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By

Laura T Ward

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Committee in charge:

Professor Nicholas J. Mills, Chair
Professor Erica Bree Rosenblum
Professor Eileen A. Lacey

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Abstract

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This dissertation combines two disparate subjects: bees and belonging. The first two chapters explore pesticide exposure for wild bees and honey bees visiting crop and non-crop plants in northern California agriculture. The final chapter utilizes surveys from a mentorship program as a case study to analyze sense of belonging among undergraduates.

The first chapter of this dissertation explores pesticide exposure for wild bees and honey bees visiting sunflower crops. Neonicotinoid seed treatments are commonly used in hybrid sunflower production, yet their impact on crop yield and pollinators, particularly wild bees, remains unclear. In this study, a matched pairs design was used to assess bee exposure to pesticides in treatment plots planted with thiamethoxam treated seed and control plots planted without thiamethoxam. Samples of field soil, sunflower pollen, sunflower nectar, pollen-foraging honey bees, nectar-foraging honey bees, and a sunflower specialist wild bee (*Melissodes agilis*) were analyzed for pesticide residues. The effects of thiamethoxam treated seed on bee diversity and crop yield was also quantified. Though thiamethoxam was only detected at low concentrations in one soil sample, the treatment still impacted bee diversity. *M. agilis* abundance and crop yield were both significantly lower in treatment plots. Conversely, wild bee richness was significantly higher in treatment plots, potentially due to resource partitioning. Furthermore, many pesticides not used in the sunflower fields were detected in the samples, some of which are known to negatively impact bees, highlighting the importance of landscape scale assessments in pesticide risk analysis. Taken together, these results suggest that thiamethoxam seed treatment in sunflower may negatively impact wild bee pollination services. Furthermore, withholding this prophylactic treatment could be economically advantageous to farmers and seed companies. Most importantly, this study exposes the necessity of including multiple metrics in pesticide risk analysis, since residue analysis for specific active ingredients may miss the broader impacts of pesticide exposure for bees.

The second chapter of this dissertation explores pesticide exposure for wild bees and honey bees visiting perimeter (i.e. non-crop) plants in an agricultural landscape. Perimeter plantings in the form of bee attractive hedgerows and forb strips are one conservation strategy to bolster bee abundance and richness in agricultural regions, but recent studies suggest that these plants may also harbor pesticides. As bee species richness is known to be higher in perimeter plantings than in the crop area itself, hedgerows and forb strips are uniquely suited for the investigation of pesticide exposure for a wide variety of bee species. In this study, a number of samples were collected within perimeter plantings for pesticide residue analysis, including: multiple species of wild bees, honey bees, flowers from four types of bee-attractive perimeter plants, and soil. This study also made use of silicone bands as passive samplers of aerial pesticide residues. A number of results arose from this study. The greatest number of pesticides were detected in aerial samples, followed by soil, however the greatest concentrations of pesticides were detected in flowers. Flowers in some cases harbored pesticides applied to crops in the greater landscape. Pesticide concentrations were also higher in bees than in soil and seemed to increase with increasing wild bee size. Honey bees and wild bees were found to be differentially exposed to pesticides and both contained pesticide mixtures of concern for bee health. These results highlight the necessity for a landscape scale approach to pesticide risk analysis, in addition to the inclusion of greater range of bee species.

The third chapter of this dissertation explores undergraduate sense of belonging as the result of a mentorship program in a large research university. Sense of belonging is a fundamental human need that precedes learning and innovation. In higher education, a strong sense of belonging is positively associated with a number of academically advantageous outcomes. Historically underrepresented student populations, such as transfer students, first-generation college students, and underrepresented minorities, are known to face greater barriers in feeling a strong sense of belonging in higher education. This study evaluated an effort to improve sense of belonging among undergraduate students through a novel academic mentoring program. The Berkeley Connect mentoring program at UC Berkeley began in 2014 and serves thousands of students each year. It is a tiered program in which faculty directors within each department mentor a small cohort of graduate students, each of whom then mentor approximately forty undergraduate students each semester. Three program elements are utilized to promote community and a sense of belonging on campus: one-on-one meetings, small-group discussions, and special events. Survey data indicated that sense of belonging increased over the course of the semester for students in the program, regardless of the demographics studied. One-on-one meetings were particularly effective for reaching program objectives. Since this mentoring program had positive impacts for large numbers of undergraduate students, including historically marginalized student populations, this may be a promising model to be applied more broadly across a range of higher education institutions.

Dedicated to my dad, Steve Brunsvold.

“You numbah one.”

I have lived on the lip

of insanity, wanting to know reasons,

knocking on a door. It opens.

I've been knocking from the inside!

- Rumi

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Introduction

This section serves to introduce two disparate topics explored in this dissertation: bees and belonging. And yet, in order to understand why they manifest alongside each other, one must first become familiar with their connecting entity: the author. Thus first, a brief introduction is presented regarding the author and her arrival at these subject matters. Then, an introduction to bees is provided as the motivation for two field studies on wild bee conservation in agriculture. Finally, a short introduction on belongingness is given as the provocation for a case study on undergraduate sense of belonging as the result of a mentorship program.

The author

Throughout the author's tenure in academia, she has sought to cultivate connection between entities thought to be detached. Connection, as it turns out, is perhaps the unsung hero of this dissertation. In her baccalaureate years, she explored the interface of philosophy and science from the ancient Greek period through the twentieth century. In her post-baccalaureate years, while she helped people with disabilities lead inclusive and engaged lives, she pondered the possibility of building her liberal arts background into a scientific career.

One crisp, clear day in early spring, she found herself under the dappled light and the generous hum of an almond orchard in full bloom. Boxes upon boxes of little white honey bee hives dotted the landscape. Agriculture heavily relies on the pollination service of this one species, transported on semi-trailer trucks from one monoculture to the next — a practice that seemed startlingly ridiculous, utterly irresponsible, and disconnected from the realities of the planet. It was there among those trees that the seed of this dissertation was first discovered.

Onward she went, meeting scientists, farmers, and activists, all working towards wild bee conservation in agriculture. As she spent more time in the field, restoring floral and nesting resources for wild bees and pioneering bee friendly practices in the horticultural industry, the paucity of knowledge on the impact of pesticides became apparent. These synthetic chemical inputs seemed to have become the norm for pest control — unnaturally second nature — in the current agricultural system and the culture at large. This disjointed system between the food that sustains us and the environment that sustains it seemed downright unsustainable. Thus, it was there in the field amidst fan clouds of pesticides trailing low swooping planes that the seed for this dissertation was sown.

She set about in her doctoral program learning the necessary subjects, designing her studies, and teaching — as grad students do to get by. For her, though, teaching was not just about getting by; it was about connecting with the students and connecting them with the world. She was recognized for outstanding teaching in a course where environmental studies were explored through the lens of both the sciences and the humanities. Eventually, she entered a mentoring fellowship to foster connections between students and the university and to cultivate a community of belonging on campus. In a desire to ground her passion for cultivating community in scientific research, she set about studying the effect of mentoring on belonging. Though, too, this investigation stemmed from her personal belief that belongingness is not only a fundamental human need, but also a necessary component of a sustainable world. Thus, it was in this way that she found herself studying two distinct, yet not entirely disconnected, subjects for her dissertation: bees and belonging. And it turns out bees and belonging bee-long together just fine.

Bees

Bees are arguably the most important pollinators of flowering plants, of which 78-94% rely on animal mediated pollination, including many important food crops (Klein et al., 2007; Ollerton et al., 2011). Having evolved from a group of hunting wasps to become what are essentially "vegetarian wasps," estimates suggest that bees originated somewhere between 140 to 110 million years ago during the same time that angiosperms became the dominant land plants (Cardinal and Danforth, 2013; Danforth et al., 2013). With over 20,000 described species worldwide across seven families (Ascher and Pickering, 2020; Danforth et al., 2013), bees are an incredibly diverse assemblage of taxa with fascinating variation in their physiology, behavior, and life history traits. While the smallest bee (*Perdita minima*) falls just shy of two millimeters in length (i.e. smaller than a grain of rice) the largest bee (*Megachile pluto*), rediscovered just recently in the wild, can be nearly four centimeters in length with a wingspan of over six centimeters (Buchman, 2020; Main, 2019). Some bees are polylectic (i.e. foraging for pollen from a variety of unrelated plants) while others are strictly oligolectic (i.e. restricted to pollen from specific species). Though it is not the dominant lifestyle, bees are perhaps best known for their adaptation of sociality, as in bumble bees and honey bees, where castes of workers support colonies with one reproductive female. In contrast, the vast majority of species display a solitary lifestyle, wherein individual females provision their own nests independently (Stephen et al., 1969). Bees exhibit an array of nesting strategies that fall into two general categories: burrowing and nonburrowing (Stephen et al., 1969). Many species burrow in soil, with some building elaborate branched channels for their brood cell chambers, while others build one tunnel in many different locations. Colloquially called "carpenter bees," some bees excavate nests in dead wood and use the wood shavings in their brood cell construction. Alternatively, nonburrowing species build their brood cells in pre-existing cavities that range from hollow stems to holes in tree trunks or old rodent nests. Brood cell material itself is highly diverse; it can be constructed from secretions (e.g. wax), collected from the environment (e.g. mud, leaves, petals, plant down, resins, and pebbles) or made from the nest substrate itself (e.g. soil and wood shavings). The intersection of these vast and distinct traits gives rise to the diversity of this assemblage and reveals the myriad of ways in which different bees interact with their environment.

Yet bee diversity and abundance is declining worldwide due to land-use change, especially agricultural intensification, which has caused significant habitat loss and increased exposure of wild bees to pesticides (Goulson et al., 2015; Potts et al., 2010). Inadvertent killing of bees through the use of pesticides, specifically insecticides, has been a concern since the 1870s (Johansen, 1977). Some insecticides are directly lethal to bees, whereas others are of concern due to their sublethal affects (e.g. deleterious changes in learning, memory, and reproduction). The use of neonicotinoids, a class of systemic insecticides that entered the global market in the 1990s, has recently been called into question for their potential sublethal effects on bees (Sgolastra et al., 2020). Neonicotinoids are the most commonly used insecticides worldwide (Bonmatin et al., 2015) and are implicated in bee declines (Lundin et al., 2015). Neonicotinoids are neurotoxic chemicals that strongly bind to the nicotinic acetylcholine receptors of insects, with little affinity for vertebrate receptors (Tomizawa and Casida, 2005). Neonicotinoids are systemic and have been found in the nectar and pollen of plants from which bees forage (Cresswell, 2011) and can also remain in field soils (Bonmatin et al., 2015), where many native bees nest (Sardiñas et al., 2016). As such, the routes of exposure to neonicotinoids for bees are multifarious.

While neonicotinoids have been in the spotlight in recent bee-pesticide literature, there is mounting evidence that bees are exposed to a number of other pesticides and pesticide combinations are of concern for bee health (Long and Krupke, 2016; Main et al., 2020; Sgolastra et al., 2020). For example, fungicides can increase the toxicity of insecticides for bees (Iverson et al., 2019; Pilling et al., 1995; Sgolastra et al., 2018; Wade et al., 2019). Even herbicides, which are generally considered to have no or low toxicity for bees, indirectly affect bee populations through removal of weedy plant species that serve as excellent sources of pollen and nectar for bees (Goulson et al., 2015; Johansen, 1977).

Most of the literature on pesticides in relation to bees is restricted to a subset of species that are conducive to laboratory research or cage studies. As such, honey bees and a few other (oftentimes commercial) taxa are among the select few species studied with regard to pesticide regulations (Sgolastra et al., 2019). However, conservationists hypothesize that honey bees (being arguably the most unique species among bees) should not be the model species used in pesticide risk analysis. Bees that visit crop plants and perimeter vegetation span a number of genera previously unexplored in the current pesticide literature (Lundin et al., 2015). A recent field study found that wild bees were negatively affected by a neonicotinoid seed treatment in oilseed rape, while honey bees were not affected (Rundlöf et al., 2015), confirming the crucial need for further studies that include a greater range of bee species. Furthermore, in accordance with the theory that indicator traits could be more useful than indicator species, there is further need for studies that analyze the response of bees to disturbance (such as pesticides) in relation to traits such as body size, nest location, sociality and trophic specialization (Williams et al., 2010). Laboratory studies have been the most common methodological approach to assess the impact of neonicotinoids on bees, whereas field realistic exposure, especially in connection with bee assemblage response, remains largely understudied (Lundin et al., 2015).

Hence the need has arisen to better understand field realistic pesticide exposure for bees in agriculture, and specifically, exposure for the many wild bee species that also offer pollination services to both crop plants and non-crop plants. Two study systems were used to investigate this further in this dissertation: a study investigating pesticide exposure for bees visiting crop plants and a study investigating pesticide exposure for bees visiting non-crop plants (specifically bee attractive perimeter plants). Firstly, a matched pairs field study in sunflower was conducted, wherein half of each field was sown with neonicotinoid treated seed and half was sown without neonicotinoid treated seed. In this system, a number of different samples were collected for pesticide residue analysis: a native sunflower specialist bee (*Melissodes agilis*), pollen foraging honey bees, nectar foraging honey bees, sunflower pollen, sunflower nectar, and soil. Secondly, a study of bees visiting bee attractive perimeter plants in the same agricultural region was conducted. In this system, the following samples were collected for pesticide residue analysis: wild bees of three size categories, honey bees, flowers of four types, soil, and air (via passive samplers). The results from this work offer better insights into field realistic pesticide exposure rates for many more bee species than is currently known.

Belonging

Sense of belonging was famously espoused by Maslow as one of the fundamental needs to attain "self-actualization" or to fulfill one's creative potential (Maslow, 1943). While there is general agreement over the importance of belongingness and the concept itself is inherently familiar, a simple definition has proven evasive. In psychology, belonging is both referred to as a force that motivates and also the effect of interpersonal relationships — it can motivate and effect cognition, emotion, behavior, health, and well-being (Baumeister and Leary, 1995). In the literature on higher education, belongingness is often considered a multidimensional construct that results from the relationship between the person and their environment, and which is heavily influenced by interpersonal relationships (Allen et al., 2018; Freeman et al., 2007; Lewis et al., 2016; Pittman and Richmond, 2008; Strayhorn, 2019). For the sake of a working definition, one could say that sense of belonging is the degree to which a person perceives that they are respected, supported, accepted, valued, and integrated into a particular community environment (Baumeister and Leary, 1995; Goodenow and Grady, 1993; Lambert et al., 2013; Lewis et al., 2016; Strayhorn, 2019).

In higher education, a strong sense of belonging is associated with a number of advantageous academic and social outcomes. Sense of belonging is positively associated with student motivation and satisfaction, decreased stress, anxiety, and depression (Civitci, 2015; Osterman, 2000; Pittman and Richmond, 2008), and it also increases student GPA and retention (Meehan and Howells, 2019; Strayhorn, 2019; Thomas, 2012). Thus, higher education institutions that transparently foster sense of belonging actually improve their students' ability to learn and succeed.

Some students face greater barriers to belonging than others. Historically marginalized student populations, including transfer students, first-generation college students, and underrepresented minority students, can encounter many challenges in higher education institutions that can cause them to feel more isolated and alienated than their peers. Transfer students are a unique subset of students that are diverse in their own right, with students ranging from age 18 to their 50s that hold various non-academic responsibilities related to work and family disproportionately compared to non-transfer students (Walker and Okpala, 2017). Transfer students often experience a type of culture shock referred to as "transfer shock" (Hills, 1965; Townley et al., 2013) as they are faced with learning how a new university operates and adjusting to (what is often experienced as) a more rigorous and competitive academic atmosphere paired with faculty that are less accessible and more impersonal (Townsend, 2008, 1995). They are faced with taking advantage of all the university has to offer in a shorter timeframe than their non-transfer counterparts. First-generation college students may begin at a university at the same time their peers, but often experience the disadvantages of a "cultural mismatch," being far less familiar with the college experience given a lack of family role models or other mentors (Palbusa and Gauvain, 2017; Pascarella et al., 2004; Phillips et al., 2020). First-generation college students are also more likely than their peers to come from low-income families, resulting in an increased financial burden and likelihood of working, reducing the amount of time they have for extracurricular activities that allow for connection with peers (Pratt et al., 2019). Microaggressions documented against first-generation college students are further isolating and detrimental to their experience (Ellis et al., 2019). Underrepresented minority students confront individual racism in the form of micro-aggressions and/or overt hostility, in addition to the effects of the systemic racism that permeates American institutions (Ingram and Wallace, 2019; Mills, 2020; Solorzano et al., 2000; Yosso et al., 2009). Many students also hold identities at the intersection of many historically marginalized student populations further exacerbating challenges in feeling a sense of belonging in higher education institutions.

The literature on the benefits and even necessity of cultivating sense of belonging for students in higher education continues to grow (Allen et al., 2018; Meehan and Howells, 2018, 2019; Thomas, 2012). The need for programs that specifically cultivate sense of belonging and also studies that assess program outcomes are apparent (Thomas, 2012). In this dissertation, an undergraduate mentorship program at UC Berkeley was utilized as a case study to test whether this intervention resulted in increased sense of belonging for its participants, with specific attention to sense of belonging for historically marginalized student demographics.

Chapter 1: Neonicotinoid seed treatment impacts wild bee biodiversity and decreases crop yield in sunflower

Abstract

Neonicotinoid seed treatments are commonly used in hybrid sunflower production even though their benefit to crop yield remains unclear, as does their impact on pollinators, particularly wild bees. Using a matched pairs design in which half of each field was sown with thiamethoxam treated seed and half without, we assessed honey bee and wild bee exposure to pesticides by analyzing pesticide residues in field soil, sunflower pollen, sunflower nectar, pollen-foraging honey bees, nectar-foraging honey bees, and a sunflower specialist wild bee (*Melissodes agilis*). We also quantified the effects of thiamethoxam treated seed on wild bee biodiversity and crop yield. Wild bee abundance was marginally lower and crop yield was significantly lower in plots with thiamethoxam added, even though this neonicotinoid was only detected at low concentrations in one soil sample. *M. agilis* abundance was also significantly lower in plots with thiamethoxam added. Conversely, wild bee richness was significantly higher and diversity marginally higher in thiamethoxam plots. Nectar volumes harvested from nectar-foraging honey bees were also significantly higher in thiamethoxam plots. Many other pesticides that were not used in the sunflower fields were detected in our samples, some of which are known to be deleterious to bee health either individually or in combination with other pesticides, highlighting the importance of the landscape scale in the assessment of pesticide exposure for bees. Overall, our results suggest that treatment of seeds with thiamethoxam may negatively impact wild bee pollination services in sunflower. Farmers and seed companies may increase profits by nearly $5400 per field by withholding neonicotinoid seed treatments in sunflower when pest presence is low. Lastly, this study highlights the necessity for the inclusion of other metrics, such as biodiversity or behavior, in pesticide risk analysis, as pesticide residue analysis as an independent metric erroneously miss the impacts of field realistic pesticide exposure.

Introduction

California currently leads the world in hybrid sunflower (*Helianthus annuus*) seed production (Long et al., 2019). Honey bees are used to pollinate hybrid sunflowers and wild bees are known to enhance pollination efficiency 5-fold (Greenleaf and Kremen, 2006). Neonicotinoid seed treatments, typically thiamethoxam, are used on California sunflower crops to control for soil pests including variegated cutworms (*Peridroma saucia*), wireworms (*Limonius* spp.), and seed corn maggot (*Delia platura*), each of which can cause serious damage to seedlings and significantly affect crop growth (Long et al., 2019). However, the effectiveness of neonicotinoid seed treatments has been called into question for many crops, including sunflowers (Bredeson and Lundgren, 2015; Hladik et al., 2018; Krupke et al., 2017), in part because neonicotinoids are known to have deleterious effects on many beneficial insects, particularly pollinators (Buszewski et al., 2019; Lundin et al., 2015).

While wild bees and honey bees are crucial pollinators for many food crops (Klein et al., 2007), they are in decline largely due to agricultural intensification and land use change, which alter the availability of floral and nesting resources and increase exposure to pesticides and pathogens (Goulson et al., 2015; Potts et al., 2010). While potentially not as toxic to bees as imidacloprid (Laycock et al., 2014), thiamethoxam has received considerably less attention than other commonly used neonicotinoids (i.e. imidacloprid and clothianidin), despite the fact that it is known to be highly toxic to honey bees and bumble bees (Lundin et al., 2015). A number of sublethal effects have also been reported for these taxa, including evidence that thiamethoxam negatively impacts learning, memory, reproduction, immune response, and colony fitness, even when individuals are only exposed as larvae (Papach et al., 2017; Sandrock et al., 2014; Stanley et al., 2015b; Thompson et al., 2019). Thiamethoxam is also known to affect honey bees through synergistic interactions with parasitic infections (*Nosema ceranae*) and pathogens (deformed wing virus) (Coulon et al., 2020; Tesovnik et al., 2020). The literature on deleterious effects to honey bees and bumble bees continues to accumulate, with effects ranging from impairment of flight, phototaxis, and thermoregulation (Potts et al., 2018; Tosi et al., 2017, 2016; Tosi and Nieh, 2017) to impairment of crop pollination services (Stanley et al., 2015a). In comparison, few studies, much less field studies, have been conducted on the exposure and toxicity of thiamethoxam to wild bees due to challenges of using wild bees as model species (Kopit & Pitts-Singer, 2018; Sgolastra et al., 2019, 2020; but see Willis Chan, Prosser, Rodríguez-Gil, & Raine, 2019), although there is some evidence that wild bees may be more severely impacted than honey bees (Rundlöf et al., 2015).

Among wild bees, *Melissodes agilis* is the most common sunflower visitor in the western US (Parker et al., 1981) although a number of other generalist and specialist species also visit sunflowers. Among these, *Anthophora urbana*, *Diadasia* spp., *Melissodes* spp., and *Svastra obliqua expurgate* provide the highest levels of pollination efficiency in sunflower crops in California (Greenleaf and Kremen, 2006). Wild bees nest in the soil of both fields and field margins of sunflower crops, with sunflower specialists, including *M. agilis*, having been found to nest only in field soils in some studies (Sardiñas et al., 2016). Thus, a number of wild bees may be exposed to thiamethoxam when used as a seed treatment in sunflowers if it is translocated to the pollen and nectar of the sunflowers and/or is retained in the soil where they nest.

The objectives of this study were to 1) to assess the exposure of honey bees and wild bees to pesticides in sunflower crops planted with thiamethoxam treated seed and 2) to quantify effects of thiamethoxam treated seed on wild bee abundance and biodiversity, and 3) to determine the effects of this pesticide on efficiency of the pollination services provided by bees in sunflower crops. We conducted a field study using a matched pairs design to test for differences in pesticide detection and concentration in soil, pollen, nectar, honey bees and *M. agilis*, and to estimate wild bee biodiversity and crop yield in hybrid sunflower fields planted with and without a thiamethoxam seed treatment. While previous studies have examined the effects of imidacloprid treatment of sunflower seeds on bee pollinators (Bonmatin et al., 2003; Stadler et al., 2003; Tasei et al., 2001), to the best of our knowledge no studies have analyzed whether thiamethoxam seed treatments in sunflower remain detectable in field soils where bees may nest (Sardiñas et al., 2016), whether they are translocated to the pollen and nectar from which bees forage (but see (Bredeson and Lundgren 2018), and whether treatment effects bee behavior and biodiversity in the field. The results from our study have the potential both to inform pest management practices with regard to their effects on wild bee conservation and to inform farmers and seed companies of the economic impacts of thiamethoxam treated sunflower seed.

Materials and Methods

Study sites and sampling design

In a matched pairs design field study near Sacramento, California, USA, we collected samples to investigate pesticide exposure for bees foraging in sunflowers (*Helianthus annuus)* planted with and without neonicotinoid seed treatments. During peak bloom (late June – early July 2016), *M. agilis*, pollen foraging honey bees, nectar foraging honey bees, sunflower pollen, sunflower nectar, and soil samples were collected from both sides of four fields, one side planted with thiamethoxam-treated seed (referred to as thiamethoxam plot) and the other side planted with seed that was not treated with thiamethoxam (referred to as control plot).

Sunflower crops are grown such that fertile parental lines that produce pollen (hereafter, "P-type" for pollen) and sterile parental lines from which the seed is harvested (hereafter, "S-type" for seed) are planted in separate rows. Although P-type and S-type sunflower plants have historically been referred to as "male" and "female" plants, respectively (Long et al., 2019), here we use gender neutral terms to describe parental lineages (Lambert and Packer, 2019). Bees foraging for pollen and nectar must move pollen from P-type flowers to S-type flowers for pollination to occur. After pollination, the P-type plants are mowed down and seed is harvested from the S-type plants. Honey bee hives are commonly rented from bee keepers and set out on field edges as P-type plants begin to flower and before S-type plants bloom. Honey bees tend to have distinct subsets of workers dedicated to pollen- versus nectar-foraging. Since P-type and S-type plants do not co-occur in the same rows in hybrid sunflower monoculture, this means that individual honey bees tend to forage on only P-type or only S-type plants during one foraging trip, which is not conducive to pollination. Wild bees forage for both pollen and nectar and their presence in the system indirectly increases pollination of sunflowers because they jostle honey bees, causing them to move back and forth between P-type and S-type rows, thereby doubling the pollination services of honey bees (Greenleaf and Kremen, 2006).

