EFFECTS AND ASSOCIATIONS OF THE

PRODUCTION OF THE BIOFUEL CROP *BRASSICA*

NAPUS ON WILD INVERTEBRATE POLLINATOR

COMMUNITIES IN THE SOUTH CENTRAL UNITED

STATES

By

SHAUN MICHAEL MCCOSHUM

Bachelor of Science in Biology
Humboldt State University
Arcata, California
2006

Master of Science in Botany
Miami University
Oxford, Ohio
2009

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2015

EFFECTS AND ASSOCIATIONS OF THE

PRODUCTION OF THE BIOFUEL CROP *BRASSICA*

NAPUS ON WILD INVERTEBRATE POLLINATOR

COMMUNITIES IN THE SOUTH CENTRAL UNITED

STATES

Dissertation Approved:

Kristen A. Baum

Barney Luttbeg

Kristopher L. Giles

Samuel D. Fuhlendorf

ACKNOWLEDGEMENTS

First and foremost I must thank my adviser for the wonderful guidance and support throughout my PhD experience. In addition to the research to earn this degree, Dr. Baum encouraged me to engage the public, take on side projects and courses to expand my research topics, and participate in both the university and local community. I also owe thanks to my committee for assisting me in designing data collection methods, and analyses.

During my time here at OSU, I have also had wonderful mentors while volunteering for Oklahoma State University. I am sincerely grateful to the Dean and Associate Deans of the Graduate College: Dr. Sheryl Tucker, Dr. Ken Clinkenbeard, Dr. Jean Van Delinder; the Vice President of Student Affairs, Dr. Lee Bird; and the interim Associate Vice President for Research Dr. Van Den Bussche, for their patience and guidance which enabled me to engage the university and local community.

These projects which make up my dissertation, as well as all of the side projects I was able to complete, were also possible through the support of my family, and I am eternally grateful. I also want to express gratitude and appreciation to my friends and colleagues who have helped me talk out ideas, relax and take breaks, as well as push me to succeed in all of my endeavors.

Name: SHAUN MICHAEL MCCOSHUM

Date of Degree: DECEMBER 2015

Title of Study: EFFECTS AND ASSOCIATIONS OF THE PRODUCTION OF THE
            BIOFUEL CROP *BRASSICA NAPUS* ON WILD INVERTEBRATE
            POLLINATOR COMMUNITIES IN THE SOUTH CENTRAL UNITED
            STATES

Major Field: ZOOLOGY

Abstract: The addition of biofuel crops to the agricultural landscape is drastically
changing habitats around the world with unknown environmental consequences.
Agricultural conversion of land negatively affects pollinators which are in decline
globally, but semi-natural lands such as pasture can have positive effects on pollinator
communities. Pollinators provide free ecological services and increase crop production of
entomophylous plants. The biofuel crop canola (*Brassica napus*) provides floral
resources that may benefit pollinators, and has higher yield when pollinated. Conversely,
insect pests can substantially reduce canola production, so 90% of canola fields are
treated with insecticides, many of which can be harmful to pollinators. In the South
Central US, canola is generally planted in rotation with winter wheat, and has increased
from 0 to 109,265 hectares since 2007. We tested whether pollinator abundance, richness,
and diversity within and around canola fields were different in areas with and without
pasture during and after canola bloom. Bees were captured (2011-2014) during April
(peak canola bloom), (2012-2014) June (post-harvest when no more flowers were
available) and (2012-2014) August (several months after canola had been harvested),
using blue vane traps for 48 hours each period. Butterflies were captured using yellow
sticky traps (2011-2013) during peak canola bloom at the same locations. Additionally,
trap nests for twig nesting bees were used in 2013 and 2014 to evaluate reproductive
success of bees during canola bloom. Fruits of a wild entomophylous plant were collected
to investigate reproductive success of wild plants after canola was harvested. Our data
show adjacent pasture did not significantly increase bee or butterfly diversity, richness or
abundance around canola fields but bumble bees were only present at sites with pasture.
Reproductive success of bees did not significantly differ between sites with and without
adjacent pasture; however, parasitism rates were highest in areas without pastures. Plant
reproductive success was significantly higher in areas where canola was produced than in
areas where it was not produced and bees were more abundant in June, after canola was
harvested. Landscape analysis showed correlations of land cover with pollinator
abundance and richness, which varied between morphospecies and time of the year.

.

TABLE OF CONTENTS

LIST OF TABLES

Table   Page

LIST OF FIGURES

Figure   Page

CHAPTER I

Overview

Loss of habitat and biodiversity is one of the most important challenges the world must address, especially as agricultural expansion occurs to meet demands of our growing population (Tilman et al. 2001, Jantz et al. 2015). Agricultural conversion is one of the leading causes of habitat loss and fragmentation, both of which reduce biodiversity (Tilman et al. 2001, Foley et al. 2005, Hoekstra et al. 2005, Jiménez-García et al. 2014, Pool et al. 2014); however, there are species that can utilize cultivated areas including birds (Cruz et al. 1985), predators of crop pests (Landis et al. 2000, Dosdall and Mason 2010), mammals (Coda et al. 2014) and pollinators (Bosch and Kemp 2002, Hoehn et al. 2008, Moron et al. 2009). Species inhabiting cultivated areas can provide free ecosystem services such as crop pest control through natural enemies or increased crop production via pollination.

Historically, we have cultivated land to provide food and some goods such as cotton and lumber. Within the past few decades, biofuel production has been added to agricultural landscapes around the world in an attempt to alleviate dependence on fossil fuels and reduce carbon emissions to lessen global climate change (Barbara 2007;

Fargione et al. 2008; Pimentel et al. 2009). The projected scale of agricultural change required to meet biofuel production goals may rival the landscape change from industrial agriculture (Altieri 2009). Biofuel can be made from oil and simple carbohydrates, which can also be used for human consumption, or from complex carbohydrates such as stalks and husks and even animal waste (Chandra et al. 2012, Koçar and Civaş 2013). In much of the Midwest, farmers have turned to *Brassica napus*, commonly known in different regions as LEAR (low euric acid rapeseed), oil seed rape (OSR), or canola (Canada oil low acid) which is the name common in North America. Canola is grown for both biofuel application, with a 2 to 1 net energy return, and for use as a cooking oil (Raymer 2002) so production is expected to continue, and likely to increase to meet demand. Production of canola is increased in the presence of pollinators, and canola provides both nectar and pollen resources for pollinators (Hoyle et al. 2007), but the addition of this crop into agricultural landscapes has unknown consequences.

