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Establishment of Pollinator Habitat within a Livestock Pasture Ecosystem

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Entomology

by

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ABSTRACT

Pollinators are important for fertilization, setting fruits, and seed development of more than 78% of the flowering plants that provide food for human beings and other species. Use of pollinators to maximize crop production is a proven agricultural practice; however, it has been less explored in livestock forage production systems. This study investigated pollinator abundance and diversity in pastures using different sampling methods and determined the impact of different pasture management practices on insect pollinators in a livestock pasture ecosystem. In Chapter 2, utility of four different colors of pan trap (blue, green, yellow, and purple) for sampling bees in a livestock pasture ecosystem of native forage species was examined. Blue color traps were the most attractive to bees followed by purple, yellow and green color traps. In Chapter 3, vanes with different colors (dark blue, bright blue, dark yellow, bright yellow, purple, and red) were designed and evaluated for their light reflectance properties and attractiveness to wild bees in livestock pasture. Bright blue traps captured the highest number of individuals and species of bees. This could be due to appropriate match between the visual spectrum of bees and light reflectance spectrum of vanes. Bees responded similarly to traps with other colors of vanes, except for red vane traps, which captured the lowest number of bees. In Chapter 4, impacts of grazing native forb and grasses on insect pollinators and other arthropod communities in a pasture system was determined. The abundance, diversity, and evenness of bee communities and other insects were greater in non-grazed plots as compared to grazed pasture plots. However, species richness, as measured by rates of species accumulation relative to sampling effort, was not different among treatments. In Chapter 5, a two-year study assessed how organic and non-organic pasture management practices affect bee abundance and diversity in pastures. Bee diversity, abundance, and species richness of bees (as measured by rarefaction curve) were

similar between pastures under organic or non-organic management. Different factors such as low availability of floral resources in organic pastures, use of synthetic fertilizers (to promote the growth of plants) and herbicides for weed management in non-organic pastures might have affected abundance and richness in organic and non-organic pastures. In Chapter 6, impact of commercial native forb/legume/grass (FLG) or warm season grasses (WSG; equal mix of *Andropogon gerardi*, *Tripsacum dactyloides*, and *Sorghastrum nutans*) on bees and non-bee insect communities in livestock pasture was determined. Total number of bees collected was higher in FLG than in WSG (3380 in FLG vs 3158 in WSG). Similarly, 3692 non-bee insects were collected from FLG whereas WSG contained 2346 non-bee insects. Findings from these studies will be helpful in selecting appropriate sampling methods for monitoring bees and other insect communities and also be helpful in developing pollinator-friendly pastures that support diversity of native bee species and other beneficial insects in livestock pasture ecosystems.

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DEDICATION

I would like to dedicate this dissertation to my husband, Mohan Acharya. Without you I would not be who I am today and for that I am so thankful.

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Buck's Hangout® (Hamilton Native outpost, Elk creek, MO)
 Tall grass Inexpensive seed mix® (Prairie Moon, Winona, MN)
 Tallgrass Exposed Clay Subsoil Seed Mix® (Prairie Moon, Winona, MN)

Butterfly and Hummingbird (Hamilton Native outpost, Elk Creek, MO)

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CHAPTER ONE

1. INTRODUCTION

Pollination is the process by which pollen grains from male anthers are transferred to the female stigmata on flowers. Pollination is essential for fertilization, setting fruits, seed development and continuation of life cycle of plants that eventually provides food for humans, livestock, and wildlife (Burns, 2011). For many angiosperms, this process is carried out by pollinators that include honeybees (*Apis* spp.), pollen bees (i.e., non-*Apis* spp.), butterflies, and several other types of insects and other animals. Although, managed honeybees are used as the main pollinators for many commercial crops, native bee species also play an important role in plant fertilization and in many cases are more efficient pollinators than honeybees (Winfree et al., 2011). Pollinators influence crop yield in different production systems (Reilly et al., 2020). A meta-analysis of numerous studies on pollinators concluded that the pollination increases yield of several pollinator-dependent specialty crops, including many fruits and vegetables, through an increase in seed production (Klein et al., 2007). Additionally, pollinators increase out-crossing and sexual reproduction in cereal crops, thereby increasing production and conserving biological diversity in natural ecosystems (Southwick & Southwick Jr, 1992; Ollerton et al., 2014); (Senapathi et al., 2015). Pollinators provide crucial ecosystem services, and about 87% of flowering plants across the globe are pollinated by insects, bats, and birds (Winfree et al., 2011). In 2005 alone, pollination services from insects added \$215 billion to the global economy by contributing to the production of 75% of crop species and assisting in the reproduction of about 94% of wild flowering plants (Vanbergen & Initiative, 2013).

In the recent past, populations of pollinators, specifically bees, have been declining across different regions (Ollerton, 2017; Zattara & Aizen, 2021). Reasons for a decrease in bee

populations are complex and interrelated. Agricultural practices such as application of pesticides and other agrochemicals negatively impact pollinators (Goulson et al., 2015). Chronic exposure to a cocktail of agrochemicals used in conventional production systems causes stress to bees and impairs immune responses (Goulson et al., 2015). Furthermore, bee populations decrease sharply if such immunocompromised bees are exposed to parasites and pathogens (Goulson et al., 2015). In farming landscapes, agronomic practices may influence pollinator populations and diversity. For instance, organic production practices tend to support more species richness for all types of pollinators compared with conventional farming (Gabriel & Tschardt, 2007; Pérez-Méndez et al., 2020), although such trends may be landscape context dependent (Rundlöf et al., 2008). Organic management may reduce some of the negative environmental impacts of intensive agriculture; however, little is known about the benefit to pollinators of biodiversity and yield under organic management of grassland meadow, pasture, and mown pastures. Grassland ecosystem shows weaker and slower response to organic management on pollinators abundance and diversity compared with agriculture croplands (Klaus et al., 2013). However, very few studies are available on the impact of organic pasture management on bees and non-bee pollinators in a livestock pasture ecosystem.

There are more than 307 million hectares of grassland in United States (Havstad et al., 2009) that provide flower (forage) and nest sites for bees (Michener, 2007) and other arthropods. Native pollinators are critical in maintaining many plant species in these rangelands but are at risk due to habitat fragmentation (Moncada, 2003). Establishment of native flowering forages in the pasture provides forage resources for pollinators, and these pollinators in turn benefit nearby agriculture crops through pollination service (Chaplin-Kramer et al., 2011). Most pastureland is managed for livestock grazing, but very rare studies are available that explained the interaction

between livestock and pollinators. Furthermore, little is known about sustainable livestock pasture systems that support pollinator habitats. It is likely due to lack of management of insect pollinators and other beneficial arthropods and their ecosystem services in the pasture system. Investigating the relationships between livestock forage species and pollinators will help in preventing loss of bees and other pollinators in livestock pasture ecosystems.

Cattle grazing in the livestock pasture impacts richness and diversity of pollinators such as beetles, flies, and hymenopterans (DeBano, 2006). Livestock grazing decreases pollinators by decreasing food resources (pollen), and destruction of nests and nesting sites (Kearns et al., 1998). Under heavy grazing, herbivores negatively affect reproductive performance of the plant, including number of flowers, and number of pollen grains per flower, negatively impacting pollinator populations (Zhang et al., 2019). A similar decrease occurred in other pollinators such as trap-nesting solitary bees, wasps, butterflies, and grasshoppers in grazed compared with ungrazed grassland (Kruess & Tscharntke, 2002). However, in a recent study, moderate grazing did not negatively affect availability of floral and nesting resources (Shapira et al., 2020). Similarly, wild bee abundance and richness, and honeybee activity was not affected by moderate grazing in this study. In contrast, it increased nectar and pollen diversity and availability to both honey bees and wild bees (Shapira et al., 2020). Effect of grazing practices on pollinators may vary from pasture to pasture and may also depend on the animal species used for grazing. While few recent studies highlight the impact of grazing practices on large animals, our knowledge about the impact of grazing native forb and grasses (by small ruminant species) on insect pollinators and other arthropod community is limited.

In order to assess pollinator populations and diversity, simple, unbiased, and reliable sampling methods are needed (Cane et al., 2000). Special focus has been given regarding the

distribution of bees in agricultural land largely due to its important role in pollination of flowering native plants and agricultural crops (Klein et al., 2007). Non-bee insects such as flies, wasps, beetles, and butterflies are also regarded as valuable pollinators though may not be as efficient as bees (Potts et al., 2016). Different sampling methods are used for assessing pollinators in various ecosystems. Passive sampling methods, (e.g., different types of traps), are easy to operate, do not require skilled manpower, and allow for data collection over a certain range of time (e.g., 24 hours; McCravy, 2018). The most common passive sampling methods used for sampling pollinator communities are malaise trap, pan and vane traps (Stephen & Rao, 2005; Campbell & Hanula, 2007). Very few studies are available that have assessed the effectiveness of colored vane traps for capturing non-bee pollinators, but it has shown great potential especially for sampling bees (Hall, 2018). These sampling methods are widely used for sampling in agricultural and related ecosystems. However, their utility for sampling bees and other insects in livestock pasture ecosystems is yet to be examined.

These passive traps are based on different colors and utilize visual cues while attracting bees. Light reflected from different colored traps have different intensity, and thus affect the number of bees and other pollinator species that are attracted to the trap (Vrdoljak & Samways, 2012; Joshi et al., 2015). A study conducted by (Moreira et al., 2016) in agricultural land and in natural or savanna-like areas found that there is a complementary relationship between colored pan traps (white, yellow or blue) on species richness. The majority of studies in this field have used blue, yellow and white-colored pan traps. The visual attractancy of several other colors for sampling bees and other arthropod insects in livestock pasture ecosystems is yet to be documented. In this context, the major focus of this study is to examine pollinator abundance and diversity in pasture ecosystems using different sampling methods, and to assess the effect of

pasture management practices and forage types on pollinator communities.

2. Review of Literature

2.1 Importance of pollinators

Use of pollinators to maximize crop production is a proven agricultural practice. Pollination results in fertilization and seed production ensuring a new generation of plants, which eventually provides food for animals and people (Klein et al., 2007). Pollinators include native bees, and honeybees, butterflies, and other insects, of which native bees are the most efficient pollinators of food crops (Klein et al., 2007). Use of native bees to maximize crop production through pollination is an inexpensive agricultural practice (James et al., 2008). The ecological service provided by wild insects which includes dung burial (\$0.38 billion), pest control (\$4.49 billion), pollination (\$3.07 billion), and recreation (\$49.96 billion) collectively could add up to \$57 billion per year in the United States alone (Losey & Vaughan, 2006). In 2005, pollination services from insects added \$215 billion to the global economy to about 75% of crop species and helped in the reproduction of about 94% of wild flowering plants (Vanbergen & Initiative, 2013).

Decrease in number of pollinators or reduction in pollination decreases production of seeds, fruits, and vegetables (Klein et al., 2007). Decreased pollination can cause reduced seed production, production of weak plant offspring, and increase inbreeding of plants, i.e. decreases in heterozygosity (Kearns et al., 1998). In a long-term scenario, reduction of seeds could ultimately lead to plant species extinction.

2.2 Conservation of pollinators

Primary pollinators such as bees depend on floral diversity, floral abundance, nectar resource diversity, and ratio of pollen to nectar for their survival (Potts et al., 2003). Flowering plants provide pollen, nectar, and nesting sites for pollinators, which is necessary for normal

survival and reproduction of pollinators, and for the development during different stages of the life cycle (Klein et al., 2007; Pywell et al., 2005; Cole et al., 2017). For example, the larval stage of bees requires body building nutrients to mature to adults, adults need energy rich nectar, and all these nutrients are obtained from a variety of floral sources (Filipiak, 2018). Perennial plants in agriculture field margins, which bloom in different times during spring, summer, and fall, can supply the floral resources to pollinators throughout the year, and thus help in conservation of pollinators in their natural habitats (Tuell et al., 2014). Growing a mixture of native wildflowers also enhances pollinator populations by delivering forage resources throughout season (Carvell et al., 2007; Tuell et al., 2014).

Diversity of flowers affects the quality of bee nutrients (lipid and protein content) (Donkersley et al., 2017). Pollinator habitats comprising a mix of different flowering species are important in conserving pollinator populations as they can provide quality food resources year-round. Flowering plants such as sunflower and other oilseeds are a very important source of pollen. However, the time period in which they provide pollen is very limited. Thus, these crops alone may not be a good source of nectar year-round. Temporary unavailability of floral resources results in movement of pollinators to different locations, decreased habitat quality, and reduction in pollinator abundance (Cole et al., 2017). Reduction in diversity of flowering plants and the decline in bees are interrelated (Filipiak, 2018). Intensively managed agricultural habitats lack flowers leading to a smaller number of pollinators (Cole et al., 2017). Intense grazing of pastures by ruminants also results in a decrease in pollinator population (Lazaro et al., 2016). Loss of pollinators in crop production leads to food scarcity (Potts et al., 2010). In the United States alone, a decrease in pollination alone can cost up to \$18 billion per year (Park et al., 2015).

Availability of appropriate pollinator habitats, nesting substrates, and nest building materials helps in the establishment of bee communities (Kearns et al., 1998). Accessibility of well-drained bare ground, nesting cavities, steep and sloping ground, abundant flowering species with pithy stems and occurrence of pre-existing cavities or burrows adds in making nests for pollinators (Potts et al., 2005). Nesting and foraging resources are necessary for structuring bee communities in an ecosystem (Potts et al., 2005). Ground nesting bees (Andrenidae, Apidae, Halictidae and Megachilidae) prefer areas with well-drained soils (Potts et al., 2005). Shrub and woodland habitat with range of post-burn age (intermediate age sites), flower richness, and moderate grazing intensities increases abundance of bees (Vulliamy et al., 2006). Development of landscape heterogeneity and boosting the quality of semi-natural habitat helps to enhance diversity and conserve pollinators (Cole et al., 2017).

2.3 Livestock Pasture and Rangeland Systems

Rangelands are a type of land on which natural vegetation is dominated by grasses, forbs and shrubs and is managed as a natural ecosystem (McMurphy et al., 1990). It includes grasslands stretching from Texas to Canada, prairie of Dakotas and Nebraska to annual grasslands of California, arid shrub-land throughout western US, the arctic tundra, mountain meadows and deserts throughout the Southwest, and forestlands and wetlands throughout North America. Rangeland can provide valuable natural resources through appropriate grazing strategies (McMurphy et al., 1990). The primary difference between rangeland and pasture is management; rangelands strive or improve persistence of natural vegetation without annual cultivation and irrigation and are harvested by grazing animals, while pastures have forage that is adapted for livestock and managed by seeding, mowing, fertilization and irrigation (McMurphy et al., 1990).

Use of native seed mixes (forbs, legume, and shrubs) in pasture may help in maintaining overall sustainability, and as a result, diversity and density of flowering plant species may increase pollinator populations in pasture ecosystem. Increasing native pollinator diversity and abundance by providing different floral food resources and suitable nesting (breeding) habitat could lead to a more sustainable production system that will eventually benefit farmers and society by ensuring food supply that relies on pollination. Moving into the future, it is important to be able to integrate conservation and livestock production to maximize biodiversity and family farm sustainability.

2.4 Livestock and Pasture Systems as Pollinator Habitat

Bee diversity and abundance in livestock pasture primarily relates to intensity of grazing, availability of nesting substrates, and post-fire age (Potts et al., 2003). Grazing livestock could alter pollinator habitat, hamper nesting sites, and reduce flowering vegetation, a food source for pollinators (Kearns et al., 1998). Deforestation to convert forest into grazing area decreases pollinator populations (Barthell et al., 1993). Wood and twigs are often removed from the pasture that in turn decreases potential nesting sites of wild bees (Kearns et al., 1998). Overgrazing and cutting existing floral food resources reduce grass height that could provide shelter for butterflies (Saarinen et al., 2005) and other beneficial insects. Sugden, (1985) observed the effect of sheep grazing on pollinator habitat in pasture with a milk vetch (*Astragalus monoensis*) and found that grazing practices destroyed nests, removed pollinator's food sources, and trampled bees. Selective grazing by livestock animals also decreases several plant species, which provides pollen and nectar to bees (Kearns et al., 1998) and other flower visiting insects.

Intense grazing early in the season is associated with significant reduction of early-and mid-season bees possibly due to reduced floral resources and altered foraging behavior of bees (Kimoto et al., 2012). A similar decrease occurs in other pollinators such as trap-nesting solitary bees, wasps, butterflies, and grasshoppers in grazed as compared to ungrazed grassland (Kruess & Tscharntke, 2002). In contrast, moderate grazing has no effect or positively increases availability of floral and nesting resources (Shapira et al., 2020). In one study, wild bee abundance and richness, and honeybee activity was not affected by moderate grazing, however, moderate grazing positively increased nectar and pollen diversity and availability to both honeybee and wild bees (Shapira et al., 2020). Modifying grazing practices could help conservation efforts in these habitats. For instance, reduction in grazing intensity preserves insect diversity, increases vegetation height, and increases the number of butterflies, trap-nesting bees, and wasps on grasslands (Kruess & Tscharntke, 2002).

Mowing is needed in the pasture to minimize weeds. Removal of weeds is necessary to increase floral resources and survival of eggs and larvae of insects (Tanis et al., 2020; Black et al., 2011). However, when the pollinators become active it is wise not to destroy bee habitat by mowing and in such situation partial mowing (mowing one third at a time) and limited grazing are beneficial for pollinators (Black et al., 2011; Tanis et al., 2020). Proper mowing practice and low grazing intensity increases plant biodiversity and increases pollinators in the pasture (Johansen et al., 2019; Ledvina et al., 2020). Thus, management practices (mowing and grazing) should be included that can benefit both livestock and pollinators in the pasture.

Establishment of native flowering forbs and grasses provides continuous food (nectar and pollen) and habitat resources for native pollinators throughout the year, therefore, benefitting farmers and society by ensuring an adequate food supply. Sustainable livestock pasture systems

that support pollinator habitat can enhance diverse communities of insect pollinators and other beneficial arthropods and their ecosystem services in pastures and adjacent farm landscapes. Bees require nectar and pollen from plants flowering from spring to fall and require specific nesting habitat. Management strategies should focus on incorporating pollinator habitats in such grasslands to benefit existing agri-environment. Inclusion of diverse seed mix such as kale, mixed cereal, linseed and legume in the pasture enhances abundance and diversity of bumblebees (Potts et al., 2009). Additional legume and forbs in grassland increases pollinator diversity (Orford et al., 2016), and use of these plants in livestock pastures could enhance establishment of native pollinator communities. Pollinators contribute pollination services and increase seed yield in annual forages such as crimson clover (*Trifolium incarnatum*) or hairy vetch (*Vicia villosa*; (Anderson et al., 2010). Also, pollinators frequently visit specific forb species such as common dandelion (*Taraxacum* spp.) and Canada thistle (*Cirsium arvense*) (Orford et al., 2016). Thus, it is important to include proper forage species in order to increase pollinator populations in pasture ecosystems.

2.5 Conventional Vs. Organic Livestock Pastures: Impact on Pollinator Health

Conventional pasture management system includes increased use of chemical fertilizers, herbicides, pesticides, and tillage practices that leads to a decrease in insect and plant biodiversity (Hirsch, 2010). Additional negative anthropogenic environment impacts include, pesticide accumulation, soil degradation, and increased greenhouse gas emission which collectively hampers balanced ecosystem (Hirsch, 2010). Organic pastures differ from conventional pasture management system in many ways, such as prohibition of the use of chemical herbicides and pesticides as well as synthetic fertilizers in forage production. Thus,

organic management may feature more environmentally sustainable production practices (USDA-NOP, 2021).

Most commonly used chemical fertilizers in conventional farming practice are phosphorus and nitrogen which benefit crop productivity (Arvin Mosier et al., 2004). However, overuse of these fertilizers, specifically nitrogen, affects reproductive traits in plant such as quality and quantity of flowers (Hoover et al., 2012). Nutrient overdosage may directly affect pollinators through loss of plant species diversity (Bobbink et al., 2010). Nectar glucose and pollen fructose levels decrease in over fertilized plants (Ceulemans et al., 2017). Consequently, the physiology, behavior, and abundance of insect pollinators are altered (Ceulemans et al., 2017). Besides fertilizers, a number of plant protection products are used including insecticides, herbicides and fungicides that could benefit agriculture (Cooper & Dobson, 2007), but there are several risks associated with their use (Cullen et al., 2019). Although these products target insect pests, they also affect non-target insects such as beneficial insect pollinators (Cullen et al., 2019).

Agriculture practices such as application of pesticides-, especially insecticides- usually have a negative impact on pollinator communities. Effect of lethal and sublethal doses of insecticides on behavior of pollinators depend on duration and route (orally or by contact) of exposure (Sponsler et al., 2019). Pesticides such as, organophosphates, carbamates, phenylpyrazoles, neonicotinoids, and pyrethroids share a common mode of action that affects central nervous system of insects, resulting in imbalance in coordination, paralysis and death of pollinating insect species (Sponsler et al., 2019). Bees contact neonicotinoid through residues in pollen, nectar, and dust after its application to forage crops (Feltham et al., 2015). Neonicotinoids may pose a threat to bumblebee colonies by negatively affecting queens (Goulson, 2013). Lethal and sublethal dosage of neonicotinoid (imidacloprid) modify the

foraging behavior of honeybees exhibited by missing bees from hive, revisiting abnormalities to the foraging source, and delay in return trip to foraging source (Yang et al., 2008). It also slows larval development and hinders learning abilities (Roulston & Goodell, 2011).

Among other pesticides, the fungicides, captan and benomyl caused *Osmia lignaria*, an orchard mason bee, to completely vanish from cherries within few days (Ladurner et al., 2005). Pesticides may also affect floral visitation in certain crops. For example, application of fungicide, bis(dithiocarbamate) decreased abundance of floral visitors in sunflower, reduced fruit weight, oil content in the seed and negatively affected the productivity of the crop (de Oliveira et al., 2019). Fungal pesticide exposure could also affect gene expression of detoxifying enzymes (Tome et al., 2020). Moreover, physiology of queen honeybees and worker behavior of workers are affected when bee's wax is exposed to the fungicide chlorothalonil and insecticide chlorpyrifos during development (Walsh et al., 2020). As a result, there was a significant reduction in egg laying rate of the adult queen and a reduction in the number of worker bees (Walsh et al., 2020). The combined effect of insecticides and fungicides can create a much more toxic environment to honeybees and bumble bees (Sanchez-Bayo & Goka, 2014). Exposure of pesticide impacts survival and health of juvenile bees and causes stress to the colony (Sanchez-Bayo & Goka, 2014). In addition, use of agrochemicals affect detoxification mechanism and hamper immune response system of bees making them susceptible to parasites (Goulson et al., 2015).

Integration of farmland with flower rich habitat, control of pesticides, and adopting sustainable farming practices are the major steps for maintaining future bee populations (Goulson et al., 2015). Organic fields support more species richness for all types of pollinators compared with conventional farming (Gabriel & Tschardtke, 2007). In an organic-strawberry

field, 45% of the strawberries were fully pollinated, whereas in conventional only 17% were pollinated by pollinators (Andersson et al., 2012). Thus, strawberry pollination occurs at a greater rate in organic settings compared with conventional farms leading to increased quality and quantity of crop yield (Andersson et al., 2012). Similarly, pollination deficit (pollination potential - actual pollination) was less, and abundance of bees was higher in organic compared with conventional field (Morandin & Winston, 2005). Native bees maintained on organic farms close to pollinator habitat can provide better pollination services compared with native bees maintained on conventional farms (Kremen et al., 2002). Offspring production and survival of female solitary bee (*Osmia lignaria*) was also decreased after isolation from natural habitat in non-organic habitat whereas organic management had little of such effects (Williams & Kremen, 2007). Relative increase in organic cropping (winter wheat field) from 5 to 20% increased bee species richness by 50%, density of solitary bee population by 60%, and bumble bee by 150% (Holzschuh et al., 2008). Organic farms also attracted 50% more bird species in comparison to conventional farms (Belfrage et al., 2005). Pollination occurs at a greater rate on some organic farms; however, such effects may be seen only after 2-4 years after conversion to organic practices (Andersson et al., 2012). Additional incorporation of landscape (patch shape, interpatch, and habitat aggregation) in organic fields enhances wild bee population in agroecosystem (Kennedy et al., 2013). Such incorporation of semi-natural vegetation in organic management increased frequency of bee visits to pollen source ultimately increasing their abundance (Klein et al., 2012).

2.6 Conservation of pollinators in livestock pastures

Agronomic crops may only attract a few bee species whereas livestock pasture comprising a wide variety of flowering forage has potential to attract many more bee species

(Gresty et al., 2018). Inclusion of bee preferred forage species in the pasture increases floral-unit abundance of forage species (Gresty et al., 2018). It is also equally important that flowering species persist throughout the season to boost the survival of wild pollinator species (Carvell et al., 2017). Besides pollinators, other beneficial arthropod species (e.g., insect predators and parasitoids) are also important in balancing an ecosystem. Farmers can maintain their landscape by optimizing abundance of these species that play a crucial role in maintaining ecosystems (Isaacs et al., 2009; Taylor et al., 2006)) determined that arthropods were more common in weedy communities not exposed to herbicides compared with monoculture communities exposed to herbicides. Thus, European agri-environment schemes have promoted establishment of flower- rich habitat in hedgerows, field-borders, and temporary flowering crops in crop boundaries (Whittingham, 2011). For the conservation of bees in urban areas, communities could add green public space, such as parks and gardens full of diversified flowers that add habitat for pollinators (Turo & Gardiner, 2019). In livestock pastures, precaution should be taken during grazing, burning pasture, mowing, and herbicide applications in order to maintain pollinator habitat.

Major plant families that are used as forage in livestock pasture are grass, forbs, and legumes. Plant-pollinator interactions have been less studied in grasslands because the dominant species are typically wind-pollinated (e.g., plants that belong to Poaceae, Cyperaceae, Chenopodeaceae, and Polygonaceae) or self-pollinated (Harmon et al., 2011). Wind- or self-pollinated plants have the ability to reproduce without the need of pollinators; however, these plants have reduced genetic diversity because of reduced chances of outcrossing (Harmon et al., 2011). That leads to poor reproductive success and decreased plant abundance and variety

(Knight et al., 2005). While grasses self-pollinate, other forage types such as forbs, legumes and flowering shrubs require cross-pollination (Harmon et al., 2011).

Pollinators such as bees, butterflies, hoverflies, and beetles respond differently to local habitat and landscape composition in livestock pasture. For example, beetle richness and abundance is positively correlated with vegetation height, hoverflies are more numerous with high forest cover in landscape, and bee abundance increased with increased number of flowers (Sjödín et al., 2008). European bumblebees favor plant species within Fabaceae (*Onobrychis viciifolia* and *Trifolium pratense*) and Scrophulariaceae (*Odontites vernus*) families. Most abundant bee species are typically less selective (polylectic), whereas rarer species tend to visit fewer flower species (oligolectic); For example, rare species such as *Bombus humilis* and *B. hortorum* gather pollen from a narrow range of plant species (Brian, 1951; Carvell, 2002; Goulson & Darvill, 2004). It is because some rarer species comes out of hibernation later and have limited time to develop and reproduce (Goodwin, 1995); therefore, highest quality of food is necessary for their brood, and hence they are more selective (Goulson & Darvill, 2004). Studies on effect of forage species to pollinators on livestock pasture ecosystem was not explored until recently.

2.7 Relationship Between Forage Species and Pollinators in Livestock Pasture

Like many crops, forbs require cross-pollination by arthropods. Cross pollination is crucial for several plant families (e.g. Scrophulariaceae and Lamiaceae) that cannot self-fertilize (Neff & Simpson, 1993). Most flowering forages need pollination for seed production and continuation of generations after the initial plant establishment (Reed, 1993). Bee-plant relationship is mutualistic, because both benefit from their interaction. While gathering nectar and pollen, bees unknowingly transfer pollen from one flower to another. This transfer results in

production of fruits and seeds. This process also reduces chances of inbreeding (Ingram et al., 1996).

Rangeland and pastures with native flowering plants can provide high quality pollinator habitat that initiates flowering early in the spring and continues till late fall and thus increases abundance of pollinators (Isaacs et al., 2009). Native floral resources increase richness and abundance of native bees (Steffan-Dewenter & Tschardtke, 2001). The important element for pollinator habitat is the inclusion of diverse flowering plants that initiate flowering early in the spring and continue till late fall (Cane, 2001). Certain species of bumblebees (*B. bimaculatus*) peak in mid-July, whereas *B. impatiens* peak later in the fall (Cane, 2001). A variety of forages that include flowering forbs and legumes as well as grasses can provide pollen to pollinators till the end of the autumn season in livestock pasture ecosystems. Bumblebee abundance, species richness, and forage activity depend on flowering plant species, vegetation structure and height (Carvell, 2002). Additional legume and forbs in a grassland pollinator community results in increased pollinator abundance (Orford et al., 2016).

2.8 Methods for Sampling Pollinators in Livestock Pasture

2.8.1 Pan trap

Different types of traps have been used for bee surveys including pan traps. Pan traps have been commonly used for sampling in agricultural insect pests (Leong & Thorp, 1999) and other arthropods in different ecosystems. Pan traps by design are an effective technique for trapping pollinators, including a large percentage of bees in the area (Wilson et al., 2008). These passive traps are more effective than other sampling methods such as, malaise trap (Campbell & Hanula, 2007) and active net sampling (Wilson et al., 2008). Samples collected from pan traps are unbiased, cost-effective, and can be collected from a large area in a short period of time

(Leong & Thorp, 1999). Pan traps allow insect pollinators to be sampled in geographical locations, including agricultural and semi-natural grasslands habitat (Roulston et al., 2007). Additionally, pan trap allows researchers to monitor pollinators across time and space without any negative impact (Gezon et al., 2015).

Bees use olfactory signals if the floral source is 30 cm away and visual cues when they are close to flowers (Streinzer et al., 2009). Light reflected from different colored traps have different intensities and thus affects number of bees and other pollinator species that are attracted to trap (Vrdoljak & Samways, 2012). Color vision of hymenopteran insects such as bumblebees and honeybees is of great importance because it impacts their attraction to the trap (Dyer et al., 2011). Bees have an ability to identify dissimilar color and discriminate color texture (fine or coarse). In the case of honeybees, visual angle depends on stimulated photoreceptor for coding the color information (Dyer et al., 2011). Color discrimination senses in bumblebees are poorly developed compared with honeybees; however, bumblebees observe stimuli with smaller visual angle in comparison with honeybees (Dyer et al., 2008).

Bee abundance, species richness and diversity in sampling sites are influenced by the color of pan traps (Joshi et al., 2015). Until recently, impact of color on pan trap to attract sample pollinators was not well understood. A study conducted by (Moreira et al., 2016) in agricultural land and in natural savanna-like areas found that there is a complementary relationship between colored pan traps (white, yellow or blue) on species richness. Yellow or blue pan traps attracted more species than white pan traps (Moreira et al., 2016). Among blue and yellow pan traps, blue attracted more bees while yellow attracted more wasps (Moreira et al., 2016). In addition, sex of bees in samples collected in pan traps varied based on the color of pan trap (Leong & Thorp, 1999). For instance, white colored pan traps attracted more male bees compared with blue

colored traps which attracted more females (Leong & Thorp, 1999).

Colored pan traps are used in monitoring the beneficial insects in different studies and used in an area where information about the relative number of insects is needed (Laubertie et al., 2006). White or yellow colored pan traps with high reflective index attracted an abundance of pollinators in lowland of Cape Floristic Region (Vrdoljak & Samways, 2012). Yellow colored pan traps may be useful for sampling large numbers of parasitic hymenopterans, but this does not always equate to high levels of diversity (Wells & Decker, 2006). Males of *Panurgus calcaratus* are attracted to white traps, and females to yellow pan traps (Heneberg & Bogusch, 2014). Among white and yellow colored pan traps, yellow was found to be more efficient in attracting a higher number and more diverse bees in comparison to white colored pan traps in open fields, riverside habitats, forests and roadside verges (Gollan et al., 2011). Wang et al. (2017) noted significantly greater pollinator diversity in yellow pan traps than white and blue pan traps. Yellow colored pan traps were more effective in collecting more individuals inside forest than blue (Abrahamczyk et al., 2010).

2.8.2 Vane trap

To sample both bee and non-bee pollinators, effective passive pollinator traps are needed. Vane traps, which are relatively newer traps, are effective in attracting bees without using insect attractant liquids or pheromones (Hall, 2018), and have been used recently used to sample wild bee populations (Gibbs et al., 2017). Blue vane traps were more effective especially for trapping wild bees in comparison to yellow vane traps (Stephen & Rao, 2005; Joshi et al., 2015). However, these studies lacked taxonomical classification of non-bee species collected in these traps. Like pan traps, vane traps are easy to install, provide an effective sampling method over time, and can be sampled over large areas for longer periods of time (Stephen & Rao, 2005).

Vane traps are considered the best method to study native bee populations in large expanse of grasslands and other habitats (Kimoto et al., 2012). However, there is a need to further customize the design of these traps and assess the effectiveness of different colored vane traps for sampling bees and non-bee species in a livestock ecosystem.

Active method of sampling pollinators has been adopted since long period of time (Taki et al., 2018). Active sampling method is time consuming and may be biased, requires skilled manpower, and experience (McCravy, 2018). However, pan traps were found to be less effective when used for sampling butterflies and moths (Vrdoljak & Samways, 2012). Colored sticky traps have been successfully used to trap arthropods, but these traps vary in their ability to trap Hymenoptera and Coleoptera and cause damage to specimens which can hinder identification (Hoback et al., 1999; Pickering & Stock, 2003). Other passive traps such as colored malaise traps are also used but are not as effective as pan traps (Campbell & Hanula, 2007). Very few studies are available that have assessed the effectiveness of colored vane traps for capturing non-bee pollinators, but it has shown great potential, especially for sampling bees (Hall, 2018). The effectiveness of vane traps to sample bees and other pollinators in livestock pasture ecosystem is yet to be tested.

3. Research objectives

Supporting pollinators to maximize crop production through enhanced pollination services is a simple and inexpensive agricultural practice that additionally benefits farmers (James et al., 2008). At present, low priority is given to sustainable livestock pasture system in commercial agriculture. It is mainly due to lack of management of insect pollinators and other beneficial arthropods and their ecosystem services in a pasture system and surrounding ecosystems. The main focus of the study was to investigate pollinator abundance and diversity in

pastures using different sampling methods and to examine the impact of native floral enhancement and pasture management practices on insect pollinators and beneficial arthropods in pastures. Specific objectives of this dissertation research include:

1. To determine the utility of different types of traps for sampling wild bees and other insects in pasture ecosystems.
2. To examine the impacts of grazing on insect pollinators and other arthropod community in native forb and grass pasture systems.
3. To study the impact of pasture production practices (i.e., organic and conventional) on pollinators.
4. To determine the impact of native floral enhancement on insect pollinators and other arthropods in livestock pasture ecosystems.

4. References

- Abrahamczyk, S., Steudel, B., & Kessler, M. (2010). Sampling Hymenoptera along a precipitation gradient in tropical forests: the effectiveness of different coloured pan traps. *Entomologia Experimentalis et Applicata*, 137(3), 262-268.
- Anderson, N., Rao, S., & Derkatch, A. (2010). Native bumble bee diversity, abundance, and pollination in crimson clover and hairy vetch seed production fields in western Oregon. *Seed Production Research*, 8.
- Andersson, G. K., Rundlöf, M., & Smith, H. G. (2012). Organic farming improves pollination success in strawberries. *PloS one*, 7(2), e31599.
- Arvin Mosier, J., Syers, K., & Freney, J. R. (2004). Agriculture and the Nitrogen Cycle: Assessing the Impacts of Fertilizer Use on Food Production and the Environment. 2004 SCOPE, 65. In: Island Press, Washington, DC.
- Barthell, J., Bradleigh Vinson, S., Frankie, G., La Salle, J., & Gauld, I. (1993). *Threats to the diversity of solitary bees in a neotropical dry forest in Central America Hymenoptera and biodiversity*. CAB International, Wallingford (RU) Natural History Museum, Londres (RU).
- Belfrage, K., Björklund, J., & Salomonsson, L. (2005). The effects of farm size and organic farming on diversity of birds, pollinators, and plants in a Swedish landscape. *AMBIO: A Journal of the Human Environment*, 34(8), 582-588.
- Black, S. H., Shepherd, M., & Vaughan, M. (2011). Rangeland management for pollinators. *Rangelands*, 33(3), 9-13.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., & Dentener, F. (2010). Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological applications*, 20(1), 30-59.
- Brian, A. D. (1951). The pollen collected by bumble-bees. *The Journal of animal ecology*, 191-194.
- Burns, D. (2011). Attracting Native Pollinators. In: North Adams, ma: Storey Publishing.
- Campbell, J. W., & Hanula, J. (2007). Efficiency of Malaise traps and colored pan traps for collecting flower visiting insects from three forested ecosystems. *Journal of Insect Conservation*, 11(4), 399-408.
- Cane, J. H. (2001). Habitat fragmentation and native bees: a premature verdict? *Conservation Ecology*, 5(1).

- Cane, J. H., Minckley, R. L., & Kervin, L. J. (2000). Sampling bees (Hymenoptera: Apiformes) for pollinator community studies: pitfalls of pan-trapping. *Journal of the Kansas entomological society*, 225-231.
- Carvell, C. (2002). Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. *Biological conservation*, 103(1), 33-49.
- Carvell, C., Bourke, A. F., Dreier, S., Freeman, S. N., Hulmes, S., Jordan, W. C., Redhead, J. W., Sumner, S., Wang, J., & Heard, M. S. (2017). Bumblebee family lineage survival is enhanced in high-quality landscapes. *Nature*, 543(7646), 547-549.
- Carvell, C., Meek, W. R., Pywell, R. F., Goulson, D., & Nowakowski, M. (2007). Comparing the efficacy of agri-environment schemes to enhance bumble bee abundance and diversity on arable field margins. *Journal of applied ecology*, 44(1), 29-40.
- Ceulemans, T., Hulsmans, E., Vanden Ende, W., & Honnay, O. (2017). Nutrient enrichment is associated with altered nectar and pollen chemical composition in *Succisa pratensis* Moench and increased larval mortality of its pollinator *Bombus terrestris* L. *Plos one*, 12(4), e0175160.
- Chaplin-Kramer, R., Tuxen-Bettman, K., & Kremen, C. (2011). Value of wildland habitat for supplying pollination services to Californian agriculture. *Rangelands*, 33(3), 33-41.
- Cole, L. J., Brocklehurst, S., Robertson, D., Harrison, W., & McCracken, D. I. (2017). Exploring the interactions between resource availability and the utilisation of semi-natural habitats by insect pollinators in an intensive agricultural landscape. *Agriculture, Ecosystems & Environment*, 246, 157-167.
- Cooper, J., & Dobson, H. (2007). The benefits of pesticides to mankind and the environment. *Crop Protection*, 26(9), 1337-1348.
- Cullen, M. G., Thompson, L. J., Carolan, J. C., Stout, J. C., & Stanley, D. A. (2019). Fungicides, herbicides and bees: A systematic review of existing research and methods. *PloS one*, 14(12), e0225743.
- de Oliveira, A. C., Junqueira, C. N., & Augusto, S. C. (2019). Pesticides affect pollinator abundance and productivity of sunflower (*Helianthus annuus* L.). *Journal of Apicultural Research*, 58(1), 2-8.
- Debano, S. J. (2006). Effects of livestock grazing on aboveground insect communities in semi-arid grasslands of southeastern Arizona. *Biodiversity & Conservation*, 15(8), 2547.
- Donkersley, P., Rhodes, G., Pickup, R. W., Jones, K. C., Power, E. F., Wright, G. A., & Wilson, K. (2017). Nutritional composition of honey bee food stores vary with floral composition. *Oecologia*, 185(4), 749-761.

- Dyer, A. G., Paulk, A. C., & Reser, D. H. (2011). Colour processing in complex environments: insights from the visual system of bees. *Proceedings of the Royal Society B: Biological Sciences*, 278(1707), 952-959.
- Dyer, A. G., Spaethe, J., & Prack, S. (2008). Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. *Journal of Comparative Physiology A*, 194(7), 617.
- Feltham, H., Park, K., Minderman, J., & Goulson, D. (2015). Experimental evidence that wildflower strips increase pollinator visits to crops. *Ecology and evolution*, 5(16), 3523-3530.
- Filipiak, M. (2018). A better understanding of bee nutritional ecology is needed to optimize conservation strategies for wild bees—the application of ecological stoichiometry. *Insects*, 9(3), 85.
- Gabriel, D., & Tschardt, T. (2007). Insect pollinated plants benefit from organic farming. *Agriculture, Ecosystems & Environment*, 118(1-4), 43-48.
- Gezon, Z. J., Wyman, E. S., Ascher, J. S., Inouye, D. W., & Irwin, R. E. (2015). The effect of repeated, lethal sampling on wild bee abundance and diversity. *Methods in Ecology and Evolution*, 6(9), 1044-1054.
- Gibbs, J., Joshi, N. K., Wilson, J. K., Rothwell, N. L., Powers, K., Haas, M., Gut, L., Biddinger, D. J., & Isaacs, R. (2017). Does passive sampling accurately reflect the bee (Apoidea: Anthophila) communities pollinating apple and sour cherry orchards? *Environmental Entomology*, 46(3), 579-588.
- Gollan, J. R., Ashcroft, M. B., & Batley, M. (2011). Comparison of yellow and white pan traps in surveys of bee fauna in New South Wales, Australia (Hymenoptera: Apoidea: Anthophila). *Australian Journal of Entomology*, 50(2), 174-178.
- Goodwin, S. (1995). Seasonal phenology and abundance of early-, mid-and long-season bumble bees in southern England, 1985–1989. *Journal of Apicultural Research*, 34(2), 79-87.
- Goulson, D. (2013). Neonicotinoids and bees: What's all the buzz? *Significance*, 10(3), 6-11.
- Goulson, D., & Darvill, B. (2004). Niche overlap and diet breadth in bumblebees; are rare species more specialized in their choice of flowers? *Apidologie*, 35(1), 55-63.
- Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347(6229).
- Gresty, C. E., Clare, E., Devey, D. S., Cowan, R. S., Csiba, L., Malakasi, P., Lewis, O. T., & Willis, K. J. (2018). Flower preferences and pollen transport networks for cavity-nesting solitary bees: Implications for the design of agri-environment schemes. *Ecology and evolution*, 8(15), 7574-7587.

- Hall, M. (2018). Blue and yellow vane traps differ in their sampling effectiveness for wild bees in both open and wooded habitats. *Agricultural and Forest Entomology*, 20(4), 487-495.
- Harmon, J. P., Ganguli, A. C., & Solga, M. J. (2011). An overview of pollination in rangelands: who, why, and how. *Rangelands*, 33(3), 4-8.
- Havstad, K., Peters, D., Allen-Diaz, B., Bartolome, J., Bestelmeyer, B., Briske, D., Brown, J., Brunson, M., Herrick, J., & Huntsinger, L. (2009). The western United States rangelands: A major resource. *Grassland quietness and strength for a new American agriculture*, 75-93.
- Heneberg, P., & Bogusch, P. (2014). To enrich or not to enrich? Are there any benefits of using multiple colors of pan traps when sampling aculeate Hymenoptera? *Journal of insect conservation*, 18(6), 1123-1136.
- Hirsch, T. (2010). *Global biodiversity outlook 3*. UNEP/Earthprint.
- Hoback, W. W., Svatos, T. M., Spomer, S. M., & Higley, L. G. (1999). Trap color and placement affects estimates of insect family-level abundance and diversity in a Nebraska salt marsh. *Entomologia experimentalis et applicata*, 91(3), 393-402.
- Holzschuh, A., Steffan-Dewenter, I., & Tschardt, T. (2008). Agricultural landscapes with organic crops support higher pollinator diversity. *Oikos*, 117(3), 354-361.
- Hoover, S. E., Ladley, J. J., Shchepetkina, A. A., Tisch, M., Gieseg, S. P., & Tylianakis, J. M. (2012). Warming, CO₂, and nitrogen deposition interactively affect a plant-pollinator mutualism. *Ecology Letters*, 15(3), 227-234.
- Ingram, M., Nabhan, G., & Buchmann, S. (1996). Our forgotten pollinators: Protecting the birds and bees. *Global Pesticide Campaigner*, 6(4), 1-12.
- Isaacs, R., Tuell, J., Fiedler, A., Gardiner, M., & Landis, D. (2009). Maximizing arthropod-mediated ecosystem services in agricultural landscapes: the role of native plants. *Frontiers in Ecology and the Environment*, 7(4), 196-203.
- James, R., James, R. R., & Pitts-Singer, T. L. (2008). *Bee pollination in agricultural ecosystems*. Oxford University Press on Demand.
- Johansen, L., Westin, A., Wehn, S., Iuga, A., Ivascu, C. M., Kallioniemi, E., & Lennartsson, T. (2019). Traditional semi-natural grassland management with heterogeneous mowing times enhances flower resources for pollinators in agricultural landscapes. *Global Ecology and Conservation*, 18, e00619.
- Joshi, N. K., Leslie, T., Rajotte, E. G., Kammerer, M. A., Otieno, M., & Biddinger, D. J. (2015). Comparative trapping efficiency to characterize bee abundance, diversity, and

- community composition in apple orchards. *Annals of the Entomological Society of America*, 108(5), 785-799.
- Kearns, C. A., Inouye, D. W., & Waser, N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual review of ecology and systematics*, 29(1), 83-112.
- Kennedy, C. M., Lonsdorf, E., Neel, M. C., Williams, N. M., Ricketts, T. H., Winfree, R., Bommarco, R., Brittain, C., Burley, A. L., & Cariveau, D. (2013). A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecology letters*, 16(5), 584-599.
- Kimoto, C., DeBano, S. J., Thorp, R. W., Taylor, R. V., Schmalz, H., DelCurto, T., Johnson, T., Kennedy, P. L., & Rao, S. (2012). Short-term responses of native bees to livestock and implications for managing ecosystem services in grasslands. *Ecosphere*, 3(10), 1-19.
- Klaus, V. H., Kleinebecker, T., Prati, D., Gossner, M. M., Alt, F., Boch, S., Gockel, S., Hemp, A., Lange, M., & Müller, J. (2013). Does organic grassland farming benefit plant and arthropod diversity at the expense of yield and soil fertility? *Agriculture, ecosystems & environment*, 177, 1-9.
- Klein, A.-M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the royal society B: biological sciences*, 274(1608), 303-313.
- Klein, A. M., Brittain, C., Hendrix, S. D., Thorp, R., Williams, N., & Kremen, C. (2012). Wild pollination services to California almond rely on semi-natural habitat. *Journal of Applied Ecology*, 49(3), 723-732.
- Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J., & Ashman, T.-L. (2005). Pollen limitation of plant reproduction: pattern and process. *Annu. Rev. Ecol. Evol. Syst.*, 36, 467-497.
- Kremen, C., Williams, N. M., & Thorp, R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences*, 99(26), 16812-16816.
- Kruess, A., & Tscharntke, T. (2002). Grazing intensity and the diversity of grasshoppers, butterflies, and trap-nesting bees and wasps. *Conservation Biology*, 16(6), 1570-1580.
- Ladurner, E., Bosch, J., Kemp, W. P., & Maini, S. (2005). Assessing delayed and acute toxicity of five formulated fungicides to *Osmia lignaria* Say and *Apis mellifera*. *Apidologie*, 36(3), 449-460.
- Laubertie, E., Wratten, S., & Sedcole, J. (2006). The role of odour and visual cues in the pan-trap catching of hoverflies (Diptera: Syrphidae). *Annals of Applied Biology*, 148(2), 173-178.

