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The influence of land use on the pollen diet of honey bee (Apis mellifera) colonies in Ellis County, Kansas

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THE INFLUENCE OF LAND USE ON THE POLLEN DIET OF HONEY BEE (*APIS MELLIFERA*) COLONIES IN ELLIS COUNTY, KANSAS

A Thesis Presented to the Graduate Faculty
of Fort Hays State University in
Partial Fulfillment of the Requirements for
the Degree of Master of Science

by

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The Master of Science Degree

by

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has been approved by

Chair, Supervisory Committee

Supervisory Committee

Supervisory Committee

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Supervisory Committee

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Chair, Department of Biological Sciences

PREFACE

This thesis follows the format of the journal *Apidologie*, to which a portion will be submitted for peer-reviewed publication.

ABSTRACT

Pollinators are integral to plant ecology as well as to worldwide food availability and security. Understanding how human-driven land use change impacts the nutrition of managed honey bees is important in pollinator conservation efforts and can contribute towards combating recent pollinator declines. The objective of this study was to determine sources of forage for honey bees across different land use types in Ellis County, Kansas, through pollen analysis and taxonomic identification. Replicate study colonies were placed in three separate land use types: Urban, Cropland, or Native/Semi-native prairie. Pollen was sampled every 7 to 14 days throughout a growing season (April 1 to September 30) to identify key resources and their availability throughout time and space. Pollen abundance (mass) varied greatly throughout time and across treatments, although Urban colonies trended toward higher pollen abundance and Cropland colonies trended toward reduced pollen abundance. A total of 41 different plant taxa were identified from experimental hives, with willows (*Salix*) and clovers (*Melilotus/Trifolium*) observed in abundance among all land use treatments. Taxonomic richness (number of taxa) of floral resources utilized by study colonies was similar between land use treatments, with the exception of a few taxa. Unique distinctions among land use treatments included the abundance of trees in Urban habitat and the prevalence of weedy species in Cropland habitat. Understanding the availability of floral resources in prairies can help to inform conservation decisions and improve understanding of community ecology and health of native pollinators in prairies. Overall colony health and overwintering success could also be improved with a better understanding of how land composition influences honey bee nutrition.

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I honestly feel that Dr. Brian Maricle is one of the most dedicated faculty within the Department of Biological Sciences, I could not have asked for a better mentor. As an advisor, Dr. Maricle instilled his positive qualities into his students, staying very organized and responsive, as well as allocating generous amounts of his personal time to helping all of his students. I can’t fully convey how much I appreciate the work and positive attitude of Dr. Maricle, without whom, I likely would have not even pursued a Master’s degree. I also thank my graduate thesis committee for their time and input.

My family and friends undoubtedly deserve acknowledgment. I thank my parents and stepparents, Eric and Nikki Engel, and Claudia Engel and Garrett Pfannenstiel. I thank my grandparents, Errol and Nadine Engel, and Fred Nicholson. I am blessed to have such a loving family which has embraced and supported all of my odd interests and passions.

Finally, I thank my life partner, Rachel Cox and her family, and my brothers and bandmates, Nathan Engel and Keith Dryden. I also thank my sisters Morgan and Ashtyn Engel for their love and support. I owe each and every one of you so much and am extremely appreciative that you’ve been a part of my life.

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INTRODUCTION

Pollination is an ecosystem service of great importance and extent (Klein et al., 2006). The production of fruits, nuts, and seeds for human and wildlife consumption, along with maintaining diverse plant communities and their associated ecosystem services, highlight just some of the ecological roles of pollinators (Fontaine et al., 2005; Breeze et al., 2011). Honey bees alone are responsible for $15 billion worth of food production annually in the United States (Sass, 2011). Certain modern agriculture markets are completely reliant upon managed pollinator populations, such as the almond industry centralized in California, where 80% of the world's almond supply originates (Henselek et al., 2018).

Both native and managed pollinator populations are declining on a global scale (Kluser and Peduzzi, 2007), which has been attributed to increased reliance upon agrochemical inputs, the spread of pollinator pests and parasites, climate change, and habitat loss and fragmentation (Potts, 2010). These declines are evident in the rapidly rising costs of commercial pollination. Fees to rent a honey bee colony in 2005 were $76 but rose to $157 in 2009 (Bond et al., 2014). This trend is largely due to high rates of winter mortality across the United States (Aizen and Harder, 2009). During 2015-2016, beekeepers in Mississippi, Massachusetts, and Kansas reported winter colony losses at 60%, 49%, and 46%, respectively, much greater than acceptable loss rates of 19% (Kulhanek et al., 2017).

Beekeepers in the United States have attributed causes of winter mortality to pesticide exposure, the presence of mites or disease, and poor nutrition (vanEngelsdorp, 2011). Colony nutrition is an especially significant factor, as it can work in synergy with other stresses exerted on bee colonies (Brodschnieder and Crailsheim, 2010; Huang 2012; Di Pasquale et al., 2013). As pollen is the sole source of amino acids, lipids, and some vitamins and minerals in the honey bee diet, deficiencies present a serious problem (Brodschnieder and Crailsheim, 2010). Poor pollen nutrition has been linked to increased *Nosema cerana* infection (Di Pasquale et al., 2013), which can result in colony collapse (Higes et al., 2008).

Colony nutrition is of utmost importance in colony health (Keller et al., 2005a; Brodschnieder and Crailsheim, 2010) and is a result of surrounding land use composition which influences the pollen sources present (Odoux et al., 2012; Donkersley et al., 2014; Alaux et al., 2017). In most cases, native or semi-native landscapes are more supportive of honey bee colonies when compared to highly transformed landscapes (Naug, 2009; Smart et al., 2016a). Apiaries located in areas with greater surrounding floral abundance tend to be less affected by pests and pathogens, highlighting the importance of diet, specifically pollen, on the fate of honey bee colonies (Brodschnieder and Crailsheim, 2010; Smart et al., 2016a).

Although bees are well evolved to use patchy resources (Pleasants and Zimmerman, 1979), current land use practices in the Great Plains of North America present many challenges for managed pollinators (Naug, 2009). Nearly 50% of the total land coverage in the state of Kansas is cropland, though trends show relatively little recent conversion (Peterson et al., 2004). Land use types resulting in reduced floral diversity, such as row-crop agricultural systems, can reduce colony survival (Naug, 2009; Smart et al., 2016b). When overall pollen and nectar collection suffers in these environments, the number of mites/pests generally increase, along with deficiencies in amino acids and lipids, leading to compromised immune systems and increased mortality (Smart et al., 2016b).

Certain agricultural landscapes provide adequate forage to maintain honey bee colonies (Requier et al., 2015). The presence of weeds makes agricultural land variable in its ability to sustain honey bee colonies, with the increased presence of weeds supporting greater biodiversity in croplands (Marshall et al., 2003). If weeds within row-cropping systems provide vital resources for pollinators (Requier et al., 2015), intensive weed management methods such as the use of herbicide in these environments could reduce the viability of supporting honey bee colonies in these landscapes. The use of glyphosate alone has increased dramatically since the introduction of genetically engineered crops (Gianessi, 2005; Benbrook, 2012). An estimated 3 billion pounds of glyphosate were used on American cropland from 1974 to 2014, with 67% occurring from 2004-2014 (Benbrook, 2016).