Pollinators are in decline globally (Biesmeijer et al. 2006, Potts et al. 2010a) and are negatively affected by agriculture and landscape homogenization (Klein et al. 2007b, Morandin et al. 2007, Jauker et al. 2009) so the agricultural intensification required to meet biofuel demands may harm pollinators. Conversely, the addition of biofuels such as canola into a landscape, which provides floral resources in the early spring when few plants are in bloom, may increase landscape heterogeneity and in turn benefit pollinators. Pollinators are extremely important because 35% of crops, globally, are at least partially dependent on animal pollination for production (Klein et al. 2007a). Crops that require pollination are critical for our nutritional health, evidenced by malnutrition overlapping with pollinator declines (Chaplin-Kramer et al. 2014, Nicole 2015). Furthermore most

wild plants depend on pollinators for reproduction (Kearns et al. 1998). Changes in pollinator communities affect wild plant communities (Biesmeijer et al. 2006) and plant communities in turn affect pollinator communities (Potts et al. 2003). Therefore, human and ecosystem health depend on how agricultural changes are affecting pollinators, plant communities and plant-pollinator interactions, as well as our ability to sustain pollinator populations.

Insects are the primary group of pollinators for wild plants and crops (Free 1970, Potts et al. 2010a). Bees (Apoidea) are the main group of pollinators for crops, whereas butterflies (Hesperiidae and Papilionoidea) are considered poor pollinators for crops (Sahli and Conner 2007), but are important for various native plants (Cruden and Hermann-Parker 1979, Jennersten 1984, Borges et al. 2003). However, some butterfly species may increase seed set for some crops more than bees (e.g., *Pieris rapae* pollination of *Brassica raphnus*; Sahli and Conner 2007) so butterflies may affect crop production more than previously thought. Both bees and butterflies are sensitive to landscape change and can be used as indicator groups for environmental changes and landscape heterogeneity (New 1997, Tscharntke et al. 1998, Hoang et al. 2011), and the addition of canola into the landscape is expected to affect these insects.

Honey bee (*Apis mellifera*) colonies are often moved to blooming crops to facilitate pollination. Native bee pollination, however, can rival services from managed hives in certain settings (Hoehn et al. 2008; Klein et al. 2007; Kremen et al. 2002, 2004; Morandin and Winston 2005). Furthermore, honey bees provide inadequate pollination for some crops, especially those that require specialized pollination services, such as

tomatoes (Banda and Paxton 1991), squash, and melons (Hoehn et al. 2008). With honey bee pollination security under threat (Winfree et al. 2007, Meixner 2010) due to the disappearance of managed and feral honey bee hives (Kraus and Page 1995, Potts et al. 2010b) from colony collapse disorder (Dainat et al. 2012, Engelsdorp et al. 2014), and the threat of mites (Martin et al. 2012, Tofilski and Oleksa 2013), plus the importance of native bees and butterflies for the pollination of certain fruits, it is crucial to increase understanding of the factors influencing wild pollinator communities to ensure crop production (Winfree et al. 2007).

Wild pollinator populations may be too small to provide adequate crop pollination in some situations (Klein et al. 2007a), but agricultural management can enhance local pollinator biodiversity and population sizes (Ricketts et al. 2008, Winfree et al. 2011, M'Gonigle et al. 2015). Therefore it is important to evaluate which pollinator species are present in agricultural areas and the affect of landscape composition on pollinator abundance and richness so that proper practices can be implemented to support pollinators and facilitate pollination services.

In the South Central United States, winter canola was recently introduced for production and Oklahoma is currently the primary canola producer in this region. Historically and presently, Oklahoma has produced mainly cereal crops (Travis and Robb 2009) which do not provide floral resources for pollinators. Long term production of cereal crops are known to negatively affect pollinators (Le Féon et al. 2013) but collections of pollinators within Oklahoma are sparse so it is unknown which species of pollinators are present and in turn may provide pollination services. Due to the

importance of pollinators for human and ecosystem health, and the sensitivity of bees and

butterflies to landscape change, we investigated how the presence of canola production

affects wild bee and butterfly communities within the South Central United States.

References

Altieri, M. A. 2009. The ecological impacts of large-scale agrofuel monoculture
　　　　production systems in the Americas. Bulletin of Science, Technology & Society
　　　　29:236-244.

Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemüller, M. Edwards, T. Peeters,
　　　　A. P. Schaffers, S. G. Potts, R. Kleukers, C. D. Thomas, J. Settele, and W. E.
　　　　Kunin. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain
　　　　and the Netherlands. Science **313**:351-354.

Borges, R., V. Gowda, and M. Zacharias. 2003. Butterfly pollination and high-contrast
　　　　visual signals in a low-density distylous plant. Oecologia **136**:571-573.

Bosch, J., and W. Kemp. 2002. Developing and establishing bee species as crop
　　　　pollinators: the example of Osmia spp. (Hymenoptera: Megachilidae) and fruit
　　　　trees. Bulletin of Entomological Research **92**:3-16.

Chandra, R., H. Takeuchi, and T. Hasegawa. 2012. Methane production from
　　　　lignocellulosic agricultural crop wastes: A review in context to second generation
　　　　of biofuel production. Renewable and Sustainable Energy Reviews **16**:1462-1476.

Chaplin-Kramer, R., E. Dombeck, J. Gerber, K. A. Knuth, N. D. Mueller, M. Mueller, G.
　　　　Ziv, and A.-M. Klein. 2014. Global malnutrition overlaps with pollinator-
　　　　dependent micronutrient production. Proceedings of the Royal Society of London
　　　　B: Biological Sciences **281**:20141799.

Coda, J., D. Gomez, A. R. Steinmann, and J. Priotto. 2014. The effects of agricultural
　　　　management on the reproductive activity of female rodents in Argentina. Basic
　　　　and Applied Ecology **15**:407-415.

Cruden, R., and S. M. Hermann-Parker. 1979. Butterfly pollination of Caesalpinia
　　　　pulcherrima, with observations on a psychophilous syndrome. The Journal of
　　　　Ecology:155-168.

Cruz, A., T. Manolis, and J. W. Wiley. 1985. The Shiny Cowbird: a brood parasite
　　　　expanding its range in the Caribbean region. Ornithological Monographs:607-620.

Dainat, B., D. van Engelsdorp, and P. Neumann. 2012. Colony collapse disorder in
　　　　Europe. Environmental Microbiology Reports **4**:123-125.