- Lazaro, A., Tscheulin, T., Devalez, J., Nakas, G., & Petanidou, T. (2016). Effects of grazing intensity on pollinator abundance and diversity, and on pollination services. *Ecological entomology*, 41(4), 400-412.
- Ledvina, J., McShea, W. J., Bourg, N. A., Herrmann, V., Akre, T., & Johnson, A. E. (2020). Management Regime and Field Age Affect Species Richness and Cover of Native Forbs and Exotic Species in Virginia Grasslands. *Ecological Restoration*, 38(2), 83-93.
- Leong, J. M., & Thorp, R. W. (1999). Colour-coded sampling: the pan trap colour preferences of oligolectic and nonoligolectic bees associated with a vernal pool plant. *Ecological Entomology*, 24(3), 329-335.
- Losey, J. E., & Vaughan, M. (2006). The economic value of ecological services provided by insects. *Bioscience*, 56(4), 311-323.
- McCravy, K. W. (2018). A review of sampling and monitoring methods for beneficial arthropods in agroecosystems. *Insects*, 9(4), 170.
- McMurphy, W., Gillen, R., Engle, D., & McCollum, F. (1990). The philosophical difference between range and pasture management in Oklahoma.
- Michener, C. D. (2007). *The bees of the world*.
- Moncada, K. (2003). The Role of Native Bees in Prairie Restoration.
- Morandin, L. A., & Winston, M. L. (2005). Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecological applications*, 15(3), 871-881.
- Moreira, E. F., da Silva Santos, R. L., Penna, U. L., Angel-Coca, C., de Oliveira, F. F., & Viana, B. F. (2016). Are pan traps colors complementary to sample community of potential pollinator insects? *Journal of insect conservation*, 20(4), 583-596.
- Neff, J. L., & Simpson, B. B. (1993). Bees, pollination systems and plant diversity.
- Ollerton, J. (2017). Pollinator diversity: distribution, ecological function, and conservation. *Annual Review of Ecology, Evolution, and Systematics*, 48, 353-376.
- Ollerton, J., Erenler, H., Edwards, M., & Crockett, R. (2014). Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. *Science*, 346(6215), 1360-1362.
- Orford, K. A., Murray, P. J., Vaughan, I. P., & Memmott, J. (2016). Modest enhancements to conventional grassland diversity improve the provision of pollination services. *Journal of Applied Ecology*, 53(3), 906-915.

- Park, M. G., Blitzer, E., Gibbs, J., Losey, J. E., & Danforth, B. N. (2015). Negative effects of pesticides on wild bee communities can be buffered by landscape context. *Proceedings of the Royal Society B: Biological Sciences*, 282(1809), 20150299.
- Pickering, C. M., & Stock, M. (2003). Insect colour preference compared to flower colours in the Australian Alps. *Nordic Journal of Botany*, 23(2), 217-223.
- Potts, S., Woodcock, B., Roberts, S., Tscheulin, T., Pilgrim, E., Brown, V., & Tallowin, J. (2009). Enhancing pollinator biodiversity in intensive grasslands. *Journal of Applied Ecology*, 46(2), 369-379.
- Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in ecology & evolution*, 25(6), 345-353.
- Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., Garibaldi, L. A., Hill, R., Settele, J., & Vanbergen, A. J. (2016). *The assessment report on pollinators, pollination and food production: summary for policymakers*. Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity.
- Potts, S. G., Vulliamy, B., Dafni, A., Ne'eman, G., & Willmer, P. (2003). Linking bees and flowers: how do floral communities structure pollinator communities? *Ecology*, 84(10), 2628-2642.
- Potts, S. G., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Ne'eman, G., & Willmer, P. (2005). Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology*, 30(1), 78-85.
- Pywell, R., Warman, E., Carvell, C., Sparks, T., Dicks, L., Bennett, D., Wright, A., Critchley, C., & Sherwood, A. (2005). Providing foraging resources for bumblebees in intensively farmed landscapes. *Biological conservation*, 121(4), 479-494.
- Pérez-Méndez, N., Andersson, G. K., Requier, F., Hipólito, J., Aizen, M. A., Morales, C. L., García, N., Gennari, G. P., & Garibaldi, L. A. (2020). The economic cost of losing native pollinator species for orchard production. *Journal of Applied Ecology*, 57(3), 599-608.
- Reed, C. (1993). Reconstruction of Pollinator Communities on Restored Prairies in Eastern Minnesota: final Report to the Minnesota Department of Natural Resources, Nongame Wildlife Program. *Minnesota Department of Natural Resources, Minnesota*.
- Reilly, J., Artz, D., Biddinger, D., Bobiwash, K., Boyle, N., Brittain, C., Brokaw, J., Campbell, J., Daniels, J., & Elle, E. (2020). Crop production in the USA is frequently limited by a lack of pollinators. *Proceedings of the Royal Society B*, 287(1931), 20200922.
- Rinehart, L. (2006). Pasture, rangeland and grazing management (ATTRA). Ed. Pull Drscoll (NACT). *National Center for Sustainable Agriculture Information Services*, 1-800.

- Roulston, T. a. H., & Goodell, K. (2011). The role of resources and risks in regulating wild bee populations. *Annual review of entomology*, 56, 293-312.
- Roulston, T. a. H., Smith, S. A., & Brewster, A. L. (2007). A comparison of pan trap and intensive net sampling techniques for documenting a bee (Hymenoptera: Apiformes) fauna. *Journal of the Kansas Entomological Society*, 80(2), 179-181.
- Rundlöf, M., Nilsson, H., & Smith, H. G. (2008). Interacting effects of farming practice and landscape context on bumble bees. *Biological conservation*, 141(2), 417-426.
- Saarinen, K., Valtonen, A., Jantunen, J., & Saarnio, S. (2005). Butterflies and diurnal moths along road verges: does road type affect diversity and abundance? *Biological Conservation*, 123(3), 403-412.
- Sanchez-Bayo, F., & Goka, K. (2014). Pesticide residues and bees—a risk assessment. *PloS one*, 9(4), e94482.
- Saunders, M. E., & Luck, G. W. (2013). Pan trap catches of pollinator insects vary with habitat. *Australian Journal of Entomology*, 52(2), 106-113.
- Senapathi, D., Biesmeijer, J. C., Breeze, T. D., Kleijn, D., Potts, S. G., & Carvalheiro, L. G. (2015). Pollinator conservation—the difference between managing for pollination services and preserving pollinator diversity. *Current Opinion in Insect Science*, 12, 93-101.
- Shapira, T., Henkin, Z., Dag, A., & Mandelik, Y. (2020). Rangeland sharing by cattle and bees: moderate grazing does not impair bee communities and resource availability. *Ecological Applications*, 30(3), e02066.
- Sjödin, N. E., Bengtsson, J., & Ekbom, B. (2008). The influence of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects. *Journal of Applied Ecology*, 763-772.
- Southwick, E. E., & Southwick Jr, L. (1992). Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. *Journal of Economic Entomology*, 85(3), 621-633.
- Sponsler, D. B., Grozinger, C. M., Hitaj, C., Rundlöf, M., Botías, C., Code, A., Lonsdorf, E. V., Melathopoulos, A. P., Smith, D. J., & Suryanarayanan, S. (2019). Pesticides and pollinators: A socioecological synthesis. *Science of the Total Environment*, 662, 1012-1027.
- Steffan-Dewenter, I., & Tschamntke, T. (2001). Succession of bee communities on fallows. *Ecography*, 24(1), 83-93.

- Stephen, W. P., & Rao, S. (2005). Unscented color traps for non-Apis bees (Hymenoptera: Apiformes). *Journal of the Kansas Entomological Society*, 373-380.
- Streinzer, M., Paulus, H. F., & Spaethe, J. (2009). Floral colour signal increases short-range detectability of a sexually deceptive orchid to its bee pollinator. *Journal of Experimental Biology*, 212(9), 1365-1370.
- Sugden, E. A. (1985). Pollinators of *Astragalus monoensis* Barneby (Fabaceae): new host records; potential impact of sheep grazing. *The Great Basin Naturalist*, 299-312.
- Tanis, M. M., Marshall, L., Biesmeijer, J. K., & van Kolfshoten, L. (2020). Grassland management for meadow birds in the Netherlands is unfavourable to pollinators. *Basic and Applied Ecology*, 43, 52-63.
- Taylor, R. L., Maxwell, B. D., & Boik, R. J. (2006). Indirect effects of herbicides on bird food resources and beneficial arthropods. *Agriculture, Ecosystems & Environment*, 116(3-4), 157-164.
- Tome, H. V., Schmehl, D. R., Wedde, A. E., Godoy, R. S., Ravaiano, S. V., Guedes, R. N., Martins, G. F., & Ellis, J. D. (2020). Frequently encountered pesticides can cause multiple disorders in developing worker honey bees. *Environmental Pollution*, 256, 113420.
- Tuell, J. K., Fiedler, A. K., Landis, D., & Isaacs, R. (2014). Visitation by wild and managed bees (Hymenoptera: Apoidea) to eastern US native plants for use in conservation programs. *Environmental entomology*, 37(3), 707-718.
- Turo, K. J., & Gardiner, M. M. (2019). From potential to practical: conserving bees in urban public green spaces. *Frontiers in Ecology and the Environment*, 17(3), 167-175.
- Vanbergen, A. J., & Initiative, t. I. P. (2013). Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment*, 11(5), 251-259.
- Vrdoljak, S. M., & Samways, M. J. (2012). Optimising coloured pan traps to survey flower visiting insects. *Journal of Insect Conservation*, 16(3), 345-354.
- Vulliamy, B., G. Potts, S., & G. Willmer, P. (2006). The effects of cattle grazing on plant pollinator communities in a fragmented Mediterranean landscape. *Oikos*, 114(3), 529-543.
- Walsh, E. M., Sweet, S., Knap, A., Ing, N., & Rangel, J. (2020). Queen honey bee (*Apis mellifera*) pheromone and reproductive behavior are affected by pesticide exposure during development. *Behavioral Ecology and Sociobiology*, 74(3), 1-14.

- Wang, M., Lu, X., Ding, S., Ren, J., Bian, Z., & Xu, Z. (2017). Pollinator diversity in different habitats of the agricultural landscape in the middle and lower reaches of the Yellow River based on the three-color pan trap method. *Acta Ecologica Sinica*, 37(3), 148-155.
- Wells, W., & Decker, T. (2006). comparison of three types of insect traps for collecting non-Formicidae Hymenoptera on the Island of Dominica. *Southwestern Entomologist*.
- Whittingham, M. J. (2011). The future of agri□environment schemes: biodiversity gains and ecosystem service delivery? *Journal of applied ecology*, 48(3), 509-513.
- Williams, N. M., & Kremen, C. (2007). Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. *Ecological applications*, 17(3), 910-921.
- Wilson, J. S., Griswold, T., & Messinger, O. J. (2008). Sampling bee communities (Hymenoptera: Apiformes) in a desert landscape: are pan traps sufficient? *Journal of the Kansas Entomological Society*, 81(3), 288-300.
- Winfree, R., Bartomeus, I., & Cariveau, D. P. (2011). Native pollinators in anthropogenic habitats. *Annual Review of Ecology, Evolution, and Systematics*, 42, 1-22.
- Yang, E., Chuang, Y., Chen, Y., & Chang, L. (2008). Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *Journal of economic entomology*, 101(6), 1743-1748.
- Zattara, E. E., & Aizen, M. A. (2021). Worldwide occurrence records suggest a global decline in bee species richness. *One Earth*, 4(1), 114-123.
- Zhang, Z., Wang, L., Liu, J., Dong, Z., Xu, W., & Wang, S. (2019). The effect of simulated sheep grazing on male and female reproductive performance in *Caragana microphylla* Lam.(Leguminosae). *Israel Journal of Ecology and Evolution*, 1(aop), 1-7.

CHAPTER TWO

Color of Pan Trap Influences Sampling of Bees in Livestock Pasture Ecosystem

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Abstract

The decline in insect pollinators has increased the importance of accurately monitoring pollinator diversity and abundance over time. Sampling techniques include the use of passive insect traps such as pan traps, yet there is still discussion over their utility and effectiveness in different ecosystems. The objective was to examine four different colors of pan traps (blue, green, yellow, and purple) for their utility in sampling bees in native forages rotationally grazed by sheep and to compare the relative abundance, richness, similarity, and community assemblage patterns among the four trap colors. Most bees were from the Halictidae family (89%). The most abundant species were *Lasioglossum imitatum* (42.2%), *Augochlorella aurata* (8.3%), *L. subviridatum* (6.8), *Agapostemon texanus* (6.4), and *L. birkmani* (4.1%). Blue color traps exhibited the highest rates of bee capture and species accumulation. Purple and yellow colored traps were moderately effective in capturing bees, while the green color pan traps were least effective. Similarly, observed and extrapolated species richness was highest in blue trap, followed by purple, yellow, and green. Notably, the blue trap captured the highest number of unique species, followed by purple, yellow and green traps. Considering the total number of insects collected (including bees and other insects), yellow and green traps captured a significantly higher number of insects than other colored traps. The light reflectance from blue, purple, green and yellow pan traps had peaks at ~450, 400, 550, and 600 nm, respectively. Since different insects respond to different light intensities, wavelengths, and reflectivity, these results could be used to guide future trapping protocols targeting certain insect groups in livestock pasture and similar ecosystems.

Keywords: pollinators; pan traps; pasture ecosystem; bees; bee vision; sampling method

1. Introduction

Bees are the most important pollinators for fruits, vegetables, nuts, forages and many other economically important crops as well as wild flowering plants. However, in recent years, intensification of agriculture has led to a decrease in foraging resources of bees and their potential nesting sites (Vanbergen & Initiative, 2013). Such intensification has negatively impacted the environment and subsequently decreased bee populations (Le Féon et al., 2010). For instance, declines in bumblebees (*Bombus* spp.) have been linked to agricultural intensification (Kells et al., 2001). Recent declines of pollinators have raised concerns not only for their conservation, but also for the potential of pollination deficits in various ecosystems (Kremen et al., 2002).

Estimates of pollinator abundance and diversity in a specific region depend on the frequency of monitoring and sampling procedures. Different types of sampling methods, including pan traps, may be used for sampling bees. Pan traps are an effective and commonly used technique for trapping insect pollinators, especially bees (Wilson et al., 2008). Additionally, pan traps can potentially allow researchers to monitor pollinator population across space and time as use of the traps does not negatively impact bee populations when sampling occurs every other week (Gezon et al., 2015).

Pan traps, also known as water traps, are commonly used for sampling agricultural insect pests (Leong & Thorp, 1999) as well as other arthropods (such as parasitoid wasps) in different ecosystems. Pan traps are passive traps that are cheaper and more effective in capturing a large number of Hymenopterans than other sampling methods such as malaise trap (Campbell & Hanula, 2007). Pan traps do not suffer from observer bias that may occur using active net sampling (Wilson et al., 2008). Although pan traps may not always provide an accurate representation of the bee fauna in a particular area (Cane et al., 2000), they still remain one of most common methods for

sampling bees as they are a cost-effective and efficient way to sample from a large area in a short period of time (Leong & Thorp, 1999). Pan traps are effective in all geographical locations, agricultural lands, and semi-natural habitats, even when few flowers are available (Roulston et al., 2007).

The intensity of light reflected differed by color of traps and thus affects the number of bees and other pollinator species that are attracted toward the traps (Joshi et al., 2015; Vrdoljak & Samways, 2012). When sampling, considering the color vision of hymenopteran insects, such as bumblebees and honeybees, it is important because it impacts their attraction toward different colored traps (Dyer et al., 2011). Bees are able to identify dissimilar colors and discriminate color textures (fine or coarse), although such ability may vary by species. In the case of honeybees, visual angle depends on stimulated photoreceptors for coding the color information (Dyer et al., 2011). Color discrimination senses in bumblebees are more poorly developed than in honeybees; however, bumblebees observe stimuli with smaller visual angle in comparison with honeybees (Dyer et al., 2008). Color intensity and chromaticity of illumination provide contextual cues that direct bees toward the source (Lotto & Chittka, 2005). Bees also use their olfactory signals if the source is 30 cm away and visual cues when they are nearer to flowers (Streinzer et al., 2009).

Measurements of abundance, species richness and diversity of insect pollinators collected by pan trapping may be influenced by the color of the pan traps being used and the type of ecosystem in which the sampling occurred. For example, the high reflective index of white or yellow pan traps attracted the greatest richness of anthophilic insects in the lowlands of the Cape Floristic Region in South Africa (Vrdoljak & Samways, 2012). Between yellow and white colored pan traps, yellow traps were found to be more efficient in attracting a higher number and diversity of bees in open fields, riverside habitats, forests and roadside verges in Australia (Gollan et al.,

2011). Overall pollinator abundance and diversity was also higher in yellow traps compared to white and blue traps in the Yellow River region of China (Wang et al., 2017). Yellow traps have also been shown to be more effective at attracting both wasps (Abrahamczyk et al., 2010; Moreira et al., 2016) and hoverflies (Laubertie et al., 2006) than other trap colors. Conversely, blue pan traps have been reported to be highly effective at trapping bees in a variety of ecosystems, including fruit orchards (Joshi et al., 2015), savannas (Moreira et al., 2016), and forested ecosystems (Campbell & Hanula, 2007). Whether pan traps are fluorescent or non-fluorescent may also affect insect capture rates (Shrestha et al., 2019). In addition to diversity measures, the sex ratios of bees in samples collected in pan traps may vary based on the color of pan trap, and differences in color preferences between males and females can vary among species (Leong & Thorp, 1999; Heneberg & Bogusch, 2014).

Abundance, diversity, and sex of bees and other pollinators could vary with color and light reflectance of the colored pan trap because the color vision of hymenopterans, such as bumblebees and honeybees, impacts their attraction to the source. Thus far, the majority of studies in this field have used blue, yellow and white-colored pan traps. However, other colored traps within the visible light spectrum for bees, such as green and purple, may also attract bees, and such visual attractancy of several other colors to bees is yet to be documented. Therefore, the main goal of this study was to investigate whether differently colored pan traps including commonly used blue and yellow traps, and less commonly used green and purple traps impact measurements of wild bee abundance and diversity, as well as bee assemblage patterns, in a livestock pasture ecosystem. The findings of this study would be useful in selecting the most appropriate type of colors for pan trapping bees in pastures, which are different than other agro-ecosystems in many ways.

2. Materials and Methods

2.1 Site Description

This study was conducted during July and August of the 2018 field season at the research farm of the USDA-ARS Dale Bumpers Small Farms Research Center in Booneville, Arkansas (35.09 °N, 93.95 °W). The soil of the site is characterized as Leadvale silt loam (fine-silty, siliceous, semiactive, thermic Typic Fragiudults) (<https://websoilsurvey.sc.egov.usda.gov>, accessed on 12 May 2021). Average temperatures at the research station during July and August of 2018 were 27.0 and 25.2 °C. The site received 96.9 mm and 151 mm rainfall during July and August, respectively.

2.2 Study Site: History and Preparation

Pasture management prior to the study included herbicide treatment: Roundup® (41% glyphosate; Monsanto, St. Louis, MO, USA; 0.764 L/ha) in June, July, September, October of 2016 and January of 2017, and Outrider (75% Sulfosulfuron; Monsanto, St. Louis, MO, USA; 0.016 L/ha) in September 2016 using a Continental Belton cluster nozzle sprayer (Continental Belton McAlester, SR: A44117, Oklahoma City, OK, USA). The site was burned in September 2016 and prepared with a tiller (Maschio Gaspardo North America Inc., SC 300, DeWitt, IA, USA) and rolled using a 12' Big Guy Roller (Graul Manufacturing, Republic, MO, USA) in October 2016. Mixtures of Tallgrass Inexpensive Seed Mix (TGI; Prairie Moon Nursery, Winona, MN, USA; 5.4 kg/acre), Buck's Hangout (BH; Hamilton Native Outpost, Elk Creek, MO, USA; 5.89 kg/acre) and Tallgrass Exposed Clay Subsoil Mix (TGE; Prairie Moon Nursery; 10.88 kg/acre) were then planted in February 2018. Plant species composition in these seed mixes at the time of sowing are available on their respective supplier websites.

Plots were grazed by sheep in late June of 2018 to provide forage to the animals, reduce weeds (mixed non-native grass species) that were palatable to sheep, and maintain native grasses in vegetative state. By early July, there were estimated to be at least three native flowering species per plot, even after being grazed by sheep. The most common flowering species visually documented during the sampling period were *Verbena hastata*, *Pycnanthemum verticillatum*, *Echinacea pallida*, *Coreopsis* spp., *Monarda fistulosa*, *Aster novae-angliae*, *Verbena* spp., *Cichorium intybus*, and *Daucus carota*. The adjacent landscape of the study site was mainly comprised of pastureland as well as some natural areas with unmanaged habitats and woods.

2.3 Pan Traps and Sampling

Four transects (~100 m long, one per plot) of four elevated pan trap platforms were established at the study site comprised of four plots (0.4 ha each). Each individual platform contained two pan traps of the same color. Within each transect, four trap colors (yellow, green, blue, and purple) were deployed, and the order of the platforms was randomly assigned. Traps consisted of 354.88 mL fluorescent colored plastic bowls with UV reflectance, in which blue (color: bright royal blue 105; Festive Occasion, East Providence, RI, USA), yellow (color: school bus yellow; Touch of Color, Creative Converting, Clintonville, WI, USA), green (color: fresh lime; Touch of Color, Creative Converting, Clintonville, WI, USA), and purple (color: purple; Touch of Color, Creative Converting, Clintonville, WI) bowls were used. Sampling began in early July and samples were collected four times per week until mid-August. Trap platforms were placed 25 m apart in transects, and the distance between transects was 15 m. The nearest trap to the fences that separated study plots was 8 m from the boundary. Pan trap platforms were placed ~1.25 m above the ground to match the height of the canopy of flowering plants in the pasture. For each sampling event, two-thirds of each bowl were filled with soapy water (Figure 1). In order to prepare soapy

water, a few drops of unscented liquid dishwashing detergent were mixed with 3.785 L of tap water. Insect samples were collected from the traps after approximately a 24 h period. Traps were set up in the morning at 7:00 a.m. to be collected on the next day around the same time.

Samples collected from each platform (two pan traps) were placed in plastic vials containing 70% ethyl alcohol before they were transported to the laboratory. In the laboratory, insects were air dried to remove ethanol, sorted, pinned, boxed and shipped for identification. Samples were identified to the species level by Drs. D. Biddinger (Department of Entomology, Penn State Fruit Research and Extension Center, Biglerville, PA, USA) and R. Jean (Senior Entomologist, Environmental Solution & Innovations, Inc., Indianapolis, IN, USA). Due to taxonomic difficulties in identifying all insects to species level, only bees were considered for analysis at species level.

2.4 Light Reflectance Analysis of Pan Traps

All colored pan traps were analyzed for their light reflectance characteristics at the Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, USA. For this purpose, a small, square-shaped piece (2 cm²) of each colored bowl or trap was kept inside the incident light window of spectrophotometer where light was reflected from the sample on the detector for 200 sec. The detector was attached to a barium sulfate coated sphere with a 60 mm integrating sphere. Total reflectance of each color of pan trap was recorded within 190–1600 nm range of spectrum using a JASCO V-780 spectrometer (JASCO Corporation, Easton, MD, USA). Intensity of light passing through the sample was recorded by using Spectra ManagerTM suite, spectroscopy software connected with Windows 7 pro (64-bit) operating system. The light reflectance and wavelength of each sample were recorded and analyzed. The baseline measurement was taken at the beginning.

2.5 Data Analyses

Measures of bee abundance, richness, similarity, and community assemblage patterns were compared among the four trap colors. The overall effect of trap color on bee abundance and total insect abundance was tested using analysis of variance (ANOVA) in JMP. A post hoc Tukey test was then conducted to identify significant differences among trap colors. For each trap color, data were summed within each of the 18 sampling dates. These abundance values were square-root transformed prior to analysis to addressing the right-skewness (i.e., preponderance of low values) in the original dataset.

Species richness of bees was compared among trap colors by developing sample-based and individual-based rarefaction curves in EstimateS v7.5 (Colwell, 2005). Rarefaction curves depict interpolated species accumulation through iterative resampling from the species-by-sample abundance data matrix. Rarefaction curves allow for direct comparison of expected species richness among treatments at a standardized number of samples or individuals collected. Differences in species accumulation rates were determined based on non-overlapping 95% confidence intervals. A Chao1 richness estimator was also used to plot extrapolated species accumulation curves based on the number of rare species in the samples. In addition, incidence-based (Sorensen Classic) and abundance-based (Chao-Sorensen raw abundance-based) similarity measures were calculated in EstimateS v7.5 (Colwell, 2005) for each pairwise combination of trap colors in order to characterize the extent of species similarity among traps colors. The number of unique species found in each trap color was also reported.

To examine community assemblage patterns associated with trap color, a constrained ordination was conducted using Canoco v4.5 (Ter Braak & Smilauer, 2002). Trap colors, coded as dummy variables, were used as environmental predictor variables. Since a large number of

bee species were found to be singletons or doubletons, bee data were aggregated at genus level. For each combination of trap color and sampling date, counts of bees within each bee genera were used as response variables. Following a detrended correspondence analysis (DCA) to evaluate data structure, a canonical correspondence analysis (CCA) was used to generate orthogonal axes representing models explaining the greatest amount of variance in the species data. Species data were squared root transformed and centered and standardized for analysis. The significance of trap color was assessed through Monte Carlo permutations ($n = 999$) and stepwise forward selection (Lepš & Šmilauer, 2003). Biplots were developed in CanoDraw (Lepš & Šmilauer, 2003) to visualize associations between bee genera and trap colors.

3. Results

3.1 Abundance and Diversity

Over the course of the study, 2327 insects were captured in pan traps. Insect capture rates differed among trap colors ($F_{3,68} = 4.24$; $p = 0.008$). Yellow traps captured the greatest number of insects, followed by green, blue and purple traps, respectively (Figure 2A). Among these insects, a total of 573 bees comprising 44 species from four families were collected (Table 1). Bee abundance (Table 2; Figure 2B) also differed among trap colors ($F_{3,68} = 12.5$; $p < 0.0001$) and was higher in blue pan traps compared to all trap colors (Figure 2B). Purple pan traps had the second highest capture rate followed by yellow and green pan traps (Figure 2B). Similarly, observed and extrapolated (Chao1) species richness was highest in blue traps, followed by purple, yellow and green traps, respectively (Table 2). Sample-based rarefaction curves revealed that species accumulation was significantly higher in blue traps relative to yellow and green traps (Figure 3A). In addition, sample-based species accumulation in purple traps was higher than in green traps

(Figure 3A). Conversely, no significant difference in species accumulation could be detected among trap colors using individual-based rarefaction (Figure 3B)

Blue traps had the highest number of unique species followed by purple, green and yellow traps, respectively (Table 2). Similarity in species composition based on pairwise comparisons was greatest among blue, purple and yellow traps, whereas green traps had the most dissimilar species composition when compared to other trap colors (Table 2). Ordination revealed distinct bee assemblages associated with blue traps ($F = 2.06$, $p = 0.023$) relative to other trap colors (Figure 4). This first axis on the biplot explained 3.5% of the species data and 52.3% of the species–environment relation. The secondary (vertical) axis explained an additional 2.1% of species data and 31.0% of the species–environment relation and depicts associations between green traps ($F = 1.49$; $p = 0.17$) and yellow and purple traps (Figure 4).

Most of the bees recorded in this study were from the Halictidae family (89%). Of the total bees, 50.9% were collected from blue traps, 26.6% from purple traps, 13.8% from yellow traps, and 9.6% from green traps. The most abundant species was *Lasioglossum imitatum* (Smith) (42.2% of total), followed by *Augochlorella aurata* (Smith) (8.3%), *L. subviridatum* (6.8% of total), *Agapostemon texanus* (Cresson) (6.4% of total) and *L. birkmani* (4.1% of total, Table 1).

3.2 Light Reflectance

Light reflectance curves varied among trap colors (Figure 5). Within the visual spectrum of bees, the blue pan trap had a peak at 450 nm with wavelength ranging from 300 to 500 nm. The frequency of wavelength of purple traps ranged from 200 to 450 nm (with a peak of 400 nm). Green traps had a peak at 550 nm with wavelength ranging from 200 to 600 nm, while yellow traps had a peak around 600 nm with a frequency range from 200 to 600 nm. Traps with higher light reflectance in the 300–500 nm range attracted the most species of bees in this study. White

platforms where traps were kept (as shown in Figure 1) had a light reflectance peak at 500 nm with a range of 200–600 nm wavelength (Figure 6).

4. Discussion

For the conservation of pollinators, it is necessary to monitor their abundance and diversity in different habitats, ideally in relation to reliable baseline records. For this, effective techniques are needed to trap them across space and time that do not negatively impact their persistence (Gezon et al., 2015). Although different trapping techniques will undoubtedly contain biases (Cane et al., 2001), pan traps remain a simple and effective pollinator sampling technique as compared with other methods such as net sampling or malaise traps (Campbell & Hanula, 2007; Joshi et al., 2015; Wilson et al., 2008). Yet there remains a need to better understand the effect of trap color and reflectance on measures of abundance and diversity of pollinators collected in different ecosystems.

Among the four different pan trap colors (blue, yellow, green and purple) that were tested in this study, bee capture rates and species accumulation rates were highest in blue pan traps compared to other colors of pan traps in livestock pasture plots. This finding agrees with other studies that have shown blue traps to be particularly attractive to bees (Joshi et al., 2015). Purple traps were also highly effective. Similarity in community composition between blue and purple traps suggest reduced complementarity; however, the fact that blue and purple traps had ten and four unique species, respectively – and distinct genera groupings revealed by ordination – suggest that including purple traps in field sampling should be considered. Overall, yellow pan traps were most effective to attract the greatest number of insects (of all groups). This difference highlights the importance of trap color selection when monitoring targeted insect groups. Green traps were the least effective of the four trap colors, which is likely due to the predominant green

pasture background, resulting in reduced color contrast. However, there could also be some impact of white color platforms (used to hold pan traps) on overall color contrast and could have influenced attractiveness of pan traps to different species of bees.

Flower color is a visual clue for detection, recognition, and memorization of food resources for bees, and other anthophilic insects (Menzel & Backhaus, 1991). The intensity of light reflected from traps was dependent on color which affects number of pollinators, especially bees attracted toward the traps (Vrdoljak & Samways, 2012). The visual spectrum of most insects usually includes UV light (Menzel & Backhaus, 1991). In most cases, the visual spectrum of bees has been reported to be in the range of 300 to 630 nm (Kevan et al., 1996). Specifically, insects in the order Hymenoptera, including bumbles bees and honeybees, are impacted by color vision (Dyer et al., 2011). In the case of honey bees (*Apis mellifera*), color vision is trichromatic having ultraviolet, blue and green photoreceptors with maximum sensitivity at 350, 440 and 540 nm (Avarguès-Weber et al., 2012).

The differences in capture rates among trap colors in the current experiment can be explained by the visual spectrum of bees and the measured light reflectance. Blue traps had highest light reflectance in 300-500 nm range, which likely played an important role in attracting different bee species. Likewise, purple traps, which had the second highest rate of bee capture and species, also had the second highest level of light reflectance in the 300-500 nm range. In general, bees discriminate blue, blue-green, violet, and yellow in the spectrum (Von Frisch, 1914). Although, bees have color spectrum from UV to orange, they may also utilize color contrast to find target objects (Hertz, 1939). For example, white flowers lack color contrast (as perceived by bees) against vegetation and other backgrounds resulting in a neutral color. Bees may thus ignore such flowers or rely on other visual cues to detect them (Kevan et al., 1996).

A total of 44 species from 15 genera and four families were collected during July and August in 2018. The number of species documented in the current study is half than that reported by (Stephenson et al., 2018) in managed emergent wetlands within the lower Mississippi Alluvial Valley of Arkansas, USA and one quarter of number of species reported by (Little, 2013) in the Arkansas River Valley. Most of the bees collected from the current study belong to Halictidae family (89%) which is similar to other findings in which pan traps were used for collecting pollinators in earlier studies (Kimoto et al., 2012) in Oregon and (Bhandari et al., 2018) in Texas. The most abundant species in the current study consisted of *L. imitatum* (42.23 %), followed by *Augochlorella aurata* (8.3 %), *L. subviridatum* (6.8 %), *Agapostemon texanus* (6.4 %) and *L. birkmani* (4.1 %). Surprisingly, in the study by (Stephenson et al., 2018) there was no report of *L. imitatum*, *L. subviridatum* and *L. birkmani* though both of the studies were conducted in the same state in Arkansas in the same season (during months of July through August), but in a different landscape and ecosystem. In another study conducted in a nearby location, *Lasioglossum* spp. was the most prevalent genus (Little, 2013). The most dominant genera reported were *Lasioglossum* spp., followed by *Megachile* spp., *Augochlorella* spp, *Bombus* spp., and *Melissodes* spp.

Pollinator species richness and functional diversity as well as species-wise distribution in livestock pasture vary during the season (Thapa-Magar et al., 2020). The current study was done during the latter half of the season (mid-July to mid-August) whereas other studies were done during the entire season (Bhandari et al., 2018; Thapa-Magar et al., 2020), and such differences in the timing and duration of sampling could be the reason we documented fewer bee species in comparison with previous studies. In addition, collection method in the current study (pan trap) was different than that used in previous studies (deep bowl trap that could hold more pollinators,

Bhandari et al., 2018; vane trap, Thapa-Magar et al., 2020) and thus could have missed capturing diverse species. However, the major dominating bees (*Bombus*, *Halictus*, *Lasioglossum*, and *Melissodes* spp.) during July-August period in the current study largely overlapped with previous studies (Bhandari et al., 2018; Thapa-Magar et al., 2020). Consistent with current study, Bhandari et al. (2018) reported 76% of Hymenoptera followed by lesser percentage of Diptera and Lepidoptera during the summertime in pastures of Southern US.

Abundance of bees in livestock pasture depends on numerous factors such as plant species composition and architecture, soil and microclimatic characteristics, and grazing intensity (Kimoto et al., 2012). Use of herbicides could decrease species richness by 71% (Cullen et al., 2019). In the current study, there was no application of herbicides up to 3 years before the sampling, and it is not likely that herbicide residues could have impacted species diversity in the current study. Trap height relative to height of canopy could impact survey of pollinators. Little has been published on pan trap height in livestock or native pastures. A study in high bush blueberries showed that traps mounted at one third the height of the canopy captured significantly higher numbers of bees than traps at higher or lower heights (Tuell & Isaacs, 2009). Traps used in the current study were relatively higher than the forage height, thus may not have been optimal for bee capture. Further research in this regard is warranted.

5. Conclusions

This study revealed that blue pan traps were the most effective pan trap color for sampling bees in a livestock pasture ecosystem. Purple traps were the second most effective, followed by yellow and green traps. These findings are supported by the reflectance value of the color trap (with or without soapy water) and the known visible light spectrum of bees. Notably, yellow and green traps were the most effective traps for sampling insect communities in general but not for sampling wild bees. In addition, the results show that livestock pasture ecosystems that include

native forages can support a wide variety of bees, which—in combination with grazing and management intensity—should be considered in pollination conservation schemes in agricultural landscapes.

Author Contributions

Conceptualization, J.B., N.K.J., and K.L.; methodology and fieldwork, R.S.A, N.K.J., E.F., and J.B.; formal analysis, R.S.A., T.L., and N.K.J., data curation, R.S.A., T.L. and N.K.J.; writing—original draft preparation, R.S.A., T.L., N.K.J., J.B., and K.L.; all authors/co-authors have reviewed and edited the manuscript draft. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement:

This study did not involve human or animal.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

6. References

- Abrahamczyk, S., Steudel, B., & Kessler, M. (2010). Sampling Hymenoptera along a precipitation gradient in tropical forests: the effectiveness of different coloured pan traps. *Entomologia Experimentalis et Applicata*, 137(3), 262-268.
- Avarguès-Weber, A., Mota, T., & Giurfa, M. (2012). New vistas on honey bee vision. *Apidologie*, 43(3), 244-268.
- Bhandari, K. B., West, C., Longing, S., Brown, C., Green, P., & Barkowsky, E. (2018). Pollinator abundance in semiarid pastures as affected by forage species. *Crop Science*, 58(6), 2665-2671.
- Campbell, J. W., & Hanula, J. (2007). Efficiency of Malaise traps and colored pan traps for collecting flower visiting insects from three forested ecosystems. *Journal of Insect Conservation*, 11(4), 399-408.
- Cane, J. H., Minckley, R. L., & Kervin, L. J. (2000). Sampling bees (Hymenoptera: Apiformes) for pollinator community studies: pitfalls of pan-trapping. *Journal of the Kansas entomological society*, 225-231.
- Colwell, R. (2005). EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5 user's guide and application published. <http://purl.oclc.org/estimates>.
- Cullen, M. G., Thompson, L. J., Carolan, J. C., Stout, J. C., & Stanley, D. A. (2019). Fungicides, herbicides and bees: A systematic review of existing research and methods. *PloS one*, 14(12), e0225743.
- Dyer, A. G., Paulk, A. C., & Reser, D. H. (2011). Colour processing in complex environments: insights from the visual system of bees. *Proceedings of the Royal Society B: Biological Sciences*, 278(1707), 952-959.
- Dyer, A. G., Spaethe, J., & Prack, S. (2008). Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. *Journal of Comparative Physiology A*, 194(7), 617.
- Gezon, Z. J., Wyman, E. S., Ascher, J. S., Inouye, D. W., & Irwin, R. E. (2015). The effect of repeated, lethal sampling on wild bee abundance and diversity. *Methods in Ecology and Evolution*, 6(9), 1044-1054.

- Gollan, J. R., Ashcroft, M. B., & Batley, M. (2011). Comparison of yellow and white pan traps in surveys of bee fauna in New South Wales, Australia (Hymenoptera: Apoidea: Anthophila). *Australian Journal of Entomology*, 50(2), 174-178.
- Heneberg, P., & Bogusch, P. (2014). To enrich or not to enrich? Are there any benefits of using multiple colors of pan traps when sampling aculeate Hymenoptera? *Journal of insect conservation*, 18(6), 1123-1136.
- Hertz, M. (1939). New experiments on colour vision in bees. *Journal of Experimental Biology*, 16(1), 1-8.
- Joshi, N. K., Leslie, T., Rajotte, E. G., Kammerer, M. A., Otieno, M., & Biddinger, D. J. (2015). Comparative trapping efficiency to characterize bee abundance, diversity, and community composition in apple orchards. *Annals of the Entomological Society of America*, 108(5), 785-799.
- Kells, A. R., Holland, J. M., & Goulson, D. (2001). The value of uncropped field margins for foraging bumblebees. *Journal of Insect Conservation*, 5(4), 283-291.
- Kevan, P., Giurfa, M., & Chittka, L. (1996). Why are there so many and so few white flowers? *Trends in Plant Science*, 1(8), 252.
- Kimoto, C., DeBano, S. J., Thorp, R. W., Taylor, R. V., Schmalz, H., DelCurto, T., Johnson, T., Kennedy, P. L., & Rao, S. (2012). Short-term responses of native bees to livestock and implications for managing ecosystem services in grasslands. *Ecosphere*, 3(10), 1-19.
- Kremen, C., Williams, N. M., & Thorp, R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences*, 99(26), 16812-16816.
- Laubertie, E., Wratten, S., & Sedcole, J. (2006). The role of odour and visual cues in the pan trap catching of hoverflies (Diptera: Syrphidae). *Annals of Applied Biology*, 148(2), 173-178.
- Le Féon, V., Schermann-Legionnet, A., Delettire, Y., Aviron, S., Billeter, R., Bugter, R., Hendrickx, F., & Burel, F. (2010). Intensification of agriculture, landscape composition and wild bee communities: a large scale study in four European countries. *Agriculture, Ecosystems & Environment*, 137(1-2), 143-150.
- Leong, J. M., & Thorp, R. W. (1999). Colour-coded sampling: the pan trap colour preferences of oligolectic and nonoligolectic bees associated with a vernal pool plant. *Ecological Entomology*, 24(3), 329-335.

- Lepš, J., & Šmilauer, P. (2003). Multivariate analysis of ecological data using CANOCO. Cambridge university press.
- Little, C. Z. (2013). Bee communities in the Arkansas River Valley. University of Central Arkansas.
- Lotto, R. B., & Chittka, L. (2005). Seeing the light: Illumination as a contextual cue to color choice behavior in bumblebees. *Proceedings of the National Academy of Sciences*, 102(10), 3852-3856.
- Menzel, R., & Backhaus, W. (1991). Colour vision in insects. *Vision and visual dysfunction*, 6, 262-293.
- Moreira, E. F., da Silva Santos, R. L., Penna, U. L., Angel-Coca, C., de Oliveira, F. F., & Viana, B. F. (2016). Are pan traps colors complementary to sample community of potential pollinator insects? *Journal of insect conservation*, 20(4), 583-596.
- Rhoades, P., Griswold, T., Waits, L., Bosque-Pérez, N. A., Kennedy, C. M., & Eigenbrode, S. D. (2017). Sampling technique affects detection of habitat factors influencing wild bee communities. *Journal of Insect Conservation*, 21(4), 703-714.
- Roulston, T. a. H., Smith, S. A., & Brewster, A. L. (2007). A comparison of pan trap and intensive net sampling techniques for documenting a bee (Hymenoptera: Apiformes) fauna. *Journal of the Kansas Entomological Society*, 80(2), 179-181.
- Shrestha, M., Garcia, J. E., Chua, J. H., Howard, S. R., Tscheulin, T., Dorin, A., Nielsen, A., & Dyer, A. G. (2019). Fluorescent pan traps affect the capture rate of insect orders in different ways. *Insects*, 10(2), 40.
- Stephenson, P. L., Griswold, T. L., Arduser, M. S., Dowling, A. P., & Kremenetz, D. G. (2018). Checklist of bees (Hymenoptera: Apoidea) from managed emergent wetlands in the lower Mississippi Alluvial Valley of Arkansas. *Biodiversity data journal* (6).
- Streinzer, M., Paulus, H. F., & Spaethe, J. (2009). Floral colour signal increases short-range detectability of a sexually deceptive orchid to its bee pollinator. *Journal of Experimental Biology*, 212(9), 1365-1370.
- Ter Braak, C. J., & Smilauer, P. (2002). CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5).
- Thapa-Magar, K. B., Davis, T. S., & Kondratieff, B. (2020). Livestock grazing is associated with seasonal reduction in pollinator biodiversity and functional dispersion but cheatgrass

- invasion is not: Variation in bee assemblages in a multi-use shortgrass prairie. *PloS one*, 15(12), e0237484.
- Tuell, J. K., & Isaacs, R. (2009). Elevated pan traps to monitor bees in flowering crop canopies. *Entomologia experimentalis et applicata*, 131(1), 93-98.
- Vanbergen, A. J., & Initiative, t. I. P. (2013). Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment*, 11(5), 251-259.
- Von Frisch, K. (1914). Der farbennsinn und formensinn der biene. Рипол Классик.
- Vrdoljak, S. M., & Samways, M. J. (2012). Optimising coloured pan traps to survey flower visiting insects. *Journal of Insect Conservation*, 16(3), 345-354.
- Wang, M., Lu, X., Ding, S., Ren, J., Bian, Z., & Xu, Z. (2017). Pollinator diversity in different habitats of the agricultural landscape in the middle and lower reaches of the Yellow River based on the three-color pan trap method. *Acta Ecologica Sinica*, 37(3), 148-155.
- Wilson, J. S., Griswold, T., & Messinger, O. J. (2008). Sampling bee communities (Hymenoptera: Apiformes) in a desert landscape: are pan traps sufficient? *Journal of the Kansas Entomological Society*, 81(3), 288-300.

7. Figures and Tables



Figure 1. A colored pan trap platform used in the study. Picture by N. Joshi.