Studying how human land use practices influence the diet and health of honey bees is crucial for conservation, yet few studies have focused efforts on analyzing the pollen diets of honey bee colonies across an entire growing season. Identifying floral resources collected by honey bees at a fine resolution would shed insight on critical resources and highlight shortcomings in specific habitat types. Previous research has explored the effect of pollen quantity on colony development (Todd and Bishop, 1940; Eckert, 1942; Keller et al., 2005b). The development of larvae, also known as brood, is strongly dependent on the presence of pollen (Keller et al., 2005b). Honey bee larvae receive nourishment from foraged pollen through two mechanisms. For the first three days upon hatching, larvae are fed a substance called royal jelly which is secreted from the hypopharyngeal glands of worker bees (Standifer, 1980). The development of the hypopharyngeal gland is largely dependent upon sufficient protein nutrition within the first week of the adult worker bee’s life (Standifer, 1967), pollen being the source of worker bee protein (Standifer, 1980). Poor pollen nutrition can reduce hypopharyngeal gland development, which would likely influence a colony’s ability to nourish larvae numbers (Standifer, 1967). After three days of a royal jelly diet, larvae are switched to a diet of pollen and nectar (Crailsheim, 1990), a mixture known as bee bread. Shortages or absences in pollen presence can force nurse bee workers to rely on bodily stores to nourish developing larvae, or in extreme cases, young larvae may be cannibalized by workers (Schmickl and Crailsheim, 2001). Deficient pollen forage impedes the growth of colonies, due to how this resource regulates larval production and development, and therefore colony growth (Allen and Jeffree, 1956; Fewell and Winston, 1992). The size of a colony is a strong indicator of overwintering probability, with larger hives more frequently making it through winter, which makes the matter of an adequate pollen diet crucial to colony fitness (Seeley and Visscher, 1985).

While the effect of pollen quantity on colony development is relatively well studied, little research has been done on the influence of pollen species richness within the diet of honey bee colonies. Pollen is easy to collect and is recognizable. The morphology of pollen grains are taxonomically distinct and the nutritive value of taxa are quite variable (Wodehouse, 1935; Levin and Haydak, 1957). Pollen quality has been shown experimentally to influence pollinator physiology (Levin and Haydak, 1957; Standifer, 1967; Di Pasquale et al., 2013). Understanding how land use influences pollen species richness is valuable, as pollen diversity has been shown to influence the physiology of parasitized honey bees (Di Pasquale et al., 2013). A pollen diet that is species rich would provide a greater array of the fatty acids, amino acids, and vitamins and minerals necessary to optimize colony health and enhance resilience to prevalent stressors affecting bee populations today (Wahl and Ulm, 1983; Schmidt et al., 1987; Di Pasquale et al., 2013).

This study assessed how human-driven land use change affects the resources used by honey bees, and therefore their nutrition. Nutrition is important to colony health (Brodschneider and Crailsheim, 2010; Huang, 2012), making this work of ecological and practical importance. Bee-collected pollen was collected and analyzed from colonies in areas that were dominated by Native/Semi-native, Urban, or Cropland land use types. Pollen abundance (mass) and species richness (number of taxa) collected by honey bees were studied to assess the diet of honey bees throughout an entire growing season to identify key resources and their availability throughout time and space. Colony nutrition is an indicator of hive success (Sponsler and Johnson, 2015); how this is influenced by prevailing land use around honey bee colonies therefore has important implications for understanding honey bee health.

As many land use modifications alter plant communities, considerable variation across land use types was expected. Colonies located in cropland were expected to see the greatest reduction in pollen taxonomic richness. Results from this research could begin to shed light on the role or forage value of common Midwestern crops such wheat or corn. Cultural practices such as mono-cropping and frequent herbicide use undoubtedly reduce floral diversity, therefore negatively impacting pollinators (Williams, 1986; Kremen et al., 2002), which was expected in this study. The impact of urban areas on pollinator forage was expected to be more varied. Greater density of flowering trees/shrubs, gardens, and horticultural plantings could provide an abundance of resources. While urban forage may be plentiful, it is likely clumped (Plascencia and Philpott, 2017). Native/Semi-native sites were predicted to provide colonies with the greatest abundance of resources (Kremen et al. 2002; Smart et al., 2016b). Consequently, it was hypothesized that land use alterations such as urbanization and row-crop agriculture would cause reductions in the mass and diversity of pollen collected by honey bee colonies. A positive relationship between the proportion of native prairie habitat and pollen mass and diversity was predicted, as potential forage resources were expected to be the least impacted by anthropogenic development.

MATERIALS AND METHODS

Study area

The area of research surrounded and included the city of Hays, located in Ellis County in western Kansas (38° 52′ N, 99° 20′ W). The pre-European settlement native habitat of the study region is mixed grass prairie (Albertson, 1937), though settlement has done much to alter this. Smaller urban areas have been established in the area, which comes with other anthropogenic development like roadways, contributing to habitat fragmentation. The most widespread land alteration in the region is the presence of monocrop agriculture, consisting of crops such as wheat and sorghum (USDA/NASS, 2017).

Study colonies of honey bees were placed in one of three separate land use types: Urban, Cropland, or Native/Semi-native. Land use treatment categories were assigned to hives by determining the dominant land use within a 1.5 km radius, as mean foraging distances are typically within this range (Steffan-Dewenter and Kuhn, 2003; Couvillon et al., 2014). Arc GIS 10.5.1 (2018 ESRI; Redlands, CA, USA) was used to determine the area of each of the broad land use categories (Urban, Cropland, Native/Semi-native) within the foraging range of each colony to calculate the relative proportion of land use. The principle investigator had access to nineteen potential study sites, of which, the area of each broad land use category was determined to select the three most representative study sites to place colonies for each land use treatment (Urban, Cropland, or Native/Semi-native). All colonies were located within Ellis County and were kept independent from one another, allowing for no overlap of the 1.5 km radius foraging ranges.

Experimental design

Individual honey bee colonies respond, behave, and grow differently due to a range of factors. This study sought to assess the impact of environmental influences while reducing the presence of other variables that may be genetic or anthropogenic in nature. To reduce the impact of natural variation in the behavior of colonies across treatments, 3 replicate colonies per treatment were established. All study colonies were started from artificial swarms (packages) to ensure equal population size of individual colonies at the point at which data collection had begun. Packages were obtained from one source and contained queens of the same Italian subspecies, *Apis mellifera ligustica*, reducing genetic variability to the greatest practical extent. All colonies were maintained by the same person to ensure management methods across all study colonies were uniform, further eliminating possible variables that could influence results of this work.

Colony population estimation

Throughout the study, colony population was estimated to standardize pollen mass measurements to colony population size, which varied between colonies. Langstroth style bee hives are used nearly universally among beekeepers, especially by the commercial beekeeping industry (Sagili and Burgett, 2011). This design contains frames, precisely spaced, which hang from the top ends of the hive. These frames house a sheet of foundation in which the bees build their comb. The wax comb contains cells which will store either pollen, honey, or developing larvae. Adult honey bees, when inside the colony, are found on the surfaces of these frames. Relative colony population was estimated by counting the number of frames which contained ≥ 75% bee coverage on both surfaces (Mueller, 2012). Frames which contained less than 75% coverage were combined to contribute to the final colony population estimate (Mueller, 2012). Frame counts were made for all study colonies during narrow windows of time (~2 hours) to eliminate bias caused by the influence of daytime on foraging behavior (Delaplane et al., 2013). All bee coverage estimates were made by the same individual to further reduce any bias using this estimation (Delaplane et al., 2013).

Study colonies were "split," as needed, in order to prevent swarming. Swarming is a colony-level reproductive behavior in which up to 50-60% of adult bees, along with the colony's old queen, leave the crowded parent colony in search of a new home (Winston, 1987). This behavior occurs most commonly as a colony's population rapidly grows during late spring and early summer (Caron and Connor, 2013). Splitting a colony is a common beekeeping management practice in which the beekeeper is deterring natural swarming behavior through manipulating the process.

During this experiment, splits were made by removing a few frames of capped brood with adhering nurse bees, transferring them from the crowded parent colony to a vacant hive, along with a frame of pollen and honey (Caron and Conner, 2013). All frames removed from the parent colony were replaced with empty combs, allowing the queen room to continue laying eggs. Colony population estimates were made before and after splits, which all occurred on June 4, 2019 (Table 1).

