BEE HEATH AND CONSERVATION

by

JENNIFER A BERRY

(Under the Direction of Kris Braman)

ABSTRACT

Bees, both wild and managed, have experienced a significant decline in recent years. Researchers have identified numerous factors contributing to this decline. To address some of these factors, this dissertation examines two approaches to managing bee decline: one on the honey bee species *Apis mellifera,* and the other on restoring wild bee habitats. In the case of *Apis mellifera,* two different methods of applying oxalic acid were studied to reduce populations of *Varroa destructor,* the primary cause of honey bee mortality worldwide. Beekeepers urgently need effective strategies for controlling Varroa. Oxalic acid (OA) has gained popularity with beekeepers in the US due to its ability to kill Varroa while still remaining relatively inexpensive. Our first study examined applying vaporized OA multiple times during the brood rearing season. We found no evidence that frequent periodic applications of OA were effective at reducing Varroa. Our second study investigated using vaporized OA after implementing a forced brood break. By caging the queen for a limited time, all the bees emerged, leaving the colony free of capped brood. We found that combining vaporization with a brood break increased mite mortality by 6 times. Our final study investigated incorporating floral resources, wildflowers, into an erosion control seed mix for wild bee habitat restoration. With habitat loss and fragmentation as the number one reason wild bee populations are in decline, reclaiming and conserving land is one way to provide food and shelter for wild bees. Research has shown that bee friendly landscapes don’t have to reside just in parks or forests, They can include urban and agricultural areas along with eroded lands. Typically, erosion control seed mixes consist of only native and non-native grasses. We compared two plots, one with a traditional grass seed mix and the other plot with wildflowers incorporated into an erosion mitigation seed mix specifically selected for their ability to attract bees, and thrive in the Piedmont region of Georgia. Our results found plots that incorporated wildflowers into the seed mix had a greater abundance, richness and diversity of bees compared to those plots that only contained grass.

INDEX WORDS: *Apis mellifera*, *Varroa destructor*, oxalic acid, bees, pollinators, beekeeping, mites, IPM, conservation, erosion, habitat restoration, wild bees, honey bees

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DEDICATION

I would like to dedicate my dissertation to my dad Lt. Colonel George D. Berry, one of the smartest people I know. It wasn't long after completing my Master's degree that my dad started asking when I was going to enroll in a PhD program. I kept saying I wasn't interested, or it wouldn't do any good. But then one day, two decades later, as I was sitting in a defense, I heard my dad say, you can do this. The next day I asked Dr. Kris Braman, the Entomology Department Head, if she would be my advisor. She immediately agreed. That night I called my dad and told him it was a go! He was very excited and so was I. That was the last time I spoke to my dad. The next day he went unresponsive and passed a few days later. Through his encouragement and gentle nudges over the years, he convinced me it was time to further my education. My dad never gave up on me! I just wish he was here today to witness my graduation.

I am also dedicating my dissertation to my husband Mark R. Davis, who stood next to me during this entire process. It was tough at times, but Mark was there helping me every step of the way. And when I lost my way, he was there guiding me back every time, with assurance, that I was going to make it to the finish line. Mark, you are wonderful and the best husband ever! I could not have completed this without your help. Thank you!

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INTRODUCTION

This dissertation has two separate yet similar parts which both explore one overarching theme; how to help our bees, those wild and those managed.

Wild Bee Conservation

Pollination, the transfer of pollen from one related flower to the another, is a crucial ecological process required by angiosperms (flowering plants) for sexual reproduction (Mitchell et at. 2009). Pollen from the male anther to the female stigma is required in order to set seed and hence reproduce (Holm, 2014). The transfer of pollen can be facilitated by wind, water or animals such as insects, birds, and bats. The color, shape, scent of flowers along with nectar sweetness have evolved over time to entice animal pollinators to do the plant's bidding. Pollination is a vital process linking plants and animals and a remarkable example of co-evolution, but it goes beyond that. Ollerton et al. (2011) estimates that 308,006 angiosperms are pollinated by animals which consists of 87.5% of all flowering plants. Out of the roughly 300 agricultural crops there are more than 80% that benefit from insect pollination, therefore, pollinators, especially bees, are crucial for successful crop production and food security in our global agricultural systems (Allsopp et al. 2008, Buchmann & Nabhan, 1996, Klein et al. 2007, National Research Council, 2007).

The economic values of Ecosystem Services (ES) provided by insects are pollination, pest management, wildlife maintenance and decomposition. Together, these services are estimated to be at least $57 billion in the US alone (Losey and Vaughan, 2006), where the economic value on pollination is estimated per year at $14.2-23.8 billion in the US (Degrandi-

Hoffman et al. 2019, Jordan et al. 2021) and $235-577 billion worldwide (IPBES, 2016). Pollination is one of the most important and highly economically impacting of the ES provided by insects. This clearly justifies conservation measures for the ecological roles they provide (Losey and Vaughan 2006).

In the United States and Canada, there are roughly 4000 species of bees grouped into 111 genera and six families. Bees come in all different shapes and sizes, from the smallest bee, *Perdita* measuring less than 0.1 inch, to our large 1+ inch long Carpenter bee, *Xylocopa,* (Wilson and Carril, 2016). Bees are sometimes confused with wasps but they differ, most notably because of their diets. Wasps are carnivores whereas almost all bees are vegetarian. Bees rely on flowering plants for sustenance and a number of flowering plants rely on bees for pollination. Without one the other wouldn’t exist. But bees are not providing this service for free. Flowers offer nectar and pollen as rewards, which many species rely on as one of or their only food source. Without these resources, many bees and other pollinator populations would decline (Kearns et al. 1998) and without the bees and other pollinators, ecosystems would suffer.

In recent decades, however, evidence is mounting that the health, diversity and abundance of wild bees are declining in North America and Europe (Goulson, Nicholls, Botías, & Rotheray, 2015). There are a number of reasons for the decline such as pesticides, pollution, invasive species, climate change and diseases (Kearns et al. 1998, Wagner et al. 2018), but habitat loss and fragmentation is by far causing the biggest impacts on bees (Decourtye et al. 2019, Potts et al. 2016). Habitat richness and reduction is due to growing human activities such as converting natural land for agriculture and grazing, harvesting of timber and commercial and residential development (Scanes, C. G. 2018), all of which reduces the area bees need for food, water, shelter, mating and nesting.

This evidence of bee decline has not been limited to the scientific community. It has also reached the public audience through countless articles and stories produced by the media. Therefore, public interest and awareness about the protection of bees and other pollinators are on the rise (Schatz et al. 2020). One critical step in slowing the decline of bees due to habitat loss is conservation and restoration of land (Winfree et al. 2009) that provides an overlapping food source throughout their active periods, an area to nest, and protection from pesticides. Finding such areas may be as easy as looking in one's own yard. Urban settings which provide floral resources can be successful environments for sustaining pollinator populations (Baldock et al. 2019, Braman & Griffin, 2022). Fielder et al. (2012), showed a 10-fold increase in bee abundance and richness due to pollinator habitat restoration impacts in an urban community. Studies have also shown the effectiveness of habitat restoration for bees in agricultural systems (Buri et al. 2014, Dicks et al. 2010, Kremen & M'Gonigle, 2015, M'Gonigle et al. 2015, Sutter et al. 2017). These studies also highlight how cities are providing refuge for bees even though their populations are in decline (Hall et al. 2017). Therefore these landscapes must not be ignored as potential habitat for pollinators.

Yet, urban and agricultural arenas have unique conservation challenges due to management practices. Periodic insecticide and herbicide applications can have negative effects on bees and don't always act alone. Goulson et al. (2015) stated that "pesticide exposure can impair both detoxification mechanisms and immune responses, rendering bees more susceptible to parasites and other diseases". Bee abundance was also shown to decline 41% with each ˚C of urban warming: these declines occurred regardless of floral density (Hamblin et al. 2018).

Other conservation challenges in urban settings are homeowner perceptions concerning possessing perfectly manicured turf lawns. Turfgrass is the most dominant vegetation in urban areas, and the number one irrigated crop in the US (Milesi et al. 2005). The vast amount of land consumed by residential yards in US cities account for 25-30% of total land and up to 50% of green spaces (Ignatieva et al. 2020, Minor et al. 2016) yet, they offer little in the way of forage opportunities for bees and other pollinators (Tonietto et al. 2011). Although grasses are largely wind-pollinated, there is minimal evidence of bees and other pollinators foraging on grass inflorescences. (Joseph et al. 2020).

Suitable nesting sites can also be a limiting resource for bee abundance and diversity in urban lawn settings. Forested or natural undisturbed areas typically supply nesting habitat for many bees with fallen dead wood, bare ground, twigs and other materials for cavity and soil nesters (Roberts et al. 2017, Van Den Berge et al. 2019). Tolerating some degree of "messiness" in yards and gardens (allowing bare patches of soil and leaving dead wood and senescent flower stems for nesting bees) is not often widely accepted by homeowners and lawn care practitioners.

Providing native bee habitat on farms and roadsides offer other opportunities for conservation. Establishment of flower-rich habitat within or around intensively farmed landscapes to increase the availability of pollen and nectar resources can include cover crops, field borders, shrubby hedgerows, and grass buffer strips (used to manage erosion and nutrient runoff) which are supplemented with flowers. Stabilization mixes are often comprised solely of native and non-native grasses which offer little in the way of food for bees. Recouping and seeding eroded land with wild flower material, included in stabilization mixtures, can provide an excellent opportunity to enhance pollinator habitat, and also benefit other ecosystem services (Wratten et al. 2012). Presently, there is a dearth of information and availability of locally adapted stabilization mixes that incorporate floral resources for pollinator conservation efforts. The objective of our wild bee habitat restoration study was to investigate the inclusion of wildflowers into a soil erosion mitigation mix to attract wild bees.

Honey Bee Health

The ES provided by managed bees has become increasingly important as the world population expands. The Western Honey Bee, *Apis mellifera,* has become the most commonly used commercial insect pollinator in the world (vanEngelsdorp and Meizner, 2010). One reason for their success lies in the ability of humans to manage them (Kearns et al. 1998). Most managed *A. mellifera* colonies are housed in hives, which can easily be transported in large numbers from one location to another or from crop to crop when pollination is required (Saex et al. 2020). Another part of their success as pollinators is that *A. mellifera* is a generalist and can thereby feed on a variety of flowering plants that benefit or rely on insects for pollination (Allsopp et. al. 2008, Aizen et. al. 2009). Pollination services are important on both a local and a global scale. To the farmer, it supports the income derived from the harvest, whereas on the global scale it is important for food security. However, in recent years populations of *A. mellifera,* along with a whole host of other pollinators, have seen a gradual, yet steady decline. This has led to increasing concerns about the stability of agriculture in the US and the world.

In the last decade, the American beekeeping industry has experienced increased colony mortality (vanEngelsdorp et al. 2017) with an annual loss of 43.7% (Bruckner et al. 2020). Causes for this decline include abiotic and biotic stressors such as intensification of agriculture, pesticide use, urbanization, nutrition, parasites and diseases (Allen-Wardell et al. 1998, Rosenkranz et al. 2010, Kevan and Phillips 2001, Zhang and Nieh 2015, Pettis et al. 2016, Branchiccela et al. 2019, Harvard et al. 2020). However, the main cause of colony mortality is due to one biotic stressor, the exotic, ectoparasitic mite, *Varroa destructor* (Rosenkranz et al. 2010).

V. destructor's original host is the Eastern Honey Bee, *Apis cerana* (Beaurepaire et al. 2015). Evolving together, an inter-specific co-adaptation between parasite and host developed and no serious damage to either species (mite or bee) occurs, (Rath, 1999) nor is treatment required. This balanced host-parasite relationship does not exist between *V. destructor* and its new host *A. mellifera*. The first report of a host shift occurred in 1957 when *V. destructor* was discovered in colonies of *A. mellifera* in Japan (Sakai and Okada, 1973). Less than two decades later, mites appeared in Europe and by the 1980's they had landed on American soil (Griffiths and Bowman, 1981). Today they are found almost everywhere in which *A. mellifera* reside. Once *V. destructor* jumped from its native host to *A. mellifera*, honey bees' and beekeeper's lives have changed forever. Since *A. mellifera* did not evolve alongside *V. destructor*, no defensive strategies to keep varroa populations from reaching damaging levels have developed, thereby making *V. destructor* the greatest threat to apiculture.

V. destructor cannot survive on its own and is intimately linked to the life cycle of honey bees (Eastern and Western). *V. destructor* has two distinct life stages: phoretic and reproductive. The phoretic stage (more recently coined, the dispersal phase), occurs when mites roam free around the colony and feed or hitch a ride on its adult host. The second stage which occurs within the sealed, capped cells of drones and workers, is the reproductive stage (Boecking et al. 2008, Mondet et al. 2018, Roth et al. 2020). It is during this phase that *V. destructor* adults and young feed upon the fat bodies of the developing immature bee, vectoring debilitating viruses (Ramsey et. al. 2019). These viruses are the root cause to colony collapse of *A. mellifera* colonies (Francis et al. 2013). The fat bodies are extremely important organs and function for insect immunity, and the storage and regulation of metabolites (Raikhel et al. 1997, Arrese and Soulages, 2010, Ramsey e. al. 2019). Feeding on the fat bodies by *V. destructor* also results in reduced weight, size, longevity, anatomical deformities and immunosuppression of individuals (Bowen-Walker and Gunn, 2001, Annoscia et al. 2012, 2019, Dainat et al. 2012, Noel et al. 2020).