Multiple companies grow sunflower seeds in central California and we partnered with one small seed company for the purposes of this study. The seed company received seed from the EU which had already been coated with two fungicides: Apron XL 350ES (Syngenta; 0.3 L mefenoxam / 100 kg seed) and Maxim 025 FS (Syngenta; 0.5 L fludioxonil / 100 kg seed). Due to the 2013 ban on the use of certain neonicotinoids in the EU, some of the seed was additionally treated in California with the neonicotinoid Cruiser 5FS (Syngenta; 0.51 L thiamethoxam / 100 kg seed). This made it possible for the company to withhold neonicotinoid seed treatment of some seed for the purpose of this study. In an effort to minimize crop damage from the lack of insecticide treatment for one half of each field, the seed company chose four field sites that were known anecdotally to have less activity by soil dwelling insect pests. Each field was sown such that all seeds were coated with the two fungicides, but only half the field had seed coated with thiamethoxam. Each field was sown in mid-April 2016 with a different cultivar; cultivars were identical between matched pairs. Three of the fields ranged from 20 to 23 ha in size, while the fourth field was smaller at 11 ha. P-type seed density was 49,420 - 56,830 seeds/ha and S-type seed density was 46,950 - 59,310 seeds/ha. Sunflower seeds were planted such that there were 6 - 10 rows of S-type, interrupted by two rows of P-type, as is the tradition for hybrid seed production. Fields were not treated with any other pesticides after planting and crops grown in these fields during the previous two years were a mix of wheat, tomatoes, safflower, and squash.

Sampling for pesticide residue analysis

For each field, pollen, nectar, and bee samples were collected along two transects in each half of the field (i.e. in the thiamethoxam plot and in the control plot), located at least 20 rows away from the center (i.e. where the two paired seed treatments met). The two transects were established such that they included both an S-type and P-type row of sunflower plants and were at least three P-type rows apart. Sampling began at least 15 m from the field edge. Soil samples, on the other hand, were collected at five random locations within each plot. All samples were placed in dry ice coolers in the field and then stored at between -14 and -20 °C until pesticide analysis. As described below, nectar foraging honey bees were placed on ice in coolers in the field prior to nectar harvesting procedures, but then stored between -14 and -20 °C until pesticide analysis.

Soil

Pesticides can be present in soils for a variety of reasons including persistence from previous applications, aerial drift, leaching from other applications, and residue from seed treatments. Many wild and native bees nest in soil; sunflower specialists are thought to sometimes nest in soils growing the crops on which they forage (Sardiñas et al., 2016). To assess pesticide exposure to crop plants and nesting bees, a soil probe 2.5 cm in diameter was inserted into the soil to a depth of 7 cm; this procedure was used to collect five samples from both the control and thiamethoxam plots of each of the four fields examined. Soil samples were collected one-month post seeding (to check for detection of seed treatments in soil) and again during bloom (to coincide with the timing of other samples, 2.5 months post seeding). The five soil cores from the same half of a field were combined into one sample based on field site, seed treatment, and sampling date and stored in Ziplock bags.

Pollen

Pesticides can be present in pollen due to either systemic uptake or spray residue. To assess pesticide exposure to bees due to pollen, pollen was collected directly from blooming P-type sunflowers. Anther dehiscence in sunflowers happens early in the morning, just as the flowers become exposed to direct sunlight, and is immediately followed by bees foraging for pollen. Thus, pollen samples were collected immediately upon dehiscence, which is apparent from anthers turning from a dull yellow brown color to a bright yellow as the pollen is exposed. Pollen was scraped from anthers into glass jars using small plastic rulers and plastic funnels. Approximately 50 mg was collected from each transect and pooled to make a 100 mg pollen sample from each field site and seed treatment. Small arthropods and other debris inadvertently collected with the pollen were removed before pesticide analysis.

Nectar

Pesticides can also be present in nectar due to either systemic uptake or spray residues. To assess pesticide exposure to bees via nectar, nectar samples were collected from nectar foraging honey bees. Honey bees observed foraging from blooming S-type sunflowers for >30 sec were collected with bug vacuums (Backyard Safari, Alex Brands, Fairfield, New Jersey, USA), transferred individually to microcentrifuge tubes, and placed on ice in a cooler in the field to immobilize them. At least 6 nectar foraging honey bees were collected from each transect. Within 24 h, the honey bees were removed from the cooler and "squeezed" to regurgitate nectar, which was collected into 20 µl capillary tubes. Capillary tubes were then sealed, wrapped in bubble wrap, and placed in a freezer between -14 and -20 °C. At least 50 µl nectar was collected for each field site and seed treatment. The actual volume of nectar collected varied between field sites and seed treatments, but was harvested from the same number of bees for both paired plots at each field site

(12 bees from plots within two field sites and 14 bees from plots within the other two field sites); the exact volume collected from each field site and seed treatment was measured prior to pesticide residue analysis.

Bees

There are many routes by which bees may be exposed to pesticides, including the pollen and nectar of crop plants. Three types of bee samples were collected to assess differential exposure to pesticides: *M. agilis* (an abundant sunflower specialist), pollen foraging honey bees, and nectar foraging honey bees. As *M. agilis* cannot be fully identified by eye in the field, at least 20 similar wild bees observed to be foraging on P-type flowers were collected and later identified, after which 10 *M. agilis* were randomly selected and pooled as a composite sample for pesticide residue analysis for each field site and seed treatment. Similarly, at least 12 honey bees observed to be foraging on P-type flowers, with pollen loads in their corbiculae as evidence of pollen foraging, were collected, as were 12 - 14 nectar foraging honey bees collected for regurgitation of nectar. For both foraging groups, 10 of the 12 - 14 individuals collected were randomly selected and pooled for each field site and seed treatment. Small brushes and gel (solidified mixture of: gelatin (50 g), glycerin (150 ml), and distilled water (175 ml)) were used to remove the majority of pollen from all bees, in an effort to analyze the pesticide load of the bee rather than that of the bee and the pollen that it carried. Nectar foraging honey bees, despite not having pollen loads, were subjected to the same pollen removal protocol as a control for potential effects of the pollen collection method.

Sampling for wild bee biodiversity

Wild bee sampling was conducted during the bloom season between 07:00 and 17:00 hours on days when weather conditions were favorable for bee flight and foraging activity (little to no cloud cover, temperatures >15.5 °C, and average wind speeds < 2.5 m/s). Timed random walks were used to collect wild bees foraging on sunflowers; bees were collected with sweep nets and bug vacuums, with the goal of sampling at least 40 individuals from each field site and seed treatment. Wild bee abundance was expressed as the number of wild bees collected per 10 min. Since we expected *M. agilis* to be a dominant species, we also calculated the abundance of *M. agilis* separately as the number of individuals collected per 10 min, standardized by the total time spent collecting bees within each field site and seed treatment. Field assistants were randomly assigned to thiamethoxam and control plots of each field site to minimize any bias due to differences in skill or style of sampling and all sampling avoided rows that had been used for pesticide sample collection. Bees were transferred into microcentrifuge tubes, placed on dry ice in the field, and later identified in the laboratory. Once identified, wild bee biodiversity was estimated as asymptotic species richness using the Chao1 estimator and as species diversity using the exponential Shannon entropy estimator from the SpadeR package in R version 3.6.1 (Chao et al., 2013; Chiu et al., 2014). Due to expected dominance of the wild bee assemblage by one sunflower specialist (*M. agilis*), species richness was also estimated without this species.

Sampling for floral resources and crop yield

To determine whether thiamethoxam seed treatment affected the floral resources present at our field sites, flower production and growth were estimated from five random S-type and five random P-type plants located at least 15 meters into each transect. S-type plants typically have just a single flower head that blooms, whereas P-type plants can have multiple flower heads that bloom. Consequently, the diameter of the entire flower head and the distance from the external edge of the flower head to the inner most open floret were measured for the single head on S-type plants and for the largest head on P-type plants. These measurements were used to estimate flower head area, flower head bloom area, and bloom area as a proportion of the full head area. We also counted the number of flower heads present on each P-type plant, the number of flower heads blooming, and then estimated the proportion of heads blooming.

To examine whether bee diversity influenced crop yield, yield measurements were estimated from photographs of flower heads at harvest. Just before seeds were harvested by the seed company, 60 photographs per seed treatment per field site were taken from randomly selected S-type heads, with a ruler placed against the flower head for scale. For each photograph the "ruler tool" from Abode Photoshop was used to measure 1 cm from the ruler and allow a grid of 2.25 cm^2 quadrats to be overlaid on the image of the flower head. Quadrats were numerically labeled in sequence and a random number generator was used to select six that fully overlapped with the sunflower head for counts of the number of viable seeds (≥ 0.25 cm in width) present. For quadrats where no seeds were visible (i.e. either absent or undeveloped), a seed count of 0.0001 was recorded and a mean viable seed count per 2.25 cm^2 quadrat was calculated for each flower head. The total area of each flower head was measured using ImageJ after adjusting for image scale and these values were used to estimate the total seed count for each flower head.

Analytical methods for pesticide detection

Pesticides were extracted from soil, pollen, and bees using pressurized solvent extraction with dispersive solid-phase extraction (SPE) clean-up (Hladik et al., 2016; Hladik and McWayne, 2012; Main et al., 2020). Nectar samples were extracted using a modified QuEChERS method of acetonitrile, hexane, water and citrate salts; with dispersive SPE clean-up the same as for the other sample types examined (Anastassiades et al., 2003). Samples were analyzed for 168 pesticides and their degradates using both gas and liquid chromatography-tandem mass spectrometry. The complete list of compounds and analytical limits of detection for each (LOD) can be found elsewhere (Hladik and Ward, *in press*). Thiamethoxam and the two fungicides (fludioxonil and mefenoxam/metalaxyl) applied by the seed company were among the pesticides analyzed.

Data analysis

To determine whether pesticide number, pesticide concentration (ng/g), floral resources, crop yield, and bee biodiversity differed with sunflower seed treatment, we used linear mixed models constructed in the lme4 package in R version 3.6.1 (Bates et al., 2015; R Core Team, 2019). For pesticide analyses, sample type and pesticide category (insecticide, herbicide, fungicide) were included in the models alongside seed treatment as fixed effects. In cases in which just one pesticide was analyzed, pesticide category was removed from the model. For all pesticide analyses, soil samples from one month post planting and during bloom were combined in the overall analysis if they were not found to be significantly different when analyzed independently. For continuous response variables, models with a Gaussian error distribution were used and, in some cases, they were transformed using natural log or natural log plus one to ensure linearity. For response variables that were counts (e.g. number of pesticides, species richness, seed counts), we used generalized linear mixed models with a Poisson error distribution. Field site was included as a random factor in all models to account for the matched pairs design. Type II Wald chi-square tests were used to determine the significance of fixed effects in the models using the Anova() function from the car package in R (Fox and Weisberg, 2019). Post hoc comparisons of marginal means among sample types within significant fixed effects were made using the emmeans() function from the emmeans package in R (Lenth, 2020).

Results

Overview of pesticide detection in samples

Of the 168 pesticide residues examined (N = 56 samples), a total of 26 pesticides and degradates were detected, of which there were 5 insecticides, 9 herbicides, 7 fungicides, and 5 degradates. When these data were examined by sample type, the total number of pesticides and degradates detected was: 7 in *M. agilis* (of which none were insecticides), 4 in honey bees (3 in nectar bees, 4 in pollen bees, of which none were fungicides), 6 in nectar (no insecticides), 6 in pollen (no insecticides), and 23 in soil (21 post seeding and 22 during bloom). Percent detection of insecticides, herbicides, and fungicides by sample type and field site is shown in **Figure 1**.

Thiamethoxam, the focal pesticide in this study, was detected in only one sample: a post seeding soil sample from a control plot, at a concentration of 0.39 ng/g. Fludioxonil and mefenoxam, the two fungicidal seed treatments applied to all seeds, were not detected in any samples. Imidacloprid, a neonicotinoid not used in our experimental seed treatment, was detected in all soil samples from both control and thiamethoxam sides of the field at one site, but not in any samples from the other three field sites. Of all the pesticides detected, only the herbicides metolachlor and propanil were detected at least once in all sample types. The fungicide boscalid was detected at least once from all sample types except honey bees, while the fungicide myclobutanil was detected only from *M. agilis* samples. The fungicide chlorothalonil was detected only from soil and sunflower pollen.

Pesticide number and concentration

Number of pesticides

The number of pesticides in our samples differed by sample type ($\chi^2 = 144.46$, df = 5, $P < 0.001$) and pesticide category ($\chi^2 = 81.45$, df = 2, $P < 0.001$), but not by seed treatment ($\chi^2 = 1.33$, df = 1, $P = 0.248$). The mean number of pesticides was generally greatest in soils compared to other samples, and there were generally more herbicides than other pesticide categories (**Figure 2**).

Pesticide concentrations for all pesticides

Concentrations of pesticides in our samples also differed by sample type ($\chi^2 = 160.79$, df = 5, $P < 0.001$) and pesticide category ($\chi^2 = 22.41$, df = 2, $P < 0.001$), but not by seed treatment ($\chi^2 = 0.01$, df = 1, $P = 0.925$). Pesticide concentrations were generally lowest in soils, similar among bees and nectar, but higher in bees than in pollen (**Figure 3**). In addition, herbicides and insecticides were generally detected in higher concentrations than fungicides.

Individual pesticide concentrations

Metolachlor concentrations did not differ by sample type ($\chi^2 = 8.30$, df = 5, $P = 0.140$) or treatment ($\chi^2 = 1.16$, df = 1, $P = 0.280$).

Propanil concentrations differed by sample type ($\chi^2 = 400.24$, df = 5, $P < 0.001$) but not by treatment ($\chi^2 = 2.20$, df = 1, $P = 0.138$). Mean propanil concentration was higher in bees (*M. agilis*, 251 ± 76.7 ng/g; nectar foraging honey bee, 493 ± 221 ng/g; pollen foraging honey bee, 304 ± 132 ng/g) than in pollen and nectar (nectar, 105 ± 20.1 ng/g; pollen, 65.1 ± 23.5 ng/g), and higher in bees and floral matrices than in soils (post seeding, 7.41 ± 0.61 ng/g) (post hoc tests $P < 0.05$).

Boscalid concentrations differed by sample type ($\chi^2 = 162.05$, df = 3, $P < 0.001$) but not treatment ($\chi^2 = 0.13$, df = 1, $P = 0.717$). Mean boscalid concentration did not differ between *M. agilis* (17.6 ± 3.08 ng/g), nectar (23.5 ± 3.29 ng/g), or pollen (8.28 ± 1.23 ng/g), but was significantly higher in these samples than in soils (1.56 ± 0.15 ng/g) (post hoc test $P < 0.001$). Boscalid concentrations in sunflower nectar was also marginally higher than in pollen ($P = 0.081$). Boscalid was not detected in either pollen or nectar foraging honey bees.

Floral resources and crop yield

The mean proportion of bloom area per P-type head ($\chi^2 = 6.44$, df = 1, $P = 0.011$) and the mean volume of nectar regurgitated by honey bees foraging for nectar from S-type plants ($\chi^2 = 11.28$, df = 1, $P < 0.001$) were both greater in the thiamethoxam treated sides of fields (**Figure 4A**). In contrast, the mean proportion of P-type heads blooming was greater in the control sides of fields ($\chi^2 = 3.88$, df = 1, $P = 0.049$; **Figure 4A**). There were no differences between control and thiamethoxam plots for all other floral resource measurements ($P > 0.25$).

While mean seed count per quadrat did not differ between seed treatments ($\chi^2 = 0.17$, df = 1, $P = 0.681$), mean harvest flower head area ($\chi^2 = 8.38$, df = 1, $P = 0.004$) and mean estimated seed count for the entire flower head were both greater in the control plots ($\chi^2 = 58.29$, df = 1, $P < 0.001$; **Figure 4B**).

Wild bee abundance and biodiversity

Wild bees collected from our field sites included 17 species representing three families (Apidae, Halictidae, and Megachilidae). The sunflower specialist *M. agilis* was the dominant species (**Figure 5**). Other sunflower specialists, including *Diadasia enavata*, *Melissodes lupina, Melissodes robustior, Melissodes tepida* and *S. obliqua expurgate*, were also present in these samples. Other genera included *Anthophora*, *Bombus*, *Ceratina*, *Halictus*, *Lasioglossum*, *Osmia* and *Peponapis*.

Mean wild bee abundance for all species combined was marginally greater in the control sides of fields ($\chi^2 = 2.78$, df = 1, $P = 0.095$), and mean abundance for *M. agilis* alone was significantly greater on control sides ($\chi^2 = 5.03$, df = 1, $P = 0.025$). In contrast, species richness (Chao1) for wild bees was greater in the thiamethoxam plots ($\chi^2 = 7.21$, df = 1, $P = 0.001$; **Figure 6**); this result persisted when the dominant species *M. agilis* was removed from the analysis ($\chi^2 = 6.58$, df = 1, $P = 0.010$). Wild bee diversity (exponential Shannon entropy) was marginally greater in the thiamethoxam plots ($\chi^2 = 3.76$, df = 1, $P = 0.052$; **Figure 6**).

Discussion

Neonicotinoid seed treatments can be effective against soil dwelling pests in sunflower fields in California (Long et al., 2019) but these substances are known to have sublethal effects on honey bees and wild bees (Buszewski et al., 2019; Lundin et al., 2015). As sunflowers rely for on honey bees and wild bees for efficient pollination, the use of neonicotinoid seed treatment on sunflower is a potential concern for bee health (Greenleaf and Kremen, 2006; Long et al., 2019). This study was designed to better assess pesticide exposure for bees that forage in thiamethoxam seed-treated sunflower in comparison to sunflower sown without thiamethoxam treatments, and to test the effect of thiamethoxam treatment on bee biodiversity and crop yield. Although a number of pesticides were detected in the various materials sampled as part of this study, the pesticides used for seed treatment were largely undetected, with thiamethoxam detected in only one soil sample from a control plot. Despite the absence of seed treatment residues in samples, our results suggest that thiamethoxam treatment marginally reduced overall wild bee abundance, significantly reduced *M. agilis* abundance and decreased crop yield, but increased wild bee species richness and diversity. Though seed treatment pesticides were rarely detected, a number of other pesticides were detected, with no difference in the number of pesticides detected nor the concentration of pesticides detected between thiamethoxam and control plots. The greatest number of pesticides were detected in soils, though pesticide concentration was lowest in soils. Pesticide concentrations were similar among bees and nectar, with a marginal trend showing higher pesticide concentrations in bees than in pollen, indicating that bees are exposed to pesticides through other plants and/or that bees are accumulating pesticides.

Three main themes arise from these results. First, thiamethoxam seed treatment in sunflowers appeared to effect bee biodiversity despite remaining undetectable in our field samples. Second, pesticide exposure for bees foraging in sunflower fields extended beyond pesticides applied within the crop itself. Third, thiamethoxam seed treatment in sunflowers decreased crop yield, perhaps due to decreased wild bee abundance, particularly for the sunflower specialist *M. agilis*. The following is a discussion of these key findings.

Effect of thiamethoxam seed treatment on bee biodiversity

Although the thiamethoxam seed treatment used in our study was undetected in all but one soil sample, this treatment still affected bee biodiversity. Wild bee abundance was marginally greater on the control sides of fields, which was driven primarily by increased *M. agilis* abundance in those plots. A greater plant visitation rate for wild bees in control sunflower fields compared to thiamethoxam treated fields has been observed previously (Bredeson and Lundgren, 2018). The greater abundance in control fields could be due to deleterious effects of the thiamethoxam seed treatment itself, however, as residues were mostly undetected in our samples, we speculate that the treatment may have indirectly impacted the availability and/or quality of floral or nesting resources.

Given that the abundance of flowers and/or pollen can impact bee abundance and species richness (Potts et al., 2003) it is possible that a greater abundance of wild bees in the control plots was influenced by the greater proportion of blooming P-type heads in these plots. This finding, however, contrasts with the greater proportion of bloom area for P-type heads in the thiamethoxam plots. It is possible that the proportion of sunflower heads in bloom is a more important factor influencing wild bee abundance in sunflower fields than the proportional area of each flower head that is in bloom (i.e. that the proportion of landing pads is more important than the proportional area of a landing pad). Similarly, nectar quality and quantity can impact bee abundance (Potts et al., 2004). Although the abundance of wild bees was greater in control plots, the volume of nectar regurgitated by honey bees foraging from S-type plants was significantly greater in thiamethoxam plots. These contrasting outcomes may indicate that honey bees were consuming more nectar from thiamethoxam plots, rather than there being greater quantities of nectar in those plots. Honey bees have been shown to consume more sucrose solution when it is laced with thiamethoxam (Kessler et al., 2015), suggesting some effect of this pesticide on foraging behavior. We did not detect thiamethoxan in the nectar extracted from honey bees collected in the thiamethoxam plots, leading us to wonder whether the nectar contained concentrations of thiamethoxam below the level of detection and/or thiamethoxam changed the chemistry or microbiology of the plants in ways that might cause honey bees to consume more nectar in foraging trips. Microbial activity is known to affect the quality and quantity of both nectar and pollen, thereby altering floral scent as well as floral rewards for pollinators (Vannette, 2020). Future studies could investigate the potential effects of neonicotinoid insecticides on floral microbes and whether and how these effects impact floral attraction and rewards for pollinators.

Wild bee abundance can also be driven by nesting substrate quantity and quality, independently or in addition to forage choice (Potts et al., 2003). Bee nest site choice is known to be influenced by a number of factors including soil moisture, soil compaction, slope, and amount of bare soil (Potts and Willmer, 1997; Sardiñas and Kremen, 2014). While it has been proposed that bees may prefer the undisturbed soils of field margins to the tilled and irrigated soil in fields, relevant data are mixed and responses seem to vary by species (Kim et al., 2006; Williams et al., 2010). Specialist squash pollinators (*Peponapis pruinose*) are known to nest at the base of crop plants (Julier and Roulston, 2009). *M. agilis* are known to nest within the sunflower fields where they forage (Sardiñas et al., 2016) and as sunflower pollination is thought to be limited by nest site location for wild bees (Sardiñas et al., 2016), it is possible that the thiamethoxam seed treatment influenced nest site choice. That is, the greater abundance of *M. agilis* in control plots may indicate that *M. agilis* preferred control sides of the field for nesting. Future work could investigate the mechanisms by which thiamethoxam seed treatment could influence this preference.

The greater richness and diversity of wild bee species in the thiamethoxam treatments may have been driven by the generally greater abundance of wild bees in control plots. Specifically, because *M. agilis* was the dominant wild bee, this dynamic may have been driven by interspecific competition, as *M. agilis* abundance was significantly higher in control plots. The greater presence of *M. agilis* in the control sides of the fields may have displaced other wild bee species to the thiamethoxam plots (i.e. niche partitioning). Bees are known to exhibit resource partitioning as an apparent response to interspecific competition (Inouye, 1978). Consequently, the greater species richness of less abundant wild bee species in the thiamethoxam plots may have been because of the large presence of *M. agilis* in the control plots.

The lack of thiamethoxam detected in our samples was unexpected, but an interesting finding in and of itself. Given that the half-life of thiamethoxam has been estimated to be 7 - 72 days in soils under field conditions (Goulson, 2013; Morrissey et al., 2015) and given that only 5% of neonicotinoid seed treatments are taken up by plants (the majority is thought to remain available in the soil or soil water: Hladik, Main, and Goulson 2018), we expected to be able to detect thiamethoxam at low concentrations in soil samples collected from the treated plots of the matched pairs at any time from planting through bloom. Instead, thiamethoxam was detected in one soil sample from one control plot one-month post planting. Given that thiamethoxam is highly soluble and is known to leach laterally or downward through soils (Kurwadkar et al., 2013), in conjunction with the flood irrigation practice at our study sites, we speculate that thiamethoxam was transported primarily offsite via water though perhaps was also transported somewhat to control sides of the field. Though some studies suggest that thiamethoxam protects plants for only 3 - 4 weeks and becomes undetectable in leaf tissue before flowering (Bredeson and Lundgren, 2015; Kohl et al., 2019), some neonicotinoids have been shown to translocate into the flower head of sunflower plants during flowering (Bonmatin et al., 2005, 2003). Thus, we anticipated the potential to detect thiamethoxam (or clothianidin, its main degradate) in pollen and nectar samples collected in the treated plots of the matched pairs. We expected higher concentrations in pollen than nectar (Dively and Kamel, 2012; Gierer et al., 2019; Jiang et al., 2018; Stoner and Eitzer, 2012) and lower concentrations in both plant matrices than in soil samples (Xu et al., 2016). Counter to our expectations, we did not detect thiamethoxam in our pollen and nectar samples, though treatment of seeds with this compound has been found to be associated with detection in the pollen and nectar of other field crops (Pilling et al., 2013). A greenhouse study of thiamethoxam seed treatment in potted sunflowers also revealed translocation into sunflower nectar (Bredeson and Lundgren, 2018). This difference in outcomes may be due in part to differences between field and greenhouse conditions, specifically the ability for pesticides to leach away from the application site in the field as well as the higher temperatures to which plants are exposed in a greenhouse, which can impact pesticide degradation.