Colony ID (Land use type)	Split or not	Pre-split population estimate (frames of adult bees)	Post-split population estimate (frames of adult bees)
Bruce (Native/Semi-native)	Split	14	8
Chuck (Native/Semi-native)	Split	18	10
Jensen (Native/Semi-native)	Split	18	11
Ellis (Urban)	Not split	6	6
Randall (Urban)	Split	16	12
Terry (Urban)	Split	18	12
Armbruster (Cropland)	Not split	9	9
Clint (Cropland)	Split	15	9
Todd (Cropland)	Not split	11	11

Table 1. A count of frames occupied by honey bees before and after splits were made to estimate colony population across land use treatments. As the colony population size between individual hives was variable, not all colonies required splitting. All splits were made on June 4, 2019

<u>Pollen collection</u>

Mann Lake 10-frame superior pollen traps (Mann Lake, Hackensack, MN) (Figs. 1 and 2), which are used to sample a portion of the pollen collected by bees (O’Neal and Waller, 1984; Odoux et al., 2012), were placed on study colonies to collect data on season long forage. A total of nine study colonies were utilized, with three replicates per land use type. Pollen traps were opened for 24 hour periods, every 7-14 days for the span of the growing season (April 1 to September 30). Pollen was collected upon trap closure for lab analysis and plant taxonomic identification.

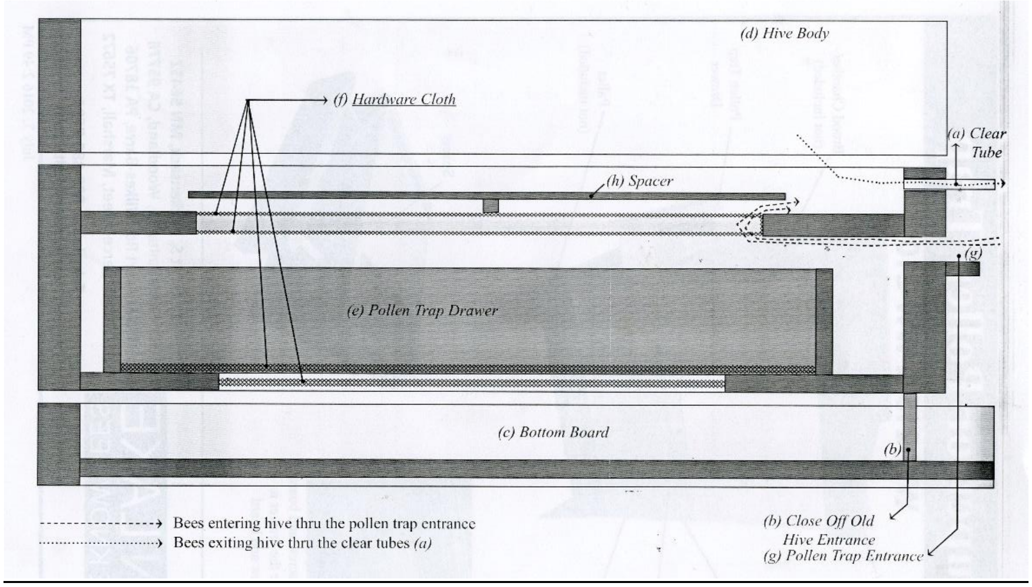

Figure 1. A diagram of the pollen collecting mechanism in *Mann Lake* 10-frame superior pollen traps used in this experiment to collect pollen during April-September 2019. When tubes are inserted, incoming bees enter through a secondary entrance, forcing them to move through screening mechanisms which remove a portion of corbicular (bee-collected) pollen. When tubes are not inserted, returning foragers can bypass the screening mechanism, retaining all corbicular pollen (diagram from Mann Lake, Hackensack, MN).

Figure 2. *Mann Lake* 10-frame superior pollen trap used during this study to collect pollen during April-September 2019 with trapping mechanism engaged. Bees exiting the hive leave using the plastic tubes, bypassing the pollen trapping mechanism. Returning foragers enter through the smaller horizontal entrance below the tubes, directing them through the screening mechanism to sample pollen. Tubes are removed to disengage trapping mechanism.

<ins>Pollen analysis</ins>

Upon arriving at the lab, mass (g) of the entire sample from each hive was determined to assess quantity of overall pollen collection. Pollen from each trap was thoroughly mixed to eliminate any spatial or temporal bias in the collection process, then one gram of this sample was used for further analysis and floral species identification.

A pollen reference library was produced directly from the local floral sources. Pollen that was collected directly from flower anthers was used to identify corbicular pollen samples. This reference aided in the overall identification of bee collected pollen, as well as gave insight to what was available to pollinators at a given time in the season. Other sources for unknown pollen identification included online pollen databases such as The Global Pollen Project (globalpollenproject.org) and dichotomous keys in Wodehouse (1935) and Erdtman (1971).

The methods used for staining pollen for the pollen reference library were modified from Kearns and Inouye (1993). Pollen grains were placed on a watch glass and stained with 0.1% Fuchsin (w/v in H_2O). Stain was rinsed with 95% ethanol until clean, then rehydrated using a 1:1 glycerol:H_2O mixture. Using a stirring rod, rehydrated pollen grains were transferred to slides containing a small amount of mounting medium (1 g gelatin, 60 mL deionized H_2O, and 70 mL glycerin). Slides were covered with a coverslip and examined under a compound light microscope at 400X. Images of pollen were captured with an Olympus DP71 digital camera (Olympus, Tokyo, Japan) mounted on an Olympus BX51 compound light microscope (Olympus, Tokyo, Japan).

The process of determining the taxonomic identities of collected pollen grains was addressed through expert identification. Fifty-four one gram subsamples of pollen pellets were shipped overnight on dry ice to the Palynology Laboratory at Texas A&M University (College Station, TX). Upon arrival, mixed pollen pellets from each subsample were homogenized into one suspension, subsamples were then extracted and mounted on slides for analysis. Approximately 300-400 pollen grains were identified and counted on slides from each subsample, as this quantity of pollen grains has previously been documented to provide a uniform representation of pollen species diversity, using the described methods of taxonomic identification (Jones and Bryant, 1998). In some cases, pollen can be identified to species. In other cases, pollen can only be identified to genus or family levels.

Taxonomic richness of pollen collected by study colonies was determined by counting the number of different taxa (species, genera, or families, as appropriate) observed per sample and calculating the mean for each respective treatment. Important pollen sources for each treatment were identified by determining the five most commonly encountered taxa throughout the growing season.

Statistical analysis

Data for pollen mass (g), pollen mass (g) per frame of bees, and taxonomic richness (number of taxa) were analyzed with repeated measures analysis of variance, with sampling time as the repeated effect. Normality of data was verified with a Shapiro-Wilk test. Comparisons were made among treatments and the treatment x time interaction. All statistical analyses were performed with SPSS Statistics 22.0 (2013 IBM Corp.; Armonk, NY, USA) at $\alpha = 0.05$.

RESULTS

Colony population size

Population size was uniform across treatments at the start of the experiment, with three frames of bees per hive (Fig. 3), as all study colonies were started from uniform packages. As spring progressed and colony population grew, there were trends with the Urban colony population growing the quickest, up to 17.5 frames of bees per hive. Native/Semi-native colonies reached 14.7 frames of bees per hive, which grew slightly faster than Cropland colonies, which only reached 13 frames of bees per hive, and were the lowest overall colony populations throughout the experiment (Fig. 3). Although these trends occurred, there was no statistically significant difference between treatments (P = 0.620), nor was there an interaction between treatment and time (P = 0.371).

