



Insecticide application prevents honey bees from realizing benefits of native forage in an agricultural landscape

Ashley L. St. Clair^{a,b,c}, Adam G. Dolezal^c, Randall P. Cass^b, Harmen P. Hendriksma^a, David S. Stein^a, Kate E. Borchardt^a, Erin W. Hodgson^b, Matthew E. O'Neal^b, Amy L. Toth^{a,b,*}

^a Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA

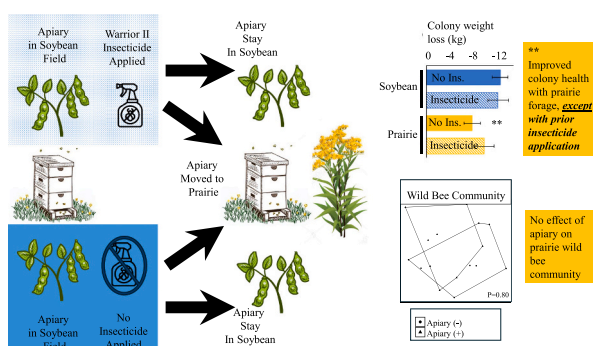
^b Department of Plant Pathology, Entomology and Microbiology, Iowa State University, Ames, IA, USA

^c Department of Entomology, University of Illinois Urbana-Champaign, IL, USA

HIGHLIGHTS

- Unknown if insecticide exposure prevents bees from benefitting from improved forage
- Examined honey bee colony responses to insecticide application in soybean fields
- No acute effects of insecticide application on colony health and survival
- Insecticide application prevented bees from later realizing benefits of prairie forage
- No detectable impact of honey bee apiaries on wild bee communities in prairies

GRAPHICAL ABSTRACT



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ABSTRACT

Health and population status of bees is negatively affected by anthropogenic stressors, many of which co-occur in agricultural settings. While pollinator habitat (often involving plantings of native forbs) holds promise to benefit both managed and wild bees, important issues remain unresolved. These include whether conventional, broad-spectrum insecticide use negates these benefits and how non-native, managed honey bees affect wild bees in these areas. We conducted a three-year replicated study in a Midwestern corn and soybean production region (i. e., Iowa, USA). We assessed acute and delayed effects of commercial-scale spraying of a commonly used, foliar-applied insecticide (λ -cyhalothrin) in soybean on the productivity of honey bee colonies kept within these fields. Colony health metrics showed no immediate significant differences between insecticide treated and untreated crop fields. As expected, health metrics declined in all colonies after soybean ceased flowering. Interestingly, the subset of colonies from untreated fields given access to restored prairies rebounded. However, colonies from insecticide-treated fields showed reduced growth, queen egg-laying, and survival, even when given access to prairies. In addition, we did not observe a detectable impact of honey bee apiaries on wild bee abundance or diversity at these prairies over a three-year period. Our findings underscore the complex interactions between agricultural practices and bee health, highlighting the necessity of integrated pest management and the

* Corresponding author at: Department of Ecology, Evolution, and Organismal Biology, 2200 Osborn Dr., 251 Bessey Hall, Ames, IA 50014.

E-mail address: amytoth@iastate.edu (A.L. Toth).

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conservation of native floral resources to support pollinator populations and sustainable beekeeping in agroecosystems.

1. Introduction

Bees are critical to the pollination of many wild and cultivated plants, providing ecosystem services that contribute to environmental health and human livelihoods (Dicks et al., 2021; Murphy et al., 2022). Many biotic and abiotic stressors currently threaten bee populations and health such as climate change (Soroye et al., 2020; Vasiliev and Greenwood, 2021), forage availability (Filipiak, 2018; Naug, 2009), pathogens (Deutsch et al., 2023; Straub et al., 2022; Evans and Schwarz, 2011; Vojvodic et al., 2012), and competition with managed bee species (Hung et al., 2019). However, in some parts of the world, the leading contributors to bee health declines are primarily associated with agricultural practices (e.g., intensive farming, monocultures, and pesticide use) (Goulson et al., 2015; Langlois et al., 2020; LeBuhn and Vargas Luna, 2021; Raven and Wagner, 2021). Although these agricultural practices increase crop yields, they also result in drastic changes to the environment that bees encounter, including less abundant or diverse floral resources and pesticide exposure, which can also have synergistic effects with other environmental stressors (Belsky and Joshi, 2019). Although several studies have demonstrated the potent effects of interacting stressors on various aspects of bee health (Botías et al., 2021; Dolezal et al., 2019a; Doublet et al., 2015; Hsieh and Dolezal, 2024) field-scale experiments which provide more realistic estimations of these synergies in the context of complex and environmentally variable scenarios are rare (Castle et al., 2022).

The availability of sufficient quality and quantity of floral resources is a major concern for both wild and managed bees, especially in highly agricultural landscapes. Landscapes that have been massively converted to monoculture farming are often areas of high honey bee colony losses (Bruckner et al., 2023) and wild bee declines (Grixti et al., 2009). The “Corn Belt” of the Midwestern United States is an example of such a landscape, where a “feast-famine” dynamic in floral resources has significant implications for honey bee health and productivity (Dolezal et al., 2019b). In this region, late summer (i.e., August) is critical for honey bee nutrition (Dolezal et al., 2019; St. Clair et al., 2022a). The widespread cultivation of annual maize and soybean, which cease blooming concomitantly with other major forage plants such as clover species, leaves little forage for bees after crops senesce. While maize and soybean are primarily wind pollinated and do not require insect pollination, soybean is considered a moderately attractive crop to both *Apis* and non-*Apis* bees (U.S. Department of Agriculture (USDA), 2017) and visitation by wild and managed bees has potential to increase soybean yield up to 24 % (Cunningham-Minnick et al., 2019). In Midwestern landscapes, honey bees prioritize visitation to soybean fields for nectar collection (Dolezal et al., 2019; Forrester et al., 2024; Lin et al., 2022) switching to alternative resources after soybean and clover senesce such as native forbs found in prairies (Carr-Markell et al., 2020; Zhang, 2021). Providing bees access to native forbs during this time can mitigate the late season declines in colony growth and increase honey bee queen egg laying (St. Clair et al., 2022a; Zhang et al., 2023). This access can be provided by moving colonies to prairies (Dolezal et al., 2019) or establishing small patches of prairie within or around crop fields (Zhang et al., 2023). This system can potentially serve as a model for the use of reconstructed native vegetation to improve floral resources and bee health in highly agricultural landscapes. Despite these benefits, there are critical knowledge gaps related to using native vegetation as a “rescue” for honey bee colony health in the context of landscapes predominantly covered by monoculture crops. Prior insecticide exposure, which may be fairly common in these landscapes, could conceivably reduce honey bees’ ability to respond to management efforts to improve resources.

Sublethal effects of pesticide exposure can diminish honey bee

reproduction (Wu-Smart and Spivak, 2016), immunity (Castelli et al., 2021), cognition (Begna and Jung, 2021), and overall physiological functioning (Dively et al., 2015), leading to suboptimal honey bee performance and population reduction. Providing bees with a high-quality diet can increase honey bee resistance to sublethal pesticide doses (Barascou et al., 2021). Conversely, bees that experience nutritional stress in combination with pesticide exposure undergo a synergistic reduction in lifespan and increased mortality (Tosi et al., 2017; Hsieh and Dolezal, 2024). Applying insecticide(s) to crop fields (e.g., through treated seed, foliar application, or both) may occur before and/or during crop bloom when resources are abundant, thus buffering an immediate response to insecticide stress. However, after crops cease blooming and bees subsequently experience forage dearth, prior sublethal pesticide exposure could result in delayed negative impacts (Castle et al., 2022).

A second major unresolved issue with placing honey bees in restored native habitats relates to their effects on wild pollinators of conservation concern. While the reconstruction of native habitats in agricultural landscapes has shown potential benefits to honey bee colony health (Zhang et al., 2023), there is concern about the potential impact of non-native honey bees on wild bees due to competition and disease (Dolezal et al., 2016; Mallinger et al., 2017; Wojcik et al., 2018). Multiple studies have found the presence of honey bees can negatively impact the abundance and diversity of wild bees (MacInnis et al., 2023; Ropars et al., 2019; Weekers et al., 2022). Several common honey bee diseases are shared with wild bees and proximity to apiaries may increase disease risk (Dalmon et al., 2021; Dolezal et al., 2016; Murray et al., 2019; Pritchard et al., 2021), and in some cases, honey bees can disrupt native plant-pollinator community interactions (Page and Williams, 2023; Valido et al., 2019; Worthy et al., 2023), though this interaction is not always significant (St. Clair et al., 2022b; Hung et al., 2019). In addition, few studies have assessed these effects over multiple years of honey bee presence. Moving forward, multi-year field-scale evaluations of how apiaries in native vegetation affect wild pollinators will be necessary for informed decision-making about balancing beekeeping and pollinator conservation.