Taken together, the paucity of thiamethoxam detections in our samples was an unexpected but important finding as it highlights the need for the inclusion of other metrics in pesticide risk analyses. Our results suggest that pesticide residue analysis alone is an insufficient measure of pesticide exposure, as bee biodiversity was affected by thiamethoxam seed treatment even when it was not detected. Future studies could be made more robust by including other metrics, such as biodiversity and behavior, to better understand field realistic pesticide exposure for bees.

Pesticide exposure for bees extends beyond materials applied within the sunflower crop

While the motivation for this study surrounded thiamethoxam use in sunflower, a number of pesticides not used in our study were detected in our samples, reinforcing the complexity of pesticide movement through the landscape. We detected the greatest number of pesticides in soil samples, though pesticide concentrations were lowest in these samples, a finding that is consistent with those of other studies (see Chapter 2). Unexpectedly, pesticide concentrations were consistently higher in all types of bees (*M. agilis*, pollen foraging honey bees, and nectar foraging honey bees) than in sunflower pollen, although concentrations in pollen and nectar did not differ. This is unexpected as previous research would suggest that pesticides detected in bees occur a concentrations lower than that found in the pollen and nectar samples due to metabolism (Rand et al., 2015) Analyses of the factors that influence pesticide residues in pollen and nectar are lacking, however many studies have found higher concentrations of pesticides in the former (Gierer et al. 2019). While detections in soil and plant matrices likely stem from pesticide drift, detections in bees and, in particular, higher concentrations in bees might be indicative of their foraging in other parts of the landscape. Bees are known to forage from both cultivated and uncultivated non-crop plants in agricultural landscapes (David et al., 2016; Long and Krupke, 2016) and bees foraging from such plants may acquire multiple pesticides (see Chapter 2).

The identity of the other pesticides detected in our study and the resulting potential mixtures of compounds raises concerns for bee health. Myclobutanil, a fungicide known to increase toxicity of bifenthrin and imidacloprid in bumble bees (Iverson et al., 2019) and to decrease metabolism in honey bees (Mao et al., 2017), was found in *M. agilis* samples (and only *M. agilis* samples). While bifenthrin and imidacloprid were not detected in bee samples, they were detected in soil samples where *M. agilis* nests, and thus may be exposed as adults and/or as larvae. Chlorothalonil, a fungicide known to result in fewer workers and smaller queens in bumble bees (Bernauer et al., 2015) as well as to predict the occurrence of the bumble bee pathogen (*Nosema bombi*), was detected in sunflower pollen and soil samples. Bumble bees do visit sunflower and were among the species observed in the fields, and as such, may be transporting pollen with chlorothalonil back to their nests. Collectively, this shows how wild bees foraging and nesting in crops are exposed to pesticides, not applied within the crop, but rather applied in the greater landscape.

Detection of non-target pesticides also varied among bee species. Although the mean number and mean concentration of pesticides did not differ among the three categories of bees in our study, the pesticides detected in *M. agilis* were not always detected in honey bees and vice versa. For example, cyhalothrin was the only insecticide detected in bees and it was detected in only one pollen foraging honey bee sample. The detection level of cyhalothrin in our bee sample was 1.9 ng/bee, and as the lethal dose to kill 50% of honey bees after 48 h of contact with cyhalothrin (LD50) is 6.1 ng/bee (USEPA, 2020), this represents a 0.31 toxicity unit (TU: concentration / LD50). Some research indicates that TU > 0.20 may contribute to toxicity, and that is without accounting for potential sublethal effects, chronic effects and/or synergism between compounds that increase toxicity (von der Ohe and de Zwart, 2013). In contrast to the cyhalothrin example, some pesticides were detected in wild bees but not honey bees. For example fungicides were detected in *M. agilis*, but not honey bees. Specifically, the fungicide, boscalid, which is known to synergistically increase toxicity of thiamethoxam (Tsvetkov et al., 2017), was detected in *M. agilis* and in the nectar regurgitated by honey bees, but not in the honey bees themselves. This may suggest differential exposure and/or metabolism among bee species, reinforcing the supposition that honey bees are not a good model for wild bee species with regard to pesticide risk assessments.

In summary, a number of pesticides and pesticide mixtures that were not used in our plots were detected in our samples. The pesticides detected in soil, pollen, and nectar samples suggest that pesticides are moving throughout the landscape via arial drift and soil leaching. Furthermore, bees themselves are moving throughout the landscape and this movement can vary by species and/or life history traits, such as foraging range (see Chapter 2). Taken together, this reinforces the importance of multiple species assessment and a landscape scale approach for pesticide risk analysis.

Effect of thiamethoxam seed treatment on crop yield

Though previous research suggested that thiamethoxam seed treatment in sunflower had no effect on crop yield (Bredeson and Lundgren, 2015), flower head area at harvest and the estimated seed yield per flower head were both greater in control plots in our study. We speculate that the difference we found may have been mediated by the greater abundance of wild bees, and specifically *M. agilis*, in these plots. While honey bees are the primary pollinators of sunflowers in California, interactions between wild bees and honey bees have been shown to effectively double the pollination services provided by honey bees in sunflower fields (Greenleaf and Kremen, 2006). Wild bees are thought to indirectly increase sunflower pollination by (1) changing honey bee behavior and (2) depositing piles of sunflower pollen onto S-type plants that honey bees then spread across the florets (Greenleaf and Kremen, 2006). When present alone, honey bees tend to forage for either nectar or pollen during a foraging trip and thus they have no reason to move pollen from the pollen producing (P-type) plants to the seed producing (S-type) plants. However, in the presence of wild bees, honey bees switch to moving between plant types more readily (Greenleaf and Kremen, 2006). Thus, the greater seed yields observed in our control plots could have resulted at least partially from the effect of the greater wild bee abundance on the efficiency of the pollination services provided by honey bees. It remains unknown whether honey bees were also more abundant in the control seed plots, as this was not quantified as part of our study. However, other studies have found no difference in plant visitation rates by honey bees in sunflower fields with and without thiamethoxam seed treatments (Bredeson and Lundgren, 2018).

Our results suggest that the cost of thiamethoxam seed treatment for production of sunflower seeds may outweigh the economic gain, at least in fields with lower observed pest activity. The mean seed set in the thiamethoxam plots (413 ± 85 seeds) was significantly lower than that in the control plots (530 ± 49 seeds). That is, without a thiamethoxam seed treatment, crop yield increased by 28%. From 2013-2018, seed companies in this region harvested an average of 1,345 kg of hybrid sunflower seeds per hectare and made $2.86 per kg, thus earning $3,846 /ha (Long et al., 2019). If we assume that the harvest of 1,345 kg/ha comes largely from fields utilizing neonicotinoid seed treatments and that crop yield could increase by 28% without such treatments, seed companies could potentially increase economic returns by $1,077 /ha, without accounting for the cost of the insecticide itself. Given that fields in this study were on average 18.75 ha, that is an increase of $5,385 per field. This finding suggests that there may be economic advantages for farmers and seed companies that abstain from the prophylactic use of thiamethoxam seed treatment in sunflower.

Conclusion

Our findings suggest that treatment of seeds with thiamethoxam impacts bee biodiversity and decreases crop yield in hybrid sunflowers despite this compound rarely being detected in soil, and not detected in nectar, pollen, or bees. This is an important finding because it reveals that pesticide residue analysis does not always capture the impacts of field realistic pesticide exposure and thus highlights the necessity for the inclusion of other metrics, such as diversity or behavior, in pesticide risk analysis. In addition, our results suggest that bees were exposed to a mixture of many other pesticides that are not applied in the sunflower crop, suggesting that pesticide exposure for bees needs to be considered at the landscape level, as even bees foraging from and nesting within sunflower crops were exposed to pesticides not applied to those individual fields. Based on the positive impacts of bee diversity on sunflower seed pollination, we suggest that sunflower seed companies and farmers assess pest presence and refrain from thiamethoxam seed treatment when pest presence is low.

Figures

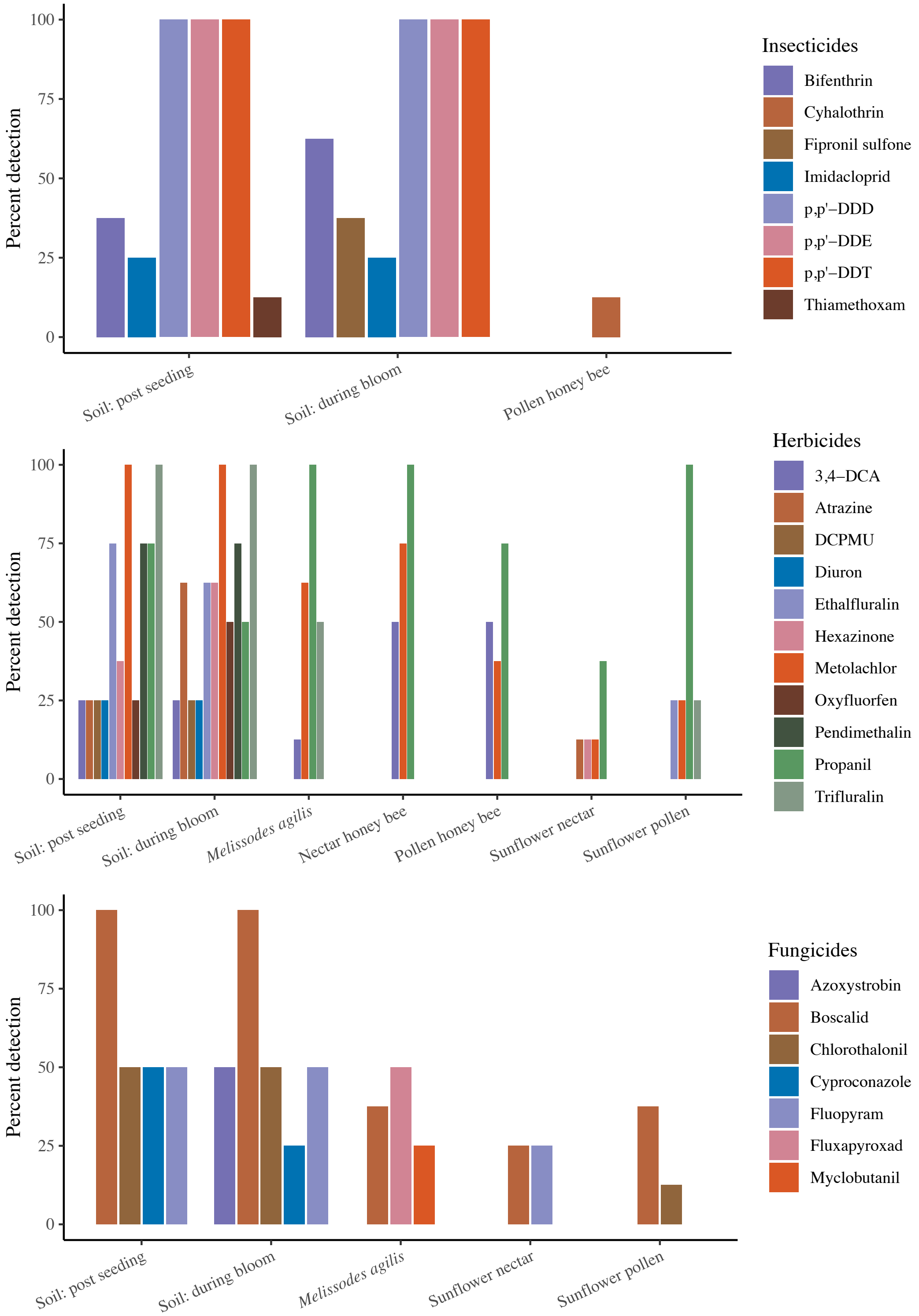


Figure 1: Pesticide detection across samples. Percent detection of insecticides, herbicides, and fungicides within each sample type.

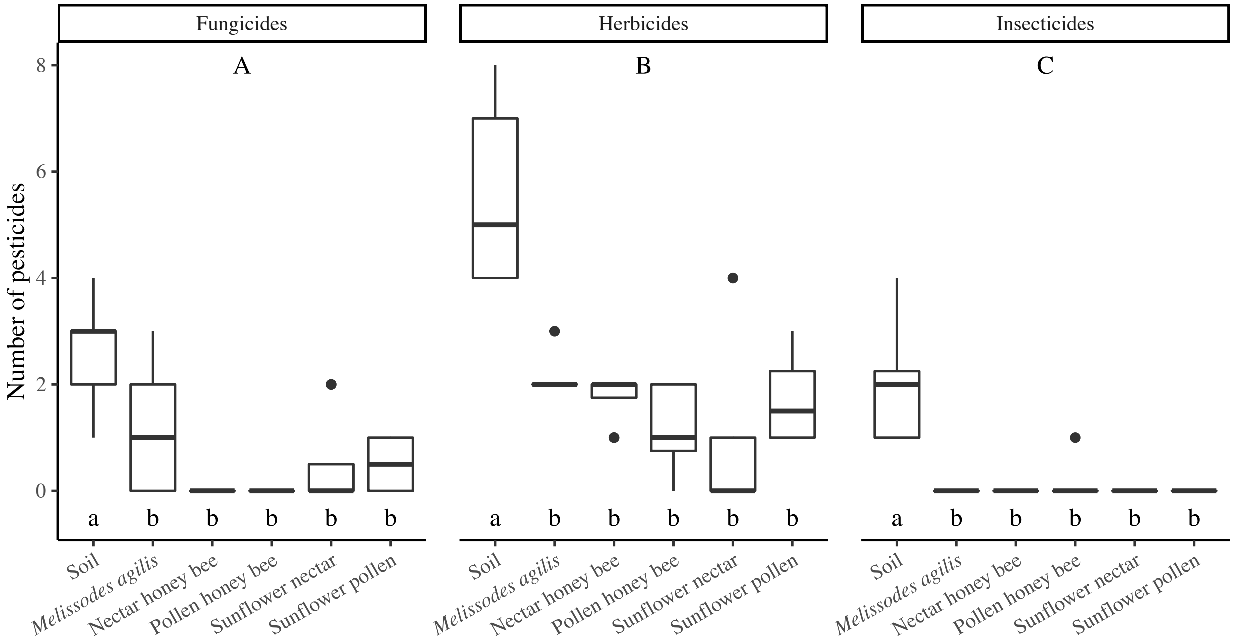


Figure 2: The influence of sample type and pesticide category on the number of pesticides detected. Bold lines depict medians, boxes depict 25th and 75th percentiles, whiskers depict 95th percentiles and dots depict outliers. Differences in mean number of pesticides between pesticide categories are indicated by capital letters ($P < 0.05$). Differences in mean number of pesticides between sample types are indicated by lower case letters ($P < 0.01$).

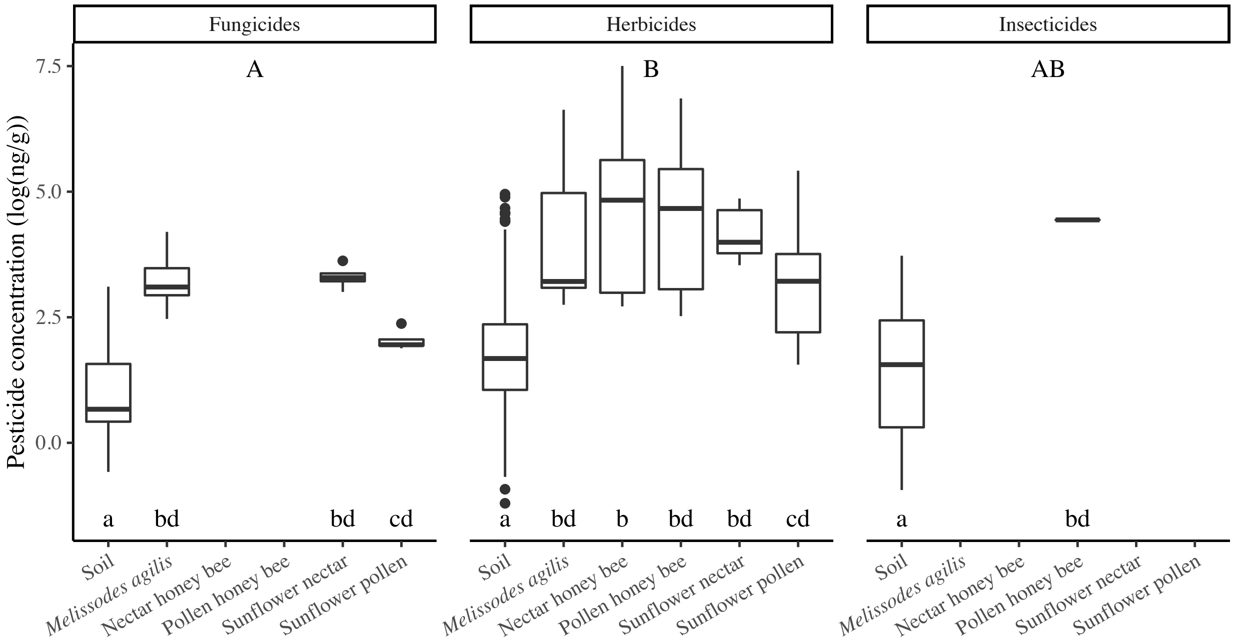
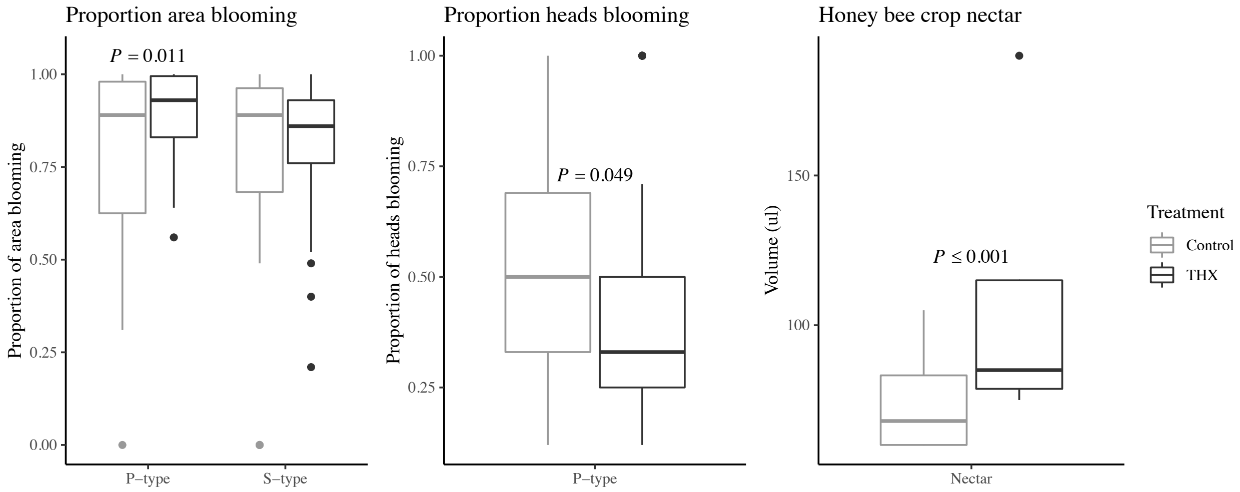


Figure 3: The influence of sample type and pesticide category on the concentration of pesticides detected. Bold lines depict medians, boxes depict 25th and 75th percentiles, whiskers depict 95th percentiles and dots depict outliers. Differences in mean number of pesticides between pesticide categories are indicated by capital letters ($P < 0.01$). Differences in mean number of pesticides between sample types are indicated by lower case letters ($P < 0.01$). Note: mean concentration marginally differed between *M. agilis* and sunflower pollen at the $P = 0.090$ level, marginally differed between the pollen foraging honey bee and sunflower pollen at the $P = 0.070$ level, and marginally differed between insecticides and fungicides at the $P = 0.053$ level, however these differences are not denoted with letters below.

(A) Floral resource measurements



(B) Crop yield measurements

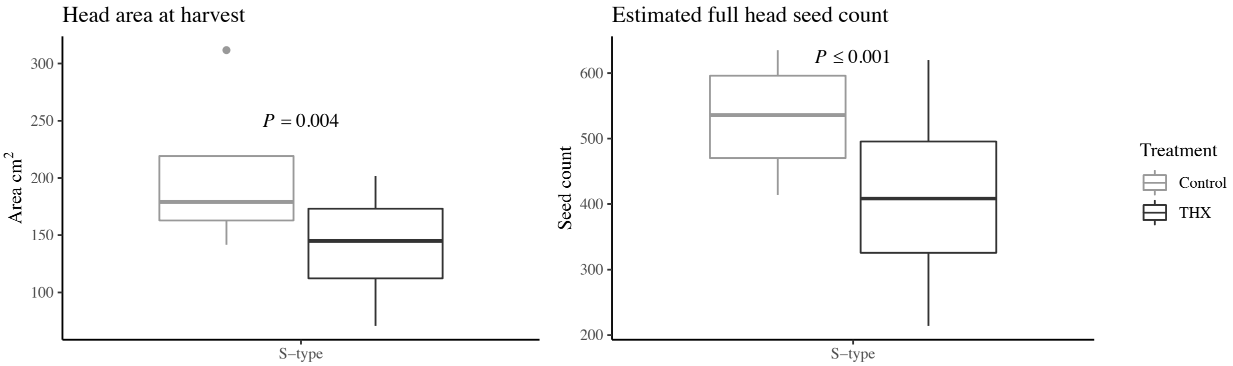


Figure 4: The influence of control and neonicotinoid seed treatments on (A) floral resource and (B) crop yield measurements. Bold lines depict medians, boxes depict 25th and 75th percentiles, whiskers depict 95th percentiles and dots depict outliers. Differences in means between treatment and control are indicated above pertinent boxplots for any $P < 0.05$. THX = thiamethoxam seed treatment.

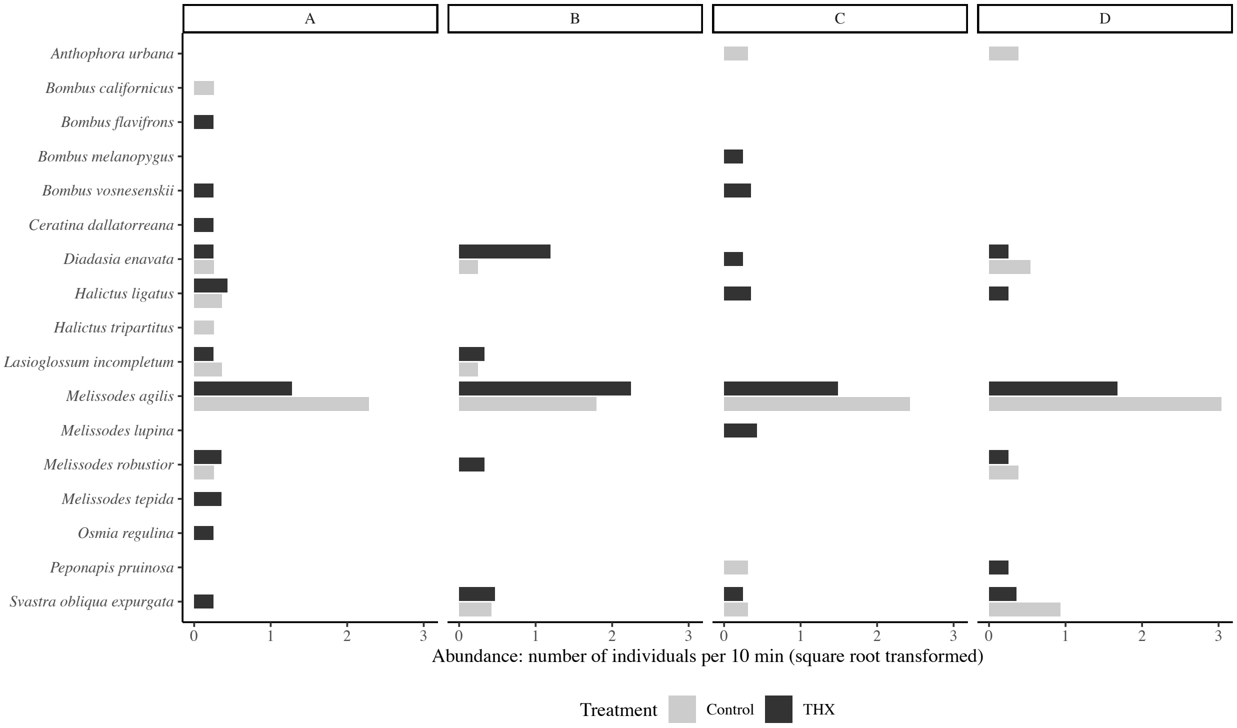


Figure 5: The influence of control and neonicotinoid seed treatments on the abundance of bee species for the four different field sites. Sites are labeled A through D. Abundance is represented by the length of bars and expressed as the square root of the number of individuals collected per 10 minutes for each species. THX = thiamethoxam seed treatment.