Dosdall, L. M., and P. G. Mason. 2010. Key pests and parasitoids of oilseed rape or
　　　　canola in North America and the importance of parasitoids in integrated
　　　　management in Biocontrol-based integrated management of oilseed rape pests.
　　　　Eds. Ingrid H. Williams. London.167-213

van Engelsdorp, D. v., J. Pettis, and W. Ritter. 2014. Colony collapse disorder. World
　　　　Organization for Animal Health, Paris, France. 157-159.

Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S.
　　　　Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, J. H. Helkowski, T. Holloway, E.

A. Howard, C. J. Kucharik, C. Monfreda, J. A. Patz, I. C. Prentice, N. Ramankutty, and P. K. Snyder. 2005. Global consequences of land use. Science **309**:570-574.

Free, J. B. 1970. Insect pollination of crops. Academic Press New York, NY USA.

Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, C. Kremen, L. G. Carvalheiro, L. D. Harder, and O. Afik. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science **339**:1608-1611.

Hoang, T. C., R. L. Pryor, G. M. Rand, and R. A. Frakes. 2011. Use of butterflies as nontarget insect test species and the acute toxicity and hazard of mosquito control insecticides. Environmental Toxicology and Chemistry **30**:997-1005.

Hoehn, P., T. Tscharntke, J. M. Tylianakis, and I. Steffan-Dewenter. 2008. Functional group diversity of bee pollinators increases crop yield. Proceedings of the Royal Society B: Biological Sciences **275**:2283-2291.

Hoekstra, J. M., T. M. Boucher, T. H. Ricketts, and C. Roberts. 2005. Confronting a biome crisis: global disparities of habitat loss and protection. Ecology Letters **8**:23-29.

Hoyle, M., K. Hayter, and J. E. Cresswell. 2007. Effect of pollinator abundance on self-fertilization and gene flow: application to GM canola. . Ecological Applications **17**:2123-2135.

Jantz, S. M., B. Barker, T. M. Brooks, L. P. Chini, Q. Huang, R. M. Moore, J. Noel, and G. C. Hurtt. 2015. Future habitat loss and extinctions driven by land-use change in biodiversity hotspots under four scenarios of climate-change mitigation. Conservation Biology **29**:1122-1131.

Jauker, F., T. Diekötter, F. Schwarzbach, and V. Wolters. 2009. Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat. Landscape Ecology **24**:547-555.

Jennersten, O. 1984. Flower visitation and pollination efficiency of some North European butterflies. Oecologia **63**:80-89.

Jiménez-García, L., G. Sánchez-Rojas, O. Villarreal, H. Bernal, and D. Jiménez-García. 2014. Agroecosystems management and biodiversity loss in an intensification gradient in traditional agriculture in Mexico.

Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics **29**:83-112.

Klein, A.-M., B. E. Vaissiere, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007a. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society of London B: Biological Sciences **274**:303-313.

Klein, A.-M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007b. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences **274**:303-313.

Koçar, G., and N. Civaş. 2013. An overview of biofuels from energy crops: Current status and future prospects. Renewable and Sustainable Energy Reviews **28**:900-916.

Kraus, B., and R. E. Page. 1995. Effect of *Varroa jacobsoni* (Mesostigmata: Varroidae) on feral *Apis mellifera* (Hymenoptera: Apidae) in California. Environmental Entomology **24**:1473-1480.

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology **45**:175-201.

Le Féon, V., F. Burel, R. Chifflet, M. Henry, A. Ricroch, B. E. Vaissière, and J. Baudry. 2013. Solitary bee abundance and species richness in dynamic agricultural landscapes. Agriculture, ecosystems & environment **166**:94-101.

M'Gonigle, L. K., L. C. Ponisio, K. Cutler, and C. Kremen. 2015. Habitat restoration promotes pollinator persistence and colonization in intensively managed agriculture. Ecological Applications **25**:1557-1565.

Martin, S. J., A. C. Highfield, L. Brettell, E. M. Villalobos, G. E. Budge, M. Powell, S. Nikaido, and D. C. Schroeder. 2012. Global honey bee viral landscape altered by a parasitic mite. Science **336**:1304-1306.

Meixner, M. D. 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. Journal of Invertebrate Pathology **103**:S80-S95.

Morandin, L. A., M. L. Winston, V. A. Abbott, and M. T. Franklin. 2007. Can pastureland increase wild bee abundance in agriculturally intense areas? Basic and Applied Ecology **8**:117-124.

Moron, D., M. Lenda, P. Skorka, H. Szentgyorgyi, J. Settele, and M. Woyciechowski. 2009. Wild pollinator communities are negatively affected by invasion of alien goldenrods in grassland landscapes. Biological Conservation **142**:1322-1332.

New, T. 1997. Are Lepidoptera an effective 'umbrella group 'for biodiversity conservation? Journal of Insect Conservation **1**:5-12.

Nicole, W. 2015. Pollinator power: nutrition security benefits of an ecosystem service. Environmental health perspectives **123**:A210.

Pool, D. B., A. O. Panjabi, A. Macias-Duarte, and D. M. Solhjem. 2014. Rapid expansion of croplands in Chihuahua, Mexico threatens declining North American grassland bird species. Biological Conservation **170**:274-281.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010a. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution **25**:345-353.

Potts, S. G., S. P. Roberts, R. Dean, G. Marris, M. A. Brown, R. Jones, P. Neumann, and J. Settele. 2010b. Declines of managed honey bees and beekeepers in Europe. Journal of Apicultural Research **49**:15-22.

Potts, S. G., B. Vulliamy, A. Dafni, G. Ne'eman, and P. Willmer. 2003. Linking bees and flowers: how do floral communities structure pollinator communities? Ecology **84**:2628-2642.

Rayner PL. 2002. Canola: an emerging oilseed crop. In: Janick J, Whipkey A, eds. Trends in new crops and new uses. Alexandria, VA: ASH Press, 122–126.

Ricketts, T. H., J. Regetz, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, A. Bogdanski, B. Gemmill-Herren, S. S. Greenleaf, A. M. Klein, M. M. Mayfield, L. A. Morandin, A. Ochieng', and B. F. Viana. 2008. Landscape effects on crop pollination services: are there general patterns? Ecology Letters **11**:499-515.

Sahli, H. F., and J. K. Conner. 2007. Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). American Journal of Botany **94**:203-209.

Tilman, D., J. Fargione, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, W. H. Schlesinger, D. Simberloff, and D. Swackhamer. 2001. Forecasting agriculturally driven global environmental change. Science **292**:281-284.

Tofilski, A., and A. Oleksa. 2013. There are still Bee Trees in Europe. Bee world **90**:18-19.