Because *A. mellifera* has no developed resistance, populations of *V. destructor* can increase rapidly, thereby severely weakening colonies and ultimately causing colony death if left unmanaged by the beekeeper. (Guzman-Novoa et al. 2010, Kulhanek et al. 2017). Due to the damage caused by these mites, beekeepers rely on Integrated Pest Management (IPM) techniques to keep infestation at non-damaging levels. (Delaplane et al. 2005). IPM strategies begin with prevention methods, such as cultural practices utilizing screen bottom boards or drone brood trapping, using tolerant stock or initiating a brood break to reduce *V. destructor* reproduction (Gregore et. al. 2017, Panziera et. al. 2017, Roth et. al. 2020). However, these methods usually only delay the inevitable colony collapse, so stronger control options in the way of chemical treatments must be utilized. Chemical treatments are part of IPM but relying solely on these applications has led to wide spread resistance to several synthetic compounds, especially coumaphos and fluvalinate (Elzen et al. 2000, Sammataro et al. 2005, Rinkevich 2020).

There are a number of natural compounds available to beekeepers for *V. destructor* control (US EPA 2016). One such compound is the essential oil, thymol, which does reduce *V. destructor* populations, but due to temperature restrictions and brood issues, it limits its effectiveness. Another natural product is oxalic acid (OA). This organic acid naturally occurs in nectar, has low probability of *V. destructor* developing resistance and has high efficacy against *V. destructor* (Bogdanov et al. 2002, Rademacher and Harz 2006, Toufailia et al. 2015, Adjlane et al. 2016). Widely used for decades in the UK, OA has just recently become popular in the United States and wasn't registered for use until 2015 (US EPA 2015). The current label includes two methods of application: trickling which is the application of OA dissolved in a sugar solution and vaporization which uses a heating device to transform the crystallized OA into a gas which is then applied to a colony (Rademacher and Harz 2006, US EPA 2015a). Even though these treatments are highly effective at killing *V. destructor* on contact, OA does not penetrate the wax cap within the brood cell where the majority of *V. destructor* resides (Adjlane et al. 2016, Ramsey et al. 2019). The best time to apply OA, and reduce *V. destructor* populations, is when the colony is “broodless” and all the mites are phoretic.

Of the two OA application methods, vaporization of OA is highly effective against *V. destructor* and has shown to be relatively safe to *A. mellifera* queens, workers and brood (Rademacher and Harz 2006, Rademacher et al., 2017). The label permits beekeepers to use OA vaporization when brood is present and “human consumable” honey is on the colony (US EPA 2015). One common heating device’s manual recommends vaporizing with OA four times, every five days (OxaVap, 2020). The concept behind this treatment schedule is to coincide with *V. destructor* emerging out of the brood cell alongside its *A. mellifera* hosts. It has become a widely used treatment protocol among mostly backyard beekeepers, however bees are still dying. The primary objective for our first two studies was to further investigate the best approach for vaporizing with OA to reduce *V. destructor* populations within *A. mellifera* colonies.

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CHAPTER 2

ASSESSING REEPEATED OXALIC ACID VAPORIZATION IN HONEY BEE (*APIS MELLIFERA*) COLONIES FOR CONTROL OF THE ECTOPARASITIC MITE *VARROA DESTRUCTOR*

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Abstract

The American beekeeping industry continually experiences colony mortality with annual losses as high as 43%. A leading cause of this is the exotic, ectoparasitic mite, *Varroa destructor*. Integrated Pest Management (IPM) options are used to keep mite populations from reaching lethal levels, however due to resistance and/or the lack of suitable treatment options, novel controls for reducing mites are warranted. Oxalic acid for controlling *V. destructor* has become a popular treatment regimen among commercial and backyard beekeepers. Vaporizing oxalic acid crystals inside a colony is a current legal application method in the USA, where mites exposed to the oxalic acid vapor die. However, if mites are in the reproductive stage and therefore under the protective wax capping, oxalic acid is ineffective. One popular method of applying oxalic is vaporizing, multiple times over several weeks to try and circumvent the problem of mites hiding in brood cells. By comparing against control colonies, we tested oxalic acid vaporization in colonies treated with seven applications separated by five days (35 days total). We tested in apiaries in Georgia and Alabama during 2019 and 2020, totaling 99 colonies. We found that adult bees, and developing brood experienced no adverse impacts from the oxalic vaporization regime. However, we did not find evidence that frequent periodic application of oxalic during brood-rearing periods is capable of bringing *V. destructor* populations below treatment thresholds.

Introduction

The western honey bee, (*Apis mellifera* L.), is a ubiquitously used insect pollinator of many agricultural crops around the world (Allsopp et al. 2008, vanEngelsdorp and Meizner, 2010) and the economic services provided by these managed bees has become increasingly important as world population expands (Degrandi-Hoffman et al. 2019). However, in recent years, populations of *A. mellifera* have seen a gradual, yet steady decline (Kulhanek et al. 2017, Potts et al. 2010, Spivak et al. 2011, vanEngelsdorp et al. 2017) with the American beekeeping industry experiencing annual losses of 43.7% (Bruckner et al. 2020). There are a number of drivers involved in colony loss, with the ectoparasitic mite *Varroa destructor* among the most important (Guzmán-Novoa et al. 2010, Le Conte et al. 2010, Rosenkranz et al. 2010).

To date there are three synthetic acaricides (amitraz, coumaphos and fluvalinate) approved for use against *V. destructor* in the USA (US EPA, 2016), but due to sub-lethal effects on honey bees along with rapidly evolving resistance in *V. destructor* (Berry et al. 2013, Elzen et al. 2000, Mathieu and Faucon, 2000, Rodríguez-Dehaibes, 2005, Rinkevich, 2020, Sammataro et al. 2005, Thompson et al. 2002), there is a need for additional efficacious active ingredients. Because of this, beekeepers have employed integrated pest management (IPM) techniques instead of relying on a single method of control. Beekeepers embracing IPM use a variety of approaches to try and keep colonies from succumbing to the detrimental effects caused by *V. destructor* (Delaplane et al. 2005). However, adequate control of this pest remains a serious challenge for many US beekeepers and chemical acaricides are still necessary as part of IPM frameworks in this system.

Non-synthetic compounds such as formic acid and thymol are effective at controlling *V. destructor*; however, their effectiveness is dependent on ambient conditions. For example, they are not effective when temperatures are too low and may kill adult and developing bees when temperatures are too high (US EPA 2019, US EPA, 2020). Another widely adopted natural compound is crystalline oxalic acid (OA) dihydrate. This organic acid naturally occurs in nectar, has putatively low likelihood of inducing *V. destructor* resistance on account of no resistance yet being observed in treated populations compared to naïve ones despite years of continuous use (Maggi et al. 2017), and has high efficacy against *V. destructor* (Adjlane et al. 2016, Bogdanov et al. 2002, Rademacher and Harz, 2006, Al Toufailia et al. 2015) in certain circumstances. Widely used for decades in Europe, (Popov et al. 1989), OA has only recently been popularized in the US and wasn't registered for legal use until 2015 (US EPA 2015). One method for applying OA is to heat the crystals using a vaporizer, creating gaseous OA that permeates the colony (Rademacher and Harz 2006, US EPA 2015). Even though these treatments are highly effective at killing *V. destructor* on contact, OA does not penetrate the wax-capped brood cells where the majority of *V. destructor* reside (Rademacher and Hartz, 2006, Rosenkranz et al. 2010). Therefore, the best time to apply OA and reduce *V. destructor* populations is when colonies are broodless, without developing larvae (Charrière and Imdorf, 2002, Gregorc and Planinc, 2001, Gregorc et al., 2016, Gregorc et al., 2017), rendering all mites phoretic on adult bees and vulnerable to the fumigant (Rademacher and Harz, 2006). However, brood-free intervals are brief or absent altogether in some warm latitudes, raising the need for alternative treatment schedules.

For treating during periods of brood rearing, instructions for one commercial vaporizer, the ProVap 110, calls for four treatments with five days between each treatment. The rationale for this 19-day interval being that this schedule exposes an entire cohort of mites bound in worker brood as the mites successively emerge with their parasitized hosts. This multiple treatment regimen has gained popularity in commercial and hobby beekeeping operations. However, the protocol has not been shown effective.

The objective of this study was to test the efficacy of a regimen of repeated OA applications against *V. destructor* during periods of brood rearing. A secondary objective was to determine if these repeated OA applications are measurably detrimental to adult bees and brood (proxies for colony viability). We hypothesized that a repeated OA treatment regimen would have a negative effect on *V. destructor* abundance while having no negative effect on *A. mellifera* colony strength, in agreement with prior demonstrations of its relative safety (Rademacher and Harz 2006).

Materials and Methods

Experimental design

Experimental *A. mellifera* colonies were established in the summer months of 2019 & 2020 and maintained in two deep Langstroth hives on research lands maintained by the University of Georgia (UGA) Bee Lab in Watkinsville, GA and the Auburn University Bee Lab in Auburn, AL. Queens, with no specific genotype, were purchased from a commercial operation in North Georgia. Prior to the beginning of the experiment, colonies were assessed and only those that were healthy with productive queens were included. Colonies were not manipulated to be 'standardized' in size or brood area beyond all being maintained with equal hive space, so as to accurately capture the variation in colony metrics observed in real apiaries.

In 2019, 13 experimental colonies were set up at the UGA Bee Lab whereas in 2020, fifty-six experimental colonies were set up at the UGA Bee Lab and 30 at the Auburn Bee Lab. All colonies had naturally occurring *V. destructor* mite infestation levels (median field-occurring PMI values at the start of each experiment: Auburn20 = 4.3; UGA19 = 5; UGA20 = 2.2). Colonies were randomly assigned to one of two treatment groups: (1) vaporized with 1g/super of OA every five days for seven applications (=7 treatments spread over 35 d) or (2) an untreated control group. The seven application regimen on days 0, 5, 10, 15, 20, 25, & 30 was chosen in order to capture both worker (21 day) and drone (24 day) developmental times.

Oxalic Acid application

OA application was administered to colonies by crystal vaporization according to label instructions of the registered product (US EPA 2015) and the user manual for the ProVap 110 Vaporizer (OxaVap, Manning, SC). Prior to vaporization and to ensure that vaporized gas would not leak from the hives, colony entrances were sealed with blue shop towels or duct tape, and screened bottom boards were sealed using corrugated plastic boards. Powered by a Champion 2000-watt gasoline generator, the vaporizer device, a Pro VAP 110, was inverted and the chamber bowl heated to 230°C. One gram of solid OA dihydrate crystals per deep brood box was placed into the separated Teflon lid and inserted into the chamber. Turning the device right side up caused the OA crystals to fall into the heated vaporizer chamber thereby generating gaseous OA. The nozzle of the device was inserted into the entrance of each UGA colony or into a pre-drilled hole in the bottom brood box of each Auburn colony, where it remained for 30 seconds to ensure that the full dose was vaporized and delivered into the colony. Once completed, the device was removed and shop towels and plastic corrugated boards left in place for an additional 10 minutes per hive. For the safety of all persons applying the OA, full face respirators with OV/P100 cartridges were worn.

Varroa destructor abundance

At the beginning (D0), mid (D21) and end of the experiment (D42), *V. destructor* levels were determined by alcohol washes. For each colony, ~ 300 adult bees were collected from the brood nest (Dietemann et al. 2013) and placed into a Varroa EasyCheck device (Mann Lake, Hackensack, MN) filled with 70% ethanol, which euthanizes adult bees and phoretic *V. destructor*. The container was sealed and shaken for 60 seconds to dislodge *V. destructor* from the adult bees. The adult bees were removed from the container by lifting out the mesh basket, any dislodged individual *V. destructor* counted, recorded and discarded. The mesh basket of bees was returned into the container, agitated for an additional 60 seconds, removed and *V. destructor* counted, recorded and discarded. This process was repeated until no *V. destructor* were recorded for two consecutive washes. For the Auburn 2020 experiments, each sample of adult bees was weighed. A subsample of 100 ethanol-drenched adult bees was then counted and weighed as a standard, and from this the expected number of bees in each sample was estimated (Dietemann et al. 2013). *V. destructors* per bee was calculated by dividing total *V. destructor* count for any given sample by the estimated number of adult bees in that sample. For the UGA 2019 and 2020 experiments, the number of bees in each alcohol sample were counted by hand, giving an exact number of adult bees per sample for calculating *V. destructor* per bee estimate.

Colony strength

For the 2020 experiments, colony strength variables were measured for each colony at the beginning and end of each experiment by two independent observers who visually estimated percent area coverage of adult bees, developing bees (capped brood), and honey on every hive frame following Delaplane et al. (2013). The total estimate for adult bee population, capped brood, and capped honey (measured in ‘full frames’) was then calculated for each colony and used in analysis.

Statistical analysis

Percent mite intensity (PMI) was calculated by measuring mites-per-bee (divided the number of mites found in a sample by the number of bees in that sample as above), and scaled this up to expected number of mites found on 100 bees from that sample. We then calculated the change in PMI for each colony between the start and end of the experiment (ΔPMI) by subtracting the PMI from the pre-treatment sample from the PMI of the post-treatment sample, giving a single ΔPMI for each colony. We calculated similar, if simpler, metrics for change in estimates of capped brood, stored honey, and adult bee population. We avoid confounds of any differences in average sizes or infestation levels between colonies randomly assigned to control or treatment by focusing on these 'change' values rather than simply comparing colony end-point measures.

All data manipulation and analyses were undertaken in the programming language R (R Core Team, 2020) version 3.6.3. We made the full analysis available as a repository on GitHub (https://github.com/LBartlett/VRTT-OA-Sublimation.git). We analyzed the data using a generalized linear mixed modelling framework (GLMMs) to account for the crossed or nested structure of repeating the experiments in multiple sites and/or apiaries and across multiple years. We used the 'afex' package (Singmann et al. 2020) which wraps around the 'lme4' package (Bates et al. 2015) to undertake type-III ANOVAs following a Kenward-Roger approximation (see afex package documentation) on linear mixed models, in order to test for significant effects of treatment on the response variables. Models were visually inspected for suitability of fit by graphically examining the distribution of residuals and residual qq-plots. Where appropriate, we used the 'emmeans' package (Lenth, 2020) for estimating approximate effect sizes and confidence intervals for plotting data.