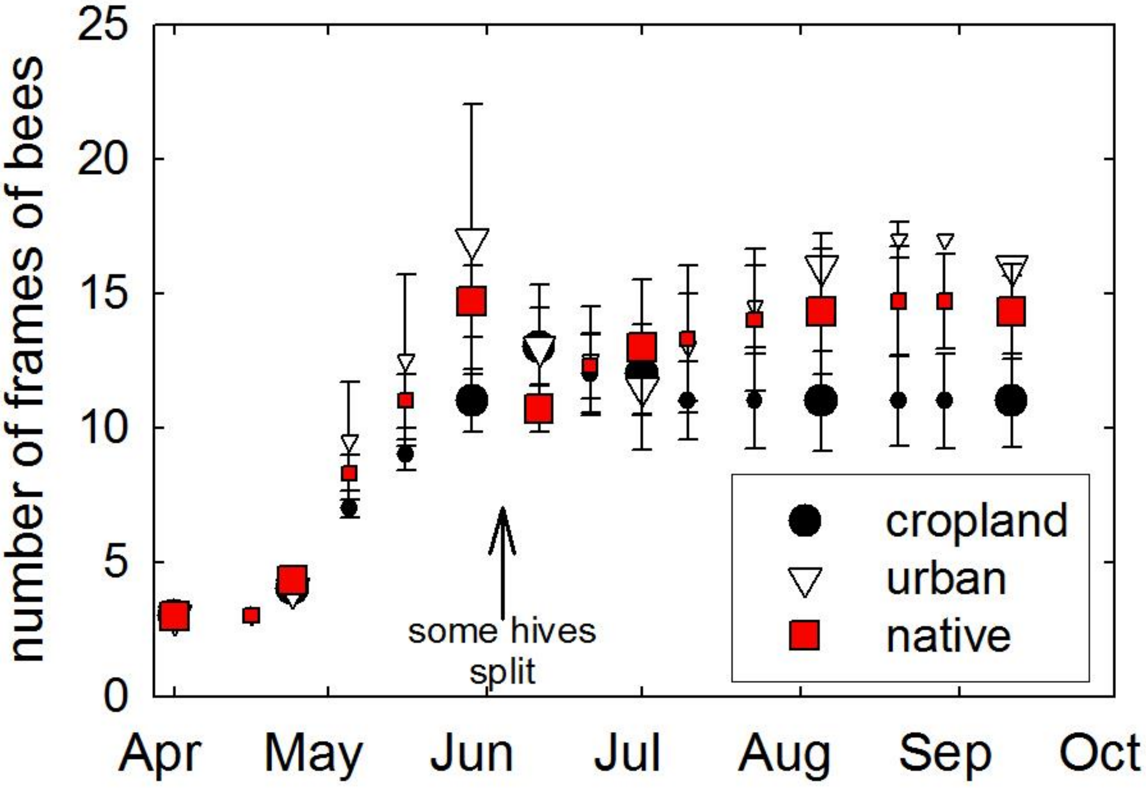

Figure 3. Colony population size estimates of honey bees throughout the 2019 growing season (April 1- September 30) in Ellis County, Kansas, calculated by counting the number of frames containing ≥75% bee coverage. Points are means from 3 replicate colonies ± SE. Larger data points represent empirically collected data while smaller points are interpolated estimates of colony size. Splits were made on colonies which required it on June 4, 2019.

Pollen analysis

Pollen mass collected per 24 hours ranged from 0.7 to 187.5 g across treatments and weeks (Fig. 4). Pollen mass varied greatly throughout time and across treatments, with no statistical significance between treatments ($P = 0.100$), nor was there an interaction between treatment and time ($P = 0.121$). There was a great deal of variation among replicate colonies, making it difficult to detect differences. The “Randall” replicate within Urban colonies, the “Armbruster” replicate within Cropland colonies, and the “Jensen” replicate within Native/Semi-native colonies responded differently than other replicate colonies within respective treatments, with consistently lower pollen mass than other colonies, contributing to the difficulty in detecting statistically significant differences. However certain trends in pollen mass were observed. On days of increased pollen collection, Urban colonies consistently displayed higher pollen mass, with pollen masses up to 187.5 g. Cropland colonies seemed to display reduced pollen mass in comparison with the other treatments, with all pollen collections being less than 30.3 g (Fig. 4). Major peaks in pollen harvest occurred in May and August, corresponding with blooming periods of willows (*Salix*) in May, maize (*Zea mays*), and various grasses (Poaceae) in August. Lesser peaks in pollen harvest occurred in every month of the growing season (Fig. 4), corresponding with blooming periods of Rosaceae in April, clovers (*Melilotus/Trifolium*) in May, June, and July, and Chenopodiaceae/Amaranthaceae in August and September (Table 2).

Processing sample #:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Treatment	Native	Cropland	Urban	Urban	Urban	Cropland	Cropland	Native	Native	Cropland	Urban	Native	Urban	Urban	Cropland	Cropland	Native	Native	Cropland	Urban	Native	Urban	Urban	Cropland	Cropland	Native	Native	Cropland	Urban	Native	Urban	Cropland	Urban	Cropland	Native	Native	Cropland	Urban	Native	Urban	Urban	Cropland	Native	Native	Cropland	Urban	Native	Urban	Urban	Cropland	Cropland	Native	Native	Cropland
Date	4/7	4/7	4/7	4/7	4/7	4/7	4/24	4/24	4/24	4/24	4/24	4/24	4/24	4/24	4/24	5/16	5/16	5/16	5/16	5/16	5/16	5/16	5/16	5/16	6/11	6/11	6/11	6/11	6/11	6/11	6/11	6/11	6/11	7/23	7/23	7/23	7/23	7/23	7/23	7/23	7/23	7/23	8/20	8/20	8/20	8/20	8/20	8/20	8/20	8/20	9/12	9/12	9/12	9/12
Acer				49	299						3			10						15		5	2																															
Agave																																														6								
Allium																										150					69																							285
Ambrosia																																											1	64			20			154				
Argemone																																		200	207	137			245															
Asteraceae-short spines (ragweed, etc.)												14																																		2					104		3	
Asteraceae-long spines (sunflower, etc.)																																		1		59		35	1															8
Asteraceae-Lactuceae (dandelion, etc.)			5				31	10	10			125	3							5																																		
Brassicaceae		250	192			54									37		114	63		1																																		
Cheno-Ams																																					309					290	2	1	3	146	57				37	122	303	7
Chilopsis linearis																																							1															
Convolvulus																																									2													
Cirsium																																				29			23															
Cornus																										53			2	25																								
Cucurbita																																						3	34															
Cynoglossum				1																																																		
Cyperaceae		25															2	22		15																																		
Diospyros										3					22																						1																	
Erodium																			4																																			
Eucalyptus																																														5								
Gleditsia																																		53	45																			
Lonicera																																				100	1				20													
Melilotus/Trifolium (all clovers)			1								1			1						50					300	114	165	300	280	300	175	290	305	7	7			276	2		6	3				1								
Mimosa																													38																									
Morus																											142																											
Oenothera																																															4					4		
Onobrychis																																		71	52		16																	
Poaceae																																								20			262	92	4	151	129	500	70	140				18
Polygonum																																																		23				
Populus	312	39		23	15	5								1																																								
Quercus																								1																														
Ranunculus			28				82	23																130																														
Ribes														12																																								
Rosaceae			100	206	8		7	16		313	5	144	211	283	77					5		5		3							48																				220	117		
Rumex																																22																						
Salix	1					255	202	268	330		314	33	114	1	173	310	196	232	310	220	300	315	300	177		1																												
Salvia																																								17												2		
Silene (Caryophyllaceae)				26																																																		
Solanum																																								70				166	350	2						149		
Tribulus terrestris																																		1	1	3	2		18		295	34		2			1							
Ulmus	23																																																					
Unknown #6					15	5																																																
Unknown #9											7																																											
Unknown #10 (Caryophyllaceae)																																						12																
Unknown #11																															20																							
Unknown #25																																								210														
Unknown #27																																											89											
Unknown #28																																																	238					
TOTAL RAW COUNTS	336	314	326	305	337	319	322	317	340	316	330	316	328	308	309	310	312	317	314	311	300	325	302	311	300	318	307	300	320	325	312	312	305	333	312	328	329	326	324	317	323	327	354	325	357	313	211	500	308	317	361	394	306	318

Table 2. Taxonomically identified pollen sources collected by honey bee colonies across time and land use treatments (Urban, Cropland, or Native/Semi-native) in Ellis County, Kansas during the 2019 growing season (April 1 – September 30). Subsamples were collected from each site and were homogenized to evenly mix all pollen sources collected within a subsample, then approximately 300 grains were counted and identified per subsample.