Travis, P. D., and J. B. Robb. 2009. Wheat. Encyclopedia of Oklahoma History and Culture. Oklahoma Historical Society www.okhistory.org.

Tscharntke, T., A. Gathmann, and I. Steffan-Dewenter. 1998. Bioindication using trap-nesting bees and wasps and their natural enemies: community structure and interactions. Journal of Applied Ecology:708-719.

Winfree, R., I. Bartomeus, and D. P. Cariveau. 2011. Native pollinators in anthropogenic habitats. Annual Review of Ecology, Evolution, and Systematics **42**:1.

Winfree, R., N. M. Williams, J. Dushoff, and C. Kremen. 2007. Native bees provide insurance against ongoing honey bee losses. Ecology Letters **10**:1105-1113.

CHAPTER II

EFFECTS OF SPRING FLOWERING CANOLA AND SURROUNDING

LANDSCAPE COVER ON WILD BEE COMMUNIITS IN THE SOUTH CENTRAL

UNITED STATES

Abstract

Demand for biofuel crops is drastically changing agricultural landscapes around

the world with unknown environmental consequences.. In the South Central US, canola

(*Brassica napus*) is generally planted in rotation with winter wheat, and is an early-

blooming, floral resource for many bee species. Canola is susceptible to insect pests that

can substantially reduce crop production, and approximately 90% of canola field are

treated with insecticides, many of which are known to be harmful to bees. Native bees

provide pollination services to canola and other crops, but many native bee species are in

decline globally, likely due to habitat loss and insecticide use. We tested the hypothesis

that bee abundance, richness, and diversity within and around canola fields will be higher

in areas with pasture compared to areas without pasture. In early spring between 2011

and 2014 we captured bees during the period of peak canola bloom using blue vane traps,

in areas with and without adjacent pasture. Additionally, trap nests for twig nesting bees

were used in 2013 and 2014 to evaluate reproductive success. Our data suggest adjacent

pasture did not significantly increase bee richness or abundance around canola fields

during peak canola bloom, but pasture did significantly increase the likelihood of bumblebee and ground nesting bees being captured in the traps. The number of total cells and cells without development in trap nests did not significantly differ between sites with and without adjacent pasture; however, parasitism rates were higher in areas without pasture. Bumblebees and ground nesting bees may occur more often in areas with pasture due to the availability of nesting resources. Study sites were located in the wheat-producing region of Oklahoma, where wheat has been produced for most of the past century, which may explain the similarity between bee communities. Bee communities of Oklahoma have not been well studied, restricting our interpretation; however, our study is informative as to which bee species occur in early spring and may increase crop yields.

Introduction

The projected scale of agricultural change required to meet biofuel production goals may rival the landscape change from the industrial revolution (Altieri 2009). This change is expected to reduce oil dependence and carbon emissions; however, the ecological implications of biofuel crop production are not yet understood (Pimentel et al. 2009). In the United States, production of biofuel crops is increasing to meet the target goal of 136 billion liters of biofuels for 2022 (White House 2010). In the South Central US, canola (*Brassica napus*) is being planted for biofuel applications and has a 2-to-1 net energy return (Canola Council of Canada website; US Canola Association website 2012), which is higher than ethanol made from corn (De Oliveira et al. 2005).

Many agricultural crops depend on the ambient pollinator community for pollination services to increase fruit and seed set (Hoehn et al. 2008, Julier and Roulston 2009). These pollination services are also important for native plant communities, which can be affected when pollinator communities are altered (Huryn 1997, Moron et al. 2009, Woods et al. 2012). Bees are considered the most important pollinator group for crop production and are responsible for pollinating 35-66% of the world's crops (Klein et al. 2007), but are also one of the more sensitive arthropod groups, of those that have been studied, to agricultural intensification (Hendrickx et al. 2007). Furthermore, native bee pollination can rival services from managed honey bee (*Apis mellifera*) colonies in certain settings (Kremen et al. 2002, Kremen et al. 2004, Morandin and Winston 2005, Klein et al. 2007, Hoehn et al. 2008) and are required for crops which honey bees cannot pollinate such as tomatoes (Banda and Paxton 1990), squash and melons (Hoehn et al. 2008). However, native bee populations and communities are in decline globally (Williams 1986, Buchmann

and Nabhan 1997, Potts et al. 2010). Possible factors contributing to this decline include competition with non-native pollinators for floral resources (Huryn 1997, Roubik and Wolda 2001) insecticides (Gill et al. 2012), and nest site and habitat loss (reviewed in (Potts et al. 2010)). Due to the ecological and economic importance of native bee communities for native plant communities and agricultural crops it is important to understand the impact of biofuel crop production on native bee communities to develop effective management strategies for agroecosystems.

Oklahoma is the primary producer of canola within the South Central US, where winter canola is planted in rotation with winter wheat. Pastures are also common within canola-wheat landscapes, and pastures have been shown to benefit bee abundance and richness by providing potential nesting and forage areas (Morandin et al. 2007, Julier and Roulston 2009). In the last 7 years, production of canola in Oklahoma has increased from 0 to 270,000 acres (109,265 hectares) (USDA NASS 2008-2015). Canola plants produce an indeterminate raceme of flowers, each of which produces relatively large amounts of nectar (0.2µl - 6µl/flower, with an average of 2µl/flower), depending on the variety (Pierre et al. 1999), as well as pollen (Morandin and Winston 2005). Flowering occurs in early spring, prior to most native plants (Berger et al. 1985, Michener 2000, Bosch and Kemp 2002) and typically lasts 3 to 5 weeks, making it attractive and potentially beneficial to native bees. Croplands with managed flowering areas have been shown to be beneficial to bumblebee (*Bombus hortorum, B. lapidarius, B. pascuorum* and *B. terrestris*) populations (Wood et al. 2015) and canola may have similar effects on other native bee species. Existing research suggests an immediate benefit to pollinators, implying canola may be beneficial to

pollinator populations (Morandin et al. 2007, Jauker et al. 2009, Diekotter et al. 2010), but few studies have investigated long-term effects on wild pollinator populations.

Although canola production has the potential to benefit native bee communities, insecticides used to control pest outbreaks in canola may harm bee communities. Canola is susceptible to a number of crop pests (Dosdall and Mason 2010) and in the South Central US, three aphid species infest canola: Turnip (*Lipaphis erysimi*), Green-Peach (*Myzus persicae*) and Cabbage aphids (*Brevicoryne brassicae*). Insecticide application is the primary method used to control aphid infestations, with approximately 90% of fields being sprayed in any given year, some more than once (Franke et al. 2009). Insecticide applications often occur around the time of flowering to control cabbage aphids that infest canola during bloom and seed-pod development (Boyles et al. 2004, Royer et al. 2004) and can significantly reduce crop production. Bifenthrin, gamma- and lambda-cyhalothrin, and methyl-parathion insecticides are used most frequently (Fact Sheet EPP-7085), all of which are considered highly toxic to bees (US EPA 2008), although new narrow-spectrum insecticides have become available in the last couple of years.