Results

For testing the efficacy of repeated 1g oxalic acid sublimation in treating for *Varroa destructor*, we analyzed data from all sites across both years using a linear mixed model with ΔPMI as the response variable, treatment as a fixed effect, year as a standalone random effect and site and apiary as nested random effects to reflect the spatial structuring of the field trials. We found a significant difference in ΔPMI values between control and treated colonies ($F_{1,88.99}$ = 9.16, p = 0.003); across the study (35 days) a typical control colony showed an increase in PMI of 4.4 (±2.6 SE), whereas a typical OA treated colony showed a very small decrease in PMI of -0.7 (±2.5SE). As shown in Figure 1, treated colonies remained at the same PMI after treatment as before (no significant change in PMI, see prior quoted effect size estimates with standard errors spanning zero).

As there was no meaningful difference in mite loads between and pre- and post-treatment treated colonies (Figure 1 – UGA 2020), there is no confounding by which *V. destructor* control indirectly improved colony health by masking or compensating for toxic effects of oxalic acid sublimation. For testing the effects of oxalic acid vaporization on overall colony health, we therefore present a detailed analysis of the UGA 2020 data (see Figure 2) as we did not gather detailed colony health data for the UGA 2019 trial data set, and the Auburn 2020 data set was more confounded by a difference in mite control between the two treatments (which will impact colony health, masking possible negative effects of the oxalic acid which compromises assessing safety) compared to the larger UGA 2020 data. Furthermore, this data set was the most replicated experiment, across the three apiaries, and represented the majority of the data.. We used a mixed modelling framework as above where response variables were either change in brood area, change in bee population, or change in honey stores, fixed effect was treatment, and random effect was apiary (yard). We found no significant differences in changes in brood ($F_{1,\ 52.06} = 0.39$, $p = 0.534$), bees ($F_{1,\ 51.23} = 0.20$, $p = 0.653$), or honey stores ($F_{1,\ 51.20} = 2.30$, $p = 0.136$) based on treatment.

Discussion

On average, after 35 days colony *V. destructor* numbers were significantly higher in non-treated controls compared to OA-treated colonies. However, this effect is wholly explained by a small *V. destructor* increase in controls while *V. destructor* levels remained unchanged in OA-treated colonies (Figure 1). OA did not reduce *V. destructor* numbers; at best, it held them static.

This effect is similar to those found by Jack et al. (2020, 2021) in which one and three applications of 1g of vaporized OA / super were also ineffective at significantly reducing *V. destructor* infestation levels while brood was present. Additionally, we observed that multiple treatments vaporizing with OA had no significant effects on overall *A. mellifera* adult bees, brood, or stored honey quantity.

Until now, there has only been anecdotal evidence that the recommended vaporizing with OA four times, 5 days apart, results in controlling *V. destructor.* Other studies that have examined the effect of repeated applications of the labeled rate of OA, either by liquid trickling or vaporization, have not shown OA to be effective during the brood rearing season (Gregorc et al. 2017, Jack et al. 2020, 2021). Studies that examined vaporizing with OA during broodless periods have documented good control of *V. destructor* (Rademacher and Harz, 2006), and for higher doses of 2.25g permissible outside the US we point to Al Toufailia et al. (2015, 2018) who also demonstrated efficacy of OA in the absence of brood by trickling, spraying and vaporizing.

Correspondingly, our first question was simply whether *V. destructor* infestation levels would be affected by the repeated OA vaporization treatment despite brood being present. Figure 1 depicts how change in percent mite infestation remained static, hovering around zero (ΔPMI in treated colonies -0.7 (±2.5 SE)). As expected, *V. destructor* levels in control colonies did increase +4.4 (±2.6 SE); hence there was a significant difference between the control colonies and those treated, with OA. However, for a *V. destructor* treatment to be successful, especially when treating colonies that have exceeded the treatment threshold as part of an IPM approach, *V. destructor* infestation must be lowered significantly and not simply remain the same. This raises the question, if colonies are treated with vaporized OA, multiple times, well before *V. destructor* levels reach the treatment threshold, can suitable *V. destructor* control be achieved (explaining anecdotal evidence from beekeepers). It is also a question whether 1 g OA / super is an effective dose. Al Toufailia et al. (2015) working in the United Kingdom found that vaporizing with 4 times the US-label rate of 1 g per brood box resulted in a 98.2% reduction in *V. destructor* levels. Recently, Jack et al. (2021) demonstrated in Florida that colonies vaporized with 4 g of OA while brood is present had significantly lower infestation levels of *V. destructor* than those vaporized with only 1 g per brood box. Future studies could investigate the efficacy of increased doses of OA on reducing *V. destructor* population levels.

It was already widely known that the most desirable time to treat with OA is when colonies are broodless (Charriere & Imdorf, 2002, Gregorc & Planinc, 2001, Gregorc et al., 2016, Gregorc et al., 2017). Unfortunately, vaporizing with OA does not penetrate the wax capping of the brood cell where *V. destructor* is reproducing (Rademacher and Harz, 2006, Rosenkranz et al. 2010) and likely accounts for much of the variance in reported success with OA. Broodless periods naturally occur when the queen seasonally stops laying eggs. Future research should investigate the practicality and effectiveness of forcing or exploiting brood breaks as part of management (Jack et al. 2020). This may be possible by caging the queen, and may be a promising avenue of future research, but this is not always convenient or possible for many beekeepers especially at commercial scales. It may also be possible to exploit brood breaks that occur incidentally as part of normal management such as making splits or requeening.

Our second objective was to determine if multiple applications of OA in a colony have measurable effects on adult bees, brood, and stored honey amounts. The overall higher exposure of OA to the colony could plausibly lead to detrimental effects, especially for developing brood (Gregorc et al. 2004, Hatjina et al. 2005, Higes et al. 1999, Terpin et al. 2019). Our results showed no significant differences in changes in adult bees, brood or stored honey when colonies were exposed to OA. This supports previous studies with gaseous OA (Jack 2020, 2021, Al

Toufailia et al. 2015). Our UGA 2020 data set is well suited for inferring the safety (or lack thereof) as the unchanging *V. destructor* parasitism levels in control and treated populations (Figure 1) removes potentially confounding effects of OA mitigating effects of the parasite. We consider our results here to be among the strongest demonstrations of the relative safety of OA to *A. mellifera*. Perhaps, future experiments may want to explore the long-term effects and over wintering ability of colonies after being treated with oxalic acid.

Based on our results, we do not recommend employing this method for controlling *V. destructor* when brood are present, especially as a summer or fall treatment option when infestation levels are at or above the treatment threshold in an IPM framework. Even though there was a difference between control and treated groups, colonies vaporized with OA multiple times did not experience a reduction in *V. destructor* infestation levels, and so treatment was ineffective by common standards. It is important for beekeepers to adopt reliable and effective treatment regimes along with realistic, IPM approaches to sustainably reduce infestation levels of *V. destructor*. In 2020 and 2021, two studies which vaporized OA, were successful in significantly reducing *V. destructor* populations. Büchler et al. (2020) vaporized with 2g of OA while incorporating a brood break and Jack (2021) vaporized with increased amounts of OA (2g & 4g) while brood was present. Because of these results, one future study could be to investigate vaporization with increased doses of OA, in conjunction with and without a brood-break.

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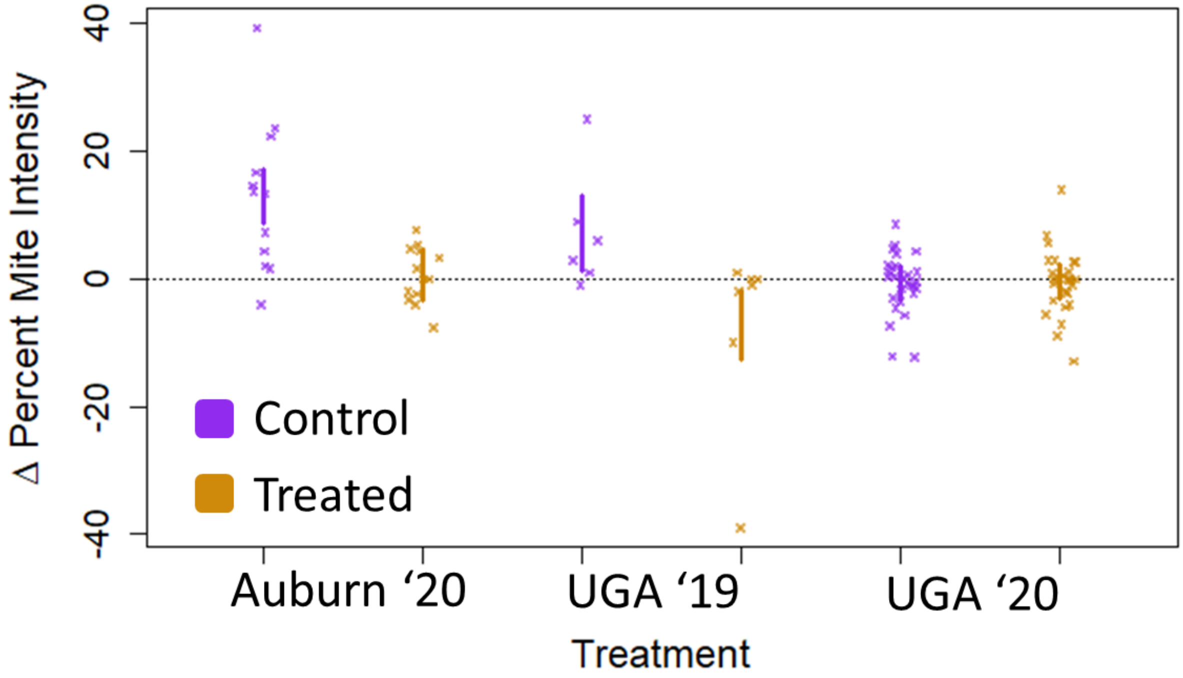

Figure 2.1 Comparison of ΔPMI by treatment across two locations and two years. Each point represents a single colony, and points are plotted alongside 95% confidence intervals, estimated from naïve linear models (note these naïve regressions are not used for statistical analyses).

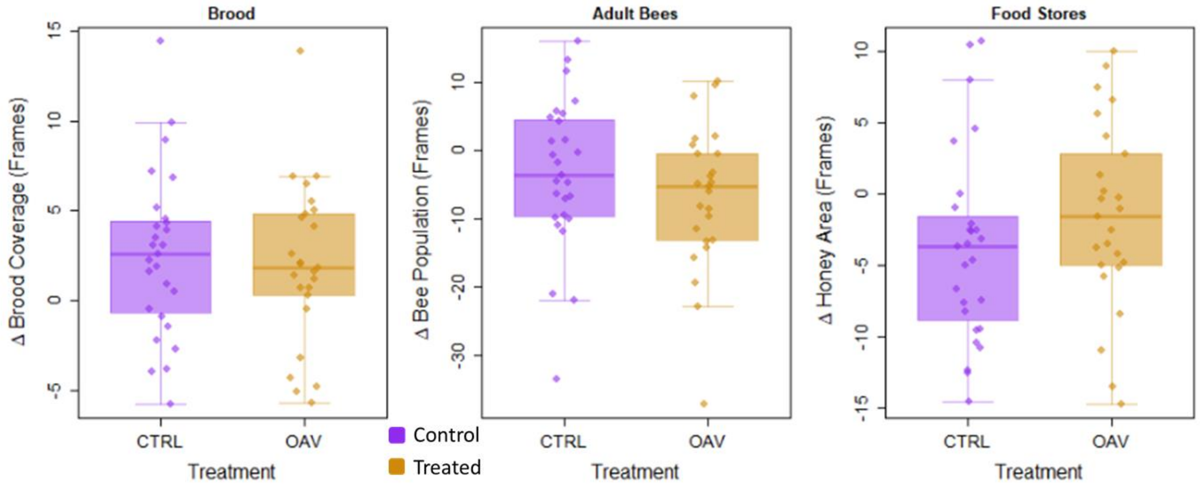

Figure 2.2 Changes in colony health metrics from the UGA 2020 data grouped by treatment. Brood coverage, adult bees, and honey stores for each colony were estimated (units: standard Langstroth deep frames) before and after the experimental period and the change (delta value) calculated for plotting here and mixed modelling analysis.

CHAPTER 3

INDUCING A SUMMER BROOD BREAK INCREASES THE EFFICACY OF OXALIC ACID VAPORIAZTION FOR *VARROA DESTRUCTOR* (MESOSTIGMATA: VARROIDAE) CONTROL IN HONEY BEE, *APIS MELLIFERA* (HYMENOPTERA: APIDAE) COLONIES IN THE SOUTHEAST US.

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[1] Berry et al. 2023. *Journal of Insect Science*. 22(1), 15.
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Abstract

The ectoparasitic mite, *Varroa destructor* (Anderson and Trueman), is the leading cause of western honey bee colony, *Apis mellifera* (L.), mortality in the United States. Due to mounting evidence of resistance to certain approved miticides, beekeepers are struggling to keep their colonies alive. To date, there are varied but limited approved options for *V. destructor* control. Vaporized oxalic acid (OA) has proven to be an effective treatment against the dispersal phase of *V. destructor* but has its limitations since the vapor cannot penetrate the protective wax cap of honey bee pupal cells where *V. destructor* reproduces. In the Southeastern US, honey bee colonies often maintain brood throughout the year, limiting the usefulness of oxalic acid. Prior studies have shown that even repeated applications of OA while brood is present are ineffective at decreasing mite populations. In the summer of 2021, we studied whether incorporating a forced brood break while vaporizing with OA would be an effective treatment against *V. destructor*. Ninety experimental colonies were divided into two blocks, one with a brood break, the other no brood break. Within the blocks, each colony was randomly assigned one of three treatments: no OA, 2g OA, or 3g OA. The combination of vaporizing with OA and a forced brood break increased mite mortality by 5x and reduced mite populations significantly. These results give beekeepers in mild climates an additional Integrated Pest Management (IPM) method for controlling *V. destructor* during the summer season.