We conducted a replicated three-year study to address the aforementioned two critical knowledge gaps related to honey bee nutritional “rescue” with native vegetation. First, we assessed whether application of an insecticide in a field-realistic setting reduced honey bees’ ability to benefit from access to native perennial, flowering vegetation. We tracked honey bee colonies placed in soybean fields, a landscape dominated by crop production, observing their responses before and after a field-level application of a commonly used insecticide, λ -cyhalothrin as formulated in Warrior II. We chose λ -cyhalothrin because it is one of the most commonly applied agrochemicals in the Midwestern landscape, predominantly in maize and soybean, to confer crop protection from insect pests (Goebel et al., 2022; U.S. Geological Survey, 2018) and has demonstrated moderate toxicity to honey bees at field realistic concentrations (Jütte et al., 2023). After the insecticide was applied and soybean ceased blooming, half the colonies were moved to a restored tallgrass prairie and colonies were closely monitored, allowing us to assess delayed impacts on bee health and colony productivity. Second, we measured the potential impacts of honey bees on wild bees in restored native vegetation by surveying the wild bee community in restored tallgrass prairies before and after apiary placement, and in prairies with and without apiaries. First, we hypothesized that exposure to pesticides would affect honey bee colonies by reducing colony success (e.g., weight, brood production, adult bee population, queen egg laying rate, and colony survival) immediately after exposure. Second, we hypothesized that access to native vegetation in prairies would “rescue” these parameters. Third, we hypothesized that apiaries would negatively

impact the abundance and richness of wild bees in prairies, and that this impact would increase over multiple years. Overall, this study addresses multiple, critical knowledge gaps necessary for crafting best management practices related to sustainable beekeeping, pesticide use, and conservation of pollinators.

2. Methods

2.1. Site selection and management

We selected three independently managed soybean fields to place apiaries in 2017 and 2018, and two fields in 2019. In 2018, the median year of this experiment, approximately 90 % (i.e., 12.4 million hectares) of the state's land area was in farm production, primarily for production of maize and soybean (U.S. Department of Agriculture (USDA), 2018). For Boone and Story counties, where our soybean fields were located, approximately 86 % of the landscape was devoted to maize and soybean production (U.S. Department of Agriculture (USDA), 2018), thus, the apiaries of honey bees were exposed to a relatively uniform agricultural matrix consisting of maize or soybean monocultures. The average field size was 39.32 ha (range 27.24–55.85 ha). Within a year, fields were located at least 3.2 km apart and maintained by Iowa State University (ISU) using conventional farming practices for this region, described below. We chose this distance to ensure independent field replication, as studies indicate that on average, foragers rarely travel farther than 2 km from their hive (Couvillon et al., 2014; Shackleton et al., 2023). Each year the fields were assigned to one of two treatments, a control in which insecticides were not used at any point during the study (INSC-; 2 controls in 2017–2018, 1 in 2019), and an experimentally treated field where foliar insecticides were applied prophylactically for management of soybean aphid (INSC+; 1 treated field in 2017–2019). The insecticide used in INSC+ soybean fields was Warrior II (Syngenta, λ -cyhalothrin at 22.8 % of formulation per SDS), as it is one of the most commonly applied insecticides for protection of corn and soybean yield, therefore representing a likely real world exposure scenario in this growing region (U.S. Geological Survey, 2018). λ -cyhalothrin is a synthetic pyrethroid insecticide which disrupts the nervous system of insects through sodium channel binding on the surface of insect cells, ultimately causing insect paralysis and death. Due to the potential for acute contact and oral toxicity to honey bees through exposure to λ -cyhalothrin (Liao et al., 2018) and potential for sublethal effects (Arthidoro de Castro et al., 2020) we ensured all applications of Warrior II followed the “Bee Rule” per the Iowa Department of Agriculture and Land Stewardship (<https://www.legis.iowa.gov/docs/iac/rule/04-23-2008.21.45.31.pdf>) requiring applications to occur before 8:00 am or after 6:00 pm when colonies are within a mile of a treated field. Across the INSC+ treatments, Warrior II applications were applied only once in a season and consistently (i.e., same formulation and application rate) across experimental fields when soybean reached growth stage of R3 (refer to Fig. S1 for a timeline Warrior II application). While many studies investigating the effects of pesticides on honey bees feed chemicals directly to bees through colony feeding or exclusion studies, we aimed to create a field-realistic scenario of potential exposure. See Table S1 for a complete list of field management practices, including seed variety and treatment, planting date, and pre- and post-emergent herbicide application.

To replicate the “prairie rescue” effect (Dolezal et al., 2019), we transported honey bee colonies in August (Fig. S1) to selected restored prairies in the Chichaqua Bottoms Greenbelt of Iowa, Polk County, approximately 40 km south of our experimental soybean fields. Prairies averaged 45.1 ha, (range 31.5–55.85 ha) and were located at least 3.2 km from one another. We selected three restored prairies in 2017, reused all of them in 2018, and reused two in 2019. Prairies with an apiary in the late season are hereafter referred to as Apiary(+) prairies.

To compare wild bee communities in Apiary(+) prairies to those without an apiary, Apiary(–), we selected additional prairies within the Chichaqua Bottoms Greenbelt (three in 2017 and 2018 and two in

2019), following the same guidelines as above (mean 63 ha, range 20.2–121.4 ha). We confirmed that there were no additional managed colonies present within 1 km by checking the Iowa voluntary hive registry (<https://driftwatch.org/>) and through visual inspection of the surrounding region. The experimental units across all three years are provided in Fig. S2, and information on prairie size, type, and seasonal management are in Table S1.

2.2. Honey bee apiary placement

Honey bee colonies started as packages or nucleus colonies and received a new laying Italian (*Apis mellifera ligustica*) queen at the start of each growing season. All colonies were housed in standard Langstroth 10-frame hive boxes and started with a mixture of undrawn and fully drawn comb. For full details on bee source, colony initiation date, and date of inspection before entering the field experiment see Tables S1 and S2.

We placed 128 honey bee colonies in soybean fields from 2017 to 2019. Each field received an apiary of 16 colonies, placed after the fields were planted, and pre- and post-crop emergent herbicides were applied to minimize effects of early-season herbicide and seed-applied insecticidal treatments. Colonies were randomly placed in soybean fields (on 2 June 2017, 8 June 2018, and 7 June 2019) with each apiary having similar average weight. We placed colonies in the centers of soybean fields to maximize their probability of exposure to our main treatment insecticide, λ -cyhalothrin, creating the potential for insecticide exposure at a field scale. To reduce bees drifting between colonies within a field, apiaries were placed at two subsites consisting of eight colonies. Subsites were at least 150 m from the closest field edge and 300 m from the adjacent sub-site. All colonies remained in the soybean fields until the second week of August, the peak in honey production within Iowa (Dolezal et al., 2019b; St. Clair et al., 2020). Half of the colonies from each sub-site ($N = 8$; on 10 August 2017, 9 August 2018, and 12 August 2019) were randomly selected and moved out of soybean fields in each treatment, randomized again, and then relocated across the three Apiary (+) prairies, previously referred to as “prairie move” treatment. Each prairie received eight colonies representing both soybean treatments (i.e., INSC- or INSC+). The remaining colonies ($N = 8$) remained in the soybean fields of their respective treatments. At the end of each season (2 October 2017, 13 October 2018, and 16 October 2019), all colonies from soybean and prairie were moved to a single ISU research field for overwintering. In October, when colonies were transported to the overwintering location, they were provided an initial supplemental feeding of either 7.5 L heavy syrup (2017, 2018) or 11 kg sugar fondant (2019).

2.3. Estimating honey bee colony growth

Colonies were inspected every other week from June to October in all three years of the experiment, following methods from (Dolezal et al., 2019b) and (St. Clair et al., 2020). Inspections included measuring capped brood area (i.e., developing pupae), estimating adult bee populations (i.e., frame sides covered in adults per Delaplane et al., 2013), and weighing colonies. Additional hive boxes were added when those present reached 75 % capacity. The weight of added equipment (i.e., hive boxes and drawn frames) was determined before placing on colonies and subtracted from the total weight of the colony resulting in estimates of added colony weight only (i.e., bee population, brood, honey and pollen stores, and newly produced wax and propolis). Due to the variation in when and how colonies were started (i.e., nucleus colonies vs packages), we estimated the starting equipment weight each year by weighing a subset of colonies before colony initiation ($N = 20$ per year). The average weight of the initial hive bodies and frames was subtracted from the total mass of each colony measured at every inspection.