Winter wheat has been a major crop in the Great Plains since before the Oklahoma land run and a dominant crop in the South Central US for more than nine decades (Travis and Robb 2009). Previous research conducted in France found consecutive years of wheat production in an area negatively affected bee communities (Le Féon et al. 2013) and the long history of production in Oklahoma has likely influenced existing bee communities in a similar manner. Current bee community composition and structure reflects the stressors of historic agricultural patterns, such as reduction of forage, as wheat does not produce floral resources and is unlikely to attract

foraging pollinators (Le Féon et al. 2013). Therefore, since bees are not expected to forage in wheat fields, insecticide application to wheat is not likely to negatively impact bees. Furthermore, less than 5% of winter wheat is treated with insecticides (Giles et al. 2003, USDA NASS 2012) so the addition of canola to the landscape represents a substantial increase in insecticide applications in a system with historically low insecticide input.

In situ studies and studies using approved concentrations of insecticide in the environment show negative consequences to bees. Imidacloprid is a neonicotinoid commonly used in canola production for foliar and seed coat application. Bumblebees (*B. terrestris*) exposed to non-lethal levels of imidacloprid: collected significantly less pollen during foraging trips, and returned to the colony with pollen less often than non-exposed individuals (Feltham et al. 2014); had 33% brood reductions, although it did not affect fecundity (Laycock et al. 2012); reduced growth and 85% reduction in queen production when exposed to realistic field concentrations of insecticides (Whitehorn et al. 2012), but some resistence has been reported and was dose dependent (Laycock and Cresswell 2013). Bumblebees (*B. terrestris*) exposed to non-lethal levels of insecticides showed accumulation within their brains and their neurons were negatively affected when exposed to imidacloprid and clothianidin (Moffat et al. 2015). Therefore, the addition of canola to the landscape could lead to higher levels of risk to bees being directly and indirectly exposed to insecticides.

In addition to foraging bees being directly exposed to insecticides, exposure can also occur via contact with contaminated pollen and/or nectar deposited in the nest

(Williams et al. 2010, Pohorecka et al. 2012, Cutler and Scott-Dupree 2014). Bees select nest sites based on biotic and abiotic factors including floral and nesting resource availability (Plowright and Laverty 1984, Potts and Willmer 1997, Gathmann and Tscharntke 2002). Most research has focused on hive nesting bees, so it is unclear how solitary bees, including species that often nest in the ground or in twigs, are affected by *in situ* exposure to insecticides. Canola has the potential to attract bees to nest near canola fields in the spring with unknown population consequences such as: increased mortality from insecticide exposure; changes in reproductive success due to differences in parasitism rates; and higher reproductive success caused by high floral resource availability, although this may also lead to lower reproductive success once canola is post-bloom.

With both commercial and industrial demands for canola (Raymer 2002), production will likely continue long into the future and it is therefore important for land managers and producers to understand how canola production impacts native bee development and community structure. In this chapter, we evaluate native bee abundance, diversity and richness around canola fields adjacent to wheat fields and in the presence or absence of adjacent pasture. We also investigate the effects of canola production on twig nesting bee reproductive success near these fields which we compare to areas without crop production.

Materials and Methods

Study Sites

From 2011 to 2014, six to eight sites were selected in Oklahoma each year, with 3 or 4 sites containing adjacent pasture and 3 or 4 sites with no adjacent pasture. The majority of sites were not the same as previous years due to crop rotation of winter wheat and winter canola. All canola fields were sprayed with insecticide during canola bloom each year. Bee sampling occurred during all four years, and bee development studies occurred during the last two years.

Bee sampling

Estimates of bee abundance and richness were obtained using blue vane traps. We selected blue vane traps because they work well in the presence of a high reward floral resource and they collect large bodied bees such as *Anthophora* and *Bombus* (Stephen and Rao 2005). Pan trapping is expected to be less effective under these conditions because capture rates tend to be lower when floral resources are abundant and large bodied bees are not adequately sampled (Roulston et al. 2007, Baum and Wallen 2011). Blue vane traps consist of a plastic container (15 cm diameter × 15 cm high) secured under two cross vanes (3 mm thick) composed of blue polypropylene sheets (24 cm × 13 cm) (SpringStar™ LLC, www.springstar.net). These traps are visually attractive to bees and collect live small to large bodied bees, while excluding honey bees (*A. mellifera)* due to honey bee foraging behavior (Stephen and Rao 2005, 2007). Utilization of these traps without killing agents allowed us to release living individuals that were easily identifiable such as *Anthophora* spp. and bumblebees (*Bombus* spp.). However, many individuals perished while in the traps, even when conditions were cool and traps were checked daily.

Blue vane traps were placed in fields for 48 h during peak canola bloom, which was classified as occurring when the majority of canola plants had green buds on the top of the inflorescence and the first flower has started to senesce on the indeterminate raceme. Transects consisting of six traps were placed perpendicular to field borders and consisted of three traps extending into the canola field and the neighboring wheat field or pasture at 50m, 150m, 300m from the crop edge (Figure 1). Sites without adjacent pasture had 1 transect (across the canola-wheat interface) and sites with adjacent pasture had two transects (one across the canola-wheat interface and the other across the canola-pasture interface) with a minimum of 600m between transects. Our sampling intensity was based on previously published research with blue vane trap sampling of bees in sunflower fields (also a high density floral resource) with three traps per field (Stephen and Rao 2005, 2007). Trapping only occurred when rain was not forecasted, winds were below 48kph, and daily high temperatures were above 10°C. Sites with and without pasture were sampled during the same days to avoid confounding weather and trapping effort (Bartholomew and Prowell 2006).