Introduction

Varroa destructor, an ectoparasitic mite, remains the leading biotic cause of western honey bee (*Apis mellifera* L.) mortality worldwide (Guzman-Novoa et al. 2010, Rosencranz et al. 2010, Kulhanek et al. 2017, Beyer et al. 2018). Controlling this pest has proven to be extremely difficult on several fronts. One, due to the limited number of chemical controls and their overuse, *V. destructor* has developed resistance to several approved miticides (Elzen et. al. 2000, Thompson et al. 2002, Rodríguez-Dehaibes et al. 2005, Gonzalez-Cabrera et al. 2016, Rinkevich 2020). Another issue, *V. destructor's* reproductive phase takes place inside the brood cell underneath a protective wax cap (Rosenkranz et al. 2010). Most miticides approved for use inside honey bee colonies do not penetrate this wax capping rendering the mites safe from treatment exposure (Rademacher and Harz 2006, Roth et al. 2020).

Chemical treatments are not the only route to help mitigate the detrimental effects of *V. destructor*. IPM, is a multi-pronged method, using biological, cultural, genetic, along with chemical, to control pests and has been successful in an array of different agricultural and non-agricultural settings (Frisbee and Luna, 1989). The concept of IPM was introduced to the beekeeping community decades ago but beekeepers were slow to implement it. However, due to *V. destructor* developing resistance to control agents and its continued impact on the industry has sparked the adoption of IPM in some beekeeping operations (Delaplane et al. 2005, Roth et al. 2020).

There are a few cultural options to limit *V. destructor* reproduction. One such is brood cycle disruption in which colonies are artificially forced to suspend reproduction and enter a period of broodlessness, forcing all *V. destructor* out of the protection of the brood cells (Gregorc et al. 2017, Jack et al. 2020). However, this and other cultural methods on their own may have limited success on reducing populations of *V. destructor* (Ellis et al. 2001, Delaplane et al. 2005, Harris 2007, Ellis et al. 2009a, Berry et al. 2012), meaning beekeeping still mostly rely on chemical control.

Organic acids, such as the crystallized form of oxalic acid dihydrate (OA), have been used for decades in Europe and Canada (Johnson et al. 2010) in beekeeping since they have proven to be extremely effective at killing *V. destructor* along with little evidence of the mite developing resistance (Adjlane et al. 2016, Maggi et al. 2016). Recently, OA was approved for use while human consumable honey supers are on colonies (EPA Reg. No. 91266-1) making it an even more popular miticidal choice among beekeepers.

Trickling, vaporizing and spraying of OA are the three methods approved for use in honey bee colonies (Rademacher and Harz, 2006). Trickling or spraying, is primarily used during winter months when there is no brood and the bees are in a cluster (Charriere and Imdorf, 2002, Rademacher and Harz, 2006). Vaporizing OA is a method whereby one heats the crystals to 101ºC using a heating device (vaporizer) which forms a gas that permeates throughout the colony (Rademacher and Harz, 2006). The latter is used historically during the winter months but has recently been adopted by beekeepers as a warm season treatment. All three of these treatment methods do not penetrate the wax capping, so affect only *V. destructor* roaming freely on the comb or on the adult bees (Adjlane et al. 2016, Ramsey et al. 2019), hence they are not effective in reducing *V. destructor* populations during the brood rearing seasons (Berry et al. 2022).

Several studies that incorporated a brood break have shown it contributes to reducing mite levels (Wagnitz and Ellis 2010, Lodesani et al. 2014, Giacomelli et al. 2016, Gregorc et al. 2017). Additionally, studies looking at oxalic acid application during natural periods of bloodlessness have highlighted that the lack of brood increases effectiveness (https://www.tandfonline.com/doi/full/10.1080/00218839.2018.1454035). However, incorporating an induced brood break alongside an OA vaporization application has been studied only once to date (Jack et al. 2020). The results were not favorable for reducing *V. destructor* populations at the legal 1g per brood chamber of vaporized OA. Also, a number of colonies died due to the queen being caged for 21 days, late in the season to induce a brood break.

The objective of this research was to determine if incorporating an earlier, shorter summer season brood break along with a higher application dose of 2g and 3g per brood chamber of OA vaporization would show effectiveness as a summertime control brood break

Materials and Methods

Experimental design

Experimental *A. mellifera* colonies were established and maintained in two 8-frame deep, Langstroth hives on research sites maintained by the research group [details given following review / acceptance]. Only queenright colonies with no additional disease stressors beyond *V. destructor* were selected for the experiment, and were headed by open mated hybrid queens.

In June of 2021, 81 experimental colonies were selected from 7 apiaries. All colonies had naturally occurring *V. destructor* infestations. Colonies were assigned to one of six total treatments, whereby colonies were either subjected to a brood break or no brood break, and dosed with either 0g, 2g, or 3g of oxalic acid vapor per brood box. Colony assignments ensured each treatment was present in every apiary at least once.

Brood break

A brood break was induced by placing the queen onto an empty drawn frame in a 8-frame deep super, above a queen excluder. The remaining seven frames of the super were completely honey bound, leaving no cells for the queen to lay eggs outside of her one designated frame. The queen remained in the deep super for 14 days. On day 14, the queen excluder was removed allowing the queen to roam freely. The frame she was given to lay eggs in was removed and destroyed. On day 21 experimental colonies receiving OA were treated. Additionally, frames in the other two supers that were isolated from the queen for 14 days were inspected for queen cells. None were detected.

Oxalic Acid application

OA application was administered to colonies by vaporization of the crystals according to label instructions of the registered product (US EPA 2015) and the user manual for the OxaVap ProVap 110 Vaporizer (OxaVap, Manning, SC). Prior to vaporization, and to ensure that vapor would not leak from the hives, colony entrances were sealed with blue shop towels and screened bottom boards were sealed using corrugated plastic boards. Powered by a Champion 2000-watt gasoline generator, the vaporizer device, an Oxavap Pro VAP 110, was inverted and the chamber bowl heated to 230°C. Two or three grams (depending on treatment group) of solid OA dihydrate crystals per deep brood box was placed into the separated Teflon lid and inserted into the chamber. Turning the device right side up caused the OA crystals to fall into the heated vaporizer chamber generating gaseous OA. The nozzle of the device was inserted into the entrance of each colony where it remained for 30 seconds to ensure that the full dose was vaporized and delivered into the colony. Once completed, the device was removed, and the shop towels and plastic corrugated boards were left in place for an additional 10 minutes per hive. For the safety of all persons applying the OA, full face respirators with OV/P100 cartridges were worn.

Varroa destructor abundance and mortality measures

We quantified the per-capita *V. destructor* parasitism levels for each colony using alcohol washes at four time points – Timepoint 1 on ‘Day 0’ prior to the induction of brood breaks, Timepoint 2 on ‘Day 14’ after the removal of queen excluders on brood break colonies, Timepoint 3 on ‘Day 24’, three days after treatment with oxalic acid, and Timepoint 4 on ‘Day 31’, a week later and a month from the start the experiment. For each alcohol wash for each colony at each timepoint, ~ 300 adult bees were collected from the brood nest (Dietemann et al. 2013) and placed into a Varroa EasyCheck device (Mann Lake, Hackensack, MN) filled with 70% ethanol, which euthanizes adult bees and attached *V. destructor*. The container was sealed and shaken for 60 seconds to dislodge *V. destructor* from the adult bees. The adult bees were removed from the container by lifting out the mesh basket. All dislodged individual *V. destructor* were counted, recorded and discarded. The mesh basket of bees was returned into the container, agitated for an additional 60 seconds, removed and *V. destructor* counted, recorded and discarded. This process was repeated until no *V. destructor* were recorded for two consecutive washes. The number of bees in each alcohol sample were counted by hand, giving an exact number of adult bees per sample for calculating *V. destructor* per bee estimate.

To measure *V. destructor* mortality rates, Dadant wood bound Varroa sticky boards were inserted into the entrance of each colony for 72h prior to OA vaporization to determine mite fall ("Day 18 – Day 21"). The day of OA vaporization ("Day 21"), the initial sticky boards were removed and new boards were inserted for an additional 72h period ("Day 21 – Day 24"including during the application of the oxalic acid for treated colonies). Total number of mites were counted on each sticky screen as a measure of *Varroa* mortality rates immediately prior to and during – following the oxalic acid treatment period. Fold-change in mite mortality was then counted as the number of mites on sticky screen 2 minus the number of mites on sticky screen 1, divided by the number of mites on sticky screen 1, for each colony.

Statistical analyses

All analyses were undertaken using the statistical programming language R v.3.6.1. We used a generalized linear mixed-modelling framework paired with type-III ANOVAs via the 'afex' package which wraps around the 'lme4' package, and compared effect sizes extracted from fit models using the 'emmeans' package. We compared pre-treatment and during/post-treatment mite mortality rates using linear mixed models with fold-change in mite mortality as a response variable, brood break and oxalic acid treatment as interacting fixed predictors, and apiary as a random effect. We examined per-capita mite parasitism using generalized mixed models with a Poisson error structure, with timepoint (continuous), brood break, and oxalic acid as interacting fixed predictors, and nested random effects of colony and apiary to account for the time series analysis and geographic grouping of the replicates. Data and code will be made available via a Zenodo-archived GitHub repository following manuscript acceptance; we are unable to make those data and scripts available for review due to the journal policy of double-blind peer review.

Results

V. destructor Knockdown

We found no evidence that the 3g dose of oxalic acid killed more *V. destructor* then the 2g dose (p = 0.854). All onward analysis therefore treated oxalic acid vaporization as a binary predictor; colonies were either treated or not treated with oxalic acid. We found strong evidence of a multiplicative interaction effect between inducing a brood break and treating with oxalic acid on *V. destructor* knockdown ($F_{71.03}$ = 7.34, p = 0.008), shown in figure 1 where the combination treatment led to *V. destructor* mortality rates 6x higher (95CI 3.9x – 8.2x) than background while treated colonies without a brood break saw 72hr mortality rates rise only 2.9x higher than background (95CI 0.70x – 5.0x). As expected, colonies which were not treated with oxalic acid showed no change in mite mortality rates during the treatment window regardless of if they experienced a brood break or not (see Figure 1). Simply stated, inducing a brood break more than doubled the effectiveness of oxalic acid treatment on our acute *V. destructor* mortality measure.

V. destructor Loads

Concordant with the *V. destructor* mortality results, we found strong evidence of a multiplicative interaction effect on colony *V. destructor* loads between inducing a brood break and oxalic acid treatment ($\chi^2 = 5.65$, $p = 0.02$), where *V. destructor* loads at the final timepoints measure were lowest in colonies experiencing the combined treatment (Figure 2). Both an induced brood break (0.15; 95CI 0.03 – 0.26) and oxalic treatment (0.18; 95CI 0.11 – 0.24) alone showed increasing rates of mite parasitism with time over the course of the 31 day experiment, however this was lower than the untreated control colonies (0.22; 95CI 0.13 – 0.31).Only in the combined treatment did per-capita mite parasitism rates decrease over the course of the experiment (-0.11; 95CI -0.19 - -0.04), see Figure 2.

Discussion

We found that combining an induced brood break with oxalic vaporization at either 2g or 3g per brood box was sufficient to reduce *V. destructor* parasitism rates in colonies (figure 2), while treating only with a brood break or oxalic acid was insufficient to prevent parasitism rates increasing during the summer. This is explained by the doubling of the effectiveness of oxalic acid vapor at killing *V. destructor* in a colony when used on conjunction with a forced brood break (figure 1). This combination of control approaches demonstrates the usefulness of the IPM framework in managing *V. destructor* in beekeeping as different control approaches can act multiplicatively.

Induction of a brood break in summer allows for expanded effectiveness of oxalic acid use in beekeeping regions where a natural brood break may not occur, such as the Southeastern United States. Other studies that have examined the effect of repeated applications of the labeled rate of OA, either by liquid trickling or vaporization, have shown OA to be ineffective during the brood rearing season (Gregorc et al. 2017, Jack et al. 2020, 2021, Berry et al. 2021) While our work demonstrated a partial solution to this problem, we did so using an oxalic dose currently above label rates. We justified this on the basis that another study, in the same region also combined a brood break late in the season with an oxalic acid treatment (Jack et al. 2020), but showed limited effectiveness in reducing *V. destructor* parasitism levels using the legal dose of 1g per brood chamber. In another study, the same authors (Jack et al. 2021) increased their dose of OA to 2g and 4g while brood was present, significantly lowering levels of *V. destructor* compared with those only vaporized with 1g per brood chamber. Al Toufailia et al. (2018) found similar results of increased mortality while vaporizing twice with 2.25 g of OA during the broodless winter months. Due to the results of these studies, we decided to incorporate doubling and tripling the amount of OA to be vaporized alongside a brood break.

Owing to the high colony mortality Jack et al. 2020 experienced due to the queen being caged for 24 days, we choose to not cage the queen, but instead restrict her into a third super above a queen excluder with full access to only one drawn frame. The other frames in the third super were completely covered in capped honey, giving the queen no cells to lay eggs in on those frames. Our thoughts were, if given the opportunity for the queen to still lay eggs, the colony may not deem themselves “queenless” and begin to construct queen cells. In summary, this approach allowed for core colony functions to continue. In addition, our approach could be done without having to necessarily find the queen, colonies can be shaken into the excluded super and the queen assumed to be therefore segregated from the lower two brood chambers. This increases the viability of the technique amongst commercial beekeepers for whom labor costs of finding and caging a queen may be preventative.