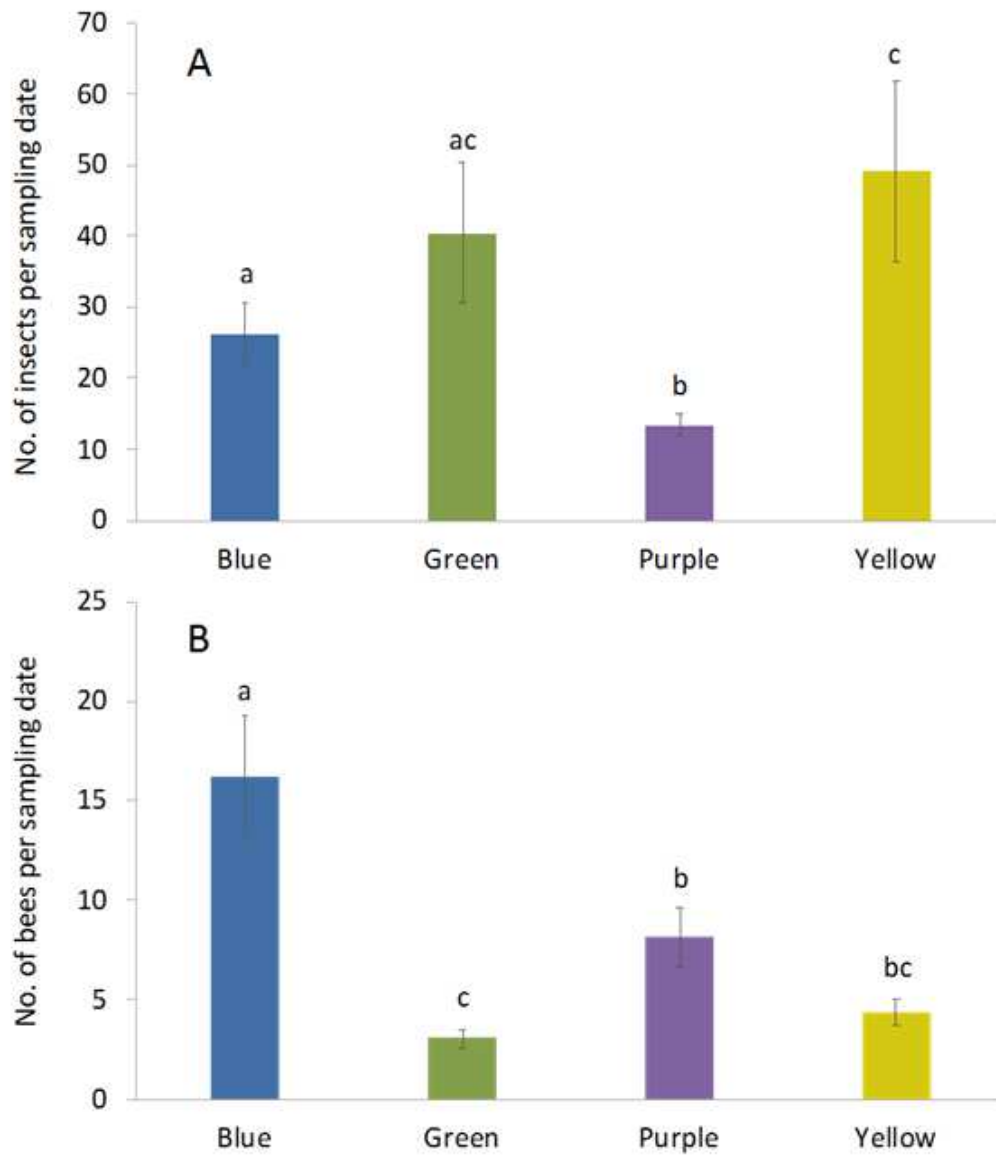


Figure 2: Differences in capture rate (±SE) of (A) total insects and (B) bees among four pan trap colors in livestock pastures. There were four trap color in each of the 4 plots. Samples were taken 4 times each week for 8 wk. Different lowercase letters indicate significant differences among trap colors ($P < 0.05$).

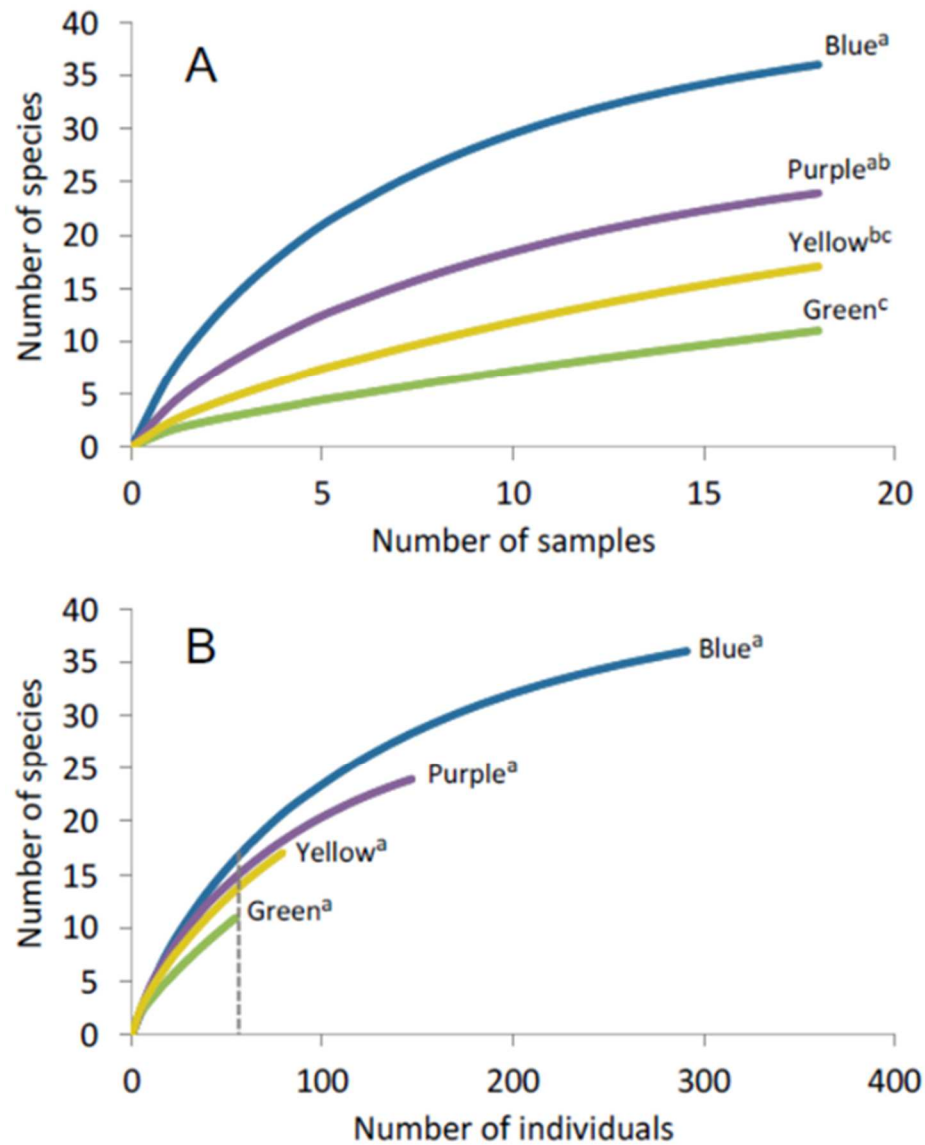


Figure 3. Rarefaction curve showing accumulation of the number of species in relation of the number of samples (A) and number of individuals (B). Dotted line in (B) indicates at what abundance value interpolated richness values were compared. Different superscript letters indicate significant differences among trap colors.

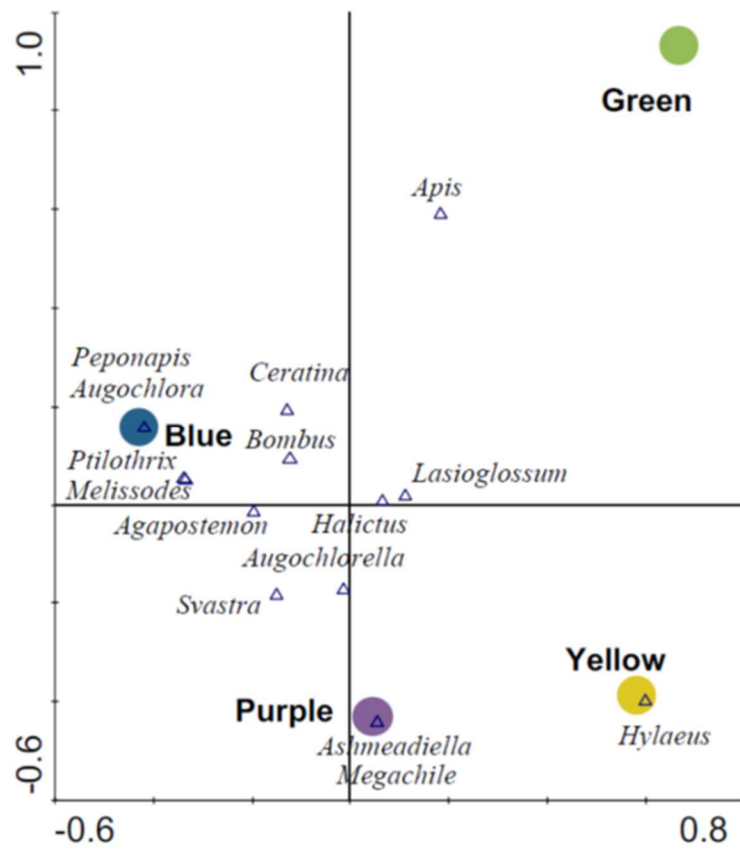


Figure 4. Redundancy analysis (RDA) biplot showing the association of wild bee species and different colored pan traps.

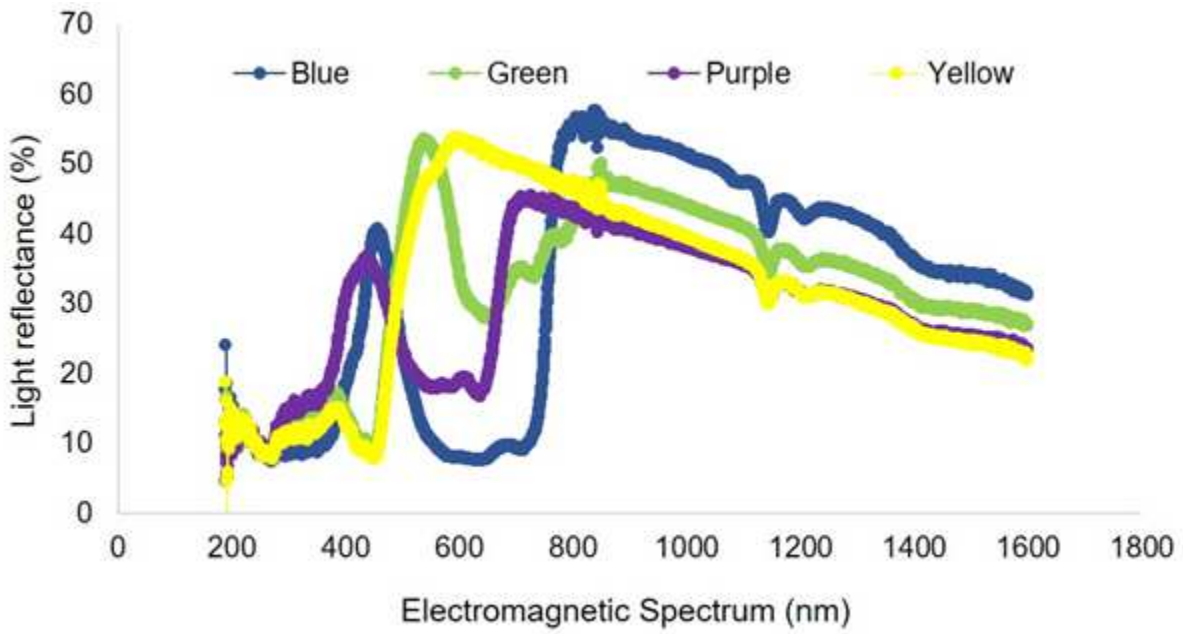


Figure 5. Light reflectance spectrum for four different colors of pan traps used for sampling pollinators and other insect communities.

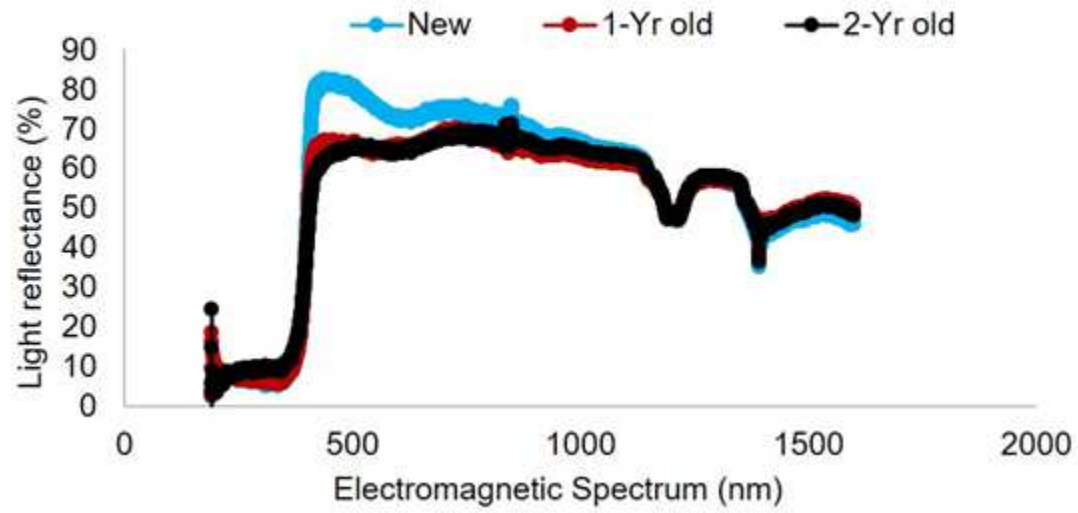


Figure 6. Light reflectance spectrum of the white color base of pan trap platform used in this study. Light reflectance spectrum of new (New), one-year-old (1-Yr old), and two-year-old (2-Yr old) platforms is presented.

Table 1. Bee species diversity (family, genus, and species) collected using different color pan traps (blue, green, purple, and yellow) in Arkansas livestock pasture ecosystem in 2018.

Diversity of Bees			Pan Trap Color			
Family	Genus	Species	Blue	Green	Purple	Yellow
Apidae	<i>Apis</i>	<i>mellifera</i>	x	x		
	<i>Bombus</i>	<i>griseocollis</i>	x		x	x
	<i>Bombus</i>	<i>pensylvanicus</i>	x	x		
	<i>Ceratina</i>	<i>strenua</i>	x	x	x	
	<i>Ceratina</i>	<i>calcarata</i>	x	x		
	<i>Melissodes</i>	<i>niveus</i>	x		x	
	<i>Melissodes</i>	<i>veroninae</i>	x			
	<i>Melissodes</i>	<i>bimaculata</i>	x			
	<i>Melissodes</i>	<i>communis</i>	x		x	
	<i>Melissodes</i>	<i>comptoides</i>	x			
	<i>Peponapis</i>	<i>timberlakei</i>	x			x
	<i>Ptilothrix</i>	<i>bombiformis</i>	x		x	x
	<i>Svastra</i>	<i>atripes</i>			x	
	<i>Svastra</i>	<i>obliqua</i>	x		x	
Colletidae	<i>Hylaeus</i>	<i>rudbeckiae</i>				x
Megachilidae	<i>Megachile</i>	<i>brevis</i>			x	
	<i>Ashmeadiella</i>	<i>floridana</i>			x	
Halictidae	<i>Agapostemon</i>	<i>texanus</i>	x	x	x	x
	<i>Agapostemon</i>	<i>splendens</i>	x		x	
	<i>Agapostemon</i>	<i>sericeus</i>	x		x	
	<i>Augochlorella</i>	<i>aurata</i>	x		x	x
	<i>Augochlorella</i>	<i>persimilis</i>	x			x
	<i>Augochlora</i>	<i>pura</i>	x			
	<i>Halictus</i>	<i>rubicundus</i>	x		x	x
	<i>Halictus</i>	<i>confusus</i>	x	x	x	x
	<i>Halictus</i>	<i>ligatus</i>	x		x	x
	<i>Halictus</i>	<i>parallelus</i>	x	x		
	<i>Lasioglossum</i>	<i>imitatum</i>	x	x	x	x
	<i>Lasioglossum</i>	<i>disparile</i>		x	x	
	<i>Lasioglossum</i>	<i>versatum</i>	x		x	x
	<i>Lasioglossum</i>	<i>coreopsis</i>	x			x
	<i>Lasioglossum</i>	<i>nr versans</i>	x		x	
	<i>Lasioglossum</i>	<i>birkmanni</i>	x		x	x
	<i>Lasioglossum</i>	<i>subviridatum</i>	x	x	x	x
	<i>Lasioglossum</i>	<i>foxii</i>			x	
	<i>Lasioglossum</i>	<i>sopinci</i>				x
	<i>Lasioglossum</i>	<i>paraforbesii</i>	x			x
	<i>Lasioglossum</i>	<i>athabascence</i>	x			
	<i>Lasioglossum</i>	<i>tegulare</i>	x		x	
	<i>Lasioglossum</i>	<i>pectorale</i>	x			
	<i>Lasioglossum</i>	<i>trigeminum</i>	x			
	<i>Lasioglossum</i>	<i>callidum</i>	x			
	<i>Lasioglossum</i>	<i>zephyrum</i>		x		
	<i>Lasioglossum</i>	<i>hitchensi</i>	x			

Table 2. A comparison of bee diversity measures among four colors of pan traps deployed in Arkansas livestock pastures.

		Pan Trap Color			
		Blue	Green	Purple	Yellow
Abundance		291	55	147	79
Richness (observed)		36	11	24	17
Richness (extrapolated; Chao1)¹		45	22	28	22
Number of unique species		10	1	4	2
Similarity Indices²	Blue	1			
	Green	0.38 (0.70)	1		
	Purple	0.63 (0.89)	0.34 (0.75)	1	
	Yellow	0.53 (0.87)	0.36 (0.76)	0.59 (0.90)	1

¹Rounded to nearest whole number.

² Sorensen classic and Chao–Sorensen raw abundance-based (in parentheses) similarity indices.

CHAPTER THREE

Wild bees respond differently to passive sampling traps with vanes of different colors and light reflectivity in a livestock pasture ecosystem

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Abstract

Wild bees are important pollinators. Different sampling techniques are used to monitor their population dynamics, evaluate species richness and develop conservation protocols. This study was conducted in a livestock pasture ecosystem with the main objective to assess the impact of different vane colors of passive trap on wild bee sampling. We recorded 2230 bees comprising 49 species, and five families. The most abundant species was *Augochlorella aurata* (Smith) (25.8%), followed by *Lasioglossum disparile* (Cresson) (18.3%), *Lasioglossum imitatum* (Smith) (10.85%), *Agapostemon texanus* (Cresson) (10.8%) and *Melissodes veroninae* (9.9%) and *Halictus ligatus* (Say) (4.7%). Traps with bright blue vanes captured the highest number of bees as well as most diverse bee species compared to traps with bright yellow, dark blue, dark yellow, and purple vanes, while red vanes captured the least diverse bee species. Out of 49 bee species, only nine were found in all vane color types. Bright blue vanes attracted the greatest number of unique species. Vanes with higher light reflectance properties (within 400-600 nm range) attracted highest number of bee species. These results suggest that bees respond differently to different light wavelength and reflectivity of vanes of passive traps, and such findings would be helpful in optimizing bee sampling methods.

Keywords: bee, color vanes, passive trap, sampling, livestock pasture, light reflection, bee vision

1. Introduction

Monitoring the status of pollinators in agricultural farmland and other habitats is important because more than 75% of flowering plants including 35% of crops depend upon insect pollinators (Klein et al., 2007; Ollerton et al., 2011). While special focus has been given regarding the distribution, diversity, and abundance of bees in agricultural farm land (Klein et al., 2007), non-bee insects such as flies, wasps, beetles, and butterflies are also regarded as valuable pollinators though may not be as efficient as bees (Potts et al., 2016). In order to regularly assess the populations of pollinators, simple, unbiased, and reliable sampling methods (Cane et al., 2000) are needed.

Active and passive sampling methods are widely used to assess pollinator populations and communities in different habitats. Sweeping, hand netting, and vacuuming are some of the active sampling methods (Jousselin & Kjellberg, 2001) that are generally time consuming, biased, require skilled manpower and experience of survey (McCravy, 2018). In contrast, passive sampling methods are considered easier as they do not require skilled manpower and allows collection of data within a specified time range (McCravy, 2018). The most common passive sampling methods used for monitoring insect pollinators are malaise traps, pan traps and vane traps (Stephen & Rao, 2005; Campbell & Hanula, 2007).

Among passive traps, pan traps have widely been used and successfully adopted for sampling pollinators (Saunders & Luck, 2013). However, capture of insects in pan traps varies with habitats (Saunders & Luck, 2013); and they are often less effective when used for sampling insects such as butterflies and moths (Vrdoljak & Samways, 2012). Colored sticky traps have been successfully used to sample arthropods, but these traps vary in their ability to trap hymenopterans and coleopterans (Hoback et al., 1999; Pickering & Stock, 2003). Other passive

traps such as colored malaise traps are also used but are not very effective for sampling different pollinators (Campbell & Hanula, 2007). Recently, vane traps have shown great potential for sampling bees as well as other pollinators (Hall, 2018). These traps are commercially available in blue and yellow colors that act as visual cues to attract bees and other insects. However, there is no information on how changing the color and design of these vanes affect overall sampling.

Visual cues help bees and other pollinators to discover the floral resources in their foraging landscapes. Bees also use their olfactory signals if the source is ~ 30 cm away and visual cues when they are closer to flowers (Streinzer et al., 2009). Color vision of bumblebees and honey bees is important because it impacts their ability to detect resources (Dyer et al., 2008). Bees are known to visit naturally preferred flower color during their first flower visit and from that experience they recognize the rewarding flowers with its colors (Gumbert, 2000). Based on the information on bee vision, researchers have used different colors in traps used for sampling bee species. Commonly used colors in passive traps are white, yellow, and blue (Vrdoljak & Samways, 2012; McCravy, 2018). Light reflected from different color traps have different intensity and thus affects number as well as species of bees and other insects that are attracted toward the trap (Vrdoljak & Samways, 2012; Joshi et al., 2015; Acharya et al., 2021).

Vane traps, which consist of a collection jar attached to a set of colored vane panels, is an effective method for capturing a wide variety of bees found in both open landscapes and woodlands (Hall, 2018) and have recently been used to sample wild bee populations in agricultural crops (Gibbs et al., 2017). Vane traps provide a simple and effective sampling method to assess relative abundance of pollinators in a large area during the entire season (Stephen & Rao, 2005). It is considered one of the best methods to study native bee populations in grasslands (Kimoto et al., 2012) and other similar habitats. However, the choice of vane

colors in currently available traps is limited to blue and yellow. Therefore, there is a need to refine these traps by optimizing vane colors and design and by assessing their effectiveness for sampling bees in different ecosystems, including livestock pastures. In this context, we tested the research hypotheses that changing the color and other characteristics of vanes affect bee species capture rate in passive traps. Vanes with different colors (viz. dark blue, bright blue, dark yellow, bright yellow, purple, and red) were designed and evaluated for their light reflectance properties and attractiveness to bees in livestock pasture.

2. Methods

2.1 Study Site Description

The research was conducted during the summer of 2017 at the USDA-ARS Dale Bumpers Small Farms Research Center in Booneville, Arkansas (35.09 °N, 93.95 °W). Soil in the research site location was Leadvale silt loam, characterized by moderately well drained, nearly level or with gentle sloped landscape (<https://websoilsurvey.sc.egov.usda.gov>). Enders silt loam (clay mixed) was predominant in higher elevation whereas Leadvale silt loam (fine silty and siliceous) type of soil was found in lower elevations (Thomas et al., 2008). Total annual rainfall during 2017 was 944.0 mm with highest rainfall during May (145.5 mm) and lowest during February (78.2 mm). Average annual temperature during same time was 17.6°C with highest during July (24 °C) and lowest during January (4 °C).

2.2 Field Preparation

A managed field site (consisting 4 study plots of total 1.6 ha) was used in which mixed native grass, forbs and legumes were established in a livestock pasture. Prior to establishment of pasture, the field was sprayed with glyphosate (41% as Roundup® , 0.764 L ha⁻¹; Ragan and Massey, Inc., Ponchatoula, LA) on June, July, September, October of 2016 and January 2017,

and with 75% Sulfosulfuron (Outrider; Monsanto, St. Louis, MO; 0.016 L ha⁻¹) on September 2016 using Continental Belton cluster nozzle sprayer (Continental Belton McAlester, SR:A44117, Oklahoma city, OK). The field was burned in September 2016 and seed bed prepared using tiller (Maschio Gaspardo North America Inc., SC 300, Des Moines, IA) and rolled using 12' Big Guy Roller (Graul Manufacturing, St. Louis, MO) on October 2016. Topsoil at 0 to 15 cm was tested for soil fertility before seeding at University of Arkansas soil test lab which determined that no supplemental fertilizer was required during the entire study period (<https://aaes.uark.edu/technical-services/soil-testing-and-research-laboratory/>). The seed-mixes were Buck's Hangout (Hamilton Native outpost, Elk creek, MO; www.hamiltonnativeoutpost.com; 14.5 kg ha⁻¹), Tallgrass Inexpensive mix and Tallgrass Exposed Clay subsoil mix (Prairie Moon, Winona, MN; www.prairiemoon.com; 13.44 kg ha⁻¹ and 26.8 kg ha⁻¹, respectively according to nursery recommendations), planted in February 2017. Species percentage per seed mixes and recommended rate are available in the websites of seed suppliers.

2.3 Vane Trap Design and Sampling Procedure

Vane traps were designed in the laboratory by selecting different colors of vane panels (Figure 1). In each trap, two panels of vanes of equal size were fitted together with panel grooves in opposite direction (Figure 1). Panel grooves were also glued together and then fitted perpendicularly into a funnel shaped lid with the help of two small steel bolts. A single panel of vane was 30.5 cm high and 18 cm wide (in the center). The base of each panel was trimmed by bending the remainder of panel to fit with the funnel-lid by steel bolts. The top of one of the panels was as wide as the center portion but contained two small holes (8 mm, one in each half of the panel) that were used to connect to a steel wire to suspend the traps from post (Figure 1),

and the top of other panel was trimmed to fit the design and was 5 cm wide. Vane panels in three colors of traps were made of plastic sheet of different colors (dark blue, dark yellow, purple; Interstate Plastics, Sacramento, CA). In another three types of traps, the plastic vane panels were covered by a micro prismatic sheeting reflective tape (bright blue, bright yellow, and red; Tape TapeCase Ltd., Elk Grove Village, IL). The vanes with funnel lids were then connected to a semi-transparent plastic jar (950 ml) for sample collection. The same type of collection jar was used in all colors of vane traps.

Four plots each of 0.4 ha were used for the study. In each plot, six different colored vane traps (dark blue, bright blue, dark yellow, bright yellow, purple, and red) were deployed in randomized order in four transects (one per plot). These traps were suspended using metallic wire from the post about 1.5 m above the ground and were placed 25 m apart. The border distance from outer post was 8 m, and adjacent area was under pasture production. Traps were deployed continuously during the entire study period (June 21 - August 11), and insect samples were collected twice every week, 48 hours after filling with soapy water. Insects were collected directly in vials containing 70% ethyl alcohol as a preservative.

Bee samples were processed and curated in the lab and were identified to the lowest taxa. Samples were identified by Drs. D. Biddinger (Penn State University, PA), and R. Jean (Environmental Solution & Innovations, Inc., IN) using dichotomous keys (Mitchell, 1962; Michener et al., 1994; Michener, 2007) and other available online taxonomic resources, such as Discover Life (www.discoverlife.org; accessed in December 2020) and Bug Guide (bugguide.net; accessed in December 2020).

2.4 Light reflectance analysis of Vane traps

Light reflectance characteristics of all types of colored vane panels and sample collection jars used in passive traps were analyzed at the Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville USA using a similar procedure as previously described¹⁸. A small square piece (~ 2 cm²) of each color vane was cut and kept inside the incident light window of spectrophotometer where light was reflected from the vane sample on the detector for 200 sec to record reflectance. Each piece of plastic was placed in front of the barium sulfate coated detector. Total reflectance of different colored vane traps was measured within 190-1600 nm range using spectrophotometer (JASCO V-780, JASCO Corporation, Easton, MD, USA). Spectrometer containing spectroscopy software related to Windows 7 pro (64-bit) operating system was used for measuring intensity of light passing through sample.

2.5 Data analyses

Bee abundance, species richness, similarity, and community assemblage patterns were analyzed and compared among all six colors of vane traps. Effect of vane color on bee abundance was examined by conducting analysis of variance (ANOVA) in R software program (R version 3.6.2). In addition, a post hoc Tukey test was conducted to detect significant differences among all six vane colors. Data represented 15 sampling dates, six vane colors, and four replicate plots for total of 360 samples. Data were squared root transformed to address non-normality (right- skewness) in the original dataset.

Bee species richness was compared among vane colors by developing sample-based and individual- based rarefaction curves in EstimateS v.9.1.0 (Colwell, 2013). A rarefaction curve depicts new species accumulated (y-axis) against number of samples or individuals collected (x-axis). Smoothed curves with 95% confidence intervals representing the statistical expectation of

species accumulation are generated through iterative resampling of the species by sample matrix. Such curves generally grow rapidly at first as most common species are collected, and soon plateau as only the rarest species are collected in successive collections. Sample based rarefaction curve estimates “species density”, i.e., number of species detected per sample, and individual based rarefaction curve estimates “species richness”, i.e., number of species per individual collected. Significant differences in diversity were determined based on lack of overlap between the 95% confidence intervals. The total number of species and the number of unique species for each vane color was reported. In addition, the number of shared species for each pairwise comparison of trap colors was calculated. Sorensen (incidence based) similarity indices were generated for each pairwise comparison of vane colors using EstimatesS v 9.1 (Colwell, 2013) in order to characterize the extent of species similarity among vane colors.

Redundancy analysis (RDA), a constrained ordination approach was used to examine community assemblage patterns associated with trap color using Canoco v4.5 (Ter Braak & Šmilauer, 2002). Vane colors were coded as dummy variables and were used as environmental predictor variables. Because of the preponderance of low abundance species, data were aggregated at genus level. Counts of bees within each bee genera were considered as response variables for each combination of trap color and plot. In the analysis, only genera higher than 1% abundance were included. Square-root transformation was done before analysis and species data were centered for visualization purposes. Significant differences among trap colors was determined using Monte Carlo permutation (n=499) and stepwise forward selection (Lepš & Šmilauer, 2003). Associations among bee genera and trap colors were visualized using biplots drawn using CanoDraw (Lepš & Šmilauer, 2003).

3. Results

3.1 Abundance and Richness

A total of 4416 insect samples were captured in all colored passive vane traps during the entire study period. Among them, 2230 samples were bees, and the remaining samples were non-bee species. All bees collected in the study belonged to five families listed in Table 1 with taxonomy up to genus and species, whereas non-bee species were excluded from further analysis due to resource limitations in identifying and processing those samples. Bee abundance (Table 1; Figure 2) differed significantly among vane colors ($F(5,354) = 17.78$; $P < 0.001$). Based on post hoc Tukey test, traps with the bright blue vanes captured the highest number of bees, while the red color vanes captured the least number of bees ($P < 0.001$; Figure 2). Differences among bright yellow, dark yellow, purple, and dark blue vanes traps were not significantly different in terms of total bee capture rate (Figure 2).

Sample-based rarefaction curve reveals significantly higher species accumulation in the traps with bright blue vanes compared to dark yellow, dark blue, and red vanes, whereas other trap vane colors (bright yellow and purple) did not differ from any of the other trap colors (Figure 3A). However, significant differences in species accumulation were not detected among vane colors using individual-based rarefaction (Figure 3B).

Ordination analysis revealed that bee assemblages differed among trap colors (Figure 4). Stepwise forward selection revealed that bee assemblages in bright blue vane traps ($F = 8.62$, $P = 0.002$) differentiated the most from other trap colors, followed by red ($F = 4.14$, $P = 0.012$) and dark blue ($F = 4.89$, $P = 0.006$). The biplot depicts the first two ordination axes, of which axis 1 explains 41.9% of the species data and Axis 2 explains an additional 21.3 % of the species data. Genus vectors on the biplot indicate that bee genera exhibited varying levels of association with

trap colors along a gradient from blues to yellows (Figure 4). *Melissodes* bees were associated with dark blue, purple and bright blue traps. *Ptilothrix*, *Lasioglossum* and *Bombus* bees showed a stronger association with bright blue traps in particular. *Agapostemon* was associated with both bright blue and bright yellow traps, whereas *Augochlorella* was more closely associated with bright yellow and dark yellow traps. *Halictus* trended toward bright blue, but the short vector suggests a weaker association. No bee genera were strongly associated with red traps.

Most of the bees recorded in this study were from the Halictidae family (77.57%). Of the total bees, 30% were collected from traps with bright blue vanes, 17.71% from bright yellow, 16.27% from dark yellow, 16.18% from purple, 13.58% from dark blue and 6.27% from traps with red vanes. The most abundant species was *Augochlorella aurata* (Smith) (25.78% of total), then *Lasioglossum disparile* (Cresson) (18.29%), *Lasioglossum imitatum* (Smith) (10.85%), *Agapostemon texanus* (Cresson) (10.85%) and *Melissodes veroninae* (9.865%) and *Halictus ligatus* (Say) (4.708%). Descriptive diversity statistics for each of the trap colors, shows that bee abundance and richness was highest in bright blue traps and followed by bright yellow traps. Abundance and richness were lowest in red traps (Table 2). Bright blue traps also had the greatest number of unique species (Table 2). Shared species and similarity index values based on pairwise comparisons of trap colors depict varying levels of complementarity (i.e. dissimilarity) among different traps colors (Table 2), although these comparisons are limited by the lowest species richness level in any pairwise comparison. The highest levels of dissimilarity were found between purple and dark yellow traps, and between dark blue and dark yellow traps (Table2). The lowest level of dissimilarity was found between purple and dark blue (Table 2).

3.2 Light Reflectance

Light reflectance spectrum of vanes of all traps used in this study were different from each other (Figure 5). The same type of collection jar was used for all traps, and the light reflectance curve of the jar showed the reflectance peak at 600 nm (Figure 6). Bright blue vane had a higher light reflectance that peaked twice in the spectrum, initially at 455 nm within the wavelength ranging from 400–600 nm, and later at 876 nm. In contrast, the light reflectance from the dark blue vane was relatively lower but peaked twice (at 450 nm and 850 nm). Bright yellow vanes had a reflectance peak at 598 nm that gradually decreased with increasing wavelength, and similar pattern was also observed in the case of dark yellow vanes (Figure 5). Purple vanes showed an initial small peak of light reflectance around 450 nm with range of frequency from 350-500 nm, but later showed higher reflectance that peaked at 879 nm. Similarly, red vanes had a very small initial peak at 390 nm but showed higher light reflectance (~48%) later at 661 nm (Figure 5). Passive traps with colored vanes of higher light reflectance (within 400-600 nm range) attracted the highest number bee species in this study.

4. Discussion

This study reveals that various measures of bee diversity-including abundance, richness, and assemblage patterns are influenced by vane color and reflectance patterns when passively collecting bees with vane traps. In particular, brightly colored vanes with higher light reflectance within 400-600 nm range attracted a greater diversity of bees in traps placed in a livestock pasture ecosystem. Effectiveness of blue and yellow vane traps had been compared previously in different ecosystems, for instance in apple orchards (Joshi et al., 2015), both woodland and open agriculture farmland (Hall, 2018), and adjacent to *Helianthus* spp. (Asteraceae) field (Stephen &

Rao, 2007). In all these studies, blue vane trap captured higher number of different bee species and 5-6 times more individuals compared to yellow vane trap.

In the current study, we tested a different design and size of vanes and a wider array of vane colors and reflectance patterns attached to sample collection jars. In particular, we used bright blue and yellow vanes that were made of plastic sheets covered with a micro-prismatic retro-reflective sheeting that provides better daytime and nighttime brightness as well as high visibility and durability. These vanes showed higher light reflectance and captured the most bees and bee species in this study (Table 2). Similar material was used on red vanes as well, but the light reflectance from those vanes was relatively lower, and as a result captured fewer bees. Traps with bright blue vanes performed especially well in terms of rates of bee capture (Figure 2; 11.1 bees per trap per sampling date) and rates of species accumulation (Figure 3). Bright yellow traps exhibited the second highest values for capture rates (Figure 2; 6.6 bees per trap per sampling date) and species accumulation (Figure 3), but these rates were not deemed significantly different from some other colors in which the reflective sheeting was not used, such as dark yellow, dark blue and purple.

Bees use visual clues for detection, recognition, and memorization of floral resources in the foraging landscape (Menzel & Backhaus, 1991; Stephen & Rao, 2005). The intensity of light reflected from different colors of vanes in traps affect number of bees attracted toward the trap (Vrdoljak & Samways, 2012). Most bees can recognize colors that fall between 300 to 600 nm visual spectrums (Kevan et al., 1996). While the information related to the vision of many solitary and wild bees is not available, in the case of honeybees (*Apis mellifera*), color vision is trichromatic with highly sensitive photoreceptors at 344 nm (ultraviolet), 436 nm (blue) and 544 nm (green) (Avarguès-Weber et al., 2012).

In this study, colored vanes at a higher light reflectance between 400 nm to 600 nm attracted the highest number of bee species in these passive traps. Capture rate differed among traps with different colored vanes in the current study, which can be explained by sensitivity of visual spectrum of bees and variation in the light reflectance of vanes of these traps. For example, bright blue vanes had two peaks of higher light reflectance, initially in 450-455 nm range and second peak with > 800 nm. Such higher reflectance peak within the optimal range of bee vision which might have played an important role in attracting abundant and diverse bee species to these passive traps. Similarly, bright yellow captured the second largest number of bees, also had higher light reflectance peak within 600 nm but gradually decreased with increasing wavelength. Though bees have a color spectrum from UV to orange (Hertz, 1939), they are sensitive to a color spectrum between blue, green and ultraviolet (Von Frisch, 1914), which is a type of trichromatic vision system (Menzel & Backhaus, 1991). In this Kühn (1924) study, red color vanes showed relatively lower light reflectance within 600 nm range, but had higher reflectance later in the spectrum, and this could be a reason why a low number of bees were collected in the traps. Past research showed contradictory views regarding the ability of bees to perceive red color. For instance, Kühn (1924), an early researcher in this field, reported that bees recognize red color objects; however, other researchers had reported inability of bees to perceive (Frisch, 1967) or discriminate red from other colors (Von Helversen, 1972). Chittka & Waser (1997) argued that the bees see up to 650 nm in the visual spectrum and may not miss red colored flowers while foraging. However, other factors such as background (vegetation) color could also be contributing to bees' ability to navigate different vane or flower colors in a livestock pasture landscape. Generally, bees use color contrast to locate flower source and hence neutral colors such as white color are usually ignored (Kevan et al., 1996).

Different species of bees responded to different colors of vane traps. Out of the 49 bee species collected in this study, only nine bee species were found in all vane color types, whereas 14 species were found in only one trap color. For instance, out of five bumble bee species, two were found in all six vane colors, one was found in five colors, and two species (*Bombus bimaculatus* and *B. fervidus*) were only found in the traps with bright blue vanes. Many of the species that were only found in one trap color- *Calloopsis adreniformis* (bright yellow), *Ceratina dupla* (bright yellow), *Diadasia afflicta* (bright blue), *Diadasia enavata* (dark blue), *Halictus rubicundus* (dark yellow), *Hylaeus mesillae* (red), *Lasioglossum tegulare* (bright blue), *Lasioglossum trigeminum* (purple), *Megachile montigava* (dark yellow), *Melitoma taurea* (bright blue), *Svastra atripes* (bright blue), and *Triepeolus lunatus* (dark yellow) were singletons and it was impossible to know if this represented a true preference or pattern. Our analysis of assemblage patterns after aggregating bees at the genus level, did show a gradient-like response in bee-color associations (Figure 4), ranging from dark blue to yellows (with no strong associations found with red vanes). These patterns may be used to guide future trapping efforts to monitor bee diversity or to target specific bee species in livestock pastures or other ecosystems. While the bright blue and yellow traps with reflective sheeting were particularly attractive to bees, dark blue and purple traps also had relatively high levels of abundance and richness and collected higher number of *Melissodes*. Purple, as a color, is less commonly used than blue and yellow traps in bee monitoring. While this study shows that purple may be a viable option for bee collection, it's similar assemblage pattern (Figure 4) and low level of complementarity with dark blue traps (Table 2) suggests that it may be redundant blue traps that are already commonly used.

Most of the bees collected in the current study were from Halictidae family (77.6%) followed by Apidae. However, few bee species in the families Andrenidae, Colletidae, and Megachilidae were collected. Consistent with our findings, (Bhandari et al., 2018) reported that bees of the Halictidae family were the most abundant bees in rangeland of Texas. The most common species found in this study were *A. aurata* (Smith), *L. disparile* (Cresson), *L. imitatum* (Smith) and *A. texanus* (Cresson). In our previous studies we have found similar bee diversity in this study region (Acharya et. al. 2021). Pollinator species richness and diversity as well as population distribution in livestock pasture vary during the season (Thapa-Magar et al., 2020). Mid-July to mid-August is the latter half of the summer season in the Southeastern USA, and the sampling period may have missed bee species that emerge earlier in the season and are reported in other studies (Bhandari et al., 2018; Thapa-Magar et al., 2020).

5. Conclusions

In this study, wild bees responded differently to passive traps with colored vanes of different light wavelength and reflectivity when deployed in a livestock pasture ecosystem. Among six different colors of vanes (dark blue, bright blue, dark yellow, bright yellow, purple and red), the bright blue traps captured the highest number of individuals and species of bees. This could be due to an appropriate match between the visual spectrum of bees and the light reflectance spectrum of vanes, which were made of a micro-prismatic retro-reflective material. Bees responded similarly to traps with other colors of vanes, except for red vane traps, which captured the lowest number of bees. A total of 49 species of wild bees belonging to five families were recorded during the study period. The most abundant species was *Augochlorella aurata* (Smith) (25.8% of total bees), followed by *Lasioglossum disparile* (Cresson) (18.3%). The findings of this study would be useful in understanding bee vision and responses to passive traps,

and, such information would help in optimizing bee sampling methods for future monitoring efforts.

6. References

- Acharya, R.S.; Leslie, T.; Fitting, E.; Burke, J.; Loftin, K.; Joshi, N.K. (2021). Color of pan trap influences sampling of bees in livestock pasture ecosystem. *Biology* 10, 445.
<https://doi.org/10.3390/biology10050445>
- Avarguès-Weber, A., Mota, T., & Giurfa, M. (2012). New vistas on honey bee vision. *Apidologie*, 43(3), 244-268.
- Bhandari, K. B., West, C., Longing, S., Brown, C., Green, P., & Barkowsky, E. (2018). Pollinator abundance in semiarid pastures as affected by forage species. *Crop Science*, 58(6), 2665-2671.
- Campbell, J. W., & Hanula, J. (2007). Efficiency of Malaise traps and colored pan traps for collecting flower visiting insects from three forested ecosystems. *Journal of Insect Conservation*, 11(4), 399-408.
- Cane, J. H., Minckley, R. L., & Kervin, L. J. (2000). Sampling bees (Hymenoptera: Apiformes) for pollinator community studies: pitfalls of pan-trapping. *Journal of the Kansas entomological society*, 225-231.
- Chittka, L., & Waser, N. M. (1997). Why red flowers are not invisible to bees. *Israel Journal of Plant Sciences*, 45(2-3), 169-183.
- Colwell, R. K. (2013). EstimateS: Statistical estimation of species richness and shared species from samples. Version 9 and earlier. User's Guide and application. Published at: <http://purl.oclc.org/estimates>.
- Dyer, A. G., Spaethe, J., & Prack, S. (2008). Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. *Journal of Comparative Physiology A*, 194(7), 617.
- Frisch, K. V. (1967). *The Dance Language and Orientation of Bees*.
- Gibbs, J., Joshi, N. K., Wilson, J. K., Rothwell, N. L., Powers, K., Haas, M., Gut, L., Biddinger, D. J., & Isaacs, R. (2017). Does passive sampling accurately reflect the bee (Apoidea: Anthophila) communities pollinating apple and sour cherry orchards? *Environmental Entomology*, 46(3), 579-588.
- Gumbert, A. (2000). Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. *Behavioral Ecology and Sociobiology*, 48(1), 36-43.
- Hall, M. (2018). Blue and yellow vane traps differ in their sampling effectiveness for wild bees in both open and wooded habitats. *Agricultural and Forest Entomology*, 20(4), 487-495.

- Hertz, M. (1939). New experiments on colour vision in bees. *Journal of Experimental Biology*, 16(1), 1-8.
- Hoback, W. W., Svatos, T. M., Spomer, S. M., & Higley, L. G. (1999). Trap color and placement affects estimates of insect family-level abundance and diversity in a Nebraska salt marsh. *Entomologia experimentalis et applicata*, 91(3), 393-402.
- Joshi, N. K., Leslie, T., Rajotte, E. G., Kammerer, M. A., Otieno, M., & Biddinger, D. J. (2015). Comparative trapping efficiency to characterize bee abundance, diversity, and community composition in apple orchards. *Annals of the Entomological Society of America*, 108(5), 785-799.
- Jousselin, E., & Kjellberg, F. (2001). The functional implications of active and passive pollination in dioecious figs. *Ecology Letters*, 4(2), 151-158.
- Kevan, P., Giurfa, M., & Chittka, L. (1996). Why are there so many and so few white flowers? *Trends in Plant Science*, 1(8), 252.
- Kimoto, C., DeBano, S. J., Thorp, R. W., Taylor, R. V., Schmalz, H., DelCurto, T., Johnson, T., Kennedy, P. L., & Rao, S. (2012). Short-term responses of native bees to livestock and implications for managing ecosystem services in grasslands. *Ecosphere*, 3(10), 1-19.
- Klein, A.-M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the royal society B: biological sciences*, 274(1608), 303-313.
- Kühn, A. (1924). Versuche über das Unterscheidungsvermögen der Bienen und Fische für Spektrallichter. *Nachrichten von der Gesellschaft der Wissenschaften zu Göttingen, Mathematisch-Physikalische Klasse*, 1924, 66-71.
- Lepš, J., & Šmilauer, P. (2003). *Multivariate analysis of ecological data using CANOCO*. Cambridge university press.
- McCravy, K. W. (2018). A review of sampling and monitoring methods for beneficial arthropods in agroecosystems. *Insects*, 9(4), 170.
- Menzel, R., & Backhaus, W. (1991). Colour vision in insects. *Vision and visual dysfunction*, 6, 262-293.
- Michener, C. (2007). *The Bees of the World* Johns Hopkins University Press. Baltimore.[Google Scholar].
- Michener, C. D., McGinley, R. J., & Danforth, B. N. (1994). *The bee genera of North and Central America (Hymenoptera: Apoidea)*. Smithsonian Institution Press.

- Mitchell, T. (1962). Bees of the eastern United States, vol. 2 (Technical Bulletin No. 152). Agricultural Experiment Station, Raleigh, NC.
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326.
- Pickering, C. M., & Stock, M. (2003). Insect colour preference compared to flower colours in the Australian Alps. *Nordic Journal of Botany*, 23(2), 217-223.
- Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., Garibaldi, L. A., Hill, R., Settele, J., & Vanbergen, A. J. (2016). The assessment report on pollinators, pollination and food production: summary for policymakers. Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity.
- Saunders, M. E., & Luck, G. W. (2013). Pan trap catches of pollinator insects vary with habitat. *Australian Journal of Entomology*, 52(2), 106-113.
- Stephen, W. P., & Rao, S. (2005). Unscented color traps for non-Apis bees (Hymenoptera: Apiformes). *Journal of the Kansas Entomological Society*, 373-380.
- Stephen, W. P., & Rao, S. (2007). Sampling native bees in proximity to a highly competitive food resource (Hymenoptera: Apiformes). *Journal of the Kansas Entomological Society*, 80(4), 369-376.
- Streinzer, M., Paulus, H. F., & Spaethe, J. (2009). Floral colour signal increases short-range detectability of a sexually deceptive orchid to its bee pollinator. *Journal of Experimental Biology*, 212(9), 1365-1370.
- Ter Braak, C. J., & Smilauer, P. (2002). CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5).
- Thapa-Magar, K. B., Davis, T. S., & Kondratieff, B. (2020). Livestock grazing is associated with seasonal reduction in pollinator biodiversity and functional dispersion but cheatgrass invasion is not: Variation in bee assemblages in a multi-use shortgrass prairie. *PloS one*, 15(12), e0237484.
- Thomas, A. L., Brauer, D. K., Sauer, T. J., Coggeshall, M. V., & Ellersieck, M. R. (2008). Cultivar influences early rootstock and scion survival of grafted black walnut. *Journal of the American Pomological Society*, 62(1), 3.
- Von Frisch, K. (1914). Der farbensinn und formensinn der biene. Рипол Классик.
- Von Helversen, O. (1972). Zur spektralen Unterschiedsempfindlichkeit der honigbiene. *Journal of comparative physiology*, 80(4), 439-472.
- Vrdoljak, S. M., & Samways, M. J. (2012). Optimising coloured pan traps to survey flower visiting insects. *Journal of Insect Conservation*, 16(3), 345-354.

