.013**	

			Estimate	SE	χ²	df	p-value	R²
		Woodland	-1.2850000	2.3594000	0.30	1	0.586	
Bumblebee activity at the end of flowering	M61	**OPPI**	**0.0007913**	**0.0003821**	**4.29**	**1**	**0.038**	18.26
	M62	Transect	0.5596000	0.3619000	2.39	1	0.122	37.28
		OPPI	**0.0007913**	**0.0003821**	**4.29**	**1**	**0.038**	
	M63	Temp	0.2596000	0.1499000	0.09	1	0.760	48.21
		Time	**-4.7180000**	**1.9768000**	**5.70**	**1**	**0.017**	

APP: *Varroa* mite counts

Response variable	Model ID	Fixed effects	Estimate	SE	χ²	df	p-value	R²
N° *Varroa* mites	M64	IPI	-0.000049	0.000042	1.39	1	0.239	12.77
	M65	OPPI	-0.000025	0.000022	1.27	1	0.278	11.83
	M66	Cropland	-1.074000	3.703500	0.08	1	0.772	0.63
	M67	Woodland	0.721800	5.041340	0.02	1	0.886	0.27

OSR: *Varroa* mite counts

Response variable	Model ID	Fixed effects	Estimate	SE	χ²	df	p-value	R²
N° *Varroa* mites	M68	IPI	-0.002297	0.002403	0.37	1	0.545	26.71
		OPPI	0.001535	0.001067	0.00	1	0.986	
		IPI.OPPI	-0.000013	0.000001	0.08	1	0.781	
		Cropland	4.902000	2.759300	3.16	1	0.076	
	M69	IPI	-0.005331	0.003187	0.37	1	0.545	26.71
		OPPI	0.001638	0.001110	0.00	1	0.986	
		IPI.OPPI	-0.000017	0.000011	0.08	1	0.781	
		Woodland	-19.170000	10.7910000	3.16	1	0.076	

APP: yield and percentage of class 1 apples

Response variable	Model ID	Fixed effects	Estimate	SE	F	ndf, ddf	p-value	R²
Apple yield	M70	IPI	0.000835	0.000812	0.00	1, 5	0.989	29.92
		Cropland	-100.100000	68.530000	2.13	1, 5	0.204	
	M71	IPI	0.000350	0.000765	0.00	1, 5	0.990	12.59
		Woodland	61.030000	71.957000	0.72	1, 5	0.435	
	M72	OPPI	0.000092	0.000347	0.09	1, 5	0.781	16.26
		Cropland	-60.200000	64.014000	0.88	1, 5	0.390	
	M73	OPPI	-0.000018	0.000321	0.08	1, 5	0.789	8.98
		Woodland	42.490000	66.053000	0.41	1, 5	0.548	
% class 1 apples	M74	IPI	-0.000340	0.0002230	1.78	1, 5	0.240	39.17
		Cropland	22.590000	18.817000	1.44	1, 5	0.284	
	M75	IPI	-0.000238	0.000212	1.39	1, 5	0.291	22.42
		Woodland	-4.488000	19.977500	0.05	1, 5	0.831	
	M76	OPPI	-0.000051	0.000109	0.18	1, 5	0.690	4.25
		Cropland	4.198000	20.171400	0.04	1, 5	0.843	
	M77	OPPI	-0.000030	0.000097	0.18	1, 5	0.689	4.76
		Woodland	5.280000	19.911100	0.07	1, 5	0.801	

OSR: yield

Response variable	Model ID	Fixed effects	Estimate	SE	F	ndf, ddf	p-value	R²
OSR yield	M78	IPI	0.001324	0.003003	0.13	1, 2	0.755	82.88
		OPPI	-0.002750	0.001087	1.77	1, 2	0.315	
		IPI.OPPI	0.000021	0.000010	3.31	1, 2	0.210	
		Cropland	-3.113000	1.704700	3.33	1, 2	0.209	
	M79	IPI	0.000111	0.002855	0.16	1, 2	0.732	79.14
		OPPI	-0.002958	0.001011	2.16	1, 2	0.279	
		IPI.OPPI	0.000021	0.000009	4.04	1, 2	0.182	
		Woodland	8.382000	5.436700	2.38	1, 2	0.263	

Part D: Additional figures not included in the chapter

Social bee activity

APP sites
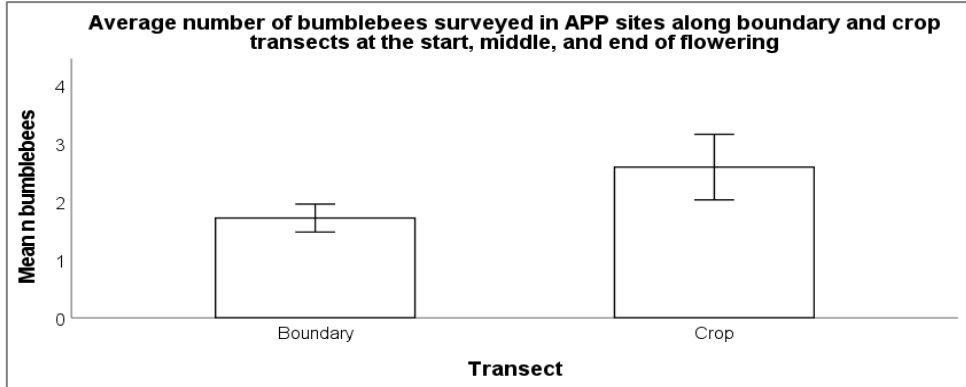
LMMs analysing average data on bumblebee activity in APP sites surveyed at the start, middle, and end of flowering revealed they were more active within crops than on boundaries (range F=5.44-6.04, ndf=1, range ddf=8.1-8.4, range p=0.038-0.040, range R^2=27.97-45.02.). Error bars: ± 1 SE from the mean.

OSR sites
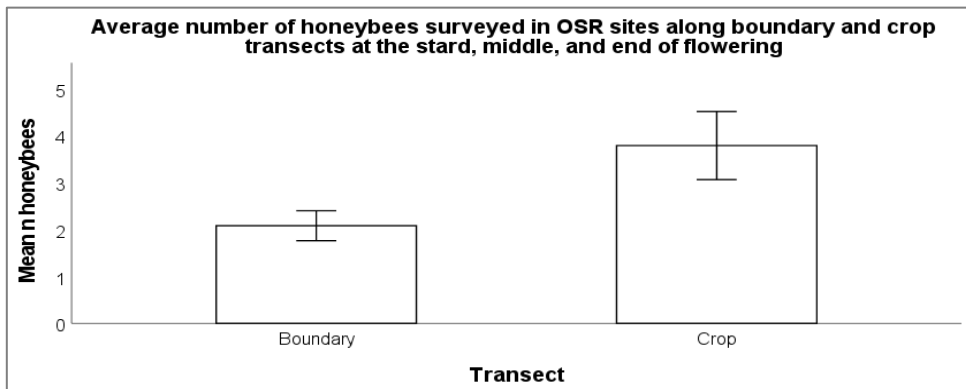
LMMs analysing average data on honeybee activity in OSR sites surveyed at the start, middle, and end of flowering revealed they were more active within crops than on boundaries (range F=5.33-5.77, ndf=1, range ddf=12-13, range p=0.032-0.040, range R^2=43.92-44.09). Error bars: ± 1 SE from the mean.
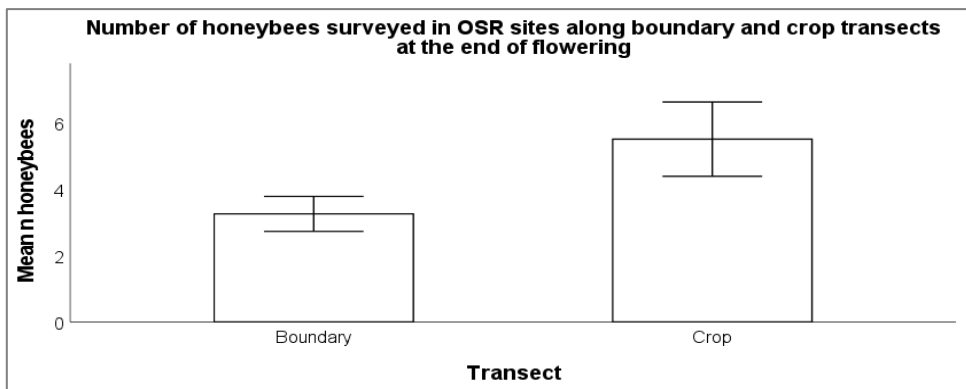
GLMMs on honeybee activity in APP sites surveyed at the end of flowering revealed they were more active within crops than on boundaries (χ^2=4.53, df=1, p=0.033, range R^2=38.61-45.69). Error bars: ± 1 SE from the mean.

Number of honeybees surveyed in OSR sites at the end of flowering and time of day

GLMMs on honeybee activity in OSR sites surveyed at the end of flowering revealed they were more active at earlier times of day (range χ^2=6.19-6.24, df=1, range p=0.012-0.013, range R^2=38.61-45.69). Error bars: ± 1 SE from the mean.

Number of bumblebees surveyed in OSR sites at the end of flowering and time of day
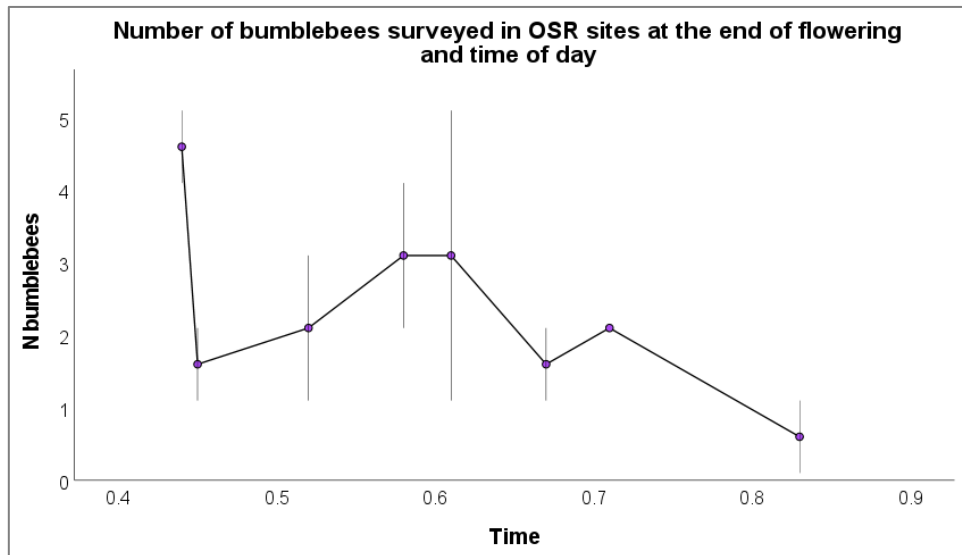
GLMMs on bumblebee activity in OSR sites surveyed at the end of flowering revealed they were more active at earlier times of day (χ^2=5.70, df=1, p=0.017, R^2=48.21). Error bars: ± 1 SE from the mean.

Chapter 3

Appendix 3.1

This appendix includes 3 parts:

- <u>Part A:</u> Protocols designed by RHUL (treatment solutions, colony development requirements).

- <u>Part B:</u> Protocols designed by UREAD (behavioural observations, yield measurements).

- <u>Part C:</u> Timeline summary.

Part A: RHUL protocols

1. Dilution protocol

Alberto Linguadoca

Dilution: sulfoxaflor 50 ppb w/w in 30% sucrose syrup

Calculations to prepare treated syrup with 0.161, 0.047, 0.14, 0.004 mg sulfoxaflor/kg are presented.

Targeting 200mg/l, we will need to dissolve the 10mg of sulfoxaflor in 50ml of distilled water:

$$200 \text{ mg} : 1000 \text{ ml} = 10 \text{ mg}: x \rightarrow x = 1000 \text{ ml} * 10 \text{ mg} / 200 \text{ mg} \quad x = 50 \text{ml}$$

This stock solution should be further diluted to reach the desired concentration. Dilution will be calculated using the following formula:

$$Ci * Vi = Cf * Vf, \text{ where:}$$

- Ci is the initial concentration of our stock

- Vi is the fraction (volume or weight) of the stock solution to be diluted

- Cf is the final concentration of the treated syrup

- Vf is the final volume (or weight) of treated syrup

Step 1: Dissolve the active ingredient in water to prepare a ˜200 mg/kg solution (Royal Holloway)

- Wear appropriate PPE (lab coat and gloves)

- Place a magnetic stirring flea in a 100 ml laboratory bottle (or a flask)

- Place the bottle on the analytical scale and tare

- Using a serological pipette, transfer the desired amount of distilled water (50ml at room temperature) into the bottle.

- Adjust the volume (add or take water) using a laboratory pipette until you read exactly 50.000g

- Transfer the bottle to the fume hood (do not turn the fume hood on as the powder is fine. Lower down the screen to protect your eyes in case of spillage)

- Use a P1000 pipette set at 0.7 ml (washing step):

    1) Take 0.7 ml water from the bottle

    2) Add it to sulfoxaflor powder in the vial

3) a. Pipette to create a suspension of sulfoxaflor powder in water (do not pipette too vigorously! you don't want to spill anything at this stage)

    b. Close the lid of the sulfoxaflor vial very well and vortex for 10 seconds at high speed

4) Transfer the suspension from the vial to the bottle. repeat this process, alternating 3a and 3b and for a total of about 5 minutes until almost all the powder has been transferred to the bottle.

- Close the vial and the bottle very well and stir the solution in the bottle at high speed on the magnetic stirrer for 30 min (it's best to cover the bottle with aluminum foil)

- After 30 minutes repeat the washing step described above. do not change the pipette tip, as there might be some residue of undissolved powder in it, which also needs to be washed out in the main stock solution

- Close the bottle very well and stir for 15 minutes

- Quickly repeat the washing step (optional, it's probably unnecessary, but I've always done it to be on the safe side)

- Close the bottle very well and stir for 15 minutes (for a total of 60 minutes - if you repeat the washing step only once, this stirring step should last 30 minutes)

- Visually check that there's no sulfoxaflor in suspension (there might be dust that looks like sulfoxaflor. If the stirring intensity is high enough, sulfoxaflor should be completely diluted at this stage. if not, stir for 15 more minutes).

- Store 1ml sample for chemical analysis

Background information	
water solubility limit (pH 7)	568 mg/l
minimum volume of solvent (distilled water) for 10 mg sulfoxaflor	17,6056338 ml

Preparation of the stock solution in water	
Desired concentration of stock solution (mg/L ≈ mg/kg)	200
Desired volume of stock solution (ml)	50
amount of technical active ingredient (mg sulfoxaflor)	10

Calculation of the quantity of sulfoxaflor needed (Royal Holloway)

Solution	Doses and respective concentrations				
	concentration (mg/kg)	number of colonies required + 10	solution per colony feeder (g)	treatment solution needed (kg)	amount of sulfoxaflor (mg)
Day 0	0,161	42	200	8,4	1,3524
Day 1	0,047	42	200	8,4	0,3948
Day 2	0,014	42	200	8,4	0,1176
Day 3	0,004	42	200	8,4	0,0336
				total amount of sulfoxaflor (mg)	1,8984

Dilution

Note: the calculation is for a final quantity of 200 g syrup (30 Brix) per colony per day, which should be enough to fill 2 gravity feeders.

N batch	N colonies per treatment*	syrup per colony (g)	Treatment solution ID	Concentration of diluted stock (1:10 v:v - 20mg/l) C_i	Spike solution (10 ml≈g)		Desired concentration of ready to use treatment solution (mg/kg) C_f	Desired final quantity of ready to use treatment solution (g) V_f	Syrup		% sucrose (w/w)	N spikes required + 3 (for mistakes)	total quantity of diluted stock (ml)
					Diluted stock (ml ≈ g) V_i	Water (ml ≈ g)			Quantity of water in the treatment solution - quantity of spike (ml ≈ g)	Quantity of sucrose (g)			
8 + pilot	2	200	SULF - Day1	20	3,22	6,78	0,161	400	270	120	30	12	39
8 + pilot	2	200	SULF - Day2	20	0,94	9,06	0,047	400	270	120	30	12	11
8 + pilot	2	200	SULF - Day3	20	0,28	9,72	0,014	400	270	120	30	12	3
8 + pilot	2	200	SULF - Day4	20	0,08	9,92	0,004	400	270	120	30	12	1
8 + pilot	2	200	SULF/CRIT - Day1	20	3,22	6,78	0,161	400	270	120	30	12	39
8 + pilot	2	200	SULF/CRIT - Day2	20	0,94	9,06	0,047	400	270	120	30	12	11
8 + pilot	2	200	SULF/CRIT - Day3	20	0,28	9,72	0,014	400	270	120	30	12	3
8 + pilot	2	200	SULF/CRIT - Day4	20	0,08	9,92	0,004	400	270	120	30	12	1
8 + pilot	2	200	CON - Day1	0	0	10	0	400	270	120	30	12	0
8 + pilot	2	200	CON - Day2	0	0	10	0	400	270	120	30	12	0
8 + pilot	2	200	CON - Day3	0	0	10	0	400	270	120	30	12	0
8 + pilot	2	200	CON - Day4	0	0	10	0	400	270	120	30	12	0
8 + pilot	2	200	CRIT - Day1	0	0	10	0	400	270	120	30	12	0
8 + pilot	2	200	CRIT - Day2	0	0	10	0	400	270	120	30	12	0
8 + pilot	2	200	CRIT - Day3	0	0	10	0	400	270	120	30	12	0
8 + pilot	2	200	CRIT - Day4	0	0	10	0	400	270	120	30	12	0

Step 2: Dilute the concentrated stock 1:10.

> - Add 15 ml concentrated stock (200 mg/L) to a flask or beaker.
>
> - Add 135 ml distilled water.
>
> - Cover the flask with cling film and stir the solution on medium speed for 2 minutes.

Now you have 150ml of diluted (20mg/l) stock, which are sufficient for up to 16 spike solution of each type.

Step 3: Prepare the solutions (Royal Holloway)

- Now there are 150 ml of diluted (20 mg/l) stock, which are sufficient for up to 16 spike solution of each type.

Step 4: (Royal Holloway)

- Freeze all tubes at -20˚C.

Step 5: (Reading)

- Defrost one tube for each treatment: leaving them at room temperature, while shielding them from direct light, until completely defrosted (15-20 minutes should be enough).
- On each day of exposure, take 4 clean, dry plastic bottles (>500 ml capacity with a good cap). Alternatively, you could use laboratory glass bottles.

- Add the quantity of distilled water reported in the table above (270 g) to each bottle (you could either measure the target volume in a volumetric tube of appropriate size and transfer the liquid to the bottle; or weigh the distilled water on a calibrated scientific scale to the nearest milligram).

- On a scientific scale weigh at nearest milligram the quantity of sucrose reported in the table above (120 g).

- Close the bottle very well and shake/stir it until the sucrose is completely dissolved. It's critical that you don't spill anything at this stage.

- Label each bottle according to the treatment and day of exposure (0, 1, 2, or 3).

- Add the 10 ml each spike to the respective bottle.

- Close the bottle very tightly and shake well.

Note: A concentration of 30% w/w sucrose of the treated syrup was chosen as reasonable worst-case for field beans. As the concentration is potentially variable, to verify that 30% is still realistic for our crop of interest a cumulative distribution of nectar concentration values for field bean, oilseed rape, and strawberry was analysed (limited amount of literature data collected in a non-systematic way) showing that 30% sucrose would be a realistic choice, although it represents the lower tail of this distribution (Figure below).
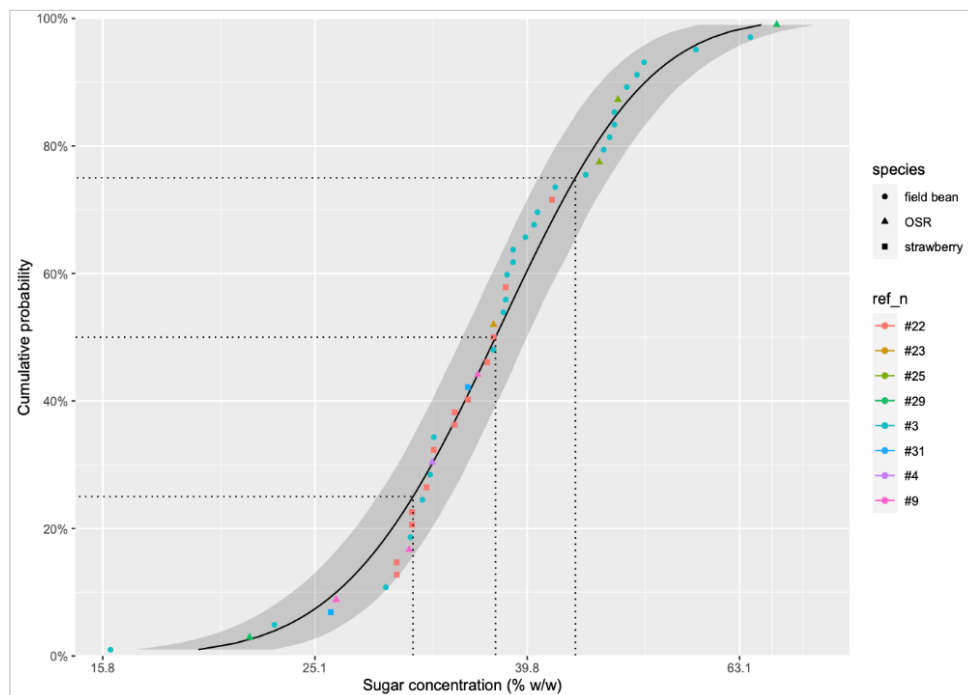
Cumulative distribution of concentrations of sugar in nectar of agricultural crops, where a concentration of 30% w/w sucrose is shown to be realistic. References: #3: Bailes et al., 2018; #4: Carruthiers et al., 2017; #9: Enkegaard et al., 2016; #22: Abrol, 1992; #23: Adegas & Couto, 1992; #25: Mohr & Jay, 1990; #29: Eisikowitch, 1981; #31: Kolev et al., 1981.

2. Colony preparation

Ed Straw

Overview

- Colonies arrive on a Wednesday.

- Colonies are checked for presence of a parasite (Colony clean check).

- Colonies are culled to 20 workers plus a queen.

- Colonies are inoculated with *Crithidia bombi* (Parasite inoculation).

- Colonies are left to develop for a week, allowing the infection to take hold.

- Colonies are screened for the presence of the parasite (Screen for parasite presence).

- Colonies are handed off to Reading (Ship).

In detail

1. Colonies arrive on a Wednesday

- 15-25 worker queenright *Bombus terrestris audax* colonies have been ordered, and a contact at Agralan will validate size in advance.

- No cotton wool cover.

2. Colonies are checked for presence of a parasite (Colony clean check)

- Any plastic items as a residual from development (i.e. small feeder) in the colony are removed.

- 20 bees per colony are removed from colony, induced to defecate with time and light agitation.

- If less than 20 bees are present all worker bees will be used.

- 10μL microcapillary tubes are used to extract the faeces.

- Faeces is pooled in labelled 1.5 ml Eppendorf tube.

- At least 16 bees faeces is used.

- This is done in the bee room next to the colony, so all bees can be removed at once as it's a fast process.

- All colonies have faeces extracted.

- Faeces is stored in the fridge at 4˚C.

- Faeces is vortexed for 5 seconds.

- Faeces is screened under a microscope for *Crithidia bombi, Nosema bombi* and *Apicystis bombi*.

- If any of the above are detected the colony is not entered into the experiment.

- Notes are taken on any other visible microbes in the faeces i.e. filamentous bacteria or yeasts.

3. Colonies are culled to 20 workers plus a queen

- A box is weighed on a field scale 0.001 g precision.

- All bees in a colony are removed from the colony bar the queen.

- The brood is photographed from a standardised distance.

- The box of bees is weighed to get a total weight of all bees.

- 20 bees are taken from the box and returned to the colony (bees for return are chosen haphazardly).

- The box of bees is weighed again to calculate the weight of returned bees.

- The whole colony and workers are weighed on a kitchen scale.

- The colony is returned to its position and feeder.
- Once all colonies are weighed they are allocated to treatments using a weight rank allocation, *i.e.* heaviest to Treatment 1, second heaviest to Treatment 2 etc. This is rotated between batches so in batch 2 the Heaviest colony is allocated to Treatment 2, second heaviest to Treatment 3 and so on.

4. Colonies are inoculated with Crithidia bombi (Parasite inoculation)

- 30-40 bees total are removed from 2 colonies of *Crithidia bombi* infected colonies. Those colonies were infected from 3 parasitised queens caught in Windsor Great Park in 2021. These bees are induced to defecate and the faeces pooled.
- *Crithidia bombi* is purified using a triangulation protocol from Cole (1970).
- *Crithidia bombi* inoculum concentration is quantified using a Neubauer haemocytometer.
- The inoculum is diluted in 1ml water to a dose of 21* 25,000 cells = 525,000 cells, equivalent to 25,000 cells per bee, a dose which induces a realistic infection.
- 4ml of 40% sucrose is added and vortexed.
- This is added to a petri dish and presented to the colonies for 24h. If not consumed a further 24h is waited.
- Control bees are exposed to 1ml water and 4ml 40% sucrose.
- Once inoculum is consumed bees are returned to their feeder.

5. Colonies are left to develop for a week, allowing the infection to take hold.

- This is long enough for a primary infection to develop and some secondary infections to be seeded.

6. Colonies are screened for the presence of the parasite (Screen for parasite presence).

- 15 bees are removed from a colony.
- They are induced to defecate.
- Their faeces is screened for *Crithidia bombi* presence/absence.
- 15 more bees are removed from a colony.
- The original 15 bees are returned to the colony.
- The second 15 bees are induced to defecate.
- The second 15 bees faeces is screened for *Crithidia bombi* presence/absence.
- If less than 20 bees are present then only 10 per batch are screened.
- If over 30 bees are present then 15 per batch are screened.
- If a prevalence of <25% is detected in a colony intentionally infected with *Crithidia bombi* that colony is discarded.
- *A priori* expectation is for a 75 ± 25% prevalence rate.
- Prevalence is recorded and the data retained.
- If a non-intentionally infected colony has a prevalence of >0% that colony is discarded.

<u>7. Colonies are handed off to Reading (Ship)</u>

- Reading arrives and collects colonies in plastic and cardboard boxes.
- Reservoirs are closed for transit.

3. Colony development requirements

Ed Straw

If we use 6 weeks of development post-exposure then at peak there will be a maximum of 60 colonies concurrently growing in the room. This will peak on the final day of the experiment, then decline from a week after this as the first set of colonies is frozen.

Room requirements

Space- A room with space for 60 colonies kept around 10 cm apart on all sides and not stacked atop one another. Colonies can get sticky due to the sugar water, so should be kept on trays (requires 60 trays), alternatively blue roll can be used and replaced every other week. Trays should be wiped with a disinfectant, washed, or swapped out every other week to prevent mould.

Temperature- 24-26°C is the preferable range. We use 2 radiators, one wall mounted and one plug in, which we fiddle with the thermostat on when in the room to keep the temperature in check. During cold snaps or hot periods its worth checking on the room to ensure it has not deviated too far.

Humidity- large amounts of sugar water can cause higher humidity, but unlikely if well ventilated. If a humidity monitor is available this should be used to check humidity does not go outside 50±20%.

Sugar water- over 6 weeks a colony could use all their reservoir. This should be checked weekly and topped up with 50% w/w sugar (mix white sugar and water 1:1 and shake) if needed. During exposure using the gravity feeders/reservoirs should be marked to link back to the colony (cannot share between due to parasite transmission). They should not be emptied in this period so as to preserve sugar water.

Pollen- over 6 weeks with 1 scoop a week 60 colonies will use around 1 kg of pollen. RHUL will provide pollen. To pollen feed a colony a heaped tablespoon of pollen should be added to the colony through the flaps in the lid (or mesh if mesh lid, if so smash down with spoon). Pollen to be kept frozen, can be served frozen as well, will remain scoopable. Please remind RHUL if no pollen given prior to experiment, should be supplied during pilot.

Freezers- UREAD will need freezer space for 60 colonies (can be taken out of their cardboard boxes as long as markings are transferred). This is a lot of freezer space for a long time (6 weeks).

Escapes- If bees escape (unlikely), they should be stamped upon/squashed/captured and frozen. Do not release due to Crithidia transmission risk to wild bees (blinded to treatments). Note of number and date

squashed can be made, but will not be able to meaningfully inform analysis unless a source colony is identified (notable holes (4mm+) in base or lid would be visible).

Part B: UREAD protocols

1. Transport and storage of *Bombus terrestris* colonies

Elena Cini

Aim

Sixty *Bombus terrestris* colonies will be transported from RHUL to UREAD to perform the flight cage experiment. Colonies will be divided into 9 batches, 8 of which will be made up of 8 colonies and 1 of 4 colonies.

Timeline

The experiment will take place over 7 weeks between May 5th – June 21st. Colonies will be transported from RHUL to UREAD 8 days after being inoculated with *Crithidia* and transportation will be made approximately every 5-6 days.

Transportation

The UREAD truck will be used to transport colonies in safe conditions. It is important to make sure of the followings:

1. Book the truck well in advance to ensure its availability for the day.
2. Be well equipped in case of bad weather (*e.g.* tarpaulin) if using the open back truck.
3. Have a sufficient number of cable ties/ropes to secure the colonies on the back of the truck, if needed.

Storage

At UREAD, colonies will be stored in two rooms with controlled temperature (24-26 °C) and humidity (50±20%) located in the Crop and Environment Laboratory at the University of Reading (CEL – Room CE17 and CE18). During the three-day observation period, colonies will be left in the flight cages overnight to facilitate the process of returning all bees to the colony the next morning and to make the experiment more field realistic. Shelters to protect the boxes from rain will be provided beforehand.

After the assessments, colonies will be stored in the CE rooms, fed with pollen, and their reservoirs will be topped up with sucrose once per week. After 6 weeks, they will be frozen and collected by RHUL for further analyses (see RHUL protocol *"Colony development requirements"*).

2. Growing field bean plants

Elena Cini

Aim and timeline

Field beans (FB) will be exposed to *Bombus terrestris* colonies in UREAD flight cages to assess if sulfoxaflor, *Crithidia*, or sulfoxaflor*Crithidia interaction may influence the behaviour of bumblebees, and will be grown in time for running the pilot (April 6th – May 2nd) and the main experiment (May 5th – June 17th).

Materials

Seeds of FB 'Fuego' variety will be supplied by Sonning Farm. 'John Innes n° 2' compost will be used to grow FB for its good nutritive content, and 3 L plastic pots (1 per plant) will be used to pot the cohorts.

Planting cohorts

One cohort of FB will be planted half-February to be in flower for the pilot experiment, while two big cohorts will be planted for the main experiment as follows:

A) First cohort: March 17th to be in flower for the first half of the experiment (4 batches of colonies)

B) Second cohort: April 7th to be in flower for the second half of the experiment (5 batches of colonies)

It is important to use plants coming from the same cohort for the same set of colonies, *e.g.* colonies in batch 3 will all be foraging on plants coming from the same cohort.

When the oldest cohorts are ready to come out, they need to be moved from the glasshouse to the isolation cage (flight cage with no pollinators) and continue potting until the desired number of plants is reached.

Plants distribution

We need to make sure that plants will be well distributed to cages so that there will always be plants in full bloom as the experiment progresses.

When a new batch arrives, one person should pick the total number of plants needed and start distributing them across the cages as follows:

1. Select the 8 most advanced plants and get 1 assigned to each cage.
2. Select the next 8 more advanced plants and get 1 assigned to each cage.
3. Continue until reaching the needed number of plants per cage.

This task needs to always be undertaken by the same person.

Summary of plant numbers

A total of 320 FB will be used as follows:

- 2 behavioural plants/cage for individual observations.
- 3 phytometer plants/cage for colony observations.

Phytometer plants will be marked with cable ties and used to assess the yield at the end of the experiment.

The same FB plants will be used in the same cage with the same colony over the 3 days of observations.