There is a critical need for additional IPM options for controlling *V. destructor* during the brood rearing months. Resistance to some and limiting factors for other miticides has left beekeepers with few options for controlling infestations of *V. destructor*. OA is becoming a chemical treatment of choice for beekeepers for many reasons already stated. Based on our results, we recommend that beekeepers employ vaporization of OA during a brood break, either naturally occurring or induced. Implementing a brood break into a beekeeper's treatment regime may be easily accomplished by most beekeepers and when combined with an OA treatment can reduce *V. destructor* populations during critical summer months when little to nothing else may be available. There are other periods during the beekeeping calendar that beekeepers can take advantage of brood break and vaporize with OA. For instance, when a colony has swarmed, when beekeepers are making splits or re-queening. Beekeepers can take these opportunities and vaporize with OA. Actually anytime when there is no capped brood and the mites are in the dispersal phase, not the reproductive, is when to best achieve good results of reducing mite populations while vaporizing with OA.

Increasing the dose of OA has also been shown to be beneficial in increasing *V. destructor* mortality in ours and other studies (Jack et al. 2021), but to date the legal amount for use in honey bee colonies is only 1g per brood chamber (EPA, 2015). We anticipate efforts to modify the OA label to approve use at higher doses.

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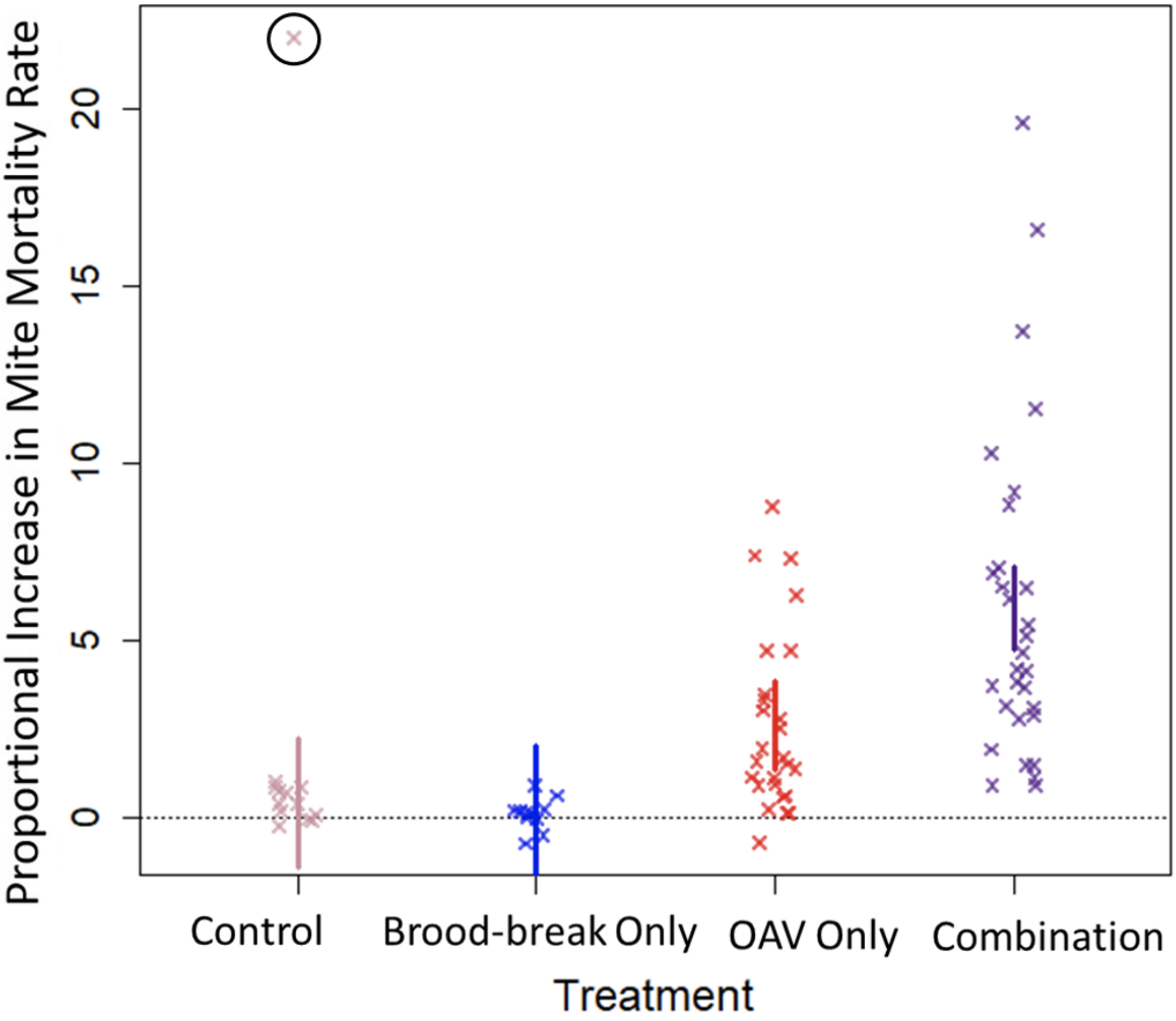

Figure 3.1 Comparison of change in mite mortality rate by treatment group (control, brood-break only, oxalic-acid vaporization [OAV] only, and a brood-break combined with OAV), calculated using mite drop 72 hours prior to treatment and 72 hours during and post- treatment. Both the OAV treatments and Combination treatment saw significant increases in inferred mite mortality, with the combination treatment significantly higher in this measure than the OAV treatment only. Each point represents a single colony. Vertical bars represent 95% confidence intervals around the mean. A ‘0’ value corresponds to no change in mite mortality, a ‘1’ value corresponds to a doubling of mite mortality, and a ‘5’ value correspond to a six-fold increase in mite mortality.

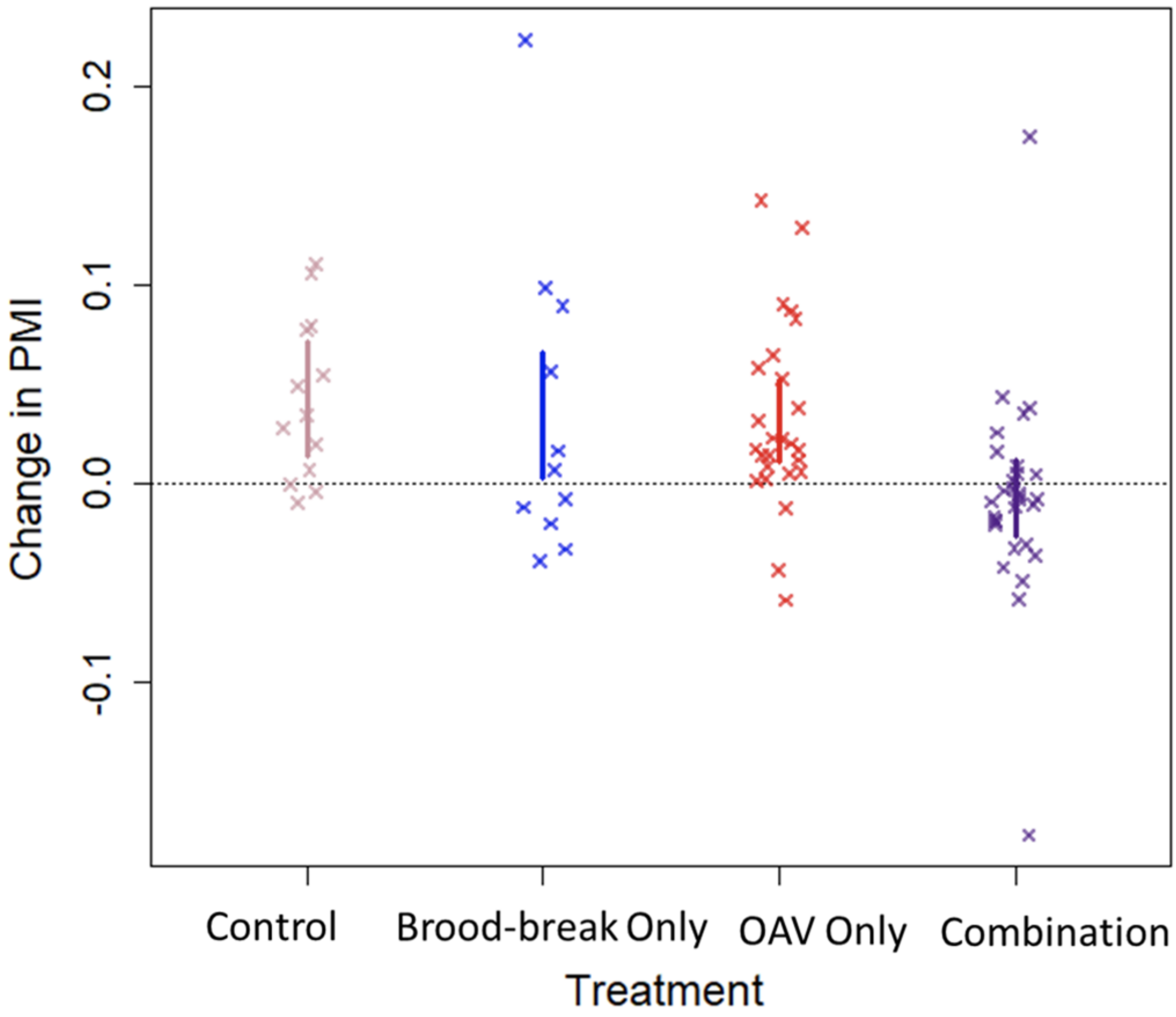

Figure 3.2 Summary of the change in percent mite intensity (‘PMI’ or mites per 100 bees) by treatment (control, brood-break only, oxalic-acid vaporization [OAV] only, and a brood-break combined with OAV), calculated using mite washes prior to and after treatment periods. Colonies experiencing a brood break alongside an OAV treatment had a significant decrease in mite infestation. Colonies with no brood break, brood break or OAV treatment showed a significant increase in mite parasitism rates of adult bees. Vertical bars represent 95% confidence intervals.

CHAPTER 4

INTEGRATING FLORAL RESOURCES INTO EROSION MITIGATION SEED MIXES FOR NATIVE BEE CONSERVATION

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Abstract

Pollination by bees plays a critical role in the viability of life on this planet from food crop productivity, to biodiversity of ecosystems, however their populations, globally, are in decline. Contributing factors include pesticides, climate change, diseases, and invasives, but most notably, are habitat degradation and fragmentation due to agricultural intensification and urbanization. Research has shown that conservation and restoration of land can help to restore bee abundance and diversity. These bee friendly landscapes don't have to reside solely in parks or forested areas, they can also be assembled in and around cities, towns or along roadsides. Another opportunity to establish habitat which will attract and provide nectar for bees is eroded lands. Recommendation for restoration of eroded land usually includes planting with a number of quick growing native and non-native grasses, which do little for bee conservation. In our study we incorporated angiosperms into an erosion mitigation seed mix specifically selected for their ability to attract bees, and thrive in the Piedmont region of Georgia. We found plots that incorporated these flowering plants had a greater abundance, richness and diversity of bees compared to those plots that only contained grass. Of the 12 angiosperms selected, the most successful were *Rudbeckia hirta* (Black eye Susan), *Chamaecrista fasciculata* (partridge pea), *Eryngium yuccifolium* (rattlesnake master), *Pycnanthemum muticum* (mountain mint), and *Coreopsis lanceolata* (Lanceleaf coreopsis). The most numerous bees collected by "bee to flower" or "sweeps" were in the genera *Lasioglossum*, *Halictus* and *Bombus.*

Introduction

Ecosystem services (ES) provided by insects include pest control, decomposition, wildlife nutrition and pollination. These services help make life possible for humans on this planet (Noriega et al., 2018; Schowalter et al., 2018). Together, these services are estimated to be at least $57 billion in the US alone (Losey and Vaughan, 2006), where the economic value on pollination, from managed and wild bees, is estimated per year at $14.2 - $34 billion in the US (Degrandi-Hoffman et al. 2019, Jordan et al. 2021) and $235 - $577 billion worldwide (IPBES, 2016). More than 75% of the worlds flowering plants (Robinson and Morse, 1989) and 35% of food plants require animal pollination (Klein et al. 2007) with bees undoubtedly being the most important of the worlds pollinators for agriculture and natural systems. (Buchmann & Nabhan, 1996, National Research Council, 2007). Global declines in bees, however, are on the rise (Dicks et al. 2021) and unfortunately, due to lack of research and resources most of the ecological impacts are unknown (Cane & Tepedino, 2001, Potts et al. 2010, Russo, 2016, Thomson, 2016).

There are a number of factors that have contributed to the decline of bees, such as pesticide use, introduction of non-native species and climate change (Kearns et al. 1998), but most notably are habitat loss and fragmentation (Decourtye et al. 2019, Potts et al. 2016). In addition, naturally, undisturbed land converted into agriculturally dominated systems have shown a reduction in bee phylogenetic diversity and pollination services (Grab et al. 2019). This clearly justifies an immediate need for habitat conservation and restoration due to the important pollination role that bees provide.