Landscape characteristics

To test for land cover effects, we used data from CropScape (Han et al. 2012, Han et al. 2014). We extracted data for land cover within a 1.5km buffer around the center of our transects because most bees forage within 1km of their nest site (and our transects extended 0.3km from the center). Furthermore, we assumed bees may travel further to find nesting and floral resource sites in the spring. Data were categorized into 8 land cover types: canola (recall it blooms before most other crops and native plants); non-

nectar producing crops which included winter wheat, corn, sorghum, etc. (these crops do not provide nectar resources for the bees); grass or pasture (these areas may contain both floral and nesting resources and were not separated as different entities through CropScape); wetland which included open water (these areas can provide mud, reeds, and other nesting resources used by bees, as well as floral resources); fallow or barren land (these areas differ from year to year and likely will not provide many resources); developed land which included homesteads and gardens, fracking platforms, and roads (these areas may provide nesting areas for ground and wood nesting bees as well as floral resources on roadsides and homesteads); forests which comprised deciduous and evergreen forest (these areas provide nesting resources for xylophylous bees); and other flowering crops such as soybeans and alfalfa which bloom in later summer months (these areas can provide nectar resources after canola senesces). These data were then converted to percent cover for the areas around each site.

Bee development studies

Two twig nesting bee boxes were placed at each site in 2013 and 2014. Each box consisted of five untreated pine wood layers, with each layer measuring 30cm x 30cm x 2.5cm. Tunnels were created in each layer using a router with a 0.318cm, 0.477cm, 0.635cm, or 1.27cm, drill bit and each tunnel was separated by 1.27cm. Each layer consisted of a single tunnel dimension and a complete nest box consisted of one layer of each tunnel size and an unrouted layer with a roof to prevent rain from leaking between layers. Layers were held together by C-clamps.

These nest boxes were designed for xylophylous bees, specifically *Ceratina*, *Megachile*, and *Osmia*, all of which were captured in our study sites during 2011 and

2012 and are known to utilize similar nest boxes (Bosch and Kemp 2000). Prior to canola flowering in the spring, two nest boxes were placed at each site in 2013 and 2014, along the field edges near canola and wheat interfaces on opposite sides of the field. Field edges were used to avoid disturbance by machinery during harvest. Nest boxes were also placed at three control sites at the Stillwater Research Range (3.2km south of Hwy 51 on Coyle Rd, Stillwater OK), which is comprised of patch-burn rangelands which are grazed with cattle at moderate stocking rates and burned on a 3 year rotation (McCollum III et al. 1999, Allred et al. 2011). No cropland is present within the immediate vicinity.

Nest boxes were removed in June of each year to reduce losses from predators such as ants and birds and to identify adults of multivoltine bee species. Nests boxes were evaluated in the lab to document cells with no-development, pupation, emergence and parasitism. Cells provisioned with pollen which did not have pupae were listed as no-development, and cells without pollen and no development were considered "false cells", which are common and hypothesized to reduce parasitism (Vinson and Frankie 1977), as well as the spread of chalkbrood fungus and mites.

Analysis

Specimens collected in blue vane traps were grouped into morphospecies and keyed to genus. Species level identifications were made when possible, and a synoptic collection was deposited at the USDA Bee Biology and Systematics Laboratory in Logan, Utah. Blue vane trap data were used to calculate abundance, richness, Simpson diversity index, and evenness and compared between crops (canola and wheat) and landscape types (with and without pasture) to evaluate temporal patterns of bee species and community structure in these landscapes from year to year. Due to sampling effort

differing between sites with and without pasture, bee abundances were standardized as the number of bees collected per transect for each landscape type (with and without pasture).

To test if the presence of adjacent pasture affected bee species presence, we used common morphospecies, which we defined as those found in both landscape types and at more than two sites. Presence was used because a severe drought occurred during our study period, and abundance of bees decreased from 2011 to 2012. The proportion of sites in which each morphospecies was captured was calculated and compared the percentage of sites with and without pasture in which the morphospecies was captured via the Z-calculator (VassarStats 2015). We also grouped morphospecies based on their common nesting habit (hive, ground, or twig) and used Chi-square to compare the abundance of each morphospecies with the same nesting habit between sites with and without pasture. Additionally, we analyzed adult parasitic species caught in our traps by landscape. Since parasitic species are only visiting traps to feed, and are not foraging and feeding at similar rates as non-parasitic species, we transformed the data to present or absent by site.

We performed ANOVA in SAS to test for the differences in mean species richness and diversity between years and sites with and without pasture. T-tests were run in SPSS to separately compare overall bee richness and abundance in landscapes with adjoining pasture to landscapes without adjoining pasture for each year. Two tailed, heteroscedastic T- tests were also performed in SPSS on nest box data for total number of cells, percent of cells with no development, and rate of parasitism. Similarity of bee

communities from sites with pasture was also compared to areas without pasture for each year using the Sørensen–Dice index.

To test how land cover from each site may have affected our data, we ran a Spearman's rank correlation in Vassar Stats to test the influence each land cover type had on the number of bee species and the overall number of specimens collected per transect at our sites.

Results

A total of 21,291 bees were captured between April 2011 and April 2014, with 68 morphospecies identified. Of the 68 morphospecies, 23 were represented by one specimen, and five were represented by only two specimens. Seventeen morphospecies occurred in both site types (with and without pasture) and of these 17 species, eight morphospecies comprised 95% of our data. The least abundant of these 8 morphospecies contributed 1.05% of the data and the most abundant morphospecies comprised 31% of the data (Table 1).

Bee diversity, abundance and richness

Adjacent pasture presence did not significantly affect bee diversity during peak canola bloom (F = 0.11; df = 1, 6; p = 0.76) or richness (F = 3.04; df = 1, 6; p = 0.09) (Figure 2). The abundance of bees per trap did not significantly differ between sites with and without pasture when compared within years (F = 0.1; df = 1, 3; p = 0.74). The number of species in each site (areas with and without pasture) also did not significantly differ (F = 2.31; df = 1, 6; p = 0.20) (Figure 3). When we grouped our transect data for all years into sites with pastures and sites without pastures, we found no difference in

diversity (t = 0.39, df = 43, p = 0.70) or richness (t = 0.56, df = 43, p = 0.58) between

transects in areas with and without pastures. Parasitic species (*Melecta* spp., *Anthidium*

porterae and *Triepeolus* sp.) alone or when grouped together did not differ in occurrence

between sites with and without pasture or when grouped together or counted as present or

absent (t = 0.854, df = 28 p = 0.20). Sites with pasture consistently had a greater

abundance of ground nesting bees (χ^2 = 436, df = 8, p < 0.001) (Table 1). Bumblebees (*B.*

pennsylvanicus and *B. auricomis*) (Z = 2.63, n = 41, p < 0.005) and the

Melissodes/Eucera (Z = -2.88, n = 41, p < 0.005) morphospecies were the only species

which were captured significantly more often in areas with pasture (Table 1).