When standardized to colony size, pollen collected per 24 hours ranged from 0.05 g to 23.4 g per frame of bees across treatments and weeks (Fig. 5). Patterns were similar to overall pollen mass, with Urban colonies trending toward greater mass of pollen per frame of bees. However, there was no statistically significant difference between treatments (*P* = 0.537), owing to small sample size and a great deal of variation. Similarly, there was no interaction between treatment and time (*P* = 0.546). Major peaks in pollen harvest occurred in April, May, and August, corresponding with blooming periods of Rosaceae in April, willows (*Salix*) in May, maize (*Zea mays*), and other grasses (Poaceae) in August (Fig. 5). Lesser peaks in pollen harvest occurred in every month of the growing season (Fig. 5), corresponding with blooming periods of maples (*Acer*) and Brassicaceae in April, clovers (*Melilotus/Trifolium*) in May, June, and July, and prickly poppies (*Argemone*) and Chenopodiaceae/Amaranthaceae in August and September (Table 2).

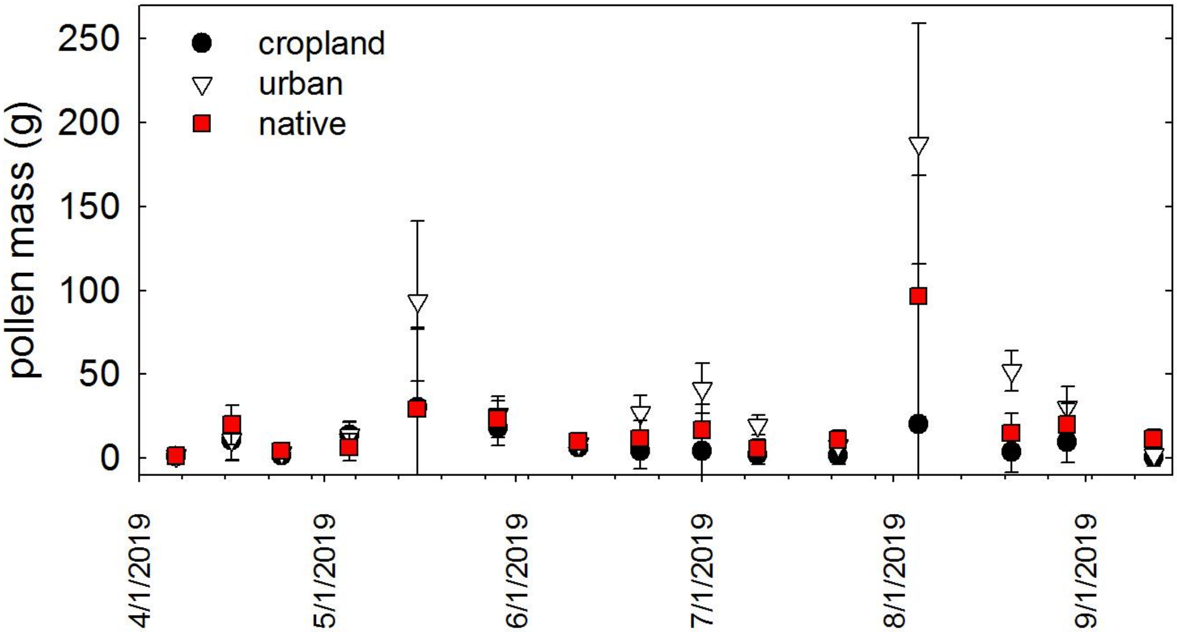

Figure 4. Pollen mass collected per 24 hours by honey bee colonies within land use treatments (Urban, Cropland, and Native/Semi-native) across the 2019 growing season (April 1 - September 20) in Ellis County, Kansas. Higher pollen masses corresponded with blooming periods of important plant species. Points are means from 3 replicate colonies ± SE.

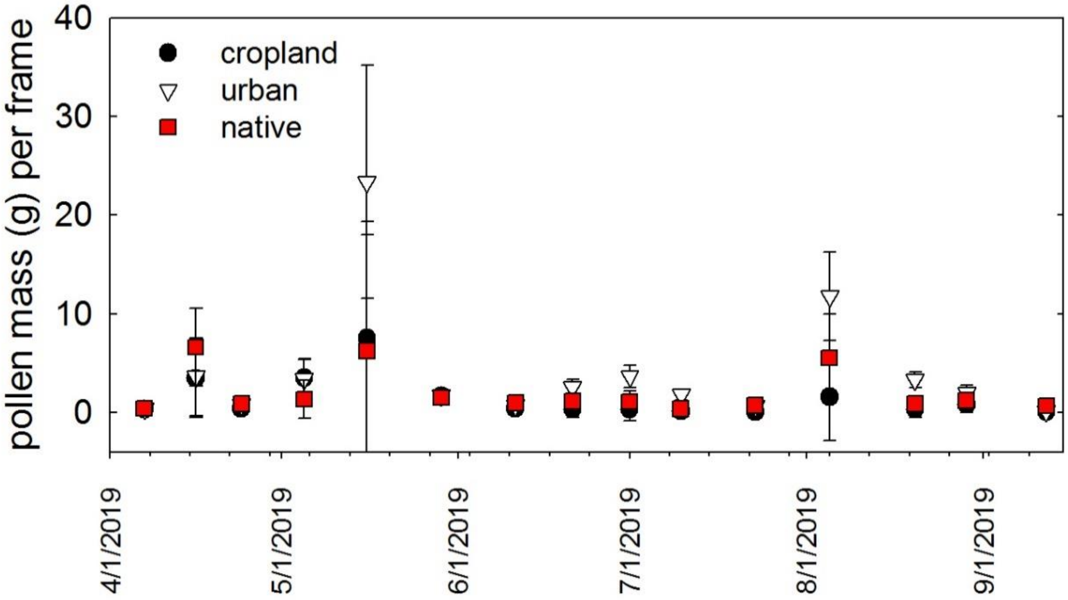

Figure 5. Pollen mass collected per frame of honey bees per 24 hours within colonies across land use treatments (Urban, Cropland, and Native/Semi-native) throughout the 2019 growing season (April 1 – September 30) in Ellis County, Kansas. This representation portrays pollen collection standardized to worker bee population within colonies. Points are means from 3 replicate colonies ± SE.

Taxonomic identification of pollen

The morphology of pollen grains have similarities based on taxonomic relationships (Erdtman, 1971). Plant families tend to contain certain morphologic characteristics which provide some distinction from other families. The number and arrangement of openings, known as apertures, are the main features that make pollen grains unique, along with the texture of the outer pollen layer, known as exine (Wodehouse, 1935). Most commonly, apertures come in the form of pores or fissures. Pores resemble small holes in the exine and fissures (colpi) are typically more elongated apertures in the exine (Wodehouse, 1935). Exine texture is quite diverse among plant families (Erdtman, 1971).

Pollen was collected directly from the flower anthers of 130 local species, spanning 45 plant families, for the pollen reference library used to aid in identification of unknown pollen grains. Observed pollen grains ranged in size from small grains (10-25 µm) in members of Lamiaceae, Fabaceae, and Brassicaceae, to large grains (100-200 µm) in members of Malvaceae, Cucurbitaceae, and Onagraceae. More diverse plant families tended to have greater diversity of pollen morphology. Though not exclusive, large spines or ridges in the exine were characteristic of Asteraceae pollen. Many of the collected mint species (Lamiaceae) had quite small pollen grains with 6 colpi and a thick exine with reticulate texture. The most commonly observed positioning of pollen grain apertures was tricolporate, which have three colpi, also containing a small pore within each of these furrows.