Conserving and enhancing pollinator habitat through restoration is a major step in slowing the decline of bees due to habitat loss (Winfree et al. 2009). Compared with larger mammals, bee pollinators have relatively small functional requirements. They need an overlapping food source throughout their active periods, an area to nest, and protection from pesticides. Hence, prioritizing high impact pollinator conservation, even in urban settings, is seen as achievable (Baldock et al. 2019, Braman & Griffin, 2022). Fielder et al. (2012), showed a 10-fold increase in bee abundance and richness due to pollinator habitat restoration impacts in an urban community. Studies have also shown the effectiveness of habitat restoration for bees in agricultural systems (Buri et al. 2014, Dicks et al. 2010, Kremen & M'Gonigle, 2015, M'Gonigle et al. 2015, Sutter et al. 2017) and how cities are providing refuge for bees even though their populations are in decline (Hall et al. 2017). These landscapes, therefore, must not be ignored as potential habitat for pollinators.

Urban agricultural settings, however, do have unique conservation challenges. Periodic insecticide and herbicide applications can have negative effects and don't always act alone. Goulson et al. (2015) stated that "pesticide exposure can impair both detoxification mechanisms and immune responses, rendering bees more susceptible to parasites and other diseases". Bee abundance was also shown to decline 41% with each ˚C of urban warming: these declines occurred regardless of floral density (Hamblin et al. 2018).

Other conservation challenges are homeowner perceptions concerning possessing perfectly manicured turf lawns. Not only is turfgrass the most dominant vegetation in urban areas, it is the number one irrigated crop in the US (Milesi et al. 2005). The vast amount of land consumed by residential yards in US cities account for 25-30% of total land and up to 50% of green spaces (Ignatieva et al. 2020, Minor et al. 2016) yet, they offer little in the way of forage opportunities for bees and other pollinators (Tonietto et al. 2011). Because grasses are largely wind-pollinated, it was thought that pollinators would not forage on turfgrasses. However, although limited, there is evidence of bees and other pollinators foraging on grass inflorescences (Joseph et al. 2020).

Suitable nesting sites can also be a limiting resource for bee abundance and richness in urban lawn settings. Forested or natural undisturbed areas typically supply nesting habitat for many bees with fallen dead wood, bare ground, twigs and other materials for cavity and soil nesters (Roberts et al. 2017, Van Den Berge et al. 2019). Tolerating some degree of "messiness" in yards and gardens (allowing bare patches of soil and leaving dead wood and senescent flower stems for nesting bees) is not often widely accepted by homeowners and lawn care practitioners.

Providing native bee habitat on farms and roadsides offer other opportunities for conservation. Establishment of flower-rich habitat within or around intensively farmed landscapes to increase the availability of pollen and nectar resources can include cover crops, field borders, and shrubby hedgerows which are supplemented with flowers. Other potential areas for incorporating pollinator and bee attracting flowers are eroded land. Soil erosion, especially in intensely farmed lands worldwide, has become a serious problem with a mean rate of 0.64 mm of soil being depleted annually (Wilkinson, 2005). Stabilization mixes from construction sites to agriculture farmland use buffer strips to manage erosion and nutrient runoff, (Baker et al. 2006). Most often these stabilization mixes are comprised solely of native and non-native, fast growing grasses which offer little in the way of food for bees. Recouping and seeding eroded land with wild flower material, included in these stabilization mixtures, can provide an excellent opportunity to enhance pollinator habitat, and also benefit other ecosystem services (Wratten et al. 2012). Presently, there is a dearth of information and availability of locally adapted stabilization mixes that incorporate floral resources for pollinator conservation efforts.

In the present study, we compared bee abundance, diversity and richness between regionally adapted, pollinator attractive plants alongside a quick growing, grass-based seed mixture typically used for erosion mitigation. The flowering plants selected were used to compliment soil stabilization and habitat restoration plus attract bee pollinators. We predicted that areas planted with pollinator plants would positively impact bee abundance, and richness. We further sought to gain insight for recommendations of flowering plants not only for pollinator habitat restoration but also for inclusion in soil stabilization seed mixtures.

Methods & Materials

Plot establishment

An five-acre, intensely eroded field along a hillside at Iron Horse Farm in Watkinsville, GA (N33°43 W83°17) was chosen for the project site. Logging and borrowing soil to create level areas for buildings at the Iron Horse Farm left the five-acre area of highly erodible saprolite (weathered bedrock) exposed on a steep slope. Although the area was seeded with cereal rye in an attempt to stabilize the slope, the highly acidic and low- nutrient saprolite did not support vegetative growth, and the area experienced extensive erosion (Figure 1). Although the erosion was a serious problem, it provided a unique opportunity to offer a living laboratory for research and extension.

The eroded area was divided into three sub-areas: shoulder and top portion of the slope where erosion began, gullied, and alluvial areas. Based on soil test recommendations, lime and poultry litter were applied to adjust soil pH and provide nutrients. Because compost socks and blankets have been successfully used to stabilize and revegetate slopes in the Southeast and elsewhere (AASHTO, 2010; Risse and Faucette, 2015; US EPA Office of Water, 2012), mulch/compost blankets were applied to retain water and stabilize the soil on the shoulder. A compost sock berm was established near the juncture of the shoulder and the slope which began to control runoff at the top of the slope. Working down the slope, blankets and berms were applied as needed. The site provided an opportunity to integrate the best management practices for erosion control and site stabilization with pollinator habitat restoration. The research area presented a confluence of five habitats on the Iron Horse Farm: restored eroded land, agricultural production, early succession (adjacent logged area), wetlands, and mature bottomland hardwoods.

Two treatments groups (Grass alone and Flower enhanced) were compared; treatment 1 (Grass) a standard grass stabilization mix and treatment 2 (Flower), a seed mix of native, southern, ecotype pollinator attractive plants. Each treatment site measured 16.8 m (55ft) X 45.7 m (150ft) with the entire site encompassing a dimension of 167.6m (550 ft) X 45.7m (150ft) (Figure 1). The entire site was planted with the GA DOT recommended grass stabilization mix and sown at 44.5 kg (100 lbs)/0.4 hectares (acre). The flower enhanced mix provided by Ernst Seed Company in Meadville, Pennsylvania, was planted in October, 2020, and again in April, 2021 into the designated flower plots at 20.4 kg (45 lbs)/0.4 hectares (acre). A complete list of the grass and flower mix by percentage and species is located in Table 1.

The site was planted in a randomized complete block (RCB) design with 5 replications of each treatment (Figure 1). Seeds were mixed with sand and placed into individual buckets and spread by hand throughout each of the randomly assigned plots. Flower seeds were also planted into individual pots and grown in the UGA Entomology greenhouse. Once the plantings had reached a suitable height and, the danger of frost was over, plants were transported to the field, divided equally among the 5 flower plots, planted and watered. Mountain mint, (*Pycnanthemum muticum*) and tickseed sunflower (*Bidens frondosa*) were also grown in the greenhouse and transplanted to the field at that time. Plots were irrigated until establishment. No insecticides or herbicides were applied. Grass plots were bush hogged approximately twice per month. Flower plots were bush hogged in the fall after establishment.

Bee Sampling

Sweep samples and bee to flower aerial net (direct observations) occurred approximately weekly during bloom periods on 19 sample dates from April 4, 2021 to August 9, 2022. Bee data collection methods for both years, 2021 & 2022, began when plants within the flower treatment group were established and blooming. Data collections continued weekly during the entire blooming season to ensure that the full seasonality of bee pollinators was represented. Bee data were collected in both grass and flower plots on sunny days once temperatures reached 16 ^{o}C during the bloom period. Two sample methods were used to collect bee specimens.

Direct observation/Bee to Flower Aerial Net Collection

Researchers positioned themselves next to a flowering plant and during a five-minute period, collected all the bees visiting the flowers with an aerial net. Only bees actively engaged (probing the flower for nectar and collecting pollen) in and on the flower surface were collected. Specimens were placed into plastic bags, tightly tied, labeled and placed into a cooler with ice packs to chill the specimens so they would not damage themselves flying inside the bag. Five observers on each date rotated among the blooming plants on each sample date.

Sweep Collection

The second method incorporated sweep (beat net) sampling across both the grass and flower plots. Starting at the upper NE corner of each plot, researchers with a sweep net in hand, walked at a steady pace making 50 sweeps transecting across the field until the lower SW corner was reached. Once complete, the contents of the sweep net were emptied into a large plastic bag. Specimens were placed into a cooler as stated above. Chilled bees were then removed, placed into vials, labeled and frozen for safe storage. Specimens from both collection methods were later pinned, labeled and identified. They were identified by Sam Droege, Eastern Ecological Science Center , and Dr. Conor Grant Fair, University of Georgia Department of Entomology, using experience, an established reference collection and a variety of printed and online resources (Mitchell, 1960, 1962; Gibbs, 2011; Gibbs et al., 2013; https://www.discoverlife.org). Voucher specimens are retained at the University of Georgia Natural History Museum.

Data Analysis

To test our hypothesis that bee abundance, richness, and diversity would be greater in the flower plots compared to the grass plots and would decrease along the proposed disturbance gradient, we fit generalized linear models using the *glm* function (R Core Team, 2023) with treatment and plot number as the independent variables. Data were pooled over both sampling years. Through testing appropriate distributions, we determined the negative binomial, Poisson, and Gaussian distributions fit best for abundance, richness, and diversity (respectively). We then completed post-hoc tests using the emmeans function (Lenth, 2022) and Tukey's Honestly Significant Difference Test (HSD). Data visualizations were completed using ggplot (Wickham, 2016) and show the box and whisker plots with the raw data plotted for reference. Different letters show significant differences between treatment groups (alpha=0.05). To analyze the plant pollinator network collected from the bee-to-flower data, interactive bipartite graphs were constructed using the *bipartite_D3* function (Terry 2021).

Results

Bee Sampling: During 2021 and 2022 aerial net (direct bee to flower observation) and beat net sweeps collected a total of 3464 specimens, representing 4 families, 19 genera, and 59 species. There were 1,524 bees collected in the sweep sampling, and 1,940 bees collected using the bee-to-flower collection methods. Bees in the genera *Lasioglossum*, *Halictus* and *Bombus* were the most numerous. A complete list of all specimens, collection method and flower (if appliable) are presented in Table 2.

Beat net sweeps; grass alone vs flower enhanced plots: Abundance, richness and diversity of bees were greater in the flower- enhanced plots compared to the grass plots (Figure 2a, b and c; $F_{1,7}$=78.5256, p value <0.001, $F_{1,7}$=43.8903, p value <0.001, $F_{1,7}$=7.875, p value =0.026, respectively). The abundance in the flower plots had an average mean of 285 specimens compared to the grass plots with an average mean of 11.2 (Figure 2a). The richness of wild bees, number of species collected in the plots, had an average mean of 26.2 for the flower plots and 5.4 for the grass plots (Figure 2b). The diversity, which is the relationship between the number of species collected and the number of individuals in each species, had an average mean of 2.19 for flower plots and 1.36 for the grass plots (Figure 2c).

Direct observation/Bee to Flower Aerial Net Collection: Of the 3464 total specimens, 1940 specimens were collected directly from the flower with 46 species represented. This means that 13 species were only found in the sweep samples and 10 species only found in the bee to flower data. There are 37 species that were found in both sweep and bee to flower samples.

The most frequently visited flowers were *Rudbeckia hirta* (731 bees), *Chamaecrista fasciculata* (617 bees), and *Eryngium yuccifolium* (348 bees). Out of 12 native ecotype Angiosperms (flowering plants) that were planted in the field, the most successful were *Rudbeckia hirta* (Black eye Susan), *Chamaecrista fasciculata* (partridge pea), *Eryngium yuccifolium* (rattlesnake master), *Pycnanthemum muticum* (mountain mint), and *Coreopsis lanceolata* (Lanceleaf coreopsis). There were little to no representations of *Baptisia alba*, (spiked wild indigo), *Bidens frondose*, (tickseed sunflower), *Chamaecrista nictitans*, (sensitive pea), *Coreopsis leavenworthii* (Leavenworth's tickseed), orange coneflower (*Rudbeckia fulgida)*, starry rosinweed (*Silphium asteriscus*), and giant ironweed (*Vernonia gigantea*). *Vicia sativa* (vetch) and *Verbena brasiliensis* (Brazilian verbena) were not planted by us, but germinated as volunteer plants that bees were obviously visiting, so are included in our observations.

We developed a visualization of a pollinator network (Figure 3) illustrating the variation in visitation among bees and flowers. *Nomia nortoni*, e.g., was only captured from *Chamaecrista fasciculata* while *Lasioglosum callidum* and *L. imitatum* were captured visiting five or six of the flower species.

Discussion

Over the past 50 years, wild bee abundance and richness in North America and Europe have been on the decline (Goulson et al. 2015). Research continues to uncover a common thread for this decline which includes pesticides, pest and pathogens, and climate change (Dicks et al., 2021; Neumann and Carreck, 2010; Ravoet et al., 2014; Soroye et al., 2020; Straub et al. 2022). However, the negative effects from habitat degradation and fragmentation, (loss of food resources and nesting sites), (Ganser et al. 2021) continue to percolate to the surface as the most debilitating reason (Winfree et al. 2009; Potts et al. 2010; Burkle et al. 2013, Marshall et al. 2017). Conservation of wild lands rich with native plants and habitat restoration, in either agricultural or residential lands, which includes incorporating native flowering plants, are both important ways we can help to reverse this declining trend.

To date, there have been few studies that have focused on the effects of incorporating flowering plants into an erosion mitigation seed mix on wild bee enhancement (Meissen et al. 2020). Our results clearly show that incorporating native, ecotype flowering plants into a traditional erosion mitigation seed mix had positive effects on bee richness and abundance. Plots that incorporated flowering plants attracted not only more bees, but 59 different species of bees. Wild bees were nearly absent from the grass plots accounting for only 3.9% of the total number of bees collected by sweeping. As expected, we found flower plots to be significantly higher in the abundance, richness and diversity of wild bees when compared to grass plots. Flower plots had an overall positive effect of bee richness, diversity and abundance when compared to grass plots.