Acclimatisation

On the first day of pesticide exposure (=day 0), bees will acclimatise to cages for 6 hours with 2 old behavioural plants coming from the same treatment, *e.g.* plant1_batch1_FB2_colonyQ and plant2_batch1_FB2_colonyQ will be used for batch2_FB2_colonyQ. For batch 1, acclimatisation plants will be spares coming from the pilot.

Pilot trial

A cohort of 50 FB will be planted in mid-February so that the flowering will happen in time for pilot observations starting on April 6th.

3. Plant exposure

Elena Cini

Introduction

This protocol is divided into 4 sections:

A) Labelling and marking materials.

B) Storing plants.

C) Bee behaviour observations.

D) Yield assessment.

A) Labelling and marking materials

Labelling colonies

1. 60 *Bombus terrestris* colonies will be labelled with a unique <u>colony ID</u> on the top of the cardboard box and colony box comprising batch number (1 to 8), plant (FB1 or FB2), and colony letter (H, F, Q, W) as follows: batch1_FB2_colonyH, batch3_FB1_colonyQ, batch7_FB1_colonyW etc. The colony ID will also be written next to the entrance on the cardboard boxes to be easily identified by the camera during colony observations, and on the reservoirs to avoid any mismatching.

2. The experiment will be blind to reduce biases, *i.e.* it will not be known which groups are the control and which the treatment ones. <u>It is extremely important to take note of the colony IDs</u> so that it will be possible to match the ID with the corresponding treatment when the experiment will be over (RHUL to provide information on matching colonies and treatments after the experiment).

Marking plant cohorts

Each day of observations plants will be labelled with <u>a unique plant ID comprising the plant number and colony ID</u>: plant1_batch1_FB2_colonyH, plant2_batch1_FB2_colonyH, plant3_ batch1_FB2colonyH, plant4_ batch1_FB2_colonyH, plant5_ batch1_FB2_colonyH. Labels will be prepared in advance during acclimatisation day.

On phytometer plants, the middle nodes on each stem will be marked with one cable tie at each end (*i.e.* nodes with the freshest and receptive flowers). One person will count the number of flowers located between cable ties to later calculate the proportion of flowers that will become pods, and the total number of flowers on each phytometer plant to be used for individual observations. Numbers will be recorded in an Excel spreadsheet. To reduce biases, the same person will always be in charge of counting flowers.

Storing plants

1. After reaching the flowering stage, plants will be moved from the glasshouse to the isolation cage (see Protocol *'Growing field bean plants'*).
2. To conduct observations and pollination assessments, every day designated plants will be moved into 8 flight cages with 1 bumblebee colony each and moved back to the isolation cage at the end of observations. <u>Beware of IDs.</u>

B) Bee behaviour observations

Logistics

Observations on bee behaviours will be based on the work of Stanley et al. (2015) and will be performed in each flight cage containing 1 colony and a cohort of plants (8 cages with FB). Since *B. terrestris* colonies will have been cut down to 20 workers by RHUL and expected to count ~ 50 workers at exposure, and that they will not remain fully open for an entire day, it will be sufficient to have 3 FB phytometer plants inside the cage during colony observations.

Over-pollination

The following observations were performed on pilot colonies foraging on field bean plants to understand how long they would averagely require to visit the whole plant (*i.e.* all its flowers) once, so as to make sure to choose a right duration of colony observations to (a) avoid over-pollination, and (b) give observers enough time to go through 8 colonies each day. Observations were performed for 5-10 minutes during warm, sunny days.

<u>17 April 2021</u>

Observations performed for 10 minutes

Colony 1	Colony 2	Colony 3
Plant 1: 37 flowers Visits: 7 7:37=x:1 X=0.19 0.19:10=1:x X=52.63 min to visit the whole plant once Plant 2: 89 flowers Visits: 5	Plant 1: 41 flowers Visits: 6 6:41=x:1 X= 0.15 0.15:10=1:x X=66.6 min to visit the whole plant once Plant 2: 17 flowers Visits: 1	Plant 1: 38 flowers Visits: 3 3:38=x:1 X=0.08 0.08:10=1:x X=125 min to visit the whole plant once Plant 2: 62 Visits: 4

5:89=x:1 X=0.06 0.06:10=1:x X=166.67 min to visit the whole plant once Mean=109.65 min	1:17=x:1 X=0.06 0.06:10=1:x X=166.67 min to visit the whole plant once Mean=116.64 min	4:62=x:1 X=0.06 0.06:10=1:x X=166.67 min to visit the whole plant once Mean=145.84 min

<u>18 April 2021</u>

Observations performed for 5 minutes

Colony 1	Colony 2
Plant 1: 40 flowers Visits: 7 7:40=x:1 X=0.175 0.18:5=1:x X= 27.78 min to visit the whole plant once Plant 2: 40 flowers Visits: 5 5:40=x:1 X= 0.13 0.13:5=1:x X= 38.46 min to visit the whole plant once Mean=33.12 min	Plant 1: 50 flowers Visits: 6 6:50=x:1 X=0.12 0.12:5=1:x X= 41.66 min to visit the whole plant once Plant 2: 30 flowers Visits: 1 1:30=x:1 X=0.03 0.03:5=1:x X=166.67 min to visit the whole plant once Mean=104.17 min

<u>21 April 2021</u>

Observations performed for 5 minutes

Colony 1	Colony 4
Plant 1: 80 flowers Visits: 18 18:80=x:1 X=0.23 0.23:5=1:x X= 21.74 min to visit the whole plant once Plant 2: 50 flowers Visits: 9 9:50=x:1 X= 0.18 0.18:5=1:x X= 27.78 min to visit the whole plant once Mean=24.76 min	Plant 1: 114 flowers Visits: 5 5:114=x:1 X=0.04 0.04:5=1:x X= 125 min to visit the whole plant once Plant 2: 86 flowers Visits: 2 2:86=x:1 X=0.02 0.02:5=1:x X=250 min to visit the whole plant once Mean=187.50 min

Average flowers on plants: approx. 55

Average time to visit the whole plant once: approx. 100 minutes

Time chosen to expose plants to colonies: 75 minutes over 3 days (25 minutes a day, of which 10 of acclimatisation and 15 of observations – 5 minutes per plant)

Cage rotation

To avoid any cage effect, a rotation must be in place to allow all colonies to always be in different cages. For instance, if batch1_colonyH will be put in cage 1, batch2_colonyH will need to be put into cage 2 and so on.

The procedure and observations explained below will be performed for each colony of each treatment group in randomised order.

Observation procedure

Record the observations together with date, time, weather, observer, task, cage, bee (1, 2 or 3) and colony ID.

Individual-level observations are estimated to take ~ 4 hours or more for 8 colonies, while colony observations may take ~2 hours.

a) Individual level observations

Individual observations will be made with the software "BORIS". Ethogram codes are reported below.

Ethogram codes used in BORIS for individual observations.

Key	Code	Type	Description	Exclude
t	Trip	State event	Time spent in cage	
n	New flower	State event	Bee forages on new flower	Move
m	Move	State event	Bee moves to next flower	New flower
p	Pollen	Point event	Bee carries pollen	
e	Error	Point event	Previous key pressed by mistake	

1. Bring 2 labelled behavioural plants into the cage.
2. Open the colony and wait for 1 bee to come out.
3. Once 1 bee is out, press 'start observations' and 't' key at the same time so that it will be easier to monitor the elapsed minutes, and quickly close the colony.
4. If the bee does not start foraging after 10 minutes, capture the bee in a tube and allow the next bee out. Do not return the bee to the colony before observations on that colony will be over.
5. Observe the bee behaviour for 15 minutes. If the bee lands on the colony entrance and tries to get back in, consider the trip to be over. If it lands anywhere else, wait for 15 minutes to elapse before ending the observations.
6. During the observation period, record:
   a. When the bee leaves the colony (key 't').
   b. When it visits a new flower (key 'n').
   c. When it moves from a flower to another (key 'm').
   d. If the bee forages for pollen (key 'p').
   e. When the bee returns to the colony/when 15 minutes elapsed (key 't').

7. The key "e" needs to be pressed after mistakenly pressing a wrong key. This will indicate that the key pressed straight before "e" is indeed a mistake and therefore such record needs to be fixed later.

8. Once 15 minutes have elapsed, catch the bee into a tube and <u>leave it aside to avoid observing the same bee twice.</u>

9. Repeat the assessment for 3 bees per colony, allowing 1 bee out at a time.

10. When done, return the bees to the colony.

11. After each observation, export the dataset to an Excel sheet to be later analysed.

With the obtained records, we aim to calculate the followings:

1. Time elapsed between each flower visit.

2. Duration of the foraging trip, *i.e.* time spent foraging (time of last visit - time of first visit).

3. Latency, *i.e.* time elapsed between the exit from the colony and the start of the foraging trip.

4. Duration of each flower visit.

5. Foraging rate (number of flowers visited divided by duration of foraging trip).

6. If the bee is foraging for pollen (yes/no. Consider "yes" if the bee is rubbing its back legs to get pollen into its pollen sacks or if pollen is visible on their legs).

NB: always remember to label the tubes used for catching the bees with the colony letter to avoid cross-contamination.

Procedure to save data

1. After opening BORIS, click on 'new observation' and name it as the following example: 21 April batch1_FB2_colonyH_bee 1. In 'description', <u>add the observer's name and weather conditions</u> as the following example: observer = EC, sunny, warm.

2. After finishing observing a bee, click on 'file → export events → tabular events' and save the data as an Excel sheet.

3. Open the exported data, correct the mistakes (see "e" keys), and write 'pollen YES' or 'pollen NO' at the top of the sheet so that it will be easier to analyse it later.

4. Note down if the bee was still foraging when 15 minutes had elapsed.

5. Go back to BORIS, click on 'start live observations → delete data' and start over observing the next bee.

6. At the most convenient time, calculate the data described above (latency, duration of foraging trip, etc.) and import data into the appropriate shared folder on Google Drive.

b) *Colony level observations*

Observations on the colony will be performed with the help of a camera recording the number of bees that enter and exit the colony. The video will be later uploaded on BORIS and analysed using the ethogram below.

Ethogram codes used in BORIS for colony observations.

Key	Code	Type	Description
l	Leave	Point event	Bee leaves colony
r	Return	Point event	Bee returns to colony

1. Observe the bees as follows:
    a. Take out the behavioural cohorts and bring in 3 phytometer plants.
    b. Prepare a sheet with date, time, weather, observer, cage number and colony ID.
    c. <u>Make sure that colony ID is clearly written next to the entrance of the colony box so that it will be visible on the recorded video.</u>
    d. Place the camera close to the colony box pointing at the entrance.
    e. Open the colony.
    f. From the moment 1 bee comes out, start recording and allow 10 minutes for bees to settle.
    g. Perform observations on the 3 selected plants (see 'section A' above), 5 minutes per plant, for a total of 15 minutes. To avoid biases, plants will be placed in a randomised order and focal observations will start from the right to the left.
    h. Stop recording.
    i. Set the colony to 'in-only' and allow bees to return to the colony.
    j. Take the plants out of the flight cages back into the isolation cage to avoid over-pollination.
    k. Move to the next cage and repeat the procedure.
2. Record:
    a. Number of entrances and exits to the colony (camera).
    b. Number of bees on each plant (live observations).
3. At the start of the day, 1 person will be counting flowers on the 3 plants we aim to observe (see 'section A' above). Using the data on flower numbers per plant, calculate visitation rate as number of visits received by plant in 5 minutes, divided by 5 minutes, divided by number of flowers on plant (*i.e.* visit per flower per minute).
4. At the end of the day, upload the video into a personal folder on the observer's laptop and code the video using BORIS at the most convenient time. When coding the video, remember to add +1 bee leaving the colony if the video started after the first exit.
5. Record the data on an Excel sheet and upload it to the shared Google Drive folder.

Contingency plan

- During colony observations, if the weather is adverse and no bee is coming out after 30 minutes, close the colony and move to the next cage.

C) Yield measurements

Logistics

To assess how treatments may affect FB yield, we will use phytometer plants (see Protocol *'Growing field bean plants'* for details).

Before starting the assessments, it is important to make sure of the followings:

1. Correctly label plant cohorts (see 'section A' above).
2. Mark the middle nodes on phytometer plants with cable ties (see 'section A' above).

Procedure

After individual and colony observations, plants will be returned to the isolation cage to prevent over-pollination. We will use the same cohort in each cage over the three days of observation.

When the phytometer plants will have reached the appropriate level of maturation, pods will be collected and the yield assessed (see Protocol *'Plant yield measurements'*).

4. Plant yield measurements

Elena Cini

Aim

The following procedures aim to investigate the impact of treatments on the yield of phytometer plants. To achieve this, measurements such as the proportion of flowers that have produced pods, the quality of fruits, and information on bean weight and quantity will be taken into account.

FB pod collection

1. After the trial period, the phytometer FB plants will be left in the isolation cage until they mature – this should happen around mid- to late August depending on the weather.
2. When the maturation approaches check regularly on the plants to prevent pods from becoming over ripe and opening before collection.
3. When plants will have reached the appropriate level of maturation collect and count the pods located between the cable ties and put them in small paper bags labelled with the plant ID. If there are no pods to collect, indicate '0 pods' in the datasheet.
4. Put the small bags into bigger paper bags labelled with the colony ID.
5. Record dates of collection on each paper bag.
6. After collection, pods will be dried in the oven for 48h at 80°C and stored in the laboratory for being processed at the most convenient time.

The whole task of pods collection and division into paper bags is estimated to take 1-2 days.

FB pod processing

1. In an Excel sheet record date, plant ID, colony ID, number of pods (counted at collection), number of flowers between cable ties (counted during trial) and weight. If there are no pods between cable ties, remember to record '0' in the datasheet. For missing data, use an asterisk (*).

2. Open each pod and record the number and weight of beans found inside.

3. Calculate the mean weight of beans per pod and return them to the appropriate paper bag.

4. Put all the information in the Excel sheet, including the recorded '0' if any.

Part C: Summary timeline

Summary table of the experiment, from colony check to freezing, applied to each experimental batch from April 25 (first colony check) to August 2 (last batch frozen), 2021.

Day	Team	Plan
Day 1	RHUL	Colony clean check
Day 2	RHUL	Colonies culled down to size
Day 3	RHUL	Parasite inoculation
Day 10	RHUL	Screen for parasite presence
Day 11	RHUL, UREAD	Shipment
Day 12	UREAD	Bees resting + sugar solution prepared
Day 13	UREAD	Pesticide + acclimatisation **(day 0)**
Day 14	UREAD	Pesticide + observations **(day 1)**
Day 15	UREAD	Pesticide + observations **(day 2)**
Day 16	UREAD	Pesticide + observations **(day 3)**
Day 18 (1 day/week)	UREAD	Feeding (pollen + top up reservoirs if needed)
Day 48	UREAD	Freezing colony

Appendix 3.2

This appendix includes 5 parts:

- <u>Part A:</u> Global models of individual, colony, and plant yield measurement analyses.
- <u>Part B:</u> Data summary (colony/individual) by treatment, experimental block, and observation day.
- <u>Part C:</u> Pearson product-moment test for correlations.
- <u>Part D:</u> Goodness-of-fit Chi-square test for poisson distribution.

Part A: Global models

Data	Response variable	Description	Random effects	Fixed effects
Individual observations	Latency	Time elapsed between bee leaving colony and start of foraging trip	'Block and colony' + 'observer'	Treatment[6] Observation day Interaction
	Duration of foraging trip	Time between start of first and end of last flower visit (max 15 minutes)	'Block and colony' + 'observer'	Treatment Observation day Interaction
	Mean duration of flower visits	Mean time spent on flowers	'Block and colony' + 'observer'	Treatment Observation day Interaction
	Mean time between visits	Mean time elapsed between two flower visits	'Block and colony' + 'observer'	Treatment Observation day Interaction
	Foraging rate	Number of flowers visited divided by duration of foraging trip	'Block and colony' + 'observer'	Treatment Observation day Interaction
	Pollen collection	Whether or not pollen was collected during foraging trip	'Block and colony' + 'observer'	Treatment Observation day Interaction
Colony observations	Mean visitation rate mean	Number of flowers visited in 5 minutes (mean of 3 phytometer plants)	'Block and colony' + 'observer'	Treatment Observation day Interaction
	Bees leaving the colony	Number of bees leaving colony	'Block and colony' + 'observer'	Treatment Observation day Interaction
	Bees entering the colony	Number of bees returning to colony	'Block and colony' + 'observer'	Treatment Observation day Interaction
Plant yield measurements	Mean number of pods	Mean pods per node	'Plant' nested within 'block and colony'	Treatment First node location
	Mean number of beans	Mean beans per pod	'Plant' nested within 'block and colony'	Treatment First node location
	Mean pod weight	Mean pod weight	'Plant' nested within 'block and colony'	Treatment First node location

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[6] Either all 4 treatments (analysis with all treatment colonies), or the percentage of *Crithidia* infection (analysis with *Crithidia*-infected colonies only).

	Mean bean weight	Mean bean weight	'Plant' nested within 'block and colony'	Treatment First node location

Part B: Data summary

Colony observations

Summary of colony observation data divided by treatment, block, and observation day.

Treatment	Observations by day			Observations by experimental block					Total
	Day 1	Day 2	Day 3	Block 1	Block 2	Block 3	Block 4	Block 5	observations
Control	7	7	9	3	5	3	6	6	**23**
Crithidia	8	7	8	2	6	6	3	6	**23**
Crithidia*sulfoxaflor	6	6	8	1	5	5	3	6	**20**
Sulfoxaflor	7	7	8	1	6	3	6	6	**22**
Total observations	**28**	**27**	**33**	**7**	**22**	**17**	**18**	**24**	**88**

Individual observations

Summary of individual observation data divided by treatment, block, and observation day.

Treatment	Observations by day			Observations by experimental block				Total observations
	Day 1	Day 2	Day 3	Block 2	Block 3	Block 4	Block 5	
Control	14	14	14	10	6	15	11	**42**
Crithidia	11	17	15	15	7	9	12	**43**
Crithidia*sulfoxaflor	9	14	10	9	6	3	15	**33**
Sulfoxaflor	10	12	9	11	6	9	5	**31**
Total	**44**	**57**	**48**	**45**	**25**	**36**	**43**	**149**

Summary of individual and colony observation data divided by colony, block, and observation day. 'Yes'= colony observation successfully performed; 'No'= colony observation not performed (*e.g.* lack of time, adverse weather conditions). '-'= Individual observations of block 1 not included in the analysis.

Experimental block	Colony	Day 1		Day 2		Day 3	
		Indiv	Colony	Indiv	Colony	Indiv	Colony
Block 1	Control	-	No	-	No	-	Yes
	Control	-	Yes	-	No	-	Yes
	Crithidia	-	No	-	No	-	No
	Crithidia	-	Yes	-	No	-	Yes
	Crithidia*sulfoxaflor	-	No	-	No	-	No
	Crithidia*sulfoxaflor	-	No	-	No	-	Yes
	Sulfoxaflor	-	No	-	No	-	No
	Sulfoxaflor	-	No	-	No	-	Yes
Block 2	Control	2	No	0	Yes	1	Yes
	Control	2	Yes	3	Yes	2	Yes
	Crithidia	1	Yes	3	Yes	3	Yes
	Crithidia	3	Yes	3	Yes	2	Yes
	Crithidia*sulfoxaflor	1	No	1	Yes	3	Yes
	Crithidia*sulfoxaflor	3	Yes	2	Yes	1	Yes
	Sulfoxaflor	3	Yes	3	Yes	2	Yes
	Sulfoxaflor	1	Yes	0	Yes	0	Yes
Block 3	Control	0	Yes	3	Yes	3	Yes
	Control	0	No	0	No	0	No
	Crithidia	0	Yes	1	Yes	3	Yes
	Crithidia	0	Yes	2	Yes	1	Yes
	Crithidia*sulfoxaflor	0	Yes	3	No	3	Yes
	Crithidia*sulfoxaflor	0	Yes	0	Yes	0	Yes
	Sulfoxaflor	0	Yes	3	Yes	3	Yes
	Sulfoxaflor	0	No	0	No	0	No
Block 4	Control	3	Yes	3	Yes	3	Yes

	Control	3	Yes	1	No	2	No
	Crithidia	3	Yes	3	Yes	3	Yes
	Crithidia	0	No	0	Yes	0	Yes
	Crithidia*sulfoxaflor	3	Yes	3	Yes	1	Yes
	Crithidia*sulfoxaflor	1	No	1	No	0	No
	Sulfoxaflor	0	Yes	2	Yes	1	Yes
	Sulfoxaflor	0	Yes	0	Yes	0	Yes
Block 5	Control	2	Yes	4	Yes	2	Yes
	Control	2	Yes	0	Yes	1	Yes
	Crithidia	2	Yes	2	Yes	0	Yes
	Crithidia	2	Yes	3	Yes	3	Yes
	Crithidia*sulfoxaflor	1	Yes	0	Yes	0	Yes
	Crithidia*sulfoxaflor	1	Yes	2	Yes	1	Yes
	Sulfoxaflor	2	Yes	3	Yes	1	Yes
	Sulfoxaflor	3	Yes	3	Yes	3	Yes
Overall total: 149	**Total by day**	**44**	**28**	**57**	**27**	**48**	**33**

Part C: Pearson product-moment correlation tests

Correlation matrixes in tables below show no correlation between any of the variables (p>0.05).

Treatment and Observation day	Visitation rate		Number of bees leaving/returning	
	All treatments	*Crithidia*-infected colonies	All treatments	*Crithidia*-infected colonies
Correlation coefficient	0.0139	0.0416	-0.0538	0.2044
P-value	0.8978	0.7913	0.6656	0.2618

Treatment and Observation day	All treatments	*Crithidia*-infected colonies
Correlation coefficient	-0.0506	0.1129
P-value	0.5171	0.3005

Treatment and Location of 1st node	All treatments	*Crithidia*-infected colonies
Correlation coefficient	-0.0759	0.0038
P-value	0.4370	0.9711

Part D: Goodness-of-fit test for Poisson distribution

The goodness-of-fit Chi-square tests for observed versus expected counts (method: Maximum Likelihood) are presented below. Significant p-values ($p<0.05$) are highlighted in bold and indicate data that do not follow a poisson distribution, for which a quasi-poisson distribution in corresponding GLMMs was adopted.

Colony observations including all treatments						
Response variable	**r**	**Number observed**	**Number expected**	**Likelihood Chi-square**	**df**	***p*-value**
Number of bees leaving colony	0-9	24	7.51	41.93	2	**< 0.001**
	9-12	10	17.93			
	12-15	7	21.53			
	15+	26	20.02			
Number of bees returning to colony	0	24	18.70	5.13	2	0.077
	1	19	23.86			
	2	11	15.23			
	3+	13	9.21			
Colony observations including *Crithidia*-infected colonies						
Response variable	**r**	**Number observed**	**Number expected**	**Likelihood Chi-square**	**df**	**p-value**
Number of bees leaving colony	0-10	11	6.33	9.56	2	**0.008**
	10-12	5	6.49			
	12-16	6	12.85			
	16+	10	6.33			
Number of bees returning to colony	0	10	8.75	0.25	1	0.615
	1	11	11.34			
	2+	11	11.91			

Appendix 3.3

This appendix includes the following parts:

- <u>Part A:</u> Model selection with AICc and ΔAICc.

- <u>Part B:</u> Table of effects of final models.

- <u>Part C:</u> Predicted means of treatments.

- <u>Part D:</u> Fisher's LSD post-hoc tests.

- <u>Part E:</u> Additional figures showing non-significant effects on response variables.

Part A: Model selection

Models with significant terms (*i.e.* visitation rate and number of bees leaving colony) were selected using AICc and ΔAICc. Final models with the lowest AICc and ΔAICc≤2 are highlighted in bold and presented from the best (ΔAICc=0) to the worst (largest ΔAICc).

Analyses on all treatment colonies

Response variable	Fixed effects	AICc	ΔAICc
Visitation rate	**Observation day**	**-294.06**	**0**
	Observation day + treatment	-268.70	25.36
	Observation day + treatment + interaction	-250.50	43.56
Number of bees leaving the colony	**Observation day**	**118.95**	**0**
	Observation day + treatment	133.41	14.46
	Observation day + treatment + interaction	142.96	24.01

Analysis on *Crithidia*-infected colonies only

Response variable	Fixed effects	AICc	ΔAICc
Visitation rate	**Observation day**	**-144.75**	**0**
	Observation day + treatment	-130.37	14.38
	Observation day + treatment + interaction	-101.25	43.50

Part B: Table of effects of final models

Tables below show the estimates, SE, analysis of variance, and R^2 of models investigating colony data, individual data, and plant yield measurements.

Colony-level observations

All treatment colonies. Random terms: 'block and colony' + observer

Visitation rate						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	**p-value**	**R²**
Observation day 2	0.02627	0.00745	9.43	2,53.9	**<0.001**	18.34
Observation day 3	0.02837	0.00713				
N bees leaving the colony						
Fixed terms	**Estimates**	**SE**	**χ²**	**df**	**p-value**	**R²**
Observation day 2	1.42300	1.11700	8.33	2	**0.016**	4.23
Observation day 3	1.72000	1.05500				
N bees returning to the colony (NS)						
Fixed terms	**Estimates**	**SE**	**χ²**	**df**	**p-value**	**R²**
Observation day 2	1.27100	1.11900	1.36	2	0.507	12.82
Observation day 3	1.78400	1.05200				

	Estimates	SE	F	ndf, ddf	p-value	
Crithidia	1.66300	1.15200	1.51	3	0.680	
Sulfoxaflor	1.57700	1.16200				
Crithidia*sulfoxaflor	1.45000	1.17000				
Observation day 2.Crithidia	-1.28350	1.28970	2.28	6	0.892	
Observation day 2.Sulfoxaflor	-1.06230	1.29070				
Observation day 2.Crithidia*Sulfoxaflor	-1.44530	1.33830				
Observation day 3.Crithidia	-1.30950	1.20390				
Observation day 3.Sulfoxaflor	-1.57570	1.22470				
Observation day 3.Crithidia*Sulfoxaflor	-1.47340	1.23020				

Crithidia-infected colonies. Random terms: 'block and colony' + observer

Visitation rate						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	**p-value**	**R^2**
Observation day 2	0.03147	0.00948	8.39	2, 25.5	**0.002**	29.56
Observation day 3	0.03371	0.00897				
N bees leaving the colony (NS)						
Fixed terms	**Estimates**	**SE**	**χ^2**	**ndf, ddf**	**p-value**	**R^2**
Observation day 2	-0.11499	0.21519	2.93	2	0.231	10.81
Observation day 3	0.16684	0.19955				
% Crithidia	0.00763	0.02418	0.30	1	0.587	
Observation day 2.% Crithidia	0.00155	0.03019	0.00	2	0.998	
Observation day 3.% Crithidia	-0.00005	0.02402				
N bees returning to the colony (NS)						
Fixed terms	**Estimates**	**SE**	**χ^2**	**ndf, ddf**	**p-value**	**R^2**
Observation day 2	0.23300	0.48760	0.19	2	0.908	26.56
Observation day 3	0.20790	0.45770				
% Crithidia	0.07085	0.06915	0.56	1	0.455	
Observation day 2.% Crithidia	-0.21430	0.09390	5.20	2	0.074	
Observation day 3.% Crithidia	-0.09420	0.06350				

Individual-level observations

All treatment colonies. Random terms: 'block and colony' + observer.

Foraging rate (NS)						
Fixed effects	**Estimates**	**SE**	**F**	**ndf, ddf**	**p-value**	**R^2**
Observation day 2	1.18720	0.74500	0.49	2, 129.6	0.614	8.69
Observation day 3	-0.04270	0.72710				
Crithidia	0.03570	0.84300	0.51	3, 21.5	0.677	
Sulfoxaflor	0.51640	0.88490				
Crithidia*sulfoxaflor	-0.89190	1.88870				
Observation day 2.Crithidia	-1.72470	1.09950	1.74	6, 102.7	0.119	
Observation day 2.Sulfoxaflor	-1.63780	1.17400				
Observation day 2.Crithidia*Sulfoxaflor	0.29010	1.12490				
Observation day 3.Crithidia	1.23180	1.06220				
Observation day 3.Sulfoxaflor	-0.42300	1.17930				
Observation day 3.Crithidia*Sulfoxaflor	0.09560	1.15920				
Duration of foraging trip (NS)						
Fixed effects	**Estimates**	**SE**	**F**	**ndf, ddf**	**p-value**	**R^2**
Observation day 2	-1.10040	1.31000	0.56	2, 124.2	0.575	8.99
Observation day 3	0.58790	1.25610				
Crithidia	0.01600	1.62100	0.81	3, 20.2	0.504	
Sulfoxaflor	-3.40100	1.69000				
Crithidia*sulfoxaflor	-1.61100	1.70600				
Observation day 2.Crithidia	1.40400	1.94700	1.66	6, 109.9	0.138	
Observation day 2.Sulfoxaflor	2.94000	2.08300				
Observation day 2.Crithidia*Sulfoxaflor	3.27600	1.97700				
Observation day 3.Crithidia	-2.47400	1.84600				
Observation day 3.Sulfoxaflor	2.31700	2.07900				
Observation day 3.Crithidia*Sulfoxaflor	0.38300	2.01700				
Latency (NS)						
Fixed effects	**Estimates**	**SE**	**F**	**ndf, ddf**	**p-value**	**R^2**
Observation day 2	0.02210	0.90450	0.61	2, 135	0.545	5.74

Fixed effects	Estimates	SE	F	ndf, ddf	p-value	R²
Observation day 3	-0.43150	0.89600				
Crithidia	-0.02770	0.96670	1.11	3, 136.3	0.346	
Sulfoxaflor	-0.21540	1.02750				
Crithidia*sulfoxaflor	0.92050	1.01750				
Observation day 2.Crithidia	0.77190	1.34380	0.63	6, 111.9	0.707	
Observation day 2.Sulfoxaflor	1.31330	1.43540				
Observation day 2.Crithidia*Sulfoxaflor	-0.29710	1.37070				
Observation day 3.Crithidia	1.58810	1.30240				
Observation day 3.Sulfoxaflor	0.16000	1.43070				
Observation day 3.Crithidia*Sulfoxaflor	-0.01790	1.42140				

Time between visits (NS)

Fixed effects	Estimates	SE	F	ndf, ddf	p-value	R²
Observation day 2	-0.02399	0.17626	0.18	2, 135.1	0.836	3.34
Observation day 3	-0.10942	0.17464				
Crithidia	-0.17545	-0.18839	0.74	3, 136.3	0.527	
Sulfoxaflor	-0.04468	0.20012				
Crithidia*sulfoxaflor	0.17248	0.19831				
Observation day 2.Crithidia	0.09269	0.26172	0.35	6, 111.8	0.906	
Observation day 2.Sulfoxaflor	0.02529	0.27952				
Observation day 2.Crithidia*Sulfoxaflor	-0.22952	0.26710				
Observation day 3.Crithidia	0.13965	0.25384				
Observation day 3.Sulfoxaflor	0.20741	0.27881				
Observation day 3.Crithidia*Sulfoxaflor	-0.07824	0.27702				

Duration of flower visit (NS)

Fixed effects	Estimates	SE	F	ndf, ddf	p-value	R²
Observation day 2	-0.02407	0.03900	0.53	2, 131	0.588	5.36
Observation day 3	0.02859	0.03843				
Crithidia	0.02359	0.04279	0.68	3, 20.6	0.576	
Sulfoxaflor	-0.04060	0.04486				
Crithidia*sulfoxaflor	0.03144	0.04514				
Observation day 2.Crithidia	0.03384	0.05720	0.77	6, 90.3	0.596	
Observation day 2.Sulfoxaflor	0.07551	0.06093				
Observation day 2.Crithidia*Sulfoxaflor	-0.04483	0.05892				
Observation day 3.Crithidia	-0.01327	0.05597				
Observation day 3.Sulfoxaflor	0.03866	0.06167				
Observation day 3.Crithidia*Sulfoxaflor	0.00541	0.06102				

Pollen collection (NS)

Fixed effects	Estimates	SE	F	ndf, ddf	p-value	R²
Observation day 2	-1.41750	0.96490	0.73	2, 124.9	0.484	19.95
Observation day 3	-0.69850	0.96600				
Crithidia	-0.04800	1.32200	0.89	3, 19.7	0.463	
Sulfoxaflor	-0.94700	1.31300				
Crithidia*sulfoxaflor	-3.41900	1.32700				
Observation day 2.Crithidia	-1.15130	1.39810	2.35	6, 35.6	0.051	
Observation day 2.Sulfoxaflor	0.92330	1.45910				
Observation day 2.Crithidia*Sulfoxaflor	3.88850	1.41390				
Observation day 3.Crithidia	-1.14740	1.39740				
Observation day 3.Sulfoxaflor	0.76910	1.54740				
Observation day 3.Crithidia*Sulfoxaflor	1.41350	1.44310				

Crithidia-infected colonies. Random terms: 'block and colony' + observer

Foraging rate (NS)

Fixed effects	Estimates	SE	F	ndf, ddf	p-value	R²
Observation day 2	0.18990	0.65330	0.38	2,69.1	0.685	2.13
Observation day 3	0.53470	0.67980				
% Crithidia	0.04518	0.07267	0.70	1,68.9	0.405	
Observation day 2.%Crithidia	-0.02167	0.09317	0.03	2,69.7	0.972	
Observation day 3.% Crithidia	-0.00848	0.11184				

Duration of foraging trip (NS)

Fixed effects	Estimates	SE	F	ndf, ddf	p-value	R²
Observation day 2	0.96040	1.01900	1.65	2,64.7	0.199	5.33
Observation day 3	-0.75820	1.07230				
% Crithidia	-0.04512	0.12400	0.42	1,12.7	0.528	

	Estimates	SE	F	ndf, ddf	p-value	R²
Observation day 2.% Crithidia	-0.02907	0.14670	0.11	2,67	0.898	
Observation day 3.% Crithidia	0.04402	0.17802				

Latency (NS)						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
Observation day 2	0.35440	0.70350	0.30	2,69.7	0.744	2.14
Observation day 3	0.50540	0.73380				
% Crithidia	0.06228	0.07853	0.01	1,69.9	0.918	
Observation day 2.% Crithidia	-0.09604	0.09995	0.46	2,69.6	0.631	
Observation day 3.% Crithidia	-0.05287	0.12025				

Time between visits (NS)						
Fixed effects	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
Observation day 2	-0.07851	0.08820	0.61	2,66.6	0.546	5.56
Observation day 3	-0.07013	0.09221				
% Crithidia	-0.01679	0.01013	1.29	1,15.2	0.273	
Observation day 2.% Crithidia	0.01320	0.01261	0.80	2,68.4	0.452	
Observation day 3.% Crithidia	0.01763	0.01520				

Duration of flower visit (NS)						
Fixed effects	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
Observation day 2	-0.01492	0.02999	0.35	2,69.4	0.704	2.61
Observation day 3	-0.02310	0.03123				
% Crithidia	-0.00274	0.00334	1.05	1,69.4	0.308	
Observation day 2.% Crithidia	0.00104	0.00427	0.06	2,69.9	0.943	
Observation day 3.% Crithidia	0.00170	0.00513				

Pollen collection (NS)						
Fixed effects	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
Observation day 2	-0.35140	0.71090	0.71	2,64.9	0.494	8.25
Observation day 3	-0.96520	0.74560				
% Crithidia	0.12460	0.09923	0.82	1,13.3	0.382	
Observation day 2.% Crithidia	-0.07581	0.10911	0.57	2,68	0.571	
Observation day 3.% Crithidia	-0.13262	0.12472				

Plant yield measurements

All treatment colonies. Random terms: 'plant' nested within 'block and colony'.