We surveyed Varroa mites (*Varroa destructor*) within colonies

monthly via the alcohol wash method (Dietemann et al., 2013) to determine when a thymol (Apiguard, Dadant and Sons Inc.) treatment was needed (mite counts provided in Fig. S3A). During inspections, we determined the presence of a queen by direct visual observation or the presence of newly laid eggs. If a queen was absent, the colony was provided a new queen within three days (summary of queen loss data provided in Fig. S3B). Queen excluders were added to restrict queens but allow workers to move freely when additional hive bodies were added to a colony. This restricted brood production to the bottom two hive deep boxes and the storage of honey and pollen to additional hive boxes.

2.4. Estimating honey bee queen egg laying rate

To quantify egg laying, we studied queens at three time points- pre-Warrior II application, post-application, and post-prairie move. Each queen was confined to a single empty frame within her colony using specialized queen cages. Cages were constructed from queen excluders and covered one side of an empty Langstroth deep frame, preventing queens from escaping but allowing workers to enter and interact with queens (illustrated in Fig. S4A). Cages remained in place for 48 h, after which egg counts were estimated (St. Clair et al., 2022a; Wu-Smart and Spivak, 2016) (as illustrated in Fig. S4B) and queens were released. We counted eggs in eight parallelograms across the frame, covering 100 cells each for a maximum of 800 possible eggs.

2.5. Estimating honey bee colony mortality

We estimated summer and winter colony mortality at the end of each growing season. Summer mortality was noted during each inspection period when colonies were in experimental fields from May to October. Winter mortality was recorded after colonies were relocated to an overwintering site and was noted every two to three weeks from October through March. Summer mortality was confirmed when no live bees were observed within the hive boxes. Additionally, a colony was considered dead if it was queenless and failed to accept a new queen for three or more inspections, ultimately having laying worker bees. To limit the exposure of bees to freezing temperatures, we did not confirm winter mortality by opening the hive boxes. Instead, we knocked on the outside wall of a hive box and used a stethoscope to verify the sound of active bees within the colony. At the end of the winter, we opened each hive box and were able to positively confirm survival or mortality of each colony.

2.6. Insecticide analysis

Pollen was collected from colonies in both INSC+ and INSC- fields to determine the presence of λ -cyhalothrin before and after the formulated product was applied in fields. For this reason, we only collected pollen through the period directly after Warrior II application, and did not collect pollen in prairie locations. Pollen collected by foraging honey bees was harvested using hive entrance pollen traps (Brushy Mountain Bee Supply, Wilsonville, USA) placed on four colonies (two per subsite) within each soybean field. To minimize the disturbance, pollen collections lasted for 24-h the week after colony inspections (see Table S3 for collection dates per site). Collected pollen was stored in a -20°C freezer until analysis. As our goal was to understand the presence of λ -cyhalothrin residues within honey bee collected pollen, not document which plants honey bees were selecting for forage (already studied in Zhang et al., 2022), we did not determine pollen composition in this experiment.

Approximately 0.2 g of pollen was subsampled from pooled pollen from each collection date to use for insecticide quantification using LC-MS (liquid chromatography-mass spectrometry). Control pollen was used to prepare calibrants, QCs, and blanks, and samples were spiked in a calibration range from 5 to 100 ppb. QC levels were 7.5, 25, and 75 ppb. Acetonitrile (LCMS Optima Grade, Fisher Scientific) was added,

and each tube was shaken for 10 min at 2500 rpm, then centrifuged (Thermo Fisher Scientific) for 5 min at 3000 rpm. The supernatant was transferred to a 2-mL dSPE tube containing 150 mg MgSO_4 , 50 mg PSA, and 50 mg C18, dSPE tubes were placed on a multi-tube shaker for 2 min at 2000 rpm and centrifuged (Eppendorf 5417C) for 5 min at 6000 rpm. The supernatant was transferred into a glass tube, dried down, then reconstituted in 100 μL of ethyl acetate containing aldrin (used as an external standard) at 50 ppb. Approximately 100 μL of sample was transferred to an autosampler vial with an insert prior to GC/ECD analysis. Samples were run on an Agilent 7890B GC with ECD detector, with a Resek Rtx-5MS w/Integra-Guard column (30 m \times 0.25 mm ID \times 0.25 μm) and flow rate of 1 mL/min. The GC gradient consisted of an initial temperature of 100°C , a hold for 1 min, a ramp at $10^{\circ}\text{C}/\text{min}$ to 180°C , a hold for 5 min, a ramp at $25^{\circ}\text{C}/\text{min}$ to 300°C , and a hold for 8 min. Injections were performed in splitless mode at 250°C with He flow at 54.5 mL/min and an injection volume of 1 μL . The GC detector was an uECD at 250°C with a flow makeup of Argon/Methane at 60 mL/min.

2.7. Assessment of wild bee populations and plant communities

The abundance and richness of the wild bee community within prairies was estimated using elevated pan traps (i.e., bee bowls) made from 3.2 oz. Solo brand cups painted either fluorescent blue, fluorescent yellow, or left white (St. Clair et al., 2020; St. Clair et al., 2022b). This color combination attracts a robust community of bees (Droege et al., 2010). We assembled each color pan trap on a stand at vegetation height (referred to herein as trap stands). Within a prairie, we placed 12 trap stands along two 60 m transects (with three trap stands at both ends of each transect). One set of trap stands was placed 30 m from the apiaries, while the three additional trap stands were placed 60 m from that point at the transect ends, resulting in the farthest traps being placed 90 m from the apiaries (illustrated in Fig. S5). We placed trap stands 10 m apart on either end of the transects and ensured they were at least 20 m from the prairie's edge. Each transect was located 60 m apart. This resulted in 36 individual pan traps placed in each prairie, both Apiary (+) and Apiary (-).

Bees were collected from pan traps once a week during a 7-week period in 2017 (26 July to 20 September 2017) and a 6-week period in 2018 and 2019 (24 July to 10 September 2018, 23 July to 16 September 2019). Pan traps were filled with 50 mL of 0.2 % soapy water solution and contents were collected after 24 h. Pan trap height was adjusted to the top of foliage each week. Three collections occurred before and after the placement of honey bee hives in Apiary (+) and Apiary (-) prairies.

Bees were pinned and identified to species utilizing keys developed by (Michener et al., 1994) and (Ascher and Pickering, 2019), excluding those belonging to genus *Lasioglossum*, which were identified to morphospecies following methods from (St. Clair et al., 2022b, St. Clair et al., 2020). 'Taxon richness' is, therefore, defined as the total number of species and *Lasioglossum* morphospecies.

Flowering forbs in all prairies were recorded weekly from July – October 2017 using a modified Pollard walk technique (Pollard, 1977) to account for potential covariation between plant and wild bee communities. In 2018, we quantified the abundance and richness of flowering forbs weekly from June to October. A transect was placed 50 m into each prairie to quantify flowering forbs. The transect was 30 m away from (but parallel to) the location of the honey bee apiaries (when present) and totaled 60 m in length. The transect was walked weekly, and the observer sight identified forbs down to species (when possible) and counted every flowering ramet located within 1 m on either side of the transect (Stein et al., 2020). No assessments of forbs were conducted in 2019 due to reduced site replication and labor in the final year of data collection, but forb composition was likely to be similar to the prior two years because the same site locations were used.

2.8. Statistical analyses

Detailed statistical methods are provided in the Supplement. All analyses were performed in SAS 9.4, except for colony mortality and insecticide levels, which were analyzed using R 3.4.1. To analyze colony data across seasons, we used generalized linear mixed effects models with AR1 correlation (ANOVA; PROC MIXED) with week, insecticide spray, and their interaction as main effects and year, field within a year, and sub-site within a field within a year as random variables.

We used least squared means to compare growth response variables between colonies in INSC+ and INSC- fields at individual weeks. After prairie move, colony growth measurements from week 33 were converted to zero and change in growth (Δ growth) was calculated as Δ growth from week 33. Bonferroni adjustments were used to compare treatments by week and least squared means to test for differences from week 33 (i.e., different from zero). We also calculated the relative rate of weight decline (from week 33) for each colony and calculated contrasts (based on Δ weight data from slopes of each treatment week 33–41). Mixed model ANOVAs were also used to analyze proportion eggs laid by the queen as the response variable, with fixed effects of insecticide spray and pre-and post-prairie move, and the random effects of year, field nested within a year, and colony within a field nested within a year. Post hoc comparisons were conducted using comparisons of least squared means.