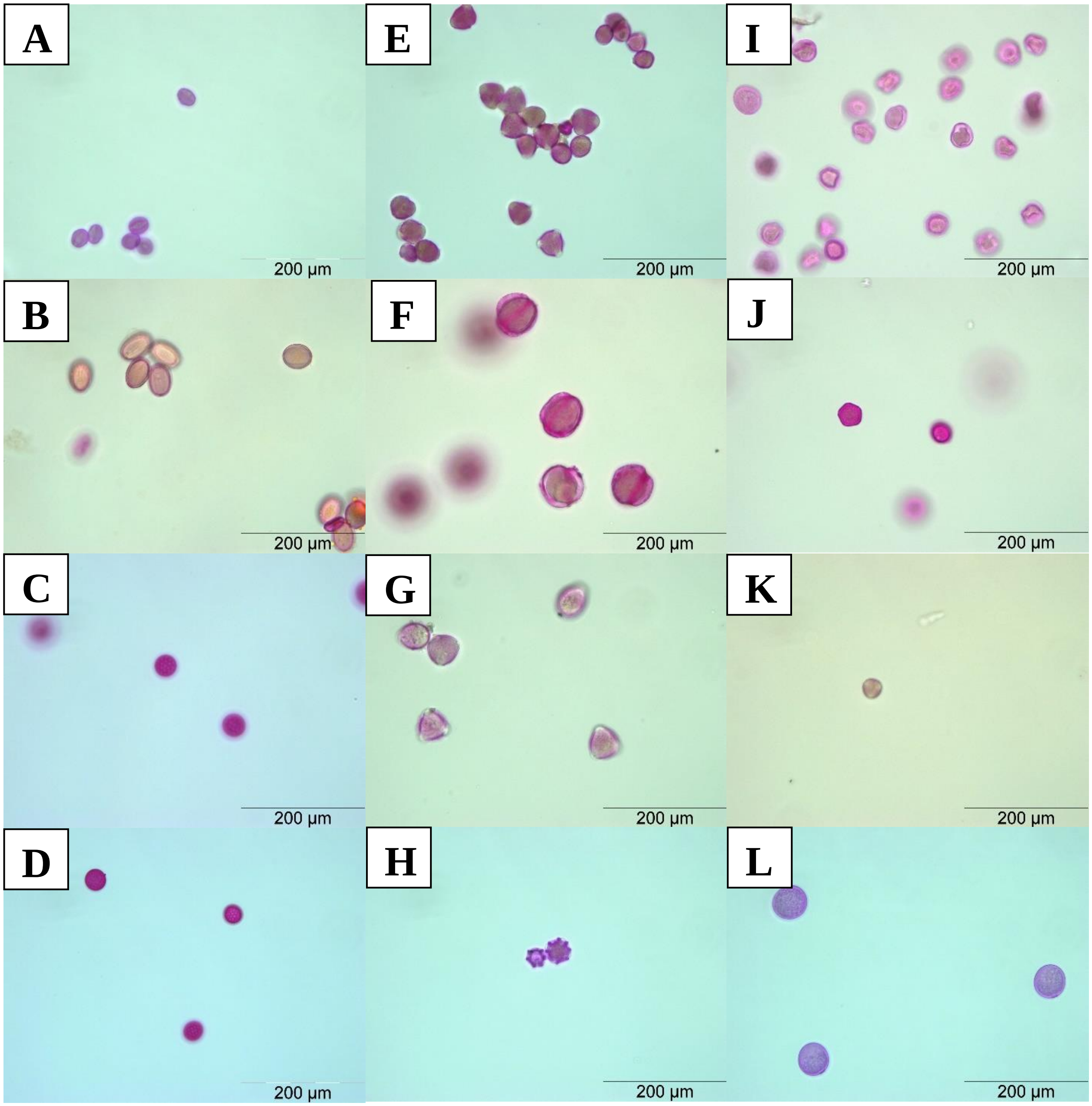

Figure 6. Pollen grains collected directly from floral sources for aid in taxonomic identification. **A.** Yellow sweet clover (*Melilotus officinalis*), **B.** Purple prairie clover (*Dalea purpurea*), **C.** Kochia (*Kochia scoparia*), **D.** Lamb's quarter (*Chenopodium album*), **E.** Bradford pear (*Pyrus calleryana*), **F.** Apple (*Malus* sp.), **G.** Sandhill plum (*Prunus angustifolia*), **H.** Dandelion (*Taraxacum* sp.), **I.** Cottonwood (*Populus*

deltoides), **J.** American elm (*Ulmus americana*), **K.** *Erysimum,* **L.** Big bluestem (*Andropogon gerardii*).

A total of 41 different taxa were identified from the experimental hives, with an additional seven pollen sources which were unable to be identified (Table 2). Of the 48 observed taxa, 26 were observed within the Cropland treatment, 29 were observed within the Native/Semi-native treatment, and 33 were observed within the Urban treatment. There were no statistically-significant differences in taxonomic richness between treatments (*P* = 0.217), nor was there an interaction between treatment and time (*P* = 0.214).

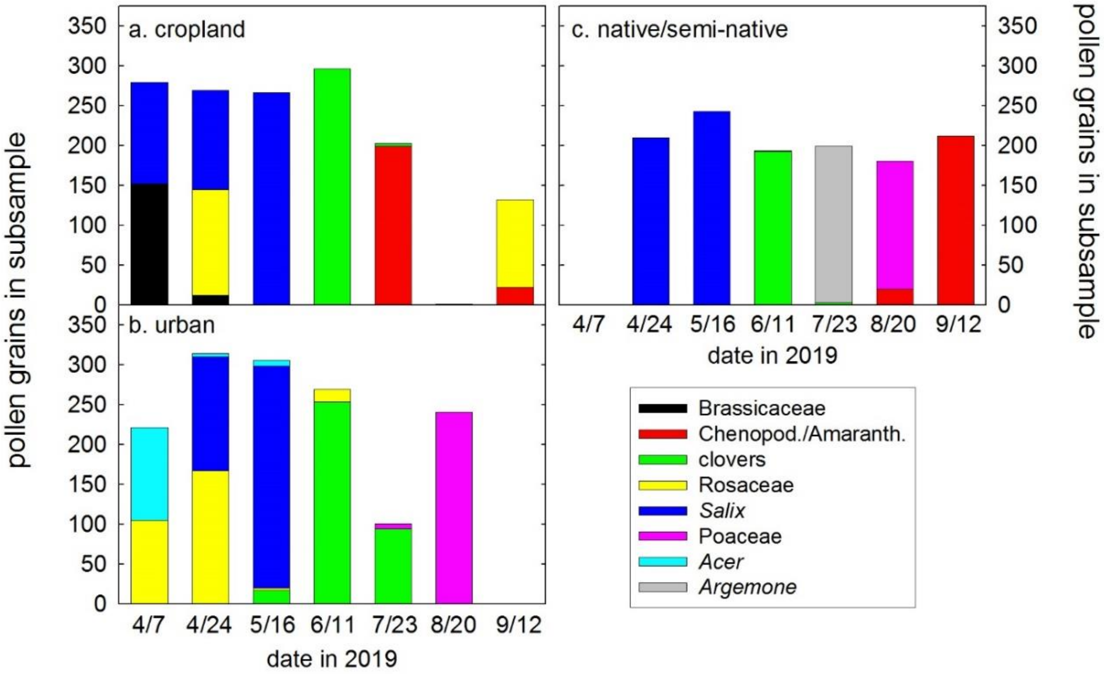

Figure 7. Key taxonomic resources across time and land use treatments (Urban, Cropland, and Native/Semi-native) throughout the 2019 growing season (April 1 – September 30). Approximately 300 pollen grains were counted and identified per

subsample. The five most abundant pollen sources per treatment were chosen as key taxonomic resources, with results shown as stacked bar graphs. Segments of bars represent proportion of pollen samples from a specific taxon. Means of 3 replicate samples are graphed for each.

Certain similarities were seen regarding floral resources between treatments (Table 2). Willows (*Salix*) provided a considerable quantity of pollen early in the year for nearly every colony, regardless of the land use treatment. It is suspected that elm species (*Ulmus*) would have been collected earlier in the growing season than willows, providing an abundant early pollen source across most land use types in the region (Keller et al., 2005a; Fortunato et al., 2006). However, the blooming period for elms was prior to data collection and as a result was not detected. Clover species (*Melilotus* and *Trifolium*) provided an abundant mid-season pollen source for all colonies. Grass (Poaceae) species provided an abundant late season source of pollen forage for most colonies across land use treatments, surprisingly, with Cropland colonies collecting the least amount of these taxa. Another unexpected finding was how little Asteraceae pollen was collected throughout the study. While aster pollen was observed within each treatment, and to some degree, throughout the entire season, it never composed a large portion of any of the analyzed samples. The most commonly observed tribe was Cichorieae, the floral source of which was likely dandelions (*Taraxacum*) or closely related species.