Average number of beans (NS)						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
Crithidia	-0.39440	0.29960	1.94	3, 27	0.147	8.01
Sulfoxaflor	-0.04630	0.30600				
Crithidia*sulfoxaflor	0.34400	0.30530				
Middle nodes	0.08130	0.22833	0.10	2, 68.3	0.907	
Late nodes	-0.04964	0.36775				

Average number of pods (NS)						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
Crithidia	-1.01140	0.47800	1.63	3, 31.6	0.203	4.99
Sulfoxaflor	-0.78430	0.47680				
Crithidia*sulfoxaflor	-0.59360	0.47810				
Middle nodes	0.02110	0.26650	0.19	2, 92.4	0.831	
Late nodes	0.28560	0.47920				

Average pod weight (NS)						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
Crithidia	-0.18905	0.24375	0.33	3, 24.9	0.803	2.83
Sulfoxaflor	0.06726	0.24802				
Crithidia*sulfoxaflor	0.04162	0.24720				
Middle nodes	-0.13510	0.17420	0.51	2, 67.7	0.604	
Late nodes	-0.24090	0.27890				

Average bean weight (NS)						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
Crithidia	-0.06640	0.07744	0.43	3, 24.7	0.734	4.22
Sulfoxaflor	0.01029	0.07832				
Crithidia*sulfoxaflor	-0.05345	0.07780				
Middle nodes	-0.03161	0.04796	0.88	2, 63.5	0.421	
Late nodes	-0.09923	0.07595				

Crithidia-infected and control colonies. Random terms: 'plant' nested within 'block and colony'.

Average number of beans (NS)						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
% Crithidia	0.00236	0.00591	0.18	1, 24.8	0.673	1.78
Middle nodes	0.15440	0.25350	0.50	2, 63.9	0.610	
Late nodes	0.39550	0.41460				

Average number of pods (NS)						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
% Crithidia	-0.01460	0.01025	1.99	1, 28.6	0.170	4.47
Middle nodes	0.60840	0.43680	1.04	2, 85.2	0.357	
Late nodes	-0.07170	0.79910				

Average pod weight (NS)						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
% Crithidia	0.00107	0.00372	0.00	1, 65	0.970	0.06
Middle nodes	0.01917	0.18685	0.02	2, 65	0.981	
Late nodes	0.05850	0.30904				

Average bean weight (NS)						
Fixed terms	**Estimates**	**SE**	**F**	**ndf, ddf**	***p*-value**	**R²**
% Crithidia	-0.00063	0.00133	0.23	1, 21.7	0.637	1.19
Middle nodes	0.02082	0.05463	0.28	2, 63.5	0.760	
Late nodes	-0.04053	0.08845				

Part C: Table of predicted means of treatments and interaction with observation day
Colony-level observations

N bees returning to colony		
Treatment	**Mean**	**S.E.**
Control	-0.3389	0.4634
Crithidia	0.4594	0.3569
Sulfoxaflor	0.3591	0.3672
Crithidia*Sulfoxaflor	0.1379	0.3944
Observation day·treatment	**Mean**	**S.E.**
Observation day 1.Control	-1.3867	1.0530
Observation day 1.Crithidia	-0.2760	0.5445
Observation day 1.Sulfoxaflor	0.1906	0.5547
Observation day 1.Crithidia*Sulfoxaflor	0.0631	0.6006
Observation day 2.Control	0.0363	0.5151
Observation day 2.Crithidia	0.4154	0.4553
Observation day 2.Sulfoxaflor	0.5513	0.4079
Observation day 2.Crithidia*Sulfoxaflor	0.0407	0.5936
Observation day 3.Control	0.3337	0.3910
Observation day 3.Crithidia	0.6869	0.4435
Observation day 3.Sulfoxaflor	0.3353	0.5163
Observation day 3.Crithidia*Sulfoxaflor	0.3100	0.4749

Individual-level observations

Duration of foraging trip			Latency		
Treatment	**Mean**	**S.E.**	**Treatment**	**Mean**	**S.E.**
Control	11.4600	0.9400	Control	1.8270	0.4540
Crithidia	11.1200	0.9400	Crithidia	2.5860	0.4500
Sulfoxaflor	9.8100	1.0000	Sulfoxaflor	2.5330	0.5040
Crithidia*Sulfoxaflor	11.0700	1.0300	Crithidia*Sulfoxaflor	2.6420	0.4950
Observation day·treatment	**Mean**	**S.E.**	**Observation day·treatment**	**Mean**	**S.E.**
Observation day 1.Control	11.6300	1.1800	Observation day 1·Control	1.9630	0.6890
Observation day 1.Crithidia	11.6400	1.3000	Observation day 1·Crithidia	1.9360	0.7680
Observation day 1.Sulfoxaflor	8.2300	1.3700	Observation day 1·Sulfoxaflor	2.1790	0.8270
Observation day 1.Crithidia*Sulfoxaflor	10.0200	1.4400	Observation day 1·Crithidia*Sulfoxaflor	2.8840	0.8500
Observation day 2.Control	10.5300	1.2200	Observation day 2·Control	1.9850	0.6900
Observation day 2.Crithidia	11.9500	1.1500	Observation day 2·Crithidia	2.7300	0.6580
Observation day 2.Sulfoxaflor	10.0700	1.2700	Observation day 2·Sulfoxaflor	3.5140	0.7420
Observation day 2.Crithidia*Sulfoxaflor	12.1900	1.2600	Observation day 2·Crithidia*Sulfoxaflor	2.6090	0.7100
Observation day 3.Control	12.2200	1.1900	Observation day 3·Control	1.5320	0.6970
Observation day 3.Crithidia	9.7600	1.1800	Observation day 3·Crithidia	3.0920	0.6750

	Mean	S.E.		Mean	S.E.
Observation day 3.Sulfoxaflor	11.1300	1.4200	Observation day 3·Sulfoxaflor	1.9070	0.8410
Observation day 3.Crithidia·Sulfoxaflor	10.9900	1.3600	Observation day 3·Crithidia*Sulfoxaflor	2.4340	0.8020

Foraging rate			**Duration of flower visit**		
Treatment	**Mean**	**S.E.**	**Treatment**	**Mean**	**S.E.**
Control	3.9260	0.4240	Control	0.1768	0.0196
Crithidia	3.7970	0.4220	Crithidia	0.2072	0.0195
Sulfoxaflor	3.7550	0.4630	Sulfoxaflor	0.1951	0.0218
Crithidia*Sulfoxaflor	3.1620	0.4670	Crithidia*Sulfoxaflor	0.1742	0.0218
Observation day·treatment	**Mean**	**S.E.**	**Observation day·treatment**	**Mean**	**S.E.**
Observation day 1.Control	3.5440	0.5950	Observation day 1·Control	0.1943	0.0296
Observation day 1.Crithidia	3.5800	0.6640	Observation day 1·Crithidia	0.2179	0.0332
Observation day 1.Sulfoxaflor	4.0610	0.7070	Observation day 1·Sulfoxaflor	0.1537	0.0353
Observation day 1.Crithidia*Sulfoxaflor	2.6520	0.7370	Observation day 1·Crithidia*Sulfoxaflor	0.2258	0.0368
Observation day 2.Control	4.7310	0.6070	Observation day 2·Control	0.1703	0.0299
Observation day 2.Crithidia	3.0420	0.5700	Observation day 2·Crithidia	0.2277	0.0280
Observation day 2.Sulfoxaflor	3.6100	0.6410	Observation day 2·Sulfoxaflor	0.2052	0.0319
Observation day 2.Crithidia*Sulfoxaflor	4.1290	0.6210	Observation day 2·Crithidia*Sulfoxaflor	0.1569	0.0305
Observation day 3.Control	3.5010	0.6010	Observation day 3·Control	0.1658	0.0298
Observation day 3.Crithidia	4.7690	0.5880	Observation day 3·Crithidia	0.1761	0.0290
Observation day 3.Sulfoxaflor	3.5950	0.7280	Observation day 3·Sulfoxaflor	0.1638	0.0364
Observation day 3.Crithidia*Sulfoxaflor	2.7050	0.6930	Observation day 3·Crithidia*Sulfoxaflor	0.2026	0.0347

Time between visits			**Pollen collection**		
Treatment	**Mean**	**S.E.**	**Treatment**	**Mean**	**S.E.**
Control	0.2323	0.0880	Control	1.2383	0.6242
Crithidia	0.1343	0.0872	Crithidia	0.4244	0.6246
Sulfoxaflor	0.3022	0.0961	Sulfoxaflor	-0.4129	0.6728
Crithidia*Sulfoxaflor	0.2652	0.0978	Crithidia*Sulfoxaflor	0.8555	0.6931
Observation day·treatment interaction	**Mean**	**S.E.**	**Observation day·treatment interaction**	**Mean**	**S.E.**
Observation day 1.Control	0.2768	0.1340	Observation day 1·Control	1.9440	0.8840
Observation day 1.Crithidia	0.1013	0.1495	Observation day 1·Crithidia	1.8960	0.9830
Observation day 1.Sulfoxaflor	0.2321	0.1609	Observation day 1·Sulfoxaflor	0.9970	0.9710
Observation day 1.Crithidia*Sulfoxaflor	0.4492	0.1654	Observation day 1·Crithidia*Sulfoxaflor	-1.4750	0.9910
Observation day 2.Control	0.2528	0.1342	Observation day 2·Control	0.5260	0.7800
Observation day 2.Crithidia	0.1700	0.1279	Observation day 2·Crithidia	-0.6730	0.7280
Observation day 2.Sulfoxaflor	0.2334	0.1443	Observation day 2·Sulfoxaflor	0.5020	0.8430
Observation day 2.Crithidia*Sulfoxaflor	0.1957	0.1380	Observation day 2·Crithidia*Sulfoxaflor	0.9960	0.8190
Observation day 3.Control	0.1673	0.1356	Observation day 3·Control	1.2450	0.7990
Observation day 3.Crithidia	0.1315	0.1313	Observation day 3·Crithidia	0.0500	0.7430
Observation day 3.Sulfoxaflor	0.3301	0.1637	Observation day 3·Sulfoxaflor	1.0670	0.9630
Observation day 3.Crithidia*Sulfoxaflor	0.2616	0.1560	Observation day 3·Crithidia*Sulfoxaflor	-0.7600	0.8880

Plant yield measurements

| **Average number of beans per pod**			**Average number of pods per node**		
Treatment	**Mean**	**S.E.**	**Treatment**	**Mean**	**S.E.**
Control	2.8670	0.1990	Control	2.1030	0.3680
Crithidia	2.6250	0.2040	Crithidia	0.9270	0.3740
Sulfoxaflor	2.8310	0.2080	Sulfoxaflor	1.0810	0.3570
Crithidia*sulfoxaflor	3.2010	0.1960	Crithidia*sulfoxaflor	1.6740	0.3600
Average weight of pods			**Average weight of individual beans**		
Treatment	**Mean**	**S.E.**	**Treatment**	**Mean**	**S.E.**
Control	1.5860	0.1490	Control	0.5414	0.0493
Crithidia	1.4440	0.1520	Crithidia	0.4871	0.0499
Sulfoxaflor	1.6070	0.1550	Sulfoxaflor	0.5258	0.5030
Crithidia*sulfoxaflor	1.6010	0.1460	Crithidia*sulfoxaflor	0.4854	0.0479

Part D: Fisher's LSD post-hoc tests

Fisher's protected LSD post-hoc tests performed on observation day after colony behaviour analyses are reported below. Significant p-values (p<0.05) are highlighted in bold.

Analysis including all treatment colonies				
Response variable	Comparison	*Difference*	*t*	*p*-value
Visitation rate	Observation day 1 vs 2	-0.02627	-3.525	**0.0008**
	Observation day 1 vs 3	-0.02837	-3.979	**0.0002**
	Observation day 2 vs 3	-0.00210	-0.290	0.7727
Number of bees leaving colony	Observation day 1 vs 2	-0.1771	-1.471	0.1497
	Observation day 1 vs 3	-0.3270	-2.834	**0.0074**
	Observation day 2 vs 3	-0.1498	-1.546	0.1306
Observation day*treatment (N.S. but close to significance, p=0.051)	*Comparisons not calculated as variance ratio for interaction between treatment and observation day is not significant.*			
Analysis including *Crithidia*-infected colonies				
Response variable	Comparison	*Difference*	*t*	*p*-value
Visitation rate	Observation day 1 vs 2	-0.03095	-2.777	**0.0106**
	Observation day 1 vs 3	-0.03450	-3.561	**0.0016**
	Observation day 2 vs 3	-0.00355	-0.352	0.7280

# Part E: Supplementary figures not included in the chapter

Colony-level observations
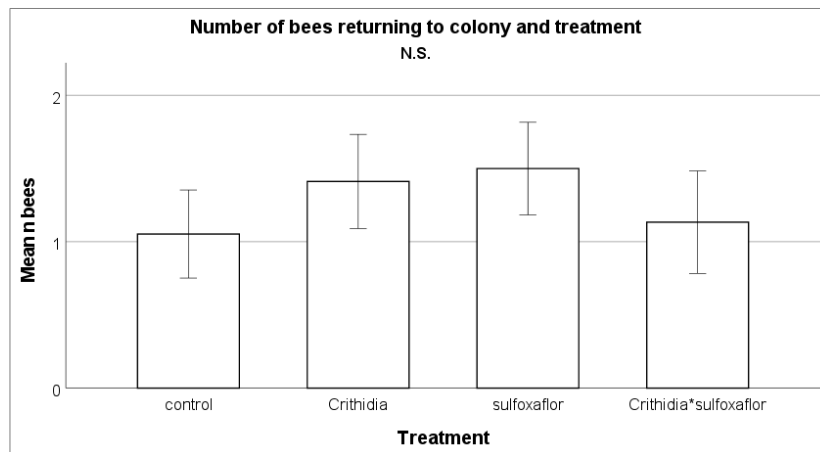
Number of bees returning to colonies during colony-level assessments is not significantly influenced by treatments (X^2=1.52, df=3, p=0.678, R^2=13.73, GLMM including all treatment colonies). N.S.= not significant. Error bars: ± 1 SE from the mean.
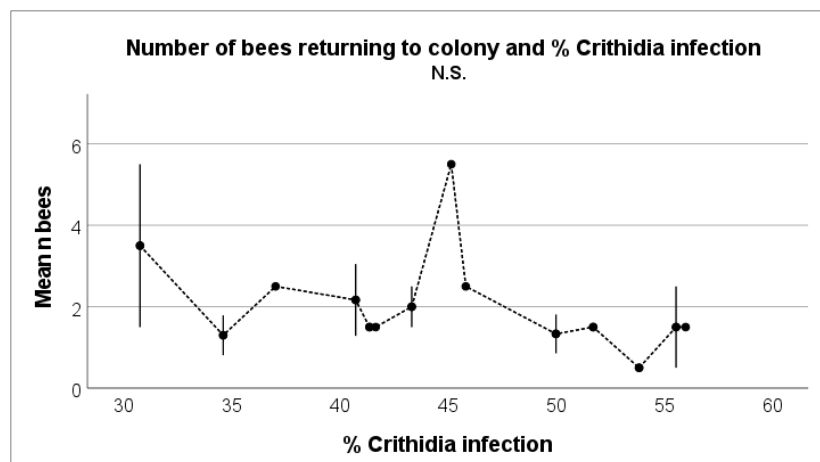

Number of bees returning to colonies during colony-level assessments is not significantly influenced by % Crithidia infection (X^2=0.56, df=1, p=0.455, R^2=26.56, GLMM including Crithidia-infected colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.
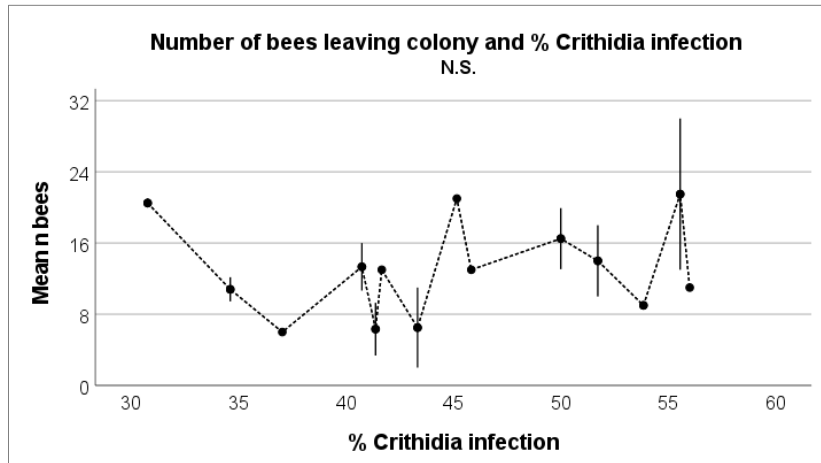
Number of bees leaving colonies during colony-level assessments is not significantly influenced by % Crithidia infection (X^2=0.30, df=1, p=0.596, GLMM including Crithidia-infected colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.

Individual observations
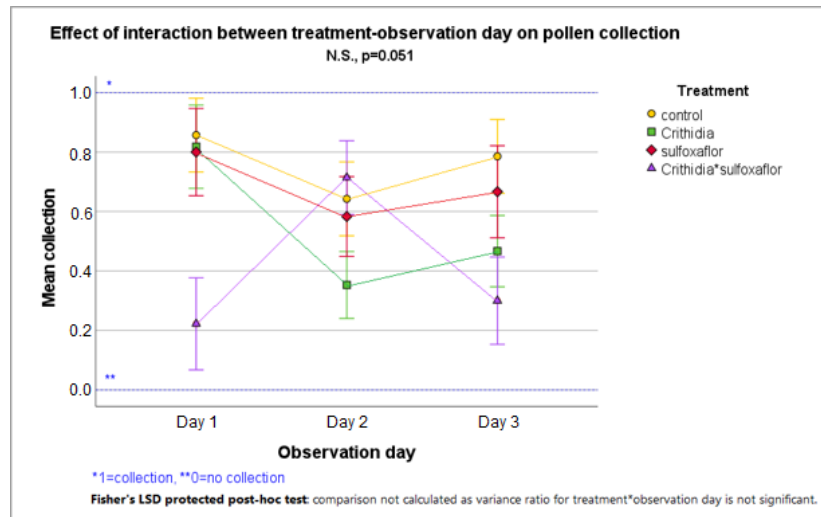
The interaction effect of treatment and observation day on pollen collection returned a p-value of 0.051, with Fisher's LSD post-hoc test showing a non-significant variance ratio (GLMM including all treatment colonies). Error bars: ± 1 SE from the mean.
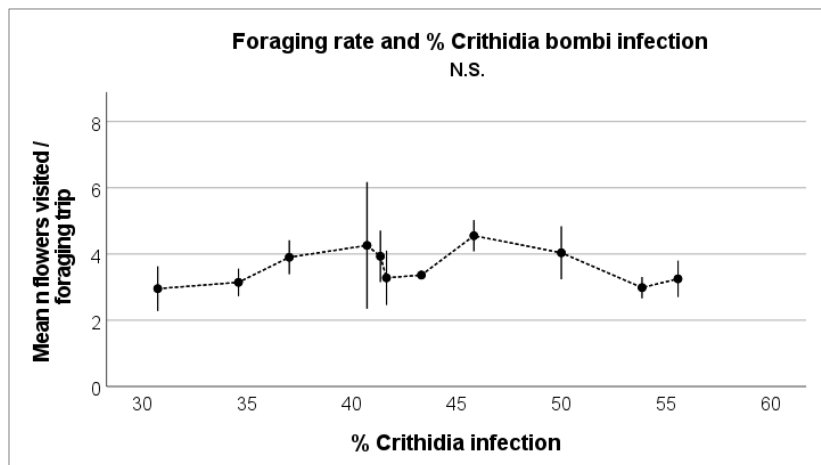
Foraging rate is not significantly influenced by % Crithidia infection ($F_{1,68.9}$=0.70, p=0.405, LMM including Crithidia-infected colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.

Duration of foraging trip and % Crithidia bombi infection
N.S.
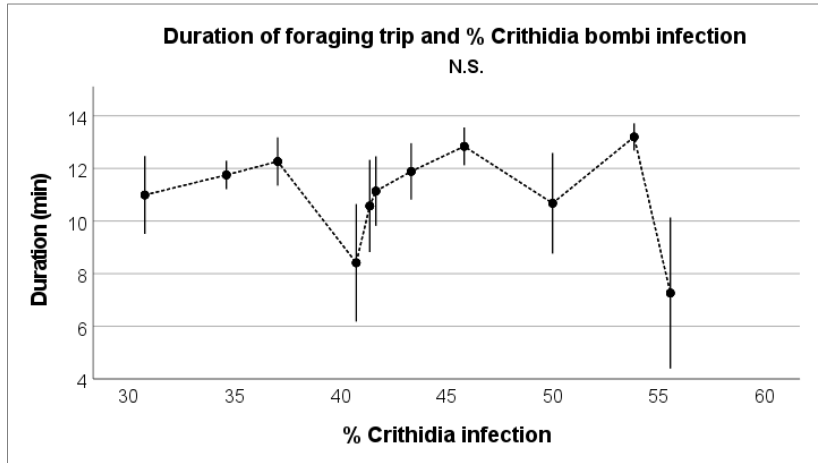
The duration of foraging trip is not significantly influenced by % Crithidia infection ($F_{1,12.7}=0.42$, $p=0.528$, LMM including Crithidia-infected colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.

Duration of flower visits and % Crithidia bombi infection
N.S.
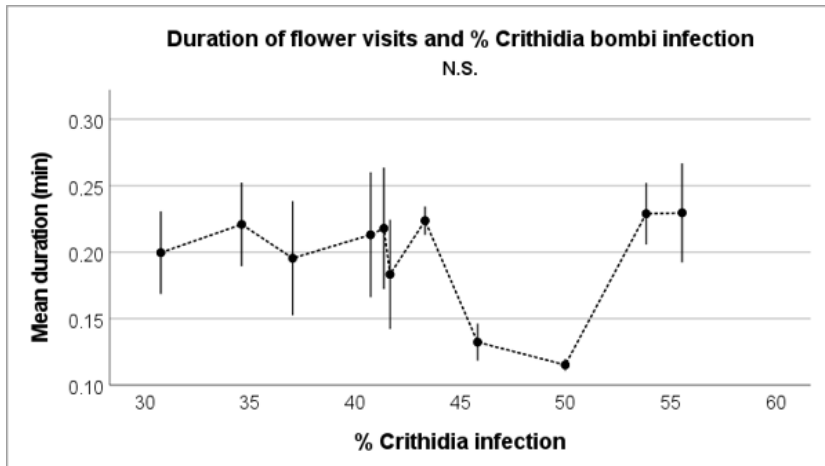
The duration of visits to flowers is not significantly influenced by % Crithidia infection ($F_{1,69.4}=1.05$, $p=0.308$, LMM including Crithidia-infected colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.


Time between flower visits and % Crithidia bombi infection
N.S.
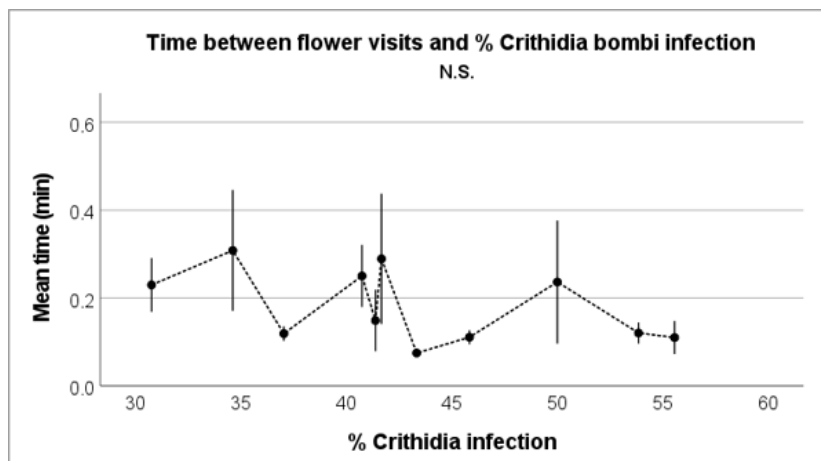
Time between flower visits is not significantly influenced by % Crithidia infection ($F_{1,15.2}=1.29$, $p=0.273$, LMM including Crithidia-infected colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.
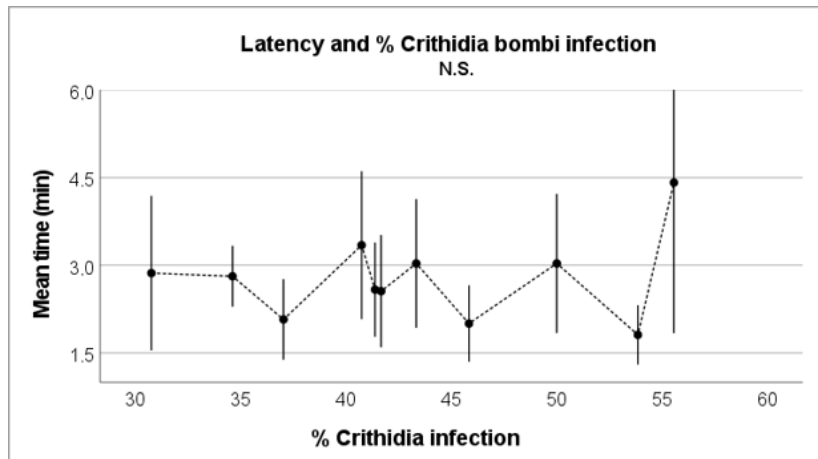
Latency is not significantly influenced by % Crithidia infection ($F_{1,69.9}=0.01$, p=0.918, LMM including Crithidia-infected colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.
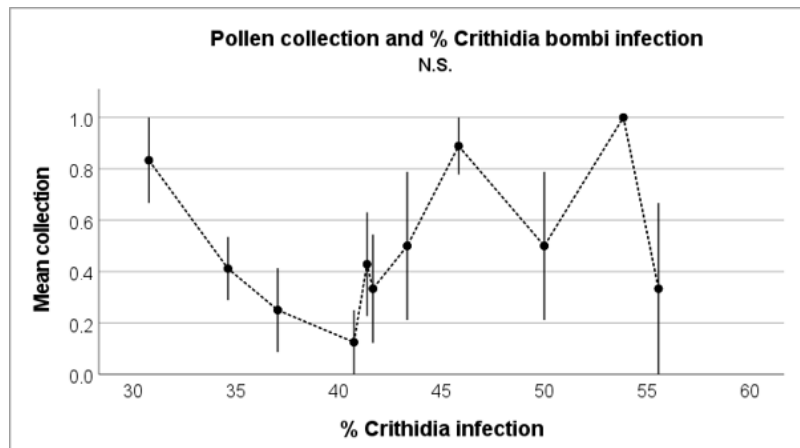

Pollen collection is not significantly influenced by % Crithidia infection ($F_{1,13.3}=0.82$, p=0.382, GLMM including Crithidia-infected colonies only). 0=no collection, 1=collection, N.S.= not significant. Error bars: ± 1 SE from the mean.