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Author contributions

Conceptualization, R.S.A, N.K.J., K.L., and J.B; methodology and fieldwork, R.S.A., N.K.J, and J.B.; formal analysis, R.S.A., T.L and N.K.J.; writing-original draft preparation, R.S.A., J.B., T.L., and K.L; all authors/co-authors have reviewed and prepared final version.

Competing interests

“The authors declare no competing interest.”

7. Figures and Tables

Figures

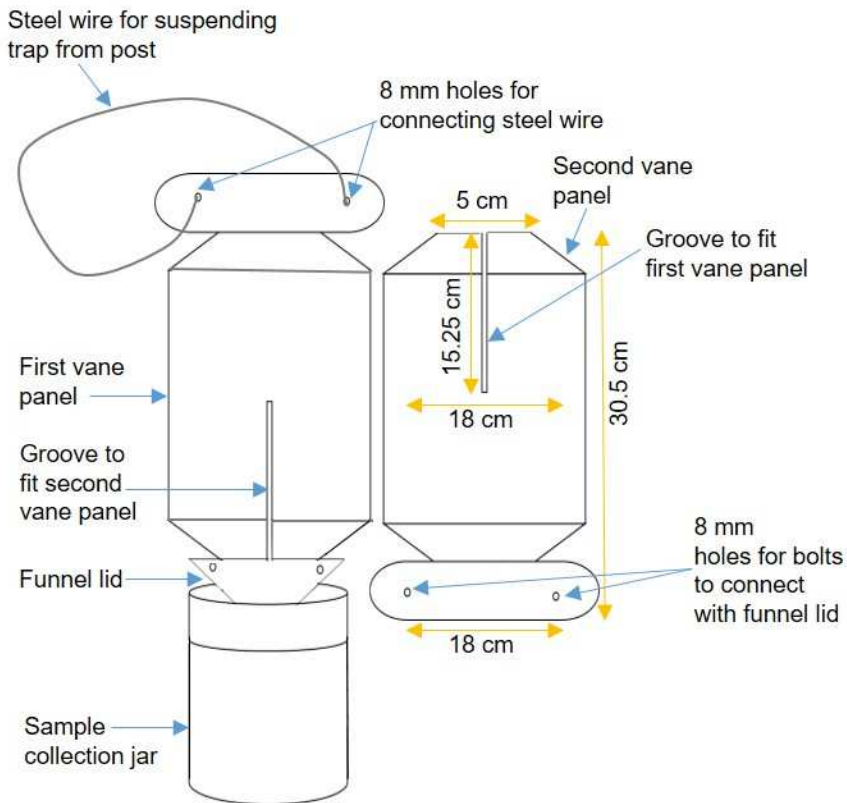


Figure 1: Design of passive vane trap used for sampling of wild bees in livestock pasture system. Design and illustration by N. Joshi.

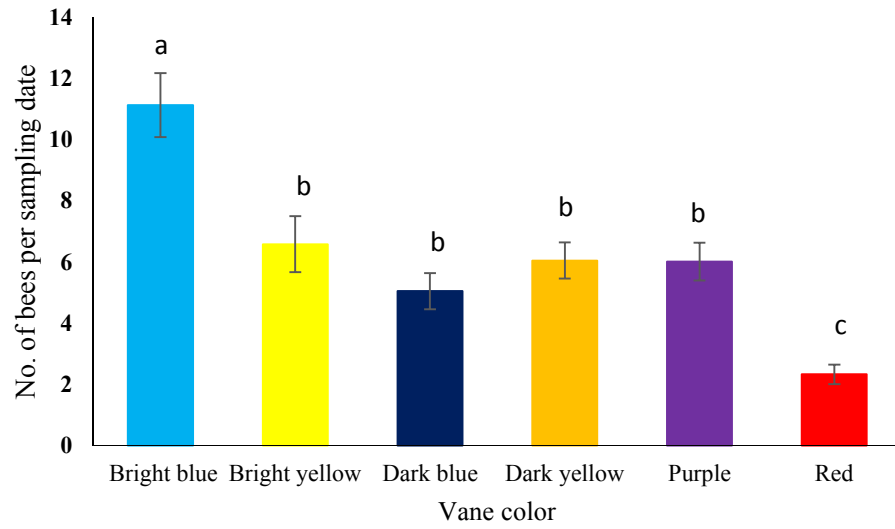


Figure 2: Differences in capture rate (\pm SE) of bees in passive traps with six different colored (bright blue, bright yellow, dark blue, dark yellow, purple, red) vanes in livestock pasture system.

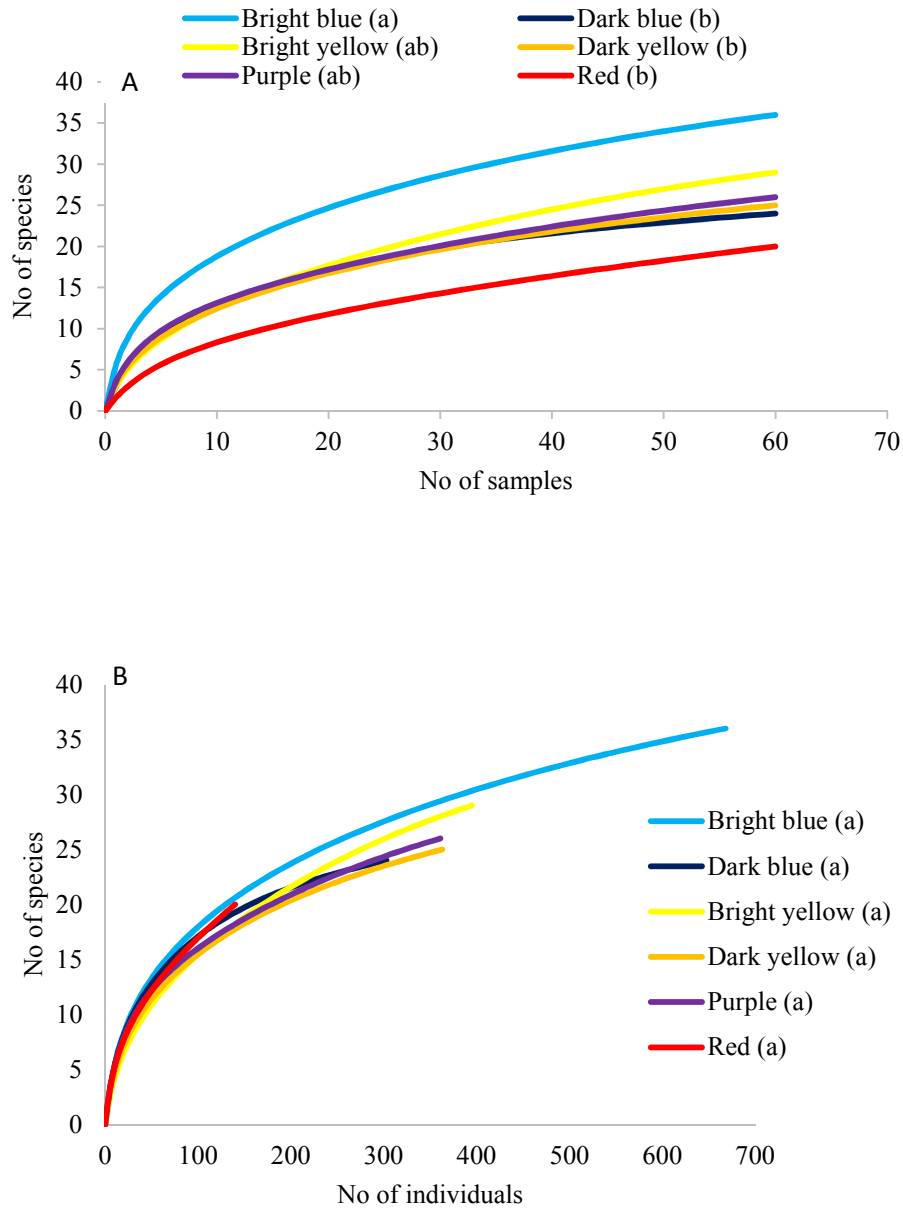


Figure 3: Rarefaction curves showing accumulation of the number of species in relation to the number of samples (A) and number of individuals (B). Different small letters after color type inside brackets indicates significant differences based on non-overlapping 95% confidence intervals.

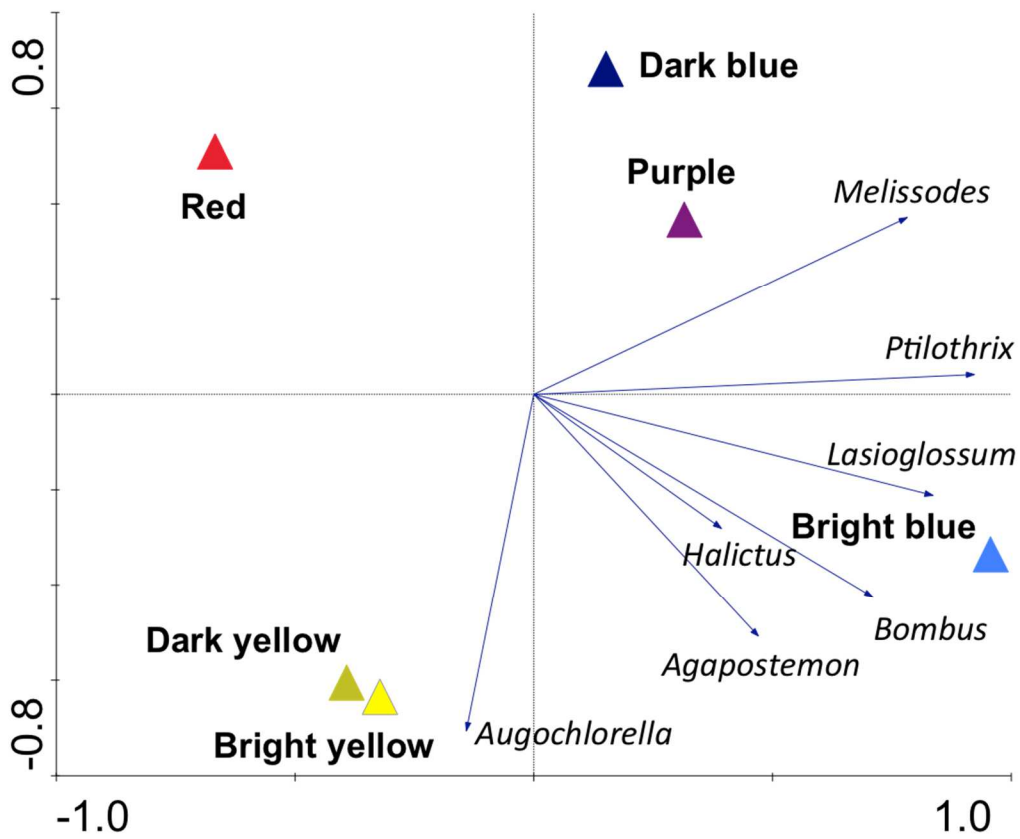


Figure 4: Ordination (redundancy analysis; RDA) biplot showing the association of bee genera and different colored vanes.

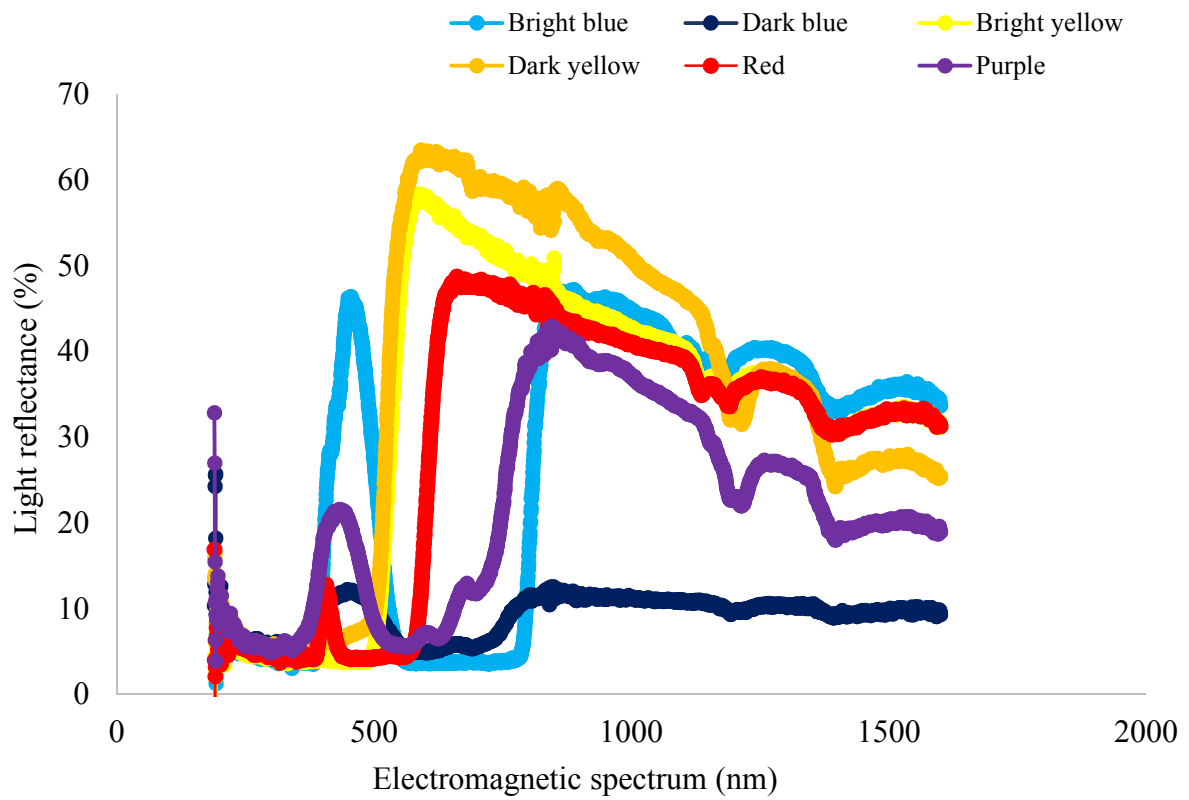


Figure 5: Light reflectance spectrum of different colored vanes of passive traps used for sampling bee communities in pasture system.

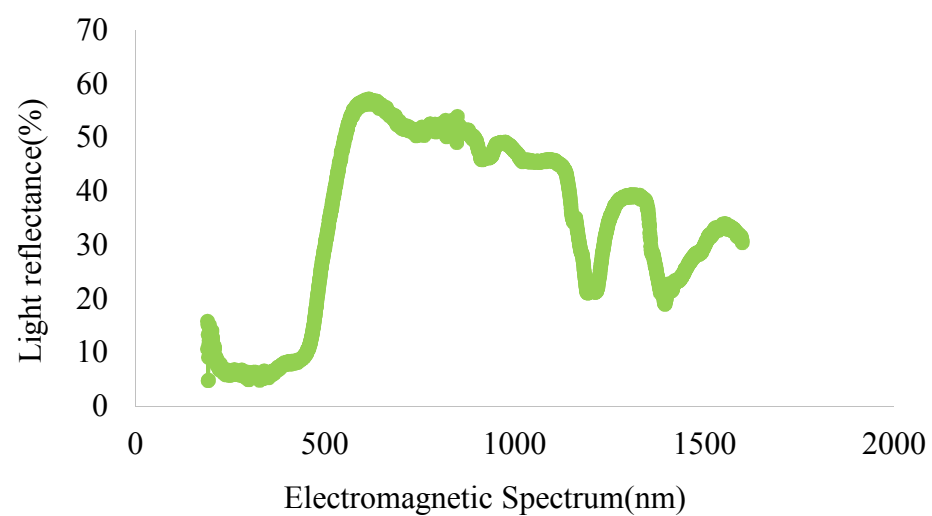


Figure 6: Light reflectance spectrum of the collection jar of the traps used for sampling bees in the study.

Table 1. Family and species list of bees collected in passive traps with different colored (bright blue, dark blue, dark yellow, bright yellow, purple, and red) vanes. * refers to the presence of species in that trap.

Family	Species	Bright blue	Dark blue	Dark yellow	Bright yellow	Purple	Red
Halictidae	<i>Agapostemon texanus</i>	*	*	*	*	*	*
	<i>Agapostemon sericeus</i>	*			*	*	
	<i>Agapostemon virescens</i>				*	*	
	<i>Augochlora pura</i>			*	*		*
	<i>Augochlorella aurata</i>	*	*	*	*	*	*
	<i>Augochloropsis metallica</i>	*		*	*		
	<i>Halictus ligatus</i>	*	*	*	*	*	*
	<i>Halictus parallelus</i>	*	*	*	*	*	*
	<i>Halictus rubicundus</i>			*			
	<i>Lasioglossum disparile</i>	*	*	*	*	*	*
	<i>Lasioglossum hitchensi</i>	*		*			
	<i>Lasioglossum imitatum</i>	*	*	*	*	*	*
	<i>Lasioglossum pectorale</i>	*	*	*	*	*	*
	<i>Lasioglossum tegulare</i>	*					
	<i>Lasioglossum lustrans</i>	*		*	*		
	<i>Halictus tripartitus</i>	*	*	*	*	*	*
	<i>Lasioglossum trigeminum</i>					*	
	<i>Lasioglossum sp.</i>	*		*	*		
	<i>Nomia nortoni</i>	*	*				
Apidae	<i>Apis mellifera</i>	*	*		*	*	*
	<i>Bombus bimaculatus</i>	*					
	<i>Bombus fervidus</i>	*					
	<i>Bombus griseocollis</i>	*	*	*	*	*	*
	<i>Bombus impatiens</i>	*	*	*	*	*	*
	<i>Bombus pensylvanicus</i>	*	*	*	*	*	
	<i>Ceratina calcarata</i>			*			*
	<i>Ceratina dupla</i>				*		
	<i>Ceratina strenua</i>	*	*	*	*	*	
	<i>Diadasia afflicta</i>	*					
	<i>Melissodes bimaculatus</i>		*		*	*	
	<i>Melissodes communis</i>	*	*	*		*	*
	<i>Melissodes comptoides</i>	*	*		*	*	*
	<i>Melissodes sp.</i>	*	*			*	*
	<i>Melissodes veroninae</i>	*	*	*	*	*	*
	<i>Melitoma taurea</i>	*					
	<i>Melissodes denticulatus</i>	*	*		*	*	
	<i>Peponapis pruinosa</i>	*				*	
	<i>Ptilothrix bombiformis</i>	*	*		*	*	
	<i>Svastra obliqua</i>				*	*	
	<i>Svastra atripes</i>	*					

Table 1 (cont.)

Family	Species	Bright blue	Dark blue	Dark yellow	Bright yellow	Purple	Red
	<i>Triepeolus lunatus</i>			*			
	<i>Xenoglossa strenua</i>	*	*				*
	<i>Xylocopa virginica</i>	*		*	*		*
Andrenidae	<i>Calliopsis andreniformis</i>				*		
Colletidae	<i>Hylaeus mesillae</i>						*
Megachilidae	<i>Megachile brevis</i>	*		*			
	<i>Megachile montigava</i>			*			
	<i>Megachile campanulae</i>		*		*	*	
	Total species	36	24	25	29	26	20

Table 2: A comparison of bee diversity and similarity measures among six colors of vane traps deployed in Arkansas livestock pastures.

	Vane color					
	Bright blue	Bright yellow	Dark blue	Dark yellow	Purple	Red
<i>Abundance</i>	668	395	303	363	361	140
No. species	36	29	24	25	26	20
No. uniques	6	2	1	3	1	1
<i>Shared species</i>						
Bright blue	–					
Bright yellow	23	–				
Dark blue	21	19	–			
Dark yellow	20	18	14	–		
Purple	22	22	21	14	–	
Red	17	15	16	15	15	–
<i>Similarity¹</i>						
Bright blue	–					
Bright yellow	0.71	–				
Dark blue	0.70	0.72	–			
Dark yellow	0.66	0.67	0.57	–		
Purple	0.71	0.80	0.84	0.55	–	
Red	0.61	0.61	0.73	0.67	0.65	–

¹Sorensen classic similarity indices.

CHAPTER FOUR

Grazing influences the diversity and community composition of bees, and other insects in livestock pastures

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Abstract

Pollinators contribute to the establishment of perennial native forages, but little is known about the effect of grazing on pollinator abundance and diversity livestock pasture ecosystems. Grazing reduces floral resources available to bees and other insects, and as a result, may influence their communities. The main objective of this study was to examine the impact of grazing on seed mixes of native forb/legume/grass (FLG; www.hamiltonnativeoutpost.com) and warm season grasses (WSG; equal seed mix of equal mix of *Andropogon gerardi*, *Tripsacum dactyloides*, and *Sorghastrum nutans*) on bees and other non-bee insects in livestock pasture in Southeastern USA. Each pasture was divided into two halves (in a split-plot design) using an electric fence in which one plot was grazed and the other plot was non-grazed. Blue vane traps and a pair of yellow and blue pan traps were used for sampling bees and other insects. Plant species composition in two types of seed mixes (FLG and WSG) was also recorded. A diverse array of bees (59 species) and other insects (at least 93 species) were found in the livestock pastures. The abundance, diversity, and evenness of bee communities and other insects were greater in non-grazed plots as compared to grazed plots. However, species richness, as measured by rates of species accumulation relative to sampling effort, was not different among treatments. Establishment of pastures using seed mixes of native forb/legume/grass and warm season grasses, respectively, was variable, with undesirable species often exceeding 50% of the plant community in both pasture types. Results show that a diverse array of bees and other insects may be found in livestock pasture systems, but that reduction in bee and insect diversity in grazed areas highlight the importance of rotational grazing regimes to allow for sufficient floral resources for pollinators at relevant scales.

Keywords: bees, grazing, livestock pasture, vane trap, pan trap, pollinators

1. Introduction

Establishment of native flowering forbs and grasses provide continuous food (nectar and pollen) and habitat resources for native pollinators throughout the year, therefore benefitting farmers and society by supporting biodiversity and pollination services. About 45% of the agricultural land in United States is classified as grassland and mostly as monoculture, which have limited value for pollinators (Hellerstein et al., 2017). In light of recent bee declines, management strategies that focus on incorporating pollinator habitats and adopting pollinator-friendly tactics within such grasslands and pastures are increasingly important. Bee communities require nectar and pollen from flowering plants from spring to fall and rely on a variety of different nesting resources. Pollinators may contribute to the establishment of perennial native forages which offer plant and ecosystem diversity, as well as benefit annuals such as crimson clover (*Trifolium incarnatum*) or hairy vetch (*Vicia villosa*) by providing sufficient pollination and seed yield planted in livestock pasture, or adjacent food crop fields (Anderson et al., 2010).

Numerous factors related to the ecology, structure, and management of rangelands and pastures may influence the diversity and activity rates of pollinators. In calcareous grassland, factors such as floristic composition, vegetative structure, grazing management, and vehicle disturbance from nearby roads affects bumblebee abundance and diversity (Carvell, 2002). Grasslands with greater number of legumes and forbs may exhibit a more diverse pollinator community, and specific forb species such as *Taraxacum* sp. and *Cirsium arvense* are especially attractive to pollinators (Orford et al., 2016). Bumblebee abundance is largely driven by floral abundance and richness, availability of nectar resources, and sward structure (Potts et al., 2009). Bumblebees may also be more abundant and diverse in a conservation mix (kale, mixed cereal,

linseed, and legume) as compared to a cereal, grass and legume mix (Potts et al., 2009). Thus, it is important to include proper forage species in order to increase pollinator population.

Grazing is a key management factor in pastures that may influence pollinators. In theory, livestock grazing should negatively impact pollinators by decreasing floral food resources (nectar and pollen), and through the destruction of nests and nesting sites (Kearns et al., 1998). In livestock pastures, pollinator visitation rates were found to be higher in non-grazed sites as compared to recently grazed or historically grazed sites, although this may not always equate to higher numbers of pollinator taxa (Robson, 2019). Under heavy grazing, herbivores negatively affect reproductive performance of the plant, including number of flowers and number of pollen grains per flower, thus negatively impacting pollinators populations (Zhang et al., 2019). Indeed, most grazing studies (primarily cattle) have found that grazing decreases richness and/or diversity of most pollinators, including various types of beetles, flies, and hymenopterans (Debano, 2006). Intense grazing during early grazing season reduces abundance, diversity, and richness of bumblebees possibly due to reduced plant diversity (Kimoto et al., 2012). A similar decrease occurred in other pollinators such as trap-nesting solitary bees, wasps, butterflies, and grasshoppers in grazed versus non-grazed grassland (Kruess & Tscharntke, 2002). However, grazing intensity is an important consideration, as moderate grazing may not affect availability of floral and nesting resources (Shapira et al., 2020). Wild bee abundance and richness, and honey bee activity was not affected by moderate grazing; instead moderate grazing positively increased nectar and pollen diversity and their availability to both honey bees and wild bees (Shapira et al., 2020). Although higher grazing intensity generally leads to reduced plant-pollinator interaction (Lazaro et al., 2016), this may not be true in all systems. In one study within shrubland and woodland habitats, with a range of post-burn time (intermediate age; 10-17 years after) and

varying intensities (lowest, moderate, and highest) of grazing, bee and flower richness both were highest at moderate to highest grazing intensities and abundance of bees increased with intensified grazing pressure (Vulliamy et al., 2006).

Grazing cattle maintained the abundance of bumble bees as they preferred forage plants such as *Centaurea nigra* and *Trifolium pratense* and removed competitive forage species (Carvell, 2002). In grasslands, snail shells provide nest habitat for solitary bees but when sheep grazed in those pastures, sheep destroyed shells (Hopfenmüller et al., 2020). Intensive grazing increased the rate of destruction, negatively affecting population of solitary bees (Hopfenmüller et al., 2020) and insect abundance (Tadey, 2015). Reduction in grazing intensity preserved insect diversity on grasslands increased vegetation height, resulting in increased butterflies, trap-nesting bees and wasps (Kruess & Tscharntke, 2002).

Grazing or mowing is necessary to maintain grasslands which otherwise become overwhelmed with weeds, but can also lead to a reduction in floral resources and pollinators (Tanis et al., 2020) and can cause direct mortality for egg and larval stage of insects (Black et al., 2011). Once pollinators become active, it is crucial not to destroy pollinator habitat especially that of bees by mowing. However, partial mowing or grazing can be beneficial to pollinator habitat and increase pollinator populations throughout year (Tanis et al., 2020). Proper mowing practice and low intensity agriculture can help address issues with pollinator loss and grassland biodiversity (Johansen et al., 2019), and improves biodiversity (Ledvina et al., 2020). Thus, proper management of pastures to foster sustainability of pollinators should consider timing of mowing and grazing to meet needs of both livestock and pollinators.

This study was conducted in livestock pastures and had three objectives: 1) establish baseline data on the diversity of bees and non-bee insects found in pasture ecosystems, 2)

examine the impacts of grazing on bees and other insects; and 3) evaluate the establishment success of native forbs and warm season grasses in these livestock pastures. The findings of this study will help inform pollinator-focused management strategies in livestock pasture systems.

2. Materials and Methods

2.1 Site Description

This study was conducted during the summer of 2018 and 2019 at the USDA-ARS Dale Bumpers Small Farms Research Center in Booneville, Arkansas (35.09 °N, 93.95 °W). The soil is characterized as Leadvale silt loam (fine-silty, siliceous, semiactive, thermic Typic Fragiudults) with water movement and plant rooting limited by a fragipan at a depth of 0.15 to 1.0 m (<https://websoilsurvey.sc.egov.usda.gov>). Details regarding the soil types of the area are available in (Thomas et al., 2008). Average temperature during July and August of 2018 were 27.0 and 25.2 °C. The site received 96.9 and 151.0 mm rainfall during July and August of 2018. In 2019, same site received 95.8 and 149.0 mm rainfall during July and August and average temperature was 29.8 and 26.0 °C.

2.2 Field Preparation

Conventionally managed plots (n =6 each of 0.4 ha size) established as a livestock pasture were used for the study. Prior to establishment of pasture, the field was sprayed with herbicides, viz: Roundup® (41% glyphosate, 4.67 l ha⁻¹) in June, July, September, and October of 2016 and January of 2017, and with Outrider (Monsanto, St. Louis, MO; 0.096 l ha⁻¹) on September 2016 using a Continental Belton cluster nozzle sprayer (Continental Belton McAlester, SR: A44117, Oklahoma city, OK). The field was tilled (Maschio Gaspardo North America Inc., SC 300, Des Moines, IA), and rolled using 12' Big Guy Roller (Grahl Manufacturing, St. Louis, MO) in October 2016. Out of six plots, three plots were planted with

mixture of native flowering plants and remaining three were planted with native warm season grasses. The Warm Season Grass seed-mix was [big bluestem (*Andropogon gerardi*), eastern gamagrass (*Tripsacum dactyloides*), and Indiangrass (*Sorghastrum nutans*); equal mix of each seed type; 8.10 kg ha⁻¹]. The native flowering plant seed-mix consisted of Buck's Hangout (Hamilton Native outpost, Elk creek, MO; www.hamiltonnativeoutpost.com; 14.5 kg ha⁻¹), Tallgrass Inexpensive, and Tallgrass Exposed Clay subsoil mix (Prairie Moon, Winona, MN; www.prairiemoon.com; 13.44 kg ha⁻¹) planted in February, 2018 and 2019. Species percentage per seed mixes are available in the aforementioned websites and supplementary file.

Each plot was further divided into two halves (0.2 ha each) using electric fence in which half of the plot was grazed by sheep and remaining half was left un-grazed. Sheep (n = 15) were allowed to graze for 4 days per week (Monday through Thursday) and traps for sampling bees were set up on Friday to collect samples on Sunday (after 48 hours). Sheep were removed from grazed plots before setting traps. In grazed plots, sheep were prevented from grazing once forb height was reduced to less than 30 cm, and minimum grazing occurred in both years.

Sampling methods

Blue vane traps and pan traps were used to sample visiting insects in each plot to compare abundance and species richness of pollinators and other insects between the grazing management treatments (grazing vs non-grazing). Samples were collected weekly from first week of July to mid-August.

2.2.1 Pan traps

Pan trapping is a passive method for collecting bees and non-bee pollinators. Pan traps are a good sampling technique for providing baseline data for unstudied ecosystems (Larsen et al., 2014). In each sub-plot, a pan trap platform was placed approximately in middle. A total of

12 elevated pan trap platforms (six in each plot type) fastened to a metal post, each placed ~1.25 m above the ground to match the height of flowering plant canopy in the pasture. For each pan trap, plastic bowls of blue (color: bright royal blue 105; Festive Occasion, East Providence, RI, USA) and yellow (color: school bus yellow; Touch of Color, Creative Converting, Clintonville, WI, USA) colored with UV light reflectance properties as reported in (Acharya et al., 2021) were placed in a platform made of plywood and white color plastic (as described in (Acharya et al., 2021)). These plastic bowls were placed in the groove of each platform at 7: 00 a.m. Soapy water was prepared by adding a few drops of unscented liquid dishwashing detergent (Dawn Dish Soap; Procter and Gamble, Cincinnati, OH) mixed with a gallon of tap water. Each bowl was filled at the two-thirds level with soapy water left in plots for 48 hours. Then, trapped insects were collected from each trap, and stored in ethanol for further processing.

2.2.2 Vane traps

A total of 12 elevated blue colored vane traps (SpringStar Inc., Woodinville, WA) were used as a measure of passive sampling of visiting insects. Vane trap consisted of two plastic vanes (10×15 cm) fitted perpendicularly to the lid connected to a 950 ml transparent plastic collecting jar. These traps were suspended from the metal stake 1 m above the ground and were placed in the middle of each split plot. Bees and other pollinators were attracted to the blue color and flew into vanes and dropped into collection jar. About 200 ml of soapy water (Dawn Dish Soap; Procter and Gamble, Cincinnati, OH) was used in each vane trap as drowning medium. These traps were deployed for 48 hours duration. All other procedures related to sample collection were same as pan trap sampling.

2.2.3 Sample processing

Samples were kept in vials containing 70% ethyl alcohol, transported to the lab, air dried, sorted, pinned and later identified up to the species level using dichotomous keys (Mitchell, 1962; Vockeroth, 1992; Michener et al., 1994; C. Michener, 2007; Miranda et al., 2013), and online taxonomic resources such as Discover Life (www.discoverlife.org) and Bug Guide (bugguide.net) by Drs. D. Biddinger (Department of Entomology, Penn State Fruit Research and Extension Center, Biglerville, PA, USA), and R. Jean (Senior Entomologist, Environmental Solution & Innovations, Inc., Indianapolis, IN, USA).

2.3 Sampling for Plant Species Composition

Plant species composition (botanical composition) was conducted every week starting from in June and July to capture the number and percentage of vegetation cover and floral resources during the sampling period following method previously described by (Vogel and Masters, 2001). For this wire frame used were obtained from wire fence panels and contained 36, $15 \times 15\text{-cm}^2$ squares. Number of cells containing one or more seeded plants were counted. Sampling was done both in east and west side of each plot and both grazed and non-grazed areas. Counts were converted into frequency of occurrence and percentage (dividing number of cells that contained a seeded plant by 100). Non-seeded species were counted as undesirable.

2.4 Statistical Analysis

Bees and non-bee insect pollinators were sampled from six plots with grazing and non-grazing treatments over two years. There were seven weekly sampling dates per year (during June and July) using both blue vane traps and paired yellow and blue pan traps on platforms. Bee and non-bee insect communities were compared between treatments based on abundance, richness, diversity, and assemblage patterns.

To test the effects of grazing on bee and non-bee insect abundance, a paired samples t-test was conducted in SPSS v27 (IBM Corp., 2020). Means comparisons were conducted on bee and non-bee abundance data from blue vane traps, pan traps, and both trap types combined. Following a Shapiro-Wilk's normality test, abundance data were $\log(x+1)$ transformed to address right skewness in the data.

We compared species richness for bees and non-bees using rarefaction curves. Here, we compared the rates of species accumulation between treatments (by trap type) by developing rarefaction curves in EstimatesS v7.5 (Colwell, 2005). For each trap type, we created a species-by-sample matrix of bee abundances for each grazing treatment. An iterative process was then used to resample from each data matrix 100 times to generate a statistical expectation of species accumulation. Significance was determined by non-overlapping 95% confidence intervals in our pairwise comparisons of treatments within trap type.

To examine treatment effects on overall diversity and evenness of bees and other insects, Shannon Diversity Index (H') and Pielou's Index of Evenness (J) were calculated for each plot within each treatment (grazed vs non-grazed), respectively. Mean diversity index values were compared between treatments using a paired samples t-test in SPSS v27 (IBM Corp., 2020).

A constrained ordination approach (redundancy analysis; RDA) was used to compare bee and non-bee insect assemblage patterns between grazed and non-grazed treatments. Due to a large number of low abundance species, data were aggregated at the genus-level for bees and family level for non-bee insects. All genera or families that represented greater than 1% of the total abundance were included in the analysis. Data were square-root transformed prior to analysis and species data were centered for visualization purposes. Significance of grazing treatment was determined through Monte Carlo permutations ($n=499$), which were restricted

within blocks (i.e., plots). Analyses were conducted in Canoco v4.5 and biplots were generated in CanoDraw.

3. Results

3.1 Bee communities

Abundance: We collected a total 1570 bees during the study, including 678 bees in grazed plots and 892 bees in non-grazed plots. *Lasioglossum imitatum* (21.3%), *Melissodes communis* (8.7%), *Bombus pensylvanicus* (13.0%), *Ceratina strenua* (8.2%), and *Augochlorella aurata* (6.0%) were the most common bee species (Table 1 and 2). A total of 59 different bee species were found during the study, with 50 species collected from non-grazed plots and 46 species collected from grazed plots (Table 3). Bee abundance (i.e., capture rates) was significantly higher in the non-grazed treatment when collected by blue vane traps ($t_{1,83}=2.35$, $p=0.021$) and by both trap types combined ($t_{1,83}=2.51$, $p=0.014$) (Figure 3A). No difference in bee abundance was found between treatments when comparing bee collected in pan traps ($t_{1,83}=1.66$, $p=0.101$) only (Figure 3 A).

For non-bee species, a total of 2,266 specimens were collected, including 958 in grazed plots and 1308 in non-grazed plots. A minimum of 93 total species (not all specimens identified to species level) collected over both years, with a minimum of 59 different species found in grazed plots and a minimum of 77 species in non-grazed plots (Table 4). The major non-bee arthropods collected were from two families of flies (Dolichopodidae and Milichiidae) and three families of beetles (Buprestidae, Meloidae, and Mordellidae), *Acmaeodera pulchella* (Coleoptera: Buprestidae), *Condyllostylus caudatus* (Diptera: Dolichopodidae), *Epicauta atrata* (Coleoptera: Meloidae), *Tabanus quinquevittatus* (Diptera: Tabanidae), and *Myzinum quinquecinctum* (Hymenoptera: Thynnidae) were the most prevalent non-bee species. Non-bee

abundance was significantly higher in the non-grazed treatment when collected by blue vane traps ($t_{1,83}=4.14$, $p<0.001$), pan traps ($t_{1,83}=2.922$, $p=0.004$), and by both trap types combined ($t_{1,83}=4.227$, $p<0.001$) (Figure 3 B).

Richness: A total 59 bee species were collected in this study, with 46 bee species found in the grazed treatment and 50 bee species in the non-grazed treatment. To compare bee and non-bee richness among treatments, rates of species accumulation for blue vane traps and pan traps (Fig. 4A and B) respectively were compared between treatments. Species accumulation did not differ between treatments based on non-overlapping 95% confidence intervals. However, for bees, the trajectories of the curves differed in a consistent fashion for both trap types (i.e., grazing had lower trajectory for both), suggesting differences in species evenness between the two treatments. When comparing the sampling methods, sample-based rarefaction curves revealed higher species accumulation rates in blue vane traps as compared to the paired yellow and blue pan traps.

3.2 Diversity and Evenness

Over the course of the study, Shannon-Wiener Diversity Index of bee communities in non-graze pastures were higher than those in graze pastures (Figure 5; $t_{1,10}=3.419$, $p=0.007$). Similarly, communities of bee collected from non-grazed plots were significantly more even than bees found in grazed pastures based on Pielou's Index of Evenness (Figure 6; $t_{1,10}=2.626$, $p=0.025$).

Likewise, non-bee communities collected in non-grazed plots were significantly higher than those in graze pastures (Figure 5; $t_{1,10}=4.27$, $p=0.0020$) and non-bee communities collected from non-grazed plots were significantly more even than bees found in grazed plots on Pielou's Index of Evenness (Figure 6; $t_{1,10}=4.02$, $p=0.0030$).

Community Assemblages: A constrained ordination revealed that bee assemblages varied between the grazed and non-grazed treatments ($F=2.16$, $p=0.044$). The position of each bee genera in relation treatment factors across the horizontal axis of the biplot represents associations between bee genera and treatment. The axis has an eigenvalue of 0.026 and explains a relatively low amount of variation in the species data (2.7%). However, the biplot indicates that bee genera such as *Augochlorella*, *Ptilothrix*, *Svastra*, *Xylocopa*, *Ceratina* and *Halictus* may be more commonly found in non-grazed plots relative to grazed plots (Figure 7).

Similarly, a constrained ordination (RDA) revealed that non-bee insect assemblages varied between grazed and non-grazed treatments ($F=3.00$, $p=0.032$). The biplot indicates that non-bee insects genera such as *Chauliognathus*, *Tabanus*, *Lygus*, *Toxomerus*, *Epicauta*, *Acmaeodera* and *Euphoria* were commonly found in non-grazed plots (Figure 8).

3.3 Botanical species composition

3.3.1 Seed mix plot

Percentage of desirable (seeded forage) and undesirable (not seeded) both were between 40 – 60% and varied between plots in seed mix plots. Most notable desirable species and undesirable species were given in Table 4. About 8.6% of the ground was bare (no forage species).

3.3.2 Warm season grass plots

Among the three major grass species all three plots majorly contained weeds (around 60-70%). The percentage of desirable plants in these plots were as low as 20-30% (*Andropogon gerardi*, *Tripsacum dactyloides*, *Sorghastrum nutans*). Details regarding desirable and undesirable plants were given in Table 4.

4. Discussion

Pastures that provide forage resources for sheep and other livestock are also important resources (habitat, food, refuge) for insect pollinators such as bees, butterflies, moths, and beetles (Enri et al., 2017). Pollinators, in return, benefit plants by providing pollination service that is essential for reproduction. Both livestock and pollinators share common resources in the livestock pasture, however grazing by livestock may impact the pollinator community. This study was done to provide baseline data on the community of bees and other insects commonly found in Arkansas livestock pastures, and the impact of sheep grazing on the bee and non-bee arthropod community.

We found a diverse array of insects in these pasture agroecosystems established with either legume/forb seed mix or native warm season grasses. The high-level of taxonomic investment in this study provides valuable baseline data on the species composition of the insect communities within these pasture systems. Our records of 59 bee species, as well as non-insect specimens comprising at least 93 different species from 5 insect orders, may be used to examine changes in the insect community in this region in response to various management tactics and even long-term temporal shifts in abundance, diversity, and community assemblages (e.g., in response to climate change). The bee community was ecologically diverse with the most abundant species representing variation in sociality and nesting habits. Availability of nesting resources, floral distribution, amount of exposed soil and pre-existing cavities in wood or stones plays key role in structuring bee communities (Cane, 1991; Eltz et al., 2002; Potts et al., 2005). In the current study, availability of floral and nesting resources, forest near plot margins, and exposed soil have added for such diversity.

Despite only collecting during the summer months of June and July, our bee diversity records are comparable to those from other pasture systems in different regions (Bhandari et al., 2018; Thapa-Magar et al., 2020). However, we may be missing some spring-active bees that are found in studies that feature full season sampling. For example, in apple orchards, more than 100 bee species have been recorded when sampling from spring through fall (Joshi et al., 2020). The abundance of floral and nesting resources, and thus opportunities for niche partitioning, within pasture systems seems to support a pollinator community that may exceed certain diversity levels in other types of agricultural systems. For example, our values of evenness (i.e., Pielou's Index) were higher than those reported in studies of other crop types, such as apple and canola, featuring species-level identification of bees, e.g., (Halinski et al., 2015; Heller et al., 2019).

Sheep grazing within our pasture systems had an effect on the abundance, diversity, and community assemblage of insects. For most measures, abundance and diversity of bees and other insects were higher in the non-grazing plots relative to the grazed plots, despite the spatial proximity of the two treatments (a split plot design was used) and the dispersal capabilities of the insects. However, differences in bee abundance could not be detected using pan traps alone, suggesting that trap type can influence characterization of bee communities. Indeed, studies have shown significant variation in capture rates and diversity measures of bees when using different colors and types of traps (Joshi et al. 2015; Acharya et al. 2021). In our own study, significantly higher species accumulation rates occurred in blue vane traps as compared to pan traps.

Although we detected differences in abundance and diversity indices between treatments, we could not detect differences in species richness when evaluating species accumulation rates. This could be due to the test of significance (non-overlapping 95% confidence intervals) used,

which is a highly conservative method, making it difficult to detect differences. However, the trajectory of the curves was consistently higher in the non-grazing treatments in accordance with our findings for the other diversity measures. Despite similarity in richness values, community assemblages of insect varied between grazing regimes. For example, certain bee genera (*Augochlorella*, *Ptilothrix*, *Svastra*, *Xylocopa*, *Ceratina* and *Halictus*) tended to be found more frequently in the non-grazed plots in comparison to grazed pastures. Similarly, certain families of non-bee insects, e.g, Meloidae, Cantharidae, Buprestidae were also found more frequently in the non-grazed setting. Other studies have examined the impact of grazing on pollinator diversity, and we found a similar trend in this study (Kruess & Tschardtke, 2002; Xie et al., 2008; Lazaro et al., 2016). In general, grazing decreases foraging resources for pollinators such as floral richness, diversity and abundance in pastures (Mitchell, 2020) leading to fewer pollinators. Such studies have reported that grazing impact wild bees by affecting nesting and foraging habitat (Sjödin, 2007), soil compaction specifically for ground dwelling bees (Murray et al., 2012), competition to foraging resource (Carvell, 2002) and trampling of bees and larvae (Sugden, 1985). Despite the negative association of grazing on pollinator studies, some studies have found contrasting results (Vulliamy et al., 2006) or have found that, when grazing intensity is considered, moderate levels of grazing may actually result in higher levels of pollinators, generally supporting the intermediate disturbance hypothesis concept (Lazaro et al., 2016).

In general, intensely managed livestock pasture provide poor foraging resources for pollinators, therefore, different seed mixes were introduced in the current study to maximize flowering resource for pollinators. For three of the six plots, our seed mixes included both legumes and forbs in order to create suitable infrastructure for pollinators (detail species list of forages in seed mix plots are provided in materials and method section and additionally in the

supplementary file). The other three plots were sown with seed of native grasses (*A. gerardi*, *T. dactyloides*, *S. nutans*). However, survival and establishment rates in both types of pasture, exhibited the difficulty in excluding undesirable species and weeds. The survival rate of most of the forb/legume seed mix was low and only few species (*E. virginicus*, *E. canadensis*, *L. capitate*, *H. grosseserratus*, and *C. grandiflora*) survived well. The survival rate of desirable species (seeds of forage that were sown during plot preparation) was below 60% and in some plots as low as 20%. In plots where, native grass seed was seeded, less than 30% of the desirable species thrived and remaining were either undesirable species or weeds (species list of undesirable species or weeds are listed in the result section). This study provides glimpse of flowering forage species that could possibly thrive in livestock pastures in Southeastern USA.