Our data from areas with and without pasture varied in similarity from year to

year. Bee data from our 2011 sites were 76.36% similar to one another. Data from 2012

had the lowest similarity at 63.16%, followed by 2013 at 67.74%. Our sites in 2014 had

the most similar bee community composition at 80.65% similarity. Bee abundance was

greatest in 2011 (63.9% of our specimens were collected that year), which also

represented the beginning of a severe drought from 2011 to 2013. Yearly data were

dominated by 3 morphospecies, *Agapostemon, Halictus,* and *Lasioglossum* (Figure 4),

none of which was consistently more abundant than the others from year to year.

Landscape effect

Our classification of sites as with or without adjacent pasture did not control for

the percent of grassy areas and pastures within 1.5km of transects. Except for canola,

which was present at all sites, no variable had moderate or strong positive correlations

with the number of bee species or the number of organisms captured per transect. The

strongest correlation was on the number of bee species (richness) per transect (r_s = -0.3902, n=28, p = 0.089) from fallow land cover. Abundance correlated most with non-nectar producing crop land cover (r_s = -0.2929, n= 28, p = 0.210) (Table 2).

Bee reproductive success

In the sites with adjacent pasture, in both 2013 and 2014, *Osmia* spp. comprised 40-43% of the bee cells. Two distinct types of Megachilidae nests were found; nests built with green leaf layers comprised 9-17% of the nest cells, whereas nests made from flower petals comprised 41-47% of the cells. Less than 1% of the cells were parasitized by flies or gregarious parasitoid wasps each year and no parasitic bee species emerged. Unidentified ant species also colonized 1 box each year and it is unclear how many nests were lost because the ants removed bee nesting materials. Evidence of mud walls and stains were observed, but it is unclear how long those walls and cells were present or if any Megachilidae nests existed before the ants invaded the nest box.

Nest boxes from sites without adjacent pasture had greater variability in numbers between years, but no statistical differences were found (p = 0.49). *Osmia* spp. made up 3-54% of the successful cells, and *Diceratosmia subfasciatus* represented 80% of the cells in 2014, but *D. subfasciatus* was absent from all boxes in 2013. Megachilidae cells made of green leaves comprised 0-5% of the data, and cells made of petals made up 7.5-36% of the data. Parasitism by flies and gregarious wasps occurred in 5% of the cells in both years.

In addition to the successful nest cells, we found no significant difference between the control rangelands (no nearby croplands), croplands with pasture and

croplands without pasture and the percent of cells with provisions and no bee

development. Both matrices with and without pasture had 8.5-8.8% of the nest cells with

provisions and no development. The sampled pollen of the cells without development

was from canola, which we also encountered in *Osmia* spp. cells which still had

developing larvae.

Discussion

Bee abundance, richness, and diversity during peak canola bloom, as well as rates

of non-development for twig nesting bees in canola-wheat landscapes, did not

significantly differ between areas with and without adjacent pasture. Land cover also did

not have any statistically significant correlations with bee abundance or richness within a

1.5km radius of our sites and our data did not reveal any discernable pattern in abundance

of the three most commonly captured morphospecies from year to year. Our findings are

likely affected by the severe drought experienced from 2011 to 2013. Bees were most

abundant in 2011 with a significant decrease in 2012, and small increases in the

following years. Therefore, environmental variables, like drought, may be strongly

influencing our results and be more important to yearly bee abundance and richness than

land cover. Although overall bee communities did not significantly differ in areas with

and without adjacent pasture, bumblebees and *Melissodes* sp. were captured significantly

more often in areas with adjacent pasture than areas without pasture (Table 1). Areas with

pasture also had more ground nesting bee morphospecies than areas without pasture,

likely due to nesting resource availability and lack of disturbance of nesting areas.

Parasitism of solitary bee nests was significantly higher in areas without pasture and all nest parasitism events were from parasitic species that are not in Apoidea. Adult parasitic bee species presence did not differ among sites with and without pasture. The difference between the rate of parasitism and the occurrence of parasite species may be due to differences in nesting resource availability in these areas. This difference may also be due to adult parasitic bees and the nest parasite species we did encounter, potentially being less attracted to our traps since they only need to feed on nectar resources and not provision for their young.

Few native bee surveys have been conducted in Oklahoma, so we cannot compare our data to early spring bee community data prior to the incorporation of canola into the landscape. Also, our current data are limited to the species that have persisted in this area of intensive wheat production over many decades, which can negatively affect bee species (Le Féon et al. 2013). Higher nest parasitism rates in areas without pasture may be due to the lower availability of nest sites for bees in these areas, which could lead to the concentration of nesting resources, and may have made it easier for parasites to find nests as well. Areas without pasture may have also had less spatial complexity, a factor which positively correlates with local persistence of parasitoid populations (Tilman and Kareiva 1997). Furthermore, we placed our trap nests on field margins, and parasitism rates are higher in these areas which has been observed for rape beetles (Thies and Tscharntke 1999, Tscharntke et al. 2002) and more generally for other species and systems (Paton 1994, Benson et al. 2013) and may be similar for bees. Our data only capture a two-year period, and these patterns may not be consistent over longer time frames or representative of patterns in non-agricultural settings.

For many decades, two to two and half million hectares of winter wheat have been produced in the South Central United States and Oklahoma. Over the past 7 years, canola production has increased each year, as it is increasingly used as a rotational crop with winter wheat (USDA NASS 2008-2015). Due to solitary bees being one of the most sensitive groups of arthropods to agricultural intensification (Hendrickx et al. 2007), historical agriculture intensity may have homogenized local bee communities. Winter wheat does not provide nectar resources for bees, and historical, large-scale production of wheat and other cereal crops is known to decrease solitary bee richness (Le Feon et al. 2013) and possibly contributed to the similarity of bee communities in our study region regardless of pasture presence. Furthermore, pasture management practices often focus on increasing graminoids by reducing forbs. For example, application of broad-leaf herbicides in pasture (Fuhlendorf et al. 2002, Hamilton 2007) may have further reduced floral resource availability in this system, although nesting resources are likely still available. Therefore, bees would need to obtain floral resources in other areas such as roadsides or fallow fields, which would likely provide similar resources in areas with and without pasture. This may explain why we found a greater abundance of ground nesting bees and higher bee community similarity in areas with pasture, where tilling is not likely to occur, compared to areas without pasture, but did not find a difference for overall bee abundance and richness. The similarity in overall bee abundance and richness may also be due to canola attracting bees to the area (Le Féon et al. 2013), which may have diluted the effects of the pasture seen in other studies.