A few interesting differences in identified pollen sources were observed between treatments (Table 2). Maple species (*Acer*) provided an abundant early source of pollen for Urban colonies, but were not detected in Cropland or Native/Semi-native colonies.

Grass pollen (Poaceae) was a major source of late-season forage for both Urban and Native/Semi-native colonies. In Cropland habitats, Brassicaceae provided a large portion of pollen foraged early in the season. While Brassicaceae pollen was observed in all treatments, it was only a minor component of early Urban and Native/Semi-native pollen forage. A similar trend was observed with Chenopodiaceae/Amaranthaceae pollen, which composed an abundant source of late-season pollen in Cropland and Native/Semi-native colonies, while playing a very minor role in the diet of Urban colonies.

Cottonwoods/poplars (*Populus*) provided a minor source of pollen for colonies in Native/Semi-native land use treatments early in the growing season. Prickly poppies (*Argemone*) made up a large portion of mid to late-season pollen for Native/Semi-native colonies, while playing a minor role in Cropland colonies and not being observed at all in Urban colonies (Table 2).

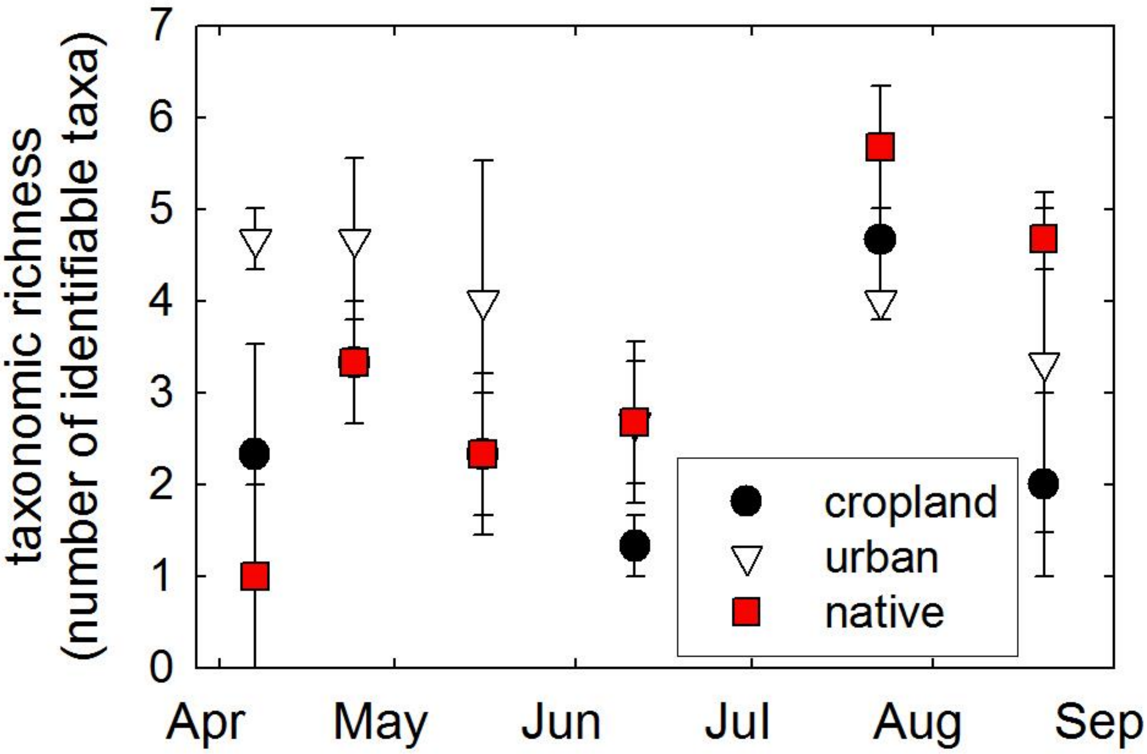

Figure 8. Taxonomic richness of floral resources collected by study colonies was determined for each treatment by calculating the mean number of taxa collected in each treatment. Points are means of three replicates ± SE.

A decrease in the number of taxa collected by colonies was observed in early June across all treatments, with means for Cropland, Urban, and Native/Semi-native colonies being 1.33, 2.67, and 2.67 taxa collected per sample, respectively. This decreased diversity was followed by a general peak in the number of taxa collected, observed in late July across all treatments, with means for Cropland, Urban, and Native/Semi-native colonies being 4.67, 4.00, and 5.67 taxa collected per sample, respectively (Fig. 8). The number of taxa collected in Urban colonies was highest early in the season and decreased over time, while the opposite trend was observed in Native/Semi-native colonies, with the least number of taxa collected at the beginning of the season and increasing over time.

DISCUSSION

Pollen was sampled from Urban, Cropland, and Native/Semi-native habitat in Ellis County, Kansas every 7 to 14 days throughout the 2019 growing season (April 1 to September 30). Pollen was identified taxonomically to provide insight on the abundance and diversity of floral resources available to honey bee colonies. While differences in the amount of pollen collection were not statistically significant, Urban colonies displayed a trend toward increased pollen collection during blooming periods of important floral sources for honey bees, such as willows (*Salix*), yellow sweet clover (*Melilotus officinalis*), and grasses (Poaceae). Similarly, no statistically significant difference was found between treatments regarding the amount of floral taxa collected. However, there were interesting distinctions between land-use types. Trees were an important pollen source in urban habitat, with pollen sources such as Maple (*Acer*) detected solely in Urban colonies. Members of Brassicaceae, likely weedy species, were more abundant early in the year in Cropland colonies in comparison to other treatments, as well as members of family Amaranthaceae, which was observed late in the season. Grasses (Poaceae) were an important source of late season forage for both Native/Semi-native and Urban colonies.

The hypothesis that land use alterations such as urbanization and row-crop agriculture would cause reductions in the mass and diversity of pollen collected by honey bee colonies was not supported. Due to small sample size, the variability among hives made detecting differences between treatments difficult. General trends observed in measured variables, such as pollen mass and colony population size, may have become more evident with an increased number of study colonies. Additionally, the hypothesized relationship between the proportion of native prairie habitat and pollen mass and diversity was not observed. Although there was a trend for reduced pollen collection and taxonomic richness in the Cropland treatment, there was a trend for increased pollen collection and taxonomic richness in the Urban treatment. Anthropogenic development seemed to have mixed effects on the resources available to honey bees.

Effects of land use on honey bee colonies

Some clear distinctions between land use treatments were observed during this study. Urban habitat appeared to have a beneficial influence on honey bee colonies in terms of the amount of pollen collected and colony population growth. As pollen is closely linked to rearing offspring (Todd and Bishop, 1940; Eckert, 1942; Keller et al., 2005b), this positive relationship is logical. Environments that provide a greater abundance of pollen would provide the additional protein, fatty acids, vitamins, and minerals to rear more individuals within a given colony. Accordingly, Urban habitat may have a positive impact on the survival of colonies, as larger colonies tend to be more likely to overwinter successfully (Seeley and Visscher, 1985). The opposite could be seen in habitats dominated by row-crop agriculture. Reductions in the amount of pollen collected in Cropland colonies could result in smaller colony populations, which would likely reduce the chances of winter survival.