Plant yield measurements
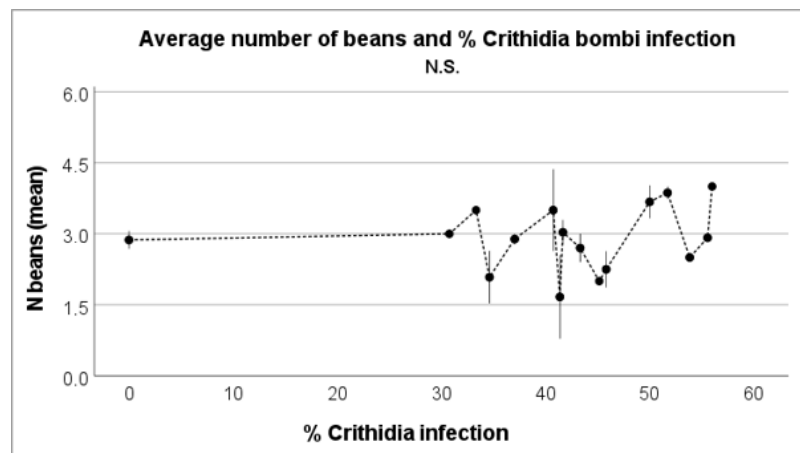
The average number of beans on plants is not significantly influenced by % infection of Crithidia bombi ($F_{1,24.8}=0.18$, p=0.673, LMM including Crithidia-infected and control colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.
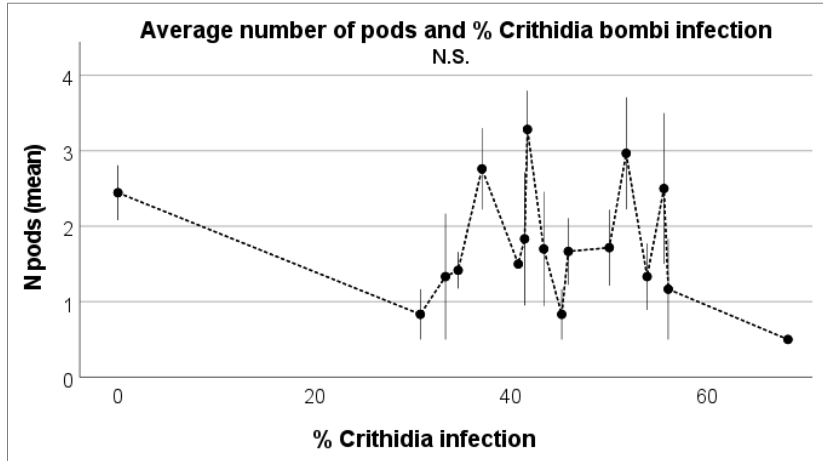
The average number of pods on field bean plants is not significantly influenced by % infection of Crithidia bombi ($F_{1,28.6}$=1.99 , p=0.637, LMM including Crithidia-infected and control colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.
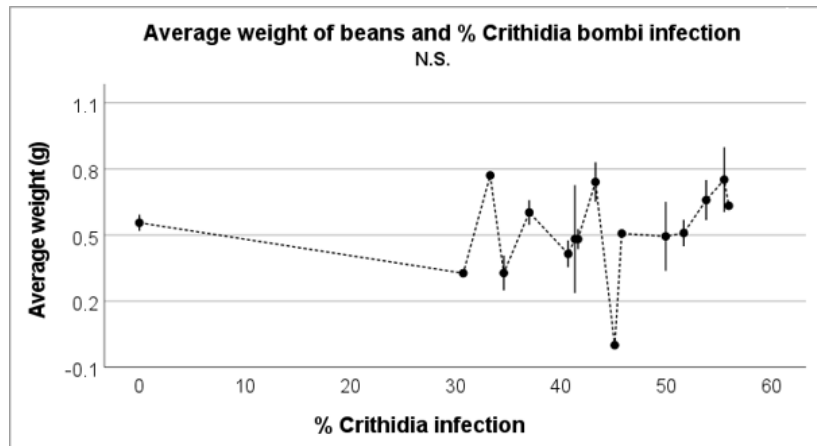
The average weight of beans is not influenced by % infection of Crithidia bombi ($F_{1,21.7}$=0.23, p=0.637, LMM including Crithidia-infected and control colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.
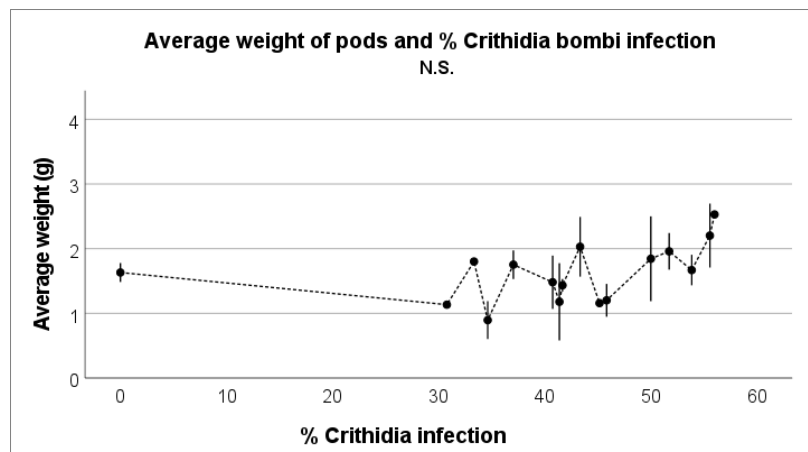
The average weight of pods is not influenced by % infection of Crithidia bombi ($F_{1,65}$=0.00, p=0.970, LMM including Crithidia-infected and control colonies only). N.S.= not significant. Error bars: ± 1 SE from the mean.

Chapter 4

Appendix 4.1

This appendix includes 3 parts:

- Part A: Extended survey questions.

- Part B: Channels used to advertise the survey in each country.

- Part C: Graphics used to advertise the survey on media platforms.

Part A: Survey questions

Final survey (18 closed questions + 1 open question) is presented below.

Question N.	Extended question
Q1	How many years have you been practicing beekeeping? • As hobby • As profession
Q2	How many hives have you kept in the last 3 years? Please indicate the average number per year (open answer)
Q3	Why do you practice beekeeping? Please tick all the options that apply. • Awareness of threats to pollinators • Environmental concerns • Personal hobby • Providing paid pollination services to growers • Selling honey, beeswax, pollen, other products • Others (please specify)
Q4_1	Are you a member of any beekeeping associations? • Yes • No
Q4_2	Please name the associations (open answer)
Q5	In a typical year, how often do you undertake a detailed check on your hives for each of the following health issues? • Diseases • Parasites • Nutrition • Chemical exposure
Q6	Please indicate what equipment and methods of hive inspection you use to monitor the issues below. If you do not use any, please skip this question. • Diseases • Parasites • Nutrition • Chemical exposure
Q7	Do you have any regular communication with growers? • Frequent (more than twice a year) • Infrequent (once or twice a year) • I am a grower myself and manage my own hives • I do not communicate with growers
Q8	How important to you are the following sources of information on beehive health? If you like, please also add the source names in the blank spaces below. • Scientific journals • Beekeeping • National bee health agencies • Newspapers • Television/radio • Social media • Online training courses • Training courses in person • Other beekeepers • NGOs • Other (please specify)

Q9	In your opinion, what are the reasons for the decline of bees?
	• The loss of natural habitats (floral and nesting resources)
	• The competition between managed and wild pollinators
	• Diseases
	• Parasites
	• Predators
	• Climate change
	• Agrochemicals
	• Genetic factors
	• Non-optimal beekeeping practices
Q10	In your opinion, what are the actions to take to reduce the decline of bees?
	• Collaborate and exchange information with growers
	• Choose hives location carefully
	• Create or manage natural habitats and flower areas
	• Monitor diseases
	• Monitor parasites
	• Monitor nutritional stress
	• Monitor exposure to agrochemicals
	• Optimal beekeeping practices
Q11	In your opinion, what are the reasons to protect the health of bees?
	• Economic (*e.g.* pollination contracts, income, etc.)
	• Legal (*e.g.* national requirements)
	• The perceptions of the public
	• The conservation of pollinators
	• The safety of consumers
	• The security of food supplies
	• The growth of different varieties of crops
Q12	If the Bee Health Card tool was commercially available, how confident would you be that it would be effective?
	• Extremely confident
	• Very confident
	• Moderately confident
	• Slightly confident
	• Not at all confident
Q13	In your opinion, what could be the barriers to using the Bee Health Card tool?
	• Poor communication with growers
	• The cost of it
	• I am not sure it is effective
	• It seems time-consuming
	• It seems difficult to use
	• I am not aware of the importance of using it
Q14	In your opinion, what could be the benefits to you to using the Bee Health Card tool?
	• Better communication with growers
	• It helps increase productivity
	• It seems quick and easy to use
	• It reduces treatment costs
	• It enhances crop pollination
	• It increases the health of bee colonies
	• It helps protect the environment
	• It helps protect pollinators
Q15	If the Bee Health Card tool was demonstrated to diagnose colony health issues efficiently and improve the colony performance, would you be interested in using it with economic incentives (*e.g.* subsidies, grants, certified products, etc.)?
	• Yes, even with extra costs to me
	• Yes, only if there were no extra costs to me
	• No
Q16	If the Bee Health Card tool was demonstrated to diagnose colony health issues efficiently and improve the colony performance, would you be interested in using it without economic incentives (*i.e.* no subsidies, grants, certified products, etc.)?
	• Yes, even with extra costs to me
	• Yes, only if there were no extra costs to me
	• No

Q17	Considering the expected benefits and cost, how many times in a typical year would you use the Bee Health Card tool with economic incentives (*e.g.* subsidies, grants, certified products, etc.)? • Regularly (at least once a month) • Irregularly (a few times a year) • Only with a reasonable suspicion • Never
Q18	Considering the expected benefits and cost, how many times in a typical year would you use the Bee Health Card tool without economic incentives (*i.e.* no subsidies, grants, certified products, etc.)? • Regularly (at least once a month) • Irregularly (a few times a year) • Only with a reasonable suspicion • Never
Q19	In your opinion, are there any specific health issues that you would like the Health Card tool to be able to detect in your colonies? (open answer)

Part B: Channels used to advertise the survey

All channels used for advertising the survey are listed below. WP1= Work Package 1, the large-scale fieldwork carried out in 8 PoshBee countries in 2019 (UK fieldwork used for thesis chapter 2).

A reminder to ask WP1 leaders to further advertise the survey was sent on October 24th, 2020.

Country	Advertisement channels
Estonia	Local Estonian beekeepers associations
Germany	Local German beekeepers associations
Ireland	'FIBKA' Facebook page and Sept 2020 newsletter
	'NIHBS' Aug 2020 News Update
	'Beekeepers of Ireland' Facebook page
	'Cork Beekeepers' Facebook page
	Twitter account of WP1 leader for Ireland
Italy	'UNAAPI' Facebook page
Spain	Twitter and Facebook accounts of WP1 leader for Spain
	'ADEA-ASAJA' contact list and Twitter account
Switzerland	Local Swiss beekeeping associations
UK	'BBKA' Facebook and Twitter pages, website
	Kent beekeepers involved in WP1
	'Barnsley BKA', circulated to members
	'Mid Bucks BKA' Aug 2020 newsletter
	'Winchester BKA' Aug 2020 newsletter
	'Bee Craft Magazine' Sept 2020 issue
	'Rustley BKA', circulated to members
	Twitter and Facebook accounts of WP1 leader for the UK
Other sources	
Pensoft	PoshBee Twitter, Facebook, website

Part C: Survey advertisements

The advertisements shown in this section include a QR code directed to growers, which were initially targeted for a second survey to investigate their interests in the Bee Health Card. However, due to the very low number of responses, the growers' survey was not included in any analysis.

EU Horizon 2020 Research and Innovation Action

PoshBee

New bee health tool:

How can it fit your needs?

Elena Cini
PhD student
University of Reading - PoshBee project

TAKE THE SURVEY TO
CONTRIBUTE TO OUR RESEARCH!

BEEKEEPERS

GROWERS

↗ https://bit.ly/38KN4qj

↗ https://bit.ly/3ffMAbj

University of **Reading**

Our research

What are we doing?

The PoshBee project is developing a new tool that gives an overview of the health status of beehives, involving 8 countries: Estonia, Germany, Ireland, Italy, Spain, Sweden, Switzerland, and the UK.

What are the benefits to beekeepers and growers?

The tool will give a rapid insight into the health of the colony. Beekeepers will be assisted with an early identification of beehive issues (pesticide exposure, diseases, malnutrition, etc.), while growers will be provided with information on adopting better farm management systems and more reliable honeybee pollination services.

Our surveys

We have prepared one survey for beekeepers and another for growers that work in one of the 8 countries involved. By filling these surveys, you can help us investigate incentives and barriers to the use of the new tool. Surveys are completely anonymous and take 10-15 minutes to complete.

Your contribution is important!

Beekeepers and growers are key to understanding motivations as well as barriers to the use of the new tool; your contribution will help us identify and tackle potential issues, refine the tool, and ensure it will be widely used.

For more information:

Elena Cini: e.cini@pgr.reading.ac.uk

↗ poshbee.eu

PoshBee

PoshBee

NEW BEE HEALTH TOOL:
HOW CAN IT FIT YOUR NEEDS?

The PoshBee project

Bees are facing multiple threats worldwide, such as pesticides, diseases, and malnutrition, that may also interact between each other. This is resulting in a decline in their populations, which needs to be investigated and halted.

The PoshBee project, funded by the EU Horizon 2020 research programme, aims to assess the exposure of bees to such threats and their interactions.

The new bee health tool

In order to improve the health of the beehives, PoshBee is developing a new tool with the involvement of 8 countries: Estonia, Germany, Ireland, Italy, Spain, Sweden, Switzerland, and the UK. Such tool is expected to be one of the main outputs of PoshBee.

Benefits to beekeepers and growers

The tool purpose is to give a rapid insight into the health of the beehives. Beekeepers will be assisted with an early identification of health issues in the beehives, while growers will be provided with information on adopting better farm management systems and more reliable honeybee pollination services.

Our surveys

Beekeepers' and growers' interests in the tool are key to ensuring its wide use. For this purpose, we have prepared one survey for beekeepers and another for growers that work in one of the 8 countries involved.

Surveys are anonymous and take around 10-15 minutes to complete. By filling them, respondents will help us investigate incentives and barriers to the use of the tool, allowing us to identify and tackle potential issues; These insights from across Europe will enable to refine the tool to fit the needs of end users.

Answer the surveys using the QR codes or the links below

BEEKEEPERS

https://bit.ly/38KN4aj

GROWERS

https://bit.ly/3ftMAbj

For more information: Elena Cini ✉ e.cini@pgr.reading.ac.uk

Appendix 4.2

This appendix shows the correlations between all survey variables, tested with Kendall's non-parametric test.

Coloured cells flag significant correlations (* correlation at α=0.05, ** correlation at α=0.01).

Kendall's non-parametric test for correlations		Country	Professional beekeeping	Years as hobbyist beekeeper	Years as professional beekeeper	N° hives per year	BKA member	Disease check	Parasite check	Nutrition check	Chemical check
Country	Coeff	1,000	-0,076	,082*	-,082*	-,082*	0,043	,082*	,079*	,110**	-,078*
	p-value		0,066	0,018	0,035	0,016	0,293	0,025	0,031	0,003	0,033
Professional beekeeping	Coeff	-0,076	1,000	-,093*	,933**	,501**	-0,065	0,081	0,078	0,003	,190**
	p-value	0,066		0,017	0,000	0,000	0,160	0,051	0,061	0,936	0,000
Years as hobbyist beekeeper	Coeff	,082*	-,093*	1,000	-,086*	,226**	-0,001	-,071*	-0,050	-0,050	-0,031
	p-value	0,018	0,017		0,020	0,000	0,976	0,040	0,154	0,151	0,382
Years as professional beekeeper	Coeff	-,082*	,933**	-,086*	1,000	,505**	-0,051	0,070	0,067	-0,009	,190**
	p-value	0,035	0,000	0,020		0,000	0,246	0,075	0,087	0,817	0,000
N° hives per year	Coeff	-,082*	,501**	,226**	,505**	1,000	-0,042	0,005	0,023	-0,047	,176**
	p-value	0,016	0,000	0,000	0,000		0,273	0,885	0,498	0,176	0,000
BKA member	Coeff	0,043	-0,065	-0,001	-0,051	-0,042	1,000	0,040	0,004	0,050	-0,078
	p-value	0,293	0,160	0,976	0,246	0,273		0,333	0,918	0,224	0,062
Disease check	Coeff	,082*	0,081	-,071*	0,070	0,005	0,040	1,000	,616**	,460**	,229**
	p-value	0,025	0,051	0,040	0,075	0,885	0,333		0,000	0,000	0,000
Parasite check	Coeff	,079*	0,078	-0,050	0,067	0,023	0,004	,616**	1,000	,364**	,271**
	p-value	0,031	0,061	0,154	0,087	0,498	0,918	0,000		0,000	0,000
Nutrition check	Coeff	,110**	0,003	-0,050	-0,009	-0,047	0,050	,460**	,364**	1,000	,190**
	p-value	0,003	0,936	0,151	0,817	0,176	0,224	0,000	0,000		0,000
Chemical check	Coeff	-,078*	,190**	-0,031	,190**	,176**	-0,078	,229**	,271**	,190**	1,000
	p-value	0,033	0,000	0,382	0,000	0,000	0,062	0,000	0,000	0,000	
Communication with growers	Coeff	-0,052	,281**	0,013	,274**	,291**	-0,038	0,029	0,069	-0,009	,223**
	p-value	0,173	0,000	0,721	0,000	0,000	0,371	0,452	0,072	0,823	0,000
Info: Journals	Coeff	-0,025	,137**	0,056	,137**	,189**	-0,027	,110**	,081*	0,034	,142**
	p-value	0,492	0,001	0,110	0,001	0,000	0,510	0,003	0,028	0,359	0,000
Info: BKA	Coeff	0,039	-,091*	-,101**	-,082*	-,132**	,263**	,151**	0,098*	,117**	0,005
	p-value	0,312	0,037	0,006	0,046	0,000	0,000	0,000	0,011	0,002	0,897
Info: NBHA	Coeff	,276**	-,087*	0,040	-,093*	-,114**	,106*	,156**	,105**	,157**	-0,005
	p-value	0,000	0,039	0,254	0,020	0,001	0,011	0,000	0,005	0,000	0,905
Info: newspapers	Coeff	-0,047	,093*	-0,038	,091*	,069*	-0,043	,145**	,101**	,085*	,134**
	p-value	0,198	0,026	0,277	0,022	0,046	0,296	0,000	0,007	0,022	0,000

Kendall's non-parametric test for correlations		Country	Professional beekeeping	Years as hobbyist beekeeper	Years as professional beekeeper	N° hives per year	BKA member	Disease check	Parasite check	Nutrition check	Chemical check
Info:TV/Radio	Coeff	-0,072	,100*	-0,030	,086*	0,044	-0,027	,084*	,083*	0,056	,171**
	p-value	0,055	0,018	0,401	0,032	0,208	0,522	0,025	0,028	0,141	0,000
Info: SM	Coeff	-0,058	,101*	-,091**	,083*	0,041	0,014	,092*	0,036	0,046	,096*
	p-value	0,117	0,016	0,009	0,036	0,234	0,734	0,013	0,332	0,212	0,010
Info: Online training	Coeff	0,058	,117**	-0,056	,095*	0,064	-0,032	,136**	,118**	0,067	,167**
	p-value	0,113	0,005	0,111	0,015	0,066	0,434	0,000	0,001	0,072	0,000
Info: training in person	Coeff	,091*	0,021	-0,058	0,012	-0,002	,106*	,171**	,110**	,109**	0,061
	p-value	0,015	0,628	0,106	0,775	0,945	0,012	0,000	0,004	0,004	0,112
Info: beekeepers	Coeff	-,082*	-0,037	-,135**	-0,045	-,121**	,107*	,098*	0,064	,105**	-0,008
	p-value	0,031	0,388	0,000	0,271	0,001	0,012	0,010	0,094	0,006	0,839
Info: NGOs	Coeff	0,003	,095*	-0,047	,089*	0,029	0,004	,141**	,106**	0,060	,156**
	p-value	0,930	0,025	0,189	0,026	0,406	0,928	0,000	0,005	0,113	0,000
Bee decline: habitat loss	Coeff	0,038	0,044	-0,053	0,035	0,021	-0,011	0,013	0,054	0,065	0,053
	p-value	0,348	0,336	0,166	0,420	0,581	0,806	0,756	0,185	0,111	0,199
Bee decline: competition wild/manage	Coeff	0,074	-0,070	-,079*	-0,074	-,113**	-0,045	,077*	0,070	0,027	0,071
	p-value	0,056	0,113	0,031	0,077	0,002	0,300	0,050	0,072	0,486	0,072

Kendall's non-parametric test for correlations		Country	Professional beekeeping	Years as hobbyist beekeeper	Years as professional beekeeper	N° hives per year	BKA member	Disease check	Parasite check	Nutrition check	Chemical check
Bee decline: diseases	Coeff	0,000	,102*	-0,058	,087*	0,021	-0,049	,129**	,143**	,125**	,111**
	p-value	0,999	0,025	0,127	0,042	0,575	0,271	0,001	0,000	0,002	0,006
Bee decline: parasites	Coeff	0,009	,129**	0,009	,118**	,093*	-0,002	,128**	,141**	,159**	,104*
	p-value	0,826	0,005	0,810	0,006	0,014	0,959	0,002	0,001	0,000	0,011
Bee decline: predators	Coeff	,103**	,105*	-,128**	,102*	0,023	-0,049	,099*	,107**	,081*	,089*
	p-value	0,008	0,017	0,000	0,013	0,525	0,255	0,011	0,006	0,038	0,023
Bee decline: climate change	Coeff	0,060	,123**	-,100**	,122**	0,032	-0,003	0,075	0,028	,079*	,119**
	p-value	0,126	0,006	0,007	0,004	0,389	0,944	0,058	0,471	0,045	0,003
Bee decline: agrochemicals	Coeff	-0,062	-0,051	-0,044	-0,034	-0,054	0,032	0,029	0,005	0,043	0,036
	p-value	0,127	0,268	0,248	0,433	0,156	0,480	0,473	0,899	0,295	0,378
Bee decline: genetics	Coeff	0,051	0,063	-,081*	0,052	-0,026	-0,003	,090*	,094*	,104**	0,074
	p-value	0,184	0,149	0,027	0,207	0,473	0,953	0,021	0,016	0,007	0,059
Bee decline: beekeeping	Coeff	0,054	0,068	0,053	0,065	,085*	0,040	0,039	0,048	0,063	,122**
	p-value	0,170	0,124	0,157	0,123	0,022	0,369	0,323	0,229	0,112	0,002
Reduce decline: growers collab	Coeff	-,150**	0,022	0,028	0,016	,103**	0,039	0,019	,106**	0,007	,135**
	p-value	0,000	0,637	0,472	0,716	0,007	0,394	0,646	0,010	0,870	0,001
Reduce decline: hive position	Coeff	-0,040	0,014	-0,022	0,011	0,028	0,008	,128**	,087*	,099*	,137**
	p-value	0,319	0,767	0,560	0,793	0,454	0,863	0,002	0,032	0,015	0,001
Reduce decline: habitat	Coeff	-0,009	-,092*	-0,025	-0,070	-0,042	,140**	,107**	,090*	,094*	0,048
	p-value	0,831	0,046	0,515	0,109	0,278	0,002	0,009	0,028	0,022	0,245
Reduce decline: monitor	Coeff	0,031	-0,014	-0,002	-0,013	-0,013	,117*	,098*	,120**	,138**	0,023
	p-value	0,449	0,759	0,956	0,764	0,737	0,011	0,017	0,003	0,001	0,585
Reduce decline: monitor	Coeff	0,052	0,022	-0,017	0,019	-0,005	0,089	,107**	,155**	,174**	0,026
	p-value	0,204	0,641	0,653	0,662	0,902	0,052	0,010	0,000	0,000	0,537
Reduce decline: monitor	Coeff	0,036	0,061	-0,065	0,057	-0,003	0,035	,114**	,145**	,254**	,131**
	p-value	0,377	0,183	0,091	0,192	0,947	0,444	0,005	0,000	0,000	0,001
Reduce decline: monitor	Coeff	-0,015	-0,005	-,121**	-0,001	-0,042	0,024	,150**	,155**	,123**	,129**
	p-value	0,709	0,921	0,002	0,979	0,267	0,605	0,000	0,000	0,003	0,002

Kendall's non-parametric test for correlations		Country	Professional beekeeping	Years as hobbyist beekeeper	Years as professional beekeeper	N° hives per year	BKA member	Disease check	Parasite check	Nutrition check	Chemical check
Reduce decline: beekeeping	Coeff	-0,002	0,010	-0,035	0,005	0,026	0,051	,143**	,113**	,115**	,088*
	p-value	0,957	0,832	0,371	0,911	0,499	0,265	0,000	0,006	0,005	0,033
Bee health: economic	Coeff	0,016	,126**	-0,053	,117**	0,019	-0,009	,122**	,147**	,131**	0,054
	p-value	0,675	0,005	0,160	0,006	0,613	0,846	0,002	0,000	0,001	0,180
Bee health: legal	Coeff	-0,006	0,018	-0,003	0,023	0,047	-0,050	0,054	,084*	,094*	,093*
	p-value	0,878	0,680	0,932	0,589	0,197	0,254	0,168	0,034	0,017	0,019
Bee health: public perception	Coeff	0,065	0,048	0,013	0,050	0,037	0,002	0,062	0,065	0,052	,083*
	p-value	0,096	0,282	0,734	0,231	0,317	0,963	0,117	0,101	0,190	0,036
Bee health: pollinators conservation	Coeff	0,003	-0,041	0,011	-0,036	-0,018	,094*	0,001	-0,009	0,007	-0,001
	p-value	0,945	0,373	0,775	0,413	0,646	0,041	0,972	0,821	0,864	0,979
Bee health: consumer safety	Coeff	-0,063	0,040	-,075*	0,044	0,054	0,065	,117**	,110**	0,042	0,062
	p-value	0,111	0,374	0,045	0,306	0,146	0,148	0,003	0,006	0,298	0,123
Bee health: food security	Coeff	-0,003	0,023	-0,070	0,029	-0,053	,099*	,112**	0,069	,093*	0,011
	p-value	0,941	0,619	0,065	0,505	0,162	0,029	0,006	0,088	0,022	0,780
Bee health: crop varieties	Coeff	-0,044	-0,002	0,028	0,011	-0,025	0,074	0,073	0,004	0,068	0,038
	p-value	0,277	0,967	0,473	0,795	0,503	0,104	0,075	0,918	0,096	0,356
BHC effectiveness: confidence	Coeff	-,078*	-0,077	-,084*	-,098*	-0,058	0,015	0,047	0,074	0,021	,118**
	p-value	0,048	0,086	0,026	0,022	0,120	0,742	0,240	0,065	0,601	0,003
BHC barriers: growers	Coeff	-0,030	0,066	0,017	0,073	0,067	0,010	0,020	0,052	0,019	,129**
	p-value	0,450	0,141	0,652	0,085	0,074	0,824	0,620	0,196	0,630	0,001
BHC barriers: cost	Coeff	0,075	-0,069	0,009	-0,060	-,080*	0,052	0,051	0,052	,086*	-0,010
	p-value	0,056	0,126	0,801	0,160	0,032	0,243	0,203	0,189	0,030	0,798

Kendall's non-parametric test for correlations		Country	Professional beekeeping	Years as hobbyist beekeeper	Years as professional beekeeper	N° hives per year	BKA member	Disease check	Parasite check	Nutrition check	Chemical check
BHC barriers: effectiveness	Coeff	0,074	0,026	0,028	0,037	0,042	0,009	-0,004	0,028	0,032	0,021
	p-value	0,055	0,549	0,440	0,374	0,246	0,844	0,910	0,478	0,406	0,587
BHC barriers: time	Coeff	0,006	0,042	0,024	0,058	0,045	0,038	0,037	0,035	0,052	0,038
	p-value	0,871	0,343	0,507	0,162	0,218	0,380	0,344	0,369	0,181	0,327
BHC barriers: difficult	Coeff	-0,011	0,069	0,022	,083*	0,060	0,000	0,036	0,054	0,036	0,049
	p-value	0,771	0,120	0,559	0,047	0,104	0,994	0,359	0,170	0,359	0,220
BHC barriers: no importance	Coeff	0,065	0,039	0,061	0,048	0,030	0,001	-0,043	-0,045	-0,010	-0,045
	p-value	0,092	0,377	0,100	0,247	0,417	0,978	0,271	0,248	0,793	0,249
BHC benefits: growers	Coeff	-0,051	,111*	-0,017	,092*	,084*	-0,057	0,040	,116**	0,001	,185**
	p-value	0,194	0,013	0,654	0,029	0,023	0,198	0,314	0,003	0,983	0,000
BHC benefits: productivity	Coeff	-0,058	,122**	-,112**	,091*	-0,012	0,024	,084*	0,063	0,062	0,061
	p-value	0,141	0,006	0,003	0,030	0,739	0,591	0,033	0,112	0,118	0,122
BHC benefits: quick and easy	Coeff	-0,009	-0,020	-0,027	-0,047	-0,043	-0,014	0,020	0,023	-0,025	-0,003
	p-value	0,811	0,650	0,469	0,262	0,241	0,760	0,622	0,567	0,525	0,934
BHC benefits: treatment cost	Coeff	-0,062	0,003	-0,065	-0,017	-0,066	0,010	0,035	0,055	0,029	0,052
	p-value	0,110	0,949	0,076	0,681	0,069	0,823	0,378	0,158	0,466	0,189
BHC benefits: crop pollination	Coeff	-0,071	-0,017	-0,055	-0,030	-0,058	-0,011	0,011	0,001	-0,002	0,052
	p-value	0,066	0,696	0,135	0,469	0,116	0,804	0,770	0,981	0,954	0,192

Kendall's non-parametric test for correlations		Communication with growers	Info: Journals	Info: BKA	Info: NBHA	Info: newspapers	Info: TV/Radio	Info: Social media	Info: Online training	Info: training in person	Info: other beekeepers	Info: NGOs	Bee decline: habitat loss	Bee decline: competition wild/managed pollinators
Country	Coeff	-0,052	-0,025	0,039	,276**	-0,047	-0,072	-0,058	0,058	,091*	-,082*	0,003	0,038	0,074
	p-value	0,173	0,492	0,312	0,000	0,198	0,055	0,117	0,113	0,015	0,031	0,930	0,348	0,056
Professional beekeeping	Coeff	,281**	,137**	-,091*	-,087*	,093*	,100*	,101*	,117**	0,021	-0,037	,095*	0,044	-0,070
	p-value	0,000	0,001	0,037	0,039	0,026	0,018	0,016	0,005	0,628	0,388	0,025	0,336	0,113
Years as hobbyist beekeeper	Coeff	0,013	0,056	-,101**	0,040	-0,038	-0,030	-,091**	-0,056	-0,058	-,135**	-0,047	-0,053	-,079*
	p-value	0,721	0,110	0,006	0,254	0,277	0,401	0,009	0,111	0,106	0,000	0,189	0,166	0,031
Years as professional beekeeper	Coeff	,274**	,137**	-,082*	-,093*	,091*	,086*	,083*	,095*	0,012	-0,045	,089*	0,035	-0,074
	p-value	0,000	0,001	0,046	0,020	0,022	0,032	0,036	0,015	0,775	0,271	0,026	0,420	0,077
N° hives per year	Coeff	,291**	,189**	-,132**	-,114**	,069*	0,044	0,041	0,064	-0,002	-,121**	0,029	0,021	-,113**
	p-value	0,000	0,000	0,000	0,001	0,046	0,208	0,234	0,066	0,945	0,001	0,406	0,581	0,002
BKA member	Coeff	-0,038	-0,027	,263**	,106*	-0,043	-0,027	0,014	-0,032	,106*	,107*	0,004	-0,011	-0,045
	p-value	0,371	0,510	0,000	0,011	0,296	0,522	0,734	0,434	0,012	0,012	0,928	0,806	0,300
Disease check	Coeff	0,029	,110**	,151**	,156**	,145**	,084*	,092*	,136**	,171**	,098*	,141**	0,013	,077*
	p-value	0,452	0,003	0,000	0,000	0,000	0,025	0,013	0,000	0,000	0,010	0,000	0,756	0,050
Parasite check	Coeff	0,069	,081*	,098*	,105**	,101**	,083*	0,036	,118**	,110**	0,064	,106**	0,054	0,070
	p-value	0,072	0,028	0,011	0,005	0,007	0,028	0,332	0,001	0,004	0,094	0,005	0,185	0,072
Nutrition check	Coeff	-0,009	0,034	,117**	,157**	,085*	0,056	0,046	0,067	,109**	,105**	0,060	0,065	0,027
	p-value	0,823	0,359	0,002	0,000	0,022	0,141	0,212	0,072	0,004	0,006	0,113	0,111	0,486
Chemical check	Coeff	,223**	,142**	0,005	-0,005	,134**	,171**	,096*	,167**	0,061	-0,008	,156**	0,053	0,071
	p-value	0,000	0,000	0,897	0,905	0,000	0,000	0,010	0,000	0,112	0,839	0,000	0,199	0,072
Communication with growers	Coeff	1,000	,220**	-0,006	-0,064	,113**	,122**	,116**	,198**	,173**	-0,017	,097*	0,001	-,104*
	p-value		0,000	0,886	0,098	0,003	0,002	0,003	0,000	0,000	0,662	0,013	0,985	0,010
Info: Journals	Coeff	,220**	1,000	,153**	,192**	,305**	,243**	,126**	,264**	,267**	0,067	,317**	0,080	0,012
	p-value	0,000		0,000	0,000	0,000	0,000	0,001	0,000	0,000	0,081	0,000	0,052	0,763
Info: BKA	Coeff	-0,006	,153**	1,000	,365**	,186**	,225**	,184**	,198**	,390**	,368**	,241**	,153**	-0,010
	p-value	0,886	0,000		0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,802
Info: NBHA	Coeff	-0,064	,192**	,365**	1,000	,170**	,249**	,078*	,212**	,314**	,132**	,284**	,082*	0,046
	p-value	0,098	0,000	0,000		0,000	0,000	0,038	0,000	0,000	0,001	0,000	0,047	0,251