In the current study, large number of diverse insect species were present in all the pasture plots irrespective of flowering forbs or native grass possibly due to habitat structure and ability to adapt in these types of ecosystems. In consistent with our results (Bhandari et al., 2018) reported presence of large number of ground nesting pollinators in livestock pastures in Texas. Ground nesting bees prefer well-drained, sparsely vegetated patches of bare ground that are favorable for nesting habitat (Warriner & Hutchins, 2016). A diverse group of perennial forages mix provides favorable nesting resources for ground nesting bees. (Tripodi & Szalanski, 2015) reported a large variation in forage species that are preferred by single *Bombus* spp. In pasture plots of this study, *Silphium integrifolium* and *Monarda fistulosa*, which are generally preferred by *Bombus* spp., were also planted, however, survivability of these forages were relatively lower in comparison to other forage species. In non-grazed plots, forages were 1-2 m high (Figure 2), were undisturbed, thus have provided constant pollen source and nesting sites for pollinators, and likely supported the abundant and diverse array of pollinators found in this study.

5. Conclusion

In summary, a diverse community of bee and non-bee insect communities were found in grazing and non-grazing pastures in Arkansas. For bees, *L. imitatum*, *M. communis*, *B. pensylvanicus*, *C. strenua*, and *A. aurata* were the most abundant bee species, whereas non-bee species belonging to families Dolichopodidae, Buprestidae, Meloidae, Milichiidae, and Mordellidae were the most abundant non-bee insects found in this study. Although capture rates of insects varied among trap types, we still found that bee and non-bee insect communities were generally more abundant and diverse in non-grazed pasture plots relative to grazed plots, even when situated in close proximity to one another. Differences in community assemblage patterns of insects may provide insight on how the community structure is influenced by grazing in pasture systems.

6. References

- Acharya, R. S., Leslie, T., Fitting, E., Burke, J., Loftin, K., & Joshi, N. K. (2021). Color of pan trap influences sampling of bees in livestock pasture ecosystem. *Biology*, 10(5), 445.
- Anderson, N., Rao, S., & Derkatch, A. (2010). Native bumble bee diversity, abundance, and pollination in crimson clover and hairy vetch seed production fields in western Oregon. *Seed Production Research*, 8.
- Bhandari, K. B., West, C., Longing, S., Brown, C., Green, P., & Barkowsky, E. (2018). Pollinator abundance in semiarid pastures as affected by forage species. *Crop Science*, 58(6), 2665-2671.
- Black, S. H., Shepherd, M., & Vaughan, M. (2011). Rangeland management for pollinators. *Rangelands*, 33(3), 9-13.
- Cane, J. H. (1991). Soils of ground-nesting bees (Hymenoptera: Apoidea): texture, moisture, cell depth and climate. *Journal of the Kansas Entomological Society*, 406-413.
- Carvell, C. (2002). Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. *Biological conservation*, 103(1), 33-49.
- Chaplin-Kramer, R., Tuxen-Bettman, K., & Kremen, C. (2011). Value of wildland habitat for supplying pollination services to Californian agriculture. *Rangelands*, 33(3), 33-41.
- Colwell, R. (2005). EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5 user's guide and application published. <http://purl.oclc.org/estimates>.
- Debano, S. J. (2006). Effects of livestock grazing on aboveground insect communities in semi-arid grasslands of southeastern Arizona. *Biodiversity & Conservation*, 15(8), 2547.
- Eltz, T., Brühl, C. A., Van der Kaars, S., & Linsenmair, E. K. (2002). Determinants of stingless bee nest density in lowland dipterocarp forests of Sabah, Malaysia. *Oecologia*, 131(1), 27-34.
- Enri, S. R., Probo, M., Farruggia, A., Lanore, L., Blanchetete, A., & Dumont, B. (2017). A biodiversity-friendly rotational grazing system enhancing flower-visiting insect assemblages while maintaining animal and grassland productivity. *Agriculture, Ecosystems & Environment*, 241, 1-10.
- Halinski, R., Dorneles, A. L., & Blochtein, B. (2015). Bee assemblage in habitats associated with *Brassica napus* L. *Revista Brasileira de Entomologia*, 59(3), 222-228.

- Heller, S., Joshi, N. K., Leslie, T., Rajotte, E. G., & Biddinger, D. J. (2019). Diversified Floral Resource Plantings Support Bee Communities after Apple Bloom in Commercial Orchards. *Scientific reports*, 9(1), 1-13.
- Hellerstein, D., Hitaj, C., Smith, D., & Davis, A. (2017). Land use, land cover, and pollinator health: A review and trend analysis.
- Hopfenmüller, S., Holzschuh, A., & Steffan-Dewenter, I. (2020). Effects of grazing intensity, habitat area and connectivity on snail-shell nesting bees. *Biological Conservation*, 242, 108406.
- Johansen, L., Westin, A., Wehn, S., Iuga, A., Ivascu, C. M., Kallioniemi, E., & Lennartsson, T. (2019). Traditional semi-natural grassland management with heterogeneous mowing times enhances flower resources for pollinators in agricultural landscapes. *Global Ecology and Conservation*, 18, e00619.
- Joshi, N. K., Leslie, T., Rajotte, E. G., & Biddinger, D. J. (2020). Environmental impacts of reduced-risk and conventional pesticide programs differ in commercial apple orchards, but similarly influence pollinator community. *Chemosphere*, 240, 124926.
- Kearns, C. A., Inouye, D. W., & Waser, N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual review of ecology and systematics*, 29(1), 83-112.
- Kimoto, C., DeBano, S. J., Thorp, R. W., Taylor, R. V., Schmalz, H., DelCurto, T., Johnson, T., Kennedy, P. L., & Rao, S. (2012). Short-term responses of native bees to livestock and implications for managing ecosystem services in grasslands. *Ecosphere*, 3(10), 1-19.
- Kruess, A., & Tschardtke, T. (2002). Grazing intensity and the diversity of grasshoppers, butterflies, and trap-nesting bees and wasps. *Conservation Biology*, 16(6), 1570-1580.
- Larsen, N., Minor, M., Cruickshank, R., & Robertson, A. (2014). Optimising methods for collecting Hymenoptera, including parasitoids and Halictidae bees, in New Zealand apple orchards. *Journal of Asia-Pacific Entomology*, 17(3), 375-381.
- Lazaro, A., Tscheulin, T., Devalez, J., Nakas, G., & Petanidou, T. (2016). Effects of grazing intensity on pollinator abundance and diversity, and on pollination services. *Ecological entomology*, 41(4), 400-412.
- Ledvina, J., McShea, W. J., Bourg, N. A., Herrmann, V., Akre, T., & Johnson, A. E. (2020). Management Regime and Field Age Affect Species Richness and Cover of Native Forbs and Exotic Species in Virginia Grasslands. *Ecological Restoration*, 38(2), 83-93.
- Michener, C. (2007). The Bees of the World Johns Hopkins University Press. *Baltimore.[Google Scholar]*.

- Michener, C. D., McGinley, R. J., & Danforth, B. N. (1994). *The bee genera of North and Central America (Hymenoptera: Apoidea)*. Smithsonian Institution Press.
- Miranda, G., Young, A., Locke, M., Marshall, S., Skevington, J., & Thompson, F. (2013). Key to the genera of Nearctic Syrphidae. *Canadian Journal of Arthropod Identification*, 23(1), 351.
- Mitchell, S. R. (2020). Impacts of Range Management Decisions on Native Pollinators: Innovative Grazing Practices and Riparian Restoration.
- Mitchell, T. (1962). Bees of the eastern United States, vol. 2 (Technical Bulletin No. 152). *Agricultural Experiment Station, Raleigh, NC*.
- Murray, T. E., Fitzpatrick, U., Byrne, A., Fealy, R., Brown, M. J., & Paxton, R. J. (2012). Local-scale factors structure wild bee communities in protected areas. *Journal of Applied Ecology*, 49(5), 998-1008.
- Orford, K. A., Murray, P. J., Vaughan, I. P., & Memmott, J. (2016). Modest enhancements to conventional grassland diversity improve the provision of pollination services. *Journal of Applied Ecology*, 53(3), 906-915.
- Potts, S., Woodcock, B., Roberts, S., Tscheulin, T., Pilgrim, E., Brown, V., & Tallwin, J. (2009). Enhancing pollinator biodiversity in intensive grasslands. *Journal of Applied Ecology*, 46(2), 369-379.
- Potts, S. G., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Ne'eman, G., & Willmer, P. (2005). Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology*, 30(1), 78-85.
- Robson, D. B. (2019). Impact of Grazing History on Pollinator Communities in Fescue Prairie. *Blue Jay*, 77(1), 10-15.
- Shapira, T., Henkin, Z., Dag, A., & Mandelik, Y. (2020). Rangeland sharing by cattle and bees: moderate grazing does not impair bee communities and resource availability. *Ecological Applications*, 30(3), e02066.
- Sjödin, N. E. (2007). Pollinator behavioural responses to grazing intensity. *Biodiversity and Conservation*, 16(7), 2103-2121.
- Sugden, E. A. (1985). Pollinators of *Astragalus monoensis* Barneby (Fabaceae): new host records; potential impact of sheep grazing. *The Great Basin Naturalist*, 299-312.
- Tadey, M. (2015). Indirect effects of grazing intensity on pollinators and floral visitation. *Ecological Entomology*, 40(4), 451-460.

- Tanis, M. M., Marshall, L., Biesmeijer, J. K., & van Kolfshoten, L. (2020). Grassland management for meadow birds in the Netherlands is unfavourable to pollinators. *Basic and Applied Ecology*, 43, 52-63.
- Thapa-Magar, K. B., Davis, T. S., & Kondratieff, B. (2020). Livestock grazing is associated with seasonal reduction in pollinator biodiversity and functional dispersion but cheatgrass invasion is not: Variation in bee assemblages in a multi-use shortgrass prairie. *PloS one*, 15(12), e0237484.
- Thomas, A. L., Brauer, D. K., Sauer, T. J., Coggeshall, M. V., & Ellersieck, M. R. (2008). Cultivar influences early rootstock and scion survival of grafted black walnut. *Journal of the American Pomological Society*, 62(1), 3.
- Tripodi, A. D., & Szalanski, A. L. (2015). The bumble bees (Hymenoptera: Apidae: Bombus) of Arkansas, fifty years later. *Journal of Melittology*(50), 1-17.
- Vockeroth, J. R. (1992). *The flower flies of the subfamily Syrphinae of Canada, Alaska, and Greenland: Diptera, Syrphidae* (Vol. 1867). Agriculture Canada.
- Vulliamy, B., G. Potts, S., & G. Willmer, P. (2006). The effects of cattle grazing on plant-pollinator communities in a fragmented Mediterranean landscape. *Oikos*, 114(3), 529-543.
- Warriner, M., & Hutchins, B. (2016). Management recommendations for native insect pollinators in Texas. *Texas Parks and Wildlife Department*.
- Xie, Z., Williams, P. H., & Tang, Y. (2008). The effect of grazing on bumblebees in the high rangelands of the eastern Tibetan Plateau of Sichuan. *Journal of Insect Conservation*, 12(6), 695-703.
- Zhang, Z., Wang, L., Liu, J., Dong, Z., Xu, W., & Wang, S. (2019). The effect of simulated sheep grazing on male and female reproductive performance in *Caragana microphylla* Lam.(Leguminosae). *Israel Journal of Ecology and Evolution*, 1(aop), 1-7.
- Öckinger, E., Franzén, M., Rundlöf, M., & Smith, H. G. (2009). Mobility-dependent effects on species richness in fragmented landscapes. *Basic and Applied Ecology*, 10(6), 573-578.
- Öckinger, E., & Smith, H. G. (2007). Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. *Journal of applied ecology*, 44(1), 50-59.

7. Figures and Tables



Figure 1. Grazed and non-grazed plots of the study site separated by wired fence. (Pictures by J. M. Burke).



Figure 2. Pan trap in grazed and non-grazed plots (Pictures by R. S. Acharya).

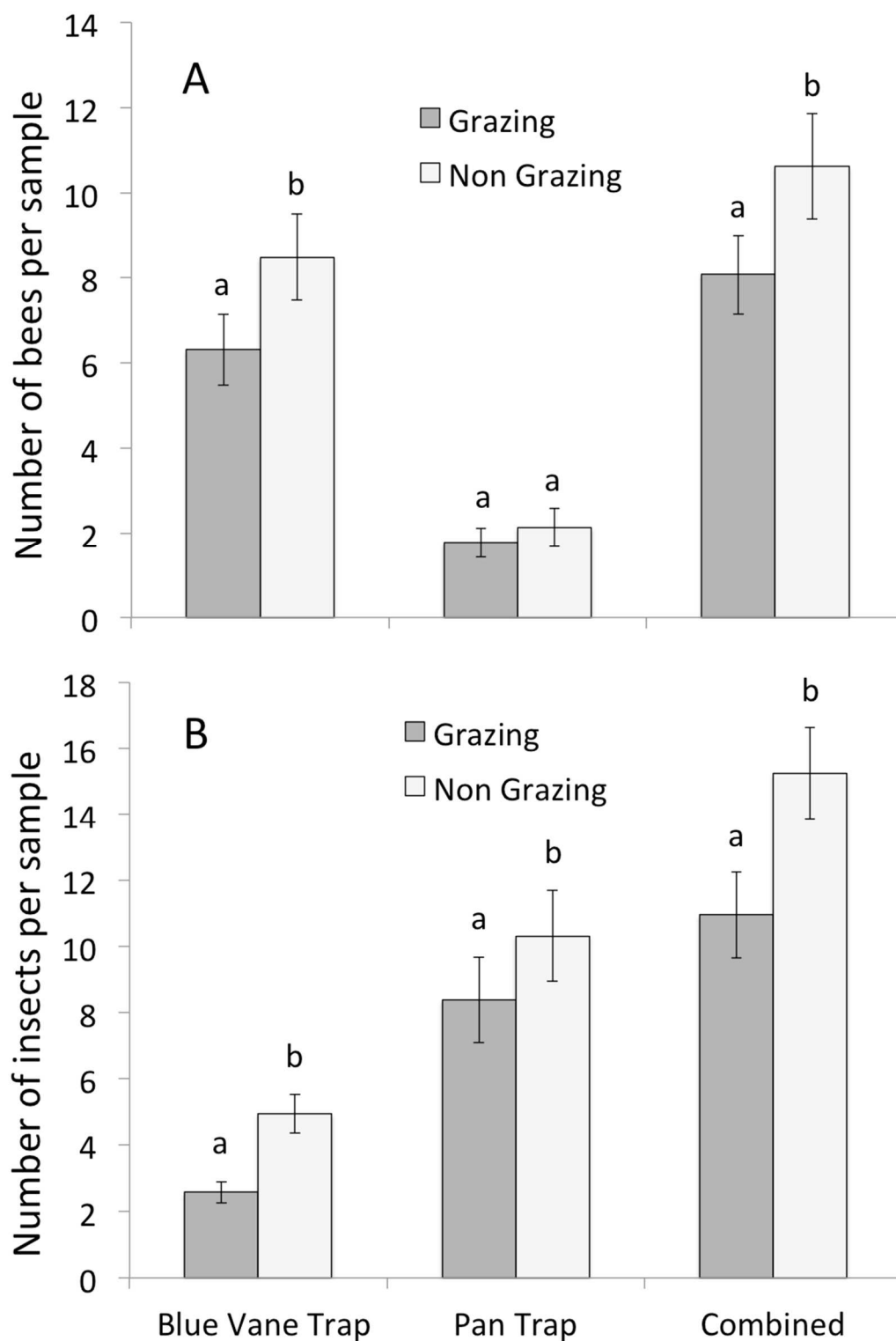


Figure 3. Capture rate of bees (A) and insects (B) among graze and non-grazed treatments for blue vane traps, pan traps and combined traps.

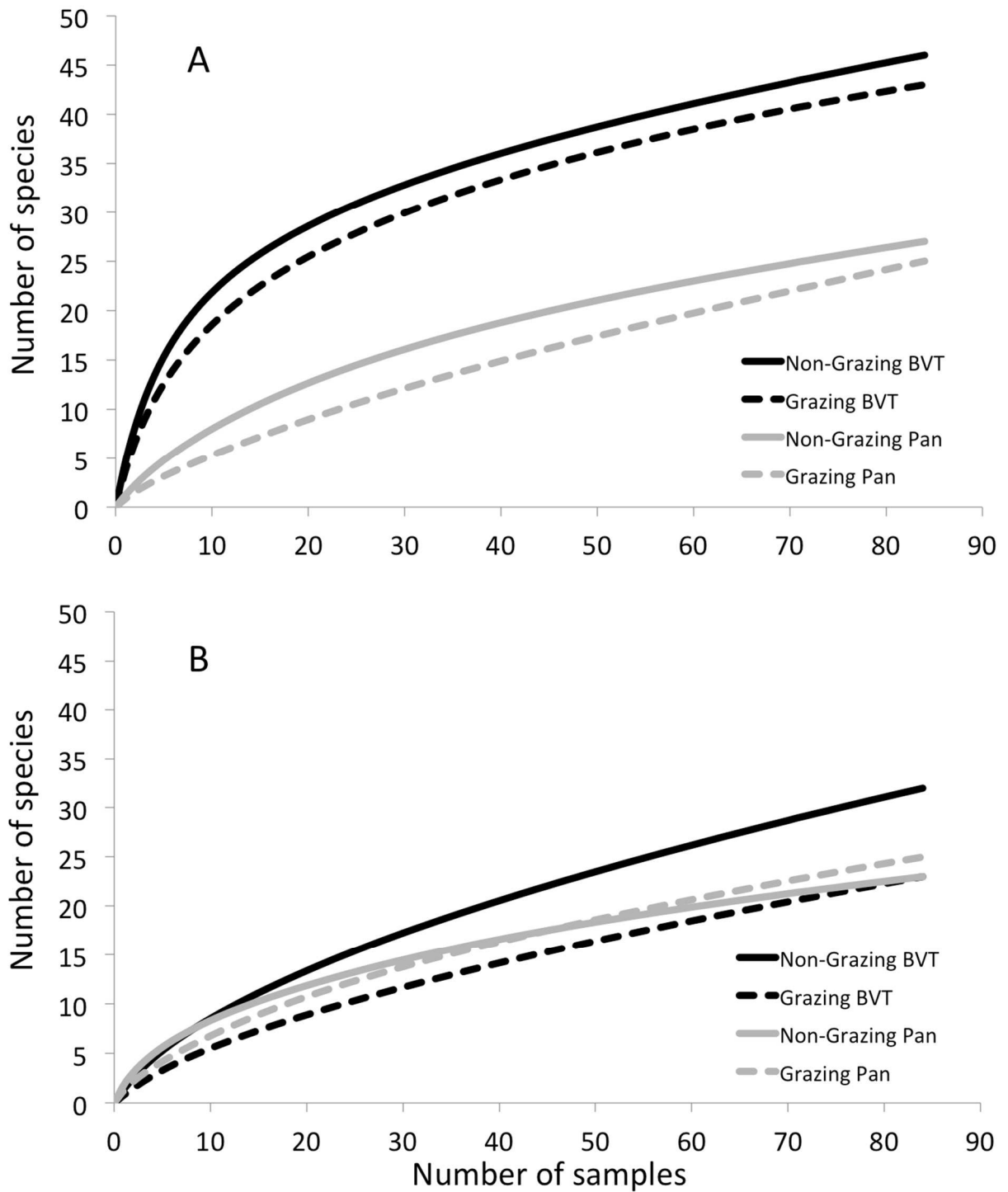


Figure 4: Rarefaction curve for bees (A) and non-bee insects (B) in pan and blue vane trap (BVT) traps from graze and non-graze pastures.

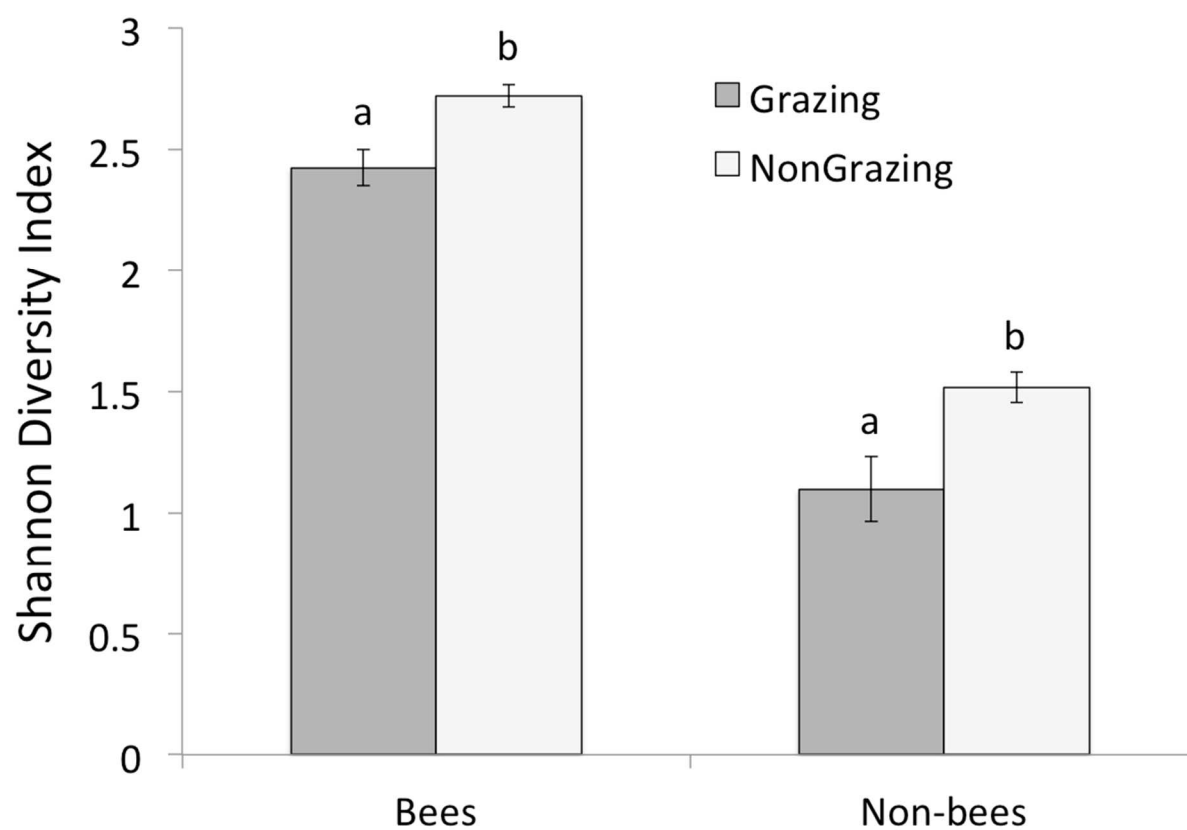


Figure 5: Mean Shannon Index values for bee and non-bee insect communities collected in grazing and non-grazing plots.

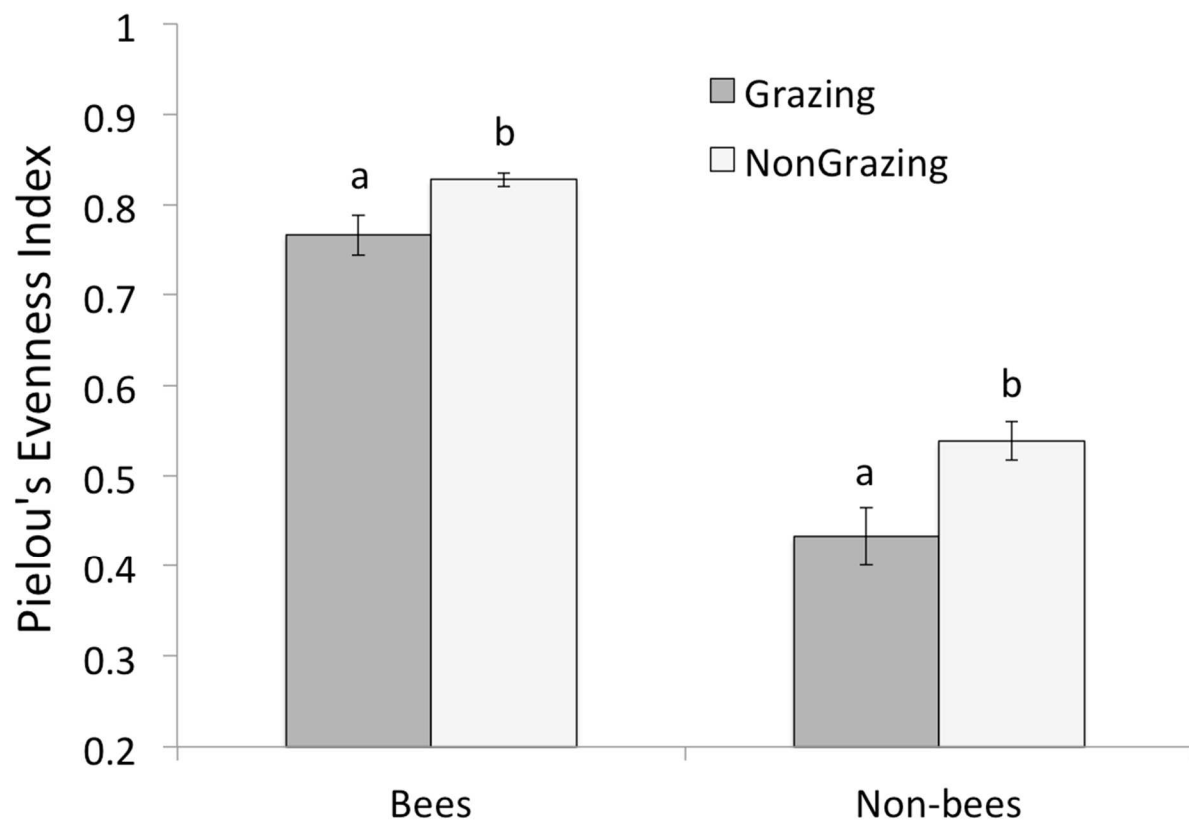


Figure 6. Mean Pielou's Index values for bee and non-bee insect communities collected in grazing and non-grazing plots.

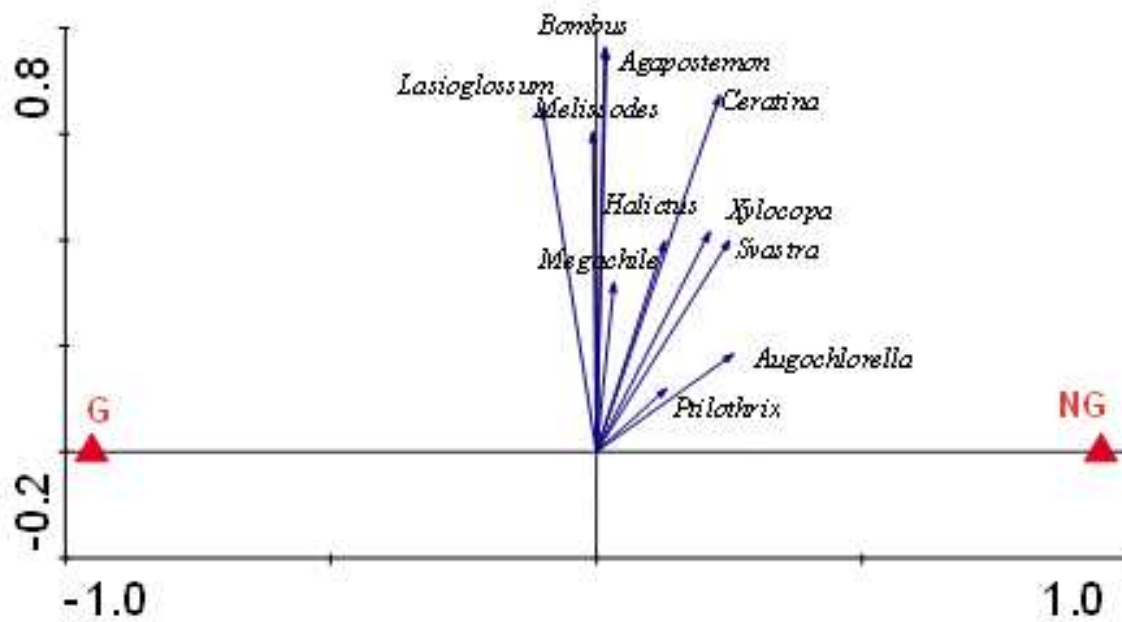


Figure 7: Redundancy analysis (RDA) biplot showing the association of bees and grazing treatments (Graze vs non-graze).

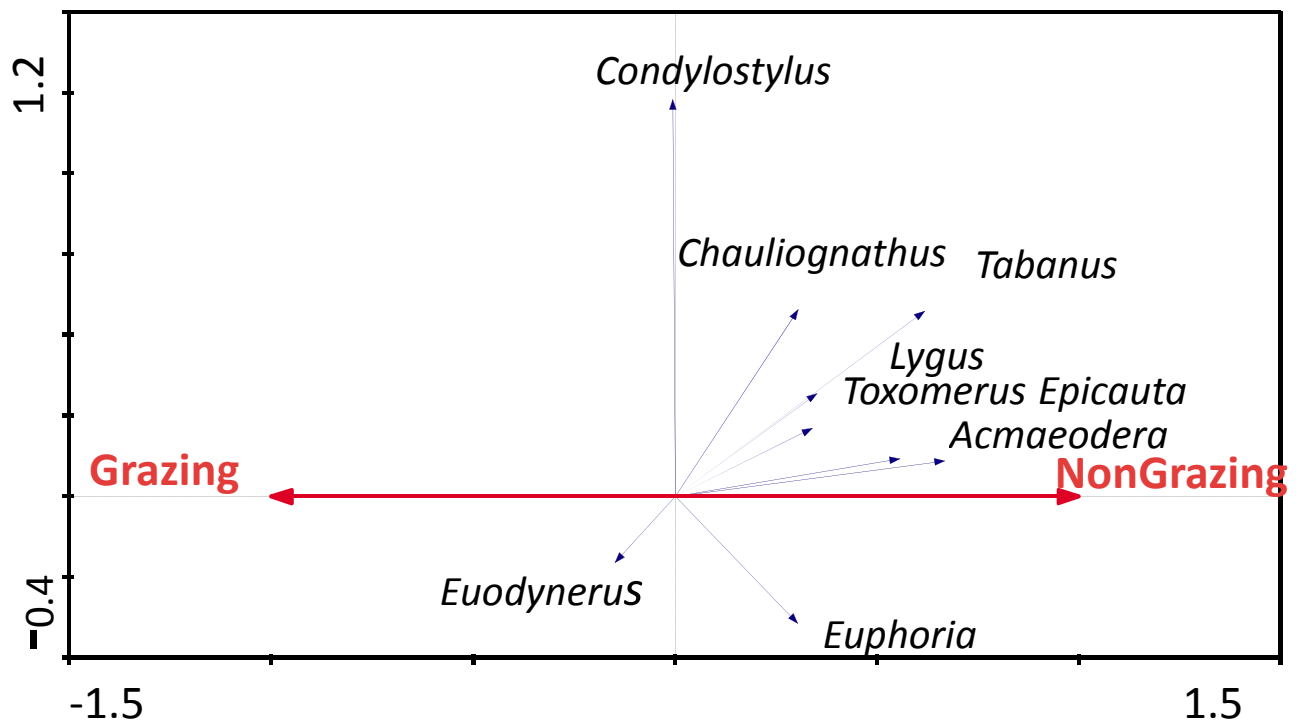


Figure 8: Redundancy analysis (RDA) biplot showing the association of non-bee insects and grazing treatments (Graze vs non-graze).

Table 1: Bee species collected from the study site in Booneville, AR (2018-2019). Total abundance and percent of total abundance are included for each species.

Species name	Author	Total	Percent total
<i>Agapostemon sericeus</i>	(Forster)	46	2.929936
<i>Agapostemon splendens</i>	(Lepeletier)	2	0.127389
<i>Agapostemon texanus</i>	(Cresson)	74	4.713376
<i>Agapostemon virescens</i>	(Fabricius)	13	0.828025
<i>Apis mellifera</i>		4	0.254777
<i>Ashmeadiella buconis</i>	(Say)	2	0.127389
<i>Augochlora pura</i>	(Say)	8	0.509554
<i>Augochlorella aurata</i>	(Smith)	94	5.987261
<i>Augochlorella persimilis</i>		1	0.063694
<i>Augochloropsis sumptuosa</i>		2	0.127389
<i>Bombus auricomis</i>	(Robertson)	5	0.318471
<i>Bombus bimaculatus</i>	(Lepeletier)	2	0.127389
<i>Bombus fervidus</i>	(F.)	2	0.127389
<i>Bombus griseocolis</i>	(DeGeer)	6	0.382166
<i>Bombus impatiens</i>	(Cresson)	3	0.191083
<i>Bombus pensylvanicus</i>	(DeGeer)	204	12.99363
<i>Ceratina dupla</i>	(Say)	31	1.974522
<i>Ceratina strenua</i>	(Smith)	128	8.152866
<i>Diadasia enavata</i>	(Cresson)	2	0.127389
<i>Halictus ligatus</i>	(Say)	39	2.484076
<i>Halictus parallelus</i>		28	1.783439
<i>Halictus rubicundus</i>	(Christ)	12	0.764331
<i>Hylaeus modestus</i>	(Say)	1	0.063694
<i>Lasioglossum cinctipes</i>	(Provancher)	1	0.063694
<i>Lasioglossum coreopsis</i>	(Robertson)	3	0.191083
<i>Lasioglossum cressonii</i>		1	0.063694
<i>Lasioglossum disparile</i>	(Cresson)	17	1.082803
<i>Lasioglossum hitchensi</i>	(Gibbs)	1	0.063694
<i>Lasioglossum imitatum</i>	(Smith)	335	21.33758
<i>Lasioglossum pectorale</i>	(Smith)	13	0.828025
<i>Lasioglossum reticulatum</i>	(Robertson)	1	0.063694
<i>Lasioglossum sp.</i>		1	0.063694
<i>Lasioglossum tegulare</i>	(Robertson)	7	0.44586
<i>Lasioglossum trigeminum</i>	(Gibbs)	1	0.063694
<i>Lasioglossum versatum</i>	(Robertson)	57	3.630573
<i>Megachile brevis</i>	(Say)	1	0.063694
<i>Megachile inimica</i>	(Cresson)	2	0.127389

Table 1 (*cont.*)

Species name	Author	Total	Percent total
<i>Megachile mendica</i>	(Cresson)	4	0.254777
<i>Megachile montivaga</i>	(Cresson)	4	0.254777
<i>Megachile petulans</i>	(Cresson)	2	0.127389
<i>Megachile policularis</i>	(Say)	1	0.063694
<i>Megachile sculpteralis</i>		1	0.063694
<i>Meliossodes niveus</i>	(Robertson)	3	0.191083
<i>Melissodes bimaculata</i>	(Lepeletier)	8	0.509554
<i>Melissodes communis</i>	(Cresson)	136	8.66242
<i>Melissodes comptoides</i>	(Robertson)	35	2.229299
<i>Melissodes denticulatus</i>	(Smith)	6	0.382166
<i>Melissodes druriellus</i>	(Kirby)	1	0.063694
<i>Melissodes veroninae</i>	(Robertson)	21	1.33758
<i>Melissodes trinodis</i>		1	0.063694
<i>Nomia nortoni</i>		2	0.127389
<i>Peponapis pruinosa</i>	(Say)	2	0.127389
<i>Perdita octomaculata</i>	(Say)	1	0.063694
<i>Ptilothrix bombiformis</i>	(Cresson)	28	1.783439
<i>Svastra atripes</i>	(Cresson)	10	0.636943
<i>Svastra obliqua</i>	(Say)	67	4.267516
<i>Trachusa dorsalis</i>		1	0.063694
<i>Xenoglossa strenua</i>	(Cresson)	13	0.828025
<i>Xylocopa virginica</i>	(L.)	73	4.649682
Grand Total		1570	100%

Table 2: Family and species of bees found in grazed (G) and non-grazed (NG) pasture plots and their relative abundance. Y refers to the species presence in that treatment.

Family	Genus	Species	Author's name	Grazed	G % Total	Non-grazed	NG % Total
Andrenidae	<i>Perdita</i>	<i>octomaculata</i>	(Say)	Y	0.0636		
Apidae	<i>Bombus</i>	<i>pensylvanicus</i>	(DeGeer)	Y	5.7961	Y	7.1337
Apidae	<i>Xenoglossa</i>	<i>strenua</i>	(Cresson)	Y	0.3821	Y	0.4458
Apidae	<i>Melissodes</i>	<i>comptoides</i>	(Robertson)	Y	1.0191	Y	1.2101
Apidae	<i>Melissodes</i>	<i>veroninae</i>	(Robertson)	Y	0.7643	Y	0.5732
Apidae	<i>Meliossodes</i>	<i>niveus</i>	(Robertson)	Y	0.0637	Y	0.1273
Apidae	<i>Melissodes</i>	<i>communis</i>	(Cresson)	Y	4.3949	Y	4.2675
Apidae	<i>Ceratina</i>	<i>strenua</i>	(Smith)	Y	3.5668	Y	4.5859
Apidae	<i>Diadasia</i>	<i>enavata</i>	(Cresson)	Y	0.0636	Y	0.0637
Apidae	<i>Xylocopa</i>	<i>virginica</i>	(L.)	Y	1.1465	Y	3.5031
Apidae	<i>Bombus</i>	<i>griseocolis</i>	(DeGeer)	Y	0.2547	Y	0.1274
Apidae	<i>Svastra</i>	<i>obliqua</i>	(Say)	Y	1.3375	Y	2.9299
Apidae	<i>Ptilothrix</i>	<i>bombiformis</i>	(Cresson)	Y	0.5732	Y	1.2101
Apidae	<i>Apis</i>	<i>mellifera</i>		Y	0.1910	Y	0.0637
Apidae	<i>Peponapis</i>	<i>pruinosa</i>	(Say)	Y	0.0637	Y	0.0637
Apidae	<i>Melissodes</i>	<i>denticulatus</i>	(Smith)	Y	0.1274	Y	0.2547
Apidae	<i>Bombus</i>	<i>bimaculatus</i>	(Cresson)	Y	0.0637	Y	0.0637
Apidae	<i>Melissodes</i>	<i>bimaculata</i>	(Lepeletier)	Y	0.2548	Y	0.2547
Apidae	<i>Svastra</i>	<i>atripes</i>	(Cresson)	Y	0.1274	Y	0.5096
Apidae	<i>Ceratina</i>	<i>dupla</i>	(Say)	Y	0.1274	Y	1.8471
Apidae	<i>Bombus</i>	<i>auricomis</i>	(Robertson)	Y	0.0637	Y	0.2547
Apidae	<i>Bombus</i>	<i>impatiens</i>	(Cresson)	Y	0.1274	Y	0.1274
Apidae	<i>Bombus</i>	<i>fervidus</i>	(F.)	Y	0.1274		
Apidae	<i>Melissodes</i>	<i>druriellus</i>	(Kirby)			Y	0.0637
Apidae	<i>Melissodes</i>	<i>trinodis</i>				Y	0.0637
Colletidae	<i>Hylaeus</i>	<i>modestus</i>	(Say)	Y	0.0637		
Halictidae	<i>Agapostemon</i>	<i>texanus</i>	(Cresson)	Y	2.1019	Y	2.6114
Halictidae	<i>Augochlorella</i>	<i>aurata</i>	(Smith)	Y	1.5287	Y	4.4586
Halictidae	<i>Lasioglossum</i>	<i>imitatum</i>	(Smith)	Y	12.038	Y	9.2993
Halictidae	<i>Lasioglossum</i>	<i>versatum</i>	(Robertson)	Y	1.5287	Y	2.1019
Halictidae	<i>Agapostemon</i>	<i>sericeus</i>	(Forster)	Y	1.1465	Y	1.7834
Halictidae	<i>Halictus</i>	<i>parallelus</i>		Y	0.7006	Y	1.0828
Halictidae	<i>Lasioglossum</i>	<i>coreopsis</i>	(Robertson)	Y	0.1274	Y	0.0637
Halictidae	<i>Halictus</i>	<i>ligatus</i>	(Say)	Y	0.8280	Y	1.6560
Halictidae	<i>Agapostemon</i>	<i>virescens</i>	(Fabricius)	Y	0.4458	Y	0.3821

Table 2 (*cont.*)

Family	Genus	species	Author's name	Grazed	G % Total	Non- grazed	NG % Total
Halictidae	<i>Lasioglossum</i>	<i>disparile</i>	(Cresson)	Y	0.4458	Y	0.6369
Halictidae	<i>Lasioglossum</i>	<i>reticulatum</i>	(Roberston)	Y	0.0637		
Halictidae	<i>Lasioglossum</i>	<i>hitchensi</i>	(Gibbs)	Y	0.0637		
Halictidae	<i>Augochloropsis</i>	<i>sumptuosa</i>		Y	0.1273		
Halictidae	<i>Augochlora</i>	<i>pura</i>	(Say)	Y	0.1910	Y	0.3184
Halictidae	<i>Augochlorella</i>	<i>persimilis</i>		Y	0.0637		
Halictidae	<i>Lasioglossum</i>	<i>tegulare</i>	(Robertson)	Y	0.2547	Y	0.1910
Halictidae	<i>Lasioglossum</i>	<i>pectorale</i>	(Smith)	Y	0.2547	Y	0.5732
Halictidae	<i>Agapostemon</i>	<i>splendens</i>	(Lepeletier)	Y	0.0637	Y	0.0637
Halictidae	<i>Lasioglossum</i>	<i>cinctipes</i>	(Provancher)			Y	0.0637
Halictidae	<i>Halictus</i>	<i>rubicundus</i>	(Christ)			Y	0.7643
Halictidae	<i>Lasioglossum</i>	<i>trigeminum</i>	(Gibbs)			Y	0.0637
Halictidae	<i>Lasioglossum</i>	<i>cressonii</i>				Y	0.0637
Halictidae	<i>Nomia</i>	<i>nortoni</i>				Y	0.1274
Megachilidae	<i>Megachile</i>	<i>montivaga</i>	(Cresson)	Y	0.2547		
Megachilidae	<i>Megachile</i>	<i>inimica</i>	(Cresson)	Y	0.0637	Y	0.0637
Megachilidae	<i>Trachusa</i>	<i>dorsalis</i>		Y	0.0637		
Megachilidae	<i>Megachile</i>	<i>petulans</i>	(Cresson)	Y	0.0637	Y	0.0637
Megachilidae	<i>Megachile</i>	<i>brevis</i>	(Say)			Y	0.0637
Megachilidae	<i>Megachile</i>	<i>sculpteralis</i>				Y	0.0637
Megachilidae	<i>Ashmeadiella</i>	<i>bucconis</i>	(Say)			Y	0.1274
Megachilidae	<i>Megachile</i>	<i>mendica</i>	(Cresson)			Y	0.2547
Megachilidae	<i>Megachile</i>	<i>policaris</i>	(Say)			Y	0.0637
Halictidae	<i>Lasioglossum</i>	<i>sp</i>				Y	0.0637

Table 3: Species of non-bee insects found in grazed and non-grazed pasture plots, during 2018-2019. Y refers to the species presence in that treatment.

Family	Genus	Species	Author's name	Grazed	Non -Grazed
Asilidae	<i>Lophria</i>	<i>species</i>		Y	Y
Asilidae	<i>Efferia</i>	<i>species</i>		Y	Y
Bibionidae					Y
Buprestidae	<i>Acmaeodera</i>	<i>pulchella</i>	(Herbst)	Y	Y
Buprestidae	<i>Acamaeoderus</i>	<i>tubulus</i>	(F.)	Y	
Cantharidae	<i>Chauliognathus</i>	<i>Marginatus.</i>		Y	Y
Cantharidae	<i>Rhagonycha</i>	<i>imbecillis</i>	(Leconte)		Y
Cerambycidae	<i>Typocerus</i>	<i>velutinus</i>	(Oliver)		Y
Cerambycidae	<i>Dectes</i>	<i>sayi</i>	(Dillon and Dillon)		Y
Chrysidae	<i>Chrysis</i>	<i>severa</i>	(Mocsary)	Y	
Chrysomelidae	<i>Colapsis</i>	<i>brunnea</i>	(F)	Y	Y
Chrysomelidae	<i>Gratiana</i>	<i>pallidulla</i>	(Boheman)	Y	
Chrysomelidae	<i>Cryptocephalus</i>	<i>calidus</i>	(suffrian)	Y	
Cicadellidae				Y	Y
Cleridae				Y	Y
Coccinellidae	<i>Harmonia</i>	<i>axyridis</i>	(Pallas)		Y
Coreidae					Y
Crabronidae	<i>Tachytes</i>	<i>Sp.</i>		Y	Y
Curculionidae				Y	Y
Cydnidae				Y	Y
Dolichopodidae	<i>Condyllostylus</i>	<i>caudatus</i>	Wiedemann	Y	Y
Dolichopodidae	<i>Condyllostylus</i>	<i>sp</i>	(F.)		Y
Dolichopodidae	<i>Condyllostylus</i>	<i>longicornis</i>	Fabricius	Y	Y
Dolichopodidae	<i>Achradocera</i>	<i>barbata</i>	Loew	Y	Y
Elateridae				Y	Y
Empididae				Y	
Ephydriidae				Y	Y
Formicidae				Y	Y
Hesperiidae				Y	Y
Ichneumonidae	<i>Ophion</i>	<i>Sp.</i>		Y	Y
Meloidae	<i>Epicauta</i>	<i>atrata(F)</i>		Y	Y
Meloidae	<i>Epiphoria</i>	<i>sepulcralis(F.)</i>			Y
Meloidae	<i>Pyrota</i>	<i>perversa</i>			Y
Micro. Lepidoptera				Y	Y

Table 3 (cont..)