Summer and winter mortality were analyzed using Cox hazard ratio survivorship analysis using the “survival”, “survminer”, and “coxme” packages in R (Therneau, 2012). The number of detectable insecticides (λ -cyhalothrin) in pollen samples from treatment groups was compared using Chi-square tests. Insecticide quantifications were compared with linear mixed model (lme in R) to test for the effects of treatment (INSC-, INSC+ and time-period (pre-spray vs. post-spray), as well as their interaction, with year and field site as random variables.

Mixed model ANOVAs were used to analyze wild bee abundance and richness, with fixed effects of insecticide spray and pre-and post-prairie move, and their interaction with year as a random effect. Post hoc comparisons were conducted using comparisons of least squared means. A linear regression was used to assess plant community richness and abundance against bee richness and abundance. Nonmetric multi-dimensional scaling was used to plot the community diversity with Bray-Curtis dissimilarity following methods from (St. Clair et al., 2020). PMANOVA was used to test for statistical differences between communities.

3. Results

3.1. Effects on honey bee colony growth

Seasonal growth of honey bee colonies observed in this study are similar to previous studies conducted in Iowa (Fig. S6A – C; Dolezal et al., 2019; St. Clair et al., 2020; Zhang et al., 2023), with colonies reaching peak weight during the first week of August (week 31), and peak brood production and adult bee population in late August (week

35). All metrics of colony growth varied by calendar week (i.e., date, Table 1). The main effect of Warrior II application did not result in significant changes in colony weight, capped brood area, or frame sides of adult bees, and there were no individual differences observed by date pre- or post-Warrior II application when colonies were in soybean fields (Fig. 1A – C, Table 1, Fig. S7).

There was a significant main effect of prairie move and a marginally significant interaction ($p = 0.0688$) of the Warrior II application with prairie move (full statistics in Table 1). Overall, we observed that colonies in the prairie landscape lost significantly less weight compared to those that remained in soybean fields (Fig. 2A, Table 1), demonstrating a “prairie rescue” effect (Dolezal et al., 2019). This rescue effect was most

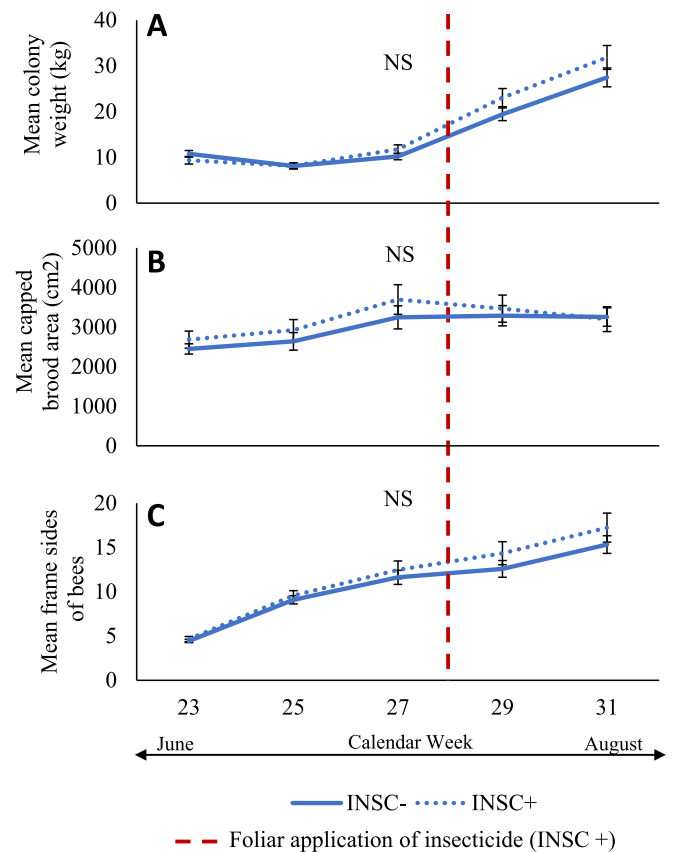


Fig. 1. Mean (A) colony weight, (B) capped brood area, and (C) frame sides of adult bees in honey bee colonies placed inside soybean fields prior to application of λ -cyhalothrin (weeks 23–27) and post application (weeks 29–31) in central Iowa during 2017, 2018, and 2019. Error bars represent one standard error of the mean. There were no significant differences between fields that were untreated (INSC- i.e., no applications of foliar λ -cyhalothrin) versus those that were treated with a foliar application of λ -cyhalothrin (INSC+) at individual weeks (Table S1).

Table 1

Results of repeated measures analysis of variance for the delta change (Δ) in weight, capped brood area, and frame sides of bees in colonies from calendar week 33 through calendar week 41 over 2017, 2018, and 2019. * $p < 0.1$, ** $p < 0.05$, *** $p < 0.001$.

Effect	Weight (kg)			Capped brood area (cm ²)			Frame sides of adult bees		
	DF	F Value	Pr > F	DF	F Value	Pr > F	DF	F Value	Pr > F
INSC spray (Warrior II)	1, 124	0.26	0.6099	1, 3.88	1.12	0.3519	1, 4.37	3.93	0.1124
Prairie move	1, 123	5.48	0.0208**	1, 148	2.61	0.1082	1, 139	0.26	0.608
INSC spray*Prairie move	1, 124	3.37	0.0688*	1, 149	0	0.9588	1, 139	0.09	0.76
Calendar week	4, 376	54.81	<0.0001***	4, 352	69.76	<0.0001***	4, 353	9.04	<0.0001***
INSC spray*Calendar week	4, 376	0.13	0.9694	4, 344	2.97	0.0196**	4, 347	0.99	0.4134
Prairie move*Calendar week	4, 376	1.78	0.1323	4, 351	2.96	0.02**	4, 353	1.05	0.3817
INSC spray*Prairie move*Calendar week	4, 376	1.81	0.1262	4, 351	1.14	0.3361	4, 352	0.33	0.857

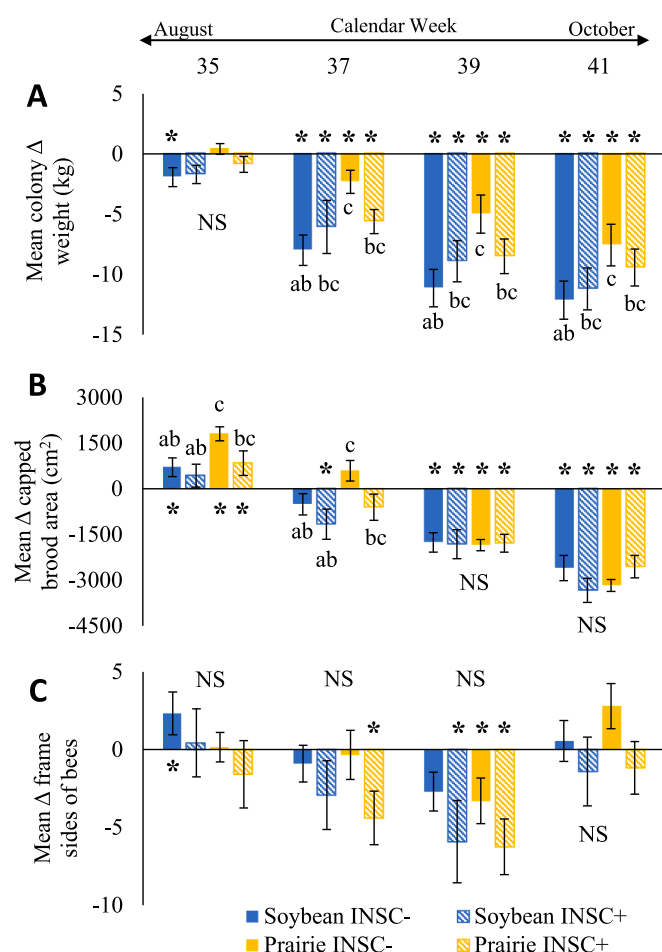


Fig. 2. Mean change (Δ/Δ) in (A) weight, (B) capped brood area, and (C) frame sides of adult bees in colonies that were either moved to prairie in August (week 33) or remained in soybean in central Iowa during 2017–2019. Delta for each metric is represented as the change from week 33 (peak colony development), which was also the first measurement after colonies were relocated to prairie or not. Error bars represent one standard error of the mean. Letters signify differences between treatments at individual sample weeks (Tables S3, S5, S7; Bonferroni adjustment $p < 0.008$). Asterisks signify a significant difference in growth compared to peak colony growth, which was also the first assessment taken post move on week 33 (Tables S3, S4, S6; treatment different from zero $p < 0.05$).

apparent in the prairie INSC- treatment (Fig. 2A weeks 37–41; Table S4), indicating that colonies provided access to native forbs are most likely to benefit when residing in crop fields where no insecticide application occurred. Colonies in all treatments were in a weight decline by mid-September compared to early August (week 33 compared to week 37, Fig. 2A; Table S5); however, those in the prairie INSC- treatment had a 2–4-week delay in this decline compared to the other treatments and consistently lost the least amount of weight during the decline period (Fig. 2A).