Anthropogenic development can not only affect the quantity of pollen collected and colony population size, but also the specific floral resources available to colonies. An increased presence of trees in urban areas provided colonies with a significant source of pollen that would largely be absent from rural areas in a prairie ecoregion. Maple (*Acer*) pollen was observed exclusively in urban habitats, providing a source of forage at a crucial time for colonies coming out of winter (Batra, 1985; Somme et al., 2016). Not only is the timing of the maple (*Acer*) bloom important, the genus also provides a high quality pollen for honey bees. *Acer pseudoplatanus* produces pollen that is rich in polypeptide content as well as phytosterols (Somme et al., 2016). When present in the diet of honey bees, certain phytosterols increase individual longevity as well as increase head protein content, which indicates greater hypopharyngeal gland development (Chakrabarti et al., 2019). High quality pollen aids in the rapid and complete development of the hypopharyngeal gland and would aid a colony's ability to rear large larval populations (Standifer, 1967), which possibly provides further explanation for the trend toward increased population size in Urban colonies. The increased presence of certain trees in urban habitat is a unique benefit to pollinators due to the increased density of floral structures when compared with herbaceous plants more typical of the flora in prairies (Somme et al., 2016). For example, the mean flower density of *Acer pseudoplatanus* in peak bloom was 7,582 flowers m^{-2}, compared to 2,883 flowers m^{-2} in alfalfa (*Medicago sativa*) and 114 flowers m^{-2} in the common poppy (*Papaver rhoeas*) (Baude et al., 2016), the latter two being plants typically included in various pollinator seed mixes. While not a statistically significant difference, there was a greater number of taxa collected by bees within the Urban treatment, likely owing to the variety of trees, gardens, weeds, and ornamentals present in an urban environment.

The presence of weeds seemed to be the largest source of pollen forage for honey bee colonies in an agricultural setting. Previous studies have shown conflicting evidence on the quality of agricultural land use on pollinator habitat (Naug, 2009; Requier et al., 2015; Smart et al., 2016b). Some of this variability is likely due to the diversity of agricultural practices, with biodiverse and organic agricultural approaches providing habitat more conducive to the needs of pollinators, resulting in increased pollinator abundance and diversity (Holzschuh et al., 2007; Rundlof et al., 2008; Nicholls and Altieri, 2013). Less diverse, conventional farming methods, such as row-crop systems, leave pollinators more reliant upon the weeds present in such systems (Marshall et al., 2003; Requier et al., 2015). This study provides further evidence for such claims, as pollen collected from spring blooming members of Brassicaceae and late summer/fall blooming Chenopodiaceae/Amaranthaceae species was more prevalent in Cropland colonies in comparison to Urban colonies. The importance of weeds for pollinators in conventional agricultural landscapes raise some concerns about intensive weed control methods currently utilized in North America, such as the rise of genetically engineered crops to tolerate regular herbicide applications (Benbrook, 2012). The combination of row-crop agriculture and intensive weed management generate homogenous landscapes (Hyvonen and Salonen, 2001). This reduction in landscape diversity was reflected in the number of floral taxa honey bees foraged upon, with Cropland colonies collecting fewer numbers of taxa when compared with other treatments.

Of all the land use types assessed, in many regards native habitat seemed to be intermediate in pollen quantity and quality. Trends occurred for Native colonies to collect less pollen than Urban colonies, but more than Cropland colonies. Possibly as a result, Native colonies had slightly smaller colony populations than Urban colonies but trended toward being larger than Cropland colonies. Floral resources which were unique to this treatment include cottonwoods (*Populus*) and prickly poppies (*Argemone*). Cottonwoods

(*Populus*), large trees native to Kansas and commonly found along rivers and streams in the western half of the state (Kellogg, 1905), provided Native/Semi-native colonies an abundant source of early forage. There is mixed evidence on the nutritional quality of *Populus* (Standifer, 1967; Schmidt, 1983; Schmidt et al., 1987). Historically, cottonwood pollen was thought to be of low quality for pollinators (Schmidt, 1983), due to a relatively low protein content (Standifer, 1967). However, when fed to individual honey bees solely, *Populus* pollen was shown to increase the mean life span of bees in comparison to many other examined pure pollen diets (Schmidt et al., 1987). The historical assessment of pollen quality was largely defined solely by protein content (Haydak and Tanquary, 1943; Standifer, 1967), leading to many misconceptions. Proteins, nitrogen, amino acids, phytosterols, lipids, and various vitamins and minerals are found in pollens (Roulston and Cane, 2000). These components undoubtedly play a role on the physiology of adult honey bees as well as larvae. Cholesterol, which is the primary sterol found in cottonwood pollen (Standifer et al., 1968), enhances larval rearing when added to worker bee diets (Herbert et al., 1980), further challenging the historic belief of cottonwood's poor pollen quality. The availability of such a pollen early in the season, which improves larval rearing ability, could be responsible for the increased population size observed in Native/Semi-native colonies when compared to Cropland colonies. A floral resource that can produce vast quantities of a high quality pollen, such as many of the trees described previously throughout the discussion, should be considered of high value to pollinators.

Management Implications

Key resources utilized by honey bees across habitat types were identified, aiding to better inform decisions related to pollinator conservation (Kearns et al., 1998). Determining nutritional deficiencies for honey bees across land use types could help to inform the best possible practices in negating whatever shortcomings a particular land use type may have (Kremen et al., 2002). Seeing deficiencies in corbicular pollen abundance or diversity collected from hives in a specific environment could indicate similar deficiencies for native pollinators (Buchmann, 1996). Comparisons could not only be made about resource availability in a given habitat, but also factors such as pesticide exposure. Corbicular pollen has been used successfully as a bioindicator, assessing pesticide exposure over relatively large areas for minimal cost (Chauzat et al., 2010; Cabrera de Oliveira et al., 2016).

The results of this work help us understand how modern land use influences the nutrition and health of honey bee colonies. Results from this project show what resources are used by honey bees in common types of land use in Kansas: Urban, Cropland, or Native/Semi-native. Understanding the quantity and quality of resources in these habitats is crucial for maintaining the health of managed and native pollinators (Di Pasquale et al., 2016). This is an important subject for beekeepers and ecologists alike, yet little work has been done in this area. This project is therefore of novel importance; a great deal has been learned about the management and ecology of honey bees from this work.

As many land use modifications drastically alter plant communities, considerable variation across land use types was expected. Given the extent to which land use has changed, it is imperative to understand the role or forage value of common Midwestern crops such as corn, wheat, or sorghum, as well as floral sources in urban settings. Colony health depends on nutrition (Brodschneider and Crailsheim, 2010; Huang, 2012; Di Pasquale et al., 2016), so understanding how land use affects honey bee forage is essential for optimal hive management. Colonies located in the cropland treatment saw a trend toward a reduction in pollen diversity. Cultural practices such as mono-cropping and heavy reliance upon herbicide undoubtedly reduce floral diversity and therefore negatively impact pollinators (Williams, 1986). The impact of urban areas on pollinator forage was more varied. Greater density of flowering trees/shrubs, gardens, and horticultural plantings provided a high diversity of resources, however these resources are likely clumped (Plascencia and Philpott, 2017). Native/Semi-native sites provided colonies with an intermediate abundance of resources (Smart et al., 2016b).

Understanding the influences of land use types on pollinator forage has major implications on the health of honey bees (Otto et al., 2016; Smart et al., 2016a; Alaux et al., 2017). Informing beekeepers on optimizing hive placement to improve overall colony health and overwintering chances could result from a better understanding of the relationship between environment and available pollinator resources (Naug, 2009). Informing beekeepers in the region of floral resources that provide significant forage could positively influence decisions pertaining to hive placement. The nutrition of bee bread within honey bee colonies is related to landscape composition, with higher protein contents observed in hives located in semi-native or native habitats (Donkersley et al., 2014). Studying the quantity and diversity of pollen collected by honey bee colonies located in varying habitats would provide insight on colony nutrition as influenced by habitat, which has previously been shown to be a potential indicator of hive success (Sponsler and Johnson, 2015).

The seriousness that lies in the issue of pollinator declines is largely due to the ecological services that are provided by pollinators (Kearns et al., 1998). Moreover, managed and native pollinator population declines have major implications towards feeding a growing global population (Sass, 2011). Little research has been done on the influence of land use on the pollen diet of honey bee colonies in prairie landscapes. Understanding how human-driven land use change impacts the nutrition of managed honey bees is important in pollinator conservation efforts and contributes towards combating pollinator declines.

Knowledge from this study could help shape restoration efforts and help to better inform land management decisions to minimize impact on pollinator populations. Results will also help to inform beekeepers on colony placement in hopes of improving colony health and reducing overwintering losses.

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