Family	Genus	Species	Author's name	Grazed	Non-Grazed
Milichiidae				Y	Y
Miridae	<i>Lygus</i>	<i>lineolaris</i>		Y	Y
Misc Heteroptera				Y	Y
Misc. Carabidae				Y	Y
Misc. Chrysomellidae				Y	Y
Misc. Scarabaeidae				Y	Y
Misc. Diptera				Y	Y
Mordellidae	<i>Hoshihananom</i>	<i>Octopunctata</i> (F.)		Y	Y
Muscidae	<i>Haematobia</i>	<i>Irritans</i> (L.)		Y	Y
Mutillidae	<i>Dasymutilla</i>	<i>vesta</i>			Y
Mutillidae	<i>Dasymutilla</i>	<i>quadrigutta</i>		Y	
Noctuidae				Y	Y
Nymphalidae	<i>Euptoieta</i>	<i>claudia</i>			Y
Nymphalidae	<i>Anaea</i>	<i>andria</i>			Y
Parasitic wasp					Y
Pentatomidae				Y	Y
Phoridae				Y	
Pieridae	<i>Colias</i>	<i>eurytheme</i>		Y	
Pipunculidae				Y	Y
Platypezidae				Y	Y
Pompilidae					Y
Pyralidae				Y	Y
Reduviidae					Y
Scarabaeidae	<i>Cotinis</i>	<i>nitida</i> (L.)			Y
Scarabaeidae	<i>Epiphoria</i>	<i>sepulcralis</i> (F.)			Y
Scoliidae	<i>Pyodasis</i>	<i>quadramaculata</i>			Y
Sespidae				Y	Y
Sphecidae	<i>Ammophila</i>	<i>spp.</i>		Y	
Sphingidae	<i>Ceratomia</i>	<i>undulosa</i>			Y
Staphylinidae				Y	
Stratiomyidae	<i>Hermetia</i>	<i>illucens</i> (L.)		Y	
Syrphidae	<i>Eristalis</i>	<i>stripator</i>		Y	
Syrphidae	<i>Toxomerus</i>	<i>geminatus</i>		Y	
Syrphidae	<i>Toxomerus</i>	<i>marginatus</i>		Y	Y

Table 3 (*cont.*)

Family	Genus	Species	Author's name	Grazed	Non-Grazed
Syrphidae	<i>Orthonevra</i>	<i>nitida</i>		Y	
Tabanidae	<i>Tabanus</i>	<i>quinquevittatus</i>	(Wiedemann)	Y	Y
Thynidae	<i>Myzium</i>	<i>obscurum</i>	(F.)	Y	Y
Thynidae	<i>Myzinum</i>	<i>quinquecinctum</i>	(Lepeletier)	Y	Y
Tiphiidae				Y	Y
Vespidae	<i>Eudynerus</i>	<i>pratensis</i>	(de Saussure)	Y	Y
Vespidae	<i>Euodynerus</i>	<i>crypticus</i>	(Say)	Y	Y
Vespidae	<i>Polistes</i>	<i>carolina</i>		Y	Y
Vespidae	<i>Parancistrocerus</i>	<i>Pedestris</i>	(de Saussure)	Y	
Vespidae	<i>Polistes</i>	<i>dorsalis</i>		Y	
Calliphoridae					Y
Carabidae	<i>Clivina</i>	<i>dentipes</i>	(Dejean)		Y
Cecidomyiidae					Y
Chloropidae					Y
Dolichopodidae	<i>Chrysotus</i>	<i>subcostatus</i>	Loew		Y
Mycetophilidae					Y
Pscoptera					Y
Scarabaeidae	<i>Trichiotinus</i>	<i>piger</i> (F)			Y
Scarabaeidae	<i>Trigonopeltastes</i>	<i>delta</i>	(Forster)		Y
Sciaridae					Y
Syrphidae	<i>Allograpta</i>	<i>exotica</i>			Y
Tabanidae	<i>Chrysops</i>	<i>sp.</i>			Y
Tabanidae	<i>Tabanus</i>	<i>lineola</i> (F.)			Y
Vespidae	<i>Parancistrocerus</i>	<i>salcularis</i>	(de Saussure)		Y
Vespidae	<i>Polistes</i>	<i>carolina</i>			Y

Table 4. Botanical species composition in different plots

Native forb legume and grass plots

Percentage of desirable plants (included in seed mix)

Elymus virginicus (22%)
Elymus canadensis (16%)
Lespedeza capitate (11%)
Helianthus grosseserratus (11%)
Coreopsis grandiflora (10%)
Rudbeckia hirta (8.6%)
Chamaecrista fasciculata (6.6%)
Parthenium integrifolium (< 1%)
Achillea millefolium (< 1%)
Chamaecrista fasciculata (< 1%)
Callirhoe involucrata (< 1%)
Penstemon digitalis (< 1%)
Echinacea pallida (< 1%)
Rudbeckia subtomentosa (< 1%)

Most dominant undesirable species include (not included in seed mix)

Ambrosia spp. (27.77%)
Lolium spp. (13.8%)
Rumex crispus (11.1%)
Rumex obtusifolius (5.5%)
Papaver somniferum (5.5%)
Bromus tectorum (< 1%)
Poa pratensis (< 1%)
Carex hirta (< 1%)
Lolium spp.. (< 1%)
Callirhoe spp. (< 1%)
Ranunculus eschscholtzii (< 1%)
Polygonum alpinum (< 1%)
Festuca arundinacea (< 1%)
Vigna unguiculata (< 1%)
Ambrosia artemisiifolia (< 1%)
Papaver somniferum (< 1%)
Trifolium campestre (< 1%)

Warm Season Grass (WSG) plots

Desirable species

Andropogon gerardi, *Tripsacum dactyloides*, *Sorghastrum nutans* (20-30%)

Most dominant undesirable species include (not included in seed mix (60-70%))

Polygonum aviculare

Cyperus rotundus

Solanum carolinense

Xanthium strumarium

Ambrosia psilostachya

Croton capitatus

Cyanodon dactylon

Festuca pratensis

Hordeum murinum

Balsamorhiza sagittata

Rumex crispus

Trifolium spp.

Note: Individual species percentage not included.

CHAPTER FIVE

Impact of Organic and Non-Organic Pasture Management on Bee Communities

Abstract

Pollinators are crucial for successful reproduction of most flowering plants but due to intensive agricultural practices and multiple interactive stressors their numbers are declining. Very little is known about the benefits of organic pasture management practices on insect pollinators, specifically bees. Therefore, the aim of study was to assess how pasture management practices affect bee abundance and diversity in organic and non-organic pastures. Organic and non-organic pasture plots were established by seeding legume, grass and forbs to provide foraging resource for bees and other pollinators. About 40-60% of seeded forage mix survived in non-organic plot whereas in organic plots establishment and persistence were low. Vane traps were used for sampling of bees during summer of 2017 and 2018. Mean number of bees per sampling date were numerically higher (not significantly) in non-organic than organic pasture. Species richness of bees (as measured by rarefaction curve) was similar between pastures under organic or non-organic management practices. Similarly, there was no difference in bee diversity between these pastures. Low floral resources in organic pasture could have caused lower bee abundance and species richness in organic pasture compared to non-organic pasture. Further studies are needed to minimize weeds to improve establishment and persistence of native flowering species to benefit pollinators in an organic pasture ecosystem.

Keywords: bee, blue vane trap, organic and non-organic pasture, pollinators

1. Introduction

Agricultural practices such as application of agrochemicals, mainly pesticides negatively impact pollinator communities in and around farm landscape. Different species of bees are important pollinators, and chronic exposure to cocktail of toxic agrochemicals causes stress to bees and impair immune response (Goulson et al., 2015). Bee populations may decrease sharply if such immunocompromised bees are also exposed to parasites and pathogens (Goulson et al., 2015). Effect of lethal and sub-lethal doses of pesticides on pollinators vary with level of exposure, duration of exposure, and route (ingestion, contact, and inhalation; (Sponsler et al., 2019). Agricultural pesticides, such as organophosphates, carbamates, phenylpyrazoles, neonicotinoids, and pyrethroids contain neurotoxic compounds that negatively impacts nervous system leading to imbalance in coordination, paralysis, and death of pollinators (Sponsler et al., 2019). Pesticides in sub-lethal doses cause impairment of foraging behavior in bees and decreases chances of returning to the nest (Roulston & Goodell, 2011). Sub-lethal doses may also slow larval development and hinder learning (Roulston & Goodell, 2011). Exposure to systemic neonicotinoid insecticide reduces the activity of bumblebee workforce and reduce colony expansion (Feltham et al., 2015). Worker bees exposed to imidacloprid insecticide carry 30% less pollen compared with those that were not exposed (Feltham et al., 2015). Additionally, neonicotinoids negatively affect activities of queen bees (Goulson, 2013).

Many insecticides and fungicides are very toxic to honeybees and bumblebees (Sanchez-Bayo & Goka, 2014). Mason bees exposed to fungicides, such as captan and benomyl, can eliminate in orchards completely vanished within a few days after application (Ladurner et al., 2005). The pesticide, bis(dithiocarbamate), when applied to a sunflower field, decreased pollinator abundance leading to reduce weight of fruit, oil content in seed, and negatively

impacted seed production (de Oliveira et al., 2019). Application of pesticides also decreases offspring production and survival of female solitary bee (*Osmia lignaria*; (Williams & Kremen, 2007). Survival and health of juvenile honeybees is also negatively impacted by the application of pesticides and thus creates stress in the colony (Williams & Kremen, 2007). In the long run, pesticides, affect gene expression of detoxifying enzyme in honeybees (Tome et al., 2020). Moreover, physiology of the queen honeybee and worker behavior are affected when the bees wax is exposed to the fungicide chlorothalonil and the insecticide chlorpyrifos during development. Exposure to some pesticides may reduce egg laying rate of adult queen and the size of worker bees (Walsh et al., 2020).

Farm management practices may influence abundance and diversity of bees and other pollinators. Increases in floral resources, improved in management of grasslands, restoration of semi-natural habitats, increases in nesting habitats, minimization of pesticides uses, and effective monitoring methods help in maintaining bee populations (Goulson et al., 2015). Crop visiting bees in arable farmland can be increased by adopting organic farming practices, planting wildflowers, and establishing grass margin strips (Kleijn et al., 2015). Organic practices support greater species richness for many types of pollinators compared with non-organic farming practices (Gabriel & Tscharntke, 2007; Pérez-Méndez et al., 2020). Pollination deficits (pollination potential - actual pollination) were less in organic compared with non-organic fields. Similarly, abundance of bees was higher in organic compared with non-organic field (Morandin & Winston, 2005). Additional landscape features or contexts (patch shape, interpatch, and habitat aggregation) near organic fields may better supports wild bee populations in agroecosystems (Kennedy et al., 2013). For example, incorporation of semi-natural vegetation in organic management increases frequency of bee visits (Klein et al., 2012). Native bees are important

because honeybees are vulnerable to agricultural pesticides and may not survive for long in all environments and may not pollinate as efficiently (Kremen et al., 2002). In organic farms near natural habitats, native bees provide effective pollination service to crops that require more frequent pollination (Kremen et al., 2002). Pollination may occur at a greater rate in organic farms; however, such effects can be seen only after 2-4 years after transition to organic farm (Anderson et al., 2010; Andersson et al., 2012). Additionally, such farm practices may increase overall farm biodiversity in the long term. For instance, the number of butterflies increased by 100%, 25 years after transition to organic management (Jonason et al., 2011).

Pollinator dependent benefits of organic farming depends on surrounding landscape, type of crop grown, type of flowers in crop field, size of farm, and its ability to provide refugia from pesticides (Brittain et al., 2010). Organic management with addition of semi-natural habitat or woody elements nearby benefits cavity-nesting bees by providing nesting and floral resources (Uzman et al., 2020). Native bees maintained in organic farms close to the pollinator habitat can provide better pollination service compared to native bees maintained in non-organic farms (Kremen et al., 2002). Natural habitat and availability of pollen have greater impact on survivability and reproduction of a female native solitary bee (*Osmia lignaria*; Williams & Kremen, 2007). Organic management provides refugia to more diverse pollinator species than non-organic farming and thus even slight decreases in yield is likely to be compensated by long-term ecosystem benefits (Smith et al., 2020).

Organic management practices reduce negative environmental impacts of intensive agriculture; however, little is known about the benefit to biodiversity and yield under organic grassland management system across meadows, pastures, and mown pastures. Unlike agricultural croplands, permanent grassland responds slower or perhaps weaker under organic

management compared to non-organic pastures in terms of net return or yield (Klaus et al., 2013). Pastures are managed in different ways and those practices may influence overall biodiversity of bees and other pollinators in these systems. Landowners often follow conventional pasture management practices in their pasturelands. However, there is very limited information available on the impact of organic and non-organic (i.e., conventional) pasture management on bees and other insects in livestock pasture ecosystem. Therefore, this study aimed to evaluate wild bee abundance and diversity in organic and non-organic pasture ecosystem.

2. Materials and Methods

2.1 Site Description

This study was conducted during summer of 2017 and 2018 at the USDA-ARS Dale Bumpers Small Farms Research Center in Booneville, Arkansas (35.09 °N, 93.95 °W). The soil at field site is characterized as Leadvale silt loam (fine-silty, siliceous, semiactive, thermic Typic Fragiudults) with water movement and plant rooting limited by a fragipan at a depth of 0.15 to 1.0 m (<https://websoilsurvey.sc.egov.usda.gov>). Details regarding the soil types of this region are available in (Thomas et al., 2008). Pasture plots with two different management practices, i.e., organic, and non-organic, were established and consistently managed since 2007. Organic pasture plots were selected within a thirty-two hectare of pasture utilized for sheep. It was managed organically since 2007 and was certified as organic pasture in 2012 (Nature's International Certification Services, Viroqua, WI). Forage type was predominantly tall fescue. Non-organic sheep pasture at same location utilized by sheep for more than 30 years was predominantly bermudagrass. Both forage types have limitations for sheep production and provide limited pollinator habitat. Plots were located with organic and non-organic pastures to be

as similar as possible initially with a combination of tall fescue and bermudagrass to be used to establish native forb and grass pollinator plots. Before plots were planted, sheep grazed at approximately ~ 10 sheep ha^{-1} according to forage availability which was seasonal and dependent on forage species present (*Festuca arundinacea*, *Cynodon dactylon*, *Lolium perenne* and other voluntary forbs and grasses). Total annual rainfall during 2017 and 2018 was 944.0 and 1213.8 mm respectively with highest during May (145.5 mm; 2017) and lowest during February (78.2 mm; 2018). Average annual temperature during same time was 17.6 and 18.1 °C respectively with highest during July (24 °C) and lowest during January (4 °C).

2.2 Field preparation

2.2.1 Pasture plot preparation for conventional non-organic forage plots

Three, 0.16-ha plots were used for the non-organic treatment in this study. For weed management, these plots were sprayed with Roundup® (41% glyphosate, 0.764 L ha^{-1}) in June, July, September, and October 2016 and January 2017, and with Outrider (75% Sulfosulfuron; Monsanto, St. Louis, MO; 0.096 l ha^{-1}) in September 2016 using a Continental Belton cluster nozzle sprayer (Continental Belton McAlester, SR: A44117, Oklahoma City, OK). The field was tilled (Maschio Gaspardo North America Inc., SC 300, Des Moines, IA), and rolled using 12' Big Guy Roller (Grahl Manufacturing, St. Louis, MO) in October 2016. Soil fertility was adjusted in past using synthetic inorganic fertilizers in accordance with the University of Arkansas soil test recommendation (<https://www.uaex.edu/publications/PDF/FSA-2153.pdf>) but was not required during the trial.

Plots were grazed by sheep to provide forage to the animals, reduce weeds (mixed non-native grass species) that were palatable to sheep, and maintain native grasses in vegetative state. By early July, there were estimated to be at least three different native flowering species per plot,

even after being grazed by sheep. The most common flowering species visually documented during the sampling period were *Verbena hastata*, *Pycnanthemum verticillatum*, *Echinacea pallida*, *Coreopsis* spp., *Monarda fistulosa*, *Aster novae-angliae*, and *Verbena* spp.

2.2.2 Organic pasture plot preparation

For organic pasture management treatment, three plots (0.4 ha each) were selected within the certified organic pasture. These plots were controlled burned using forestry drip torch after establishing a firebreak around the plots for initial weed management and field preparation. Cover crop of oats (*Avena sativa*) was planted the winter before spring planting native seed mixes.

Seeds were planted into a prepared seed bed using a Brillion planter (Brillion Farm Equipment, Sure Stand Model SSP-8, Brillion, WI) at a 1.25 cm depth. Historically, soil fertility was adjusted in these plots using poultry litter (4.4 Mg ha⁻¹; fresh weight basis) in accordance with the University of Arkansas soil test recommendation (<https://www.uaex.edu/publications/PDF/FSA-2153.pdf>) but was not required in 2017 and 2018 due to low grazing pressure and desire to establish native forage.

The seed mixes used in this study were Buck's Hangout (Hamilton Native outpost, Elk creek, MO; www.hamiltonnativeoutpost.com/ 14.5 kg ha⁻¹), Tallgrass Inexpensive or Tallgrass Exposed Clay subsoil mix (Prairie Moon, Winona, MN; www.prairiemoon.com/ 13.44 kg ha⁻¹ and 26.8 kg ha⁻¹), and "Butterfly and Hummingbird" Mix from Hamilton Native outpost (Elk Creek, MO; www.hamiltonnativeoutpost.com/; at 8.5 kg ha⁻¹; species composition and percentage of each species are available in the above website and additionally in the supplementary file).

2.3 Sheep grazing

The plots were dual purpose for pollinator benefit and sheep forage, and grazing was cognizant to achieve both objectives. Plots were grazed if they became weedy (with undesirable plant species such as *Chenopodium album* and *Ambrosia artemisiifolia*), or if forage height was over 1 m. Grazing of the non-organic plots occurred to remove excess forage and weeds, but not to detriment of the native plant species. Stocking rate of each plot was different based on visual assessment of forage availability which included plant height, density of unwanted and desired plants. And grazing of organic plots was avoided during summer months because of the fragility of the native species in balance to the forage base and the desire to allow native bees to nest.

2.4 Bee Sampling

In 2017, blue and yellow colored vane traps were used for passive sampling. Description of vane traps is given in the previous chapter. Four vane traps (2 blue and 2 yellow) were deployed in each plot. Traps were placed at 25 and 50 m from the border of each plot. Distance between rows was 13.5 m, and the border distance was 7-8 m. Traps were suspended from a post about 1.5 m above the ground. Traps were deployed continuously during the entire research period (July-August 2017) and samples were collected every week for a month. Ethyl glycol and soapy water were maintained in traps to collect bees and other insects for 7 days. Ethyl glycol diluted in tap water (50:50) was used as a drowning and preservative medium in collection jars of vane traps. Samples collected from vane traps were placed in plastic vials containing ethyl alcohol until they could be sorted, pinned and labeled.

In 2018, two blue vane traps were placed in each plot. Blue vane traps were placed at the center of plot. Traps were placed at 50 m from the border of the plot. Distance between two traps was 13.5 m. Traps were placed in plots and samples were collected every week, 48 hours after

filling with soapy water. The plot sites, location and number were same as in 2017 for both farming practices (organic and non-organic).

In the lab, bees were dried, pinned, and labelled for identification of bee taxa. Bees were identified to the species level using dichotomous keys (Mitchell, 1960; Michener et al., 1994; Michener, 2000) and online taxonomic resource, such as Discover Life (www.discoverlife.org) and Bug Guide (bugguide.net) by Drs. D. Biddinger (Department of Entomology, Penn State Fruit Research and Extension Center, Biglerville, PA, USA), and R. Jean (Senior Entomologist, Environmental Solution & Innovations, Inc., Indianapolis, IN, USA).

2.5 Statistical analysis

There were two treatments (organic and conventional non-organic) and three replication per treatment (3 study plots per treatment) and the study was conducted for two years. There were four weekly sampling events in 2017 and five in 2018. Bee communities were analyzed and compared between treatments based on their abundance, richness, and diversity.

Bee abundance was compared between treatments using a two-sample independent t-test in R software program (R version 3.6.2). The number of bees were summed for each plot within each treatment across both years separately), and mean abundance per weekly sampling date was calculated. Before analysis Shapiro Wilks and Levene's tests were conducted to check normality and constant variance assumption in datasets and data were found to be normally distributed.

Bee richness between organic and conventional non-organic plots was compared by using rarefaction curves. In particular, for each year, we compared the rates of species accumulation between these two differently managed pastures by developing rarefaction curves in EstimateS v7.5 (Colwell, 2005). Species-by-sample matrix of bee abundances for each treatment was created with trap type for each year analyzed separately. Rarefaction then used an

iterative process to resample from each data matrix 100 times to generate a statistical expectation of species accumulation. Significance was determined by non-overlapping 95% confidence intervals.

The Shannon Diversity Index (H'), which accounts for both species richness and evenness, was calculated for each plot within each pasture treatment (organic vs non-organic) for both years. Mean diversity index values were compared between treatments and within years using two sample t-test to examine the effect of conventional non-organic and organic pasture management practices on bee diversity. In addition, species evenness was measured using Pielou's Index of Evenness and index values were compared between treatments by an independent sample t- test.

3. Results

3.1 Bee Species and Abundance in Organic and Non-Organic Pastures

In total, 1783 (1090 in non-organic & 693 in organic) and 360 (205 in non-organic & 155 in organic) bees were collected in 2017 and 2018, respectively. The most common species in 2017 were *Lasioglossum imitatum*, *Augochlorella aurata*, and *Lasioglossum disparile* (Table 1 & 2). However, in 2018, *Bombus pensylvanicus*, *Lasioglossum imitatum*, and *Augochlorella aurata* were the most pre-dominant species found during sampling period. Out of six bumblebee species, five were found in organic pastures and four were found in non-organic pastures. *Bombus auricomis* was only observed in non-organic pastures whereas *Bombus griseocollis* and *Bombus perplexus* were only found in organic pastures. Similarly, *Apis mellifera* was documented in organic but not in non-organic pastures.

There was no significance difference between mean number of bees collected per sampling event for non-organic (73.75 bees) and organic pasture (44.83 bees) ($t = 1.55$, $P = 0.227$) (Figure

2 a) in 2017. Likewise, there was no significance difference between mean number of bees collected per sampling event between non-organic (13.67 bees) and organic (10.33bees) ($t = 0.85737$, $P = 0.4396$) (Figure 2 b) in 2018.

Rarefaction curves to assess the bee species richness and the rates of species accumulation between organic and non-organic pasture within each year were compared. From sample-based rarefaction curve no significant difference was found between two treatments (Figure 3a and b).

3.2 Diversity Index and Evenness

Over the course of this study, there were no significant differences between bee communities in organic and in non-organic pastures in both years (Figure 4 A) ($t = 0.34895$, $P = 0.7459$). Likewise, for evenness there were no differences between organic pasture and non-organic pasture (Figure 4 B) ($t = 0.891$, $P = 0.457$).

3.3 Species composition of forage species in plots

Plots varied in number of desirable species (seeded forage), ranging from 40-60%. Most notable species were *Elymus virginicus* (22%), *Elymus canadensis* (16%), *Lespedeza cuneata* (11%), *Helianthus grosseserratus* (11%), *Coreopsis grandiflora* (10%), *Rudbeckia hirta* (8.6%), and *Chamaecrista fasciculata* (6.6%). Other species documented were, *Parthenium integrifolium*, *Achillea millefolium*, *Callirhoe involucrata*, *Penstemon digitalis*, *Echinacea pallida*, and *Rudbeckia subtomentosa*. Species list was not prepared for organic plots but in 2017 only three flowering species were documented (in 1 out of 3 plots) and only covered less than 40% but eventually invasive plants outcompeted these plants during study period. Remaining two plots and all plots in 2018 had nominal number of desirable forage species.

4. Discussion

Over 90% of flowering plants in natural and semi-natural habitats relay on pollinators (mainly different species of bees) for pollination (Corbet et al., 1991; Buchmann & Nabhan, 1996). However, in last decades pollinator populations have been decreased due to agricultural intensification (Allen-Wardell et al., 1998) and other related interactive factors. Various ecosystems, for example grasslands provide main habitat for pollinators by providing food and nesting sites (Osborne et al., 1991). Agronomic practices, such as organic management can enhance pollinator diversity due to restrictions in use of synthetic pesticides and mineral fertilizers (Hole et al., 2005). Effects of organic management on pollinators in agricultural crops have been documented in previous studies (Brittain et al., 2010), but little is known about the impact of such management practices in livestock pastures. In this context this study examined impacts of organic and non-organic pasture management on abundance and diversity of bees. The findings of this study reveal no significant differences in bee abundance and diversity between pasture management practices.

In this study, pastures under non-organic management did not receive any insecticide applications. However, herbicides were applied multiple times in these pasture plots. Negative impact of herbicides on pollinators has been documented in recent studies (Tome et al., 2020; Uzman et al., 2020; Walsh et al., 2020). While exposure to herbicides may not cause bee mortality on short term, chronic exposure of these chemicals may cause stress, nervous system impairment, reproduction failure, and death of bees and other pollinators (Goulson, 2013; Goulson et al., 2015; Sponsler et al., 2019). However, those studies were mostly conducted in the laboratory environment where bees were orally exposed to herbicides rather than field realistic contact exposure in the field, and there was no evidence of a direct toxic effect of herbicides on

pollinators in a field environment (Cullen et al., 2019). Herbicides (for example sulfosulfuron) easily breakdown to less toxic metabolites after few weeks of spray leaving no toxic effect in soil (Singh & Kulshrestha, 2007). Similarly, the harmful effect of glyphosate disappears after three months of application in the soil (Roy et al., 1989). Sampling in this current study started after plants were established and it was after months of herbicide application in conventional non-organic plots. For instance, in 2017 sampling was started seven months after the last application of these herbicides and after more than a year in 2018. Thus, in this study, negative effect of these herbicides in non-organic plots was less likely. On other hand, organic pastures support more pollinator in comparison to non-organic only if flowering diversity is higher (Power et al., 2012; Power et al., 2016). This is in line with our findings that floral abundance in non-organic pastures was higher and thus mean raw values of bee richness and diversity was seen than organic plots however differences were not significant.

Intensely managed grassland in general provides poor foraging resources for pollinators therefore, forage mixes were included in both treatments (organic vs non-organic) to maximize the number flowering species that provide foraging sources for pollinators. These forage mixes contained legumes, grasses and forbs to create a suitable infrastructure to enhance pollinators. From the assessment of botanical composition in non-organic plots, we found that desirable species (species included in the seed mix) varied between plots ranging from 40-60% (detailed in the result section). Surprisingly, only few species such as *E. virginicus*, *E. canadensis*, *L. capitata*, *H. grosseserratus*, and *C. grandiflora* established and dominated in the pasture whereas other species were either few or did not establish at all. In organic plots, only one plot (out of 3) had substantial number of flowering plants survive (up to 40% of the total area) and persisted in 2017 but eventually other invasive plants outcompeted the desirable native plants (Figure 1). In

the other two organic plots in 2017, and all organic plots in 2018, were nominal in numbers of flowering plants. Most of the seeds in organic plots did not establish. Briefly, plots were burned, plant cover (oats) were planted, and burned again. It may be because seed bed of previously established forage (*Festuca arundinacea*) and weeds was too great for native species to compete. Also, several factors such as inadequate moisture, presence of greater diversity of wildlife (birds, rodents) eating seeds had hampered establishment of forages in the organic plots. Additionally, without the use of herbicides to control the non-native grasses and weeds before planting, the seed mix in organic plots did not establish well. Fewer flowering plants in organic than non-organic pasture plots might have resulted in less availability of foraging sources for bees leading less abundance and diversity of bees in organic pastures.

The number of bee species documented in the current study in livestock pasture ecosystem is less than those reported from nearby regions (Stephenson et al., 2018; Little, 2013). Different ecosystems, use of different sampling methods, and duration of collection/sampling period could have impacted such difference between studies. Bees that are more dominant during the early season may not be available during later part and vice versa (Thapa-Magar et al., 2020). The current study was conducted during July-August, which is the latter half of the bee season with a high probability of missing earlier emerging seasonal bees (Bhandari et al., 2018; Thapa-Magar et al., 2020). In 2017, *Lasioglossum imitatum* was the most prevalent bee species both in organic (60.78%) and non-organic (44.63%) while in 2018 *Bombus pensylvanicus* was the most dominant in both treatment plots (41.93% in organic and 23.90% in non-organic). Other most prevalent species were *Augochlorella aurata*, *Ptilothrix bombiformis*, and *Lasioglossum disparile* differing in their relative percentage in different pasture treatments and year. Surprisingly, the two most dominant species (*L. imitatum* and *B. pensylvanicus*) in the current

study were not documented previously in Arkansas (Stephenson et al., 2018). However, (Little, 2013) documented *L. imitatum* as one of the dominant species whereas *B. pennsylvanicus* was not found in that study.

5. Conclusion

This study showed that the species diversity and abundance measures such as the mean number of bees per sampling event, species richness of bees (as measured by rarefaction curve), and bee diversity were similar between organic or non-organic pasture management. Low floral resources in organic pastures could have masked the beneficial effect of organic management on bee species abundance and diversity. Further studies should focus on finding effective methods to control weeds for optimal establishment of native flowering species to obtain pollinator benefits in an organic pasture ecosystem.

6. Reference

- Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P. A., Dalton, V., Feinsinger, P., & Ingram, M. (1998). The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation biology*, 8-17.
- Anderson, N., Rao, S., & Derkatch, A. (2010). Native bumble bee diversity, abundance, and pollination in crimson clover and hairy vetch seed production fields in western Oregon. *Seed Production Research*, 8.
- Andersson, G. K., Rundlöf, M., & Smith, H. G. (2012). Organic farming improves pollination success in strawberries. *PloS one*, 7(2), e31599.
- Bhandari, K. B., West, C., Longing, S., Brown, C., Green, P., & Barkowsky, E. (2018). Pollinator abundance in semiarid pastures as affected by forage species. *Crop Science*, 58(6), 2665-2671.
- Brittain, C., Bommarco, R., Vighi, M., Settele, J., & Potts, S. G. (2010). Organic farming in isolated landscapes does not benefit flower-visiting insects and pollination. *Biological Conservation*, 143(8), 1860-1867.
- Buchmann, S., & Nabhan, G. (1996). The forgotten pollinators. Washington DC/Covelo. In: Shearwater Books/California Island Press. Edward M. Spevak.
- Colwell, R. (2005). EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5 user's guide and application published. <http://purl.oclc.org/estimates>.
- Corbet, S. A., Williams, I. H., & Osborne, J. L. (1991). Bees and the pollination of crops and wild flowers in the European Community. *Bee world*, 72(2), 47-59.
- Cullen, M. G., Thompson, L. J., Carolan, J. C., Stout, J. C., & Stanley, D. A. (2019). Fungicides, herbicides and bees: A systematic review of existing research and methods. *PloS one*, 14(12), e0225743.
- de Oliveira, A. C., Junqueira, C. N., & Augusto, S. C. (2019). Pesticides affect pollinator abundance and productivity of sunflower (*Helianthus annuus* L.). *Journal of Apicultural Research*, 58(1), 2-8.
- Feltham, H., Park, K., Minderman, J., & Goulson, D. (2015). Experimental evidence that wildflower strips increase pollinator visits to crops. *Ecology and evolution*, 5(16), 3523-3530.
- Gabriel, D., & Tschardtke, T. (2007). Insect pollinated plants benefit from organic farming. *Agriculture, Ecosystems & Environment*, 118(1-4), 43-48.

- Goulson, D. (2013). Neonicotinoids and bees: What's all the buzz? *Significance*, 10(3), 6-11.
- Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347(6229).
- Hole, D. G., Perkins, A., Wilson, J., Alexander, I., Grice, P., & Evans, A. D. (2005). Does organic farming benefit biodiversity? *Biological conservation*, 122(1), 113-130.
- Jonason, D., Andersson, G. K., Öckinger, E., Rundlöf, M., Smith, H. G., & Bengtsson, J. (2011). Assessing the effect of the time since transition to organic farming on plants and butterflies. *Journal of Applied Ecology*, 48(3), 543-550.
- Kennedy, C. M., Lonsdorf, E., Neel, M. C., Williams, N. M., Ricketts, T. H., Winfree, R., Bommarco, R., Brittain, C., Burley, A. L., & Cariveau, D. (2013). A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecology letters*, 16(5), 584-599.
- Klaus, V. H., Kleinebecker, T., Prati, D., Gossner, M. M., Alt, F., Boch, S., Gockel, S., Hemp, A., Lange, M., & Müller, J. (2013). Does organic grassland farming benefit plant and arthropod diversity at the expense of yield and soil fertility? *Agriculture, ecosystems & environment*, 177, 1-9.
- Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L. G., Henry, M., Isaacs, R., Klein, A.-M., Kremen, C., M'gonigle, L. K., & Rader, R. (2015). Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nature communications*, 6(1), 1-9.
- Klein, A. M., Brittain, C., Hendrix, S. D., Thorp, R., Williams, N., & Kremen, C. (2012). Wild pollination services to California almond rely on semi-natural habitat. *Journal of Applied Ecology*, 49(3), 723-732.
- Kremen, C., Williams, N. M., & Thorp, R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences*, 99(26), 16812-16816.
- Ladurner, E., Bosch, J., Kemp, W. P., & Maini, S. (2005). Assessing delayed and acute toxicity of five formulated fungicides to *Osmia lignaria* Say and *Apis mellifera*. *Apidologie*, 36(3), 449-460.
- Little, C. Z. (2013). *Bee communities in the Arkansas River Valley*. University of Central Arkansas.
- Morandin, L. A., & Winston, M. L. (2005). Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecological applications*, 15(3), 871-881.

- Osborne, J. L., Williams, I. H., & Corbet, S. A. (1991). Bees, pollination and habitat change in the European community. *Bee world*, 72(3), 99-116.
- Power, E. F., Jackson, Z., & Stout, J. C. (2016). Organic farming and landscape factors affect abundance and richness of hoverflies (Diptera, Syrphidae) in grasslands. *Insect Conservation and Diversity*, 9(3), 244-253.
- Power, E. F., Kelly, D. L., & Stout, J. C. (2012). Organic farming and landscape structure: effects on insect-pollinated plant diversity in intensively managed grasslands. *PLoS One*, 7(5), e38073.
- Pérez-Méndez, N., Andersson, G. K., Requier, F., Hipólito, J., Aizen, M. A., Morales, C. L., García, N., Gennari, G. P., & Garibaldi, L. A. (2020). The economic cost of losing native pollinator species for orchard production. *Journal of Applied Ecology*, 57(3), 599-608.
- Roulston, T. a. H., & Goodell, K. (2011). The role of resources and risks in regulating wild bee populations. *Annual review of entomology*, 56, 293-312.
- Roy, D. N., Konar, S. K., Banerjee, S., Charles, D. A., Thompson, D. G., & Prasad, R. (1989). Persistence, movement, and degradation of glyphosate in selected Canadian boreal forest soils. *Journal of Agricultural and Food Chemistry*, 37(2), 437-440.
- Sanchez-Bayo, F., & Goka, K. (2014). Pesticide residues and bees—a risk assessment. *PloS one*, 9(4), e94482.
- Singh, S. B., & Kulshrestha, G. (2007). Determination of sulfosulfuron residues in soil under wheat crop by a novel and cost-effective method and evaluation of its carryover effect. *Journal of Environmental Science and Health Part B*, 42(1), 27-31.
- Smith, O. M., Cohen, A. L., Reganold, J. P., Jones, M. S., Orpet, R. J., Taylor, J. M., Thurman, J. H., Cornell, K. A., Olsson, R. L., & Ge, Y. (2020). Landscape context affects the sustainability of organic farming systems. *Proceedings of the National Academy of Sciences*, 117(6), 2870-2878.
- Sponsler, D. B., Grozinger, C. M., Hitaj, C., Rundlöf, M., Botías, C., Code, A., Lonsdorf, E. V., Melathopoulos, A. P., Smith, D. J., & Suryanarayanan, S. (2019). Pesticides and pollinators: A socioecological synthesis. *Science of the Total Environment*, 662, 1012-1027.
- Stephenson, P. L., Griswold, T. L., Arduser, M. S., Dowling, A. P., & Krementz, D. G. (2018). Checklist of bees (Hymenoptera: Apoidea) from managed emergent wetlands in the lower Mississippi Alluvial Valley of Arkansas. *Biodiversity data journal*(6).
- Thapa-Magar, K. B., Davis, T. S., & Kondratieff, B. (2020). Livestock grazing is associated with seasonal reduction in pollinator biodiversity and functional dispersion but cheatgrass

- invasion is not: Variation in bee assemblages in a multi-use shortgrass prairie. *PloS one*, 15(12), e0237484.
- Thomas, A. L., Brauer, D. K., Sauer, T. J., Coggeshall, M. V., & Ellersieck, M. R. (2008). Cultivar influences early rootstock and scion survival of grafted black walnut. *Journal of the American Pomological Society*, 62(1), 3.
- Tome, H. V., Schmehl, D. R., Wedde, A. E., Godoy, R. S., Ravaiano, S. V., Guedes, R. N., Martins, G. F., & Ellis, J. D. (2020). Frequently encountered pesticides can cause multiple disorders in developing worker honey bees. *Environmental Pollution*, 256, 113420.
- Uzman, D., Reineke, A., Entling, M. H., & Leyer, I. (2020). Habitat area and connectivity support cavity-nesting bees in vineyards more than organic management. *Biological Conservation*, 242, 108419.
- Walsh, E. M., Sweet, S., Knap, A., Ing, N., & Rangel, J. (2020). Queen honey bee (*Apis mellifera*) pheromone and reproductive behavior are affected by pesticide exposure during development. *Behavioral Ecology and Sociobiology*, 74(3), 1-14.
- Williams, N. M., & Kremen, C. (2007). Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. *Ecological applications*, 17(3), 910-921.

7. Figures and tables



Figure 1: Pictures of three organic pasture plots at USDA Agriculture Research Services, Booneville, Arkansas. Pictures were taken during summer of 2017 by Roshani Sharma Acharya.

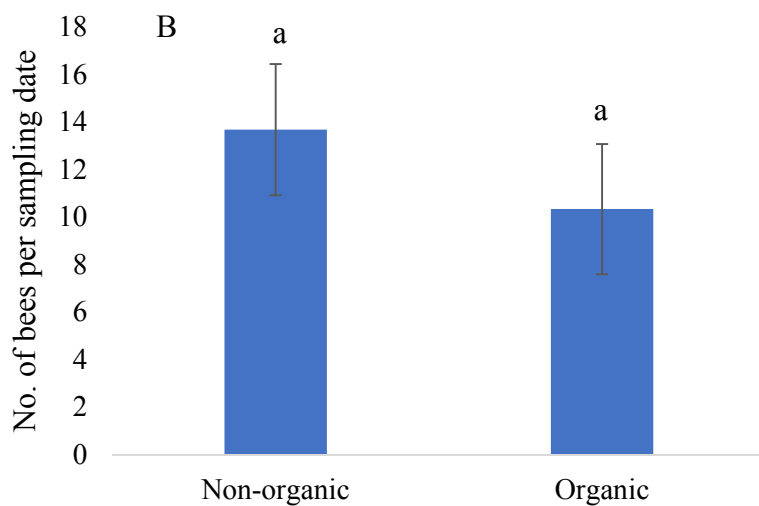
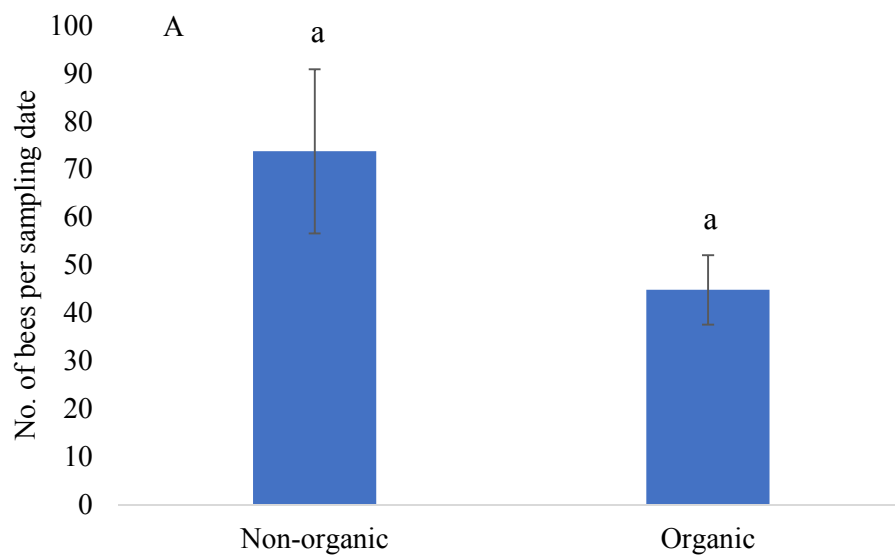


Figure 2: Comparison of mean number of bees per sampling date between non-organic and organic. Samples were taken during summer of (A) 2017 and (B) 2018 at USDA Agriculture Research Station in Booneville, AR.

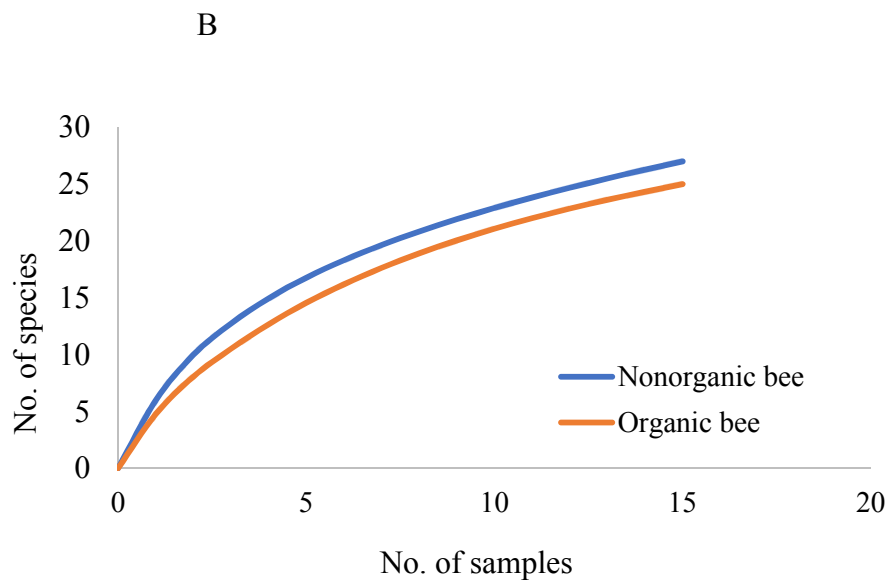
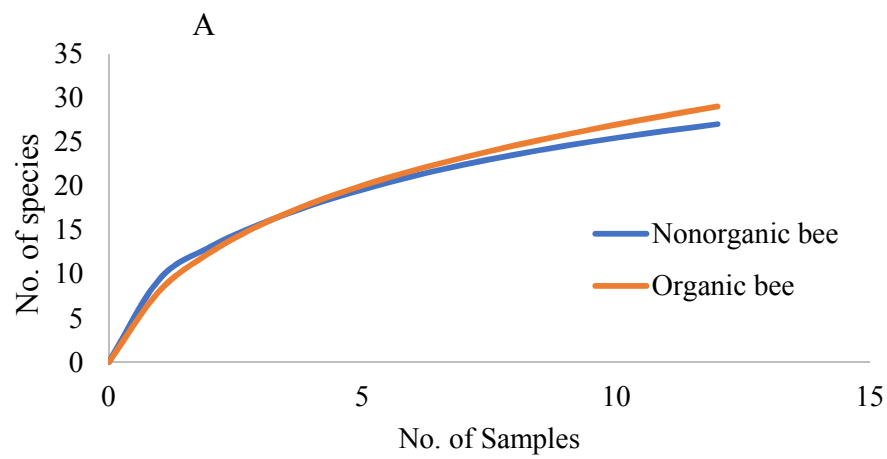


Figure 3: Rarefaction curve for bees in non-organic and organic pastures. Samples were collected for 4 weeks during July -August of 2017 (A) and for 5 weeks during July-August of 2018 (B) in Booneville, AR.

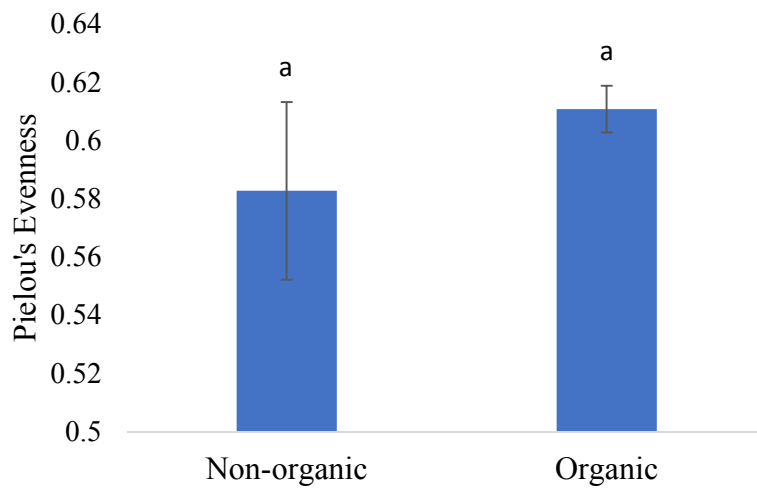
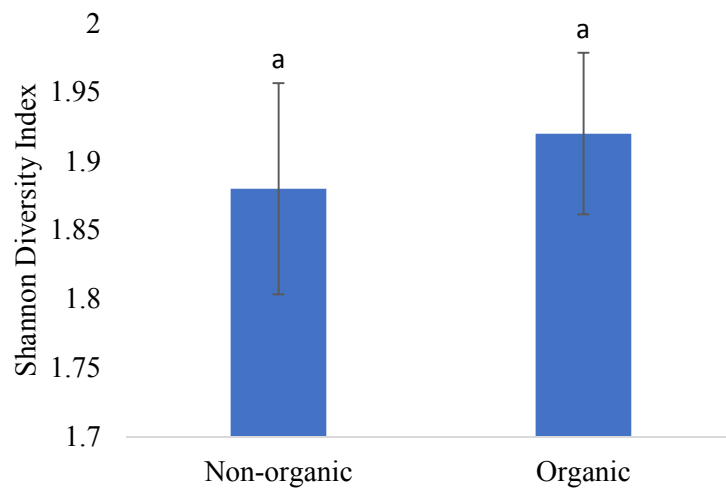


Figure 4. Shannon diversity Index values of bee communities collected in Organic and Non-Organic plots in both years.

Table 1. Bee species diversity in organic and non-organic livestock pastures during 2017-2018. Y refers to the species presence in that treatment.