For another perspective on this weight loss, we compared the overall rate of weight change per colony during the post-prairie move period (week 33 – week 41). Colonies moved to a prairie from a soybean field that did not receive a Warrior II application (i.e., prairie INSC-) declined in weight at a significantly lower rate compared to colonies that remained in soybean fields in both INSC+ and INSC- treatments (Fig. 3; Table S6). In contrast, the rate of weight change in colonies moved to a prairie which came from a Warrior II treated soybean field (i.e., prairie INSC+) was not different from those that remained in soybean fields (Fig. 3; Table S6), demonstrating a lack of the “prairie rescue” effect when coming from an environment where insecticides were previously

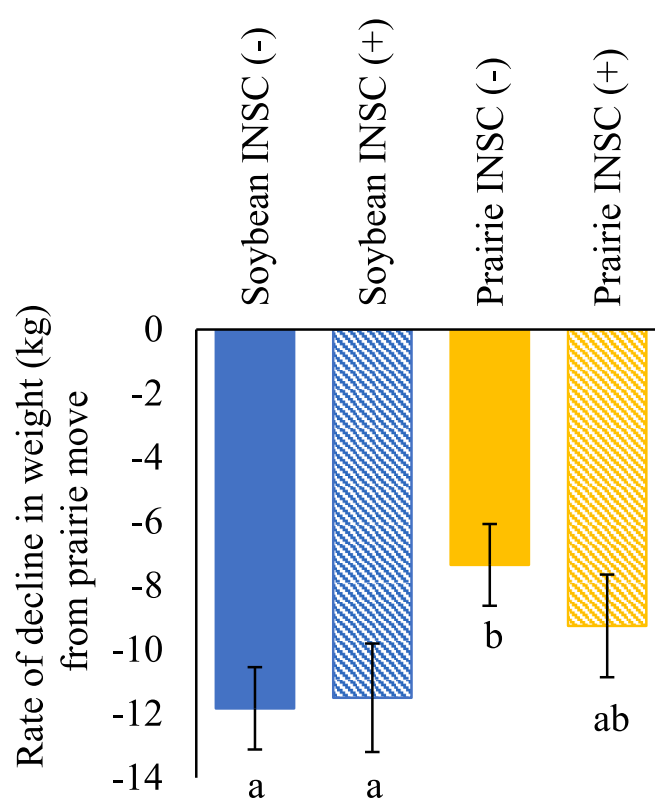


Fig. 3. Rate of decline in weight of honey bee colonies placed in soybean fields or moved to prairies in central Iowa in 2017–2019. Decline was based on delta mass (Δ) from the peak mass (week 33; first inspection after 50 % of colonies were moved to prairie) through pre-overwintering (week 41). Letters indicate significant differences in mean rate of decline $p < 0.05$.

used.

Although there was year to year variation and a trend towards more brood in colonies moved to prairies, prairie move had no significant overall effect on capped brood area (Table 1). However, there were interactions of prairie move with date, as well as INSC treatment with date (Fig. 2B; Table 1). The lack of significant main effects is likely due to a seasonal reduction in brood, observed at week 39 in all treatments, part of a transition to an overwintering state in temperate regions (Fig. 2B; Table S7). Although the effects on colonies from Warrior II treated soybean fields were subtle, several weekly patterns support that the exposure environment from the insecticide application prevented a “prairie rescue” for colony brood production. First, colonies moved into prairies from the INSC+ soybean fields (i.e., prairie INSC+) did not exhibit the same significant increase in brood production as colonies moved from the INSC- fields (i.e., prairie INSC-) (Fig. 2B; Table S8). By week 37, colonies remaining in soybean INSC+ were the only treatment with a significant decrease in capped brood compared to week 33 (Fig. 2B; Table S7). Second, colonies moved to a prairie from soybean INSC- fields (i.e., prairie INSC-) had higher capped brood area compared to colonies remaining in soybeans in both the INSC- and INSC+ treatments on weeks 35 and 37 (Fig. 2B; Table S8). By week 39, all treatments had similarly declining populations of capped brood, suggesting that colonies moved to prairies in a non-insecticidal environment (i.e., prairie INSC-) may experience a ~ 6-week boost in brood production (Fig. 2B; Table S7). Third, colonies remaining in INSC+ soybean fields were the only treatment not to experience an increase in capped brood area in week 35 compared to week 33 (Fig. 2B; Table S7).

Frame sides of adult bees only varied by date (Fig. 2C, Table 1). Populations fluctuated on specific weeks compared to week 33, but ultimately, colonies from each treatment went into the winter on week 41 with adult bee populations that were not significantly different from

when they were moved to a prairie (week 33) (Fig. 2C; Table S9). Furthermore, no significant differences were observed between treatments on any week post-prairie move (Table S10).

3.2. Honey bee queen egg production

There were no overall main effects of Warrior II application or application date (pre- vs post-application) on queen egg production, nor were there any interactions of date and Warrior II application ($F_{1, 9.36} = 1.29, p = 0.29$; $F_{1, 66.3} = 0.04, p = 0.85$; $F_{1, 66.5} = 1.17, p = 0.28$, Fig. 4A for Warrior II treatment, date, and their interaction respectively). Later in the season, there was also no overall effect of prairie move or Warrior II application on queen egg production ($F_{1, 32.9} = 1.69, p = 0.20$, $F_{1, 56.2} = 1.28, p = 0.26$; Fig. 4B for prairie move and Warrior II application respectively), and no interaction ($F_{1, 56.3} = 2.16, p = 0.15$). However, queens who were given access to prairie after being in INSC- soybean fields (i.e., prairie INSC-) laid significantly more eggs than those that remained in those fields (Fig. 4B; Table S11). Furthermore, queens in colonies in the prairie INSC- treatment laid marginally significantly more eggs compared to colonies that experienced the Warrior II application environment earlier in the season (i.e., soybean INSC+ and prairie INSC+; Fig. 4B; Table S11), further supporting that the prairie rescue may not be possible if colonies experience an exposure event even months earlier in the season.

3.3. Honey bee colony mortality

Across all treatments, 75 % or more of colonies survived the summer. Survivorship was numerically highest (≥ 95 %) in the prairie INSC- treatment (Fig. 5A), and we observed a significant difference in survivorship across treatments throughout the summer (Fig. 5A; $p = 0.0013$). Before application of Warrior II in INSC+ fields (weeks 23 to 27) there were no summer colony mortalities observed, however, one month after application (week 31), we began to observe colony mortalities starting in the soybean INSC+ treatment (Fig. 5A), suggesting potential delayed lethal effects of insecticide exposure on the colony level. Overall, colonies that were moved to prairies (regardless of whether they came from soybean fields with Warrior II or not), had significantly higher survival compared to colonies that remained in either soybean treatment, indicating late-season access to native forbs can increase the likelihood of survival in this region. At the end of the summer, all surviving colonies were moved to an overwintering site and winter mortality was tracked. We observed no differences in winter survival among the four treatments across all three years (Fig. 5B; $p = 0.47$). At the end of the winter, when colonies were checked for final mortality, <25 % of all colonies had survived.

Further investigation of treatment effects using Cox hazard ratio

models shows that the main effect of Warrior II application (spray) on week 28 was not on summer survivorship (Fig. 6A). However, there was a significant effect of prairie move; those moved to prairie had a lower risk of mortality compared to those that remained in soybean fields (Fig. 6A). To make a comparison of all treatments, we set the prairie INSC- as the reference to which all other treatments were compared, as prairie INSC- had the highest predicted survival. From this comparison, colonies remaining in soybeans regardless of Warrior II application (INSC+ and INSC-) had significantly higher hazard (i.e., risk of colony mortality) compared to the reference treatment of prairie INSC-, which had the lowest mortality overall (Fig. 6B).