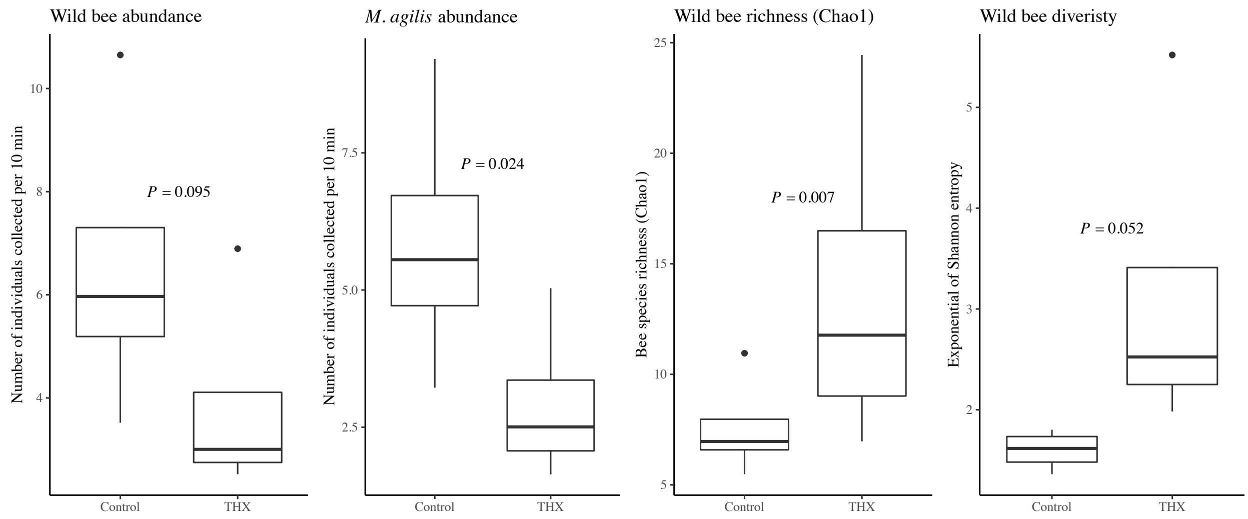


Figure 6: The influence of control and neonicotinoid seed treatments on wild bee abundance and diversity. Bold lines depict medians, boxes depict 25th and 75th percentiles, whiskers depict 95th percentiles and dots depict outliers. Differences in means between treatment and control are indicated above boxplots. THX = thiamethoxam seed treatment.

Chapter 2: Pesticide exposure for wild bees and honey bees foraging from perimeter flowers in intensively managed agriculture

Abstract

Bees are critical for food crop pollination, yet their populations are declining as agricultural practices intensify. Pollinator attractive perimeter plantings are one practice utilized in agricultural areas to conserve bee species, but recent studies suggest that these plants may harbor pesticides. Actual pesticide exposure rates for wild bees in agricultural areas remains largely unknown, but are key to informing agricultural practices and pesticide regulations with regard to bee conservation. In this study, we collected multiple species of wild bees, honey bees, flowers from four types of bee-attractive perimeter plants, and soil samples for pesticide residue analyses. Silicone bands were also utilized as passive aerial samplers of pesticide residues. We detected the greatest number of pesticides in bands, followed by soil, and found that the flowers of perimeter plants harbor pesticides that are applied to crops in the greater landscape. Pesticide concentrations detected were lower in bees than in flowers but higher in bees than in soils and increased with increasing wild bee size. Honey bees and wild bees foraging from perimeter plants were differentially exposed to pesticides, and both contained various pesticide mixtures that are known to contribute to sublethal effects. The results from this study highlight the need for pesticide regulations to consider a greater range of bee species and exposure at the landscape level.

Introduction

Wild bees and honey bees are crucial organisms in agroecosystems because of their key role in pollination services that ensure global food security (Klein et al., 2007). However, bees are declining worldwide due to land-use change, especially agricultural intensification, which has caused significant habitat loss and increased exposure of bees to pesticides (Goulson et al., 2015; Potts et al., 2010).

One practice to promote bee conservation in agricultural areas has been the restoration of bee-attractive perimeter plants, such as hedgerows and flower strips (Albrecht et al., 2020; M'Gonigle et al., 2015; Morandin and Kremen, 2013; Ponisio et al., 2016; Venturini et al., 2017). This is considered a cost-effective strategy (Morandin et al., 2016) that both provides continuous floral resources for bees in the otherwise forage limited monocultural landscapes (Blaauw and Isaacs, 2014) and can also increase crop pollination and crop yield (Boyle et al., 2020; Garibaldi et al., 2014). Recent evidence, however, suggests that these cultivated, bee-attractive perimeter plants harbor pesticides that have been applied to adjacent crops (Botías et al., 2015; David et al., 2016). Uncultivated perimeter plants (i.e. weeds) have also been found to harbor many pesticides applied within the greater landscape (Long and Krupke, 2016). While flower strips and hedgerows can improve crop pollination, pest control, and biodiversity (Albrecht et al., 2020), it is important to understand whether they may be a source of pesticide exposure for honey bees and wild bees, since significant exposure from perimeter plants could counteract the intended conservation benefits of this land use practice. Also, as these perimeter plantings are known to support higher bee species richness than crop fields (Sardiñas and Kremen, 2015) they are uniquely suited to assess pesticide exposure for a wide variety of bee species.

Wild bees contribute to pollination services in many agricultural areas (Pitts-Singer and James, 2008), yet pesticide research with bees has largely focused on honey bees (though see

(Kopit and Pitts-Singer, 2018; Sgolastra et al., 2019). Bees are incredibly diverse with thousands of species and thus exposure and sensitivity to pesticides likely varies between species, especially for those with differing life histories (Kopit and Pitts-Singer, 2018). Though more efforts have been made to include a select number of other managed and wild bee species as models for pesticide risk assessment (e.g. Botías et al. 2017), this research is still limited (Sgolastra et al., 2019). Furthermore, few studies have looked at pesticide exposure for wild bees in the field (though see Hladik et al., 2016; Longing et al., 2020; Main et al., 2020); most have been laboratory or cage studies (Lundin et al., 2015) using a subset of species that can be cultivated for laboratory research (Kopit and Pitts-Singer, 2018; Sgolastra et al., 2019). Routes of exposure and particularly variation in field-realistic exposure rates remain largely unknown for the majority of bee species, yet this information is essential for understanding pesticide risk to bees.

To better understand pesticide exposure for different kinds of bees in an agricultural landscape, we examined pesticide residues at field sites with both perennial and annual bee-attractive perimeter plants, with pesticide residues sampled in different matrices including wild bees, honey bees, flowers, soil, and air (via passive samplers). The objectives of this study were to determine (1) whether the flowers of perimeter plants harbor pesticides, (2) whether the composition and/or concentration of pesticides differ between bees, flowers, soil, and air, and (3) whether pesticide exposure differs between flower types and/or bee types. This study is one of the first to examine pesticide exposure for a wide variety of bees visiting perimeter plants in an agricultural landscape by comparing pesticides detected in wild bee and honey bees bodies to samples from the crop environment that could be sources of pesticide exposure for bees, namely, flowers, soil, and air. Understanding the pesticide profile of honey bees and wild bees foraging from perimeter plants in agricultural areas compared to the pesticide profile of potential sources of exposure can help to inform pesticide regulations and conservation practices.

Materials and Methods

Study Sites

Samples were collected at field sites on eight farms in intensively managed agricultural areas in Yolo & Colusa Counties, CA. Each site had a pollinator hedgerow, a forb strip, and weedy areas, all at the perimeter of an agricultural crop. Hedgerows were generally mature (>10 years since establishment), 3-6m in width and 350m long, consisting largely of native perennial trees and shrubs (M'Gonigle et al., 2015; Morandin et al., 2016; Morandin and Kremen, 2013; Ponisio et al., 2016). Forb strips had been established (i.e. seeded) in the previous three to four years and consisted of primarily native annual herbaceous plants in rectangular plots (800 m^2) either directly adjacent to, or in one case, interdigitated with the hedgerows (Williams, 2016). Weedy areas included patches directly adjacent to hedgerows and forb strips, in addition to patches adjacent to crops, in irrigation ditches or in otherwise fallow land within the farm site. In general, pesticides were not applied directly to the hedgerow and forb strips during the sampling period and for at least three to four years before sampling began. Occasionally, the herbicides glyphosate and clethodim were spot applied to weedy areas in the forb strips, to help the pollinator plants become established (Williams, 2016). While the agricultural region consists primarily of conventionally managed row crops, vineyards, and orchards, the crops are relatively diverse. Crops occupying five percent or more of the region within a radius of four kilometers of the sampling sites included: alfalfa, almonds, hay (non-alfalfa), oats, olives, rice, sunflower, tomatoes, walnut, and winter wheat.

Sampling Design

Samples were collected from the eight sites between mid-April and June 2016, with collection focusing on peak bloom times for two distinct sets of bee-visited flowering plants. Period one spanned from mid-April to mid-May and period two ranged from mid-May to July. In order to better understand the different routes of pesticide exposure for bees, multiple matrices were sampled: wild bees, honey bees, flowers, soil, and silicone bands (as aerial passive samplers). Sampling methods are described below for each sample type. Not all samples could be collected from each of the eight field sites during the two sampling periods. In period one, only six medium wild bee samples, seven small and large wild bee samples, six woody flower samples, and seven phacelia and poppy flower samples were analyzed for pesticide residues. In period two, only five large wild bee samples, and seven gum flower samples were analyzed for pesticide residues.

Wild bees and honey bees

Both honey bees and wild bees visit perimeter plants in agricultural fields, however they have very different behavioral and life history patterns that could lead to differing exposure to pesticides (Kopit and Pitts-Singer, 2018; Sponsler et al., 2019). Thus, both honey bees and wild bees were collected by sweep netting at each site. Since bee size measured by intertegular span (IT-span) is correlated with foraging distance (Greenleaf et al., 2007), we divided the wild bees collected into three size categories (small, IT span < 2.12 mm; medium, IT span 2.13 – 2.87 mm; large, IT span > 2.87 mm) based on their foraging distance (small, < 1 km; medium, 1 – 2 km; large, > 2km). All bees were collected via sweep net, transferred to micro-centrifuge tubes, and placed on dry ice in the field, then stored at between -14 to -20 °C in the laboratory prior to pesticide analysis. Small brushes and gel (solidified mixture of: gelatin (50 g), glycerin (150 ml), and distilled water (175 ml)) were used to remove the majority of pollen from the surface of each bee, in an effort to analyze the pesticide load of the bee itself rather than the bee and the pollen it carried.

The number of individuals in each wild bee sample varied due to the need for a minimum sample weight of 1 g for pesticide analysis (small, 28-118; medium, 6-18; large: 2-11). It was not feasible to sample a fixed number of individuals; we sometimes needed > 100 small bees to reach the minimum sample weight for chemical analysis and sampling that many large bees would have been impractical and potentially detrimental to local populations of large bodied species. Bees were typically collected when observed foraging on flowers. To achieve the minimum number of individuals required for pesticide analysis, bees were occasionally collected in flight in flower patches. For one small wild bee sample in the first sampling period, five of the bees included in the total composite sample of 28 bees were collected one month prior to the official sampling period; these were included to be able to achieve the minimum weight for the sample. At one site, three bumble bees that had been observed to be entering and/or exiting an underground nest were collected. These three bees were excluded from statistical analysis and are reported separately in the results.

While individual wild bees forage for both pollen and nectar, individual honey bees tend to have distinct castes of pollen or nectar foragers (Free, 1963). To avoid biasing our data, we attempted to collect an equal number of pollen and nectar foraging honey bees. During sweep netting, honey bees were collected and labeled as pollen foragers if they had large pollen loads in their corbicula. Honey bees were collected and labeled as nectar foragers if they had been observed foraging for nectar for at least 30 seconds. A total of 10 honey bees were collected per site per time period.

Flowers

Pesticides applied to crops can be present in perimeter plants due to both aerial drift and uptake from the soil (Bonmatin et al., 2015; Botías et al., 2016; David et al., 2016; Krupke et al., 2012). Pesticide uptake and dissipation can vary with multiple plant characteristics (growth, acidity, shape, leaf texture, and transpiration) and pesticides can be present in differing amounts in distinct parts of a plant (stem, leaves, petals, pollen, and nectar), leading to the expectation that pesticide detection and concentration could differ among herbaceous and woody plants (Bonmatin et al., 2015; Fantke and Juraske, 2013). Bees consume nectar from flowers and collect pollen to feed to their progeny. To estimate pesticide exposure for bees visiting perimeter flowering plants, four types of flowers were collected during each sampling period; flowers from two herbaceous plants, one woody plant, and one weedy plant (see **Table 1** for species). Flower petals and sepals were reduced as much as possible with scissors and/or cork borers to isolate the pollen and nectar structures. Flowers from each plant type were placed into glass containers and transferred to coolers in the field before storage at between -14 to -20 °C for pesticide analysis. To achieve a minimum sample weight of 0.3 g for pesticide analysis, the number of individual flowers per sample was inversely related to flower size. When possible, flowers were collected from different plants at each site.

Soil

Pesticides applied to crops can leach through soils and be taken up by perimeter plants (Bonmatin et al., 2015; Botías et al., 2015, 2016; David et al., 2016). Also, many wild bees nest in soil (Kopit and Pitts-Singer, 2018). To understand exposure of perimeter plants and nesting bees, five soil cores (2.5 cm diameter and 7 cm depth) were collected across each site from locations where other bee and flower samples were collected. The five soil samples were collected once during each sampling period, combined, and stored in Ziplock bags. Bags were placed in coolers in the field, then stored at between -14 to -20 °C for pesticide analysis.

Silicone bands

As bees can encounter pesticides via aerial spray drift and dust (Krupke et al 2012), silicone bands (100% silicone wristbands from 24HourWristbands.com, 1.27 cm with 6.7 cm inner diameter) were deployed as passive samplers to collect aerial deposits of pesticides. One band was used per site per sampling period. Pre-cleaned bands (O'Connell et al, 2014) were removed from foil storage on site, cut into pieces, and each piece was stapled vertically to separate above-ground stakes at a height between 1.25 - 1.75 m at the beginning of the sampling period. The stakes were spread across each site, such that their locations corresponded with where flower, bee, and soil samples were collected. At the end of each sampling period, bands were recovered, stored in foil, placed in a cooler, and then stored at between -14 to -20 °C for pesticide analysis. Bands from each site were stored separately.

Analytical Methods for Pesticide Detection

Silicone bands were extracted via sonication with ethyl acetate (detailed protocol in Swanson et al., 2018) while the other types of samples (soil, flowers, bees) were extracted via pressurized liquid extraction and solid phase extraction cleanup (Hladik et al., 2016; Hladik and McWayne, 2012; Main et al., 2020). Samples were analyzed for 168 pesticides and degradates using both gas and liquid chromatography-tandem mass spectrometry. The complete list of compounds and analytical limits of detection (LOD) can be found elsewhere (Hladik and Ward, *in press*). While a wide range of pesticides were included in the analysis, some exceptions include the herbicides glyphosate, 2,4-D, and triclopyr. Although these herbicides are widely used, herbicides are considered less toxic to pollinators in general than insecticides or fungicides (Goulson et al., 2015).

Data Analysis

Generalized linear mixed models and linear mixed models were run using the lme4 package in R version 3.6.1 (Bates et al., 2015; R Core Team, 2019). Type II Wald chi square tests were used to determine the significance of fixed effects in the models using the Anova() function from the car package in R (Fox and Weisberg, 2019). Post hoc comparisons of marginal means among sample types within significant fixed effects were made using the emmeans() function from the emmeans package in R (Lenth, 2020).

To determine the relationship between the number of pesticides detected and sample type (i.e. wild bees, honey bees, flowers, soil, and silicone bands) generalized linear mixed models with a Poisson error distribution were used, with sample type, pesticide category (fungicide, herbicide or insecticide), and their interaction as fixed effects. We also included field site and time period as random effects to account for the repeated sampling (two time periods). We conducted this analysis for all sample types, in addition to a subset of wild bee samples alone to examine the effect of bee size, and a subset of flower samples alone, to examine the effect of flower type. For analyses of the number of pesticides, degradates, and parent compounds were combined and counted as just one pesticide when their association with each other was indisputable. As such, the degradates DDE and DDD and parent compound DDT were combined and counted only once, and the degradate DCPMU and parent compound diuron were combined and counted only once. We removed the degradate 3,4-DCA from these analyses because it is a degradate of both propanil and diuron and so it could not be assigned to a single parent compound.

To determine the relationship between the concentrations of the pesticides detected and sample type, linear mixed models based on a Gaussian error distribution were used. Sample type and pesticide category and their interaction were included as fixed effects and field site and time period as random effects. In cases where just one pesticide was analyzed (i.e. bifenthrin), the fixed effect of pesticide category was removed from the model. Concentration was analyzed in units of nanogram of pesticide per gram sample (ng/g). Due to the fact the pesticide concentration in silicone bands is more dependent on surface area than mass, band concentration is reported in nanograms of pesticide per band (ng/band) and was thus not included as a sample type in the concentration analysis. Pesticide concentrations in the three size categories of wild bees were also analyzed separately, with concentration expressed both as ng/g and as ng/bee for each composite sample. The latter expression of pesticide concentration was obtained from the former by multiplying by the mass of the sample and dividing by the number of bees in the sample. Pesticide concentrations were also analyzed for flowers alone to examine the effect of flower type. For these analyses, pesticide degradates and parent compounds were treated as distinct pesticides (i.e. they were not combined, as in the number analysis). In all cases, analyses were restricted to only those pesticides that were detected, and concentration was transformed with natural logs to linearize the data.

As an indicator of potential routes of exposure for bifenthrin, linear mixed models with Gaussian errors were used to test whether the concentration of bifenthrin in wild bees was associated with sample type and either wild bee size or flower type. The same analysis was also conducted for honey bees. In these concentration by concentration analyses, sample type was included as a fixed effect in the model and field site was included as a random effect. Concentrations were scaled so that silicone bands could be included as a sample type despite the different unit of measurement for concentration.

Results

Overview of Pesticide Detection in Samples

Of the 148 samples analyzed for the potential occurrence of 168 pesticide residues, a total of 37 pesticides and degradates were detected, of which there were 8 insecticides, 13 herbicides, and 12 fungicides, and 4 degradates. There was a total of 17 pesticides and degradates detected in wild bees overall (16 in small bees, 17 in medium bees, and 12 in large bees), 10 in honey bees, 17 in flowers overall (12 in herb 1, 11 in herb 2, 12 in weeds, and 12 in woody), 25 in soil, and 24 in silicone bands. Percent detection of insecticides, herbicides, and fungicides within sample types are depicted in **Figure 1**.

The three most frequently detected pesticides in all samples were the insecticide bifenthrin and the herbicides thiobencarb and metolachlor, which together were detected in greater than 37% of samples. Pesticides that were detected in over 20% of all samples included the insecticide bifenthrin, the herbicides metolachlor, pendimethanlin, propanil and thiobencarb, and the fungicides boscalid, carbendazim and fluopyram. For wild bees, the insecticide bifenthrin and the herbicides hexazinone, metolachlor, propanil, and thiobencarb were detected in over 25% of samples. For honey bees the insecticide bifenthrin, the herbicide thiobencarb, and the fungicides carbendazim and fluropyram were detected in over 25% of samples.

Among the insecticides examined, neonicotinoids are known to be particularly harmful to bees. Neonicotinoids were infrequently detected at our field sites; imidacloprid was the sole neonicotinoid detected (**Figure 1**). Imidacloprid was detected in 20% of flower samples at concentrations of 8.0 to 28.7 ng/g. It was also detected in 13% of soil samples at a concentration of 0.3 to 0.9 ng/g (at only one field site during both sampling periods). All imidacloprid detections in flowers were in herbaceous or weedy plants sampled during period one (*Phacelia* spp., *Eschscholzia californica*, and Brassicaceae); imidacloprid was not detected in woody plant flowers in either sampling period. At the site where imidacloprid was detected in the soil, it was only also detected in weedy flowers, not in the herbaceous flowers.

On the other hand, the insecticide bifenthrin was detected at all sites and was the most frequently detected pesticide in our samples in general. Bifenthrin was detected in 29% of wild bee samples, 31% of honey bee samples, 40% of flower samples, 63% of soil samples, and 100% of the silicone band samples. Of the flower samples, bifenthrin was notably detected in all of yellow star thistle samples (weed, period 2) and all but one sample of toyon (woody, period 2). Bifenthrin is a pyrethroid insecticide and the lethal dose required to kill 50% of honey bees

($LD50_{Hb}$) is 14.6 ng/bee (Sanchez-Bayo and Goka, 2014). None of our bee samples contained bifenthrin in concentrations close to the $LD50_{Hb}$, however 60% of honey bee samples and 55% of wild bee samples with bifenthrin detections exhibited a toxic unit (TU, concentration detected/LD50) ≥ 0.07 (von der Ohe and de Zwart, 2013). The greatest TU for a honey bee was 0.16 and the greatest TU for a wild bee was 0.20 (large wild bee). The sample of three bumble bees collected above the underground nest at one farm site had a TU of 0.37 for bifenthrin.

Of the fungicides known to be harmful to bees either independently or synergistically, three were detected in our samples: chlorothalonil, myclobutanil, and propiconazole (**Figure 1**). Chlorothalonil was detected at all sites and in 88% of silicone band samples, 19% of soil samples, 10% of wild bee samples, and 10% of flower samples (only *Phacelia* spp., and *Frangula californica*). Myclobutanil was detected at 63% of sites in only bee samples; in 19% of honey bee and 10% of wild bee samples. In two cases (one honey bee and one large bee sample), myclobutanil was present in the same sample as bifenthrin. Propiconazole was detected at all sites and in all silicone band samples, 15% of wild bee samples, 1 flower sample, and 25% of soil samples.

Number of Pesticides

Sample Type and Pesticide Category

A total of 33 pesticides were detected in our samples after combination of parent materials and degradates. The number of pesticides detected differed by pesticide category and sample type ($\chi^2_{\text{pesticide category}} = 66.86$, df = 1, $P < 0.001$; $\chi^2_{\text{sample type}} = 422.15$, df = 4, $P < 0.001$) with no significant interaction between these factors ($\chi^2_{\text{interaction}} = 13.97$, df = 8, $P = 0.083$). Typically, less than two pesticides from each category were detected in each sample type; the one exception was the silicone bands, which detected from 6 to 8 herbicides and fungicides (**Figure 2a**). A greater number of pesticides was detected in soil samples than in flower or bee samples, but less than in silicone band samples. The number of pesticides detected generally increased from insecticides to fungicides to herbicides.

Wild Bee Size

Among wild bees, the mean number of pesticides detected differed by pesticide category ($\chi^2_{\text{pesticide category}} = 26.73$, df = 2, $P < 0.001$), following a similar trend as for the overall dataset. The number of pesticides detected did not differ by wild bee size ($\chi^2_{\text{wild bee}} = 2.52$, df = 2, $P = 0.284$; **Figure 2b**) and there no interaction ($\chi^2_{\text{interaction}} = 1.77$, df = 4, $P = 0.778$).

Flower Type

Among the different flowers examined, the mean number of pesticides detected differed by pesticide category and flower type ($\chi^2_{\text{pesticide category}} = 12.15$, df = 2, $P = 0.002$; $\chi^2_{\text{flower type}} = 17.78$, df = 3, $P = 0.001$, **Figure 2c)**, with no interaction ($\chi^2_{\text{interaction}} = 5.15$, df = 6, $P = 0.525$). Herbaceous plants in general, and specifically Herb 2 (*Eschscholzia californica*) had fewer distinct pesticides detected than other plant types.

Pesticide Concentration

Sample Type and Pesticide Category

Analyses of pesticide concentrations revealed a significant interaction between pesticide category and sample type ($\chi^2_{\text{interaction}} = 21.91$, df = 6, $P = 0.001$) and thus each pesticide category was examined separately. The mean concentration of pesticides differed among sample types for all three pesticide categories examined ($\chi^2_{\text{insecticides}} = 27.54$, $\chi^2_{\text{herbicides}} = 162.73$, $\chi^2_{\text{fungicides}} = 90.68$ with df = 3 and $P < 0.001$ for all). For all pesticide categories, pesticide concentrations tended to be greatest in flowers and least in soils (**Figure 3a**). While pesticide concentrations detected in wild bees and honey bees did not differ, concentrations detected in wild bees were typically lower than in flowers for all pesticide categories (**Figure 3a**); in contrast, insecticide and fungicide concentrations detected in honey bees were similar to those detected in flowers.

Wild Bee Size

For data from wild bees, using concentrations expressed as mean pesticide concentration (ng/bee) increased with increasing bee size ($\chi^2_{\text{wild bee size}} = 180.35$, df = 2, $P < 0.001$; **Figure 3b**) and differed by pesticide category ($\chi^2_{\text{pesticide category}} = 29.27$, df = 2, $P < 0.001$), with no significant interaction between these factors ($\chi^2_{\text{interaction}} = 1.84$, df = 4, $P = 0.765$). Although mean pesticide concentration did not differ with wild bee size for concentrations expressed as ng/g ($\chi^2_{\text{wild bee}} = 1.33$, df = 2, $P = 0.514$; **Figure 3c**), this measure did differ by pesticide category ($\chi^2_{\text{pesticide category}} = 21.60$, df = 2, $P < 0.001$); again, there was no significant interaction between these factors ($\chi^2_{\text{interaction}} = 1.15$, df = 4, $P = 0.887$).