Of the seven bee families, only five are common in Georgia; Andrenidae, Apidae, Colletidae, Halictidae, and Megachilidae. Melittidae have been identified in the Southeast but are extremely rare and Stenotritidae are only found in Australia. Of the common families in Georgia, we only collected four of the five. We did not collect any Andrenidae or mining bees in either sweep or bee to flower methods even though they are quite common. This was probably due to the fact that Andrenidae are active in the early spring months and are rare to find in summer of fall (Herrera et al. 2023). Our collection timeframe was just outside of their active period which is why we believe none of these wild bees were collected in our plots.

The angiosperms we planted that thrived in the field were *Rudbeckia hirta* (Black eye Susan), *Chamaecrista fasciculata* (partridge pea), *Eryngium yuccifolium* (rattlesnake master), *Pycnanthemum muticum* (mountain mint), and *Coreopsis leavenworthii* (Leavenworth's tickseed). Their success was primarily due to these plants being unsavory to deer browsing. The other Angiosperms that were planted *Baptisia alba,* (spiked wild indigo), *Bidens frondose*, (tickseed sunflower), *Chamaecrista nictitans*, (sensitive pea), *Coreopsis leavenworthii* (Leavenworth's tickseed), *Rudbeckia fulgida,* (orange coneflower*), Silphium asteriscus,* (starry rosinweed) and *Vernonia gigantea,* (giant ironweed) were quickly eaten and mostly destroyed by deer. Unfortunately, due to the severity of the deer predation, only a few remained but never bloomed enough during our collection period to gather bee to flower data.

The flowers chosen to be present in the flower plots, were based on consultation with Ernst Seed Company to represent native ecotypes. *Rudbeckia hirta* and *Chamaecrista fasciculata* were by far the most prominent of the flowering species which may be one reason they attracted more than twice as many bees than the other angiosperms. However, when comparing wild bee richness, *Eryngium yuccifolium* and *Pycnanthemum muticum* rivaled the former winners, Table 3.

Future research not only needs to address how to incorporate flowering plants into disturbed habitats, but also which flowering plants are best to include (Purvis, 2021). There are a number of wildflower seeds mixes available, but with closer investigation, a number of these do not contain native species. Non-native species may attract our generalist bees, like honey bees, but a number of our wild bees are unable to extract the floral resource necessary to sustain their populations. Mixes also need to have a diversity of native flowering plants that have a succession of bloom time, from early spring to late fall to provide necessary nutrition for our early arriving pollinators. Nesting sites and distance to floral resources for our wild bees also need to be considered when restoring natural areas.

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Table 4.1 A complete list of the grass and flower seed mix by percentage and species

	Percent Mix	**Common Name**	**Species & Ecotype**
Grass Mix	33%	Pensacola Bahiagrass	*Paspalum notatum*
	33%	Un-hulled Common Bermuda	*Cynodon dactylon*
	34%	Browntop Millet	*Urochloa ramosa*
Flower Mix	46.7%	Little Bluestem 'Prairie View'	*Schizachyrium scoparium* IN Ecotype
	24.5%	Beaked Panicgrass	*Panicum anceps* GA Ecotype
	21.8%	Virginia Wildrye	*Elymus virginicus* PA Ecotype
	1.8%	Black-eyed Susan	*Rudbeckia hirta* Coastal Plain NC Ecotype
	1.3%	Partridge Pea	*Chamaecrista fasciculata* FL Ecotype
	1.3%	Lanceleaf Coreopsis	*Coreopsis lanceolata*
	0.7%	Orange Coneflower	*Rudbeckia fulgida* Northern VA Ecotype
	0.5%	Sensitive Pea	*Chamaecrista nictitans* NC Ecotype
	0.5%	Rattlesnake Master	*Eryngium yuccifolium* SC Ecotype
	0.3 %	Giant Ironweed	*Vernonia gigantea* FL Ecotype
	0.2%	Spiked Wild Indigo	*Baptisia alba* SC Ecotype
	0.2%	Starry Rosinweed	*Silphium asteriscus* GA Ecotype
	0.2%	Leavenworth's Tickseed	*Coreopsis leavenworthii* FL Ecotype.

Table 4.2 Complete bee species list and abundance from both collection methods: Bee to Flower and Sweeps.

	Bee to Flower								**Sweeps**	
Species	Black eye Susan	Brazilian Verbena	Coreopsis	Mountain Mint	Rattlesnake master	Sensitive pea	Tickseed sunflower	Vetch	Grass	Flower
Agapostemon splendens	0	0	0	1	0	0	0	0	0	0
Agapostemon virescens	1	0	0	0	0	0	0	0	0	0
Apis mellifera	2	0	0	1	0	50	0	0	0	21
Augochlora pura	0	0	0	0	0	0	0	0	0	2
Bombus bimaculatus	0	0	1	0	0	0	0	7	0	1
Bombus fraternus	1	0	0	9	0	34	0	0	0	7
Bombus griseocollis	9	0	0	9	3	0	0	0	0	0
Bombus impatiens	4	1	0	34	2	335	0	0	1	12
Bombus pensylvanicus	0	0	0	0	0	8	0	0	0	4
Ceratina cockerelli	0	0	1	0	0	0	0	0	0	2
Ceratina dupla	0	0	0	0	1	0	0	0	0	0
Coelioxys dolichos	0	0	0	1	0	0	0	0	0	2
Coelioxys mexicanus	0	0	0	0	0	0	0	0	0	2
Coelioxys sayi	0	0	0	0	0	0	0	0	0	3
Colletes nudus	0	0	0	0	0	0	0	0	0	1
Dieunomia heteropoda	0	0	0	0	0	1	0	0	0	1
Eucera hamata	0	0	0	0	0	0	0	0	0	1
Eucera rosae	0	0	0	0	0	0	0	0	0	1
Halictus confusus	2	0	0	0	1	0	0	0	0	1
Halictus ligatus/poeyi	570	0	75	9	11	3	6	1	9	366
Halictus parallelus	2	0	0	33	10	0	0	0	0	2
Halictus rubicundus	0	0	0	2	1	0	0	0	0	0

Lasioglossum admirandum	0	0	0	0	1	0	0	0	0	4
Lasioglossum callidum	28	0	1	14	35	57	1	0	15	331
Lasioglossum coreopsis	5	0	0	0	1	0	0	0	5	9
Lasioglossum disparile	5	0	1	0	0	1	0	0	0	5
Lasioglossum gotham	2	0	0	0	0	2	0	0	0	3
Lasioglossum hitchensi	1	0	0	1	1	4	0	0	1	31
Lasioglossum illinoense	0	0	0	0	0	21	0	0	3	75
Lasioglossum imitatum	28	0	4	2	263	41	1	0	3	233
Lasioglossum leucocomus	0	0	0	0	0	0	0	0	0	2
Lasioglossum lionotum	1	0	0	0	1	0	0	0	0	1
Lasioglossum longifrons	0	0	0	0	0	0	0	0	3	4
Lasioglossum lustrans	5	0	0	1	0	0	0	0	0	2
Lasioglossum pilosum	11	0	5	1	0	1	1	0	1	12
Lasioglossum pruinosum	1	0	0	0	0	1	0	0	1	5
Lasioglossum tegulare	3	0	0	0	0	2	0	0	5	23
Lasioglossum trigeminum	7	0	2	0	20	4	0	0	8	67
Lasioglossum zephyrum	0	0	0	0	0	3	0	0	0	13
Megachile brevis	0	0	0	0	0	0	0	0	0	2
Megachile campanulae	0	0	0	0	0	1	0	0	0	0
Megachile mendica	4	0	0	3	0	3	0	0	1	17
Megachile petulans	0	0	0	0	0	0	0	0	1	0
Megachile sculpturalis	1	0	0	1	0	0	0	0	0	0
Megachile xylocopoides	0	0	0	2	0	0	0	0	0	1
Melissodes bimaculatus	0	0	0	2	0	4	0	0	0	3
Melissodes comptoides	0	0	0	0	0	3	0	0	0	1
Melissodes tepaneca	1	0	0	2	0	0	0	0	0	3
Nomia nortoni	0	0	0	0	0	4	0	0	0	4
Osmia georgica	0	0	1	0	0	0	0	0	0	3

Ptilothrix bombiformis	1	0	0	0	0	0	0	0	0	0
Sphecodes antennariae	0	0	0	1	0	0	0	0	0	0
Sphecodes atlantis/cressonii	0	0	0	0	0	0	0	0	0	0
Sphecodes dichrous	0	0	0	0	0	0	0	0	0	1
Svastra aegis	0	0	0	0	0	0	0	0	0	1
Svastra obliqua	7	0	0	0	0	1	0	0	0	4
Svastra petulca	1	0	0	0	0	1	0	0	0	1
Xylocopa micans	0	0	0	1	0	7	0	0	0	0
Xylocopa virginica	1	0	0	9	4	9	0	0	0	2

Table 4.3 Abundance and Richness of wild bees collected on individual flowers or by sweeps

Flower	**Abundance of Wild Bees**	**Richness of Wild Bees**
Black Eye Susan	731	27
Brazilian verbena	1	1
Coreopsis	87	9
Mountain Mint	143	21
Rattlesnake Master	348	15
Sensitive Pea	617	26
Tickseed Sunflower	6	5
Vetch	7	1
Sweep	1534	49
Total	3464	59

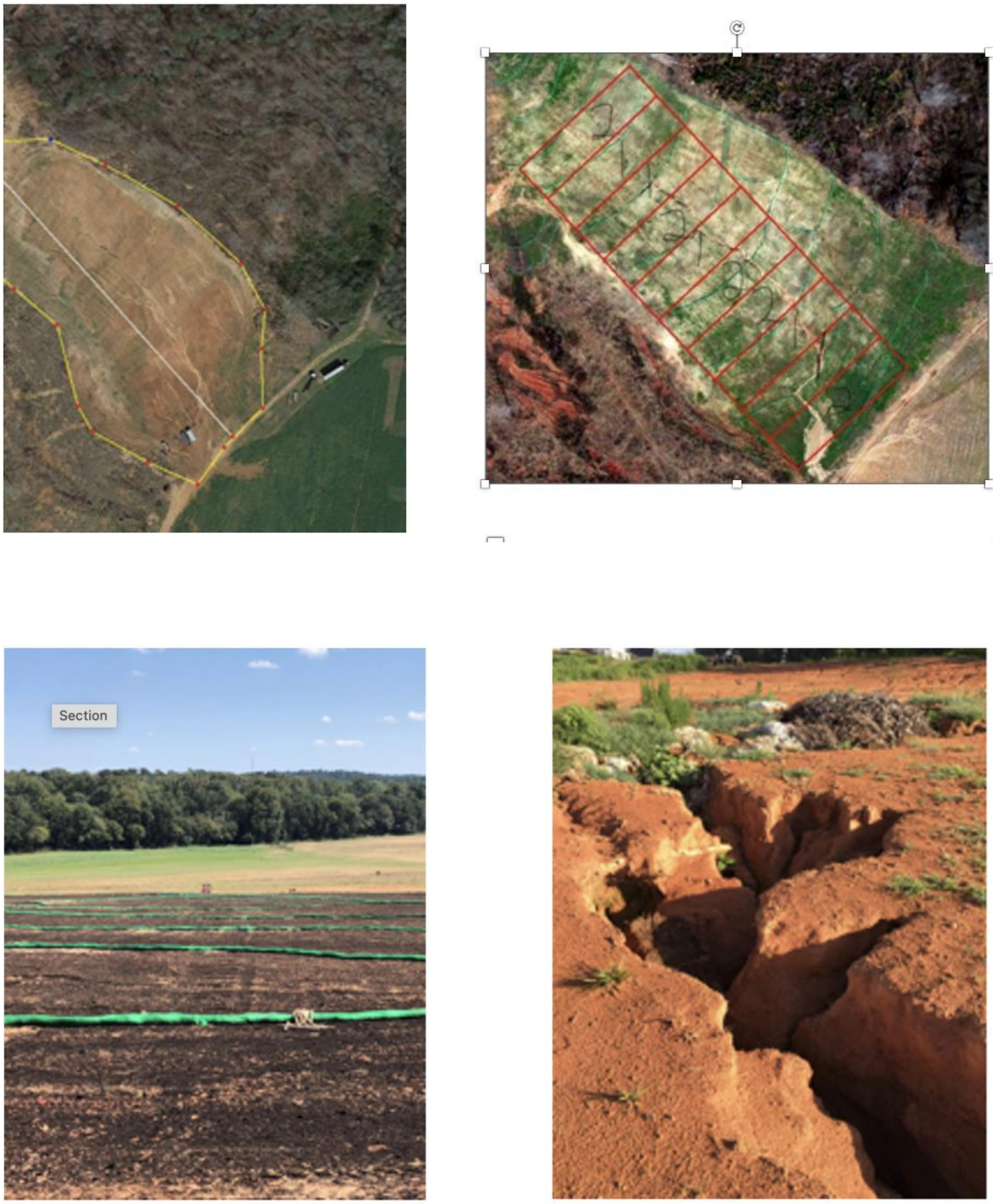

Figure 4.1 Aerial and ground photos of the field site at Iron Horse Farm.