3.4. Pesticide quantification

To address exposure risk in soybean fields which were sprayed with Warrior II (INSC+) compared to fields with no direct insecticide application (INSC-), we sampled bee collected pollen from a subset of hives in all three years of the study. Because we knew the exact timing of application, we focused on quantification of λ -cyhalothrin levels in the immediate pre- and post-spray periods each year (sampling dates and spray dates listed in Table S3, and note we were unable to sample the post-spray period in 2019). There was no overall difference in the main effect of spray application (INSC- vs INSC+; $F_{1,5} = 2.089, p = 0.208$), or between application date (pre- vs post-spray; $F_{1,26} = 0.670, p = 0.435$), and no interaction was observed ($F_{1,26} = 0.000012, p = 0.997$; Table S3). However, when we compared λ -cyhalothrin within a time point using Chi-Square analysis, we observed the proportion of samples with detectable λ -cyhalothrin in bee collected pollen was significantly higher across all three years in the post-spray samples from soybean INSC+ fields (71.4 % with detectable λ -cyhalothrin) compared to only 18.2 % in soybean INSC- fields (Chi-Square = 5.103, $df = 1, p = 0.024$; Table 2). There was no difference in the proportion of detectable samples during the pre-spray period (INSC- 32.7 %, INSC+ 44.4 %, Chi-Square = 0.040, $df = 1, p = 0.307$; Table 2). While we did detect the presence of λ -cyhalothrin in bee-collected pollen prior to our experimental application of Warrior II in INSC+ fields, indicating that environmental exposure external to the fields or drift within the field may have occurred, there was significantly higher λ -cyhalothrin detects in INSC+ fields post application, suggesting that our treatment created a different exposure scenario compared to INSC- treatments.

3.5. Wild bee communities

Over the three years of the experiment, we collected 1338 wild bees in prairies and assigned these to 92 different species (including 26 *Lasioglossum* sp. morphotypes (St. Clair et al., 2022b) (Table S12). Of those collected, 65 species were identified in Apiary(-) prairies and 75

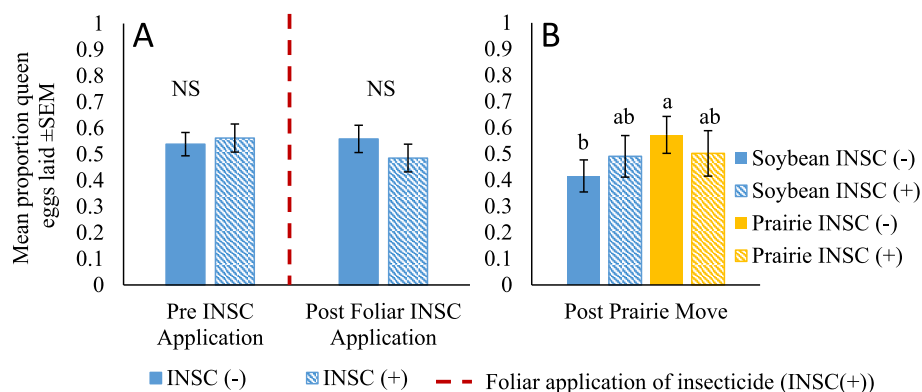


Fig. 4. Queen egg-laying estimates comparing (A) colonies in soybean fields pre- and post- λ -cyhalothrin application as formulated in Warrior II within the INSC- and INSC+ treatments, and (B) soybean fields and prairie sites after movement to the prairies and prior λ -cyhalothrin exposure (INSC-/+). Letters indicate significant differences in mean queen egg laying between treatments $p < 0.05$.

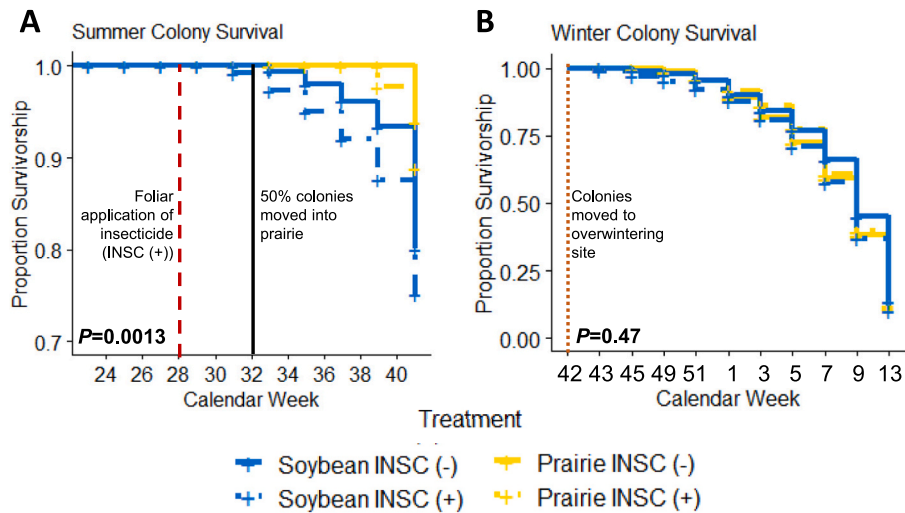


Fig. 5. (A) Proportion summer survivorship (May through October) of honey bee colonies placed in soybeans either sprayed or left untreated from λ -cyhalothrin and with half the colonies either staying in soybeans in the late season or being relocated to a prairie. Survival curves represent survivorship across the summers of 2017–2019 combined. (B) Proportion winter survivorship (October – March) of honey bee colonies after all experimental colonies have been relocated to a single overwintering site. Survival curves represent survivorship across the winters of 2017–2019 combined.

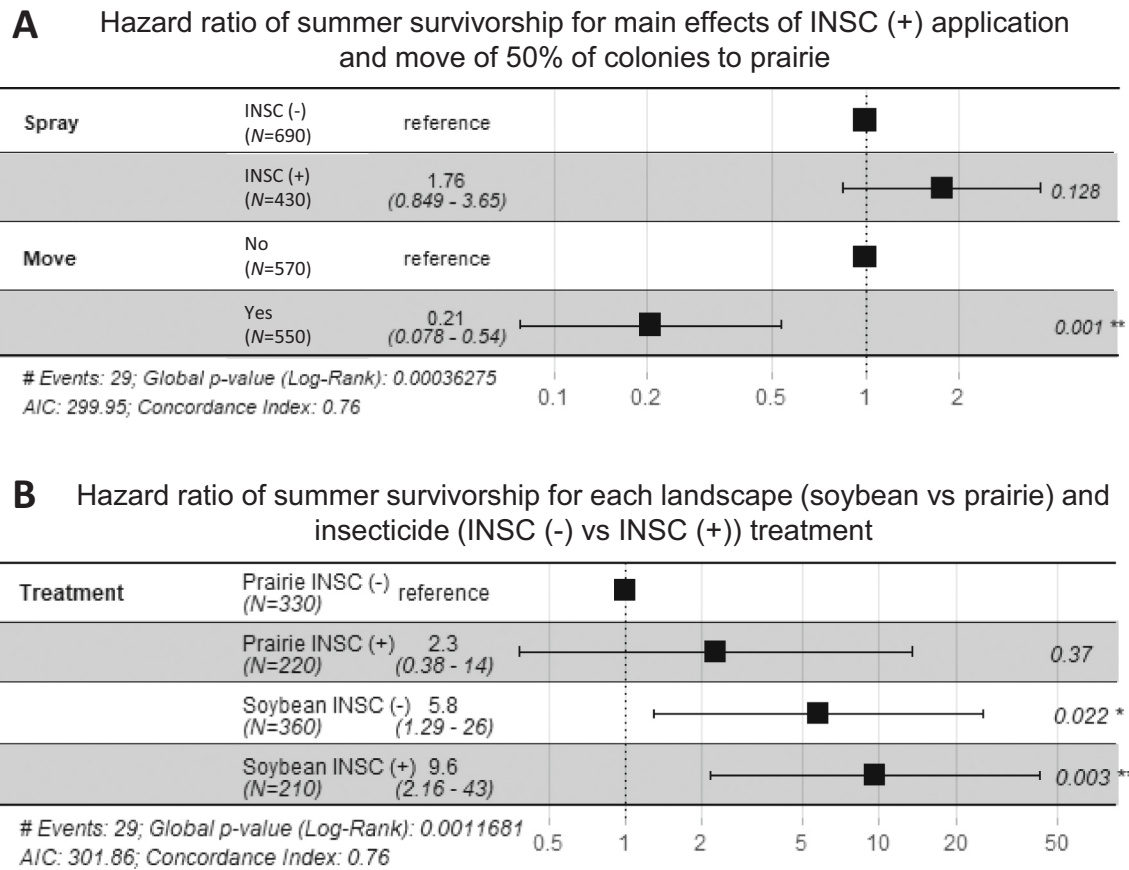


Fig. 6. Cox hazard ratios for the proportion survivorship of honey bee colonies across the summers of 2017, 2018, and 2019. (A) Hazard ratios for the main effects of λ -cyhalothrin Warrior II spray and of prairie move. (B) Hazard ratios comparing each individual landscape and λ -cyhalothrin treatment combination. Prairie INSC-, with the highest proportion survivorship and most favorable landscape conditions, was set as the reference to which all other treatments were compared.