Flower Type

For data from flowers, there was a significant interaction between pesticide category and flower type ($\chi^2_{\text{interaction}} = 19.40$, df = 6, $P = 0.004$) and thus we analyzed each pesticide category separately. The mean concentration of pesticides detected differed among flower types for all three pesticide categories ($\chi^2_{\text{insecticides}} = 8.94$, $\chi^2_{\text{herbicides}} = 9.85$, $\chi^2_{\text{fungicides}} = 10.04$ with df = 3 and $P < 0.05$ for all; **Figure 3d**). Insecticide concentrations did not differ across flower types, although the concentration of herbicides and fungicides detected in weeds were lower than in one of the cultivated plants (**Figure 3d**).

Bifenthrin

Mean bifenthrin concentration differed significantly among sample types ($\chi^2 = 84.85$, df = 3, $P < 0.001$). Bifenthrin concentration generally increased from soil, to bees, to flowers (**Figure 4a**). For wild bees with concentration expressed as(ng/bee), bifenthrin concentration increased with wild bee size ($\chi^2 = 23.49$, df = 2, $P < 0.001$; **Figure 4b)**. In contrast, when analyzed for bifenthrin concentration expressed as ng/g there was no difference between bee sizes ($\chi^2 = 3.64$, df = 2, $P = 0.162$; **Figure 4c**). Bifenthrin concentration differed by flower type ($\chi^2 =25.51$, df = 3, $P < 0.001$) and was higher in weeds than other plant types (**Figure 4d**). Concentration by concentration analyses showed a significant association between bifenthrin concentrations in honey bees and those in both woody plants ($\chi^2 = 10.35$, df = 1, $P < 0.001$) and herb 1 ($\chi^2 = 4.87$, df = 1, $P < 0.027$), while bifenthrin concentrations in wild bees were significantly associated with those in herb 2 ($\chi^2 = 5.59$, df = 1, $P < 0.018$) and marginally associated with those in weeds ($\chi^2 = 3.82$, df = 1, $P < 0.051$).

Discussion

Hedgerows and perimeter plants have been suggested to be effective ways for restoring and maintaining pollinator communities in intensive agricultural regions, but these plants harbor pesticides that are detrimental to bees (Botías et al., 2015; David et al., 2016). We found that the number of pesticides detected was greater in aerial (i.e. silicone band) and soil samples than in flowers or bees. Pesticide concentrations were generally lower in bees than flowers, higher in bees than in soils, varied considerably among flower types, and were greater in larger wild bees than smaller wild bees. While many studies have focused on neonicotinoid insecticides alone, we found many other pesticides in our study system, including those that are known to be harmful to bees on their own and synergistically with others (Iverson et al., 2019). Collectively our findings suggest that (1) bee-attractive, flowering perimeter plants indirectly harbor a number of pesticides that are applied to crops in the greater landscape, (2) biology and ecology influence pesticide exposure for bees, lending support to the suggestion that wild bees need to be considered in addition to honey bees, and (3) beyond neonicotinoids, bees are exposed to a mixture of other pesticides under field conditions that are known to have negative sublethal effects on these pollinators.

Pesticide Exposure and Perimeter Plants

The flowers that we sampled from the perimeter of agricultural crops harbored a number of pesticides. Other studies on perimeter plants have also found that these plants contain pesticides even though these products have not been applied directly to perimeter plants (Botías et al., 2015; David et al., 2016; Long and Krupke, 2016). Pesticides can be deposited on plants indirectly via aerial drift or dust (Krupke et al., 2012). Water soluble pesticides that have systemic properties can also be taken up indirectly by plants via soil leaching (Bonmatin et al., 2015). Thus, pesticides may be present on the surface of plant structures (as residue deposits), and/or within plant tissues and products (such as pollen, nectar and guttation fluid) even in the absence of direct exposure. Consequently, the pesticides detected in our flower samples likely came from various exposure routes, including drift (aerial) and leaching (soil).

Some of the pesticides detected in our study were likely applied to crops grown at some distance from the field sites. For example, the herbicides clomazone, propanil, and thiobencarb are applied exclusively in rice crops in this agricultural region (Hladik et al, 2020). While 75% of our field sites had rice growing within 4 km, rice fields represented only 1.33% (± 0.66) of the surrounding landscape and none of our field sites were directly adjacent to rice crops (LW, personal observation). This suggests that the exposure of bees to pesticides needs to be considered at the landscape scale, not just the farm scale. In this context it would be valuable to examine the pesticides known to have been applied within the foraging range of bees sampled at our field sites versus those detected in our pesticide analyses.

The number of pesticides present in flowers varied by flower type, with woody and weedy plants having a greater number of pesticides than herbaceous plants. This may reflect the tendency for woody plants to be present in the landscape for longer than annual, herbaceous plants, which may allow the former to accumulate more pesticides. It is unclear why weedy plants at our field sites had a greater number of pesticides as they were also herbaceous annuals, but this finding is consistent with some other studies (Long and Krupke, 2016). Differences in pesticide concentration between flower types did not show a consistent pattern across pesticide categories. Taken together, this suggests a multifaceted interplay of pesticide chemistry and plant anatomy and physiology and indicates that studies of acquisition of specific active ingredients across different plant types are needed to better understand flowers as a route of pesticide exposure for bees.

The frequency of detection and concentration of bifenthrin specifically was strikingly different among flower types. Bifenthrin was detected in all eight weedy plant samples (yellow star thistle) and in seven of eight woody samples (toyon), during sampling period two, yet in only one to two of the other flower samples. Bifenthrin concentrations were also significantly higher in the flowers of weedy plants than all other plants, driven largely by its presence in yellow star thistle. Given that bifenthrin is neither absorbed by nor translocated through plants, it is possible that the difference in the frequency of detection across flower types indicates that the residues from aerial drift are unevenly distributed, potentially due to differing flower heights and/or their location in the landscape. Additionally, the association of bifenthrin concentrations detected in honey bees and wild bees varied by flower types, with honey bee concentrations associated with nearly the opposite flower types as for wild bees. Thus, it is important to consider plant-pollinator relationships in regard to pesticide exposure for different bee species in a landscape.

Effects of Bee Biology and Ecology

The factors contributing to pesticide exposure for bees in agricultural landscapes are complex. In addition to being exposed to pesticides while foraging, bees can encounter pesticides during most activities that they engage in: during flight (via aerial sprays or the dust from planting of treated seeds), while walking or resting on the surface of plants and soils, during nesting (in soil, leaves, resins, wax and other materials), and while feeding and developing as larvae (Boyle and Sheppard, 2017; Gradish et al., 2019; Sgolastra et al., 2019). Concentrations of pesticides in bees were generally lower than in flowers but higher than in soil, suggesting that adult bees are exposed to greater concentrations of pesticides while foraging rather than nesting and that they are either not exposed to the full pesticide load presented in flowers and/or detoxification in bees leads to lower concentrations. However, as our samples were a composite of bees with various nesting strategies, future studies might consider comparing pesticide profiles in flowers and soil to that of cavity nesting vs soil nesting species to better understand the relationship between exposure via foraging versus nesting. Since we did not analyze the concentration of pesticides in the silicone bands per unit mass, we cannot compare concentrations in bees to those in air. However, we suspect that exposure from flying through dust or drift patches would be an infrequent occurrence. While our results suggest that flowers are an important source of pesticide exposure, our findings also underscore the difficulty of generalizing sources of exposure for bees as a whole.

We had expected to detect a greater number of pesticides in honey bees compared to wild bees given that bee keepers transport honey bee hives over large distances from different landscapes and geographic regions to pollinate crops in California. Instead, we found no difference between the mean number of pesticides detected in wild versus honey bees. However, the total number of pesticides detected only in wild bees was greater than the number detected only in honey bees. While this outcome may reflect that typically more individuals were collected per sample for wild bees compared to honey bees, the same pattern was evident in samples for large-bodied wild bees, which had on average fewer individuals per composite sample than honey bees. It is likely that differences in life history traits between wild bees and honey bees affect routes of exposure and thus the number and composition of pesticides encountered by different bee species.

A number of traits are relevant to pesticide exposure for bees including: sociality, fecundity, nest type (e.g. substrate, materials and period), flower preferences, adult and larval food, body size, and other anatomical and physiological characteristics (e.g. how pollen is transported and metabolism) (Sgolastra et al., 2019). In this study, we capitalized on the fact that bee size is correlated with foraging distance to analyze pesticide exposure for bees with differing foraging ranges (Greenleaf et al., 2007). We expected that because bee size is correlated with foraging distance, larger bees that forage over greater distances would be exposed to a greater number of pesticides. Despite being intensively managed, the agricultural region where we conducted our study has greater crop diversity than other regions where similar studies have been conducted (e.g. (Main et al., 2020) and thus, bees foraging over greater distances at our study sites may have been exposed to a greater number of pesticides due to the diversity of crops encountered. However, the number of pesticides detected in wild bees did not differ among size categories, a result that contrasts with those from other studies. For example, individual bees collected from non-crop patches in a region dominated by cotton revealed a general trend toward increased neonicotinoid detection with average bee body mass (Longing et al., 2020). The effects of body size and foraging distance on pesticide exposure may be more pronounced in areas with reduced crop diversity, although we caution against drawing strong conclusions given that the number of bees in our composite samples was indirectly related to bee size. We suggest that in areas of greater crop diversity, the number of pesticides detected may also increase with bee size if composite samples had been standardized by number rather than mass of bees.

On the other hand, larger bees in our study did tend to harbor a greater amount of pesticides on average than smaller bees. This trend was apparent when concentration was expressed per bee, but not when it was expressed per unit body mass (ng/g). Theories on how body size may affect exposure levels are mixed. Since body size and surface area are inversely related, smaller bees may experience greater contact exposure per unit body mass than larger bees. Furthermore, the inverse relationship between body size and mass-specific metabolic rates (Heinrich, 1993) suggests that smaller bees may experience greater oral exposure because they ingest more nectar and/or pollen per unit body mass than large bees (Sgolastra et al., 2019). Yet, for example, Botias et al (2017) found that bumble bees with lower mass had lower amounts of pesticide residue and suggested this may result from smaller bees consuming less than larger bees. The inverse relationship between body size and mass-specific metabolic rates may also mean that smaller bees detoxify pesticides more quickly, so while their exposure may be higher, it is possible that detection frequency and possibly sensitivity could be lower in smaller bees. Taken together bee biology and ecology likely lead to differences in pesticide exposure, detection ability, and pesticide sensitivity that are relevant to not just lethal effects, but also to sublethal effects. This highlights the drawbacks of categorizing bees as a single and uniform entity, best represented by honey bees as a model organism, when it comes to risk assessment from pesticide exposure.

Beyond Neonicotinoids

Neonicotinoids were not frequently detected in our samples, though they are applied in the region (e.g. aerial spray on walnuts, seed treatments for sunflower: Pesticide Use Report Database; R. Long et al. 2019). This outcome may reflect a lower usage of this class of insecticides in our study region as compared to studies of bees in areas that are dominated by neonicotinoid-treated crops (e.g. oil-seed rape, corn, soybeans: David et al. 2016; Botías et al. 2015; Main et al. 2020). While neonicotinoid research has been highly beneficial in revealing the necessity of updating pesticide risk assessments, especially with regard to (1) sublethal effects and the (2) consideration of a greater range of bee species (Sgolastra et al., 2020), more research is needed to understand bee exposure and sensitivity to a greater range of the pesticides that are used in the field.

Bifenthrin was the most frequently detected insecticide in our samples, and was detected alongside the fungicide, myclobutanil. Bifenthrin can be harmful to bees on its own (Sanchez-Bayo and Goka, 2014) and is known to react synergistically with the triazole SBI fungicides difenoconazole and myclobutanil, increasing its toxicity to bumble bees (Iverson et al., 2019). No bee samples collected had concentrations near the LD50 for bifenthrin, as would be expected because only live bees were collected in our samples, however at least one wild bee sample had a TU = 0.20. While TU < 0.20 is unlikely to contribute to acute toxicity, it may contribute to sublethal effects, chronic effects and/or synergism between compounds that increase toxicity (von der Ohe and de Zwart, 2013). Myclobutanil was present in two samples that also contained bifenthrin, suggesting that synergistic effects may have been possible. Difenoconazole was detected in silicone band samples, providing additional evidence that interactions among pesticides may have occurred at our study sites.

Fungicides on their own can also be harmful to bees. For example, myclobutanil, which was detected in bee samples and only bee samples, has been found to inhibit detoxification pathways in honey bees (Mao et al., 2017). Chlorothalonil, which was detected in many sample types including wild bees but not honey bees, has been associated with bumble bee colonies that produce fewer workers and smaller queens (Bernauer et al., 2015) and found to be strongly associated with pathogen presence (*Nosema bombi*) in declining bumble bee species (McArt et al., 2017). Further, insecticides that are not generally considered toxic to bees have been shown to increase in toxicity in the presence of fungicides. For example, we detected chlorantraniliprole and propiconazole together in one small wild bee sample and also in one medium wild bee sample. While the relatively new diamide insecticide chlorantraniliprole has low toxicity to bees, a recent study found increased honey bee larval mortality and adult toxicity when chlorantraniliprole exposure was combined with the fungicide propiconazole (Wade et al., 2019). Collectively, these findings underscore the importance of considering multiple pesticides and pesticide mixtures when assessing effects on wild bees. Several previous studies have reported a range of pesticides in bees or bee related samples, including several of the same insecticides, herbicides, and fungicides detected in our study (Botías et al., 2017; David et al., 2016; Hladik et al., 2016; Krupke et al., 2012; Main et al., 2020; Mullin et al., 2010). To date, however, pesticide regulations typically consider only one compound at a time, indicating that additional work is needed to assess potential interactions and synergistic effects (Sgolastra et al., 2020).

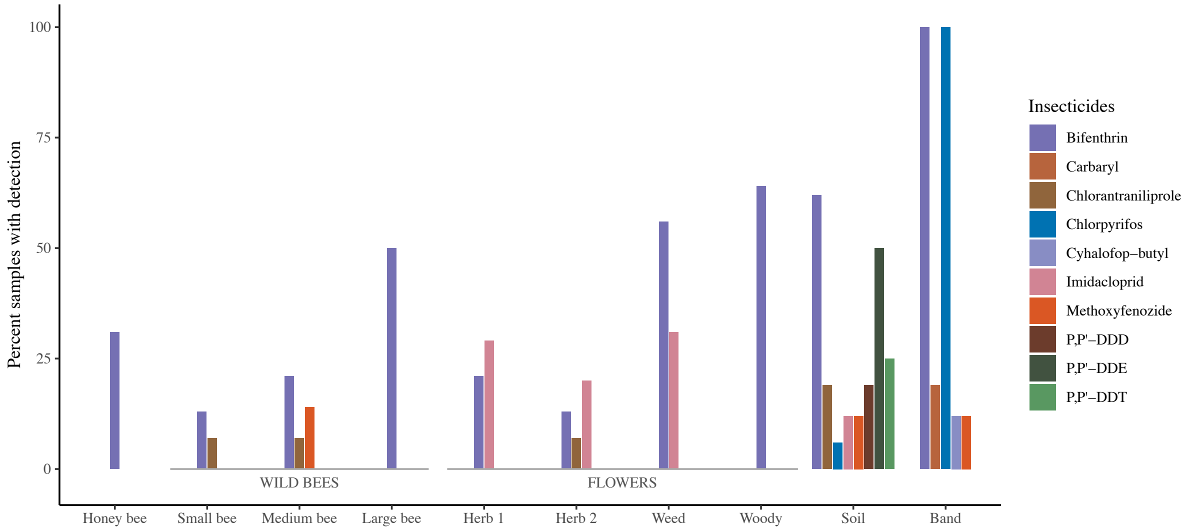
Conclusion

Much remains to be learned about routes of pesticide exposure, frequency of exposure, and toxicity in relation to conservation of wild bees and honey bees foraging in agricultural landscapes. The results of our study suggest that bee-attractive perimeter plants in agricultural areas harbor many pesticides, despite the fact that pesticides are not applied directly to these plants. Additionally, the number and concentration of pesticides differ between plant types. Bees typically contained fewer pesticides and lower concentrations of pesticides than flowers, albeit still at levels of concern, particularly for sublethal effects (e.g. bifenthrin), as well as in combinations that can potentially have synergistic effects on the toxicity of different compounds. Studies that analyze only a subset of pesticides and a subset of bee species may overlook evidence for larger ecological consequences. While this study generates important insights for pesticide exposure of bees in an intensively managed agricultural landscape, more research is needed to understand the sensitivity of a range of bee species from exposure to realistic field concentrations and mixtures of pesticides.

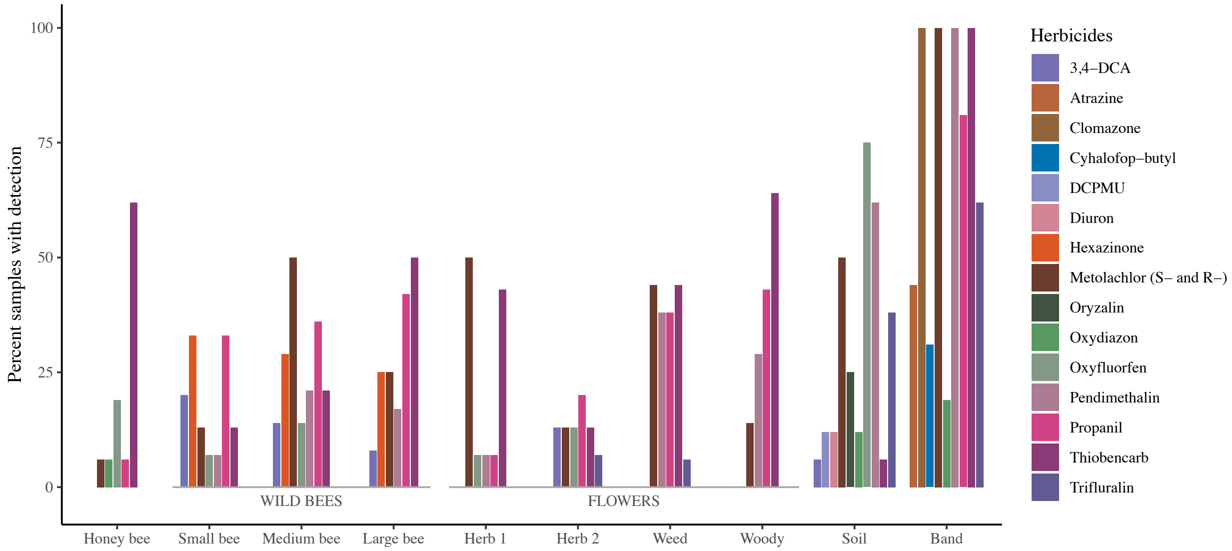
Figures

	Herb 1	Herb 2	Weed	Woody
P1	Phacelia (*Phacelia* spp. mostly *tanacetifolia*)	California poppy (*Eschscholzia californica*)	Mustard (Brassicaceae: *Brassica* spp., *Hirschfeldia* spp., *Raphnus* spp.)	Coffee berry (*Frangula californica)*
P2	Gum (*Grindelia* spp. mostly *camporum*)	California poppy (*Eschscholzia californica*)	Yellow star-thistle (*Centaurea solstitialis*)	Toyon (*Heteromeles arbutifolia)*

Table 1: Flower species sampled during each sampling period (P1 = period one and P2 = period two).



A) Insecticide detection



B) Herbicide detection

C) Fungicide detection

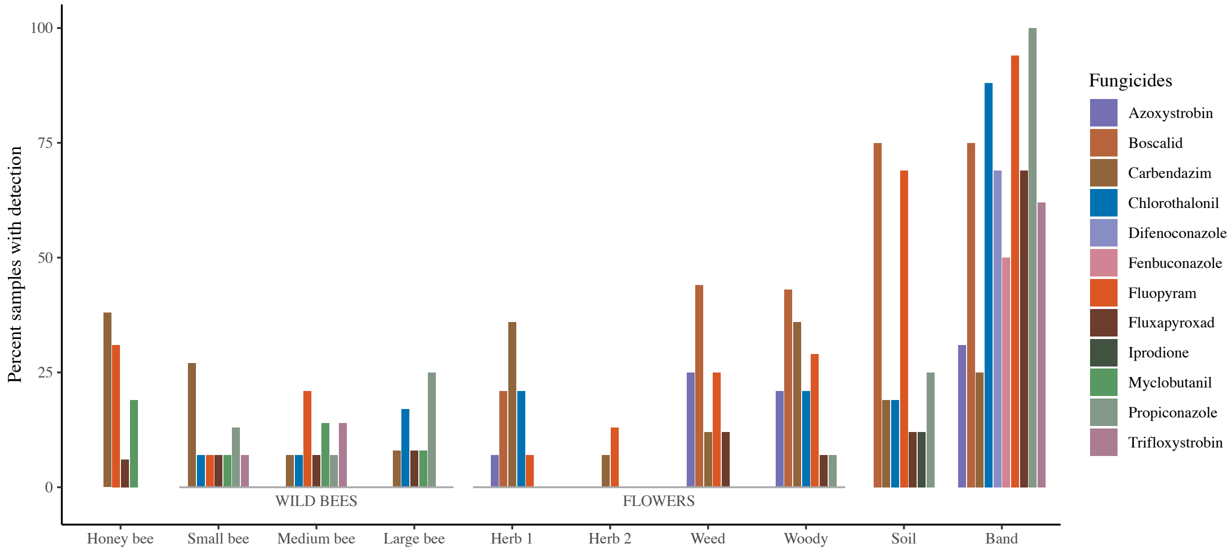
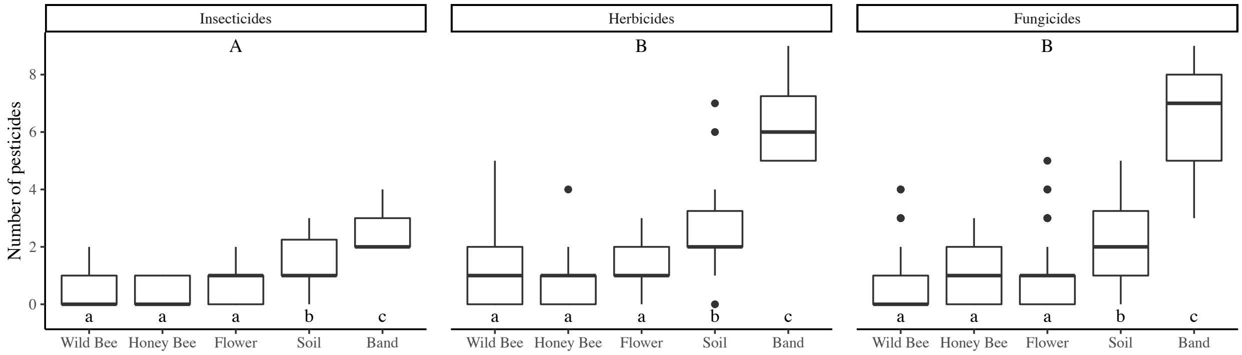
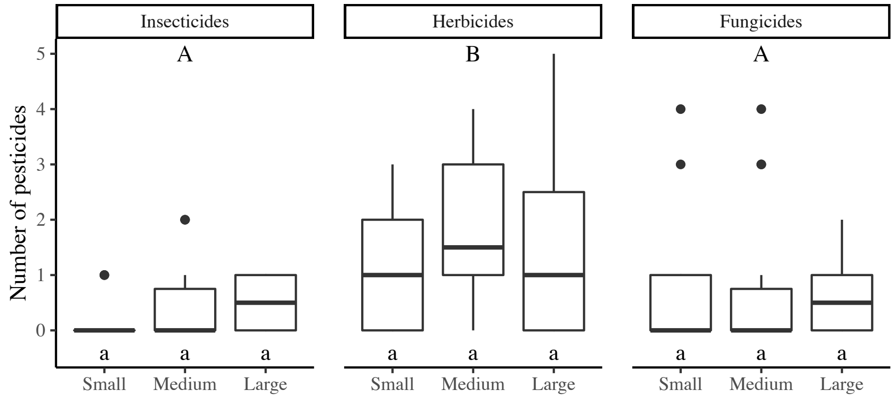


Figure 1: Percent detection of (A) insecticides, (B) herbicides, and (C) fungicides and their degradates for all sample types.