Kendall's non-parametric test for correlations		Communication with growers	Info: Journals	Info: BKA	Info: NBHA	Info: newspapers	Info: TV/Radio	Info: Social media	Info: Online training	Info: training in person	Info: other beekeepers	Info: NGOs	Bee decline: habitat loss	Bee decline: competition wild/managed pollinators
Info: newspapers	Coeff	,113**	,305**	,186**	,170**	1,000	,453**	,263**	,303**	,191**	,128**	,270**	0,031	0,070
	p-value	0,003	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,001	0,000	0,455	0,075
Info:TV/Radio	Coeff	,122**	,243**	,225**	,249**	,453**	1,000	,414**	,348**	,239**	,185**	,381**	,084*	,112**
	p-value	0,002	0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000	0,043	0,005
Info: SM	Coeff	,116**	,126**	,184**	,078*	,263**	,414**	1,000	,371**	,227**	,178**	,270**	,159**	0,070
	p-value	0,003	0,001	0,000	0,038	0,000	0,000		0,000	0,000	0,000	0,000	0,000	0,073
Info: Online training	Coeff	,198**	,264**	,198**	,212**	,303**	,348**	,371**	1,000	,395**	,185**	,310**	,081*	0,074
	p-value	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,048	0,058
Info: training in person	Coeff	,173**	,267**	,390**	,314**	,191**	,239**	,227**	,395**	1,000	,291**	,250**	,085*	0,044
	p-value	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,000	0,000	0,041	0,277
Info: beekeepers	Coeff	-0,017	0,067	,368**	,132**	,128**	,185**	,178**	,185**	,291**	1,000	,202**	0,074	0,049
	p-value	0,662	0,081	0,000	0,001	0,001	0,000	0,000	0,000	0,000		0,000	0,078	0,226
Info: NGOs	Coeff	,097*	,317**	,241**	,284**	,270**	,381**	,270**	,310**	,250**	,202**	1,000	,094*	,125**
	p-value	0,013	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,023	0,002
Bee decline: habitat loss	Coeff	0,001	0,080	,153**	,082*	0,031	,084*	,159**	,081*	,085*	0,074	,094*	1,000	,143**
	p-value	0,985	0,052	0,000	0,047	0,455	0,043	0,000	0,048	0,041	0,078	0,023		0,001
Bee decline: competition wild/manage	Coeff	-,104*	0,012	-0,010	0,046	0,070	,112**	0,070	0,074	0,044	0,049	,125**	,143**	1,000
	p-value	0,010	0,763	0,802	0,251	0,075	0,005	0,073	0,058	0,277	0,226	0,002	0,001	

Kendall's non-parametric test for correlations		Communication with growers	Info: Journals	Info: BKA	Info: NBHA	Info: newspapers	Info: TV/Radio	Info: Social media	Info: Online training	Info: training in person	Info: other beekeepers	Info: NGOs	Bee decline: habitat loss	Bee decline: competition wild/managed pollinators
Bee decline: diseases	Coeff	0,028	0,011	,106*	0,078	0,068	,112**	0,076	,101*	0,062	0,041	,148**	-0,060	0,016
	p-value	0,499	0,792	0,012	0,058	0,094	0,007	0,059	0,013	0,133	0,331	0,000	0,180	0,709
Bee decline: parasites	Coeff	0,031	0,026	0,058	0,056	0,008	0,021	0,018	0,051	0,056	0,023	,083*	-0,011	0,016
	p-value	0,462	0,527	0,170	0,173	0,838	0,611	0,653	0,208	0,180	0,581	0,044	0,812	0,706
Bee decline: predators	Coeff	-0,015	-0,021	0,035	0,039	0,068	,164**	,167**	,164**	0,045	,081*	,098*	,089*	,183**
	p-value	0,707	0,598	0,387	0,321	0,084	0,000	0,000	0,000	0,260	0,044	0,013	0,037	0,000
Bee decline: climate change	Coeff	0,073	,110**	,153**	0,072	,097*	,132**	,130**	,223**	,178**	,089*	,222**	,204**	,123**
	p-value	0,072	0,006	0,000	0,073	0,015	0,001	0,001	0,000	0,000	0,029	0,000	0,000	0,003
Bee decline: agrochemicals	Coeff	-0,054	-0,020	0,082	-0,009	0,069	0,056	0,068	0,079	0,022	0,057	,089*	,237**	0,075
	p-value	0,203	0,623	0,055	0,825	0,094	0,181	0,095	0,054	0,592	0,177	0,032	0,000	0,080
Bee decline: genetics	Coeff	0,038	0,037	0,020	,100*	,097*	,147**	,134**	,088*	0,067	0,067	,161**	0,063	,194**
	p-value	0,342	0,341	0,616	0,012	0,014	0,000	0,001	0,024	0,093	0,099	0,000	0,144	0,000
Bee decline: beekeeping	Coeff	,103*	,178**	-0,034	,101*	0,072	,092*	0,058	,116**	0,063	-0,024	,119**	-0,012	,115**
	p-value	0,012	0,000	0,406	0,012	0,069	0,023	0,147	0,003	0,124	0,558	0,003	0,788	0,006
Reduce decline: growers collab	Coeff	,227**	,135**	,125**	0,034	,135**	,182**	0,068	,138**	0,099	,125**	,109**	,090*	0,015
	p-value	0,000	0,001	0,004	0,418	0,001	0,000	0,099	0,001	0,018	0,003	0,009	0,046	0,727
Reduce decline: hive position	Coeff	,118**	,144**	,109**	,091*	,085*	,151**	,089*	,150**	,148**	,089*	,104*	0,046	0,031
	p-value	0,005	0,000	0,010	0,028	0,038	0,000	0,029	0,000	0,000	0,035	0,012	0,307	0,471
Reduce decline: habitat	Coeff	-0,033	0,031	,087*	0,016	-0,035	-0,040	0,027	0,015	0,060	,150**	0,024	,233**	0,041
	p-value	0,443	0,457	0,042	0,702	0,397	0,340	0,521	0,721	0,155	0,000	0,566	0,000	0,342
Reduce decline: monitor	Coeff	0,022	0,076	,098*	,142**	0,033	,140**	0,063	,100*	0,066	-0,041	,142**	0,034	0,079
	p-value	0,606	0,064	0,022	0,001	0,428	0,001	0,126	0,016	0,121	0,330	0,001	0,447	0,070
Reduce decline: monitor	Coeff	0,050	0,067	,102*	,158**	0,038	,115**	,086*	0,071	0,075	0,002	,148**	0,057	0,081
	p-value	0,243	0,105	0,018	0,000	0,354	0,006	0,037	0,084	0,076	0,970	0,000	0,211	0,062
Reduce decline: monitor	Coeff	,093*	,138**	,137**	,162**	0,074	,111**	,127**	,152**	,131**	0,061	,130**	,112*	,086*
	p-value	0,028	0,001	0,001	0,000	0,070	0,008	0,002	0,000	0,002	0,149	0,002	0,013	0,046
Reduce decline: monitor	Coeff	0,015	0,025	,139**	0,076	,094*	,133**	0,071	,155**	,137**	,127**	,127**	,177**	,143**
	p-value	0,724	0,542	0,001	0,069	0,022	0,001	0,083	0,000	0,001	0,003	0,002	0,000	0,001

Kendall's non-parametric test for correlations		Communication with growers	Info: Journals	Info: BKA	Info: NBHA	Info: newspapers	Info: TV/Radio	Info: Social media	Info: Online training	Info: training in person	Info: other beekeepers	Info: NGOs	Bee decline: habitat loss	Bee decline: competition wild/managed pollinators
Reduce decline: beekeeping	Coeff	0,030	,151**	0,083	,124**	,155**	,123**	0,039	,141**	0,079	0,082	,099*	0,060	0,053
	p-value	0,483	0,000	0,053	0,003	0,000	0,003	0,348	0,001	0,062	0,053	0,018	0,186	0,226
Bee health: economic	Coeff	-0,044	-0,005	0,017	0,030	0,018	0,042	,079*	0,055	-0,038	-0,042	,140**	0,030	,113**
	p-value	0,286	0,904	0,690	0,462	0,652	0,296	0,047	0,165	0,354	0,308	0,001	0,493	0,007
Bee health: legal	Coeff	0,056	,185**	,086*	,117**	,156**	,202**	,080*	,148**	,097*	-0,006	,180**	,095*	,212**
	p-value	0,173	0,000	0,037	0,003	0,000	0,000	0,045	0,000	0,016	0,879	0,000	0,029	0,000
Bee health: public perception	Coeff	,091*	,097*	0,075	,161**	,207**	,258**	,141**	,195**	,133**	0,027	,199**	,106*	,086*
	p-value	0,026	0,014	0,070	0,000	0,000	0,000	0,000	0,000	0,001	0,507	0,000	0,015	0,039
Bee health: pollinators conservation	Coeff	0,026	,128**	,107*	0,066	0,066	,104*	,112**	,130**	0,078	0,063	,094*	0,069	0,076
	p-value	0,534	0,002	0,013	0,112	0,110	0,013	0,007	0,002	0,064	0,140	0,024	0,129	0,080
Bee health: consumer safety	Coeff	0,060	0,006	,091*	0,046	0,070	,168**	,107**	,147**	0,066	0,001	,122**	,099*	0,073
	p-value	0,147	0,878	0,030	0,257	0,081	0,000	0,008	0,000	0,107	0,972	0,003	0,025	0,086
Bee health: food security	Coeff	0,037	0,024	,125**	0,067	0,067	,150**	,147**	,140**	0,065	-0,032	,140**	,092*	,137**
	p-value	0,382	0,552	0,003	0,102	0,101	0,000	0,000	0,001	0,119	0,447	0,001	0,040	0,001
Bee health: crop varieties	Coeff	,108*	0,047	,182**	0,079	,144**	,098*	,095*	,127**	,099*	,093*	,119**	0,012	0,048
	p-value	0,010	0,255	0,000	0,056	0,000	0,018	0,020	0,002	0,019	0,027	0,004	0,788	0,269
BHC effectiveness: confidence	Coeff	,100*	,099*	,153**	0,076	,128**	,235**	,164**	,247**	,083*	,161**	,145**	,111*	,098*
	p-value	0,015	0,014	0,000	0,062	0,001	0,000	0,000	0,000	0,043	0,000	0,000	0,012	0,021
BHC barriers: growers	Coeff	,120**	,082*	,089*	-0,002	0,073	,114**	0,069	,130**	,100*	-0,050	,139**	,139**	0,062
	p-value	0,004	0,041	0,034	0,965	0,070	0,005	0,084	0,001	0,015	0,230	0,001	0,002	0,145
BHC barriers: cost	Coeff	-,108**	-0,032	-0,018	,106**	0,027	0,047	0,001	-0,042	-0,013	-0,046	0,031	0,032	,088*
	p-value	0,008	0,430	0,666	0,009	0,504	0,247	0,989	0,289	0,743	0,266	0,451	0,467	0,038

Kendall's non-parametric test for correlations		Communication with growers	Info: Journals	Info: BKA	Info: NBHA	Info: newspapers	Info: TV/Radio	Info: Social media	Info: Online training	Info: training in person	Info: other beekeepers	Info: NGOs	Bee decline: habitat loss	Bee decline: competition wild/managed pollinators
BHC barriers: effectiveness	Coeff	0,016	-0,014	-0,054	-0,071	-0,050	-,103**	-0,054	-,082*	0,024	-0,031	-,083*	0,031	0,002
	p-value	0,699	0,722	0,182	0,073	0,203	0,010	0,172	0,035	0,555	0,435	0,036	0,469	0,970
BHC barriers: time	Coeff	0,003	0,053	0,034	0,015	0,030	0,048	0,036	-0,004	,097*	0,031	0,051	0,065	0,074
	p-value	0,949	0,172	0,410	0,700	0,447	0,226	0,362	0,916	0,016	0,441	0,201	0,131	0,071
BHC barriers: difficult	Coeff	0,019	0,032	0,028	-0,004	0,045	0,046	0,001	-0,032	0,068	-0,025	0,022	0,005	0,057
	p-value	0,648	0,419	0,496	0,917	0,254	0,247	0,979	0,415	0,094	0,540	0,577	0,904	0,171
BHC barriers: no importance	Coeff	-0,009	-0,056	-0,016	-0,030	0,040	0,050	-0,007	-0,054	-0,018	-0,043	-0,044	0,058	0,063
	p-value	0,822	0,152	0,691	0,442	0,306	0,212	0,863	0,172	0,660	0,283	0,266	0,181	0,127
BHC benefits: growers	Coeff	,162**	,134**	0,047	0,017	,180**	,238**	,174**	,211**	,091*	0,051	,163**	0,073	0,069
	p-value	0,000	0,001	0,253	0,665	0,000	0,000	0,000	0,000	0,025	0,215	0,000	0,094	0,097
BHC benefits: productivity	Coeff	0,028	0,030	0,079	0,022	0,039	,215**	,213**	,113**	0,016	0,020	,155**	0,045	0,079
	p-value	0,488	0,446	0,056	0,580	0,320	0,000	0,000	0,004	0,700	0,620	0,000	0,303	0,059
BHC benefits: quick and easy	Coeff	0,013	0,042	0,044	0,038	0,041	,140**	,101*	,093*	0,009	0,036	,092*	0,073	0,046
	p-value	0,749	0,293	0,293	0,349	0,303	0,001	0,012	0,019	0,825	0,378	0,023	0,096	0,275
BHC benefits: treatment cost	Coeff	0,042	0,010	,092*	,100*	0,074	,229**	,132**	,113**	0,069	0,032	,195**	0,052	,105*
	p-value	0,295	0,808	0,024	0,012	0,058	0,000	0,001	0,004	0,088	0,436	0,000	0,227	0,011
BHC benefits: crop pollination	Coeff	0,029	0,044	,106*	,095*	,130**	,209**	,171**	,135**	0,073	0,066	,188**	0,074	,117**
	p-value	0,479	0,267	0,010	0,017	0,001	0,000	0,000	0,001	0,071	0,102	0,000	0,088	0,005

Kendall's non-parametric test for correlations		Bee decline: diseases	Bee decline: parasites	Bee decline: predators	Bee decline: climate change	Bee decline: agrochemicals	Bee decline: genetics	Bee decline: beekeeping	Reduce decline: growers collab	Reduce decline: hive position	Reduce decline: habitat	Reduce decline: monitor diseases	Reduce decline: monitor parasites
Country	Coeff	0,000	0,009	,103**	0,060	-0,062	0,051	0,054	-,150**	-0,040	-0,009	0,031	0,052
	p-value	0,999	0,826	0,008	0,126	0,127	0,184	0,170	0,000	0,319	0,831	0,449	0,204
Professional beekeeping	Coeff	,102*	,129**	,105*	,123**	-0,051	0,063	0,068	0,022	0,014	-,092*	-0,014	0,022
	p-value	0,025	0,005	0,017	0,006	0,268	0,149	0,124	0,637	0,767	0,046	0,759	0,641
Years as hobbyist beekeeper	Coeff	-0,058	0,009	-,128**	-,100**	-0,044	-,081*	0,053	0,028	-0,022	-0,025	-0,002	-0,017
	p-value	0,127	0,810	0,000	0,007	0,248	0,027	0,157	0,472	0,560	0,515	0,956	0,653
Years as professional beekeeper	Coeff	,087*	,118**	,102*	,122**	-0,034	0,052	0,065	0,016	0,011	-0,070	-0,013	0,019
	p-value	0,042	0,006	0,013	0,004	0,433	0,207	0,123	0,716	0,793	0,109	0,764	0,662
N° hives per year	Coeff	0,021	,093*	0,023	0,032	-0,054	-0,026	,085*	,103**	0,028	-0,042	-0,013	-0,005
	p-value	0,575	0,014	0,525	0,389	0,156	0,473	0,022	0,007	0,454	0,278	0,737	0,902
BKA member	Coeff	-0,049	-0,002	-0,049	-0,003	0,032	-0,003	0,040	0,039	0,008	,140**	,117*	0,089
	p-value	0,271	0,959	0,255	0,944	0,480	0,953	0,369	0,394	0,863	0,002	0,011	0,052
Disease check	Coeff	,129**	,128**	,099*	0,075	0,029	,090*	0,039	0,019	,128**	,107**	,098*	,107**
	p-value	0,001	0,002	0,011	0,058	0,473	0,021	0,323	0,646	0,002	0,009	0,017	0,010
Parasite check	Coeff	,143**	,141**	,107**	0,028	0,005	,094*	0,048	,106**	,087*	,090*	,120**	,155**
	p-value	0,000	0,001	0,006	0,471	0,899	0,016	0,229	0,010	0,032	0,028	0,003	0,000
Nutrition check	Coeff	,125**	,159**	,081*	,079*	0,043	,104**	0,063	0,007	,099*	,094*	,138**	,174**
	p-value	0,002	0,000	0,038	0,045	0,295	0,007	0,112	0,870	0,015	0,022	0,001	0,000
Chemical check	Coeff	,111**	,104*	,089*	,119**	0,036	0,074	,122**	,135**	,137**	0,048	0,023	0,026
	p-value	0,006	0,011	0,023	0,003	0,378	0,059	0,002	0,001	0,001	0,245	0,585	0,537
Communication with growers	Coeff	0,028	0,031	-0,015	0,073	-0,054	0,038	,103*	,227**	,118**	-0,033	0,022	0,050
	p-value	0,499	0,462	0,707	0,072	0,203	0,342	0,012	0,000	0,005	0,443	0,606	0,243
Info: Journals	Coeff	0,011	0,026	-0,021	,110**	-0,020	0,037	,178**	,135**	,144**	0,031	0,076	0,067
	p-value	0,792	0,527	0,598	0,006	0,623	0,341	0,000	0,001	0,000	0,457	0,064	0,105
Info: BKA	Coeff	,106*	0,058	0,035	,153**	0,082	0,020	-0,034	,125**	,109**	,087*	,098*	,102*
	p-value	0,012	0,170	0,387	0,000	0,055	0,616	0,406	0,004	0,010	0,042	0,022	0,018
Info: NBHA	Coeff	0,078	0,056	0,039	0,072	-0,009	,100*	,101*	0,034	,091*	0,016	,142**	,158**
	p-value	0,058	0,173	0,321	0,073	0,825	0,012	0,012	0,418	0,028	0,702	0,001	0,000

Kendall's non-parametric test for correlations		Bee decline: diseases	Bee decline: parasites	Bee decline: predators	Bee decline: climate change	Bee decline: agrochemicals	Bee decline: genetics	Bee decline: beekeeping	Reduce decline: growers collab	Reduce decline: hive position	Reduce decline: habitat	Reduce decline: monitor diseases	Reduce decline: monitor parasites
Info: newspapers	Coeff	0,068	0,008	0,068	,097*	0,069	,097*	0,072	,135**	,085*	-0,035	0,033	0,038
	p-value	0,094	0,838	0,084	0,015	0,094	0,014	0,069	0,001	0,038	0,397	0,428	0,354
Info:TV/Radio	Coeff	,112**	0,021	,164**	,132**	0,056	,147**	,092*	,182**	,151**	-0,040	,140**	,115**
	p-value	0,007	0,611	0,000	0,001	0,181	0,000	0,023	0,000	0,000	0,340	0,001	0,006
Info: SM	Coeff	0,076	0,018	,167**	,130**	0,068	,134**	0,058	0,068	,089*	0,027	0,063	,086*
	p-value	0,059	0,653	0,000	0,001	0,095	0,001	0,147	0,099	0,029	0,521	0,126	0,037
Info: Online training	Coeff	,101*	0,051	,164**	,223**	0,079	,088*	,116**	,138**	,150**	0,015	,100*	0,071
	p-value	0,013	0,208	0,000	0,000	0,054	0,024	0,003	0,001	0,000	0,721	0,016	0,084
Info: training in person	Coeff	0,062	0,056	0,045	,178**	0,022	0,067	0,063	,099*	,148**	0,060	0,066	0,075
	p-value	0,133	0,180	0,260	0,000	0,592	0,093	0,124	0,018	0,000	0,155	0,121	0,076
Info: beekeepers	Coeff	0,041	0,023	,081*	,089*	0,057	0,067	-0,024	,125**	,089*	,150**	-0,041	0,002
	p-value	0,331	0,581	0,044	0,029	0,177	0,099	0,558	0,003	0,035	0,000	0,330	0,970
Info: NGOs	Coeff	,148**	,083*	,098*	,222**	,089*	,161**	,119**	,109**	,104*	0,024	,142**	,148**
	p-value	0,000	0,044	0,013	0,000	0,032	0,000	0,003	0,009	0,012	0,566	0,001	0,000
Bee decline: habitat loss	Coeff	-0,060	-0,011	,089*	,204**	,237**	0,063	-0,012	,090*	0,046	,233**	0,034	0,057
	p-value	0,180	0,812	0,037	0,000	0,000	0,144	0,788	0,046	0,307	0,000	0,447	0,211
Bee decline: competition wild/manage	Coeff	0,016	0,016	,183**	,123**	0,075	,194**	,115**	0,015	0,031	0,041	0,079	0,081
	p-value	0,709	0,706	0,000	0,003	0,080	0,000	0,006	0,727	0,471	0,342	0,070	0,062

Kendall's non-parametric test for correlations		Bee decline: diseases	Bee decline: parasites	Bee decline: predators	Bee decline: climate change	Bee decline: agrochemicals	Bee decline: genetics	Bee decline: beekeeping	Reduce decline: growers collab	Reduce decline: hive position	Reduce decline: habitat	Reduce decline: monitor diseases	Reduce decline: monitor parasites
Bee decline: diseases	Coeff	1,000	,615**	,257**	,166**	0,047	,203**	,167**	,123**	,167**	-0,045	,262**	,255**
	p-value		0,000	0,000	0,000	0,292	0,000	0,000	0,006	0,000	0,320	0,000	0,000
Bee decline: parasites	Coeff	,615**	1,000	,174**	,150**	,091*	,146**	,130**	,094*	,118**	0,037	,220**	,361**
	p-value	0,000		0,000	0,001	0,041	0,001	0,003	0,036	0,008	0,410	0,000	0,000
Bee decline: predators	Coeff	,257**	,174**	1,000	,313**	0,082	,247**	,126**	,090*	,154**	0,011	,161**	,151**
	p-value	0,000	0,000		0,000	0,057	0,000	0,002	0,037	0,000	0,808	0,000	0,000
Bee decline: climate change	Coeff	,166**	,150**	,313**	1,000	,234**	,190**	0,005	0,049	0,050	0,052	,179**	,218**
	p-value	0,000	0,001	0,000		0,000	0,000	0,913	0,258	0,254	0,236	0,000	0,000
Bee decline: agrochemicals	Coeff	0,047	,091*	0,082	,234**	1,000	0,045	-0,044	0,064	0,012	,191**	,098*	0,075
	p-value	0,292	0,041	0,057	0,000		0,297	0,314	0,154	0,797	0,000	0,031	0,100
Bee decline: genetics	Coeff	,203**	,146**	,247**	,190**	0,045	1,000	,242**	0,047	,123**	-0,017	,128**	,139**
	p-value	0,000	0,001	0,000	0,000	0,297		0,000	0,276	0,004	0,694	0,003	0,001
Bee decline: beekeeping	Coeff	,167**	,130**	,126**	0,005	-0,044	,242**	1,000	,116**	,287**	0,032	,180**	,106*
	p-value	0,000	0,003	0,002	0,913	0,314	0,000		0,008	0,000	0,460	0,000	0,016
Reduce decline: growers collab	Coeff	,123**	,094*	,090*	0,049	0,064	0,047	,116**	1,000	,160**	,110*	,157**	,207**
	p-value	0,006	0,036	0,037	0,258	0,154	0,276	0,008		0,000	0,015	0,001	0,000
Reduce decline: hive position	Coeff	,167**	,118**	,154**	0,050	0,012	,123**	,287**	,160**	1,000	,104*	,236**	,191**
	p-value	0,000	0,008	0,000	0,254	0,797	0,004	0,000	0,000		0,021	0,000	0,000
Reduce decline: habitat	Coeff	-0,045	0,037	0,011	0,052	,191**	-0,017	0,032	,110*	,104*	1,000	,131**	,131**
	p-value	0,320	0,410	0,808	0,236	0,000	0,694	0,460	0,015	0,021		0,004	0,004
Reduce decline: monitor	Coeff	,262**	,220**	,161**	,179**	,098*	,128**	,180**	,157**	,236**	,131**	1,000	,696**
	p-value	0,000	0,000	0,000	0,000	0,031	0,003	0,000	0,001	0,000	0,004		0,000
Reduce decline: monitor	Coeff	,255**	,361**	,151**	,218**	0,075	,139**	,106*	,207**	,191**	,131**	,696**	1,000
	p-value	0,000	0,000	0,000	0,000	0,100	0,001	0,016	0,000	0,000	0,004	0,000	
Reduce decline: monitor	Coeff	,176**	,179**	,134**	,215**	0,065	,191**	,217**	,137**	,244**	,094*	,276**	,349**
	p-value	0,000	0,000	0,002	0,000	0,151	0,000	0,000	0,002	0,000	0,037	0,000	0,000
Reduce decline: monitor	Coeff	0,024	0,032	,110*	,194**	,430**	0,037	-0,004	,199**	,133**	,191**	,179**	,151**
	p-value	0,594	0,482	0,011	0,000	0,000	0,398	0,924	0,000	0,003	0,000	0,000	0,001

Kendall's non-parametric test for correlations		Bee decline: diseases	Bee decline: parasites	Bee decline: predators	Bee decline: climate change	Bee decline: agrochemicals	Bee decline: genetics	Bee decline: beekeeping	Reduce decline: growers collab	Reduce decline: hive position	Reduce decline: habitat	Reduce decline: monitor diseases	Reduce decline: monitor parasites
Reduce decline: beekeeping	Coeff	,161**	,196**	,121**	0,077	0,013	,137**	,378**	,156**	,320**	,126**	,262**	,263**
	p-value	0,000	0,000	0,005	0,080	0,774	0,002	0,000	0,001	0,000	0,006	0,000	0,000
Bee health: economic	Coeff	,151**	,205**	,156**	,159**	0,034	0,076	0,056	0,027	0,048	0,034	,166**	,205**
	p-value	0,000	0,000	0,000	0,000	0,437	0,069	0,192	0,546	0,277	0,440	0,000	0,000
Bee health: legal	Coeff	0,071	,095*	,105*	,159**	-0,018	0,075	,160**	,094*	,116**	0,044	,167**	,129**
	p-value	0,098	0,029	0,011	0,000	0,684	0,070	0,000	0,032	0,008	0,318	0,000	0,003
Bee health: public perception	Coeff	0,043	0,073	,140**	,142**	0,006	,121**	,124**	,158**	0,083	-0,004	,150**	,106*
	p-value	0,314	0,092	0,001	0,001	0,897	0,004	0,003	0,000	0,056	0,927	0,001	0,015
Bee health: pollinators conservation	Coeff	0,022	0,071	0,050	,120**	,165**	0,033	0,025	0,081	,106*	,218**	,162**	,162**
	p-value	0,623	0,117	0,245	0,007	0,000	0,449	0,575	0,076	0,019	0,000	0,000	0,000
Bee health: consumer safety	Coeff	,133**	,125**	,209**	,171**	,089*	,119**	0,036	,116**	,168**	0,067	,220**	,229**
	p-value	0,002	0,005	0,000	0,000	0,045	0,005	0,397	0,009	0,000	0,133	0,000	0,000
Bee health: food security	Coeff	,153**	,133**	,204**	,218**	,103*	,127**	,115**	0,088	,188**	0,086	,246**	,251**
	p-value	0,001	0,003	0,000	0,000	0,022	0,003	0,008	0,050	0,000	0,057	0,000	0,000
Bee health: crop varieties	Coeff	0,064	0,080	0,048	,127**	,091*	0,059	0,060	,165**	0,088	,174**	,189**	,190**
	p-value	0,151	0,074	0,265	0,004	0,043	0,168	0,165	0,000	0,050	0,000	0,000	0,000
BHC effectiveness: confidence	Coeff	,090*	0,022	,151**	,151**	0,054	,122**	,158**	,204**	,088*	0,031	,138**	,174**
	p-value	0,038	0,616	0,000	0,000	0,224	0,004	0,000	0,000	0,045	0,491	0,002	0,000
BHC barriers: growers	Coeff	,089*	0,045	0,060	,149**	,118**	,107*	0,056	,191**	,147**	0,081	,103*	,105*
	p-value	0,042	0,301	0,151	0,000	0,007	0,011	0,187	0,000	0,001	0,069	0,021	0,018
BHC barriers: cost	Coeff	0,049	0,046	0,020	-0,074	0,084	0,043	0,053	-0,021	0,018	0,047	,109*	0,084
	p-value	0,262	0,295	0,635	0,084	0,055	0,305	0,215	0,630	0,677	0,287	0,014	0,057

Kendall's non-parametric test for correlations		Reduce decline: monitor nutrition	Reduce decline: monitor chemicals	Reduce decline: beekeeping	Bee health: economic motives	Bee health: legal motives	Bee health: public perception	Bee health: pollinators conservation	Bee health: consumer safety	Bee health: food security	Bee health: crop varieties	BHC effectiveness: confidence
Country	Coeff	0,036	-0,015	-0,002	0,016	-0,006	0,065	0,003	-0,063	-0,003	-0,044	-,078*
	p-value	0,377	0,709	0,957	0,675	0,878	0,096	0,945	0,111	0,941	0,277	0,048
Professional beekeeping	Coeff	0,061	-0,005	0,010	,126**	0,018	0,048	-0,041	0,040	0,023	-0,002	-0,077
	p-value	0,183	0,921	0,832	0,005	0,680	0,282	0,373	0,374	0,619	0,967	0,086
Years as hobbyist beekeeper	Coeff	-0,065	-,121**	-0,035	-0,053	-0,003	0,013	0,011	-,075*	-0,070	0,028	-,084*
	p-value	0,091	0,002	0,371	0,160	0,932	0,734	0,775	0,045	0,065	0,473	0,026
Years as professional beekeeper	Coeff	0,057	-0,001	0,005	,117**	0,023	0,050	-0,036	0,044	0,029	0,011	-,098*
	p-value	0,192	0,979	0,911	0,006	0,589	0,231	0,413	0,306	0,505	0,795	0,022
N° hives per year	Coeff	-0,003	-0,042	0,026	0,019	0,047	0,037	-0,018	0,054	-0,053	-0,025	-0,058
	p-value	0,947	0,267	0,499	0,613	0,197	0,317	0,646	0,146	0,162	0,503	0,120
BKA member	Coeff	0,035	0,024	0,051	-0,009	-0,050	0,002	,094*	0,065	,099*	0,074	0,015
	p-value	0,444	0,605	0,265	0,846	0,254	0,963	0,041	0,148	0,029	0,104	0,742
Disease check	Coeff	,114**	,150**	,143**	,122**	0,054	0,062	0,001	,117**	,112**	0,073	0,047
	p-value	0,005	0,000	0,000	0,002	0,168	0,117	0,972	0,003	0,006	0,075	0,240
Parasite check	Coeff	,145**	,155**	,113**	,147**	,084*	0,065	-0,009	,110**	0,069	0,004	0,074
	p-value	0,000	0,000	0,006	0,000	0,034	0,101	0,821	0,006	0,088	0,918	0,065
Nutrition check	Coeff	,254**	,123**	,115**	,131**	,094*	0,052	0,007	0,042	,093*	0,068	0,021
	p-value	0,000	0,003	0,005	0,001	0,017	0,190	0,864	0,298	0,022	0,096	0,601
Chemical check	Coeff	,131**	,129**	,088*	0,054	,093*	,083*	-0,001	0,062	0,011	0,038	,118**
	p-value	0,001	0,002	0,033	0,180	0,019	0,036	0,979	0,123	0,780	0,356	0,003
Communication with growers	Coeff	,093*	0,015	0,030	-0,044	0,056	,091*	0,026	0,060	0,037	,108*	,100*
	p-value	0,028	0,724	0,483	0,286	0,173	0,026	0,534	0,147	0,382	0,010	0,015
Info: Journals	Coeff	,138**	0,025	,151**	-0,005	,185**	,097*	,128**	0,006	0,024	0,047	,099*
	p-value	0,001	0,542	0,000	0,904	0,000	0,014	0,002	0,878	0,552	0,255	0,014
Info: BKA	Coeff	,137**	,139**	0,083	0,017	,086*	0,075	,107*	,091*	,125**	,182**	,153**
	p-value	0,001	0,001	0,053	0,690	0,037	0,070	0,013	0,030	0,003	0,000	0,000
Info: NBHA	Coeff	,162**	0,076	,124**	0,030	,117**	,161**	0,066	0,046	0,067	0,079	0,076
	p-value	0,000	0,069	0,003	0,462	0,003	0,000	0,112	0,257	0,102	0,056	0,062