species in Apiary(+). The abundance of honey bees in pan traps was significantly affected by apiary treatment (i.e., Apiary (-)/(+) ($F_{3, 28} = 15.11, p = 0.0006$), movement of the Apiary to prairies in the late season ($F_{3, 28} = 18.71, p = 0.0002$), and interaction of apiary treatment and movement occurred ($F_{3, 28} = 11.15, p = 0.0024$) (Fig. 7A). Placing an

apiary significantly increased the abundance of honey bees captured in pan traps, indicating that the presence of free-flying honey bees in prairies was experimentally increased in the Apiary(+) treatment (Fig. 7A; Table S13). Species richness ($F_{3, 28} = 1.17, p = 0.34$; Fig. 7B) and the abundance of wild bees ($F_{3, 28} = 0.72, p = 0.55$; Fig. 7C) did not

Table 2

Number of positive detections out of total analyzed samples, for λ -cyhalothrin in samples from all three years in either λ -cyhalothrin sprayed (INSC(+)) or not sprayed (INSC(−)) soybean fields. Data were lumped from multiple pre-spray and post-spray collection dates. A full list of individual detects can be found in Table S3.

Year	Pre-spray	Post-spray	Pre-spray	Post-spray
	INSC (−)	INSC (−)	INSC (+)	INSC (+)
2017	9/33 (27.2 %)	0/7 (0 %)	5/14 (35.7 %)	2/3 (66.6 %)
2018	4/8 (50 %)	2/4 (50 %)	3/5 (60 %)	3/4 (75 %)
2019	3/8 (37.5 %)	–	4/8 (50 %)	–
Total	32.7 %	18.2 %	44.4 %	71.4 %

differ significantly between the Apiary(−) and Apiary(+) treatments. Wild bee richness and abundance varied across years, with the highest richness and abundance in 2017 (Fig. S8, Tables S14 – S15). However, we did not observe a treatment by year interaction, and all sites including Apiary(−) sites showed lower metrics in later years, suggesting these were annual (possibly climatic; Zhang et al., 2022) fluctuations that were not driven by multiple years of honey bee presence.

There was no significant difference in the overall dissimilarity of wild bee communities (Bray-Curtis) in the Apiary(−) prairie locations when comparing pre- to post-apiary placement times ($F_{1,14} = 1.58$, $p = 0.11$; Fig. 8A). This is unsurprising, considering we did not place apiaries in these locations. However, we also observed no significant difference between the bee communities pre- and post-movement for the Apiary(+) locations ($F_{1,14} = 1.49$, $p = 0.11$; Fig. 8B). Focusing on just the sampling points after the apiaries had been moved to prairies in the Apiary(+) treatment, we also observed no difference in the dissimilarity in the community in Apiary(−) compared to Apiary(+) locations ($F_{1,14} = 1.49$, $p = 0.11$; Fig. 8C). In terms of prairie forb communities, we documented 25 species of plants within our transects at our prairie sites over two years of sampling (Table S16). There were no significant relationships between forb abundance and forb richness and the abundance/richness of wild bees (graphs and statistical results in Fig. S9).

4. Discussion

This field-scale study provides evidence that a commonly used, broad-spectrum insecticide formulation containing λ -cyhalothrin (Warrior II) can cause delayed detrimental health effects on bees. In the timeframe directly following the application of Warrior II in INSC+ soybean fields, we did not observe any observable differences in colony health or growth compared to untreated fields. Despite no evidence of an immediate impact, colonies from soybean fields treated with Warrior II failed to respond to an improvement in forage availability weeks later, at a critical time in the lifecycle of the colony. Specifically, colonies that resided in fields where Warrior II was applied were less likely to survive the summer compared to other treatments, had significantly higher rates of weight decline compared to those moved to prairies, and failed to receive any boost in late season brood production compared to other treatments.

Previous studies in Iowa, USA show honey bee colonies lose weight after soybeans cease flowering (Dolezal et al., 2019; Zhang et al., 2023). Soybeans are a significant source of nectar for honey bees not only in Iowa, but elsewhere in the US (Lin et al., 2022) and other countries (Blettler et al., 2018). Efforts to address this forage dearth have revealed that several species of plants found in the native biome of the North Central US (i.e., prairie) can rescue honey bees from this potential famine (i.e., prairie rescue effect; Dolezal et al., 2019). In the current study, we found that this rescue failed if colonies had resided in soybean fields with a Warrior II application. In this way, we observed an interaction between insecticide use and the ability to respond to future forage availability. In addition, queens kept in insecticide-treated soybean fields laid fewer eggs when given access to prairies than those from fields that were not treated. This is remarkable as the insecticide was applied

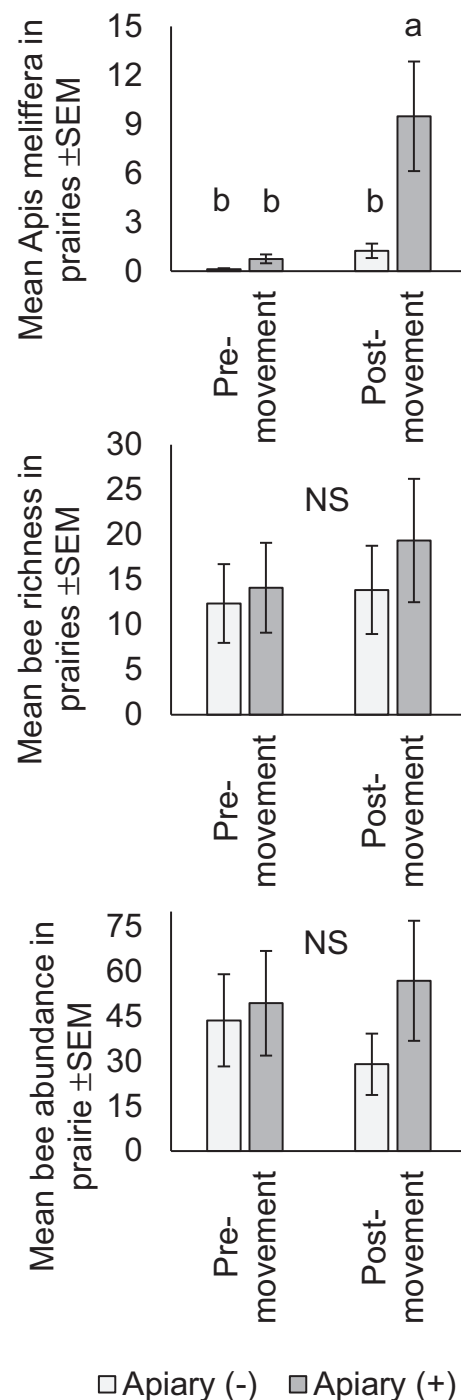


Fig. 7. (A) Mean *Apis mellifera* captured in pan traps over the sampling period (July – Sept.) in Apiary(−) and Apiary(+) prairies prior to and post movement of the apiaries to locations over 2017–2019. Mean wild bee richness (B) and abundance (C) as represented by pan trap captures over the sampling period (July – Sept.) in Apiary(−) and Apiary(+) prairies prior to and post movement of the apiaries to locations over 2017–2019. Letters signify significant differences among treatments, $p < 0.05$.

several weeks before the forage was made available, suggesting prolonged consequences from a discrete insecticide use event (Stuligross and Williams, 2021). We also found Warrior II application combined with lack of late season prairie forage was associated with lower summer colony survival. However, this was not accompanied by detectable reductions in worker population, thus suggesting “sublethal” insecticide effects on the worker level could lead to delayed lethal effects on a