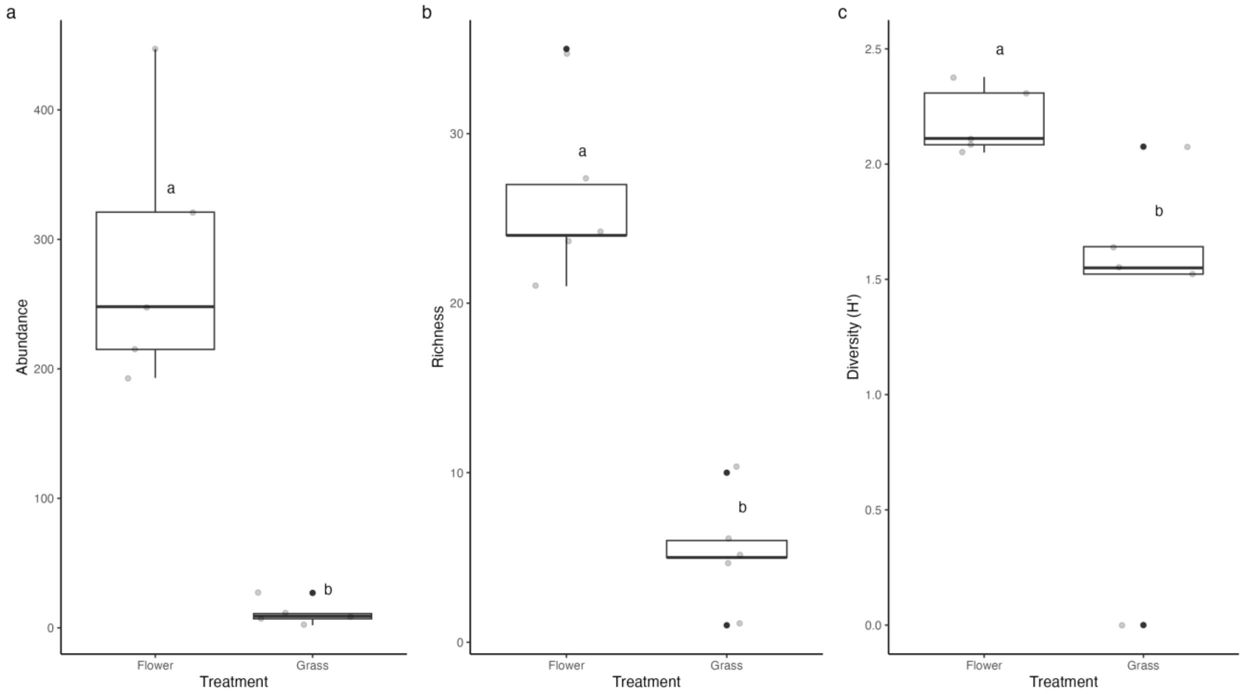

Figure 4.2 Bee abundance (a), richness (b) and diversity (c) in grass alone versus flower enhanced plots in an erosion mitigation plant mix

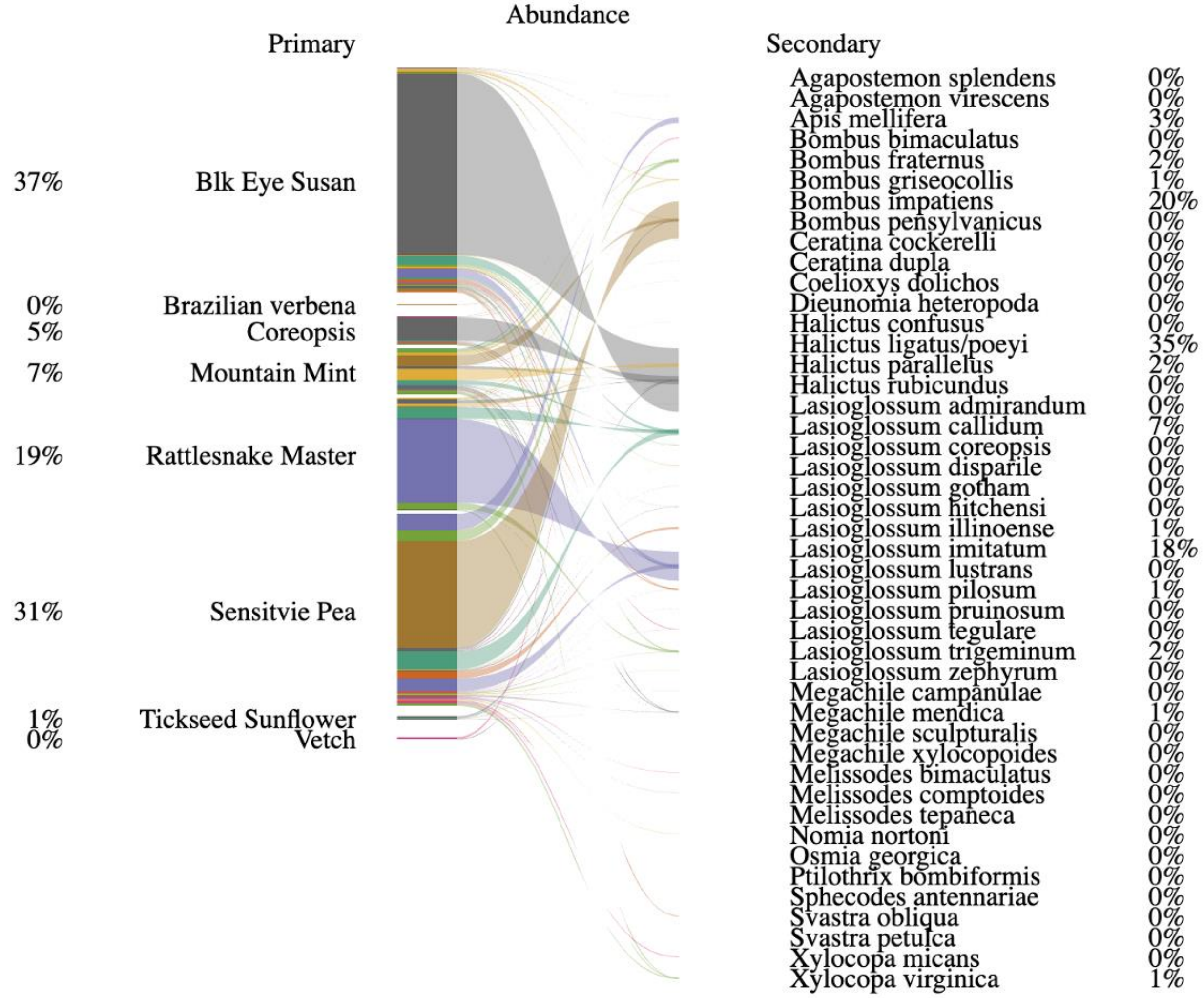

Figure 4.3 Network of flowers and the bees that visited them in the flower-enhanced erosion mitigation mix evaluated for pollinator conservation during 2021 and 2022 in Georgia, USA.

CHAPTER 5

CONCLUSIONS

My research examined the honey bee and wild bee arena with one overarching objective in mind; how can we help them survive. In the first two chapters we explored how to control *Varroa destructor,* an ectoparasitic mite, in *Apis mellifera* colonies, using different methods of applying oxalic acid. Varroa has become the number one reason why *A. mellifera* are dying globally (Guzmán-Novoa et al. 2010, Le Conte et al. 2010, Rosenkranz et al. 2010). Varroa causes little harm to the Eastern honey bee, *Apis cerana* (Grindrod & Martin, 2023), but this is not the case with the European honey bee, *Apis mellifera*. Varroa and *Apis mellifera* have only had a short history of coevolution, hence *A. mellifera* has developed no defense strategy to combat this formidable pest (Noel et al. 2020). If populations of Varroa are not controlled, honey bee colonies usually do not survive. Therefore, beekeepers are desperate for treatments which not only kill Varroa, but are easy to use, don't require a license and will not harm the bees they are treating. However, this has not been an easy task. Adverse impacts of certain in-hive acaricides on bees, and developing resistance in Varroa have made a number of miticides (organic and synthetic) undesirable (Berry et al. 2013, Elzen et al. 2000, Mathieu and Faucon, 2000, Rodríguez-Dehaibes, 2005, Rinkevich, 2020, Sammataro et al. 2005, Thompson et al. 2002).

Recently, an organic acid, oxalic acid dihydrate (OA), has made its way across the pond and into the hands of beekeepers in the US. OA has been a popular choice for controlling Varroa in Europe and Canada (Johnson et al. 2010), and in 2015, three application methods of OA were approved for use in the United States (US EPA, 2015). Out of the three methods, vaporization, heating the crystals which then forms a gas, has become the most widely used among backyard and commercial beekeepers. Vaporization of OA is highly effective at killing Varroa when it comes in direct contact with these mites but does not penetrate the wax capping were most Varroa reside (Rademacher and Hartz, 2006, Rosenkranz et al. 2010).

Beekeepers are a clever group and are continuously developing new or creative methods for treating Varroa, however, a number of these have not been approved or tested. One such method is treating colonies with vaporized OA multiple times over several days/weeks to coincide with the bee's reproductive cycle. The rationale is to expose the entire cohort of mites to OA gas within a colony as they emerge alongside their parasitized hosts. Unfortunately, our results found that applying OA multiple times while brood was present was not effective at reducing Varroa populations (Berry et al. 2022).

Because of these results, we decided to take a different approach at applying OA that beekeepers could easily incorporate into their management scheme. We sequestered the queen for a period of time which kept her from laying eggs. By doing this, we created a brood break which forced all Varroa outside the brood cells and from underneath the protection of the wax capping. Once the colonies were free of capped brood, we vaporized with OA exposing many of the Varroa to the gaseous OA. Our results were more favorable than the first study. We found when colonies are vaporized with OA in addition to the "forced" brood break they had a significant increase in Varroa mortality than colonies that only had a brood break or OA vaporization. At this time, we recommend that beekeepers only use OA in conjunction with a "forced" brood break or during times when colonies naturally have little to no capped brood which can occur in winter or during periods when they are being re-queened.

My third chapter examined how integrating floral resources into an erosion control seed mix can help to restore habitat for wild bees. In today's world, with eight billion people, land is at a premium. It is needed to feed and house the many. Conserving and restoring land for bees and other pollinators sounds great, but how can it be justified with such growing human demands. That is why we need to develop creative ways to enhance flower rich habitats for bees.

Residential and city landscapes offer a perfect opportunity for the addition of flowering plants and habitat which then can support a number of bees and other pollinators. Studies have shown increases in bee abundance and richness in urban and agricultural communities when pollinator habitat has been restored (Buri et al. 2014, Dicks et al. 2010, Fielder et al. 2012, Hall et al. 2017, Kremen & M'Gonigle, 2015, M'Gonigle et al. 2015, Sutter et al. 2017). Other areas which have been targeted for conservation and/or habitat restoration are lands alongside farms, roads, and parking lots. But one area that has been barely explored for pollinator habitat restoration is eroded land.

Soil erosion caused by human activates is not a new problem and continues to be a major threat to the sustainability of agriculture globally (Lal & Stewart, 1990). It began when man started to cultivate the land for crops and livestock. Since that point, erosion has steadily stripped the rich surface topsoil and degraded much of the world's agricultural land. In the US some estimates are as high as 30% of the farmland or around 100 million hectares have been permanently destroyed due to erosion (Borrelli et al., 2017, Butzer, 1997, Pimentel & Harvey, 1999). Restoring these undesirable lands is not an easy task, but with proper planning, site stabilization and implementation, some lands can be restored. Our study is one example.

In 2019, an intensely eroded, 5-acre plot of land located at the Iron Horse Farm in Watkinsville, was selected as a study site for wild bee habitat restoration. Over several months, the area was graded, limed, fertilized, composted and mulched in order to stabilize the soil surface for future planting. The site was randomly divided into 10 plots with 5 replications of two treatment planting groups - grass alone and flower enhanced. In the fall of 2019 and the spring of 2020, plots were seeded and planted with the assigned seed mix. The grass mix comprised of a recommended grass stabilization mix and the flower enhanced mix was established by the Ernst Seed Company.

After the plots had adequate bloom in years 2021 and 2022 we began to collect bee specimens using two different methods. The first method researchers collected bees visiting a flower with an aerial net**.** The second method incorporated sweep (beat net) sampling across both the grass and flower plots. After specimens were collected, they were pinned, labeled and then identified. In the two-year period we collected 3464 specimens, representing 4 families, 19 genera and 59 species. In both sweep and bee to flower collections, abundance, richness and diversity of bees were greater in the flower plots compared to the grass plots, with *Lasioglossum*, *Halictus* and *Bombus* being the most abundant genera collected.

There were 12 flowering plants selected for this study. Of these, only *Rudbeckia hirta* (Black eye Susan), *Chamaecrista fasciculata* (partridge pea), *Eryngium yuccifolium* (rattlesnake master), *Pycnanthemum muticum* (mountain mint), and *Coreopsis leavenworthii* (Leavenworth's tickseed) survived. The others were either eaten or destroyed by deer. But the ones that did survive, flourished. As a follow up study, it would be beneficial to examine which of the 5 plants continue to flourish and for how long. Another area for consideration is developing pollinator/bee/wildflower seed mixes that are more regionally friendly. When examining seed mixes, a number of them contain species that are not native to the US, or regionally adaptive. What may grow well in Michigan may not in grow well in Georgia or vise versa. What may feed bees in California, may not feed bees in Georgia. More research needs to examine which flowering plants are best for the area, not only in the ability to grow but their ability to attract, feed and shelter the local bees and other pollinators. While on this subject, more education and information needs to be available to homeowners and urban landscapers about which flowering plants actually feed something and which flowering plants don’t. Years ago, I too thought all pretty flowers fed bees and butterflies. This is not the case.

The results of this study – flower plots attracted more bees than grass plots - may not be earth shattering, but it does offer more sustenance to the area of habitat restoration for bees and other pollinators. The 5-acre plot prior to being planted, wasn’t feeding or sheltering much if anything. It was weathered bedrock with not much to offer. But after just two years, it provided habitat to at least 59 species of bees. If estimates are correct, and there are millions of hectares of eroded, unused land laying fallow just in the US, think of all the possibilities and the bees that can be fed with a little (or a lot) of effort.

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