Kendall's non-parametric test for correlations		Reduce decline: monitor nutrition	Reduce decline: monitor chemicals	Reduce decline: beekeeping	Bee health: economic motives	Bee health: legal motives	Bee health: public perception	Bee health: pollinators conservation	Bee health: consumer safety	Bee health: food security	Bee health: crop varieties	BHC effectiveness: confidence
Info: newspapers	Coeff	0,074	,094*	,155**	0,018	,156**	,207**	0,066	0,070	0,067	,144**	,128**
	p-value	0,070	0,022	0,000	0,652	0,000	0,000	0,110	0,081	0,101	0,000	0,001
Info:TV/Radio	Coeff	,111**	,133**	,123**	0,042	,202**	,258**	,104*	,168**	,150**	,098*	,235**
	p-value	0,008	0,001	0,003	0,296	0,000	0,000	0,013	0,000	0,000	0,018	0,000
Info: SM	Coeff	,127**	0,071	0,039	,079*	,080*	,141**	,112**	,107**	,147**	,095*	,164**
	p-value	0,002	0,083	0,348	0,047	0,045	0,000	0,007	0,008	0,000	0,020	0,000
Info: Online training	Coeff	,152**	,155**	,141**	0,055	,148**	,195**	,130**	,147**	,140**	,127**	,247**
	p-value	0,000	0,000	0,001	0,165	0,000	0,000	0,002	0,000	0,001	0,002	0,000
Info: training in person	Coeff	,131**	,137**	0,079	-0,038	,097*	,133**	0,078	0,066	0,065	,099*	,083*
	p-value	0,002	0,001	0,062	0,354	0,016	0,001	0,064	0,107	0,119	0,019	0,043
Info: beekeepers	Coeff	0,061	,127**	0,082	-0,042	-0,006	0,027	0,063	0,001	-0,032	,093*	,161**
	p-value	0,149	0,003	0,053	0,308	0,879	0,507	0,140	0,972	0,447	0,027	0,000
Info: NGOs	Coeff	,130**	,127**	,099*	,140**	,180**	,199**	,094*	,122**	,140**	,119**	,145**
	p-value	0,002	0,002	0,018	0,001	0,000	0,000	0,024	0,003	0,001	0,004	0,000
Bee decline: habitat loss	Coeff	,112*	,177**	0,060	0,030	,095*	,106*	0,069	,099*	,092*	0,012	,111*
	p-value	0,013	0,000	0,186	0,493	0,029	0,015	0,129	0,025	0,040	0,788	0,012
Bee decline: competition wild/manage	Coeff	,086*	,143**	0,053	,113**	,212**	,086*	0,076	0,073	,137**	0,048	,098*
	p-value	0,046	0,001	0,226	0,007	0,000	0,039	0,080	0,086	0,001	0,269	0,021

Kendall's non-parametric test for correlations		Reduce decline: monitor nutrition	Reduce decline: monitor chemicals	Reduce decline: beekeeping	Bee health: economic motives	Bee health: legal motives	Bee health: public perception	Bee health: pollinators conservation	Bee health: consumer safety	Bee health: food security	Bee health: crop varieties	BHC effectiveness: confidence
Bee decline: diseases	Coeff	,176**	0,024	,161**	,151**	0,071	0,043	0,022	,133**	,153**	0,064	,090*
	p-value	0,000	0,594	0,000	0,000	0,098	0,314	0,623	0,002	0,001	0,151	0,038
Bee decline: parasites	Coeff	,179**	0,032	,196**	,205**	,095*	0,073	0,071	,125**	,133**	0,080	0,022
	p-value	0,000	0,482	0,000	0,000	0,029	0,092	0,117	0,005	0,003	0,074	0,616
Bee decline: predators	Coeff	,134**	,110*	,121**	,156**	,105*	,140**	0,050	,209**	,204**	0,048	,151**
	p-value	0,002	0,011	0,005	0,000	0,011	0,001	0,245	0,000	0,000	0,265	0,000
Bee decline: climate change	Coeff	,215**	,194**	0,077	,159**	,159**	,142**	,120**	,171**	,218**	,127**	,151**
	p-value	0,000	0,000	0,080	0,000	0,000	0,001	0,007	0,000	0,000	0,004	0,000
Bee decline: agrochemicals	Coeff	0,065	,430**	0,013	0,034	-0,018	0,006	,165**	,089*	,103*	,091*	0,054
	p-value	0,151	0,000	0,774	0,437	0,684	0,897	0,000	0,045	0,022	0,043	0,224
Bee decline: genetics	Coeff	,191**	0,037	,137**	0,076	0,075	,121**	0,033	,119**	,127**	0,059	,122**
	p-value	0,000	0,398	0,002	0,069	0,070	0,004	0,449	0,005	0,003	0,168	0,004
Bee decline: beekeeping	Coeff	,217**	-0,004	,378**	0,056	,160**	,124**	0,025	0,036	,115**	0,060	,158**
	p-value	0,000	0,924	0,000	0,192	0,000	0,003	0,575	0,397	0,008	0,165	0,000
Reduce decline: growers collab	Coeff	,137**	,199**	,156**	0,027	,094*	,158**	0,081	,116**	0,088	,165**	,204**
	p-value	0,002	0,000	0,001	0,546	0,032	0,000	0,076	0,009	0,050	0,000	0,000
Reduce decline: hive position	Coeff	,244**	,133**	,320**	0,048	,116**	0,083	,106*	,168**	,188**	0,088	,088*
	p-value	0,000	0,003	0,000	0,277	0,008	0,056	0,019	0,000	0,000	0,050	0,045
Reduce decline: habitat	Coeff	,094*	,191**	,126**	0,034	0,044	-0,004	,218**	0,067	0,086	,174**	0,031
	p-value	0,037	0,000	0,006	0,440	0,318	0,927	0,000	0,133	0,057	0,000	0,491
Reduce decline: monitor	Coeff	,276**	,179**	,262**	,166**	,167**	,150**	,162**	,220**	,246**	,189**	,138**
	p-value	0,000	0,000	0,000	0,000	0,000	0,001	0,000	0,000	0,000	0,000	0,002
Reduce decline: monitor	Coeff	,349**	,151**	,263**	,205**	,129**	,106*	,162**	,229**	,251**	,190**	,174**
	p-value	0,000	0,001	0,000	0,000	0,003	0,015	0,000	0,000	0,000	0,000	0,000
Reduce decline: monitor	Coeff	1,000	,296**	,279**	,163**	,152**	,150**	0,066	,191**	,250**	,189**	,103*
	p-value		0,000	0,000	0,000	0,000	0,001	0,148	0,000	0,000	0,000	0,019
Reduce decline: monitor	Coeff	,296**	1,000	,150**	,166**	,093*	0,081	,259**	,218**	,210**	,237**	,178**
	p-value	0,000		0,001	0,000	0,034	0,063	0,000	0,000	0,000	0,000	0,000

Kendall's non-parametric test for correlations		Reduce decline: monitor nutrition	Reduce decline: monitor chemicals	Reduce decline: beekeeping	Bee health: economic motives	Bee health: legal motives	Bee health: public perception	Bee health: pollinators conservation	Bee health: consumer safety	Bee health: food security	Bee health: crop varieties	BHC effectiveness: confidence
Reduce decline: beekeeping	Coeff	,279**	,150**	1,000	0,073	,200**	,143**	,161**	,136**	,161**	,124**	,133**
	p-value	0,000	0,001		0,100	0,000	0,001	0,000	0,002	0,000	0,006	0,003
Bee health: economic	Coeff	,163**	,166**	0,073	1,000	,333**	,195**	0,085	,269**	,299**	,207**	0,064
	p-value	0,000	0,000	0,100		0,000	0,000	0,055	0,000	0,000	0,000	0,136
Bee health: legal	Coeff	,152**	,093*	,200**	,333**	1,000	,431**	0,085	,225**	,194**	,200**	,108*
	p-value	0,000	0,034	0,000	0,000		0,000	0,053	0,000	0,000	0,000	0,011
Bee health: public perception	Coeff	,150**	0,081	,143**	,195**	,431**	1,000	,181**	,208**	,211**	,197**	,104*
	p-value	0,001	0,063	0,001	0,000	0,000		0,000	0,000	0,000	0,000	0,015
Bee health: pollinators conservation	Coeff	0,066	,259**	,161**	0,085	0,085	,181**	1,000	,167**	,298**	,262**	,121**
	p-value	0,148	0,000	0,000	0,055	0,053	0,000		0,000	0,000	0,000	0,006
Bee health: consumer safety	Coeff	,191**	,218**	,136**	,269**	,225**	,208**	,167**	1,000	,539**	,250**	,148**
	p-value	0,000	0,000	0,002	0,000	0,000	0,000	0,000		0,000	0,000	0,001
Bee health: food security	Coeff	,250**	,210**	,161**	,299**	,194**	,211**	,298**	,539**	1,000	,414**	,126**
	p-value	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,000	0,004
Bee health: crop varieties	Coeff	,189**	,237**	,124**	,207**	,200**	,197**	,262**	,250**	,414**	1,000	,128**
	p-value	0,000	0,000	0,006	0,000	0,000	0,000	0,000	0,000	0,000		0,004
BHC effectiveness: confidence	Coeff	,103*	,178**	,133**	0,064	,108*	,104*	,121**	,148**	,126**	,128**	1,000
	p-value	0,019	0,000	0,003	0,136	0,011	0,015	0,006	0,001	0,004	0,004	
BHC barriers: growers	Coeff	,087*	,181**	0,075	,121**	,131**	,143**	0,053	,167**	,226**	,179**	,100*
	p-value	0,049	0,000	0,090	0,005	0,002	0,001	0,232	0,000	0,000	0,000	0,021
BHC barriers: cost	Coeff	0,077	,115**	0,020	,095*	0,009	0,076	-0,010	0,018	,099*	0,071	-,124**
	p-value	0,082	0,009	0,655	0,026	0,836	0,076	0,820	0,681	0,023	0,106	0,004

Kendall's non-parametric test for correlations		Reduce decline: monitor nutrition	Reduce decline: monitor chemicals	Reduce decline: beekeeping	Bee health: economic motives	Bee health: legal motives	Bee health: public perception	Bee health: pollinators conservation	Bee health: consumer safety	Bee health: food security	Bee health: crop varieties	BHC effectiveness: confidence
BHC barriers: effectiveness	Coeff	0,020	-0,056	-0,085	-0,073	-0,040	0,019	-0,061	-0,026	-0,029	-0,040	-,398**
	p-value	0,648	0,193	0,050	0,081	0,339	0,648	0,159	0,544	0,494	0,350	0,000
BHC barriers: time	Coeff	0,031	0,029	-,086*	-0,040	0,026	,118**	-0,074	0,008	0,020	0,027	-,243**
	p-value	0,475	0,507	0,047	0,340	0,537	0,005	0,088	0,849	0,648	0,536	0,000
BHC barriers: difficult	Coeff	0,022	-0,009	-0,052	-0,044	0,040	,087*	-0,042	-0,003	0,031	0,022	-,222**
	p-value	0,605	0,839	0,231	0,299	0,346	0,038	0,333	0,948	0,473	0,615	0,000
BHC barriers: no importance	Coeff	-0,068	-0,063	-,097*	-0,035	0,016	,117**	-0,014	-0,071	-0,041	0,005	-,276**
	p-value	0,117	0,145	0,026	0,399	0,705	0,005	0,747	0,095	0,334	0,905	0,000
BHC benefits: growers	Coeff	,094*	,187**	,121**	,183**	,161**	,186**	,098*	,204**	,127**	,137**	,332**
	p-value	0,031	0,000	0,006	0,000	0,000	0,000	0,025	0,000	0,003	0,002	0,000
BHC benefits: productivity	Coeff	0,062	,107*	0,033	,239**	,126**	,093*	0,071	,233**	,224**	,140**	,307**
	p-value	0,158	0,014	0,453	0,000	0,003	0,027	0,108	0,000	0,000	0,001	0,000
BHC benefits: quick and easy	Coeff	0,017	,133**	,092*	,101*	0,057	0,036	,134**	,144**	,119**	0,060	,409**
	p-value	0,694	0,003	0,037	0,019	0,182	0,390	0,002	0,001	0,006	0,168	0,000
BHC benefits: treatment cost	Coeff	,114**	,088*	0,050	,124**	0,074	0,028	0,041	,134**	,155**	0,074	,368**
	p-value	0,008	0,042	0,252	0,003	0,076	0,508	0,346	0,002	0,000	0,087	0,000
BHC benefits: crop pollination	Coeff	0,052	,133**	0,058	,125**	0,061	,108*	,091*	,195**	,180**	,107*	,314**
	p-value	0,232	0,002	0,186	0,003	0,146	0,010	0,038	0,000	0,000	0,013	0,000

Kendall's non-parametric test for correlations		BHC barriers: growers	BHC barriers: cost	BHC barriers: effectiveness	BHC barriers: time	BHC barriers: difficulty	BHC barriers: no importance	BHC benefits: growers	BHC benefits: productivity	BHC benefits: quick and easy	BHC benefits: treatment cost
Country	Coeff	-0,030	0,075	0,074	0,006	-0,011	0,065	-0,051	-0,058	-0,009	-0,062
	p-value	0,450	0,056	0,055	0,871	0,771	0,092	0,194	0,141	0,811	0,110
Professional beekeeping	Coeff	0,066	-0,069	0,026	0,042	0,069	0,039	,111*	,122**	-0,020	0,003
	p-value	0,141	0,126	0,549	0,343	0,120	0,377	0,013	0,006	0,650	0,949
Years as hobbyist beekeeper	Coeff	0,017	0,009	0,028	0,024	0,022	0,061	-0,017	-,112**	-0,027	-0,065
	p-value	0,652	0,801	0,440	0,507	0,559	0,100	0,654	0,003	0,469	0,076
Years as professional beekeeper	Coeff	0,073	-0,060	0,037	0,058	,083*	0,048	,092*	,091*	-0,047	-0,017
	p-value	0,085	0,160	0,374	0,162	0,047	0,247	0,029	0,030	0,262	0,681
N° hives per year	Coeff	0,067	-,080*	0,042	0,045	0,060	0,030	,084*	-0,012	-0,043	-0,066
	p-value	0,074	0,032	0,246	0,218	0,104	0,417	0,023	0,739	0,241	0,069
BKA member	Coeff	0,010	0,052	0,009	0,038	0,000	0,001	-0,057	0,024	-0,014	0,010
	p-value	0,824	0,243	0,844	0,380	0,994	0,978	0,198	0,591	0,760	0,823
Disease check	Coeff	0,020	0,051	-0,004	0,037	0,036	-0,043	0,040	,084*	0,020	0,035
	p-value	0,620	0,203	0,910	0,344	0,359	0,271	0,314	0,033	0,622	0,378
Parasite check	Coeff	0,052	0,052	0,028	0,035	0,054	-0,045	,116**	0,063	0,023	0,055
	p-value	0,196	0,189	0,478	0,369	0,170	0,248	0,003	0,112	0,567	0,158
Nutrition check	Coeff	0,019	,086*	0,032	0,052	0,036	-0,010	0,001	0,062	-0,025	0,029
	p-value	0,630	0,030	0,406	0,181	0,359	0,793	0,983	0,118	0,525	0,466
Chemical check	Coeff	,129**	-0,010	0,021	0,038	0,049	-0,045	,185**	0,061	-0,003	0,052
	p-value	0,001	0,798	0,587	0,327	0,220	0,249	0,000	0,122	0,934	0,189
Communication with growers	Coeff	,120**	-,108**	0,016	0,003	0,019	-0,009	,162**	0,028	0,013	0,042
	p-value	0,004	0,008	0,699	0,949	0,648	0,822	0,000	0,488	0,749	0,295
Info: Journals	Coeff	,082*	-0,032	-0,014	0,053	0,032	-0,056	,134**	0,030	0,042	0,010
	p-value	0,041	0,430	0,722	0,172	0,419	0,152	0,001	0,446	0,293	0,808
Info: BKA	Coeff	,089*	-0,018	-0,054	0,034	0,028	-0,016	0,047	0,079	0,044	,092*
	p-value	0,034	0,666	0,182	0,410	0,496	0,691	0,253	0,056	0,293	0,024
Info: NBHA	Coeff	-0,002	,106**	-0,071	0,015	-0,004	-0,030	0,017	0,022	0,038	,100*
	p-value	0,965	0,009	0,073	0,700	0,917	0,442	0,665	0,580	0,349	0,012
Info: newspapers	Coeff	0,073	0,027	-0,050	0,030	0,045	0,040	,180**	0,039	0,041	0,074
	p-value	0,070	0,504	0,203	0,447	0,254	0,306	0,000	0,320	0,303	0,058

Kendall's non-parametric test for correlations		BHC barriers: growers	BHC barriers: cost	BHC barriers: effectiveness	BHC barriers: time	BHC barriers: difficulty	BHC barriers: no importance	BHC benefits: growers	BHC benefits: productivity	BHC benefits: quick and easy	BHC benefits: treatment cost
Info:TV/Radio	Coeff	,114**	0,047	-,103**	0,048	0,046	0,050	,238**	,215**	,140**	,229**
	p-value	0,005	0,247	0,010	0,226	0,247	0,212	0,000	0,000	0,001	0,000
Info: SM	Coeff	0,069	0,001	-0,054	0,036	0,001	-0,007	,174**	,213**	,101*	,132**
	p-value	0,084	0,989	0,172	0,362	0,979	0,863	0,000	0,000	0,012	0,001
Info: Online training	Coeff	,130**	-0,042	-,082*	-0,004	-0,032	-0,054	,211**	,113**	,093*	,113**
	p-value	0,001	0,289	0,035	0,916	0,415	0,172	0,000	0,004	0,019	0,004
Info: training in person	Coeff	,100*	-0,013	0,024	,097*	0,068	-0,018	,091*	0,016	0,009	0,069
	p-value	0,015	0,743	0,555	0,016	0,094	0,660	0,025	0,700	0,825	0,088
Info: beekeepers	Coeff	-0,050	-0,046	-0,031	0,031	-0,025	-0,043	0,051	0,020	0,036	0,032
	p-value	0,230	0,266	0,435	0,441	0,540	0,283	0,215	0,620	0,378	0,436
Info: NGOs	Coeff	,139**	0,031	-,083*	0,051	0,022	-0,044	,163**	,155**	,092*	,195**
	p-value	0,001	0,451	0,036	0,201	0,577	0,266	0,000	0,000	0,023	0,000
Bee decline: habitat loss	Coeff	,139**	0,032	0,031	0,065	0,005	0,058	0,073	0,045	0,073	0,052
	p-value	0,002	0,467	0,469	0,131	0,904	0,181	0,094	0,303	0,096	0,227
Bee decline: competition wild/manage	Coeff	0,062	,088*	0,002	0,074	0,057	0,063	0,069	0,079	0,046	,105*
	p-value	0,145	0,038	0,970	0,071	0,171	0,127	0,097	0,059	0,275	0,011

Kendall's non-parametric test for correlations		BHC barriers: growers	BHC barriers: cost	BHC barriers: effectiveness	BHC barriers: time	BHC barriers: difficulty	BHC barriers: no importance	BHC benefits: growers	BHC benefits: productivity	BHC benefits: quick and easy	BHC benefits: treatment cost
Bee decline: diseases	Coeff	,089*	0,049	-0,050	-0,071	0,028	-0,064	,199**	,238**	,121**	,190**
	p-value	0,042	0,262	0,240	0,094	0,519	0,135	0,000	0,000	0,005	0,000
Bee decline: parasites	Coeff	0,045	0,046	-0,062	-0,004	0,037	-0,079	,122**	,196**	0,042	,151**
	p-value	0,301	0,295	0,147	0,922	0,390	0,066	0,005	0,000	0,339	0,000
Bee decline: predators	Coeff	0,060	0,020	-0,007	0,064	0,064	-0,005	,118**	,156**	0,066	,106**
	p-value	0,151	0,635	0,861	0,120	0,122	0,896	0,005	0,000	0,117	0,010
Bee decline: climate change	Coeff	,149**	-0,074	-0,034	0,018	0,013	-0,061	,095*	,111**	0,079	,126**
	p-value	0,000	0,084	0,416	0,660	0,753	0,145	0,025	0,008	0,063	0,003
Bee decline: agrochemicals	Coeff	,118**	0,084	-0,025	0,075	0,083	0,010	,140**	0,050	0,008	0,072
	p-value	0,007	0,055	0,562	0,080	0,057	0,825	0,001	0,250	0,864	0,094
Bee decline: genetics	Coeff	,107*	0,043	0,013	0,013	0,032	0,011	,173**	,155**	,095*	,270**
	p-value	0,011	0,305	0,756	0,753	0,438	0,787	0,000	0,000	0,024	0,000
Bee decline: beekeeping	Coeff	0,056	0,053	-0,036	0,006	0,020	-,088*	,128**	0,074	0,035	,120**
	p-value	0,187	0,215	0,383	0,890	0,631	0,036	0,002	0,079	0,410	0,004
Reduce decline: growers collab	Coeff	,191**	-0,021	-,091*	-0,017	-0,049	-,154**	,222**	0,070	,125**	,090*
	p-value	0,000	0,630	0,036	0,693	0,259	0,000	0,000	0,110	0,005	0,038
Reduce decline: hive position	Coeff	,147**	0,018	-0,029	-0,003	-0,004	-,104*	,112**	,094*	0,013	0,042
	p-value	0,001	0,677	0,492	0,949	0,924	0,016	0,010	0,030	0,770	0,331
Reduce decline: habitat	Coeff	0,081	0,047	0,031	0,043	-0,009	0,010	0,049	-0,034	0,005	-0,027
	p-value	0,069	0,287	0,471	0,322	0,846	0,812	0,264	0,440	0,909	0,533
Reduce decline: monitor diseases	Coeff	,103*	,109*	-,092*	-0,021	-0,018	-0,077	,209**	,191**	,139**	,176**
	p-value	0,021	0,014	0,034	0,634	0,678	0,077	0,000	0,000	0,002	0,000
Reduce decline: monitor parasites	Coeff	,105*	0,084	-,176**	-0,069	-,088*	-,130**	,159**	,176**	,163**	,194**
	p-value	0,018	0,057	0,000	0,110	0,044	0,003	0,000	0,000	0,000	0,000
Reduce decline: monitor nutrition	Coeff	,087*	0,077	0,020	0,031	0,022	-0,068	,094*	0,062	0,017	,114**
	p-value	0,049	0,082	0,648	0,475	0,605	0,117	0,031	0,158	0,694	0,008
Reduce decline: monitor chemicals	Coeff	,181**	,115**	-0,056	0,029	-0,009	-0,063	,187**	,107*	,133**	,088*
	p-value	0,000	0,009	0,193	0,507	0,839	0,145	0,000	0,014	0,003	0,042

Kendall's non-parametric test for correlations		BHC barriers: growers	BHC barriers: cost	BHC barriers: effectiveness	BHC barriers: time	BHC barriers: difficulty	BHC barriers: no importance	BHC benefits: growers	BHC benefits: productivity	BHC benefits: quick and easy	BHC benefits: treatment cost
Reduce decline: beekeeping	Coeff	0,075	0,020	-0,085	-,086*	-0,052	-,097*	,121**	0,033	,092*	0,050
	p-value	0,090	0,655	0,050	0,047	0,231	0,026	0,006	0,453	0,037	0,252
Bee health: economic	Coeff	,121**	,095*	-0,073	-0,040	-0,044	-0,035	,183**	,239**	,101*	,124**
	p-value	0,005	0,026	0,081	0,340	0,299	0,399	0,000	0,000	0,019	0,003
Bee health: legal	Coeff	,131**	0,009	-0,040	0,026	0,040	0,016	,161**	,126**	0,057	0,074
	p-value	0,002	0,836	0,339	0,537	0,346	0,705	0,000	0,003	0,182	0,076
Bee health: public perception	Coeff	,143**	0,076	0,019	,118**	,087*	,117**	,186**	,093*	0,036	0,028
	p-value	0,001	0,076	0,648	0,005	0,038	0,005	0,000	0,027	0,390	0,508
Bee health: pollinators conservation	Coeff	0,053	-0,010	-0,061	-0,074	-0,042	-0,014	,098*	0,071	,134**	0,041
	p-value	0,232	0,820	0,159	0,088	0,333	0,747	0,025	0,108	0,002	0,346
Bee health: consumer safety	Coeff	,167**	0,018	-0,026	0,008	-0,003	-0,071	,204**	,233**	,144**	,134**
	p-value	0,000	0,681	0,544	0,849	0,948	0,095	0,000	0,000	0,001	0,002
Bee health: food security	Coeff	,226**	,099*	-0,029	0,020	0,031	-0,041	,127**	,224**	,119**	,155**
	p-value	0,000	0,023	0,494	0,648	0,473	0,334	0,003	0,000	0,006	0,000
Bee health: crop varieties	Coeff	,179**	0,071	-0,040	0,027	0,022	0,005	,137**	,140**	0,060	0,074
	p-value	0,000	0,106	0,350	0,536	0,615	0,905	0,002	0,001	0,168	0,087
BHC effectiveness: confidence	Coeff	,100*	-,124**	-,398**	-,243**	-,222**	-,276**	,332**	,307**	,409**	,368**
	p-value	0,021	0,004	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
BHC barriers: growers	Coeff	1,000	0,084	-0,033	,123**	,123**	0,001	,326**	,137**	0,059	,126**
	p-value		0,052	0,428	0,004	0,004	0,977	0,000	0,001	0,173	0,003
BHC barriers: cost	Coeff	0,084	1,000	,203**	,260**	,217**	,205**	-,085*	-0,003	-,095*	-,108*
	p-value	0,052		0,000	0,000	0,000	0,000	0,047	0,944	0,026	0,011

Kendall's non-parametric test for correlations		BHC barriers: growers	BHC barriers: cost	BHC barriers: effectiveness	BHC barriers: time	BHC barriers: difficulty	BHC barriers: no importance	BHC benefits: growers	BHC benefits: productivity	BHC benefits: quick and easy	BHC benefits: treatment cost
BHC barriers: effectiveness	Coeff	-0,033	,203**	1,000	,396**	,367**	,392**	-,116**	-,158**	-,312**	-,233**
	p-value	0,428	0,000		0,000	0,000	0,000	0,005	0,000	0,000	0,000
BHC barriers: time	Coeff	,123**	,260**	,396**	1,000	,658**	,372**	-0,053	-,139**	-,386**	-,139**
	p-value	0,004	0,000	0,000		0,000	0,000	0,204	0,001	0,000	0,001
BHC barriers: difficult	Coeff	,123**	,217**	,367**	,658**	1,000	,395**	0,005	-0,060	-,383**	-0,074
	p-value	0,004	0,000	0,000	0,000		0,000	0,907	0,150	0,000	0,077
BHC barriers: no importance	Coeff	0,001	,205**	,392**	,372**	,395**	1,000	-,092*	-,177**	-,331**	-,170**
	p-value	0,977	0,000	0,000	0,000	0,000		0,028	0,000	0,000	0,000
BHC benefits: growers	Coeff	,326**	-,085*	-,116**	-0,053	0,005	-,092*	1,000	,393**	,267**	,321**
	p-value	0,000	0,047	0,005	0,204	0,907	0,028		0,000	0,000	0,000
BHC benefits: productivity	Coeff	,137**	-0,003	-,158**	-,139**	-0,060	-,177**	,393**	1,000	,352**	,443**
	p-value	0,001	0,944	0,000	0,001	0,150	0,000	0,000		0,000	0,000
BHC benefits: quick and easy	Coeff	0,059	-,095*	-,312**	-,386**	-,383**	-,331**	,267**	,352**	1,000	,394**
	p-value	0,173	0,026	0,000	0,000	0,000	0,000	0,000	0,000		0,000
BHC benefits: treatment cost	Coeff	,126**	-,108*	-,233**	-,139**	-0,074	-,170**	,321**	,443**	,394**	1,000
	p-value	0,003	0,011	0,000	0,001	0,077	0,000	0,000	0,000	0,000	
BHC benefits: crop pollination	Coeff	,160**	-0,034	-,133**	-0,060	-0,025	-,144**	,415**	,451**	,316**	,508**
	p-value	0,000	0,421	0,001	0,149	0,549	0,001	0,000	0,000	0,000	0,000

Kendall's non-parametric test for correlations		BHC benefits: crop pollination	BHC benefits: bee health	BHC benefits: environment protection	BHC benefits: pollinator protection
Country	Coeff	-0,071	-0,061	-0,034	-,086[*]
	p-value	0,066	0,122	0,389	0,031
Professional beekeeping	Coeff	-0,017	-0,067	-0,014	-0,042
	p-value	0,696	0,137	0,749	0,358
Years as hobbyist beekeeper	Coeff	-0,055	-,111[**]	-,088[*]	-,114[**]
	p-value	0,135	0,003	0,019	0,003
Years as professional beekeeper	Coeff	-0,030	-,087[*]	-0,025	-0,050
	p-value	0,469	0,042	0,557	0,245
N° hives per year	Coeff	-0,058	-,137[**]	-,088[*]	-,076[*]
	p-value	0,116	0,000	0,017	0,043
BKA member	Coeff	-0,011	0,036	-0,035	0,015
	p-value	0,804	0,415	0,433	0,734
Disease check	Coeff	0,011	0,041	0,047	0,057
	p-value	0,770	0,312	0,233	0,156
Parasite check	Coeff	0,001	0,026	-0,027	0,015
	p-value	0,981	0,515	0,501	0,710
Nutrition check	Coeff	-0,002	0,060	0,034	0,049
	p-value	0,954	0,136	0,395	0,218
Chemical check	Coeff	0,052	-0,035	0,016	0,044
	p-value	0,192	0,387	0,684	0,279
Communication with growers	Coeff	0,029	-0,053	0,048	0,020
	p-value	0,479	0,200	0,246	0,633
Info: Journals	Coeff	0,044	-0,044	0,013	0,018
	p-value	0,267	0,280	0,751	0,661
Info: BKA	Coeff	,106[*]	0,082	,130[**]	,124[**]
	p-value	0,010	0,051	0,002	0,003
Info: NBHA	Coeff	,095[*]	0,076	,085[*]	0,055
	p-value	0,017	0,060	0,034	0,178
Info: newspapers	Coeff	,130[**]	0,065	,119[**]	,114[**]
	p-value	0,001	0,106	0,003	0,005

Kendall's non-parametric test for correlations		BHC benefits: crop pollination	BHC benefits: bee health	BHC benefits: environment protection	BHC benefits: pollinator protection
Info:TV/Radio	Coeff	,209[**]	,134[**]	,172[**]	,175[**]
	p-value	0,000	0,001	0,000	0,000
Info: SM	Coeff	,171[**]	,111[**]	,153[**]	,157[**]
	p-value	0,000	0,006	0,000	0,000
Info: Online training	Coeff	,135[**]	,087[*]	,127[**]	,135[**]
	p-value	0,001	0,030	0,001	0,001
Info: training in person	Coeff	0,073	0,029	0,050	,089[*]
	p-value	0,071	0,475	0,222	0,032
Info: beekeepers	Coeff	0,066	0,033	0,039	,087[*]
	p-value	0,102	0,420	0,339	0,036
Info: NGOs	Coeff	,188[**]	,155[**]	,148[**]	,197[**]
	p-value	0,000	0,000	0,000	0,000
Bee decline: habitat loss	Coeff	0,074	0,009	,116[**]	0,077
	p-value	0,088	0,846	0,008	0,081
Bee decline: competition wild/manage	Coeff	,117[**]	,086[*]	,086[*]	0,070
	p-value	0,005	0,043	0,042	0,100