A) Sample type



B) Wild bee size



C) Flower type

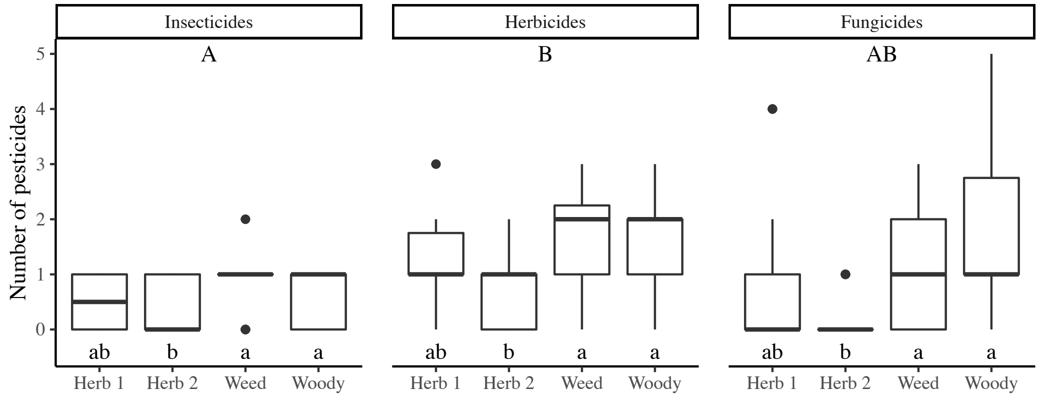
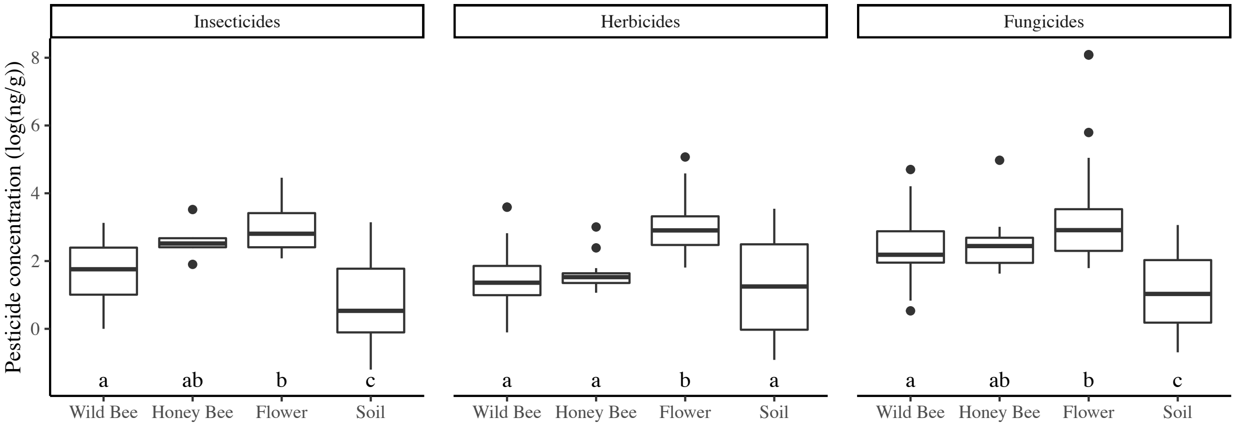
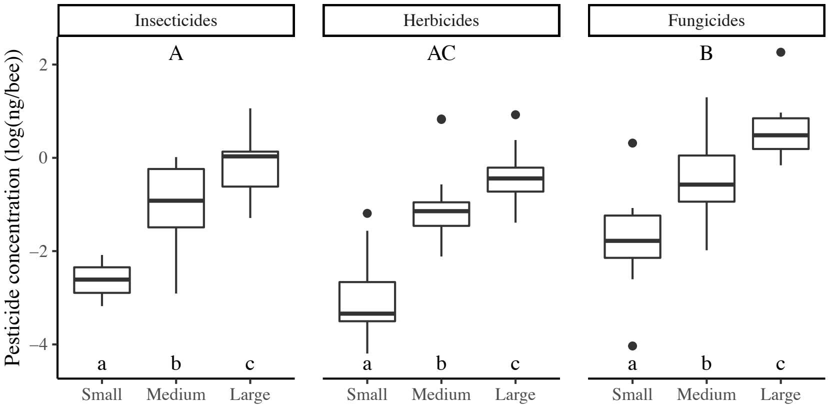


Figure 2: Number of pesticides detected in relation to pesticide category for (A) sample type, (B) wild bee size, and (C) flower type. Bold lines depict medians, boxes depict 25th and 75th percentiles, whiskers depict 95th percentiles and dots depict outliers. Differences in mean number of pesticides between pesticide categories are indicated by capital letters ($P < 0.01$). Differences in mean number of pesticides within sample types, wild bee sizes, and flower types are indicated by lower case letters ($P < 0.01$). Note that the mean number of herbicides and fungicides, were also marginally different for herb 1 and herb 2 ($P = 0.07$).

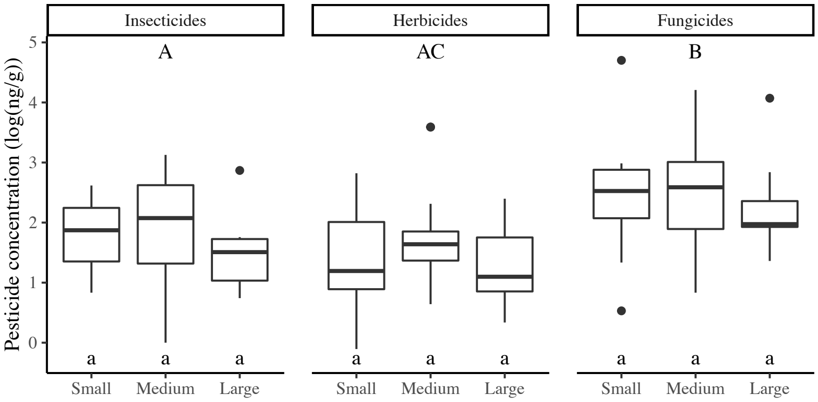
A) Sample type (ng/g)



B) Wild bee size (ng/bee)



C) Wild bee size (ng/g)



D) Flower type (ng/g)

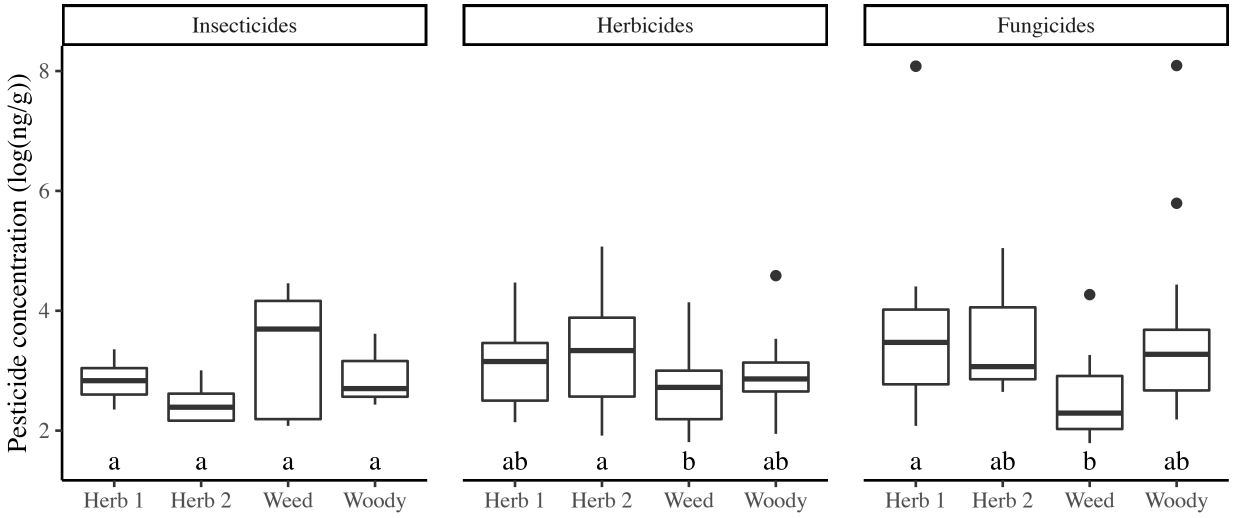
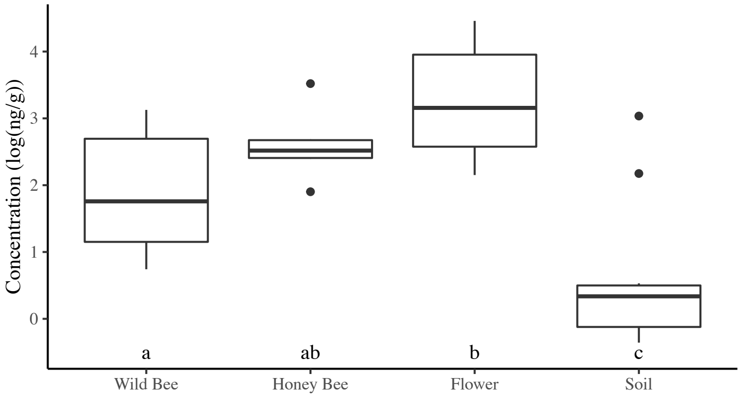


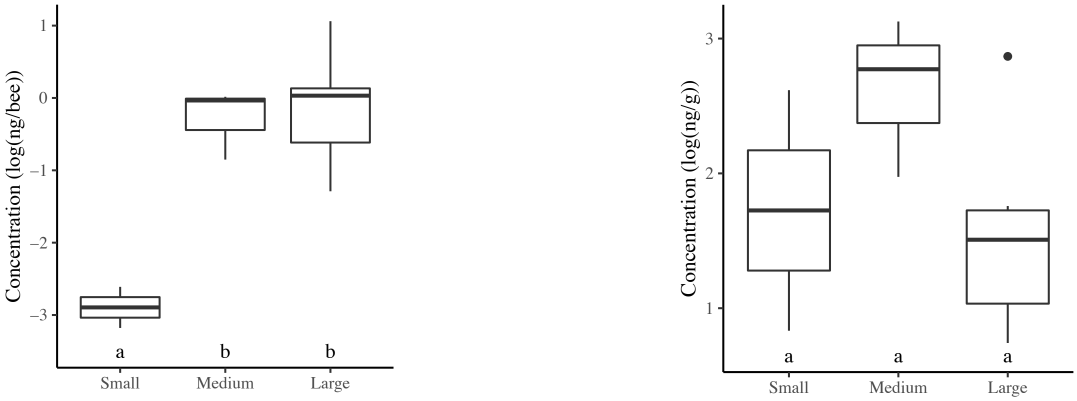
Figure 3: Pesticide concentrations detected in relation to pesticide category and (A) sample type (ng/g), (B) wild bee size (ng/bee), (C) wild bee size (ng/g) and (D) flower type (ng/g). Bold lines depict medians, boxes depict 25th and 75th percentiles, whiskers depict 95th percentiles and dots depict outliers. Differences in mean concentration between pesticide categories are indicated by capital letters, for cases when no interaction was present (*P* < 0.05). Differences in mean concentration within sample types, wild bee sizes, and flower types for each pesticide category are indicated by lower case letters (*P* < 0.05).

A) Sample type (ng/g)



B) Wild bee size (ng/bee)

C) Wild bee size (ng/g)



D) Flower type (ng/g)

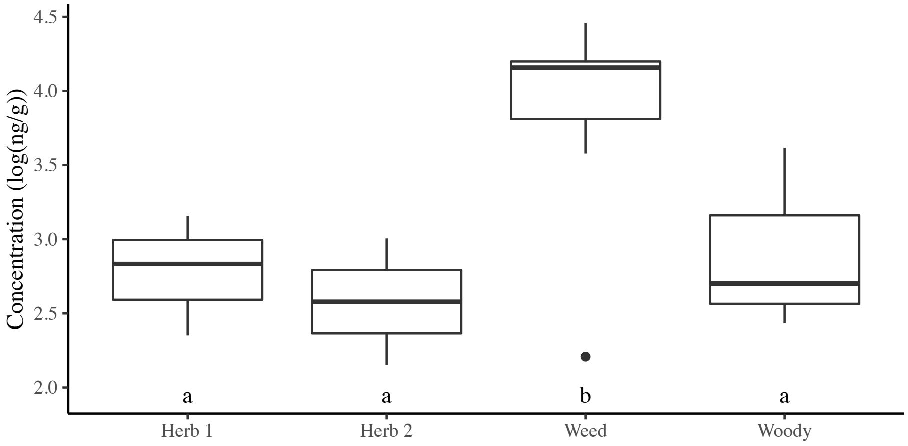


Figure 4: Concentration of bifenthrin detected in relation to (A) sample type (ng/g), (B) wild bee size (ng/bee), (C) wild bee size (ng/g) and (D) flower type (ng/g). Bold lines depict medians, boxes depict 25th and 75th percentiles, whiskers depict 95th percentiles and dots depict outliers. Differences in mean concentration are indicated by lower case letters ($P < 0.05$).

Chapter 3: You belong here: a mentoring program fosters sense of belonging for undergraduates at a large research university

Abstract

Sense of belonging is a critical human need that precedes learning and innovation. In higher education, sense of belonging increases retention and academic success. Some student populations (e.g. transfer students, first-generation college students, and underrepresented minorities) face greater barriers in developing a sense of belonging based on their own experiences and systemic bias in institutional structures. This study evaluates efforts to improve sense of belonging among undergraduate students at a large research university via an academic mentoring program. Berkeley Connect is a mentoring program at UC Berkeley serving thousands of students each year. In each participating academic department, a faculty director mentors a small cohort of graduate students, each of whom in turn mentors up to forty undergraduate students per semester. The program utilizes three program elements: one-on-one meetings, small-group discussions, and special events to promote community building and sense of belonging. Survey data indicate that sense of belonging among undergraduates increased over the course of a semester for Berkeley Connect students, with potentially greater impacts in populations that entered the program with the lowest levels of sense of belonging. All program elements were synergistically effective in increasing belonging and other outcomes, with one-on-one meetings being particularly impactful. Given the positive impacts of this mentoring model in achieving desired outcomes for large numbers of students, we suggest that this program be applied more broadly across a range of higher education institutions.

Introduction

Sense of belonging is not only a fundamental human need (Maslow, 1954), but a key precursor to academic success in higher education (Strayhorn, 2019; Thomas, 2012). Sense of belonging can be defined as the degree to which a person perceives that they are respected, supported, accepted, valued, and integrated into a particular community (Baumeister and Leary, 1995; Goodenow and Grady, 1993; Lambert et al., 2013; Lewis et al., 2016; Strayhorn, 2019). It is a cardinal motivation that affects cognition, emotion, behavior, health, and well-being (Baumeister and Leary, 1995). In higher education, sense of belonging is positively associated with student GPA and retention (Meehan and Howells, 2019; Strayhorn, 2019; Thomas, 2012). Students who feel a sense of belonging are more engaged and motivated in learning communities, more committed to school, perform better, and have higher quality of learning (Osterman, 2000). Sense of belonging is influenced by many factors including social connection, satisfaction with college friendships, and feeling successful in class (Bowman et al., 2019). Sense of belonging also impacts perceived stress and life satisfaction (Civitci, 2015). Increased sense of belonging for university students is associated with decreased anxiety, depression, and somatic issues (Pittman and Richmond, 2008). Thus, universities that actively foster a sense of belonging improve their student's ability to learn, innovate, and succeed.

In academia, a key component to sense of belonging is that students feel that they are valued by and matter to faculty, graduate student instructors, administrators, and peers (Flett et al., 2019; Stachl and Baranger, 2020; Strayhorn, 2019). Studies suggest that intentionally cultivating belonging on campus and in the classroom supports students' ability to learn and succeed academically (Strayhorn, 2019; Wilson et al., 2015). Sense of belonging can be facilitated by creating a welcoming community of faculty, staff, and peers that offer relationships built on trust and genuine interest in a student's academic journey (Meehan and Howells, 2019; O'Keefe, 2013; Thomas, 2012). A student with a strong sense of belonging does not feel like an anonymous number, token, or tolerated guest, and rather speaks about their university with a sense of proud ownership (Ballantyne, 2012).

Although a strong sense of belonging is important to the well-being and success of undergraduates, cultivating sense of belonging can be difficult, particularly at large research universities. Sense of belonging is not automatic or formulaic (Strayhorn, 2019); it requires attention, intention, and effort. Students from different backgrounds and holding different identities enter college with distinct needs and varying levels of vulnerability to feelings of isolation and imposter syndrome. At intensive research universities, historically marginalized student populations such as transfer students, first-generation college students, and underrepresented minorities (URMs) tend to face greater barriers to sense of belonging (O'Keefe, 2013; Strayhorn, 2019). Further, sense of belonging must be reinforced continuously as stable relationships with frequent interactions best strengthen sense of belonging in higher education (Baumeister and Leary, 1995). Therefore, efforts to welcome students and bolster belonging for entering students (e.g. orientation, freshman seminars, etc.) need to be complemented by other interventions throughout the college experience (Strayhorn, 2019; Thomas, 2012).

One way to improve a sense of belonging for students is through a mechanism that already exists at every research university: mentoring (Crisp et al., 2017; Jacobi, 1991; Lunsford et al., 2017). Some undergraduates seek out mentoring relationships with their professors, receive mentoring from graduate students and post-docs, gain mentorship by joining research projects, and/or avail themselves of peer-to-peer mentoring programs (Crisp et al., 2017). However, students with a lower sense of belonging—those students who would arguably derive the greatest benefit from personalized mentoring—may be more likely to believe that these opportunities are "not for them" (Strayhorn, 2019).

With the goal of increasing undergraduates' sense of belonging, the University of California Berkeley established an academic mentoring program called Berkeley Connect in 2014. This program is open to all undergraduates and all students are invited to participate. It is neither an honors nor a remedial program, has no prerequisites, and no recommendations or referrals are necessary. Undergraduates who wish to participate in Berkeley Connect enroll in a one-unit (pass/no pass) semester-long course in the participating department aligned with their academic interests; the program is open to all students regardless of major or year. Participation is the primary requirement to pass; there are no homework assignments, readings, or tests. Originally developed by faculty in the English department in 2010, Berkeley Connect now operates in 15 departments across the arts, humanities, social sciences, and STEM fields, and serves over 2,000 students each year. The Berkeley Connect mentoring model is detailed in **Box 1**.

In this study, we evaluate the success of the three elements of the Berkeley Connect program with respect to the seven objectives outlined in **Box 1**. In particular, we consider whether sense of belonging for participating students increases over the course of the Berkeley Connect semester, with particularly attention on transfer students, first-generation college students, and URM students using a pre/post survey model. Our findings suggest that this mentorship model is highly effective at increasing undergraduate students' sense of belonging, among other positive outcomes.

Materials and Methods

Study system: student demographics at University of California, Berkeley

This study examines the effectiveness of a mentorship program developed at the University of California, Berkeley to increase students' sense of belonging using survey data. UC Berkeley is a large research university with an undergraduate population of over 30,000 (University of California, Berkeley 2020). Like most research universities, it is a complex institution that can be difficult for students to navigate. It has the benefit and challenge of serving a diverse study body. UC Berkeley is a public land-grant university with a mission to educate California residents. While it does attract out-of-state and international students, about 75% of UC Berkeley undergraduates are from California; in 2019, 26% of the undergraduate population were transfer or re-entry students, most of whom transferred to Berkeley from California community colleges. In 2019, 22% of freshmen entrants and 43% of transfer entrants were Pell grant eligible (low income), and these percentages match those of first-generation college students within each population (22% and 44% respectively). In 2019, the demographic make-up of Berkeley's student body was 40% Asian, 24% White, 16% Latinx, 12% International, 3% African American, <1% Native American, and <1% Pacific Islander (with 4% declining to state their demographic) (University of California, Berkeley 2019). Informal surveys often find that while Berkeley alumni deeply appreciate the quality of the education they received, many report that the university could feel overwhelming or impersonal.

Data sources

We used data from two sources to assess the impact of Berkeley Connect on student sense of belonging: (1) a mandatory exit survey administered to undergraduate students who participated in Berkeley Connect every semester since 2014 (BC Exit Survey 2014-2019) and (2) a pre/post survey administered to undergraduate students who participated in the program in 2019 (BC Pre/Post Survey). Student respondents do not represent a randomized sample across the entire university as surveys were only collected from students who voluntarily enrolled in the Berkeley Connect program.

BC Exit Survey

The purpose of the exit survey conducted by the Berkeley Connect program every semester was to assess the impact of the overall program as well as each program element with regard to the seven desired outcomes (see above). The overall survey has typically been composed of 50 to 60 questions, with a mixture of Likert style items and open text responses. Twenty-six questions relevant to our research objectives were utilized for this analysis; question wording has been relatively stable since Fall 2014, with slight variations across semesters (main questions listed in **Appendix X** with language variations in **Appendix Z**). This subset of 26 questions applied a three-point Likert items, with response choices: Not true, Somewhat true, and Very True.

Data from Fall 2014 through Fall 2019 (ten semesters) were analyzed. Eleven departments participated during Fall 2014; in Fall 2019, the following fourteen departments participated: African American Studies, Architecture, Comparative Literature, Computational Biology, English, Environmental Science Policy and Management, Ethnic Studies, History, Math, Music, Philosophy, Physics, Sociology, and Social Welfare. Because completion of the Berkeley Connect exit survey was a mandatory requirement for passing the course, the student response rate was nearly 100% with approximately 12,100 students surveys completed in our dataset.

BC Pre/Post Survey

In 2019, in support of this research project, Berkeley Connect expanded its program assessment to include pre/post measurement of students' self-reported status before and after participating in Berkeley Connect. This expansion entailed administering a new start-of-semester survey (pre-survey) that students were asked to complete before attending their second Berkeley Connect small-group meeting. The post-survey, asking the same style of questions as the pre-survey, was incorporated into the existing exit survey administered at the end of the semester.

The overall pre/post survey included 23 questions of which six were utilized for this analysis (questions listed in **Appendix Y**). One set of questions on the pre/post survey asked about the seven desired program outcomes without tying them specifically to Berkeley Connect (see **Appendix Y**). These questions applied a three-point Likert items, with response choices: Not true, Somewhat true, and Very True. The pre/post survey also included questions designed to measure the student's mental/emotional state at the time of response relating to sense of belonging, feeling supported, and feeling stressed. These mental/emotional state questions applied a five-point Likert items, with response choices: Very untrue, Somewhat untrue, Neither true nor untrue, Somewhat true, Very true. In an effort to understand the impact of Berkeley Connect specifically on historically marginalized student populations, the pre/post survey also included three demographic questions about transfer status, whether students were the first in their immediate family to attend a four-year college, and whether students identified as African-American, Chicano/Latinx, and/or Native American/Alaska Native. The latter questions were intended to identify first-generation college students and URM students, as defined by UC Berkeley. These definitions have some limitations with respect to identifying all students that may have historically been marginalized. For example, the URM categories did not explicitly include people who identify as Black but are not of African descent and did not include questions about other aspects of identity (e.g. gender, sexuality, religion, and socioeconomic status) that may contribute to students feeling marginalized.

In Spring 2019, departments could choose whether or not to administer the pre/post survey. Five departments voluntarily chose to administer the pre/post survey (English, Environmental Science Policy and Management, Math, Social Welfare, and Sociology). In Fall 2019, all fourteen departments participating in Berkeley Connect were required to administer the pre/post survey (African American Studies, Architecture, Comparative Literature, Computational Biology, English, Environmental Science Policy and Management, Ethnic Studies, History, Math, Music, Philosophy, Physics, Sociology, and Social Welfare). Students were encouraged to take the pre-survey but were not penalized if they did not complete it, whereas the post-survey was incorporated into the mandatory exit survey. Survey responses were pooled across semesters and departments for analysis. There were approximately 1600 and 1700 student responses for each question in the pre- and post-survey datasets respectively, which represents an estimated 94% response rate for the pre-survey. Individual student responses students were not paired for analysis.

Survey platform

The exit survey and the pre/post survey were administered via bCourses, UC Berkeley's version of the Canvas learning management system (Instructure, 2020). Students were instructed to complete the surveys using the Quiz function on the Berkeley Connect bCourses site. The portal allowed faculty directors to track student completion of the survey, while also allowing responses to remain anonymous.

Data analysis

The Berkeley Connect program administrator exported the survey results from the bCourses website as .csv files, removed any accidental identifiers, and shared the otherwise raw data with the authors. Raw data were then imported to the R software environment for statistical analysis. In all cases, individual responses marked "Not Applicable" and/or questions that were left unanswered were removed from the analysis. Depending on which subset of data was analyzed, the NAs removed represented 0.06-8% of the data.

BC Exit Survey

To test whether participation in Berkeley Connect helped to achieve the seven desired outcomes, we used a composite score analysis to test for differences in answering tendencies for questions related to the seven desired outcomes (see **Figure 2A** and **2B**) (Boone and Boone, 2012). First, we counted the number of times each of the three answer categories was selected across all students for each of the seven Likert items associated with each desired outcome (**Appendix X**, questions 1 - 7). Then, we used an ANOVA to test for differences in the mean number of students responding to each answer category across all seven Likert items. One item/question (**Appendix X**, question 3) was removed from this analysis because it was not asked in all semesters. A post-hoc Tukey test was utilized to identify significant contrast between answer categories.