Family	Genus Species	Organic	Non-organic
Apidae	<i>Apis mellifera</i>	Y	
	<i>Bombus auricomis</i>		Y
	<i>Bombus fervidus</i>	Y	Y
	<i>Bombus griseocollis</i>	Y	
	<i>Bombus impatiens</i>	Y	Y
	<i>Bombus pensylvanicus</i>	Y	Y
	<i>Bombus perplexus</i>	Y	
	<i>Ceratina dupla</i>		Y
	<i>Ceratina strenua</i>	Y	Y
	<i>Melissodes bimaculatus</i>	Y	Y
	<i>Melissodes communis</i>	Y	Y
	<i>Melissodes comptoides</i>	Y	Y
	<i>Melissodes veroninae</i>	Y	
	<i>Melissodes agilis</i>		Y
	<i>Melissodes denticulatus</i>	Y	
	<i>Melitoma taurea</i>	Y	
	<i>Peponapis pruinosa</i>	Y	Y
	<i>Ptilothrix bombiformis</i>	Y	Y
	<i>Svastra obliqua</i>	Y	Y
	<i>Svastra atripes</i>	Y	Y
	<i>Svastra obliqua</i>		Y
	<i>Svastra aegis</i>	Y	Y
	<i>Xenoglossa strenua</i>	Y	Y
	<i>Xylocopa virginica</i>	Y	Y
Halictidae	<i>Agapostemon sericeus</i>	Y	Y
	<i>Agapostemon texanus</i>	Y	Y
	<i>Agapostemon virescens</i>		Y
	<i>Augochlora pura</i>		Y
	<i>Augochlorella aurata</i>	Y	Y
	<i>Augochloropsis metallica</i>	Y	Y
	<i>Halictus ligatus</i>	Y	Y
	<i>Halictus parallelus</i>	Y	Y
	<i>Halictus rubicundus</i>		Y
	<i>Lasioglossum disparile</i>	Y	Y
	<i>Lasioglossum hitchensi</i>	Y	
	<i>Lasioglossum imitatum</i>	Y	Y
	<i>Lasioglossum pectorale</i>	Y	Y

Table 1 (Cont..)

Family	Genus species	Organic	Non-organic
Halictidae	<i>Lasioglossum spp.</i>		Y
	<i>Lasioglossum admirandum</i>		Y
	<i>Lasioglossum tegulare</i>	Y	Y
	<i>Lasioglossum trigeninum</i>		Y
	<i>Lasioglossum lustrans</i>	Y	
	<i>Lasioglossum versatum</i>		Y
Megachilidae	<i>Dianthidium curvatum</i>	Y	
	<i>Megachile bevis</i>	Y	
	<i>Megachile campanulae</i>		Y
	<i>Megachile mendica</i>		Y
	<i>Megachile polcaris</i>		Y
Colletidae	<i>Hylaeus modestus</i>	Y	

Table 2. Five most dominant bee species in organic and non-organic pastures during study years (2017-2018) at the USDA Agriculture Research Services, Booneville, AR.

Year	Treatment	Genus species	% of total
2017	Organic	<i>Lasioglossum imitatum</i>	60.78
		<i>Augochlorella aurata</i>	19.88
		<i>Ptilothrix bombiformis</i>	2.60
		<i>Lasioglossum pectorale</i>	2.60
		<i>Agapostemon texanus</i>	2.04
			87.37%
2018		<i>Bombus pensylvanicus</i>	41.93
		<i>Ptilothrix bombiformis</i>	9.67
		<i>Melissodes veroninae</i>	4.51
		<i>Melissodes comptoides</i>	4.51
		<i>Svastra obliqua</i>	4.51
			60.63%
2017	Non-organic	<i>Lasioglossum imitatum</i>	44.63
		<i>Augochlorella aurata</i>	33.10
		<i>Lasioglossum disparile</i>	8.24
		<i>Halictus ligatus</i>	3.276
		<i>Bombus pensylvanicus</i>	2.03
			91.28%
2018		<i>Bombus pensylvanicus</i>	23.90
		<i>Lasioglossum imitatum</i>	14.63
		<i>Augochlorella aurata</i>	14.14
		<i>Svastra obliqua</i>	8.78
		<i>Lasioglossum versatum</i>	7.31
			68.76%

CHAPTER SIX

Impact of native flowering forages and warm season grasses on biodiversity of bees and other non-bee insects in livestock pasture

Abstract

About 75% of all the flowering plants depend on plant-pollinator interactions for pollination, but our understanding of interactions between forage species and pollinators in livestock pasture ecosystem is far from complete. The objective of this study was to determine the species of bees and non-bee insects in livestock pastures seeded with a commercial native forb/legume/grass seed mix (FLG) or warm season grasses (WSG; equal mix of *Andropogon gerardi*, *Tripsacum dactyloides*, and *Sorghastrum nutans*). Total of six plots were used for the study, 1) three 0.4-ha plots were seeded with a native flowering seed mix and 2) three 0.4-ha plots were seeded with warm season grasses in February 2017 and same month in 2018. Bees and non-bee insects were sampled by using pan and vane traps in May and June of 2017 and 2018. A total of 3308 bees and 3692 non-bee insects were collected from native forb/legume/grass mix plots whereas in warm season grass plots there were 3158 bees and 2346 non-bees in both years (2017 and 2018). Most of the bees and non-bee insects collected fall under five orders (Coleoptera, Diptera, Hymenoptera, Lepidoptera, Hemiptera) and 56 families. These findings will serve as a guideline for selection of forage species in livestock pasture in order to provide additional ecosystem services that support native pollinators.

Keywords: Warm season grass, seed mix, bees, non-bee pollinators, livestock pasture

1. Introduction

Pollination is the process by which pollen is transferred from anthers to stigma to fertilize ovules in flowering plants. Pollination is carried out by wind, water, insects and other animal species. About 87% of flowering plants across the globe are pollinated by insects and birds (Winfree et al., 2011). In 2005 alone, pollination services from insects added \$215 billion to the global economy by pollinating ~ 75% of crop species and 94% of wild flowering plants (Vanbergen & Initiative, 2013).

About 45% of the agricultural land (307 million hectares) in the U.S. is classified as grassland (Havstad et al., 2009) that provides refuge (natural habitat and food resources) to more than 4,000 species of arthropod pollinators (C. D. Michener, 2007). Livestock graze grasses, forbs, legumes and shrubs. Plant-pollinator interaction in the livestock pasture ecosystem has been less studied in the past because most species in these ecosystems are wind-pollinated (e.g., plants that belong to Poaceae, Cyperaceae, Chenopodeaceae, and Polygonaceae) or are self-pollinated (Harmon et al., 2011). Advantage of wind-or self-pollination includes ability to reproduce without depending on pollinators while disadvantage includes reduced chance for genetic diversity (Harmon et al., 2011). Additionally, absence of insect pollinators leads to poor reproductive success, lower plant abundance and population variability as well as impaired floral traits (Knight et al., 2005). While grasses self-pollinate, forbs and flowering shrubs on the other hand require cross-pollination by insect pollinators (Harmon et al., 2011). Cross-pollination is especially important for plants of Scrophulariaceae and Lamiaceae families that are incapable of self-fertilization (Neff & Simpson, 1993). Other forages need pollination for seed production and continuation of next generation after initial plantation (Reed, 1993). The relationship between bees and plants is mutualistic because bees depend on plants for nectar while plants

need bees for cross-pollination and seed production. During cross-pollination, insects (mainly bees and other flower-visiting species) transfer pollen from stamen to stigma; in doing so plants not only produce seeds but also decrease chance for inbreeding. Lack of pollination results in low yield of seeds in forb species (Ingram et al., 1996). Reduced seed production leads to negative effects on seedling recruitment, and could further lead to the phase of species extinction (Harris & Johnson, 2004).

Native pollinators are critical in maintaining many plants in native grasslands but are at risk due to habitat fragmentation (Moncada, 2003). Agriculture intensification, crop monoculture, excessive use of fertilizers, pesticides, decrease of semi-natural habitat all reduce the abundance and diversity of pollinating insects (Landis et al., 2005; Flynn et al., 2009). Other additional factors such as loss of natural forests, grassland habitat, decrease of field margin, and riparian zones decrease number of certain insect species (Ewers, 2004; Öckinger et al., 2009; Diekötter et al., 2010). Excessive use of nitrogen fertilizer in pastures facilitates dominance of competitive plants leading to monoculture, decreasing forage species richness, and a decline in insect-pollinated plants (Flynn et al., 2009). Breakage of plant-pollinator interaction may not be noticed for long period though such disruptions are serious (Janzen, 1971).

Incorporating native floral resources in livestock pastures and rangelands has potential to provide critical support to pollinators and other beneficial arthropod species. Pastures that serve a dual purpose of providing forage for livestock and ecosystem benefits require a diversity of native forages that are palatable to livestock and provide floral food resources such as pollen and nectar to pollinators till the end of the season. Conservation of pasture with native flowering plants and grasses provide nesting habitat and even pollen for some species of native pollinators

(Isaacs et al., 2009). Most bees need multiple forage species that persist during entire foraging season for bees from early spring to late fall.

Agronomic monoculture crops attract only a few bee species, often honeybees and various species of bumblebees. In contrast, livestock pastures with flowering plants have the potential to attract greater number of bee species (Gresty et al., 2018). Inclusion of specific plant species for foraging resources provides a strong positive effect on floral-unit abundance of specific bee species (Gresty et al., 2018). Persistence of flowering plant species throughout the season and landscape management is necessary for the survival of wild pollinator species (Carvell et al., 2017). In addition, insect pollinators and other beneficial arthropod species (e.g., insect predators and parasitoids) are also important in balancing ecosystem. A study has shown that bumblebees were more abundant and diverse in a conservation forage mix (kale, mixed cereal, linseed, and legume) in comparison with cereal, grass and legume mix (Potts et al., 2009). In general, incorporation of additional legume and forbs in grassland increases pollinator community (Orford et al., 2016).

Pollinators and other insects respond differently to local habitat and landscape composition in the livestock pasture. For instance, beetle richness and abundance was positively correlated with vegetation height, hoverflies were more abundant in forest landscapes, and bee abundance and richness were significantly correlated with availability of flowering species (Sjödin et al., 2008). Specifically, bumblebees were more attracted to plant species belonging to Fabaceae (*Onobrychis viciifolia*, *Melilotus cf. altissimus*, *Trifolium pratense*) and Scrophulariaceae (*Odontites vernus*) families in a study that occurred in southern England UK (Goulson et al., 2005). Most abundant bee species are less selective than rarer species; for example, *B. humilis* and *B. hortorum* gather pollen from a narrow range of plant species. Several

studies reported that *B. humilis* collect pollen almost exclusively from *T. pratense* (Brian, 1951; Carvell, 2002; Goulson & Darvill, 2004). One of the potential factors behind such association could be that these bee species emerges from hibernation late and have less time to develop and reproduce (Goodwin, 1995), thus highest quality of food is needed for their brood and hence they are more selective (Goulson & Darvill, 2004). Farmers can maintain their landscape by optimizing abundance of these species that play a crucial role in maintaining ecosystems. Thus, it is important to include proper forage species in order to increase pollinator population in pasture ecosystems. Establishment of native flowering forbs and grasses not only provide continuous food (i.e., nectar and pollen) and habitat resources for pollinators but also other ecosystem services such as livestock forage, control of soil erosion, carbon sequestration, and pollination services to nearby cropland, thus, benefitting farmers and society by ensuring an adequate food supply (Chaplin-Kramer et al., 2011).

Interaction between livestock and pollinators in a livestock pasture ecosystem are not well documented. Little information is available on sustainable pasture systems that support pollinators. Therefore, there is a need to conduct studies on conservation of pollinators and other beneficial species in livestock pasture systems with a focus on growing diverse forage species to help to retain floral resources for spring, summer and fall. Thus, the main objective of this study was to determine differences between a mixed forb/legume/grass mix and a more common livestock pasture of grasses alone in their ability to affect abundance and diversity of bees and other pollinators in the livestock pasture system.

2. Materials and Methods

2.2 Site Description

This study was conducted during summer of 2017 and 2018 at the USDA-ARS Dale Bumpers FLG all Farms Research Center in Booneville, Arkansas (35.09 °N, 93.95 °W). Gentle slope land topography in which upper part contained clayey mixed (enders silt loam) soil type, whereas lower part contained silty siliceous (leadvale silt loam) soil. Soil was a deep, well-drained, moderate to high water-holding capacity, and relatively low fertility for pasture establishment (Thomas et al., 2008). Precipitation at the site averaged 1078 mm annually (2017 and 2018) with a maximum in May (145.5 mm) and minimum in February (78.2 mm; average between years). Temperature averaged 17.9 °C (a minimum of 4 °C during January and maximum of 24 °C during July).

2.2.1 Pasture plot preparation for commercial native forb/legume/grass seed mix

Three plots (0.4-ha × 0.4-ha) were planted with FLG (commercial native forb/legume/grass seed mix) forage (forage species attached in the supplementary files). In June, July, September, and October of 2016 and January of 2017, sites were sprayed with Roundup® (41% glyphosate, 0.764 L ha⁻¹) and Outrider (75% Sulfosulfuron; Monsanto, St. Louis, MO; 0.016 L ha⁻¹) using a Continental Belton cluster nozzle sprayer (Continental Belton McAlester, SR: A44117, Oklahoma City, OK). The seedbed was prepared by intensive tillage (Maschio Gaspardo North America Inc., SC 300, Des Moines, IA), then rolled with 12' Big Guy Roller (Graul Manufacturing, St. Louis, MO) in October 2016. Half of each plot (0.16-ha plot) was seeded with Buck's Hangout seed mix (Hamilton Native outpost, Elk creek, MO; www.hamiltonnativeoutpost.com/ 14.5 kg ha⁻¹) and other half with Tallgrass Inexpensive or Tallgrass Exposed Clay subsoil mix (Prairie Moon, Winona, MN; www.prairiemoon.com/ 13.44

kg ha⁻¹ and 26.8 kg ha⁻¹, respectively according to commercial nursery recommendations) in early February 2017 and 2018. Plots were part of a larger study to compare pollinator activity and forage establishment and persistence among native seed types and grazing activity; seed types were randomized to include two different seed mixes in each plot. Soil fertility was adjusted during seeding period following soil fertility recommendation of University of Arkansas lab (<https://www.uaex.edu/publications/PDF/FSA-2153.pdf>).

2.2.2 Pasture plot preparation for native warm season grass

Three 0.4 ha plots were used to establish native warm-season grasses. Herbicide application, tillage and field preparations were similar to that described above for FLG. Plots were planted with native warm-season grasses in March 2017 [equal percentage of each species; 13.2 kg ha⁻¹]; big bluestem (*A. gerardi*), eastern gamagrass (*T. dactyloides*), and Indiangrass (*S. nutans*)] and burned in 2018. Seed was drilled onto a prepared seed bed using a Brillion planter.

2.2.3 Sheep grazing

Sheep flash grazed plots when forage height reached more than 25 cm or were excessively weedy, generally remaining in plots for 4 to 8 hours. Weeds included *Chenopodium album* and *Ambrosia artemisiifolia*. There were 15-20 sheep per plot during the time of grazing which occurred weekly as needed between May and September. Sheep were observed to graze weeds and native forages, but damaged stems of wildflowers, many of which subsequently recovered.

2.2.4 Soil sample collection and analysis of physiochemical analysis

Soil samples were collected in July and August of 2017 and 2018. Samples were collected from 0-15 cm, packed in sterile whirl-pack bags and stored at -20°C until analysis. Before analysis, samples were dried at 70 °C for 48 h and then ground with mortar and pestle to

pass through 2 mm sieve. Mehlich-3 extractable nutrients (i.e., Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Ti, and Zn) were determined using 1:10 soil volume: extractant solution volume ratio (Tucker, 1992) and analyzed by inductively coupled argon-plasma spectrometry using 7300 IPC-OES DV (Perlin-Elmer, Waltham, MA). Carbon and N concentration were analyzed via dry combustion using a Vario Max CN combustion analyzer (Elementar Americas Inc., Mt. Laurel, NJ). Soil pH was determined using symphony B30PCI probe (VWR International, Atlanta, GA).

2.3 Sampling methods

2.3.1 Pan and vane trap collection

In each plot, 3 pan trap platforms and 3 vane traps were placed in two 100 m long transects in east and west side of the plot. These traps were placed at the distance of 25, 50, and 100 m from the edge of plots. Border distance at the beginning of each plot was 7-8 m. Traps were set up every week (at 7:00 am) and samples were collected after 48 h. In both years, weekly sampling occurred between May15 and July 21. Pan trap provides an effective sampling technique to find relative abundance of pollinators in unstudied ecosystem (Larsen et al., 2014). During the study, pan trap platforms were placed at the distance of 25, 50, and 100 m from the edge (Acharya et al., 2021). Pan trap platform was placed ~1.25 m above the ground to match the height of flowering plant canopy in pasture, which consisted of a pair of pan traps with two colors (yellow and blue). Colored plastic bowls with UV light reflectance were kept in each groove of platform. In the beginning of each sampling event, two-thirds of the bowls were filled with soapy water, and insect samples were collected from the traps after 48 h. Traps were set up at 7:00 am to be collected after 2 d around the same time. To prepare soapy water, a few drops of liquid dishwashing detergent were mixed in a 3.785 l of tap water.

Blue colored vane traps were used for the passive sampling of insects. Vane traps were suspended using a metal wire attached to a metal fence post about 1.5 m above the ground. A plastic lid connected to a collecting jar (950 ml). Traps were placed at the same distance as pan traps, but in a separate transect 15 m apart. The border distance was 8 m. Traps remained in plots and samples were collected every week, 48 h after filling with soapy water.

Samples collected from the pan and vane traps were placed in plastic vials containing 70% ethyl alcohol before they were transported to the laboratory. In the laboratory, insects were air dried, pinned and sorted for identification of bee taxa. Samples were identified to the species level by Drs. David Biddinger (Department of Entomology, Penn State Fruit Research and Extension Center, Biglerville, PA) and Robert Jean (Senior Entomologist, Environmental Solution & Innovations, Inc.) using dichotomous keys (Mitchell, 1962; Michener et al., 1994; Michener, 2007), Discover life ID guides (www.discoverlife.org), and Bug Guide (bugguide.net).

2.4 Plant species composition

Forage species composition was assessed every week during the entire study period using the wire frame method described by (Vogel & Masters, 2001). Wire frame used in the study contained 36 $15 \times 15 \text{ cm}^2$ squares. During the process of assessing species composition, number of cells containing one or more of the desirable established native plants originating from the commercial seed mix were counted. Sampling was done in three random locations in east and west sides of each plot. Number of forage species was converted into frequency of occurrence and percentage. Species that were not identified as desirable were counted and listed as weeds or undesirable species.

3. Results

3.1 Chemical properties of soil (pH, nutrient and mineral content)

Mean, standard deviation, minimum, and maximum values for each soil parameter from WSG and FLG are given in Table 1. The results demonstrate that pH, nutrient, and mineral content in the two different forage plots were similar. All the nutrients were within the range required for growth of forage.

3.2 Abundance and diversity

A total of 3308 bees and 3692 non-bee insects were collected from FLG plots whereas WSG plots contained 3158 bees and 2346 other non-bee insects in 2017 and 2018, respectively. It shows that higher number of bees and pollinators were collected in FLG and less in WSG. Both types of forage contained all five bee families, such as Apidae, Andrenidae, Colletidae, Halictidae, and Megachilidae (Table 2). In total there were 66 species of bees, where WSG and FLG plots had 43 and 59 different bee species respectively (Table 2). Most abundant bee species collected in both plots were *Lasioglossum imitatum* (47.33%), *Lasioglossum disparile* (14.03%), *Augochlorella aurata* (3.918%), *Halictus rubicundus* (3.918%), *Eucera hamata* (4.32%), and *Agapostemon texanus* (3.62%). Two species belonging to Colletidae (*Hylaeus mesillae* and *Hylaeus affinis*) were only found in WSG plots whereas one species (*Hylaeus modestus*) was only found in FLG plots. Total eight different species of megachilid bees were collected from this study. Seed mix plots had seven species viz., *Osmia pumila*, *O. cornifrons*, *Megachile addenda*, *M. mendica*, *M. exilis*, *Ashmeadiella buconis*, and *Heriades leavitti*, while one species *M. petulans* was found in WSG plots.

3.3 Abundance and Diversity of Non-bee Insects

Among non-bee insects, there were predominantly *Dipteran* spp., *Coleopteran* spp., *Lepidopteran* spp., and some *Hemipteran* spp. In total there were 95 species of non-bee insects where WSG and FLG plots had 68 and 75 different non-bee insects respectively (Table 3). Total 54 families of non-bee insects were found in this study where FLG plots contain 45 and WSG plots contain 40 families of non-bee insects, respectively. Details regarding all bees and non-bee species listed in Table 2 & 3. Also, most abundant bee species in native forb/legume/grass and warm season grass plots were listed in Table 4.

3.4 Botanical species composition

3.4.1 Seed mix with legume/ forbs /grass plots

Percentage of desirable (seeded forage) and undesirable (not seeded) both were between 40 – 60% and varied between plots in seed mix plots (FLG). Details regarding desirable and undesirable species are listed in Table 5. About 8.6% of the ground was bare (no forage species).

3.4.2 Warm season grass plots

Among the three major grass species all three plots majorly contained weeds (around 60-70% and the percentage of desirable plants in these plots were as low as 20-30%. It was observed (but forage species not examined) in 2018 that a greater proportion of grasses were present in plots. Details about desirable and undesirable species are listed in Table 5.

4. Discussion

Pollinators are important for growth and reproduction of more than 80% of the world's flowering plants including flowering forage species (Ollerton et al., 2011). An important food source for pollinator is forages grown in hay and pasture field (Kevan et al., 1996; Potts et al., 2009). Benefit of inclusion of flowering forage species on pollinators in livestock pasture ecosystem is less recognized in the past. To develop pollinator-friendly pasture system, it is

important to find right forage species for given geographical location. The seed mixes used in this study contained both legume and forbs to create suitable infrastructure to enhance pollinators. However, establishment of several forage species both in FLG and WSG was poor. In FLG plots, only few species such as *E. virginicus*, *E. canadensis*, *L. capitata*, *R. hirta*, *H. grosseserratus*, *D. illinoensis*, and *C. grandiflora* dominated and were still below 40-60% and of the total desirable species. Similarly, in warm season plots the desirable species represented less than 30% of the plot coverage. The presence of more weeds and less desirable species in both plots could have impacted the overall results.

This observational data provides the evidence for the type of flowering or grass forage species that could thrive in livestock pasture in Arkansas (or similar regions) and that could potentially provide foraging resources for pollinators. We found that *E. virginicus*, *E. canadensis*, *L. capitata*, *H. grosseserratus*, and *C. grandiflora* established well. About 75% of all the flowering plants depends on plant-pollinator interaction (Committee et al., 2007), and insect assisted pollination plays an obvious and impressive role in maintaining plant diversity and conservation (Travers et al., 2011).

Bumblebees are effective native pollinators because of their floral preference, large body size and their role in buzz pollination (Goulson & Darvill, 2004). In this study, five bumblebee species i.e., *B. griseocollis*, *B. pensylvanicus*, *B. impatiens*, *B. bimaculatus*, and *B. fervidus* were documented. A few studies done in the past in Arkansas grasslands found seven to eight bumblebee species (Chandler & McCoy Jr, 1965; Warriner, 2011). Studies conducted in between 2000-2015 also documented a total of seven species of bumblebees (Warriner, 2011; Tripodi & Szalanski, 2015). In current study, *B. variabilis* and *B. fraternus* were not found, but these species were documented in the past in Arkansas (Chandler & McCoy Jr, 1965; Warriner, 2011).

Consistent with our study (Warriner, 2011) failed to document *B. variabilis* during his seven years long survey in Arkansas's native grassland. Warriner (2011) suggested potential extirpation of two other species *B. auricomus* and *B. fraternus* that were not found in our study region. In a different study, Tripodi & Szalanski, (2015) has listed *B. fraternus* and *B. variabilis* as a rare species in the Southeastern United States and a need for their conservation.

Non-bee insects such as butterflies and moths (Lepidoptera), wasps (Hymenoptera), beetles (Coleoptera), flies (Diptera) were also documented and identified up to the species level in the current study. Many of these non-bee insects also help in pollination of flowering plants. The contribution of non-bee pollinators for pollination has large been unnoticed but it has been shown that non-bee pollinators contribute as much as 25% of the total flower visitation and pollination (Rader et al., 2011). Non-bee taxa, in comparison to bees, can forage in extreme weather conditions, and in some case can transfer pollen to farther distance in comparison to bees and thus are important in livestock forage pasture (Cutler et al., 2012; Rader et al., 2011). Literatures regarding non-bee pollinators in livestock pastures is extremely rare and their pollination contribution has not been explored. Similar to our findings, a study done in rangeland in Texas documented 21 insect families from 4 orders (Coleoptera, Diptera, Lepidoptera, and Hymenoptera) a three-year period (Bhandari et al., 2018).

Non-syrphid Diptera have long been overlooked in many pollination studies (Acharya et al., 2021; Joshi et al., 2015; Zawislak et al., 2019) because of difficulties in identification to the species level and largely underestimated role in pollination service. In some ecosystem such as high altitudes (alpine and subarctic ecosystem) the proportion of non-syrphid Diptera increases with increase in latitude providing efficient pollination service (Elberling & Olesen, 1999). Non-syrphid Diptera are also major pollinator of sapromyophilic flowers that are less attractive for

bees (Larson et al., 2001). Agriculture intensification has affected honeybees and other bee populations. However, such declines are rare for many species for Diptera due to differences in ecology. Thus, Diptera can provide alternative pollination services that may buffer bee losses (Kearns, 2001). Several families of Diptera have bristles, furry, hairs, long tongue that enables them to carry pollen-load as equivalent or slightly less than Diptera making them second most efficient pollinator after bees (Skevington & Dang, 2002).

Syrphid flies (Syrphidae: Diptera), commonly known as hoverflies are the largest families of true flies consisting of 6674 species (review by (Dunn et al., 2020) and are regarded as important pollinators in agriculture systems. Due to morphological limitations, syrphids feed only flowers with a short corolla (such as *Coriandrum sativum*) but they can carry viable pollen up to 400 m (Rader et al., 2011). Unlike bees, syrphids can tolerate change in land-use and foraging sources (Holzschuh et al., 2016) and the larvae of some species can survive by feeding on aphid (Gilbert, 2005).

Non-bee pollinators are taxonomically diverse and include butterflies, beetles, flies, moths and thrips. Bees depend on floral resources from larvae to adult stage whereas flies and other non-bee pollinators survive on aphids, organic materials, and larvae and eggs of insects making non-bees better adaptive to extreme weather conditions (Rader et al., 2020). Non-bees such as flies, butterflies and beetles travel at a relative to longer distance for food resources and do not maintain nests (Rader et al., 2020) thus can pollinate earlier than bees which requires nesting preferably in semi-natural habitat outside the agricultural cropland (Cusser et al., 2021). Butterflies are well known for their great contribution in pollinating tubular flowers (Faegri & Van der Pijl, 2013). Butterfly and fly pollinators visit temporally and spatially distinct flowers

that are left out by bees (Cusser et al., 2021), and their efficiency in pollinating flowers of different plant species could be studied in future research.

5. Conclusion

In the current study we compared difference in pollinators (both bees and non-bees) captured by pan or vane traps in livestock pasture plots planted with either FLG or WSG (*A. gerardi*, *T. dactyloides*, and *S. nutans*). Total number of bees was higher in FLG and less in WSG. Similarly, 3692 non-bee insects were collected from FLG whereas WSG contained 2346 non-bee insects. This could be due to presence of mixture of native forbs/ legume/grass than seed mix with warm season grasses only. This finding indicates prevalence of native bee species and non-bee insects in a livestock pasture in Southeastern USA. Additional research is needed to find the specific plant that have potential to attract bees and non-bee insects in the livestock pasture.

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6. References

- Abrahamczyk, S., Steudel, B., & Kessler, M. (2010). Sampling Hymenoptera along a precipitation gradient in tropical forests: the effectiveness of different coloured pan traps. *Entomologia Experimentalis et Applicata*, 137(3), 262-268.
- Acharya, R. S., Leslie, T., Fitting, E., Burke, J., Loftin, K., & Joshi, N. K. (2021). Color of Pan Trap Influences Sampling of Bees in Livestock Pasture Ecosystem. *Biology*, 10(5), 445.
- Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Ingram, M. (1998). The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation biology*, 8-17.
- Anderson, N., Rao, S., & Derkatch, A. (2010). Native bumble bee diversity, abundance, and pollination in crimson clover and hairy vetch seed production fields in western Oregon. *Seed Production Research*, 8.
- Andersson, G. K., Rundlöf, M., & Smith, H. G. (2012). Organic farming improves pollination success in strawberries. *PloS one*, 7(2), e31599.
- Arvin Mosier, J., Syers, K., & Freney, J. R. (2004). Agriculture and the Nitrogen Cycle: Assessing the Impacts of Fertilizer Use on Food Production and the Environment. 2004 SCOPE, 65. In: Island Press, Washington, DC.
- Barthell, J., Bradleigh Vinson, S., Frankie, G., La Salle, J., & Gauld, I. (1993). *Threats to the diversity of solitary bees in a neotropical dry forest in Central America Hymenoptera and biodiversity*. CAB International, Wallingford (RU) Natural History Museum, Londres (RU).
- Belfrage, K., Björklund, J., & Salomonsson, L. (2005). The effects of farm size and organic farming on diversity of birds, pollinators, and plants in a Swedish landscape. *AMBIO: A Journal of the Human Environment*, 34(8), 582-588.
- Bhandari, K. B., West, C., Longing, S., Brown, C., Green, P., & Barkowsky, E. (2018). Pollinator abundance in semiarid pastures as affected by forage species. *Crop Science*, 58(6), 2665-2671.
- Black, S. H., Shepherd, M., & Vaughan, M. (2011). Rangeland management for pollinators. *Rangelands*, 33(3), 9-13.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., . . . Dentener, F. (2010). Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological applications*, 20(1), 30-59.
- Brian, A. D. (1951). The pollen collected by bumble-bees. *The Journal of animal ecology*, 191-194.

- Brittain, C., Bommarco, R., Vighi, M., Settele, J., & Potts, S. G. (2010). Organic farming in isolated landscapes does not benefit flower-visiting insects and pollination. *Biological Conservation*, 143(8), 1860-1867.
- Buchmann, S., & Nabhan, G. (1996). The forgotten pollinators. Washington DC/Covelo. In: Shearwater Books/California Island Press. Edward M. Spevak.
- Burns, D. (2011). Attracting Native Pollinators. In: North Adams, ma: Storey Publishing.
- Campbell, J. W., & Hanula, J. (2007). Efficiency of Malaise traps and colored pan traps for collecting flower visiting insects from three forested ecosystems. *Journal of Insect Conservation*, 11(4), 399-408.
- Cane, J. H. (1991). Soils of ground-nesting bees (Hymenoptera: Apoidea): texture, moisture, cell depth and climate. *Journal of the Kansas Entomological Society*, 406-413.
- Cane, J. H. (2001). Habitat fragmentation and native bees: a premature verdict? *Conservation Ecology*, 5(1).
- Cane, J. H., Minckley, R. L., & Kervin, L. J. (2000). Sampling bees (Hymenoptera: Apiformes) for pollinator community studies: pitfalls of pan-trapping. *Journal of the Kansas entomological society*, 225-231.
- Carvell, C. (2002). Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. *Biological conservation*, 103(1), 33-49.
- Carvell, C., Bourke, A. F., Dreier, S., Freeman, S. N., Hulmes, S., Jordan, W. C., Heard, M. S. (2017). Bumblebee family lineage survival is enhanced in high-quality landscapes. *Nature*, 543(7646), 547-549.
- Carvell, C., Meek, W. R., Pywell, R. F., Goulson, D., & Nowakowski, M. (2007). Comparing the efficacy of agri-environment schemes to enhance bumble bee abundance and diversity on arable field margins. *Journal of applied ecology*, 44(1), 29-40.
- Ceulemans, T., Hulsmans, E., Vanden Ende, W., & Honnay, O. (2017). Nutrient enrichment is associated with altered nectar and pollen chemical composition in *Succisa pratensis* Moench and increased larval mortality of its pollinator *Bombus terrestris* L. *Plos one*, 12(4), e0175160.
- Chandler, L., & McCoy Jr, C. E. (1965). Bumble Bees of Arkansas (Hymenoptera, Apidae, Bombinae). *Journal of the Arkansas Academy of Science*, 19(1), 46-53.
- Chaplin-Kramer, R., Tuxen-Bettman, K., & Kremen, C. (2011). Value of wildland habitat for supplying pollination services to Californian agriculture. *Rangelands*, 33(3), 33-41.

- Cole, L. J., Brocklehurst, S., Robertson, D., Harrison, W., & McCracken, D. I. (2017). Exploring the interactions between resource availability and the utilisation of semi-natural habitats by insect pollinators in an intensive agricultural landscape. *Agriculture, Ecosystems & Environment*, 246, 157-167.
- Colwell, R. (2005). EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5 user's guide and application published. <http://purl.oclc.org/estimates>.
- Committee, S. o. P. i. N. A., America, C. o. t. S. o. P. i. N., Press, N. A., Staff, N. A. P., Council, N. R., Earth, D. o, Staff, N. R. C. (2007). *Status of pollinators in North America*. National Academy Press.
- Cooper, J., & Dobson, H. (2007). The benefits of pesticides to mankind and the environment. *Crop Protection*, 26(9), 1337-1348.
- Corbet, S. A., Williams, I. H., & Osborne, J. L. (1991). Bees and the pollination of crops and wild flowers in the European Community. *Bee world*, 72(2), 47-59.
- Cullen, M. G., Thompson, L. J., Carolan, J. C., Stout, J. C., & Stanley, D. A. (2019). Fungicides, herbicides and bees: A systematic review of existing research and methods. *PloS one*, 14(12), e0225743.
- Cusser, S., Haddad, N. M., & Jha, S. (2021). Unexpected functional complementarity from non-bee pollinators enhances cotton yield. *Agriculture, Ecosystems & Environment*, 314, 107415.
- Cutler, G. C., Reeh, K. W., Sproule, J. M., & Ramanaidu, K. (2012). Berry unexpected: nocturnal pollination of lowbush blueberry. *Canadian Journal of Plant Science*, 92(4), 707-711.
- de Oliveira, A. C., Junqueira, C. N., & Augusto, S. C. (2019). Pesticides affect pollinator abundance and productivity of sunflower (*Helianthus annuus* L.). *Journal of Apicultural Research*, 58(1), 2-8.
- Debano, S. J. (2006). Effects of livestock grazing on aboveground insect communities in semi-arid grasslands of southeastern Arizona. *Biodiversity & Conservation*, 15(8), 2547.
- Diekötter, T., Wamser, S., Wolters, V., & Birkhofer, K. (2010). Landscape and management effects on structure and function of soil arthropod communities in winter wheat. *Agriculture, ecosystems & environment*, 137(1-2), 108-112.
- Donkersley, P., Rhodes, G., Pickup, R. W., Jones, K. C., Power, E. F., Wright, G. A., & Wilson, K. (2017). Nutritional composition of honey bee food stores vary with floral composition. *Oecologia*, 185(4), 749-761.

- Dunn, L., Lequerica, M., Reid, C. R., & Latty, T. (2020). Dual ecosystem services of syrphid flies (Diptera: Syrphidae): pollinators and biological control agents. *Pest management science*, 76(6), 1973-1979.
- Dyer, A. G., Paulk, A. C., & Reser, D. H. (2011). Colour processing in complex environments: insights from the visual system of bees. *Proceedings of the Royal Society B: Biological Sciences*, 278(1707), 952-959.
- Dyer, A. G., Spaethe, J., & Prack, S. (2008). Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. *Journal of Comparative Physiology A*, 194(7), 617.
- Elberling, H., & Olesen, J. M. (1999). The structure of a high latitude plant-flower visitor system: the dominance of flies. *Ecography*, 22(3), 314-323.
- Eltz, T., Brühl, C. A., Van der Kaars, S., & Linsenmair, E. K. (2002). Determinants of stingless bee nest density in lowland dipterocarp forests of Sabah, Malaysia. *Oecologia*, 131(1), 27-34.
- Enri, S. R., Probo, M., Farruggia, A., Lanore, L., Blanchetete, A., & Dumont, B. (2017). A biodiversity-friendly rotational grazing system enhancing flower-visiting insect assemblages while maintaining animal and grassland productivity. *Agriculture, Ecosystems & Environment*, 241, 1-10.
- Ewers, R. M. (2004). The extent of forest fragmentation in New Zealand and its effects on arthropod biodiversity.
- Faegri, K., & Van der Pijl, L. (2013). Principles of pollination ecology.
- Feltham, H., Park, K., Minderman, J., & Goulson, D. (2015). Experimental evidence that wildflower strips increase pollinator visits to crops. *Ecology and evolution*, 5(16), 3523-3530.
- Filipiak, M. (2018). A better understanding of bee nutritional ecology is needed to optimize conservation strategies for wild bees—the application of ecological stoichiometry. *Insects*, 9(3), 85.
- Flynn, D. F., Gogol-Prokurat, M., Nogeire, T., Molinari, N., Richers, B. T., Lin, B. B., . . . DeClerck, F. (2009). Loss of functional diversity under land use intensification across multiple taxa. *Ecology letters*, 12(1), 22-33.
- Gabriel, D., & Tschardtke, T. (2007). Insect pollinated plants benefit from organic farming. *Agriculture, Ecosystems & Environment*, 118(1-4), 43-48.

- Gezon, Z. J., Wyman, E. S., Ascher, J. S., Inouye, D. W., & Irwin, R. E. (2015). The effect of repeated, lethal sampling on wild bee abundance and diversity. *Methods in Ecology and Evolution*, 6(9), 1044-1054.
- Gibbs, J., Joshi, N. K., Wilson, J. K., Rothwell, N. L., Powers, K., Haas, M., . . . Isaacs, R. (2017). Does passive sampling accurately reflect the bee (Apoidea: Anthophila) communities pollinating apple and sour cherry orchards? *Environmental Entomology*, 46(3), 579-588.
- Gilbert, F. (2005). Syrphid aphidophagous predators in a food-web context. *European Journal of Entomology*, 102(3), 325.
- Gollan, J. R., Ashcroft, M. B., & Batley, M. (2011). Comparison of yellow and white pan traps in surveys of bee fauna in New South Wales, Australia (Hymenoptera: Apoidea: Anthophila). *Australian Journal of Entomology*, 50(2), 174-178.
- Goodwin, S. (1995). Seasonal phenology and abundance of early-, mid-and long-season bumble bees in southern England, 1985–1989. *Journal of Apicultural Research*, 34(2), 79-87.
- Goulson, D. (2013). Neonicotinoids and bees: What's all the buzz? *Significance*, 10(3), 6-11.
- Goulson, D., & Darvill, B. (2004). Niche overlap and diet breadth in bumblebees; are rare species more specialized in their choice of flowers? *Apidologie*, 35(1), 55-63.
- Goulson, D., Hanley, M. E., Darvill, B., Ellis, J., & Knight, M. E. (2005). Causes of rarity in bumblebees. *Biological conservation*, 122(1), 1-8.
- Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347(6229).
- Gresty, C. E., Clare, E., Devey, D. S., Cowan, R. S., Csiba, L., Malakasi, P., . . . Willis, K. J. (2018). Flower preferences and pollen transport networks for cavity-nesting solitary bees: Implications for the design of agri-environment schemes. *Ecology and evolution*, 8(15), 7574-7587.
- Halinski, R., Dorneles, A. L., & Blochtein, B. (2015). Bee assemblage in habitats associated with *Brassica napus* L. *Revista Brasileira de Entomologia*, 59(3), 222-228.
- Hall, M. (2018). Blue and yellow vane traps differ in their sampling effectiveness for wild bees in both open and wooded habitats. *Agricultural and Forest Entomology*, 20(4), 487-495.
- Harmon, J. P., Ganguli, A. C., & Solga, M. J. (2011). An overview of pollination in rangelands: who, why, and how. *Rangelands*, 33(3), 4-8.
- Harris, L. F., & Johnson, S. D. (2004). The consequences of habitat fragmentation for plant-pollinator mutualisms. *International Journal of Tropical Insect Science*, 24(1), 29-43.

- Havstad, K., Peters, D., Allen-Diaz, B., Bartolome, J., Bestelmeyer, B., Briske, D., Huntsinger, L. (2009). The western United States rangelands: A major resource. *Grassland quietness and strength for a new American agriculture*, 75-93.
- Heller, S., Joshi, N. K., Leslie, T., Rajotte, E. G., & Biddinger, D. J. (2019). Diversified Floral Resource Plantings Support Bee Communities after Apple Bloom in Commercial Orchards. *Scientific reports*, 9(1), 1-13.
- Hellerstein, D., Hitaj, C., Smith, D., & Davis, A. (2017). Land use, land cover, and pollinator health: A review and trend analysis.
- Heneberg, P., & Bogusch, P. (2014). To enrich or not to enrich? Are there any benefits of using multiple colors of pan traps when sampling aculeate Hymenoptera? *Journal of insect conservation*, 18(6), 1123-1136.
- Hirsch, T. (2010). *Global biodiversity outlook 3*. UNEP/Earthprint.
- Hoback, W. W., Svatos, T. M., Spomer, S. M., & Higley, L. G. (1999). Trap color and placement affects estimates of insect family-level abundance and diversity in a Nebraska salt marsh. *Entomologia experimentalis et applicata*, 91(3), 393-402.
- Hole, D. G., Perkins, A., Wilson, J., Alexander, I., Grice, P., & Evans, A. D. (2005). Does organic farming benefit biodiversity? *Biological conservation*, 122(1), 113-130.
- Holzschuh, A., Dainese, M., González-Varo, J. P., Mudri-Stojnić, S., Riedinger, V., Rundlöf, M., Bommarco, R. (2016). Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. *Ecology letters*, 19(10), 1228-1236.
- Holzschuh, A., Steffan-Dewenter, I., & Tschardt, T. (2008). Agricultural landscapes with organic crops support higher pollinator diversity. *Oikos*, 117(3), 354-361.
- Hoover, S. E., Ladley, J. J., Shchepetkina, A. A., Tisch, M., Gieseg, S. P., & Tylianakis, J. M. (2012). Warming, CO₂, and nitrogen deposition interactively affect a plant-pollinator mutualism. *Ecology Letters*, 15(3), 227-234.
- Hopfenmüller, S., Holzschuh, A., & Steffan-Dewenter, I. (2020). Effects of grazing intensity, habitat area and connectivity on snail-shell nesting bees. *Biological Conservation*, 242, 108406.
- Ingram, M., Nabhan, G., & Buchmann, S. (1996). Our forgotten pollinators: Protecting the birds and bees. *Global Pesticide Campaigner*, 6(4), 1-12.
- Isaacs, R., Tuell, J., Fiedler, A., Gardiner, M., & Landis, D. (2009). Maximizing arthropod-mediated ecosystem services in agricultural landscapes: the role of native plants. *Frontiers in Ecology and the Environment*, 7(4), 196-203.

- James, R., James, R. R., & Pitts-Singer, T. L. (2008). *Bee pollination in agricultural ecosystems*. Oxford University Press on Demand.
- Janzen, D. H. (1971). The deflowering of central America.
- Johansen, L., Westin, A., Wehn, S., Iuga, A., Ivascu, C. M., Kallioniemi, E., & Lennartsson, T. (2019). Traditional semi-natural grassland management with heterogeneous mowing times enhances flower resources for pollinators in agricultural landscapes. *Global Ecology and Conservation*, 18, e00619.
- Jonason, D., Andersson, G. K., Öckinger, E., Rundlöf, M., Smith, H. G., & Bengtsson, J. (2011). Assessing the effect of the time since transition to organic farming on plants and butterflies. *Journal of Applied Ecology*, 48(3), 543-550.
- Joshi, N. K., Leslie, T., Rajotte, E. G., & Biddinger, D. J. (2020). Environmental impacts of reduced-risk and conventional pesticide programs differ in commercial apple orchards, but similarly influence pollinator community. *Chemosphere*, 240, 124926.
- Joshi, N. K., Leslie, T., Rajotte, E. G., Kammerer, M. A., Otieno, M., & Biddinger, D. J. (2015). Comparative trapping efficiency to characterize bee abundance, diversity, and community composition in apple orchards. *Annals of the Entomological Society of America*, 108(5), 785-799.
- Kearns, C. A. (2001). North American dipteran pollinators: assessing their value and conservation status. *Conservation Ecology*, 5(1).
- Kearns, C. A., Inouye, D. W., & Waser, N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual review of ecology and systematics*, 29(1), 83-112.
- Kennedy, C. M., Lonsdorf, E., Neel, M. C., Williams, N. M., Ricketts, T. H., Winfree, R., . . . Cariveau, D. (2013). A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecology letters*, 16(5), 584-599.
- Kevan, P., Giurfa, M., & Chittka, L. (1996). Why are there so many and so few white flowers? *Trends in Plant Science*, 1(8), 252.
- Kimoto, C., DeBano, S. J., Thorp, R. W., Taylor, R. V., Schmalz, H., DelCurto, T., . . . Rao, S. (2012). Short-term responses of native bees to livestock and implications for managing ecosystem services in grasslands. *Ecosphere*, 3(10), 1-19.
- Klaus, V. H., Kleinebecker, T., Prati, D., Gossner, M. M., Alt, F., Boch, S., . . . Müller, J. (2013). Does organic grassland farming benefit plant and arthropod diversity at the expense of yield and soil fertility? *Agriculture, ecosystems & environment*, 177, 1-9.

- Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L. G., Henry, M., Isaacs, R., . . . Rader, R. (2015). Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nature communications*, 6(1), 1-9.
- Klein, A.-M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the royal society B: biological sciences*, 274(1608), 303-313.
- Klein, A. M., Brittain, C., Hendrix, S. D., Thorp, R., Williams, N., & Kremen, C. (2012). Wild pollination services to California almond rely on semi-natural habitat. *Journal of Applied Ecology*, 49(3), 723-732.
- Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., . . . Ashman, T.-L. (2005). Pollen limitation of plant reproduction: pattern and process. *Annu. Rev. Ecol. Evol. Syst.*, 36, 467-497.
- Kremen, C., Williams, N. M., & Thorp, R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences*, 99(26), 16812-16816.
- Kruess, A., & Tscharntke, T. (2002). Grazing intensity and the diversity of grasshoppers, butterflies, and trap-nesting bees and wasps. *Conservation Biology*, 16(6), 1570-1580.
- Ladurner, E., Bosch, J., Kemp, W. P., & Maini, S. (2005). Assessing delayed and acute toxicity of five formulated fungicides to *Osmia lignaria* Say and *Apis mellifera*. *Apidologie*, 36(3), 449-460.
- Landis, D. A., Menalled, F. D., Costamagna, A. C., & Wilkinson, T. K. (2005). Manipulating plant resources to enhance beneficial arthropods in agricultural landscapes. *Weed Science*, 53(6), 902-908.
- Larsen, N., Minor, M., Cruickshank, R., & Robertson, A. (2014). Optimising methods for collecting Hymenoptera, including parasitoids and Halictidae bees, in New Zealand apple orchards. *Journal of Asia-Pacific Entomology*, 17(3), 375-381.
- Larson, B., Kevan, P., & Inouye, D. W. (2001). Flies and flowers: taxonomic diversity of anthophiles and pollinators. *The Canadian Entomologist*, 133(4), 439-465.
- Laubertie, E., Wratten, S., & Sedcole, J. (2006). The role of odour and visual cues in the pan-trap catching of hoverflies (Diptera: Syrphidae). *Annals of Applied Biology*, 148(2), 173-178.
- Lazaro, A., Tscheulin, T., Devalez, J., Nakas, G., & Petanidou, T. (2016). Effects of grazing intensity on pollinator abundance and diversity, and on pollination services. *Ecological entomology*, 41(4), 400-412.