Kendall's non-parametric test for correlations		BHC benefits: crop pollination	BHC benefits: bee health	BHC benefits: environment protection	BHC benefits: pollinator protection
Bee decline: diseases	Coeff	,194**	,160**	,098*	,137**
	p-value	0,000	0,000	0,024	0,002
Bee decline: parasites	Coeff	,120**	,153**	,134**	,144**
	p-value	0,006	0,000	0,002	0,001
Bee decline: predators	Coeff	,103*	0,066	,114**	,107*
	p-value	0,013	0,120	0,006	0,012
Bee decline: climate change	Coeff	0,075	,116**	,145**	,153**
	p-value	0,076	0,007	0,001	0,000
Bee decline: agrochemicals	Coeff	,099*	0,061	,089*	,140**
	p-value	0,023	0,170	0,042	0,002
Bee decline: genetics	Coeff	,191**	,130**	,120**	,096*
	p-value	0,000	0,002	0,004	0,023
Bee decline: beekeeping	Coeff	,089*	0,065	0,015	-0,001
	p-value	0,034	0,131	0,729	0,972
Reduce decline: growers	Coeff	,160**	,094*	,122**	,186**
	p-value	0,000	0,035	0,006	0,000
Reduce decline: hive position	Coeff	,095*	0,023	0,029	0,015
	p-value	0,028	0,600	0,512	0,728
Reduce decline: habitat	Coeff	0,040	0,031	0,017	0,020
	p-value	0,362	0,488	0,703	0,652
Reduce decline: monitor	Coeff	,180**	,214**	,120**	,142**
	p-value	0,000	0,000	0,007	0,002
Reduce decline: monitor	Coeff	,161**	,224**	,121**	,181**
	p-value	0,000	0,000	0,006	0,000
Reduce decline: monitor	Coeff	0,052	0,054	0,042	-0,001
	p-value	0,232	0,220	0,335	0,974
Reduce decline: monitor	Coeff	,133**	,178**	,103*	,192**
	p-value	0,002	0,000	0,020	0,000
Reduce decline: beekeeping	Coeff	0,058	,095*	,107*	,089*
	p-value	0,186	0,033	0,015	0,046

Kendall's non-parametric test for correlations		BHC benefits: crop pollination	BHC benefits: bee health	BHC benefits: environment protection	BHC benefits: pollinator protection
Bee health: economic	Coeff	,125**	,167**	,147**	,110*
	p-value	0,003	0,000	0,001	0,011
Bee health: legal	Coeff	0,061	0,059	0,078	0,036
	p-value	0,146	0,168	0,066	0,401
Bee health: public perception	Coeff	,108*	-0,034	,110**	0,015
	p-value	0,010	0,426	0,010	0,727
Bee health: pollinators conservation	Coeff	,091*	,094*	0,063	,150**
	p-value	0,038	0,034	0,152	0,001
Bee health: consumer safety	Coeff	,195**	,129**	,182**	,139**
	p-value	0,000	0,003	0,000	0,001
Bee health: food security	Coeff	,180**	,204**	,215**	,173**
	p-value	0,000	0,000	0,000	0,000
Bee health: crop varieties	Coeff	,107*	,121**	,163**	,168**
	p-value	0,013	0,006	0,000	0,000
BHC effectiveness: confidence	Coeff	,314**	,392**	,385**	,405**
	p-value	0,000	0,000	0,000	0,000
BHC barriers: growers	Coeff	,160**	,153**	,201**	,202**
	p-value	0,000	0,000	0,000	0,000
BHC barriers: cost	Coeff	-0,034	-0,026	-0,082	-0,075
	p-value	0,421	0,549	0,055	0,085

Kendall's non-parametric test for correlations		BHC benefits: crop pollination	BHC benefits: bee health	BHC benefits: environment protection	BHC benefits: pollinator protection
BHC barriers: effectiveness	Coeff	-,133**	-,297**	-,239**	-,248**
	p-value	0,001	0,000	0,000	0,000
BHC barriers: time	Coeff	-0,060	-,228**	-,151**	-,160**
	p-value	0,149	0,000	0,000	0,000
BHC barriers: difficult	Coeff	-0,025	-,221**	-,126**	-,174**
	p-value	0,549	0,000	0,003	0,000
BHC barriers: no importance	Coeff	-,144**	-,271**	-,204**	-,264**
	p-value	0,001	0,000	0,000	0,000
BHC benefits: growers	Coeff	,415**	,293**	,344**	,328**
	p-value	0,000	0,000	0,000	0,000
BHC benefits: productivity	Coeff	,451**	,427**	,380**	,368**
	p-value	0,000	0,000	0,000	0,000
BHC benefits: quick and easy	Coeff	,316**	,396**	,297**	,338**
	p-value	0,000	0,000	0,000	0,000
BHC benefits: treatment cost	Coeff	,508**	,447**	,404**	,390**
	p-value	0,000	0,000	0,000	0,000
BHC benefits: crop pollination	Coeff	1,000	,444**	,476**	,411**
	p-value		0,000	0,000	0,000

Appendix 4.3

This appendix contains further analyses not illustrated in the chapter.

Number of beehives

The table below shows the average number of beehives kept in the last 3 years by each participant. The number varies across countries, with the majority of Italian beekeepers having the highest average (50/year), and Ireland and the UK the lowest (3/year). This may be explained by the fact that the majority of Irish and UK participants are hobbyist, while Italians are mainly professionals (see table 'Hobbyist vs. professional beekeepers' below).

Average number per year	Respondents															
	Estonia		Germany		Ireland		Italy		Spain		Switzerland		UK		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
0	0	0.00	1	3.03	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
1	1	3.13	1	3.03	12	10.43	1	1.52	0	0.00	0	0.00	5	3.68	20	4.22
1.5	0	0.00	0	0.00	1	0.87	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
2	0	0.00	2	6.06	19	16.52	0	0.00	0	0.00	0	0.00	18	13.24	39	8.23
2.5	0	0.00	1	3.03	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
3	1	3.13	1	3.03	22	19.13	3	4.55	0	0.00	1	1.92	29	21.32	57	12.03
3.5	0	0.00	0	0.00	1	0.87	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
4	1	3.13	1	3.03	9	7.83	2	3.03	0	0.00	2	3.85	17	12.50	32	6.75
5	2	6.25	4	12.12	7	6.09	3	4.55	1	2.50	1	1.92	12	8.82	30	6.33
6	0	0.00	5	15.15	5	4.35	3	4.55	0	0.00	2	3.85	12	8.82	27	5.70
7	0	0.00	1	3.03	5	4.35	1	1.52	1	2.50	1	1.92	2	1.47	11	2.32
8	1	3.13	0	0.00	1	0.87	0	0.00	1	2.50	1	1.92	6	4.41	10	2.11
8,5	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.74	1	0.21
9	0	0.00	0	0.00	0	0.00	1	1.52	1	2.50	0	0.00	0	0.00	2	0.42
10	4	12.50	2	6.06	9	7.83	3	4.55	3	7.50	7	13.46	9	6.62	37	7.81
11	0	0.00	0	0.00	1	0.87	1	1.52	0	0.00	0	0.00	0	0.00	2	0.42
12	0	0.00	3	9.09	3	2.61	1	1.52	1	2.50	3	5.77	1	0.74	12	2.53
13	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
14	1	3.13	1	3.03	2	1.74	0	0.00	1	2.50	1	1.92	0	0.00	6	1.27
15	0	0.00	3	9.09	3	2.61	3	4.55	5	12.50	1	1.92	1	0.74	16	3.38
16	0	0.00	2	6.06	0	0.00	1	1.52	0	0.00	1	1.92	2	1.47	6	1.27
16.5	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
17	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
17.5	0	0.00	0	0.00	0	0.00	0	0.00	1	2.50	0	0.00	0	0.00	1	0.21
18	0	0.00	0	0.00	0	0.00	2	3.03	0	0.00	3	5.77	1	0.74	6	1.27
19	0	0.00	0	0.00	2	1.74	0	0.00	0	0.00	0	0.00	0	0.00	2	0.42
20	0	0.00	1	3.03	3	2.61	3	4.55	1	2.50	7	13.46	4	2.94	19	4.01
22	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	1.92	0	0.00	1	0.21
24	0	0.00	1	3.03	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
25	2	6.25	0	0.00	0	0.00	4	6.06	1	2.50	4	7.69	4	2.94	15	3.16
30	1	3.13	0	0.00	2	1.74	0	0.00	5	12.50	7	13.46	3	2.21	18	3.80
32	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	1	1.92	0	0.00	2	0.42
35	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	2	3.85	1	0.74	3	0.63
40	0	0.00	1	3.03	2	1.74	1	1.52	2	5.00	2	3.85	1	0.74	9	1.90
45	2	6.25	0	0.00	0	0.00	1	1.52	1	2.50	0	0.00	0	0.00	4	0.84
50	1	3.13	1	3.03	0	0.00	7	10.61	2	5.00	3	5.77	1	0.74	15	3.16
54	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.74	1	0.21
60	0	0.00	0	0.00	1	0.87	1	1.52	0	0.00	0	0.00	0	0.00	2	0.42
65	0	0.00	0	0.00	1	0.87	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
70	1	3.13	0	0.00	0	0.00	0	0.00	2	5.00	0	0.00	0	0.00	3	0.63
75	0	0.00	0	0.00	0	0.00	2	3.03	0	0.00	0	0.00	1	0.74	3	0.63
80	1	3.13	0	0.00	0	0.00	0	0.00	1	2.50	1	1.92	1	0.74	4	0.84
85	0	0.00	0	0.00	1	0.87	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21

	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
100	2	6.25	1	3.03	1	0.87	3	4.55	6	15.00	0	0.00	3	2.21	16	3.38
119	0	0.00	0	0.00	1	0.87	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
120	1	3.13	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	2	0.42
149	0	0.00	0	0.00	0	0.00	0	0.00	1	2.50	0	0.00	0	0.00	1	0.21
150	1	3.13	0	0.00	0	0.00	5	7.58	0	0.00	0	0.00	0	0.00	6	1.27
155	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
160	2	6.25	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	2	0.42
170	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
180	0	0.00	0	0.00	1	0.87	1	1.52	0	0.00	0	0.00	0	0.00	2	0.42
200	2	6.25	0	0.00	0	0.00	1	1.52	2	5.00	0	0.00	0	0.00	5	1.05
230	0	0.00	0	0.00	0	0.00	2	3.03	0	0.00	0	0.00	0	0.00	2	0.42
250	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
280	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
350	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
500	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
600	0	0.00	0	0.00	0	0.00	0	0.00	1	2.50	0	0.00	0	0.00	1	0.21
1000	0	0.00	0	0.00	0	0.00	2	3.03	0	0.00	0	0.00	0	0.00	2	0.42
1500	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
N/A	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
Total	**32**		**33**		**115**		**66**		**40**		**52**		**136**		**474**	

Hobbyist vs. professional beekeepers

The following percentages were obtained from Q1 ('How many years have you been practicing beekeeping as hobby and as profession?'). If years of profession = 0, beekeepers were counted only as hobbyists. If years of profession > 0, beekeepers were counted among professionals. The majority of recruited respondents are hobbyist (most common answers in bold).

Respondents	Estonia	Germany	Ireland	Italy	Spain	Switzerland	UK	Total
Hobbyists	43.75%	87.88%	**89.57%**	37.88%	**55.00%**	**82.69%**	85.29%	**74.26%**
Professionals	**50.00%**	9.09%	10.43%	**60.61%**	45.00%	11.54%	13.97%	24.05%

Years of beekeeping

These tables show the number of years participants have been practicing beekeeping as a hobby (A) and profession (B).

A: Years of beekeeping as hobby	Estonia		Germany		Ireland		Italy		Spain		Switzerland		UK		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
0	5	15.63	1	3.13	1	0.87	7	10.61	1	2.50	0	0.00	0	0.00	15	3.18
0.3	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.74	1	0.21
0.5	0	0.00	0	0.00	1	0.87	0	0.00	0	0.00	0	0.00	1	0.74	2	0.42
1	1	3.13	1	3.13	12	10.43	6	9.09	2	5.00	0	0.00	10	7.41	32	6.78
1.5	0	0.00	0	0.00	1	0.87	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
2	4	12.50	1	3.13	12	10.43	8	12.12	3	7.50	0	0.00	16	11.85	44	9.32
3	0	0.00	5	15.62	11	9.57	6	9.09	2	5.00	2	3.85	8	5.93	34	7.20
4	1	3.13	2	6.25	20	17.39	8	12.12	1	2.50	2	3.85	14	10.37	48	10.1
5	4	12.50	0	0.00	6	5.22	13	19.70	5	12.50	4	7.69	12	8.89	44	9.32
6	4	12.50	3	9.38	4	3.48	4	6.06	2	5.00	2	3.85	10	7.41	29	6.14
7	1	3.13	1	3.13	5	4.35	2	3.03	4	10.00	2	3.85	6	4.44	21	4.45
8	2	6.25	2	6.25	6	5.22	1	1.52	1	2.50	3	5.77	3	2.22	18	3.81
9	0	0.00	1	3.13	4	3.48	1	1.52	0	0.00	3	5.77	3	2.22	12	2.54
10	2	6.25	1	3.13	11	9.57	0	0.00	0	0.00	5	9.62	11	8.15	30	6.36
11	0	0.00	2	6.25	0	0.00	0	0.00	0	0.00	1	1.92	3	2.22	6	1.27
12	0	0.00	0	0.00	2	1.74	1	1.52	1	2.50	2	3.85	4	2.96	10	2.12
13	1	3.13	2	6.25	2	1.74	1	1.52	0	0.00	2	3.85	1	0.74	9	1.91
14	0	0.00	1	3.13	2	1.74	0	0.00	0	0.00	2	3.85	1	0.74	6	1.27
15	1	3.13	1	3.13	4	3.48	3	4.55	4	10.00	7	13.46	3	2.22	23	4.87

	Estonia n	%	Germany n	%	Ireland n	%	Italy n	%	Spain n	%	Switzerland n	%	UK n	%	Total n	%
16	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	1.92	1	0.74	2	0.42
17	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	1.92	0	0.00	1	0.21
18	0	0.00	0	0.00	1	0.87	0	0.00	0	0.00	1	1.92	0	0.00	2	0.42
19	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	1.92	1	0.74	2	0.42
20	0	0.00	0	0.00	2	1.74	2	3.03	4	10.00	1	1.92	4	2.96	13	2.75
21	0	0.00	2	6.25	1	0.87	0	0.00	0	0.00	0	0.00	0	0.00	3	0.64
22	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.74	1	0.21
23	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.74	1	0.21
24	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
25	0	0.00	0	0.00	1	0.87	0	0.00	2	5.00	2	3.85	2	1.48	7	1.48
26	0	0.00	0	0.00	0	0.00	0	0.00	1	2.50	0	0.00	1	0.74	2	0.42
28	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.74	2	0.42
30	1	3.13	1	3.13	1	0.87	0	0.00	6	15.00	0	0.00	3	2.22	12	2.54
31	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.74	2	0.42
32	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.74	1	0.21
35	0	0.00	0	0.00	1	0.87	0	0.00	0	0.00	0	0.00	3	2.22	4	0.85
38	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
40	0	0.00	0	0.00	2	1.74	1	1.52	1	2.50	1	1.92	2	1.48	7	1.48
42	0	0.00	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
43	0	0.00	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
45	1	3.13	1	3.13	1	0.87	0	0.00	0	0.00	3	5.77	0	0.00	6	1.27
47	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
48	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	2	1.48	2	0.42
50	0	0.00	1	3.13	1	0.87	0	0.00	0	0.00	0	0.00	2	1.48	4	0.85
59	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.74	1	0.21
60	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	1.92	1	0.74	2	0.42
65	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	1.92	0	0.00	1	0.21
N/A	1	3.13	1	3.13	0	0.00	0	0.00	0	0.00	2	3.85	0	0.00	4	0.85
Total	**32**		**32**		**115**		**66**		**40**		**52**		**135**		**472**	

B: Years of beekeeping as profession	Estonia n	%	Germany n	%	Ireland n	%	Italy n	%	Spain n	%	Switzerland n	%	UK n	%	Total n	%
0	14	43.75	29	87.88	103	89.57	25	37.88	22	55.00	43	82.69	116	85.29	352	74.26
1	0	0.00	1	3.03	2	1.74	5	7.58	4	10.00	0	0.00	4	2.94	17	3.59
2	1	3.13	1	3.03	2	1.74	1	1.52	2	5.00	1	1.92	3	2.21	11	2.32
3	1	3.13	0	0.00	1	0.87	6	0.09	4	10.00	2	3.85	5	3.68	19	4.01
4	1	3.13	0	0.00	0	0.00	2	3.03	1	2.50	0	0.00	0	0.00	4	0.84
5	4	12.50	0	0.00	3	2.61	3	4.55	0	0.00	1	1.92	1	0.74	12	2.53
6	1	3.13	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	2	0.42
7	1	3.13	0	0.00	0	0.00	4	6.06	0	0.00	0	0.00	0	0.00	5	1.05
8	0	0.00	0	0.00	1	0.87	1	1.52	0	0.00	1	1.92	1	0.74	4	0.84
9	0	0.00	0	0.00	0	0.00	0	0.00	1	2.50	1	1.92	0	0.00	2	0.42
10	1	3.13	0	0.00	1	0.87	5	7.58	1	2.50	0	0.00	1	0.74	9	1.90
11	1	3.13	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	2	0.42
12	0	0.00	0	0.00	0	0.00	0	0.00	1	2.50	0	0.00	0	0.00	1	0.21
13	0	0.00	1	3.03	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
15	1	3.13	0	0.00	1	0.87	0	0.00	1	2.50	0	0.00	0	0.00	3	0.63
17	0	0.00	0	0.00	0	0.00	0	0.00	1	2.50	0	0.00	0	0.00	1	0.21
18	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
21	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
22	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
25	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
30	1	3.13	0	0.00	1	0.87	3	4.55	0	0.00	0	0.00	3	2.21	8	1.69
35	1	3.13	0	0.00	0	0.00	2	3.03	0	0.00	0	0.00	0	0.00	3	0.63
36	1	3.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.21
37	0	0.00	0	0.00	0	0.00	0	0.00	1	2.50	0	0.00	0	0.00	1	0.21
42	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	0	0.00	0	0.00	1	0.21
45	1	3.13	0	0.00	0	0.00	1	1.52	1	2.50	0	0.00	0	0.00	3	0.63
50	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.74	1	0.21

N/A	2	6.25	1	3.03	0	0.00	1	1.52	0	0.00	3	5.77	1	0.74	8	1.69
Total	**32**		**33**		**115**		**66**		**40**		**52**		**136**		**474**	

Reasons to practice beekeeping

The 13.71% of respondents (65 beekeepers) listed additional reasons to practice beekeeping. Reasons suggested more frequently are highlighted in bold.

Other reasons to practice beekeeping	Respondents	
	N	**%**
Sustainability	1	1.54
Enjoyment	3	4.62
Bee health	4	6.15
Teaching/helping other beekeepers	4	6.15
Make own products for personal use	5	7.69
Self-learning	**10**	**15.38**
Own farm pollination	2	3.08
Make own products as gift	4	3.08
Job	6	9.23
Conservation	3	4.62
Own garden pollination	3	4.62
Crop pollination	3	4.62
Fascination for bees/nature	**18**	**27.69**
Queen rearing	1	1.54
Inheritance	6	9.23
Selling bees	1	1.54
Environmental concerns	2	3.08

Frequency of communication with growers

The highest percentages per country are highlighted in bold.

Communication with growers	Country							
	Estonia	Germany	Ireland	Italy	Spain	Switzerland	UK	Total
Frequent communication (more than twice a year)	21.88%	27.27%	17.39%	**40.91%**	**47.50%**	**57.69%**	12.50%	27.22%
I am a grower myself and manage my own hives on my lands	15.63%	0.00%	7.83%	19.70%	20.00%	3.85%	11.76%	11.18%
I do not communicate with growers	21.88%	**39.39%**	**62.61%**	18.18%	22.50%	3.85%	**67.65%**	**43.67%**
Infrequent communication (once or twice a year)	**40.63%**	33.33%	12.17%	21.21%	10.00%	32.69%	8.09%	17.72%
Only when taking payments for professional pollination services	0.00%	0.00%	0.00%	0.00%	0.00%	1.92%	0.00%	0.21%

Sources of information on beehive health

The highest percentages per source per country are highlighted in bold.

Country	Source of information	Importance of sources of information				
		Extremely important	Very important	Moderately important	Slightly important	Not at all important
Estonia	Beekeeping associations	15.63%	**43.75%**	31.25%	9.38%	0.00%
	Other beekeepers	18.75%	**37.50%**	**37.50%**	6.25%	0.00%
	Training in person	6.25%	**34.38%**	31.25%	21.88%	6.25%
	National bee health agencies	3.13%	21.88%	**31.25%**	12.50%	31.25%
	Journals	**28.13%**	18.75%	25.00%	21.88%	6.25%
	Social media	0.00%	15.63%	28.13%	**37.50%**	18.75%
	Online training	3.13%	15.63%	28.13%	**37.50%**	15.63%

	Newspapers	6.25%	28.13%	**37.50%**	21.88%	6.25%
	TV/Radio	3.13%	6.25%	31.25%	**34.38%**	25.00%
	NGOs	3.13%	3.13%	**31.25%**	25.00%	37.50%
Germany	Beekeeping associations	27.27%	**39.39%**	18.18%	12.12%	3.03%
	Other beekeepers	**39.39%**	**39.39%**	12.12%	3.03%	6.06%
	Training in person	15.15%	**51.52%**	12.12%	9.09%	12.12%
	National bee health agencies	12.12%	21.21%	15.15%	18.18%	**33.33%**
	Journals	15.15%	**48.48%**	9.09%	15.15%	12.12%
	Social media	3.03%	9.09%	15.15%	24.24%	**48.48%**
	Online training	6.06%	9.09%	18.18%	24.24%	**42.42%**
	Newspapers	6.06%	24.24%	27.27%	**30.30%**	12.12%
	TV/Radio	0.00%	6.06%	15.15%	**39.39%**	**39.39%**
	NGOs	3.03%	9.09%	9.09%	18.18%	**60.61%**
Ireland	Beekeeping associations	**54.78%**	34.78%	6.09%	3.48%	0.87%
	Other beekeepers	41.74%	**42.61%**	11.30%	2.61%	1.74%
	Training in person	33.91%	**38.26%**	14.78%	6.09%	6.96%
	National bee health agencies	31.30%	**35.65%**	13.91%	10.43%	8.70%
	Journals	19.13%	**27.83%**	23.48%	16.52%	13.04%
	Social media	13.91%	20.87%	**21.74%**	**21.74%**	**21.74%**
	Online training	10.43%	22.61%	21.74%	17.39%	**27.83%**
	Newspapers	7.83%	23.48%	**26.96%**	20.00%	21.74%
	TV/Radio	8.70%	6.96%	21.74%	30.43%	**32.17%**
	NGOs	6.96%	12.17%	20.00%	26.96%	**33.91%**
Italy	Beekeeping associations	**45.45%**	33.33%	15.15%	4.55%	1.52%
	Other beekeepers	**40.91%**	33.33%	18.18%	6.06%	1.52%
	Training in person	**42.42%**	39.39%	13.64%	4.55%	0.00%
	National bee health agencies	21.21%	**31.82%**	25.76%	12.12%	9.09%
	Journals	30.30%	**50.00%**	15.15%	4.55%	0.00%
	Social media	7.58%	21.21%	**40.91%**	21.21%	9.09%
	Online training	22.73%	**33.33%**	28.79%	10.61%	4.55%
	Newspapers	13.64%	**37.88%**	28.79%	13.64%	6.06%
	TV/Radio	6.06%	16.67%	18.18%	**36.36%**	22.73%
	NGOs	12.12%	9.09%	**28.79%**	**28.79%**	21.21%
Spain	Beekeeping associations	35.00%	**47.50%**	12.50%	5.00%	0.00%
	Other beekeepers	27.50%	**42.50%**	15.00%	12.50%	2.50%
	Training in person	32.50%	**47.50%**	15.00%	5.00%	0.00%
	National bee health agencies	10.00%	**37.50%**	32.50%	12.50%	7.50%
	Journals	17.50%	**37.50%**	20.00%	17.50%	7.50%
	Social media	7.50%	25.00%	**40.00%**	17.50%	10.00%
	Online training	17.50%	**37.50%**	35.00%	10.00%	0.00%
	Newspapers	0.00%	25.00%	27.50%	**35.00%**	12.50%
	TV/Radio	2.50%	12.50%	30.00%	**32.50%**	22.50%
	NGOs	2.50%	5.00%	12.50%	**37.50%**	42.50%
Switzerland	Beekeeping associations	**50.00%**	38.46%	7.69%	3.85%	0.00%
	Other beekeepers	28.85%	**53.85%**	5.77%	9.62%	1.92%
	Training in person	**63.46%**	32.69%	1.92%	0.00%	1.92%
	National bee health agencies	**46.15%**	42.31%	9.62%	0.00%	1.92%
	Journals	**28.13%**	18.75%	25.00%	21.88%	6.25%
	Social media	0.00%	23.08%	23.08%	**30.77%**	23.08%
	Online training	11.54%	**30.77%**	**30.77%**	15.38%	11.54%
	Newspapers	17.31%	**30.77%**	26.92%	15.38%	9.62%
	TV/Radio	1.92%	15.38%	**30.77%**	28.85%	23.08%
	NGOs	3.85%	19.23%	28.85%	11.54%	**36.54%**
UK	Beekeeping associations	**47.06%**	30.88%	13.97%	5.15%	2.94%
	Other beekeepers	**42.65%**	33.82%	15.44%	3.68%	4.41%
	Training in person	28.68%	**38.24%**	16.18%	8.09%	8.82%
	National bee health agencies	25.74%	**39.71%**	22.06%	7.35%	5.15%
	Journals	18.38%	19.85%	**25.00%**	17.50%	17.65%
	Social media	9.56%	8.09%	22.79%	20.59%	**38.97%**
	Online training	8.82%	17.65%	**30.15%**	20.59%	22.79%
	Newspapers	5.15%	11.76%	19.85%	17.65%	**45.59%**
	TV/Radio	7.35%	0.74%	17.65%	25.00%	**49.26%**

	NGOs	10.29%	13.24%	**30.88%**	18.38%	27.21%

Reasons for bee decline

The highest percentages per reason per country are highlighted in bold.

Country	Reasons for bee decline	Agreement				
		Strongly agree	**Agree**	**Neutral**	**Disagree**	**Strongly disagree**
Estonia	Loss of natural habitats	25.00%	**40.63%**	21.88%	9.38%	3.13%
	Competition wild/managed	3.13%	15.63%	25.00%	**37.50%**	18.75%
	Diseases	40.63%	**53.13%**	3.13%	3.13%	0.00%
	Parasites	**56.25%**	34.38%	6.25%	3.13%	0.00%
	Predators	12.50%	21.88%	**34.38%**	31.25%	0.00%
	Climate change	6.25%	34.38%	**40.63%**	12.50%	6.25%
	Genetics	3.13%	34.38%	**46.88%**	15.63%	0.00%
	Non-optimal beekeeping	31.25%	**50.00%**	9.38%	9.38%	0.00%
	Agrochemicals	34.38%	**50.00%**	12.50%	3.13%	0.00%
Germany	Loss of natural habitats	**48.48%**	42.42%	3.03%	3.03%	3.03%
	Competition wild/managed	3.03%	15.15%	30.30%	**42.42%**	9.09%
	Diseases	18.18%	**33.33%**	30.30%	15.15%	3.03%
	Parasites	30.30%	30.30%	**33.33%**	3.03%	3.03%
	Predators	0.00%	3.03%	21.21%	**51.52%**	24.24%
	Climate change	12.12%	21.21%	**33.33%**	27.27%	6.06%
	Genetics	0.00%	15.15%	**39.39%**	27.27%	18.18%
	Non-optimal beekeeping	6.06%	36.36%	**30.30%**	18.18%	9.09%
	Agrochemicals	40.63%	**50.00%**	6.25%	3.13%	0.00%
Ireland	Loss of natural habitats	**66.09%**	28.79%	3.48%	0.87%	0.87
	Competition wild/managed	3.48%	11.30%	**52.17%**	22.61%	10.43%
	Diseases	22.61%	**53.04%**	22.61%	0.87%	0.87%
	Parasites	32.17%	**47.83%**	17.39%	2.61%	0.00%
	Predators	1.74%	13.04%	**44.35%**	33.91%	6.96%
	Climate change	15.65%	**43.48%**	33.04%	7.83%	0.00%
	Genetics	4.35%	20.87%	**56.52%**	18.26%	0.00%
	Non-optimal beekeeping	9.57%	30.43%	**46.96%**	11.30%	1.74%
	Agrochemicals	**65.22%**	30.43%	2.61%	1.74%	0.00%
Italy	Loss of natural habitats	**62.12%**	36.36%	0.00%	1.52%	0.00%
	Competition wild/managed	12.12%	10.61%	**36.36%**	30.30%	10.61%
	Diseases	**40.91%**	37.88%	16.67%	4.55%	0.00%
	Parasites	**50.00%**	39.39%	7.58%	3.03%	0.00%
	Predators	13.64%	**33.33%**	24.24%	21.21%	7.58%
	Climate change	**63.64%**	25.76%	7.58%	3.03%	0.00%
	Genetics	4.55%	16.67%	**43.94%**	30.30%	4.55%
	Non-optimal beekeeping	15.15%	**39.39%**	25.76%	12.12%	7.58%
	Agrochemicals	**75.76%**	18.18%	6.06%	0.00%	0.00%
Spain	Loss of natural habitats	40.00%	**45.00**%	7.50%	5.00%	2.5%
	Competition wild/managed	7.50%	17.50%	15.00%	**35.00%**	25.00%
	Diseases	**57.50%**	27.50%	12.50%	2.50%	0.00%
	Parasites	**57.50%**	30.00%	12.50%	0.00%	0.00%
	Predators	12.50%	**30.00%**	27.50%	25.00%	5.00%
	Climate change	**47.50%**	35.00%	10.00%	5.00%	2.50%
	Genetics	10.00%	22.50%	**30.00%**	30.30%	7.50%
	Non-optimal beekeeping	22.50%	**35.00%**	22.50%	12.50%	7.50%
	Agrochemicals	**57.50%**	32.50%	7.50%	2.50%	0.00%
Switzerland	Loss of natural habitats	**61.54%**	28.85%	3.85%	3.85%	1.92%
	Competition wild/managed	3.85%	9.62%	30.77%	**40.38%**	15.38%
	Diseases	17.31%	**42.31%**	19.23%	19.23%	1.92%
	Parasites	**36.54%**	28.85	15.38%	17.31%	4.01%
	Predators	1.92%	3.85%	25.00%	**42.31%**	26.92%
	Climate change	9.62%	**30.77%**	21.15%	26.92%	11.54%
	Genetics	7.69%	21.15%	**30.77%**	28.85%	11.54%

	Non-optimal beekeeping	23.08%	**42.31%**	26.92%	7.69%	0.00%
	Agrochemicals	32.00%	**48.00%**	14.00%	6.00%	0.00%
UK	Loss of natural habitats	**67.65%**	22.06%	7.35%	1.47%	1.47%
	Competition wild/managed	5.88%	21.32%	**40.44%**	23.53%	8.82%
	Diseases	26.47%	**51.47%**	18.38%	2.94%	0.74%
	Parasites	38.24%	**44.85%**	13.97%	2.21%	0.74%
	Predators	4.41%	30.88%	**35.29%**	19.85%	9.56%
	Climate change	22.06%	**40.44%**	22.79%	11.76%	2.94%
	Genetics	8.09%	25.74%	**49.26%**	14.71%	2.21%
	Non-optimal beekeeping	17.65%	**38.24%**	35.29%	6.62%	2.21%
	Agrochemicals	**53.33%**	35.56%	8.89%	2.22%	0.00%

Reasons to reduce bee decline

The highest percentages per reason per country are highlighted in bold.