To determine whether program elements (one-on-one meetings, small group sections, or special events) had an effect on student responses with regard to each of the seven desired outcomes (**Appendix X**, questions 8 - 26), the kruskal.test() scrip was used to perform Kruskal-Wallis rank sum tests (Hollander and Wolfe, 1973). In cases where program elements had a significant effect on an individual desired outcome, we further tested which program elements were most effective. For these post-hoc tests, we used the dunnTest() scrip from the FSA package in R to perform Dunn Kruskal-Wallis multiple comparison with p-values adjusted using Bonferroni corrections (Dunn, 1964). In two cases, desired outcomes were associated with only two program elements (i.e. the survey questions associated with educational opportunities and meeting peers were not analyzed with regards to special events). For these cases where desired outcomes associated with only two program elements, the wilcox.test() scrip from the stats package in R to perform a Mann-Whitney U test (Bauer, 1972; Hollander and Wolfe, 1973). We did not utilize post-hoc tests for these analyses as there were only two program elements. For all of these tests, the three response choices from each Likert item were converted to a numeric score for analysis (with one corresponding with "not true" up through three corresponding with "very true"). However, even when response choices were converted to numeric scores, these data are still ordinal, which is why we opted for the non-parametric tests detailed above.

To determine whether the three program elements differed in effectiveness overall, we used an ANOVA to examine variation in scores among the three program elements. For this purpose, numeric scores were for each Likert item to create a composite score (Boone and Boone, 2012). This analysis was limited to the five desired outcomes associated with all three program elements. We also limited the analysis to surveys of students that had answered all five questions. Thus an individual's summed score ranged from five (answered “not true” for each item) to fifteen (answered “very true” for each item). We used a Type III correction with the ANOVA as the summed scores were expected to be unbalanced due to fewer students attending events than sections and one-on-one meetings. A post-hoc Tukey test was utilized to determine which program elements differed significantly.

BC Pre/Post Survey

To understand differences in responses in pre and post surveys relating students’ mental/emotional state (i.e. sense of belonging, feeling supported, and feeling stressed; **Appendix Y**, questions 1 - 3), Mann-Whitney U tests were used. The five response choices from these Likert items were converted to a numeric score for analysis (with one corresponding to “very untrue” up through five corresponding to “very true”). These data were then subsetted to test for pre/post response differences with respect to specific demographic attributes: transfer students, non-transfer students, first-generation college students, non-first-generation college students, URMs, and non-URMs.

Results

BC Exit Survey

Approximately 12,100 students completed the exit survey during our study period. For all seven desired program outcomes, a numerical majority of students responded “somewhat true” or “very true”. The proportion of positive responses indicates that Berkeley Connect meets the stated programmatic goals of: increasing student sense of belonging, increasing comfort approaching professors, deepening student knowledge of their academic discipline, helping students meet with peers with shared academic interests, increasing awareness of educational opportunities, increasing confidence in success, and giving students new ideas about what to do after graduation (**Figure 2A**). A composite analysis showed that the cumulative responses for each answer category was significantly different (ANOVA, $p < 0.0001$). A post-hoc Tukey showed that the mean count of “very true” and “somewhat true” responses were significantly higher than “not true,” and the mean count of “somewhat true” responses was also significantly higher than “very true” (**Figure 2B**, Tukey, somewhat true > very true, very true & somewhat true > not true; $p = 0.006, 0.000, 0.000$ respectively).

Answer scores were significantly different between program elements for all desired outcomes (Kruskal-Wallis Chi-square (df = 2) results for: Sense of belonging = 740.67; Confidence in academic success = 790.73; Comfort approaching professors = 1166.6; Disciplinary knowledge = 197.15; Awareness of career opportunities = 315.67; Mann Whitney U results for: Awareness of educational opportunities 80,725,360; Peer networks with shared academic interests = 65,906,230; $P < 0.001$ for all). The three program elements (one-on-one meetings, small-group meetings, and special events) were differentially effective at meeting desired outcomes (**Figure 5**). One-on-one meetings between graduate student mentors and undergraduate participants were significantly more effective than other program elements at achieving four of the seven outcomes: sense of belonging, increased confidence, comfort approaching professors, and awareness of educational opportunities. Small-group discussion sessions facilitated by the mentors were significantly more effective than one-one-one meetings at helping students to meet peers with shared interests. Small-group discussion sessions were significantly less effective than one-on-one meetings, but significantly more effective than events, at achieving: sense of belonging, increased confidence, and comfort approaching professors. Special events, which often featured faculty and alumni guest speakers, were significantly more effective than other program elements at helping students understand career opportunities. Lastly, one-on-one meetings and special events were significantly more effective than small-group meetings at increasing student's disciplinary knowledge. The exact number of student responses varied among Likert items; the approximate number of student responses across the desired outcomes was: 12,000 for one-one-one meetings, 12,000 for small group sessions, and 9,900 for special events.

When combining responses for program objective items, the composite mean score (which could vary from five to fifteen) was 11.88 (± 0.02) for one-on-one meetings, 11.19 (± 0.02) for small group sessions, and 11.29 (± 0.03) special events. Despite these similarities, composite analysis showed that program elements had a significant effect on composite score (ANOVA: $p < 0.001$). A post hoc test showed that one-on-one meetings had a significantly higher effect on scores than both other elements (Tukey: $p < 0.001$ for each), and special events also had a significantly higher effect than small group meetings (Tukey: $p = 0.014$).

BC Pre/Post Survey

Approximately 1,600 and 1,700 students completed the pre and post surveys, respectively. The pre/post survey revealed that sense of belonging (**Figure 3A**) and feeling supported (**Figure 3B**) increased significantly over the course of the semester for Berkeley Connect participants. Stress also increased significantly over the course of the semester, although the change in mean score was small (**Figure 3C**). Mann-Whitney U tests showed that the median score (for belonging, support, and stress categories) was always significantly higher in the post survey than the pre survey ($W_{\text{belong}} = 1,076,843$, $P < 0.001$; $W_{\text{support}} = 1,129,238$, $P < 0.001$; $W_{\text{stress}} = 1,298,219$, $P = 0.007$).

Of the 1,600 students that completed the pre survey, 50% were transfer students, 34% were first generation college students, and 26% were URM (**Figure 1**). Twenty-eight percent of transfer students and 46% of first-generation students identified as URM (**Figure 1**). 21% of transfer students identified as first-generation college students (**Figure 1**). Eight percent of students identified as transfer students, first generation college students and URMs (**Figure 1**). Approximately 3-3.5% of students reported that they preferred not to state whether they were first-generation college students and/or URMs; these responses were removed from the analysis.

Mann-Whitney U tests showed that the median score for sense of belonging was significantly higher in the post survey than the pre survey for all demographics analyzed (**Figure 4 A, B, C;** $W_{\text{transfer}} = 261,510$, $W_{\text{not-transfer}} = 272,395$, $W_{\text{1st-generation}} = 110,420$, $W_{\text{not-1st-generation}} = 444,632$, $W_{\text{URM}} = 68,406$, $W_{\text{not-URM}} = 539,177$ with $P < 0.001$ for all). Furthermore, the numerical mean score for sense of belonging in the pre-survey was always higher for the background population (i.e. non-transfer, non-first generation, and/or non-URM), however, the positive change in numerical mean from the pre- to post-survey was higher for transfer students, first-generation college students, and URMs (**Table 1** and **Figure 4 D**). Although we could not test whether the magnitude of positive change differed significantly among subsets of students (due to the ordinal nature of the data), it is an important trend.

Discussion

Fostering a sense of belonging for undergraduates in universities with high student-to-faculty ratios can be challenging. However, our results suggested that multifaceted mentoring programs, particularly those that combine one-on-one and small-group interactions between undergraduate and graduate students, can have significant impacts on sense of belonging while reaching large numbers of students efficiently. Survey results indicate that the Berkeley Connect mentoring program is quite successful at meeting programmatic goals. Moreover, the increase in sense of belonging over the Berkeley Connect semester was robust to changes in other measured factors, like stress level. Further, students who initially reported a low sense of belonging – including transfer, first generation, and URM students – appear to accrue a particularly strong benefit from mentoring. Overall, we found that relatively modest amounts of dedicated time can produce a substantial increase in sense of belonging. Below we discuss our key findings.

Mentoring and the importance of multifaceted formats

Our results demonstrated that a mentoring model that connects undergraduate students and graduate student mentors in multiple formats is effective for increasing sense of belonging. Although one-on-one meetings, small-group discussions, and special events each were effective in advancing different desired outcomes, one-on-one meetings were the most effective program element overall. Two fifteen-minute meetings, one near the beginning of the semester, and one near the end of the semester, helped mentors to better understand the unique needs of each student. Mentors could then tailor their approach to facilitating small-group discussions – and building community among students – based on the known interests and needs of participating students. At a large university, it is not uncommon for a student to pass through an entire semester without having a meaningful one-on-one interaction with an instructor or advisor. We expect that the brief but highly personalized meetings with mentors through Berkeley Connect help students to feel that they “matter”. Mattering is when people feel valued as individuals (Flett, 2018) and is one of the core elements promoting sense of belonging in higher education (Strayhorn, 2019).

Although one-on-one meetings were highly impactful, we anticipate that this element alone would not have had the same impact as it does in conjunction with other program elements. In the Berkeley Connect mentoring model, mentors and students build trust-based relationships through structured, ongoing contact in a variety of settings throughout the semester. Berkeley Connect integrates academic and social interaction by fostering an informal, convivial atmosphere to encourage strong social connections outside the classroom. Mentors facilitate small-group discussions about specific academic topics and the context of academia itself. Field trips and special events provide additional enrichment. Thus, small and large group activities may contribute to the effectiveness of one-on-one meetings. Other studies have shown that social and academic peer interactions have a positive association with belonging and mattering, respectively (Cole et al., 2020). That is, when students discuss their beliefs and opinions with each other (social interactions), they are more likely to build friendships and feel like they belong. Similarly, when students discuss their projects, progress, and pitfalls, with one another (academic interactions), this results in a sense of mattering (Cole et al., 2020). Thus, the integration of academic and social interactions may contribute to the increased sense of belonging in students who participate in Berkeley Connect.

Belonging, support, and stress

Sense of belonging and feeling supported increased significantly over the course of the semester for Berkeley Connect students, even while levels of stress also increased. This is especially noteworthy given that some studies suggest that student sense of belonging may actually decline over their time in college (Hausmann et al., 2007). Given the prevalence of stress and anxiety among college students (ACHA, 2016; Kitzrow, 2011), increased sense of belonging may help students to cope with - rather than alleviate - their stress. Some studies have suggested that adequate coping skills should reduce levels of student stress (Matheny et al., 2008) and that stress is negative correlated with student satisfaction (Rezaei and Mousanezhad Jeddi, 2020). Therefore, it is somewhat perplexing to see both stress and sense of belonging increase simultaneously in our results. The fact that students report higher stress levels in the post-survey is not necessarily unexpected. Among other reasons, students responded to the survey just before or during final exam week. Regardless, it is noteworthy that sense of belonging can remain strong even during periods of stress.

Belonging and student demographics

Sense of belonging significantly increased over the course of the semester for all Berkeley Connect demographics studied, that is, for both those who identified as underrepresented minorities, first generation college students, and transfers students, and those who did not. While this finding is promising, any interpretations should be taken cautiously, as the numerical mean score for sense of belonging still appeared to be lower overall for students from historically marginalized groups (**Figure 4**). Other studies suggest that a lower sense of belonging in these identity groups is common (Packard, 2016; Soria et al., 2013; Spiegler and Bednarek, 2013; Strayhorn, 2019; Walton and Cohen, 2007), and the heightened risk for loneliness, alienation, and imposter syndrome is well recognized (Bravata et al., 2020; Chang et al., 2019; McGee, 2016). Furthermore, many students hold identities at the intersection of multiple marginalized populations, thereby making it potentially even more challenging to develop a sense of belonging. In fact, 34% of Berkeley Connect students identified with two or more of the historically marginalized populations in this study (**Figure 1**).

An ideal mentoring model would provide all students with a similar sense of belonging after the mentorship intervention. That said, it is notable that Berkeley Connect did significantly increase sense of belonging for historically marginalized student populations (despite overall lower reported sense of belonging). In fact, the numerical increase in sense of belonging for under-represented minorities, first generation, and transfer students was higher than for the background population (Table 1). That is, though these students entered the semester with a lower sense of belonging than their peers, they appear to have left with a greater change in their sense of belonging. The positive impact of this mentoring program on historically marginalized student populations (**Table 2**) is consistent with other recent research (Gillen-O'Neel, 2019; Murphy and Zirkel, 2015). Moreover, sense of belonging has been shown to be associated with numerous positive outcomes for historically marginalized student populations, including metrics of academic success (like GPA and retention) and increased health and well-being (Murphy et al., 2020; Strayhorn, 2019; Walton and Cohen, 2011).

We speculate that the increase in sense of belonging for historically marginalized populations and other students may be in part due to the personalized nature of this mentorship model via one-on-one meetings. Additionally, Berkeley Connect tends to attract a greater proportion of students from historically marginalized populations and thus can create comradery around shared experience. As universities work to address institutional legacies of exclusion and oppression, it is more important than ever to increase sense of belonging for all students. Programs like Berkeley Connect may be able to contribute as early and authentic interventions to promote diversity, equity, and inclusion.

Future directions

One limitation of this study is that students were only surveyed over the course of the semester that they participated in Berkeley Connect. We do not know whether student sense of belonging persists, increases, or declines after one semester of participation in Berkeley Connect. Theoretical models suggest that sense of belonging must be reinforced as conditions change over the course of a student's undergraduate career (Strayhorn, 2019). Future studies should employ surveys that are administered multiple times with anonymous identifiers to track student experience before, during, and after mentorship interventions. In addition, future studies could more directly address additional factors that contribute to sense of belonging. For example, students participating in Berkeley Connect may also have been engaged in other activities during the semester that could increase sense of belonging. Therefore, students within and outside the program could be surveyed simultaneously to provide a clear control group. Future research should also seek to understand which program elements have shorter versus longer term impacts. A more longitudinal study of the same students could also reveal the changing needs of students over time and how mentoring interventions can be tailored to changing conditions. Many factors influence sense of belonging (Bowman et al., 2019), both related to mentorship interventions (e.g., program elements) and other variables (e.g., socioeconomic background, whether students are working, identity intersectionality, etc.) (Museus et al., 2016).

Conclusion

Sense of belonging supports and enhances student success and well-being. Our results indicate that the Berkeley Connect mentorship program increased a sense of belonging for all participating undergraduate student demographics studied, among other positive outcomes. All program elements (one-on-one meetings, small-group discussion sessions, and special events) were synergistically effective, with one-on-one meetings being notably impactful relative to the amount of time they required. Traditional mentoring programs rely on very low mentor-to-mentee ratios and thus can be labor/resource-intensive, with benefits to only relatively small groups of students. The multifaceted mentoring model employed by Berkeley Connect is both effective and efficient in achieving positive results for a large number of students per semester, thus providing a way to deploy mentoring at research universities with large undergraduate populations. This model is distinctive in that it harnesses the experience of graduate students to extend the reach of the program, while providing graduate students with fellowship support and faculty mentoring. We suggest that this model could be easily adapted at other institutions. We encourage other universities to collaborate on piloting this approach and assessing its impact, in an effort to improve the sense of belonging and thus the success of students in higher education, particularly those students who face the greatest barriers to belonging.

Figures

Box 1: The Berkeley Connect mentoring model.

Berkeley Connect uses a tiered mentoring model: in each academic department, a faculty director is appointed to mentor a small cohort of graduate students, each of whom in turn mentors up to 40 undergraduate students per semester, both one-on-one and in small groups. Berkeley Connect mentoring is accomplished through three main program elements:

(1) one-on-one meetings during which students can talk with their mentors about any topics related to their academic life: goals, ideas, questions, concerns
(2) small-group discussion sessions, facilitated by the mentors, during which students engage in interactive conversations about topics related to a specific discipline or to the research university in general, in a congenial, ungraded setting
(3) special events/field trips that familiarize students with resources available to them on campus and introduce them to a network of faculty and alumni

Mentors hold short one-on-one meetings with each student at least two times during the semester (and hold additional optional mentoring hours) and meet with their students in small groups (up to 20 students each) an average of eight times a semester. The combination of one-on-one and small-group mentoring allows Berkeley Connect to provide individualized attention to students on a large scale; for example, 30 mentors can reach 1200 students a semester. The graduate student mentors are selected through a competitive application process; selected graduate students are awarded a research fellowship with a service obligation to mentor undergraduates interested in their field of study. Graduate students report spending an average of 6 hours per week on mentorship and the rest of their time on their own research and professional development.

Berkeley Connect is explicitly designed to increase students' sense of belonging. Its motto is "You belong here." The program has identified seven desired outcomes for students. These are as follows:

(1) To increase sense of belonging at UC Berkeley
(2) To feel better prepared to approach professors in office hours
(3) To deepen knowledge of an academic discipline
(4) To meet peers with shared academic interests
(5) To increase awareness of educational opportunities and resources on campus
(6) To increase their confidence they can succeed at UC Berkeley
(7) To learn about post-college career opportunities

Together, these goals help students weave themselves into the fabric of the university experience by equipping them with a navigational awareness of resources available to them and empowering them with the confidence to engage.

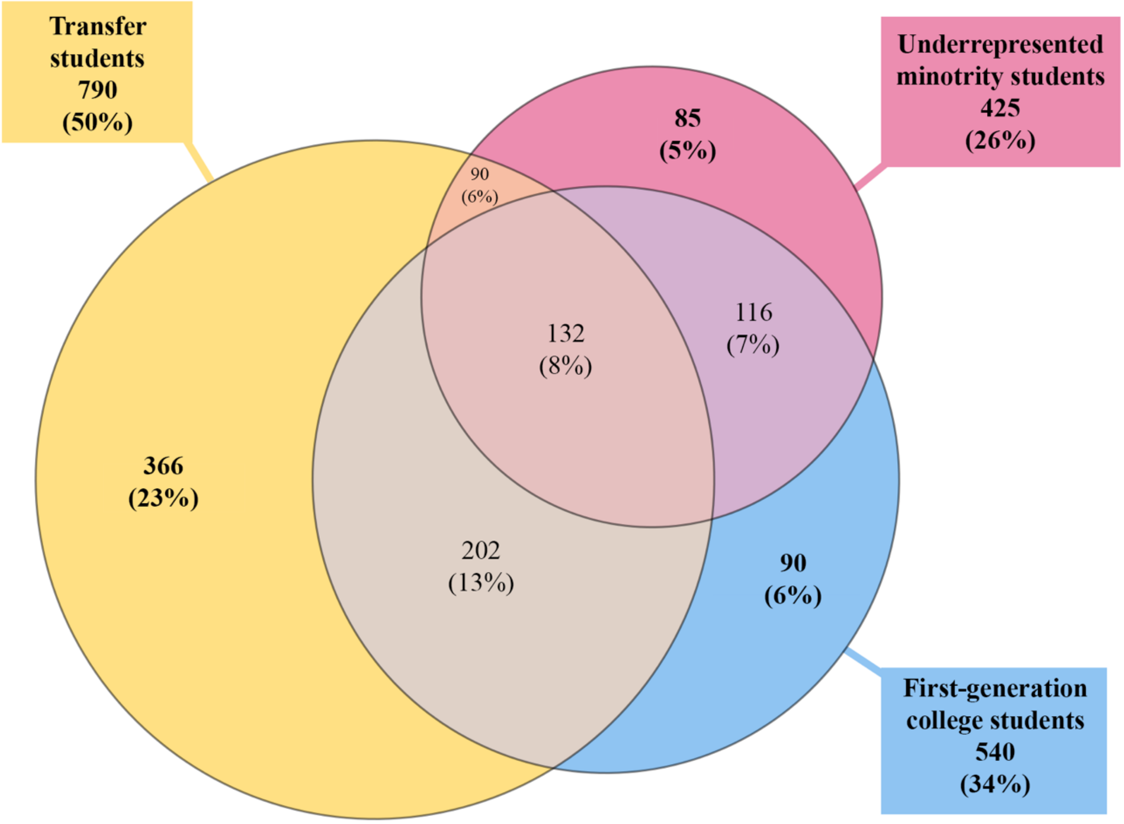
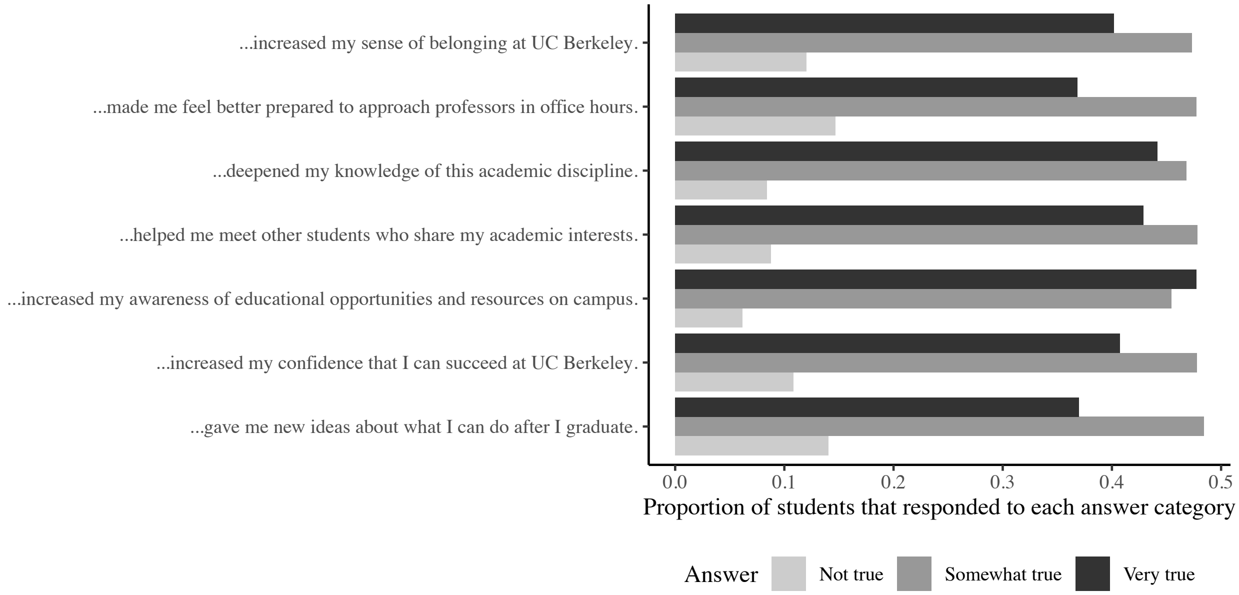


Figure 1: Berkeley Connect 2019 exit survey demographic and intersectionalities. Berkeley Connect served transfer students, first-generation college students, and underrepresented minority students and the intersections among these identities. Out of the 1600 students that completed the pre-survey, only 32 percent did not identify with any of these three groups. Notably, there is strong overlap among these identity groups and all are represented in greater proportion in Berkeley Connect than in the background population at UC Berkeley.

A) Participating in Berkeley Connect…



B) Mean number of responses for each answer type

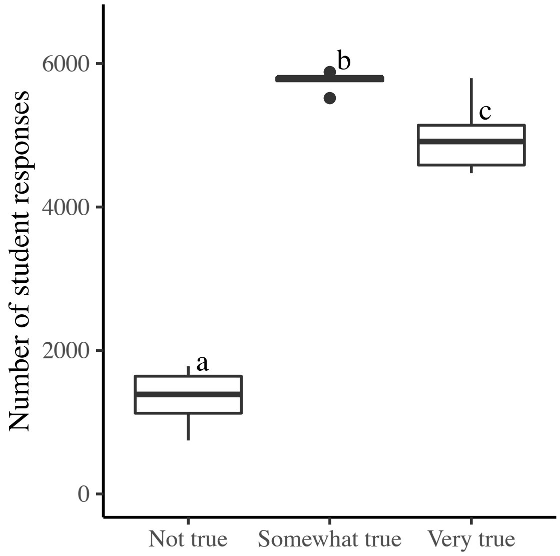
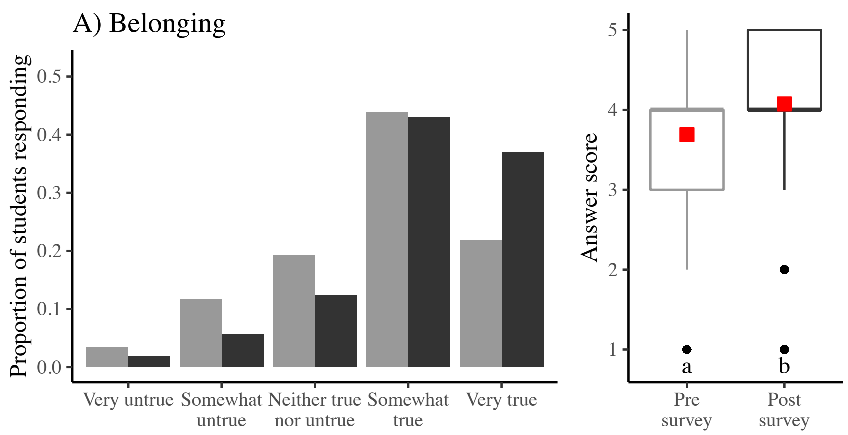
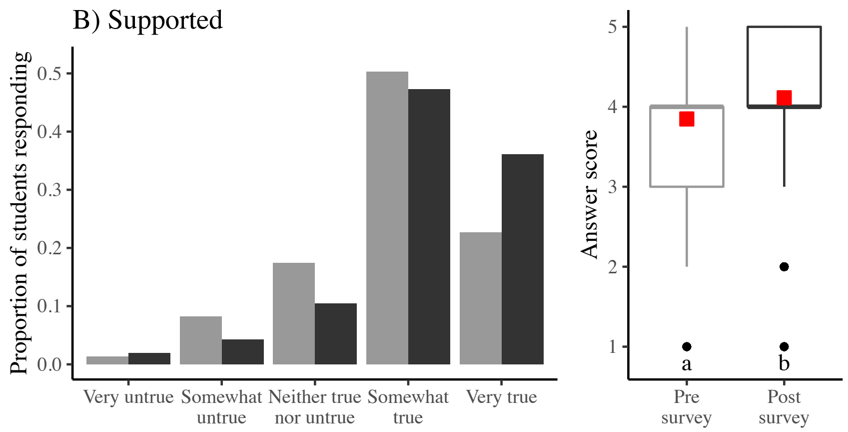


Figure 2: Berkeley Connect desired program outcomes. Students were asked about their Berkeley Connect experience with Likert items corresponding to the seven desired program objectives in the exit survey. In **Figure 2A,** the proportion of students that responded to each answer category for each Likert item are shown. A numerical majority of students reported that participation in Berkeley Connect helped achieve these objectives. In **Figure 2B**, bold lines depict medians, boxes depict 25th and 75th percentiles, whiskers depict 95th percentiles and dots depict outliers. Differences in mean number of student responses between answer categories are indicated by lower case letters ($P < 0.01$).