- Ledvina, J., McShea, W. J., Bourg, N. A., Herrmann, V., Akre, T., & Johnson, A. E. (2020). Management Regime and Field Age Affect Species Richness and Cover of Native Forbs and Exotic Species in Virginia Grasslands. *Ecological Restoration*, 38(2), 83-93.
- Leong, J. M., & Thorp, R. W. (1999). Colour-coded sampling: the pan trap colour preferences of oligolectic and nonoligolectic bees associated with a vernal pool plant. *Ecological Entomology*, 24(3), 329-335.
- Little, C. Z. (2013). *Bee communities in the Arkansas River Valley*. University of Central Arkansas.
- Losey, J. E., & Vaughan, M. (2006). The economic value of ecological services provided by insects. *Bioscience*, 56(4), 311-323.
- McCravy, K. W. (2018). A review of sampling and monitoring methods for beneficial arthropods in agroecosystems. *Insects*, 9(4), 170.
- McMurphy, W., Gillen, R., Engle, D., & McCollum, F. (1990). The philosophical difference between range and pasture management in Oklahoma.
- Michener, C. (2007). *The Bees of the World* Johns Hopkins University Press. *Baltimore*. [Google Scholar].
- Michener, C. D. (2007). *The bees of the world*.
- Michener, C. D., McGinley, R. J., & Danforth, B. N. (1994). *The bee genera of North and Central America (Hymenoptera: Apoidea)*. Smithsonian Institution Press.
- Miranda, G., Young, A., Locke, M., Marshall, S., Skevington, J., & Thompson, F. (2013). Key to the genera of Nearctic Syrphidae. *Canadian Journal of Arthropod Identification*, 23(1), 351.
- Mitchell, S. R. (2020). Impacts of Range Management Decisions on Native Pollinators: Innovative Grazing Practices and Riparian Restoration.
- Mitchell, T. (1962). Bees of the eastern United States, vol. 2 (Technical Bulletin No. 152). *Agricultural Experiment Station, Raleigh, NC*.
- Moncada, K. (2003). The Role of Native Bees in Prairie Restoration.
- Morandin, L. A., & Winston, M. L. (2005). Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecological applications*, 15(3), 871-881.

- Moreira, E. F., da Silva Santos, R. L., Penna, U. L., Angel-Coca, C., de Oliveira, F. F., & Viana, B. F. (2016). Are pan traps colors complementary to sample community of potential pollinator insects? *Journal of insect conservation*, 20(4), 583-596.
- Murray, T. E., Fitzpatrick, U., Byrne, A., Fealy, R., Brown, M. J., & Paxton, R. J. (2012). Local scale factors structure wild bee communities in protected areas. *Journal of Applied Ecology*, 49(5), 998-1008.
- Neff, J. L., & Simpson, B. B. (1993). Bees, pollination systems and plant diversity.
- Ollerton, J. (2017). Pollinator diversity: distribution, ecological function, and conservation. *Annual Review of Ecology, Evolution, and Systematics*, 48, 353-376.
- Ollerton, J., Erenler, H., Edwards, M., & Crockett, R. (2014). Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. *Science*, 346(6215), 1360-1362.
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326.
- Orford, K. A., Murray, P. J., Vaughan, I. P., & Memmott, J. (2016). Modest enhancements to conventional grassland diversity improve the provision of pollination services. *Journal of Applied Ecology*, 53(3), 906-915.
- Osborne, J. L., Williams, I. H., & Corbet, S. A. (1991). Bees, pollination and habitat change in the European community. *Bee world*, 72(3), 99-116.
- Park, M. G., Blitzer, E., Gibbs, J., Losey, J. E., & Danforth, B. N. (2015). Negative effects of pesticides on wild bee communities can be buffered by landscape context. *Proceedings of the Royal Society B: Biological Sciences*, 282(1809), 20150299.
- Pickering, C. M., & Stock, M. (2003). Insect colour preference compared to flower colours in the Australian Alps. *Nordic Journal of Botany*, 23(2), 217-223.
- Potts, S., Woodcock, B., Roberts, S., Tscheulin, T., Pilgrim, E., Brown, V., & Tallowin, J. (2009). Enhancing pollinator biodiversity in intensive grasslands. *Journal of Applied Ecology*, 46(2), 369-379.
- Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in ecology & evolution*, 25(6), 345-353.
- Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., . . . Vanbergen, A. J. (2016). *The assessment report on pollinators, pollination and food production: summary for policymakers*. Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity.

- Potts, S. G., Vulliamy, B., Dafni, A., Ne'eman, G., & Willmer, P. (2003). Linking bees and flowers: how do floral communities structure pollinator communities? *Ecology*, 84(10), 2628-2642.
- Potts, S. G., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Ne'eman, G., & Willmer, P. (2005). Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology*, 30(1), 78-85.
- Power, E. F., Jackson, Z., & Stout, J. C. (2016). Organic farming and landscape factors affect abundance and richness of hoverflies (Diptera, Syrphidae) in grasslands. *Insect Conservation and Diversity*, 9(3), 244-253.
- Power, E. F., Kelly, D. L., & Stout, J. C. (2012). Organic farming and landscape structure: effects on insect-pollinated plant diversity in intensively managed grasslands. *PLoS One*, 7(5), e38073.
- Pywell, R., Warman, E., Carvell, C., Sparks, T., Dicks, L., Bennett, D., . . . Sherwood, A. (2005). Providing foraging resources for bumblebees in intensively farmed landscapes. *Biological conservation*, 121(4), 479-494.
- Pérez-Méndez, N., Andersson, G. K., Requier, F., Hipólito, J., Aizen, M. A., Morales, C. L., . . . Garibaldi, L. A. (2020). The economic cost of losing native pollinator species for orchard production. *Journal of Applied Ecology*, 57(3), 599-608.
- Rader, R., Cunningham, S., Howlett, B., & Inouye, D. (2020). Non-bee insects as visitors and pollinators of crops: Biology, ecology, and management. *Annual review of entomology*, 65, 391-407.
- Rader, R., Edwards, W., Westcott, D. A., Cunningham, S. A., & Howlett, B. G. (2011). Pollen transport differs among bees and flies in a human-modified landscape. *Diversity and Distributions*, 17(3), 519-529.
- Reed, C. (1993). Reconstruction of Pollinator Communities on Restored Prairies in Eastern Minnesota: final Report to the Minnesota Department of Natural Resources, Nongame Wildlife Program. *Minnesota Department of Natural Resources, Minnesota*.
- Reilly, J., Artz, D., Biddinger, D., Bobiwash, K., Boyle, N., Brittain, C., Elle, E. (2020). Crop production in the USA is frequently limited by a lack of pollinators. *Proceedings of the Royal Society B*, 287(1931), 20200922.
- Rinehart, L. (2006). Pasture, rangeland and grazing management (ATTRA). Ed. Pull Drscoll (NACT). *National Center for Sustainable Agriculture Information Services*, 1-800.
- Robson, D. B. (2019). Impact of Grazing History on Pollinator Communities in Fescue Prairie. *Blue Jay*, 77(1), 10-15.

- Roulston, T. a. H., & Goodell, K. (2011). The role of resources and risks in regulating wild bee populations. *Annual review of entomology*, 56, 293-312.
- Roulston, T. a. H., Smith, S. A., & Brewster, A. L. (2007). A comparison of pan trap and intensive net sampling techniques for documenting a bee (Hymenoptera: Apiformes) fauna. *Journal of the Kansas Entomological Society*, 80(2), 179-181.
- Roy, D. N., Konar, S. K., Banerjee, S., Charles, D. A., Thompson, D. G., & Prasad, R. (1989). Persistence, movement, and degradation of glyphosate in selected Canadian boreal forest soils. *Journal of Agricultural and Food Chemistry*, 37(2), 437-440.
- Rundlöf, M., Nilsson, H., & Smith, H. G. (2008). Interacting effects of farming practice and landscape context on bumble bees. *Biological conservation*, 141(2), 417-426.
- Saarinen, K., Valtonen, A., Jantunen, J., & Saarnio, S. (2005). Butterflies and diurnal moths along road verges: does road type affect diversity and abundance? *Biological Conservation*, 123(3), 403-412.
- Sanchez-Bayo, F., & Goka, K. (2014). Pesticide residues and bees—a risk assessment. *PloS one*, 9(4), e94482.
- Saunders, M. E., & Luck, G. W. (2013). Pan trap catches of pollinator insects vary with habitat. *Australian Journal of Entomology*, 52(2), 106-113.
- Senapathi, D., Biesmeijer, J. C., Breeze, T. D., Kleijn, D., Potts, S. G., & Carvalheiro, L. G. (2015). Pollinator conservation—the difference between managing for pollination services and preserving pollinator diversity. *Current Opinion in Insect Science*, 12, 93-101.
- Shapira, T., Henkin, Z., Dag, A., & Mandelik, Y. (2020). Rangeland sharing by cattle and bees: moderate grazing does not impair bee communities and resource availability. *Ecological Applications*, 30(3), e02066.
- Singh, S. B., & Kulshrestha, G. (2007). Determination of sulfosulfuron residues in soil under wheat crop by a novel and cost-effective method and evaluation of its carryover effect. *Journal of Environmental Science and Health Part B*, 42(1), 27-31.
- Sjödin, N. E. (2007). Pollinator behavioural responses to grazing intensity. *Biodiversity and Conservation*, 16(7), 2103-2121.
- Sjödin, N. E., Bengtsson, J., & Ekbom, B. (2008). The influence of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects. *Journal of Applied Ecology*, 763-772.
- Skevington, J. H., & Dang, P. (2002). Exploring the diversity of flies (Diptera). *Biodiversity*, 3(4), 3-27.

- Smith, O. M., Cohen, A. L., Reganold, J. P., Jones, M. S., Orpet, R. J., Taylor, J. M., . . . Ge, Y. (2020). Landscape context affects the sustainability of organic farming systems. *Proceedings of the National Academy of Sciences*, 117(6), 2870-2878.
- Southwick, E. E., & Southwick Jr, L. (1992). Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. *Journal of Economic Entomology*, 85(3), 621-633.
- Sponsler, D. B., Grozinger, C. M., Hitaj, C., Rundlöf, M., Botías, C., Code, A., Suryanarayanan, S. (2019). Pesticides and pollinators: A socioecological synthesis. *Science of the Total Environment*, 662, 1012-1027.
- Steffan-Dewenter, I., & Tscharntke, T. (2001). Succession of bee communities on fallows. *Ecography*, 24(1), 83-93.
- Stephen, W. P., & Rao, S. (2005). Unscented color traps for non-Apis bees (Hymenoptera: Apiformes). *Journal of the Kansas Entomological Society*, 373-380.
- Stephenson, P. L., Griswold, T. L., Arduser, M. S., Dowling, A. P., & Kremen, D. G. (2018). Checklist of bees (Hymenoptera: Apoidea) from managed emergent wetlands in the lower Mississippi Alluvial Valley of Arkansas. *Biodiversity data journal*(6).
- Streinzer, M., Paulus, H. F., & Spaethe, J. (2009). Floral colour signal increases short-range detectability of a sexually deceptive orchid to its bee pollinator. *Journal of Experimental Biology*, 212(9), 1365-1370.
- Sugden, E. A. (1985). Pollinators of *Astragalus monoensis* Barneby (Fabaceae): new host records; potential impact of sheep grazing. *The Great Basin Naturalist*, 299-312.
- Tadey, M. (2015). Indirect effects of grazing intensity on pollinators and floral visitation. *Ecological Entomology*, 40(4), 451-460.
- Tanis, M. M., Marshall, L., Biesmeijer, J. K., & van Kolschoten, L. (2020). Grassland management for meadow birds in the Netherlands is unfavourable to pollinators. *Basic and Applied Ecology*, 43, 52-63.
- Taylor, R. L., Maxwell, B. D., & Boik, R. J. (2006). Indirect effects of herbicides on bird food resources and beneficial arthropods. *Agriculture, Ecosystems & Environment*, 116(3-4), 157-164.
- Thapa-Magar, K. B., Davis, T. S., & Kondratieff, B. (2020). Livestock grazing is associated with seasonal reduction in pollinator biodiversity and functional dispersion but cheatgrass invasion is not: Variation in bee assemblages in a multi-use shortgrass prairie. *PloS one*, 15(12), e0237484.

- Thomas, A. L., Brauer, D. K., Sauer, T. J., Coggeshall, M. V., & Ellersieck, M. R. (2008). Cultivar influences early rootstock and scion survival of grafted black walnut. *Journal of the American Pomological Society*, 62(1), 3.
- Tome, H. V., Schmehl, D. R., Wedde, A. E., Godoy, R. S., Ravaiano, S. V., Guedes, R. N., Ellis, J. D. (2020). Frequently encountered pesticides can cause multiple disorders in developing worker honey bees. *Environmental Pollution*, 256, 113420.
- Travers, S. E., Fauske, G. M., Fox, K., Ross, A. A., & Harris, M. O. (2011). The hidden benefits of pollinator diversity for the rangelands of the Great Plains: Western prairie fringed orchids as a case study. *Rangelands*, 33(3), 20-26.
- Tripodi, A. D., & Szalanski, A. L. (2015). The bumble bees (Hymenoptera: Apidae: Bombus) of Arkansas, fifty years later. *Journal of Melittology*(50), 1-17.
- Tuell, J. K., Fiedler, A. K., Landis, D., & Isaacs, R. (2014). Visitation by wild and managed bees (Hymenoptera: Apoidea) to eastern US native plants for use in conservation programs. *Environmental entomology*, 37(3), 707-718.
- Turo, K. J., & Gardiner, M. M. (2019). From potential to practical: conserving bees in urban public green spaces. *Frontiers in Ecology and the Environment*, 17(3), 167-175.
- Uzman, D., Reineke, A., Entling, M. H., & Leyer, I. (2020). Habitat area and connectivity support cavity-nesting bees in vineyards more than organic management. *Biological Conservation*, 242, 108419.
- Vanbergen, A. J., & Initiative, t. I. P. (2013). Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment*, 11(5), 251-259.
- Vockeroth, J. R. (1992). *The flower flies of the subfamily Syrphinae of Canada, Alaska, and Greenland: Diptera, Syrphidae* (Vol. 1867). Agriculture Canada.
- Vogel, K. P., & Masters, R. A. (2001). Frequency grid--a simple tool for measuring grassland establishment. *Rangeland Ecology & Management/Journal of Range Management Archives*, 54(6), 653-655.
- Vrdoljak, S. M., & Samways, M. J. (2012). Optimising coloured pan traps to survey flower visiting insects. *Journal of Insect Conservation*, 16(3), 345-354.
- Vulliamy, B., G. Potts, S., & G. Willmer, P. (2006). The effects of cattle grazing on plant pollinator communities in a fragmented Mediterranean landscape. *Oikos*, 114(3), 529-543.
- Walsh, E. M., Sweet, S., Knap, A., Ing, N., & Rangel, J. (2020). Queen honey bee (*Apis mellifera*) pheromone and reproductive behavior are affected by pesticide exposure during development. *Behavioral Ecology and Sociobiology*, 74(3), 1-14.

- Wang, M., Lu, X., Ding, S., Ren, J., Bian, Z., & Xu, Z. (2017). Pollinator diversity in different habitats of the agricultural landscape in the middle and lower reaches of the Yellow River based on the three-color pan trap method. *Acta Ecologica Sinica*, 37(3), 148-155.
- Warriner, M., & Hutchins, B. (2016). Management recommendations for native insect pollinators in Texas. *Texas Parks and Wildlife Department*.
- Warriner, M. D. (2011). Bumblebees (Hymenoptera: Apidae) of remnant grasslands in Arkansas. *Journal of the Kansas Entomological Society*, 84(1), 43-50.
- Wells, W., & Decker, T. (2006). comparison of three types of insect traps for collecting non-Formicidae Hymenoptera on the Island of Dominica. *Southwestern Entomologist*.
- Whittingham, M. J. (2011). The future of agri□environment schemes: biodiversity gains and ecosystem service delivery? *Journal of applied ecology*, 48(3), 509-513.
- Williams, N. M., & Kremen, C. (2007). Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. *Ecological applications*, 17(3), 910-921.
- Wilson, J. S., Griswold, T., & Messinger, O. J. (2008). Sampling bee communities (Hymenoptera: Apiformes) in a desert landscape: are pan traps sufficient? *Journal of the Kansas Entomological Society*, 81(3), 288-300.
- Winfree, R., Bartomeus, I., & Cariveau, D. P. (2011). Native pollinators in anthropogenic habitats. *Annual Review of Ecology, Evolution, and Systematics*, 42, 1-22.
- Xie, Z., Williams, P. H., & Tang, Y. (2008). The effect of grazing on bumblebees in the high rangelands of the eastern Tibetan Plateau of Sichuan. *Journal of Insect Conservation*, 12(6), 695-703.
- Yang, E., Chuang, Y., Chen, Y., & Chang, L. (2008). Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *Journal of economic entomology*, 101(6), 1743-1748.
- Zattara, E. E., & Aizen, M. A. (2021). Worldwide occurrence records suggest a global decline in bee species richness. *One Earth*, 4(1), 114-123.
- Zawislak, J., Adamczyk, J., Johnson, D. R., Lorenz, G., Black, J., Hornsby, Q., . . . Joshi, N. (2019). Comprehensive survey of area-wide agricultural pesticide use in southern United States row crops and potential impact on honey bee colonies. *Insects*, 10(9), 280.
- Zhang, Z., Wang, L., Liu, J., Dong, Z., Xu, W., & Wang, S. (2019). The effect of simulated sheep grazing on male and female reproductive performance in *Caragana microphylla* Lam.(Leguminosae). *Israel Journal of Ecology and Evolution*, 1(aop), 1-7.

Öckinger, E., Franzén, M., Rundlöf, M., & Smith, H. G. (2009). Mobility-dependent effects on species richness in fragmented landscapes. *Basic and Applied Ecology*, 10(6), 573-578.

Table 1. Mean, standard deviation (SD), minimum, and maximum values of chemical properties of soil in warm season grass and forb/legume/grass plots. Soil samples were collected from a depth of 0-15 cm.

Soil parameters	Warm season grass plots				Seed mix plots			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
Nitrogen (g kg ⁻¹)	0.15	0.02	0.14	0.16	0.13	0.01	0.11	0.14
Carbon (g kg ⁻¹)	1.39	0.15	1.19	1.61	1.17	0.08	1.09	1.24
C:N ratio	9.37	0.85	8.22	10.7	8.93	0.71	7.99	10.3
pH	6.86	0.6	6.04	7.46	6.74	0.56	5.86	7.36
Aluminum	333	38.4	273	394	337	29.9	285	380
Arsenic	0.11	0.02	0.08	0.13	0.10	0.02	0.07	0.12
Boron	30.8	0.7	30.2	32.3	31.1	0.4	30.6	31.8
Calcium	1180	340	1580	793	972	218	1190	655
Cadmium	0.04	0.00	0.03	0.04	0.04	0.01	0.03	0.04
Cobalt	1.20	0.14	1.08	1.53	1.21	0.18	1.04	1.53
Chromium	0.09	0.01	0.06	0.10	0.08	0.01	0.06	0.09
Copper	0.69	0.13	0.56	0.92	0.61	0.08	0.51	0.77
Iron	162	28.1	99	193	123.8	9.6	106	136
Potassium	98.5	13.2	81.1	115	90.5	11.7	73.0	108
Magnesium	58.4	5.03	51.2	65.1	53.5	2.94	50.0	57.7
Manganese	185	21.4	146.0	221.0	168.3	19.5	139.0	193.0
Molybdenum	0.01	0.0	0.01	0.02	0.01	0.0	0.01	0.02
Sodium	103	8.1	95.2	118.0	105.5	8.42	95.8	123.0
Nickel	0.52	0.09	0.38	0.63	0.45	0.10	0.31	0.63
Phosphorus	26.8	4.9	19.5	29.4	18.5	1.7	16.8	21.0
Lead	2.6	0.3	2.02	2.87	2.6	0.1	2.29	2.54
Sulphur	9.76	1.40	8.2	11.5	7.76	0.95	6.5	9.5
Selenium	0.07	0.02	0.03	0.10	0.04	0.04	0.00 ¹	0.08
Titanium	0.45	0.13	0.34	0.75	0.41	0.06	0.35	0.45
Zinc	1.39	0.31	1.08	1.98	2.11	0.99	1.00	3.64

All units mg kg⁻¹ unless otherwise noted

¹Values of 0.00 were below the level of detection.

Table 2. List of bee species collected from livestock pastures established with warm season grasses (WSG) and forb/legume/grass (FLG) at USDA ARS, Booneville, AR in 2017-2018. Y refers to the species presence in that treatment.

Family	Diversity of Bees			Forage type	
	Genus	Species	Authors	WSG	FLG
Andrenidae	<i>Andrena</i>	<i>beameri</i>	La Berg	Y	
	<i>Perdita</i>	<i>octomaculata</i>	Say		Y
Apidae	<i>Apis</i>	<i>mellifera</i>		Y	Y
	<i>Eucera</i>	<i>hamata</i>	Bradely	Y	Y
	<i>Bombus</i>	<i>griseocollis</i>		Y	Y
	<i>Ceratina</i>	<i>dupla</i>		Y	Y
	<i>Ceratina</i>	<i>strenua</i>	Smith		Y
	<i>Xylocopa</i>	<i>virginica</i>	(L.)	Y	Y
	<i>Bombus</i>	<i>pennsylvanicus</i>	DeGeer	Y	Y
	<i>Svastra</i>	<i>obliqua</i>	(Say)	Y	Y
	<i>Melissodes</i>	<i>communis</i>	Cresson		Y
	<i>Melitoma</i>	<i>taurea</i>	(Say)	Y	Y
	<i>Bombus</i>	<i>impatiens</i>		Y	Y
	<i>Bombus</i>	<i>bimaculatus</i>		Y	Y
	<i>Ptilothrix</i>	<i>bombiformis</i>		Y	Y
	<i>Bombus</i>	<i>fervidus</i>		Y	Y
	<i>Melissodes</i>	<i>veroninae</i>	Robertson	Y	
	<i>Melissodes</i>	<i>comptoides</i>	(Smith)	Y	Y
	<i>Melissodes</i>	<i>trinodis</i>	Robertson	Y	Y
	<i>Peponapis</i>	<i>pruinosa</i>			Y
	<i>Xenoglossa</i>	<i>strenua</i>		Y	
	<i>Melissodes</i>	<i>intorta</i>	Cresson	Y	Y
	<i>Melissodes</i>	<i>tepaneca</i>	Cresson	Y	
	<i>Diadasia</i>	<i>rinconis</i>	Cockerell		Y
	<i>Anthophora</i>	<i>walshii</i>	Cresson	Y	Y
	<i>Eucera</i>	<i>rosae</i>	Robertson	Y	
	<i>Melissodes</i>	<i>denticulatus</i>	Smith		Y
	<i>Svastra</i>	<i>cressoni</i>	Dalla torre		Y
Halictidae	<i>Lasioglossum</i>	<i>disparile</i>	Cresson	Y	Y
	<i>Lasioglossum</i>	<i>imitatum</i>	Smith	Y	Y
	<i>Augochlorella</i>	<i>aurata</i>	Smith	Y	Y
	<i>Lasioglossum</i>	<i>tegulare</i>	Robertson	Y	Y
	<i>Lasioglossum</i>	<i>pectorale</i>		Y	Y

Table 2 (continued)

Family	Diversity of Bees			Forage type	
	Genus	Species	Authors	WSG	FLG
Colletidae	<i>Lasioglossum</i>	<i>hitchensi</i>			Y
	<i>Halictus</i>	<i>parallelus</i>	(Say)	Y	Y
	<i>Agapostemon</i>	<i>virescens</i>	(F.)	Y	Y
	<i>Halictus</i>	<i>ligatus</i>		Y	Y
	<i>Halictus</i>	<i>rubicundus</i>	Christ	Y	Y
	<i>Agapostemon</i>	<i>texanus</i>	Cresson	Y	Y
	<i>Augochlora</i>	<i>pura</i>		Y	Y
	<i>Lasioglossum</i>	<i>bruneri</i>	Crawford		Y
	<i>Agapostemon</i>	<i>sericeus</i>	Forster	Y	
	<i>Lasioglossum</i>	<i>versatum</i>	Robertson	Y	Y
	<i>Augochloropsis</i>	<i>metallica</i>	(F.)	Y	Y
	<i>Lasioglossum</i>	<i>cressonii</i>	Robertson	Y	
	<i>Lasioglossum</i>	<i>admirandum</i>	Sandhouse		Y
	<i>Lasioglossum</i>	<i>lineatulum</i>	Crawford		Y
	<i>Lasioglossum</i>	<i>trigeminum</i>	Gibbs		Y
	<i>Lasioglossum</i>	<i>obscurum</i>	Robertson		Y
	<i>Lasioglossum</i>	<i>coreopsis</i>			Y
	<i>Lasioglossum</i>	<i>nelumbonis</i>	Robertson		Y
	<i>Lasioglossum</i>	<i>cinctipes</i>	Provancher		Y
	<i>Sphecodes</i>	<i>sp.</i>			Y
	<i>Hylaeus</i>	<i>mesillae</i>	Cockerell	Y	Y
	<i>Hylaeus</i>	<i>affinis</i>	(Smith)	Y	
	<i>Hylaeus</i>	<i>modestus</i>	Say		
Megachilidae	<i>Osmia</i>	<i>pumila</i>			Y
	<i>Osmia</i>	<i>cornifrons</i>			Y
	<i>Megachile</i>	<i>addenda</i>	Cresson		Y
	<i>Megachile</i>	<i>mendica</i>	Cresson		Y
	<i>Megachile</i>	<i>exilis</i>	Cresson		Y
	<i>Megachile</i>	<i>petulans</i>	Cresson	Y	Y
	<i>Ashmeadiella</i>	<i>bucconis</i>	Say		Y
	<i>Heriades</i>	<i>leavitti</i>	Crawford		Y

Table 3. List of species of non-bee insects collected from livestock pastures established with warm season grasses (WSG) and forb/legume/grass (FLG) at USDA ARS Booneville AR (2017 – 2018). Y refers to the species presence in that treatment.

Family	Diversity of non-bee insects		Authors	Forage type	
	Genus	Species		WSG	FLG
Anthocoridae	<i>Orius</i>	<i>Sp.</i>		Y	
Arctiidae	<i>Cisseps</i>	<i>fulvicollis</i>			Y
Asilidae	<i>Promachus</i>	<i>bastardii</i>	Macquart		Y
	<i>Atomosia</i>	<i>puella</i>		Y	Y
Buprestidae	<i>Acmaeodera</i>	<i>pulchella</i>	Herbst	Y	Y
	<i>Acmaeodera</i>	<i>ornata</i>	(F.)	Y	Y
	<i>Agrilus</i>	<i>Sp.</i>		Y	
Cantharidae	<i>Chauliognathu</i>	<i>marginatus</i>		Y	Y
Carabidae	<i>Lebia</i>	<i>analisis</i>	Dejean	Y	Y
	<i>Tachys</i>	<i>Sp.</i>		Y	Y
	<i>Paraclivina</i>	<i>bipustulata</i>	(F.)	Y	Y
	<i>Harpalus</i>	<i>Sp.</i>		Y	
	<i>Stenolophus</i>	<i>comma</i>	(F.)	Y	
	<i>Stenolophus</i>	<i>lineola</i>	(F.)	Y	
	<i>Stenolophus</i>	<i>ochropepus</i>	Say	Y	Y
Crabronidae	<i>Oxybelus</i>	<i>marginatus</i>	Say	Y	Y
Cerambycidae	<i>Typocerus</i>	<i>octonotatus</i>	Halderman	Y	Y
				Y	Y
	<i>Typocerus</i>	<i>Lunulatus texanus</i>	Linsley & Chemsak	Y	Y
Cercopidae					Y
Chrysomelidae	<i>Disonycha</i>	<i>glabrata</i>	(F.)	Y	
	<i>Diabrotica</i>	<i>undecimpunctata</i>		Y	Y
	<i>Epitrix</i>	<i>hirtipennis</i>	Melsheimer	Y	Y
	<i>Systema</i>	<i>elongata</i>	(F.)		Y
Cicadellidae	<i>Oncometopia</i>	<i>orbona</i>			Y
Cleridae	<i>Priocera</i>	<i>castanea</i>	Newman	Y	
Coccinellidae	<i>Harmonia</i>	<i>axyridis</i>		Y	Y
	<i>Coleomegilla</i>	<i>maculata</i>	DeGeer	Y	Y
	<i>Cocinella</i>	<i>septumpunctata</i>		Y	
	<i>Hippodamia</i>	<i>convergens</i>		Y	Y
	<i>Coleomegilla</i>	<i>maculata</i>	Timberlake	Y	Y
Coreidae	<i>Leptoglossus</i>	<i>phyllopus</i>	(F.)		Y

Table 3 (Continued)

Diversity of non-bee insects				Forage type	
Family	Genus	Species	Authors	WSG	FLG
Curculionidae	<i>Rhodobaenus</i>	<i>quinquepunctatus</i>	Say (F.)	Y	Y
	<i>Odontocorynus</i>	<i>umbellae</i>		Y	
Cydnidae				Y	Y
Dolichopodidae	<i>Condylostylus</i>	<i>caudatus</i>	Wiedeman n	Y	Y
Elateridae	<i>Conoderus</i>	<i>Sp.</i>		Y	Y
	<i>Melanotus</i>	<i>species</i>		Y	Y
	<i>Anpedus</i>	<i>rubricus</i>	Say		Y
	<i>Conoderus</i>	<i>bellus</i>	Say	Y	
Erotylidae				Y	Y
Formicidae					Y
Hesperiidae	<i>Lerema</i>	<i>accius</i>		Y	Y
	<i>Atalopedes</i>	<i>campestris</i>		Y	Y
	<i>Problema</i>	<i>byssus</i>		Y	
Lampyridae				Y	Y
Melandryidae	<i>Dircaea</i>	<i>liturata</i>	LeConte	Y	
Meliodae	<i>Epicauta</i>	<i>vittata</i>		Y	
	<i>Epicauta</i>	<i>atrata</i>	(F.)	Y	Y
Membracidae	<i>Micrutalis</i>	<i>Sp.</i>			Y
Miridae	<i>Lygus</i>	<i>lineolaris</i>	Palisot de Beauvois	Y	Y
Misc.Carabidae					Y
Misc.Scarabaeidae					Y
Mordelidae					Y
Mordelidae	<i>Falsomordellist a</i>	<i>bihamata</i>		Y	
	<i>Hoshihananom</i>	<i>octopunctata</i>	(F.)	Y	Y
Muscidae				Y	Y
Noctuidae				Y	
Notonectidae				Y	
Nymphalidae	<i>Limenitis</i>	<i>andria</i>			Y
Arctiidae	<i>Anaea</i>	<i>thoracica</i>		Y	
Oedemeridae	<i>Oxycopsis</i>	<i>asterius</i>	(F.)	Y	Y
Papilionidae	<i>Polyxenes</i>	<i>glaucus</i>	Stoll	Y	
	<i>Papilio</i>	<i>pugnax</i>			Y
Pentatomidae	<i>Oebalus</i>	<i>Sp.</i>			Y
Pompilidae	<i>Tachypompilus</i>	<i>serripes</i>		Y	Y

Table 3 Continued

Diversity of non-bees				Forage type	
Family	Genus	Species	Authors	WSG	FLG
Reduviidae				Y	
Pyralidae					Y
Rhyparochromidae	<i>Myodocha</i>	<i>plebejus</i>	Oliviera	Y	
	<i>Myodocha</i>	<i>serripes</i>	Oliviera		Y
	<i>Heraeus</i>	<i>Sp.</i>		Y	Y
Scarabaeidae	<i>Ataenius</i>	<i>liberta</i>		Y	
	<i>Diploptaxis</i>	<i>sepulchralis</i>	Germar	Y	
	<i>Euphoria</i>	<i>borealis</i>	(F.)	Y	
	<i>Cyclocephala</i>	<i>sepulchralis</i>	Arrow	Y	Y
	<i>Euphoria</i>	<i>affinis</i>	(F.)		Y
	<i>Trichiotinus</i>	<i>piger</i>	Gory Percheron	Y	Y
	<i>Trichiotinus</i>	<i>delta</i>		Y	Y
	<i>Trigonopeltastes</i>	<i>lunulatus</i>	Forster	Y	Y
	<i>Trichiotinus</i>	<i>Sp.</i>	(F.)	Y	
	<i>Phyllophaga</i>	<i>trifasciata</i>		Y	Y
Scoliidae	<i>Compsomeris</i>	<i>californicum</i>	Saussure	Y	
Sphecidae	<i>Chalybion</i>	<i>caementarium</i>			Y
	<i>Sceliphron</i>	<i>pennsylvanicus</i>			Y
	<i>Sphex</i>	<i>Sp.</i>		Y	Y
	<i>Ammophila</i>	<i>illucens</i>		Y	
Stratiomyidae	<i>Hermetia</i>	<i>hieroglyphicus</i>	(L.)		Y
	<i>Psellidothus</i>	<i>geminatus</i>			Y
Syrphidae	<i>Toxomerus</i>	<i>exotica</i>		Y	Y
	<i>Allograpta</i>	<i>marginatus</i>		Y	Y
	<i>Toxomerus</i>	<i>ejuncida</i>			Y
	<i>Xylota</i>	<i>nitida</i>			Y
	<i>Orthonevra</i>	<i>politus</i>	Wiedemann		Y
	<i>Toxomerus</i>	<i>americanus</i>			Y
	<i>Eupeodes</i>	<i>americanus</i>			Y
	<i>Allograpta</i>	<i>obliqua</i>		Y	
	<i>Palpada</i>	<i>furcata</i>	Wiedemann	Y	
Tabanidae	<i>Tabanus</i>	<i>quinquevittatus</i>	Wiedemann	Y	Y
	<i>Tabanus</i>	<i>vivax osten</i>			Y
	<i>Tabanus</i>	<i>sparus</i>	Whitney		Y
	<i>Chrysops</i>	<i>Sp.</i>			Y
Tenebrionidae	<i>Helops</i>	<i>farctus</i>	LeConte		Y

Table 3: Continue

Diversity of non-bees				Forage type	
Family	Genus	Species	Authors	WSG	FLG
	<i>Alleculinae</i>	<i>species</i>			Y
Thynnidae	<i>Myzinum</i>	<i>quinquecinctum</i>		Y	Y
Thyreocoidae	<i>Corimelaena</i>	<i>lateralis</i>	(F.)	Y	Y
	<i>Corimelaena</i>	<i>Sp.</i>			Y
Lygaeidae	<i>Lygaeus</i>	<i>kalmii</i>	Stal		Y
Tetrigidae				Y	Y
Tiphiidae	<i>Tiphia</i>	<i>sp.</i>		Y	Y
Vespidae	<i>Polistes</i>	<i>fuscatus</i>	Christ	Y	Y
	<i>Stenodynerus</i>	<i>pulvinatus</i>	Bohart	Y	Y
	<i>Polistes</i>	<i>carolinach</i>			Y

Table 4: Relative abundance of bees in plots with legume/forbs/grass (FLG) and warm season grasses (WSG).

Treatment	Genus	Species	% of total
FLG	<i>Lasioglossum</i>	<i>imitatum</i>	50.48
	<i>Lasioglossum</i>	<i>disparile</i>	14.38
	<i>Augochlorella</i>	<i>aurata</i>	5.98
	<i>Eucera</i>	<i>hamata</i>	3.96
	<i>Melissodes</i>	<i>communis</i>	3.11
			77.91%
WSG	<i>Lasioglossum</i>	<i>imitatum</i>	45.07
	<i>Lasioglossum</i>	<i>disparile</i>	13.93
	<i>Halictus</i>	<i>rubicundus</i>	5.44
	<i>Agapostemon</i>	<i>texanus</i>	5.06
	<i>Eucera</i>	<i>hamata</i>	4.78
			69.22%

Table 5. Botanical species composition in different plots

Native forb legume and grass (FLG) plots

Percentage of desirable plants (included in seed mix)

Elymus virginicus (22%)
Elymus canadensis (16%)
Lespedeza capitate (11%)
Helianthus grosseserratus (11%)
Coreopsis grandiflora (10%)
Rudbeckia hirta (8.6%)
Chamaecrista fasciculata (6.6%)
Parthenium integrifolium (< 1%)
Achillea millefolium (< 1%)
Chamaecrista fasciculata (< 1%)
Callirhoe involucrata (< 1%)
Penstemon digitalis (< 1%)
Echinacea pallida (< 1%)
Rudbeckia subtomentosa (< 1%)

Most dominant undesirable species include (not included in seed mix)

Ambrosia spp. (27.77%)
Lolium spp. (13.8%)
Rumex crispus (11.1%)
Rumex obtusifolius (5.5%)
Papaver somniferum (5.5%)
Bromus tectorum (< 1%)
Poa pratensis (< 1%)
Carex hirta (< 1%)
Lolium spp. (< 1%)
Callirhoe spp. (< 1%)
Ranunculus eschscholtzii (< 1%)
Polygonum alpinum (< 1%)
Festuca arundinacea (< 1%)
Vigna unguiculata (< 1%)
Ambrosia artemisiifolia (< 1%)
Papaver somniferum (< 1%)
Trifolium campestre (< 1%)

Warm Season Grass (WSG) plots

Desirable species

Andropogon gerardi, *Tripsacum dactyloides*, *Sorghastrum nutans* (20-30%)

Most dominant undesirable species include (not included in seed mix (60-70%))

Polygonum aviculare

Cyperus rotundus

Solanum carolinense

Xanthium strumarium

Ambrosia psilostachya

Croton capitatus

Cyanodon dactylon

Festuca pratensis

Hordeum murinum

Balsamorhiza sagittata

Rumex crispus

Trifolium spp.

Individual species percentage not included.

CONCLUSION

The main aim of this study was to assess pollinator abundance and diversity in pasture ecosystems using different sampling methods, and to examine the effect of pasture management practices and forage types on pollinator communities.

Effective sampling methods for monitoring bees and non-bee insects in livestock pastures were determined by conducting two studies. In the first study, blue pan traps were found as the most effective pan trap color for sampling bees in a livestock pasture ecosystem. Purple traps were the second most effective, followed by yellow and green traps. These findings are supported by the reflectance value of the color trap and the known visible light spectrum of bees. Notably, yellow and green traps were the most effective traps for sampling insect communities in general but not for sampling wild bees. In addition, the results show that livestock pasture ecosystems that include native forages can support a wide variety of bees, which—in combination with grazing and management intensity—should be considered in pollination conservation schemes in agricultural landscapes.

In the second study, wild bees responded differently to passive traps with colored vanes of different light wavelength and reflectivity when deployed in a livestock pasture ecosystem. Among six different colors of vanes (dark blue, bright blue, dark yellow, bright yellow, purple and red), the bright blue traps captured the highest number of individuals and species of bees. This could be due to an appropriate match between the visual spectrum of bees and the light reflectance spectrum of vanes, which were made of a micro-prismatic retro-reflective material. Bees responded similarly to traps with other colors of vanes, except for red vane traps, which captured the lowest number of bees. In this study, the most abundant species was *Augochlorella aurata* (Smith) (25.8% of total bees), followed by *Lasioglossum disparile* (Cresson) (18.3%).

These findings would be useful in understanding bee vision and responses to passive traps, and such information would help in optimizing bee sampling methods for future monitoring efforts.

Diverse community of bees and non-bee insects were found in pasture plots under grazing and non-grazing in Arkansas. Although capture rates of insects varied among trap types used for sampling, bee and non-bee insect communities were generally more abundant and diverse in non-grazed plots as compared to grazed plots. Additionally, bee and non-bee communities showed higher evenness in non-grazed compared to grazed pastures. These findings suggest that grazing influences the diversity and community composition of bees and other insects in livestock pastures. Bee genera such as *Augochlorella*, *Ptilothrix*, *Svastra*, *Xylocopa*, *Ceratina* and *Halictus* were more commonly found in non-grazed plots relative to grazed plots. Further studies should examine differences in bee species abundance and diversity using other sampling methods such as visual counts and active net collection of bees and other arthropods to determine more prominent effects of grazing and non-grazing practices in livestock pastures.

In another study, impact of organic and non-organic pasture management was examined. Bee diversity, mean number of bees per sampling date, and species richness of bees (as measured by rarefaction curve) were similar between pastures under organic or non-organic management. Low floral resources in organic could have masked the beneficial effect of organic management on bee species abundance and diversity. Further studies should focus to find ways to control weeds for the better establishment of native species in organic plots and for the pollinator benefits in organic pasture ecosystem.

Pollinator (both bees and non-bee insects) communities in livestock pasture plots seeded with either a flowering seed mix of forb/legume/grass or warm season grasses (*A. gerardi*, *T.*

dactyloides, and *S. nutans*) were recorded. The establishment of some species (*E. virginicus*, *E. canadensis*, *H. grosseserratus*, and *C. grandiflora*) was better than other species included in the seed mixes; however, these species still covered only 40-60% of the ground and the remaining area was mainly weeds. The establishment of warm season grasses (*Andropogon gerardi*, *Tripsacum dactyloides*, and *Sorghastrum nutans*) was below 30%. More bees were collected in forb/legume/grass seed mixes compared to warm season grasses mix (3380 in FLG vs 3158 in WSG; total from 2017 & 2018). Similarly, 3692 non-bee insects were collected from FLG whereas WSG contained 2346 non-bee insects. These findings will provide important information on the types of forage plants to use in developing pollinator-friendly pastures that support diversity of native bee species and non-bee insects in the livestock pasture in the Southeastern USA.

Supplementary file

Buck's Hangout® (Hamilton Native outpost, Elk creek, MO)

Chamaecrista fasciculata

Chasmanthium latifolium

Coreopsis lanceolata

Coreopsis tinctoria

Dalea purpureum

Desmanthus illinoensis

Elymus virginicus

Heliopsis helianthoides

Lespedeza virginica

Penstemon digitalis

Ratibida pinnata

Rudbeckia hirta

Solidago nemoralis

Strophostyles leiosperma

Tridens flavus

* Total percentage by weight for each species is not available

Tall grass inexpensive seed mix® (Prairie Moon, Winona, MN)

Wildflowers

Aster novae-angllae (1.01%)
Astragalus Canadensis (0.51%)
Dalea purpurea (1.52%)
Echinacea pallida (4.54%)
Echinacea purpurea (2.53%)
Eryngium yuccifolium (2.88%)
Gentiana flavida (1.01%)
Heliopsis helianthoides (1.52%)
Lespedeza capitate (1.10%)
Monarda fistulosa (1.01%)
Parthenium integrifolium (2.53%)
Penstemon digitalis (1.52%)
Ratibida pinnata (1.52%)
Rudbeckia hirta (4.05%)
Rudbeckia subtomentosa (0.51%)
Rudbeckia triloba (1.52%)
Silphium integrifolium (1.10%)
Silphium laciniatum (1.01%)
Solidago rigida (0.51%)
Zizia aurea (2.02%)

Total % of wildflowers: 33.92%

Grasses, sedges and rushes

Andropogon gerardii (22.03%)
Elymus canadensis (11.01%)
Panicum virgatum (1.10%)
Sorghastrum nutans (31.94%)

Total percentage by weight for grasses, sedges and rushes: 66.08%

Tallgrass Exposed Clay Subsoil Seed Mix® (Prairie Moon, Winona, MN)
Wildflowers
<i>Agastache foeniculum</i> (1.23%)
<i>Allium canadense</i> (1.79%)
<i>Aster laevis</i> (1.35%)
<i>Aster novae-angliae</i> (0.90%)
<i>Astragalus canadensis</i> (0.67%)
<i>Baptisia alba</i> (0.90%)
<i>Cacalia atriplicifolia</i> (0.90%)
<i>Chamaecrista fasciculata</i> (14.31%)
<i>Dalea candida</i> (1.95%)
<i>Dalea purpurea</i> (2.93%)
<i>Echinacea purpurea</i> (5.38%)
<i>Gaura biennis</i> (2.06%)
<i>Heliopsis helianthoides</i> (1.35%)
<i>Kuhnia eupatorioides</i> (1.12%)
<i>Lespedeza capitata</i> (2.24%)
<i>Monarda fistulosa</i> (1.35%)
<i>Penstemon digitalis</i> (1.35%)
<i>Pycnanthemum virginianum</i> (1.35%)
<i>Ratibida pinnata</i> (1.35%)
<i>Rudbeckia hirta</i> (3.59%)
<i>Rudbeckia subtomentosa</i> (0.45%)
<i>Rudbeckia triloba</i> (0.98%)
<i>Silphium laciniatum</i> (1.35%)
<i>Silphium terebinthinaceum</i> (0.90%)
<i>Solidago rigida</i> (0.45%)
<i>Verbena hastata</i> (1.35%)
<i>Verbena stricta</i> (0.90%)
Total % by wt. wildflowers: 54.45%
Trees, shrubs and vines
<i>Amorpha canescens</i> (1.95%)
Total % by wt. trees, shrubs and vines: 1.95%
Grasses, sedges and rushes
<i>Andropogon gerardii</i> (9.77%)
<i>Elymus canadensis</i> (9.77%)
<i>Elymus virginicus</i> (7.17%)
<i>Juncus dudleyi</i> (0.45%)
<i>Panicum virgatum</i> (0.98%)
<i>Sorghastrum nutans</i> (13.67%)
<i>Sphenopholis obtusata</i> (1.79%)
Total % by wt. grasses, sedges and rushes: 43.60%

Butterfly and Hummingbird (Hamilton Native outpost, Elk Creek, MO)

Amorpha canescens
Asclepias tuberosa
Asclepias incarnate
Asclepias syriaca
Bidens aristosa
Chamaecrista fasciculata
Coreopsis grandiflora
Coreopsis lanceolata
Coreopsis tinctoria
Dalea candidum
Dalea purpureum
Desmanthus illinoensis
Echinacea purpurea
Helianthus grosseserratus
Heliopsis helianthoides
Lespedeza virginica
Liatris pycnostachya
Lobelia cardinalis
Ludwigia alternifolia
Mimosa quadrivalvis
Monarda fistulosa
Penstemon digitalis
Pycnanthemum verticillatum
Ratibida columnifera
Rudbeckia hirta
Senna marilandica
Silphium perfoliatum
Solidago nemoralis
Solidago rigida
Verbena stricta
Verbesina helianthoides
Zizia aurea

* Total percentage by weight for each species is not available