Country	Reasons to reduce bee decline	Agreement				
		Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Estonia	Collab with growers	41.94%	**48.39%**	9.68%	0.00%	0.00%
	Hive position	40.63%	**59.38%**	0.00%	0.00%	0.00%
	Natural habitats/flower areas	**53.13%**	40.63%	6.25%	0.00%	0.00%
	Monitor diseases	**59.38%**	37.50%	3.13%	0.00%	0.00%
	Monitor parasites	**68.75%**	25.00%	6.25%	0.00%	0.00%
	Monitor nutrition	28.13%	**46.88%**	21.88%	3.13%	0.00%
	Monitor agrochemicals	37.50%	**46.88%**	15.63%	0.00%	0.00%
	Optimal beekeeping	40.63%	**53.13%**	6.25%	0.00%	0.00%
Germany	Collab with growers	**54.55%**	39.39%	3.03%	0.00%	3.03%
	Hive position	32.26%	**45.16%**	16.13%	6.45%	0.00%
	Natural habitats/flower areas	**65.63%**	31.25%	3.13%	0.00%	0.00%
	Monitor diseases	37.50%	**40.63%**	21.88%	0.00%	0.00%
	Monitor parasites	**46.88%**	28.13%	25.00%	0.00%	0.00%
	Monitor nutrition	15.15%	**54.55%**	27.27%	0.00%	3.03%
	Monitor agrochemicals	**53.13%**	34.38%	12.50%	53.13%	0.00%
	Optimal beekeeping	35.48%	**51.61%**	12.90%	0.00%	0.00%
Ireland	Collab with growers	**46.09%**	**46.09%**	7.83%	0.00%	0.00%
	Hive position	26.96%	**55.65%**	14.78%	2.61%	0.00%
	Natural habitats/flower areas	**62.61%**	33.91%	1.74%	1.74%	0.00%
	Monitor diseases	43.48%	**53.04%**	3.48%	0.00%	0.00%
	Monitor parasites	44.35%	**51.30%**	4.35%	0.00%	0.00%
	Monitor nutrition	39.13%	**43.48%**	15.65%	0.87%	0.87%
	Monitor agrochemicals	**68.70%**	26.09%	4.35%	0.87%	0.00%
	Optimal beekeeping	**42.61%**	41.74%	15.65%	0.00%	0.00%
Italy	Collab with growers	**60.94%**	31.25%	7.81%	0.00%	0.00%
	Hive position	39.06%	**46.88%**	14.06%	0.00%	0.00%
	Natural habitats/flower areas	**74.24%**	24.24%	0.00%	1.52%	0.00%
	Monitor diseases	**45.31%**	42.19%	12.50%	0.00%	0.00%
	Monitor parasites	**48.44%**	45.31%	6.25%	0.00%	0.00%
	Monitor nutrition	34.85%	**40.91%**	19.70%	4.55%	0.00%
	Monitor agrochemicals	**66.67%**	30.30%	3.03%	0.00%	0.00%
	Optimal beekeeping	**51.52%**	33.33%	15.15%	0.00%	0.00%
Spain	Collab with growers	45.00%	**52.50%**	2.50%	0.00%	0.00%
	Hive position	35.00%	**42.50%**	17.50%	5.00%	0.00%
	Natural habitats/flower areas	35.00%	**45.00%**	20.00%	0.00%	0.00%
	Monitor diseases	**60.00%**	32.50%	7.50%	0.00%	0.00%
	Monitor parasites	**57.50%**	32.50%	10.00%	0.00%	0.00%
	Monitor nutrition	**37.50%**	**37.50%**	17.50%	7.50%	0.00%
	Monitor agrochemicals	**55.00%**	37.50%	7.50%	0.00%	0.00%
	Optimal beekeeping	**56.41%**	33.33%	10.26%	0.00%	0.00%
Switzerland	Collab with growers	**50.00%**	48.08%	1.92%	0.00%	0.00%
	Hive position	**48.08%**	36.54%	7.69%	7.69%	0.00%
	Natural habitats/flower areas	**63.46%**	30.77%	5.77%	0.00%	0.00%

		Strongly agree	Agree	Neutral	Disagree	Strongly disagree
	Monitor diseases	**48.08%**	40.38%	11.54%	0.00%	0.00%
	Monitor parasites	**46.15%**	42.31%	11.54%	0.00%	0.00%
	Monitor nutrition	**42.31%**	34.62%	19.23%	3.85%	0.00%
	Monitor agrochemicals	**50.00%**	36.54%	9.62%	3.85%	0.00%
	Optimal beekeeping	**52.94%**	45.10%	1.96%	0.00%	0.00%
UK	Collab with growers	29.85%	**46.27%**	22.39%	0.00%	1.49%
	Hive position	27.21%	**51.47%**	19.12%	2.21%	0.00%
	Natural habitats/flower areas	**71.32%**	24.26%	4.41%	0.00%	0.00%
	Monitor diseases	**54.07%**	41.48%	4.44%	0.00%	0.00%
	Monitor parasites	**53.68%**	41.18%	5.15%	0.00%	0.00%
	Monitor nutrition	**41.91%**	40.44%	17.65%	0.00%	0.00%
	Monitor agrochemicals	**57.04%**	33.33%	8.89%	0.74%	0.00%
	Optimal beekeeping	**54.07%**	31.11%	14.81%	0.00%	0.00%

Reasons to protect bee health

The highest percentages per reason per country are highlighted in bold.

Country	Reasons to protect bee health	Agreement				
		Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Estonia	Economic reasons	28.13%	**43.75%**	25.00%	3.13%	0.00%
	Legal reasons	18.75%	**40.63%**	31.25%	9.38%	0.00%
	Public perception	15.63%	21.88%	**43.75%**	12.50%	6.25%
	Pollinators conservation	**65.63%**	28.13%	6.25%	0.00%	0.00%
	Consumer safety	**43.75%**	40.63%	15.63%	0.00%	0.00%
	Food security	37.50%	**56.25%**	6.25%	0.00%	0.00%
	Crop varieties	34.38%	**43.75%**	18.75%	3.13%	0.00%
Germany	Economic reasons	12.12%	**33.33%**	**33.33%**	15.15%	6.06%
	Legal reasons	12.12%	30.30%	**36.36%**	12.12%	9.09%
	Public perception	18.18%	30.30%	**36.36%**	6.06%	9.09%
	Pollinators conservation	**59.38%**	37.50%	3.13%	0.00%	0.00%
	Consumer safety	9.09%	30.30%	**33.33%**	18.18%	9.09%
	Food security	24.24%	**33.33%**	27.27%	12.12%	3.03%
	Crop varieties	**51.52%**	36.36%	9.09%	3.03%	0.00%
Ireland	Economic reasons	25.22%	**40.87%**	26.09%	6.09%	1.74%
	Legal reasons	13.91%	31.30%	**36.52%**	13.91%	4.35%
	Public perception	18.26%	**33.04%**	32.17%	13.91%	2.61%
	Pollinators conservation	**77.39%**	20.00%	1.74%	0.87%	0.00%
	Consumer safety	30.43%	**44.35%**	16.52%	7.83%	0.87%
	Food security	**56.52%**	33.04%	7.83%	1.74%	0.87%
	Crop varieties	42.61%	**43.48%**	12.17%	1.74%	0.00%
Italy	Economic reasons	25.76%	**43.94%**	25.76%	3.03%	1.52%
	Legal reasons	18.18%	31.82%	**42.42%**	6.06%	1.52%
	Public perception	21.21%	30.30%	**31.82%**	9.09%	7.58%
	Pollinators conservation	**81.82%**	13.64%	3.03%	1.52%	0.00%
	Consumer safety	**39.39%**	37.88%	15.15%	6.06%	1.52%
	Food security	**57.58%**	28.79%	9.09%	4.55%	0.00%
	Crop varieties	39.39%	**43.94%**	13.64%	3.03%	0.00%
Spain	Economic reasons	27.50%	**35.00%**	22.50%	15.00%	0.00%
	Legal reasons	10.00%	32.50%	**40.00%**	12.50%	5.00%
	Public perception	10.00%	**37.50%**	35.00%	15.00%	2.50%
	Pollinators conservation	**60.00%**	35.00%	5.00%	0.00%	0.00%
	Consumer safety	**40.00%**	**40.00%**	17.50%	2.50%	0.00%
	Food security	**42.50%**	40.00%	15.00%	2.50%	0.00%
	Crop varieties	30.00%	**50.00%**	17.50%	2.50%	0.00%
Switzerland	Economic reasons	9.62%	**30.77%**	26.92%	25.00%	7.69%
	Legal reasons	13.46%	**48.08%**	26.92%	11.54%	0.00%
	Public perception	21.15%	**48.08%**	23.08%	7.69%	0.00%
	Pollinators conservation	**57.69%**	36.54%	5.77%	0.00%	0.00%
	Consumer safety	17.31%	28.85%	**44.23%**	7.69%	1.92%
	Food security	23.08%	**38.46%**	30.77%	7.69%	0.00%

	Crop varieties	34.62%	**42.31%**	19.23%	3.85%	0.00%
UK	Economic reasons	**38.24%**	33.09%	20.59%	6.62%	1.47%
	Legal reasons	12.50%	28.68%	**47.06%**	9.56%	2.21%
	Public perception	16.18%	34.56%	**38.97%**	9.56%	0.74%
	Pollinators conservation	**78.68%**	16.91%	4.41%	0.00%	0.00%
	Consumer safety	24.26%	**36.03%**	32.35%	5.88%	1.47%
	Food security	**52.21%**	33.09%	11.03%	2.21%	1.47%
	Crop varieties	**40.44%**	39.71%	16.18%	2.21%	1.47%

Frequency of health checks performed on beehives

The highest percentages for each check in each country are highlighted in bold.

Country	Frequency	Checks			
		Diseases	**Parasites**	**Nutrition**	**Chemicals**
Estonia	Weekly	**31.25%**	25.00%	28.13%	18.75%
	Fortnightly	9.38%	12.50%	15.63%	6.25%
	Monthly	18.75%	18.75%	15.63%	9.38%
	More than once a year	21.88%	**34.38%**	**31.25%**	15.63%
	Yearly	3.13%	3.13%	3.13%	6.25%
	Only with a reasonable suspicion	15.63%	6.25%	6.25%	**43.75%**
	Never	0.00%	0.00%	0.00%	0.00%
Germany	Weekly	12.12%	6.06%	9.09%	0.00%
	Fortnightly	15.15%	15.15%	18.18%	3.03%
	Monthly	9.09%	9.09%	15.15%	3.03%
	More than once a year	27.27%	**45.45%**	**33.33%**	6.06%
	Yearly	12.12%	12.12%	6.06%	9.09%
	Only with a reasonable suspicion	21.21%	9.09%	9.09%	**48.48%**
	Never	3.03%	3.03%	9.09%	30.30%
Ireland	Weekly	20.87%	14.78%	32.17%	10.43%
	Fortnightly	**32.17%**	26.09%	**34.78%**	8.70%
	Monthly	19.13%	20.00%	17.39%	3.48%
	More than once a year	20.87%	**30.43%**	4.35%	2.61%
	Yearly	0.87%	1.74%	0.87%	2.61%
	Only with a reasonable suspicion	6.09%	6.09%	5.22%	28.70%
	Never	0.00%	0.87%	5.22%	**43.48%**
Italy	Weekly	**37.88%**	27.27%	24.24%	21.21%
	Fortnightly	27.27%	25.76%	**31.82%**	15.15%
	Monthly	21.21%	19.70%	6.06%	12.12%
	More than once a year	9.09%	22.73%	18.18%	10.61%
	Yearly	0.00%	0.00%	1.52%	7.58%
	Only with a reasonable suspicion	4.55%	4.55%	15.15%	**24.24%**
	Never	0.00%	0.00%	3.03%	9.09%
Spain	Weekly	7.50%	5.00%	10.00%	10.00%
	Fortnightly	20.00%	12.50%	17.50%	5.00%
	Monthly	**30.00%**	**40.00%**	22.50%	7.50%
	More than once a year	25.00%	20.00%	**27.50%**	10.00%
	Yearly	12.50%	17.50%	5.00%	2.50%
	Only with a reasonable suspicion	5.00%	2.50%	10.00%	20.00%
	Never	0.00%	2.50%	7.50%	**45.00%**
Switzerland	Weekly	19.23%	19.23%	19.23%	5.77%
	Fortnightly	**34.62%**	25.00%	**30.77%**	9.62%
	Monthly	25.00%	**32.69%**	25.00%	1.92%
	More than once a year	15.38%	13.46%	21.15%	13.46%
	Yearly	0.00%	0.00%	0.00%	3.85%
	Only with a reasonable suspicion	5.77%	9.62%	3.85%	**46.15%**
	Never	0.00%	0.00%	0.00%	10.29%
UK	Weekly	**31.62%**	24.26%	**40.44%**	2.21%
	Fortnightly	21.32%	17.65%	26.47%	2.21%
	Monthly	18.38%	26.47%	17.65%	4.41%
	More than once a year	22.06%	23.53%	7.35%	2.21%
	Yearly	1.47%	1.47%	0.74%	**44.85%**

	Only with a reasonable suspicion	4.41%	6.62%	5.88%	33.82%
	Never	0.74%	0.00%	1.47%	10.29%

Potential barriers to using the Bee Health Card

The highest percentages per barrier per country are highlighted in bold.

Country	Barriers	Agreement				
		Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Estonia	No communication with growers	18.75%	**46.88%**	25.00%	9.38%	0.00%
	Cost	25.81%	**38.71%**	22.58%	12.90%	0.00%
	Effectiveness	6.25%	12.50%	**50.00%**	28.13%	3.13%
	Time	6.25%	15.63%	**40.63%**	34.38%	3.13%
	Difficulty	3.13%	12.50%	**40.63%**	31.25%	12.50%
	No importance in being used	3.13%	3.13%	**40.63%**	31.25%	21.88%
Germany	No communication with growers	9.09%	**42.42%**	36.36%	3.03%	9.09%
	Cost	15.63%	**43.75%**	34.38%	6.25%	0.00%
	Effectiveness	21.21%	18.18%	**33.33%**	27.27%	0.00%
	Time	3.03%	21.21%	**45.45%**	27.27%	3.03%
	Difficulty	0.00%	12.12%	**51.52%**	33.33%	3.03%
	No importance in being used	18.18%	15.15%	**39.39%**	18.18%	9.09%
Ireland	No communication with growers	21.74%	**39.13%**	31.30%	6.96%	0.87%
	Cost	25.44%	**40.35%**	26.32%	7.89%	0.00%
	Effectiveness	2.61%	32.17%	**42.61%**	19.13%	3.48%
	Time	2.61%	20.00%	**41.74%**	29.57%	6.09%
	Difficulty	0.87%	11.30%	**42.61%**	39.13%	6.09%
	No importance in being used	3.48%	12.17%	**43.48%**	26.96%	13.91%
Italy	No communication with growers	**42.42%**	34.85%	15.15%	6.06%	1.52%
	Cost	18.46%	**33.85%**	32.31%	15.38%	0.00%
	Effectiveness	10.61%	28.79%	**33.33%**	22.73%	4.55%
	Time	7.58%	24.24%	**36.36%**	31.82%	0.00%
	Difficulty	6.06%	18.18%	34.85%	**39.39%**	1.52%
	No importance in being used	4.55%	9.09%	28.79%	**37.88%**	19.70%
Spain	No communication with growers	30.00%	**40.00%**	20.00%	5.00%	5.00%
	Cost	20.00%	**32.50%**	**32.50%**	15.00%	0.00%
	Effectiveness	12.50%	17.50%	**35.00%**	32.50%	2.50%
	Time	0.00%	12.50%	**45.00%**	27.50%	15.00%
	Difficulty	0.00%	7.50%	**50.00%**	35.00%	7.50%
	No importance in being used	10.00%	7.50%	**37.50%**	27.50%	17.50%
Switzerland	No communication with growers	7.69%	**48.08%**	30.77%	7.69%	5.77%
	Cost	17.65%	**52.94%**	27.45%	1.96%	0.00%
	Effectiveness	15.38%	**40.38%**	28.85%	11.54%	3.85%
	Time	9.62%	**34.62%**	**34.62%**	19.23%	1.92%
	Difficulty	9.62%	23.08%	**36.54%**	23.08%	7.69%
	No importance in being used	15.38%	19.23%	**44.23%**	11.54%	9.62%
UK	No communication with growers	25.00%	30.15%	**39.71%**	2.21%	2.94%
	Cost	33.82%	**38.97%**	19.85%	7.35%	0.00%
	Effectiveness	11.76%	26.47%	**42.65%**	16.91%	2.21%
	Time	4.41%	20.59%	**36.03%**	**36.03%**	2.94%
	Difficulty	2.21%	8.09%	**42.65%**	41.18%	5.88%
	No importance in being used	3.68%	20.59%	**36.03%**	30.88%	8.82%

Potential benefits of using the Bee Health Card

The highest percentages per benefit per country are highlighted in bold.

Country	Benefits	Agreement				
		Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Estonia	Communication with growers	9.38%	**50.00%**	31.25%	6.25%	3.13%

	Productivity	6.25%	**43.75%**	40.63%	6.25%	3.13%
	Quick and easy	3.13%	40.63%	**50.00%**	3.13%	3.13%
	Lower treatment cost	3.13%	40.63%	**43.75%**	9.38%	3.13%
	Better crop pollination	3.13%	31.25%	**59.38%**	3.13%	3.13%
	Increases bee health	9.38%	**65.63%**	21.88%	0.00%	3.13%
	Environment protection	3.13%	**43.75%**	40.63%	9.38%	3.13%
	Pollinators protection	3.13%	**65.63%**	25.00%	3.13%	3.13%
Germany	Communication with growers	9.09%	**36.36%**	33.33%	6.06%	15.15%
	Productivity	3.03%	15.15%	**51.52%**	21.21%	9.09%
	Quick and easy	0.00%	**42.42%**	39.39%	9.09%	9.09%
	Lower treatment cost	0.00%	15.15%	**60.61%**	18.18%	6.06%
	Better crop pollination	0.00%	27.27%	**42.42%**	21.21%	9.09%
	Increases bee health	9.09%	**54.55%**	21.21%	9.09%	6.06%
	Environment protection	6.06%	**42.42%**	33.33%	15.15%	3.03%
	Pollinators protection	9.09%	**45.45%**	33.33%	9.09%	3.03%
Ireland	Communication with growers	14.78%	35.65%	**37.39%**	9.57%	2.61%
	Productivity	14.78%	**45.22%**	33.04%	5.22%	1.74%
	Quick and easy	9.57%	**43.48%**	41.74%	4.35%	0.87%
	Lower treatment cost	9.57%	33.04%	**46.09%**	10.43%	0.87%
	Better crop pollination	10.43%	32.17%	**48.70%**	7.83%	0.87%
	Increases bee health	33.91%	**42.61%**	21.74%	0.87%	0.87%
	Environment protection	26.09%	31.30%	**39.13%**	2.61%	0.87%
	Pollinators protection	29.57%	**46.96%**	20.87%	1.74%	0.87%
Italy	Communication with growers	21.21%	**43.94%**	25.76%	6.06%	3.03%
	Productivity	15.15%	**39.39%**	33.33%	12.12%	0.00%
	Quick and easy	7.58%	33.33%	**48.48%**	10.61%	0.00%
	Lower treatment cost	4.55%	30.30%	**40.91%**	19.70%	4.55%
	Better crop pollination	6.06%	22.73%	**50.00%**	18.18%	3.03%
	Increases bee health	21.21%	**48.48%**	19.70%	7.58%	3.03%
	Environment protection	19.70%	**45.45%**	25.76%	4.55%	4.55%
	Pollinators protection	22.73%	**59.09%**	15.15%	1.52%	1.52%
Spain	Communication with growers	25.00%	**35.00%**	30.00%	7.50%	2.50%
	Productivity	25.00%	**50.00%**	15.00%	7.50%	2.50%
	Quick and easy	22.50%	**40.00%**	32.50%	2.50%	2.50%
	Lower treatment cost	20.00%	**35.00%**	25.00%	15.00%	5.00%
	Better crop pollination	17.50%	22.50%	**47.50%**	7.50%	5.00%
	Increases bee health	35.00%	**37.50%**	20.00%	5.00%	2.50%
	Environment protection	22.50%	**42.50%**	27.50%	5.00%	2.50%
	Pollinators protection	**30.00%**	40.00%	22.50%	5.00%	2.50%
Switzerland	Communication with growers	19.23%	26.92%	**36.54%**	13.46%	3.85%
	Productivity	3.85%	13.46%	**46.15%**	30.77%	5.77%
	Quick and easy	5.77%	34.62%	**38.46%**	17.31%	3.85%
	Lower treatment cost	5.77%	11.54%	**42.31%**	32.69%	7.69%
	Better crop pollination	3.85%	17.31%	**48.08%**	17.31%	13.46%
	Increases bee health	13.46%	28.85%	**38.46%**	13.46%	5.77%
	Environment protection	7.69%	25.00%	**42.31%**	11.54%	13.46%
	Pollinators protection	15.38%	28.85%	**42.31%**	7.69%	5.77%
UK	Communication with growers	15.44%	27.21%	**44.85%**	8.09%	4.41%
	Productivity	9.56%	28.68%	**52.94%**	5.88%	2.94%
	Quick and easy	11.03%	38.24%	**39.71%**	9.56%	1.47%
	Lower treatment cost	7.35%	24.26%	**48.53%**	16.91%	2.94%
	Better crop pollination	8.82%	18.38%	**58.09%**	11.76%	2.94%
	Increases bee health	29.41%	**40.44%**	22.79%	5.15%	2.21%
	Environment protection	18.38%	33.09%	**39.71%**	6.62%	2.21%
	Pollinators protection	25.74%	**36.76%**	29.41%	5.88%	2.21%

Health issues to be detected by the Bee Health Card

The 46.41% of respondents (220 beekeepers) suggested some bee health issues that they would like the BHC to detect. Most suggested issues are highlighted in bold.

Health issues	Respondents	
	n	%
Acarine	4	1.82%
Bacterial infections	2	0.91%
Bee health improvements	1	0.45%
Black Queen Cell Virus	1	0.45%
Brood diseases	2	0.91%
Chalkbrood	5	2.27%
Chilled brood	1	0.45%
Chronic Bee Paralysis Virus	22	10.00%
Colony Collapse Disorder	3	1.36%
Deformed Wing Virus	16	7.27%
Diseases	31	14.09%
Fat body	1	0.45%
Foulbroods	**52**	**23.64%**
Fungal infections	2	0.91%
Gut diseases	1	0.45%
Issues that cannot be detected by visual inspections	2	0.91%
Mated queen fertility	1	0.45%
Nosema	41	18.64%
Nutritional issues	17	7.73%
Parasites	18	8.18%
Parasitic Mite Syndrome	1	0.45%
Pathogens	4	1.82%
Pesticides	**47**	**21.36%**
Pollution	5	2.27%
Queen health	1	0.45%
Resilience index	1	0.45%
Sac brood	6	2.73%
Sour brood	2	0.91%
Spiroplasma	1	0.45%
Stress	2	0.91%
Tracheal mites	2	0.91%
Varroa and viruses linked to it	**57**	**25.91%**
Viruses	41	18.64%

Appendix 4.4

This appendix is divided into 3 parts:

- Part A: Global models of the binary logistic regressions.

- Part B: Model selection using BIC.

- Part C: Table of coefficients obtained from the binary logistic regressions.

Part A: Global models

Global models before and after removing terms with a Variance Inflation Factor (VIF) ≥5 are shown below.

Response variable	Global model before removing terms with VIF ≥5	Global model after removing terms with VIF ≥5
Willingness to use the BHC with incentives	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i bhc.be.pp.ep.qe bhc.be.g.bh.cp	bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i bhc.be.pp.ep.qe bhc.be.g.bh.cp
Willingness to use the BHC without incentives	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i bhc.be.pp.ep.qe bhc.be.g.bh.cp	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.t.e.d.i bhc.be.pp.ep.qe bhc.be.g.bh.cp
Willingness to accept BHC extra costs with incentives	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i bhc.be.pp.ep.qe bhc.be.g.bh.cp	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i
Willingness to accept BHC extra costs without incentives	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i bhc.be.pp.ep.qe bhc.be.g.bh.cp	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i
Frequency of BHC use with incentives	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i bhc.be.pp.ep.qe bhc.be.g.bh.cp	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i
Frequency of BHC use without incentives	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i bhc.be.pp.ep.qe bhc.be.g.bh.cp	Country bhc.e bhc.be.p bhc.be.tc bhc.ba.c bhc.ba.t.e.d.i

Part B: BIC model selection

Model selection tables below show candidate models for each analysis from the best (ΔBIC=0) to the worst model (largest BIC). Models in bold are the final, selected models with the lowest BIC and ΔBIC≤2.

Willingness to use the BHC with economic incentives		
Terms	**BIC**	**ΔBIC**
bhc.e + bhc.be.p + bhc.ba.t.e.d.i	**288.28**	**0**
bhc.e + bhc.be.p + bhc.ba.c + bhc.ba.t.e.d.i	295.14	6.86
bhc.e + bhc.be.p + bhc.ba.c + bhc.ba.t.e.d.i + bhc.be.pp.ep.qe	303.77	15.49
bhc.e + bhc.be.p + bhc.be.tc + bhc.ba.c + bhc.ba.t.e.d.i + bhc.be.pp.ep.qe	313.11	24.83
bhc.e + bhc.be.p + bhc.be.tc + bhc.ba.c + bhc.ba.t.e.d.i + bhc.be.pp.ep.qe + bhc.be.g.bh.cp	325.38	37.1
Willingness to use the BHC without economic incentives		
Terms	**BIC**	**ΔBIC**
bhc.e + bhc.ba.t.e.d.i + bhc.be.pp.ep.qe	**313.92**	**0**
Country + bhc.e + bhc.ba.t.e.d.i + bhc.be.pp.ep.qe	339.15	25.23
Country + bhc.e + bhc.be.tc + bhc.ba.t.e.d.i + bhc.be.pp.ep.qe	349.79	35.87
Country + bhc.e + bhc.be.p + bhc.be.tc + bhc.ba.t.e.d.i + bhc.be.pp.ep.qe	361.51	47.59
Country + bhc.e + bhc.be.p + bhc.be.tc + bhc.ba.t.e.d.i + bhc.be.pp.ep.qe + bhc.be.g.bh.cp	373.76	59.84
Willingness to accept BHC extra costs with economic incentives		
Terms	**BIC**	**ΔBIC**
bhc.e + bhc.ba.t.e.d.i	**615.39**	**0**
bhc.e + bhc.be.p + bhc.ba.t.e.d.i	623.91	8.52
bhc.e + bhc.be.p + bhc.ba.c + bhc.ba.t.e.d.i	632.36	16.97
bhc.e + bhc.be.p + bhc.be.tc + bhc.ba.c + bhc.ba.t.e.d.i	643.44	28.05
Country + bhc.e + bhc.be.p + bhc.be.tc + bhc.ba.c + bhc.ba.t.e.d.i	676.64	61.25
Frequency of BHC use with economic incentives		
Terms	**BIC**	**ΔBIC**
bhc.e + bhc.ba.c	**481.11**	**0**
bhc.e + bhc.be.p + bhc.ba.c	488.02	6.91
bhc.e + bhc.be.p + bhc.ba.c + bhc.ba.t.e.d.i	497.26	16.15
Country + bhc.e + bhc.be.p + bhc.ba.c + bhc.ba.t.e.d.i	526.07	44.96
Country + bhc.e + bhc.be.p + bhc.be.tc + bhc.ba.c + bhc.ba.t.e.d.i	537.33	56.22
Frequency of BHC use without economic incentives		
Terms	**BIC**	**ΔBIC**
bhc.e + bhc.ba.c	**546.23**	**0**
Country + bhc.e + bhc.ba.c	574.27	28.04
Country + bhc.e + bhc.be.p + bhc.ba.c	585.79	39.52
Country + bhc.e + bhc.be.p + bhc.be.tc + bhc.ba.c	597.62	51.39
Country + bhc.e + bhc.be.p + bhc.be.tc + bhc.ba.c + bhc.ba.t.e.d.i	609.53	63.30

Part C: Table of coefficients

The following table shows coefficients, SE, z-values, p-values, and VIFs of final model terms. Variables used as reference values are 'fair confidence' and 'neutral' answers. Significant p-values (<0.050) are highlighted in bold and indicate significant differences from the reference variables.

Willingness to use the BHC with incentives	Coefficient values				
Term	**Coeff**	**SE**	**Z-Value**	**P-Value**	**VIF**
Confidence level in effectiveness					
High confidence	0.706	0.536	1.32	0.188	1.19
No confidence	-1.630	0.418	-3.90	**<0.001**	1.09
Productivity as benefit					
Disagree	-0.791	0.391	-2.03	**0.043**	1.14
Agree	0.963	0.468	2.06	**0.040**	1.17
Time, effectiveness, difficulty, and importance as barriers					
Disagree	0.065	0.563	0.11	0.908	1.19
Agree	-1.163	0.366	-3.18	**0.001**	1.16
Willingness to use the BHC without incentives	**Coefficient values**				

Term	Coeff	SE	Z-Value	P-Value	VIF
Confidence level in effectiveness					
High confidence	1.535	0.651	2.36	**0.018**	1.15
No confidence	-1.366	0.413	-3.31	**0.001**	1.07
Time, effectiveness, difficulty, and importance as barriers					
Disagree	-0.313	0.492	-0.64	0.524	1.18
Agree	-0.918	0.345	-2.66	**0.008**	1.16
Pollinator protection, environment protection, and easy to use the tool as benefits					
Disagree	-1.097	0.466	-2.36	**0.018**	1.11
Agree	1.058	0.393	2.69	**0.007**	1.17
Willingness to accept BHC extra costs with incentives	**Coefficient values**				
Term	Coeff	SE	Z-Value	P-Value	VIF
Confidence level in effectiveness					
High confidence	0.475	0.215	2.21	**0.027**	
No confidence	-1.902	0.552	-3.45	**0.001**	1.02
Time, effectiveness, difficulty, and importance as barriers					
Disagree	0.778	0.255	3.05	**0.002**	1.10
Agree	-0.894	0.268	-3.34	**0.001**	1.09
Willingness to accept BHC extra costs without incentives	**Coefficient values**				
Term	Coeff	SE	Z-Value	P-Value	VIF
Confidence level in effectiveness					
High confidence	0.306	0.216	1.42	0.156	1.12
No confidence	-1.866	0.555	-3.36	**0.001**	1.02
Cost as barrier					
Disagree	0.624	0.403	1.55	0.122	1.23
Agree	-0.381	0.227	-1.68	**0.094**	1.21
Time, effectiveness, difficulty, and importance as barriers					
Disagree	0.655	0.257	2.54	**0.011**	1.15
Agree	-0.593	0.267	-2.22	**0.026**	1.13
Frequency of use of the BHC with incentives	**Coefficient values**				
Term	Coeff	SE	Z-Value	P-Value	VIF
Confidence level in effectiveness					
High confidence	0.837	0.260	3.22	**0.001**	1.04
No confidence	-1.262	0.476	-2.65	**0.008**	1.03
Cost as barrier					
Disagree	-0.310	0.482	-0.64	0.520	1.33
Agree	-0.734	0.296	-2.48	**0.013**	1.31
Frequency of use of the BHC without incentives	**Coefficient values**				
Term	Coeff	SE	Z-Value	P-Value	VIF
Confidence level in effectiveness					
High confidence	0.877	0.221	3.96	**<0.001**	1.03
No confidence	-1.273	0.586	-2.17	**0.030**	1.02
Cost as barrier					
Disagree	0.287	0.428	0.67	0.503	1.23
Agree	-0.742	0.249	-2.98	**0.003**	1.23

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