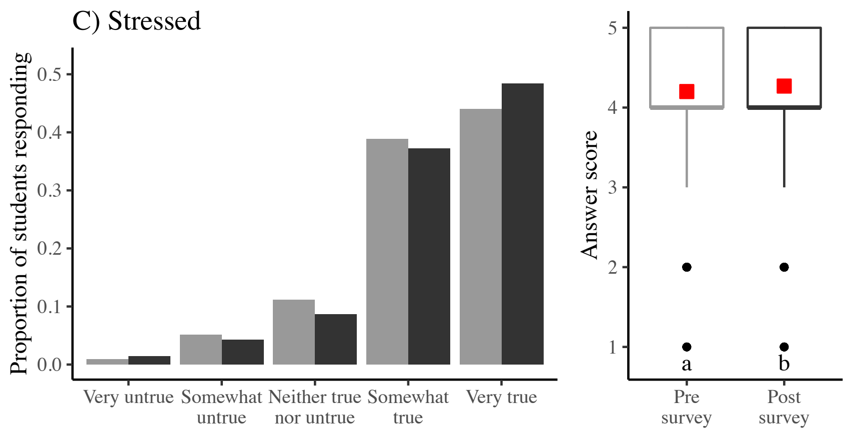
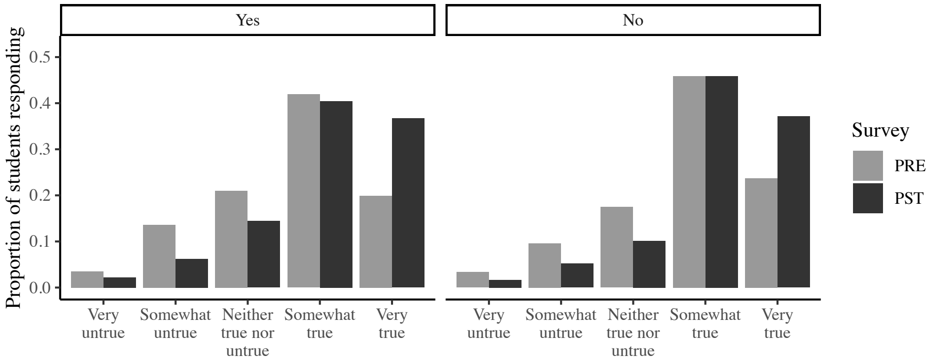
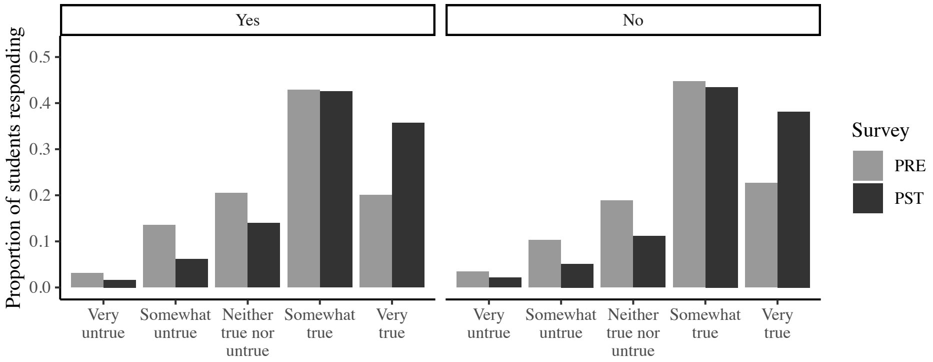


Figure 3: Proportion of students responding to each answer category and corresponding mean answer scores for (A) belonging, (B) support, and (C) stress. For all plots, pre survey results are depicted in light grey and post survey in dark grey. For boxplots, bold lines depict median scores, boxes depict 25th and 75th percentiles, whiskers depict 95th percentiles, dots depict outliers, and red squares depict mean scores. Significant differences in median scores between pre and post surveys are indicated by lower case letters in boxplots (*P* < 0.01). Answer score numbers correspond to different answer categories (e.g. 1= “Very untrue” and 5= “Very true”).

A) Belonging for transfer and non−transfer students



B) Belonging for first generation and non−first generation students



C) Belonging for URM and non−URM students

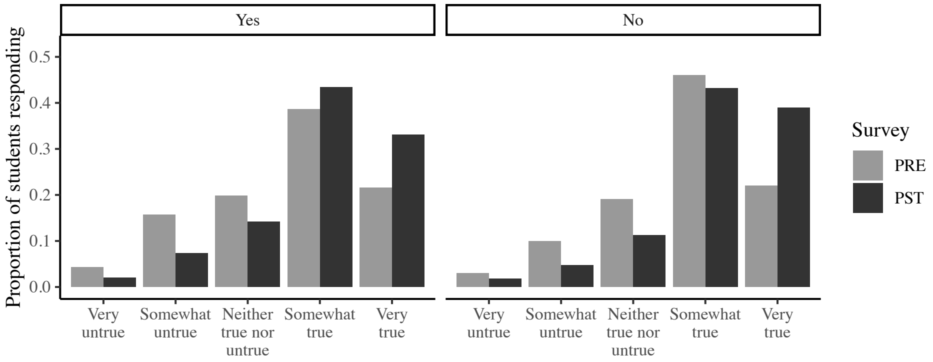


Figure 4: Proportion of students responding to each answer category regarding (A) Transfer and non-transfer student belonging, (B) First-generation and non-first generation student belonging, and (C) URM and non-URM student belonging. Proportions were calculated within each demographic. “Yes” and “no” describe how the student answered the demographic question (i.e. “Are you a transfer student?”)**.** “PRE” indicates pre survey and “PST” indicates post survey.

Demographic		*Number of students in demographic*	*Mean Score Pre*	*Mean Score Post*	*Change in Mean Score*
Transfer student	Yes	1652	3.611	4.032	0.421
	No	1648	3.769	4.116	0.347
First-generation college student	Yes	1070	3.633	4.045	0.412
	No	2131	3.727	4.104	0.377
Underrepresented minority student	Yes	832	3.574	3.982	0.408
	No	2365	3.739	4.128	0.389

Table 1: Change in sense of belonging. Summary of statistical results for change in sense of belonging over the course of a semester for Berkeley Connect students from various student demographics. "Yes" and "no" describe how the student answered the demographic question (i.e. "Are you a transfer student?")

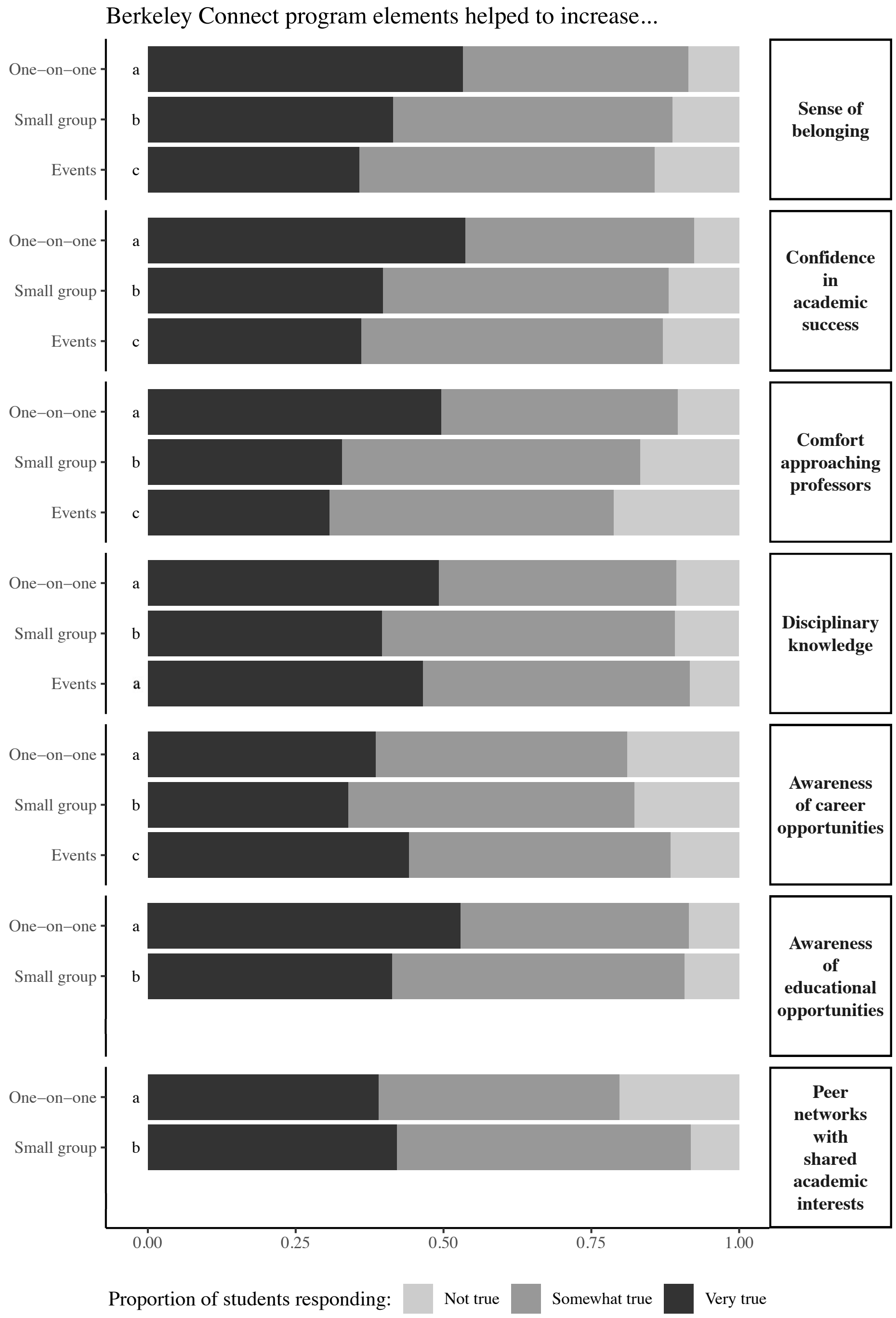


Figure 5: Berkeley Connect program element effectiveness. Proportion of students responding not true, somewhat true, and very true for each program element for each desired outcome. Educational opportunities and meeting peers were not analyzed in regards to special events. Lower case letters indicate significant differences between program elements within each desired outcome ($P < 0.001$).

Conclusions

This section serves as a discussion of results and future directions for the two disparate topics explored in this dissertation: bees and belonging. First, results from two field studies on wild bee conservation in agriculture and future directions will be discussed. Subsequently, results from a case study on undergraduate sense of belonging as the result of a mentorship program and future directions will be discussed.

Bees

Bees are an incredibly diverse assemblage of taxa, crucial in their role as pollinators across the planet, yet are declining worldwide (Potts et al., 2010). While this is likely due to a number of interacting factors, pesticides are largely implicated in their decline (Goulson et al., 2015). Insecticides are specifically concerning for bee health, along with pesticides that synergistically increase the toxicity of insecticides for bees (Iverson et al., 2019; Pilling et al., 1995; Sgolastra et al., 2020, 2018). The first two chapters of this dissertation explored pesticide exposure for different kinds of wild bees and honey bees in an agricultural context. Four key findings arise from this work. First, the results indicate that wild bees and honey bees visiting crop and non-crop plants are exposed to a number of pesticides from the greater landscape. Second, pesticides detected in bee tissues are sometimes present in mixtures that are concerning for bee health. Third, pesticides can impact bee biodiversity and crop yield even when they are undetected in the environment. And lastly, bees are not a monolith; different bees are differentially exposed to pesticides. Taken together, this work reveals that pesticide exposure for bees in agricultural is an ecologically dynamic dilemma. As such, the regulation of pesticides in regard to their impact on bee health must take multiple, potentially synergistic, factors into account. More accurate assessments of pesticides will only be made possible with studies and assessments that include several bee species and realistic pesticide mixtures using various metrics at multiple spatial and temporal scales.

Although the findings from these field studies have provided some valuable new insights into the question of realistic rates and impacts of pesticide exposure among wild and honey bees in agricultural settings, further research should address the following concerns. Firstly, any future work would be made more robust by conducting multiyear studies in similar systems across geographic regions. Importantly, studies that explore pesticide residues for specific bee species are encouraged. The sunflower system would be especially well suited for this work as a number of wild bee species are reasonably abundant including: *Melissodes agilis*, *Melissodes robustior*, *Diadasia enavata*, and *Svastra obliqua expurgate*. In cases where specific species are not available in the quantities needed for pesticide residue analysis, submitting composite samples grouped by other life history traits may help to generalize pesticide exposure trends across bees with a similar ecology. Wild bees collected by the same methods detailed in this dissertation could be identified (without pinning) and subsequently grouped by a number of different traits prior to residue analysis including nest location, nesting substrates, sociality, and trophic specialization.

Notably, as residue detection is only one aspect of pesticide exposure, it would be prudent for future research on the susceptibility of different wild bee species to particular insecticides to combine pesticide residue analysis with measures of behavior (e.g. nesting, foraging and flight) and diversity (e.g. species richness and evenness). For the sunflower system specifically, it could prove insightful to test whether the same trends in bee abundance and richness are found if matched pairs are not directly adjacent to one another (i.e. if the suggested niche partitioning operates at just the field scale or also at the landscape scale), and also whether honey bee abundance follows similar or different trends as wild bee abundance.

Belonging

Sense of belonging is a fundamental human need and a growing body of literature calls for higher education institutions to intentionally cultivate a culture of belonging on campus due to the numerous positive social and academic outcomes for students (Baumeister and Leary, 1995; Maslow, 1954; Meehan and Howells, 2019; Strayhorn, 2019; Thomas, 2012). Sense of belonging can be fostered in many ways and one way is through mentorship (Crisp et al., 2017; Graham and McClain, 2019; Lunsford et al., 2017). The final chapter of this dissertation explored sense of belonging in the context of an undergraduate mentorship program at the University of California, Berkeley. The results indicate that the mentorship program had a number of advantageous outcomes. One important finding was that undergraduate participants experienced an increase in their sense of belonging whether they identified as transfer students, first-generation college students, underrepresented minorities, or not. Given the barriers to belonging faced by historically marginalized student populations in academia, this result suggests that this mentorship intervention may be a promising model for increasing sense of belonging among a number of differing student demographics across higher education institutions.

Although the findings from this study have provided some new insights into the effects of this mentorship intervention on sense of belonging for undergraduate students, further research should address the following concerns. Firstly, any future work would be made more robust by conducting multiyear studies in higher education institutions across geographic regions with varying demographics. Surveys administered before, during, and after the mentorship program are encouraged, with anonymous identifiers that allow pre/post analysis to be paired at the individual level. Administering the same surveys to a control group of students not receiving the intervention is also recommended. A number of other demographics factors relevant to belonging would be prudent to study, including: gender, sexual orientation, socioeconomic status, financial obligations, and family/parenting obligations. Investigations into how the program impacts belonging for graduate student mentors and faculty directors could help to better understand the potential reciprocal nature of mentorship programs on belonging. In this regard, investigating mentor traits as factors in mentee belonging could also be beneficial. Piloting a similar program to compare belongingness across different cultures and different industries (e.g. academia, non-profits, government, and industry) could also be beneficial in an effort to understand whether and how belonging operates differently across communities with differing values and goals.

Final remarks

As long as humans walk the face of this planet, sustainable ecosystems and sustainable human systems will be intimately interconnected. Sustainable ecosystems benefit from the conservation of ecosystem services and sustainable human systems benefit from the cultivation of belongingness between people and between people and their environments (Stern, 2018). Both are critical to the continued stability of Earth as a home for *Homo sapiens* and many other species. Overall, this dissertation has served to advance the understanding of both the conservation of bees and the cultivation of belongingness to inform sustainability practices for the benefit of humans and the environment.

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Appendices

Appendices for Chapter 3

Appendix X: Exit Survey Questions used for analysis.

Below are the questions utilized for this study from the exit survey. Variations in language across semesters can be found in Appendix Z.

<u>Berkeley Connect Overall</u> (Response options: *Very true, Somewhat true, Not true*)

1. Participating in Berkeley Connect helped me meet other students who share my academic interests.
2. Participating in Berkeley Connect increased my awareness of educational opportunities and resources on campus.
3. Participating in Berkeley Connect deepened my knowledge of this academic discipline.
4. Participating in Berkeley Connect made me feel better prepared to approach professors in office hours.
5. Participating in Berkeley Connect gave me new ideas about what I can do after I graduate.
6. Participating in Berkeley Connect increased my sense of belonging at UC Berkeley.
7. Participating in Berkeley Connect increased my confidence that I can succeed at UC Berkeley.

<u>Small group</u> (Response options: *Very true, Somewhat true, Not true*)

8. In the small-group meetings, I met other students who share my academic interests.
9. The small-group meetings increased my awareness of educational opportunities and resources on campus.
10. The small-group meetings deepened my knowledge of this academic discipline.
11. The small-group meetings helped me feel better prepared to talk to professors.
12. In the small-group meetings, I learned about different career options.
13. The small-group meetings helped me feel like I belong at UC Berkeley.
14. The small-group meetings increased my confidence that I can succeed at UC Berkeley.

<u>One-on-one</u> (Response options: *Very true, Somewhat true, Not true*)

15. Through one-on-one meetings with my mentor, I met others who share my academic interests.
16. The one-on-one meetings with my mentor increased my awareness of educational opportunities and resources on campus.
17. The one-on-one meetings with my mentor deepened my knowledge of this academic discipline.
18. The one-on-one meetings with my mentor helped me feel better prepared to talk to professors.
19. In one-on-one meetings with my mentor, I learned about different career options.
20. The one-on-one meetings with my mentor helped me feel like I belong at UC Berkeley.
21. The one-on-one meetings with my mentor increased my confidence that I can succeed at UC Berkeley.

Special Events (Response options: *Very true, Somewhat true, Not true*)

22. Some or all of these events deepened my knowledge of this academic discipline.
23. Some or all of these events helped me feel better prepared to talk to professors.
24. Some or all of these events helped me learn about different career options.
25. Some or all of these events helped me feel like I belong at UC Berkeley.
26. Some or all of these events increased my confidence that I can succeed at UC Berkeley.

Appendix Y: Pre/Post Survey Questions used for analysis.

Below are the questions utilized for this study from the pre/post survey.

Feeling (Response options: *Very untrue, Somewhat untrue, Neither true nor untrue, Somewhat true, Very true*)

1. I feel a sense of belonging at UC Berkeley.
2. I feel supported.
3. I feel stressed.

Demographics (Response options: *Yes, No, Prefer not to state*[a])

4. Are you a transfer student?
5. Are you the first in your immediate family to attend a four-year college?
6. Do you identify as African-American, Chicanx/Latinx[b], and/or Native American/Alaska Native?

Notes:
a: "Prefer not to state" was not an option for the transfer student question.
b: One semester used "Chicanx/Latinx" while the other used "Chicano/Latinx."

Appendix Z: All forms of questions asked in exit surveys

ID	*Program Element*	*Program Objective*	*Question Language*
16	BC Experience	Careers	Participating in Berkeley Connect gave me new ideas about what I can do after I graduate.
17	BC Experience	Confidence	Participating in Berkeley Connect increased my confidence that I can succeed at UC Berkeley.
19	BC Experience	Academic Discipline	Participating in Berkeley Connect deepened my knowledge of this academic discipline.
19	BC Experience	Academic Discipline	Participating in Berkeley Connect deepened my knowledge of this major.

22	BC Experience	Professors	Participating in Berkeley Connect made me feel more comfortable approaching professors in office hours.
22	BC Experience	Professors	Participating in Berkeley Connect made me feel better prepared to approach professors in office hours.
28	BC Experience	Educational Opportunities	Participating in Berkeley Connect increased my awareness of educational opportunities and resources on campus.
31	BC Experience	Belonging	Participating in Berkeley Connect increased my sense of belonging at UC Berkeley.
31	BC Experience	Belonging	Participating in Berkeley Connect increased my sense of belonging at UC Berkeley
33	BC Experience	Peers	Participating in Berkeley Connect helped me meet other students who share my academic interests.
91	One-on-One	Careers	In one-on-one meetings with my mentor, I learned about different career options.
92	One-on-One	Academic Discipline	The one-on-one meetings with my mentor deepened my knowledge of the department & academic discipline.
92	One-on-One	Academic Discipline	The one-on-one meetings with my mentor deepened my knowledge of this academic discipline.
92	One-on-One	Academic Discipline	The one-on-one meeting with my mentor deepened my knowledge of this academic discipline.
92	One-on-One	Academic Discipline	The one-on-one meetings with my mentor deepened my knowledge of this major.
94	One-on-One	Professors	The one-on-one meetings with my mentor helped me feel more comfortable talking to professors.
94	One-on-One	Professors	The one-on-one meetings with my mentor helped me feel better prepared to talk to professors.
94	One-on-One	Professors	The one-on-one meetings with my mentor helped me feel better prepared to talk to professors.
96	One-on-One	Educational Opportunities	The one-on-one meetings with my mentor increased my awareness of educational opportunities and resources on campus.
97	One-on-One	Confidence	The one-on-one meetings with my mentor increased my confidence that I can succeed at UC Berkeley.
97	One-on-One	Confidence	The one-on-one meeting with my mentor increased my confidence that I can succeed at UC Berkeley.
98	One-on-One	Belonging	The one-on-one meetings with my mentor helped me feel like I belong at UC Berkeley.
102	One-on-One	Peers	Through one-on-one meetings with my mentor, I met others who share my academic interests.

102	One-on-One	Peers	Through one-on-one meetings with my mentor, I connected with some one who shared my¬†academic interests.
102	One-on-One	Peers	Through one-on-one meeting with my mentor, I met others who share my academic interests.
107	Small-group	Careers	In the small-group meetings, I learned about different career options.
108	Small-group	Academic Discipline	The small-group meetings deepened my knowledge of the department & academic discipline.
108	Small-group	Academic Discipline	The small-group meetings deepened my knowledge of this academic discipline.
108	Small-group	Academic Discipline	The small-group meetings deepened my knowledge of this major.
110	Small-group	Professors	The small-group meetings helped me feel more comfortable talking to professors.
110	Small-group	Professors	The small-group meetings helped me feel better prepared to talk to professors.
115	Small-group	Educational Opportunities	The small-group meetings increased my awareness of educational opportunities and resources on campus.
116	Small-group	Confidence	The small-group meetings increased my confidence that I can succeed at UC Berkeley.
117	Small-group	Belonging	The small-group meetings helped me feel like I belong at UC Berkeley.
118	Small-group	Peers	In the small-group meetings, I met other students who share my academic interests.
124	Special Events	Careers	This event helped me learn about different career options.
124	Special Events	Careers	Some or all of these events helped me learn about different career options.
125	Special Events	Academic Discipline	This event deepened my knowledge of the department & academic discipline.
125	Special Events	Academic Discipline	Some or all of these events deepened my knowledge of this academic discipline.
125	Special Events	Academic Discipline	Some or all of these events deepened my knowledge of this major.
137	Special Events	Professors	This event helped me feel more comfortable talking to professors.
137	Special Events	Professors	Some or all of these events helped me feel better prepared to talk to professors.
137	Special Events	Professors	Some or all of these events helped me feel more comfortable talking to professors.

137	Special Events	Professors	Some or all of these events helped me feel better prepared to talk to professors.
139	Special Events	Confidence	Some or all of these events increased my confidence that I can succeed at UC Berkeley.
140	Special Events	Belonging	This event helped me feel like I belong at UC Berkeley.
140	Special Events	Belonging	Some or all of these events helped me feel like I belong at UC Berkeley.