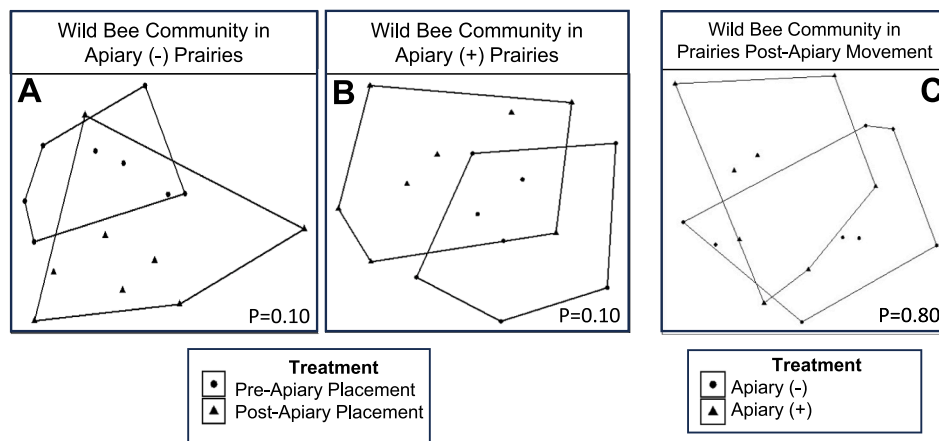


Fig. 8. Nonmetric multidimensional scaling (NMDS) plot of the Bray-Curtis dissimilarities between the wild bee communities prior to and post placing honey bee apiaries in Apiary(-) prairies (A) and Apiary(+) prairies (B) in central Iowa during the summers of 2017–2019. Wild bee communities are based on a 7 week sampling period through the end of July into early September each year. Apiaries were moved to respective sites after the third sampling week (i.e., 3 weeks of prior and 4 weeks of post). (C) NMDS plot of the Bray-Curtis dissimilarities between the wild bee communities during the post-apiary move time period (4 sample weeks) for colonies in Apiary(-) and Apiary(+) treatments over 2017–2019. Significance based on pMANOVA of the dissimilarities for the main effects of apiary placement timepoint (A, B) and apiary treatment (C); $\alpha = 0.05$.

colony level due to compromised queen health or other indirect causes (e.g. reduced immunity). These delayed effects could have been the result of continued/late consumption of contaminated food stores (Traynor et al., 2021), or more immediate health deficits that only became apparent at the colony level on longer time scales.

While we did not directly feed colonies insecticides, or assess individual bees to confirm body burden levels across treatments, we instead opted to test a field-realistic scenario in which exposure to a broadly applied insecticide (λ -cyhalothrin formulated as Warrior II) was likely. We note that colonies could potentially have also been exposed to pesticides in the neighboring landscape matrix (as evidenced by the ~30 % λ -cyhalothrin detected in the pre-spray pollen samples assessed across both treatments). Despite the complexity of this field-realistic experimental design, several lines of evidence lend confidence to the notion that colonies in treated fields were indeed exposed to λ -cyhalothrin. First, we directly quantified λ -cyhalothrin, an insecticide with no persistence in soil or water, immobility in soil, and an on-plant half-life of approximately 5 days (~30 days in soil), in bee collected pollen. We found evidence of increased detection in samples from insecticide treated fields and in samples collected after spraying compared to before spraying. Although mean levels did not differ, this is not unexpected, as previous studies suggest considerable variation in insecticide levels in bee-collected pollen, even in experimental conditions (Pecenka et al., 2021). Thus, it is likely that λ -cyhalothrin application on these soybean fields resulted in the entry of this insecticide at a higher frequency into honey bee hives and honey bee bodies compared to untreated fields. Although we did not test honey bee collected nectar, it could have also been contaminated with λ -cyhalothrin and served as another source of exposure. Second, we found multiple significant interactive health effects after the prairie move, and Warrior II application had a significant negative impact on summer colony survival. Thus, our results suggest nuanced and long-term effects of colonies residing in an insecticide application environment that can affect bee health in ways that are not immediately detectable, even when insecticides are applied according to legal guidelines and regulations. Given these cautionary results, beekeepers may wish to take precautions such as closing their colonies during known insecticide spray periods, and/or place their colonies further from known insecticide treated soybean fields.

Our results suggest that insecticide exposure from field applications may prevent honey bee colonies from realizing the benefits of improved forage and nutrition. The mechanism of this effect is still unknown, but insecticides, including λ -cyhalothrin, and formulated products have

been documented to have non-lethal negative impacts on honey bee immunity and disease resistance (Frizzera et al., 2024), learning and memory (Siviter et al., 2018) (leading to less efficient foraging and reduced honey and pollen collection, (Schneider et al., 2012)), disruptions to the gut microbiome (Al Naggar and Wubet, 2024), and reduced queen reproductive function (reviewed in Tosi et al., 2022). If bees in insecticide-treated soybean fields suffered from such deficiencies at a colony scale, this could lead to a reduced ability to take advantage of prairie forage. For example, if λ -cyhalothrin caused foragers to have reduced lifespan or energetic capacity, learning and memory deficits, and/or lower brood production leading to lower stimulation to forage (Pankiw et al., 1998), these could all result in reduced colony growth (Sagili and Pankiw, 2009) and survival.

In the absence of insecticide use, we replicated the previously demonstrated “prairie rescue” effect (Dolezal et al., 2019) on honey bee colonies, i.e., decreased mortality and increased colony weight and brood, and increased queen egg laying. However, if beekeepers are to take advantage of native vegetation as a source of forage, it is essential to consider potential impacts on the wild bee community that also uses these habitats. Placing non-native, managed bees in areas of restored native vegetation used for wildlife conservation is controversial (Geldmann and González-Varo, 2018; González-Varo and Geldmann, 2018). Two previous studies in Iowa failed to find direct negative impacts of honey bees on wild bee communities in agricultural fields (St. Clair et al., 2022b; St. Clair et al., 2020) and reconstructed prairies (Pritchard et al., 2021). However, these prior studies used small apiaries and were shorter in duration (1–2 years), and did not visit the same locations in recurring years. Nonetheless, the current study also failed to find any negative impacts of apiaries of intermediate size ($N = 8$ colonies) on wild bee abundance, richness, and community structure, and this pattern remained consistent over three consecutive years. Instead, these communities fluctuated substantially across years and seasons, which may be driven by climatic variation or differences in floral resource quantity and quality (Zhang et al., 2022). However, we did not find significant relationships between wild bee communities and prairie forb communities in this study. It is possible trends were not detected because we sampled plants for a relatively short period, or the fact that all restored prairie sites are likely floral resource rich compared to the surrounding agricultural matrix, thus minor variations in the addition of floral diversity/abundance did not lead to significant relationships in our data. Thus, our specific conclusion is that small to medium-sized apiaries, present during the late summer, do not have any noticeable

negative impacts on the wild bee community in this region. However, this conclusion should not be extrapolated beyond the specific scenario outlined—it is still possible that there could be negative impacts on specific wild pollinator taxa, with longer-term honey bee residence and with large apiary sizes. Further, these evaluations should be repeated in different regions and environmental contexts to make informed decisions for sustainable beekeeping and conservation management.

5. Conclusion

Our study suggests a comprehensive protection plan that addresses insecticides and forage is necessary for bees to realize health improvements in an agricultural landscape. The incorporation of native vegetation can be a viable option for honey bee health in agroecosystems, but such efforts may fail if insecticide exposure not also addressed. In addition, our results suggest there is the potential for wild bees and honey bees to coexist and thrive in these environments. Still, results must not be extrapolated indiscriminately—i.e., the current research only explored one of several possible scenarios. Further exploration of how wild and honey bees interact across seasons and different types of environments will be important to inform specific beekeeping and conservation practices. This work outlines the importance of a holistic view of managed and wild bee health that considers multiple stressors' long-term and interactive effects.

CRediT authorship contribution statement

Ashley L. St. Clair: Writing – review & editing, Writing – original draft, Project administration, Investigation, Formal analysis, Data curation. **Adam G. Dolezal:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Randall P. Cass:** Writing – review & editing, Supervision, Investigation, Data curation. **Harmen P. Hendriksma:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation. **David S. Stein:** Writing – review & editing, Investigation. **Kate E. Borchardt:** Writing – review & editing, Investigation. **Erin W. Hodgson:** Writing – review & editing, Funding acquisition, Conceptualization. **Matthew E. O'Neal:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Amy L. Toth:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.178146>.

Data availability

Raw project data are available for public download on the following website: <https://iastate.box.com/s/ptgths8trne7kos5bl47t81mp7lipxdud>.

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