Previous research has shown that natural lands (Greenleaf et al. 2007), pasture, and forested areas (Mandelik et al. 2012) within agricultural landscapes increase bee

diversity and abundance in croplands (Kim et al. 2006, Morandin et al. 2007, Julier and

Roulston 2009). Crop pollination services, however, are not significantly increased by

rare species, but are provided by a subset of common bees from the local bee community

(Kleijn et al. 2015b) so it is important to provide areas for bees to increase abundance and

in turn pollination services (Deguines et al. 2014). Our study shows that *Agapostemon,*

Lasioglossum, and *Halictus* are the most abundant bees in our study area, which may be

of interest to land managers and producers trying to increase pollinator abundance. The

increase in diversity and abundance in previous studies likely reflects more availability of

nesting resources, such as undisturbed ground for ground nesting bees, woody vegetation

for twig nesting bees and diversity of floral resources throughout the year (Potts and

Willmer 1997, Kim et al. 2006). Previous studies, however, focused on later season crops

and bee communities which may have more richness and abundance than early spring bee

communities. It is also unclear which of the previously studied systems have been heavily

farmed in the past, which crops were grown historically in those regions, and if refugia

existed to sustain the bee community as agricultural production intensified.

Although the overall bee abundance and richness did not significantly differ, our

data show bumblebees and ground nesting bee species were more common in the

presence of pasture, but further research is needed to understand how farming intensity

on a landscape scale influences bee communities in these areas. We were not able to

control for dirt roads and roadside areas which may have also contributed to patch

similarities. Roadsides, especially those restored to prairie or those not treated with

herbicide, have been identified as important resources for bee communities (Hopwood

2008). Common pollinators are able to survive in agricultural areas and are enhanced

with simple conservation practices such as hedgerows (Kleijn et al. 2015a). Our roadsides were mostly unmanaged, but sometimes mowed, with various floral resources present in late spring and early summer (unpublished data) and may function as refugia by providing sufficient nesting and floral resources to sustain spring bee communities in years when canola was not planted, or after canola senesces which could support bee community persistence in these areas.

We found canola pollen in our trap nest boxes which suggests these bees foraged mainly in canola fields and is congruent with previous research on *B. napus* (Le Féon et al. 2013). There was no difference in the percent of cells without larval development between sites with and without pasture. Since our canola-wheat and canola-wheat-pasture landscapes were both treated with insecticides, we expected higher rates of no development or incomplete development in areas with canola compared to our control (non-crop) landscapes. Our data suggest native bees have similar rates of non-development in areas producing canola and areas not producing canola. Further research is needed on *in situ* exposure of insecticides on solitary bees, since most existing research has focused mainly on hive nesting bees (Feltham et al. 2014, Moffat et al. 2015). Some of the effects of non-lethal pesticide exposure on bumblebees were decreases in colony and worker size and reduction of queen production (Whitehorn et al. 2012). This suggests negative effects may be observed in the next generation, or insecticides could affect adult weight, foraging capability, and the number of offspring per exposed adult which our research did not address. The similar rates of cells without larval development in our nest traps between sites in canola producing and non-producing areas suggests canola pollen

is a suitable food resource for solitary bees. However, we did not test for effects of sublethal pesticide exposure or pesticide residue within the trap nests.

Although many agroecosystems within the United States do not provide year round floral resources, native bee communities do benefit from agricultural planning that provides consistent floral resources throughout the year (Mandelik et al. 2012). Our data suggest canola potentially is a suitable forage for spring bees, and could be beneficial if integrated into crop rotations. However, additional research is needed to evaluate the implications of insecticide applications in canola on native bees, both in adult and larval stages.

Tables and Figures

Table 1: The percent of sites in which bee morphospecies were captured out of the total number of each site type (areas with and without pasture). Data are only provided for the 17 morphospecies encountered in 2 or more sites, with z-scores and p-values for the differences in occurrences. Nesting habitat for each morphospecies is indicated by color, and the total percent of the data which the morphospecies represented is provided in the last row. Grey shaded cells in the last row identify the 8 most abundant morphospecies. NaN is reported for numbers that could not be tested due to 100% presence.

Morphospecies	Nesting	Div	Sim	Z-score	p-value	% of total data
Agapostemon	Ground	100	100	NaN	0.00	0.32
Anthophora 1	Ground	73.08	93.33	-1.58	0.11	26.15
Anthophora 2	Ground	46.15	53.33	-0.44	0.66	3.13
Anthophora 3	Ground	30.77	53.33	-1.43	0.15	1.39
Bombus	Both	69.23	26.67	2.63	0.0085	2.62
Colletes 1	Ground	42.31	53.33	-0.68	0.50	0.48
Colletes 2	Ground	34.62	26.67	0.53	0.60	0.01
Diceratosmia subfasciatus	Twig	34.62	26.67	0.53	0.60	28.96
Halictus ligatus	Ground	84.62	93.33	-0.82	0.41	0.21
Lasioglossum group	Ground	96.15	100	-0.77	0.44	31.16
Lasioglossum/dialictus	Ground	42.31	46.67	-0.27	0.79	1.05
Melissodes	Both	42.31	33.33	0.57	0.57	0.34
Melissodes/Eucera	Both	26.92	73.33	-2.88	0.0040	1.4
Megachile 1	Twig	42.31	40.00	0.14	0.89	0.3
Megachile 2	Twig	11.54	33.33	-1.70	0.09	0.06
Megachile 3	Twig	3.85	13.33	-1.12	0.26	0.14
Osmia 1	Twig	3.85	13.33	-1.12	0.26	0.28

Nesting: Twig, Ground, Both, Top 8

Table 2: Spearman's rank correlation test values for percent land cover of sites within a 1.5km radius from the center of transects and the number of species (richness) and number of bees (abundance) collected per transect. Reported p-values are for a 2-tailed test. The average percent cover of each land cover category is also reported.

|		Non-nectar producing	Grass/ pasture	Canola	Nectar producing	Developed/ open space	Fallow	Forest	Wetland
Abundance	Rs	-0.2929	0.2063	0.3283	-0.2169	-0.1476	-0.2899	0.0696	0.0132
	p-value	0.21	0.385	0.159	0.36	0.537	0.217	0.768	0.953
Richness	Rs	-0.1288	0.223	0.2516	-0.2855	-0.0362	-0.3902	0.0953	-0.315
	p-value	0.589	0.345	0.286	0.224	0.882	0.089	0.687	0.898
Percent ± SE		56.71 ± 3.15	23.73 ± 2.27	12.01 ± 1.94	2.02 ± 0.32	3.77 ± 0.24	0.61 ± 0.39	1.10 ± 0.36	0.04 ± 0.02
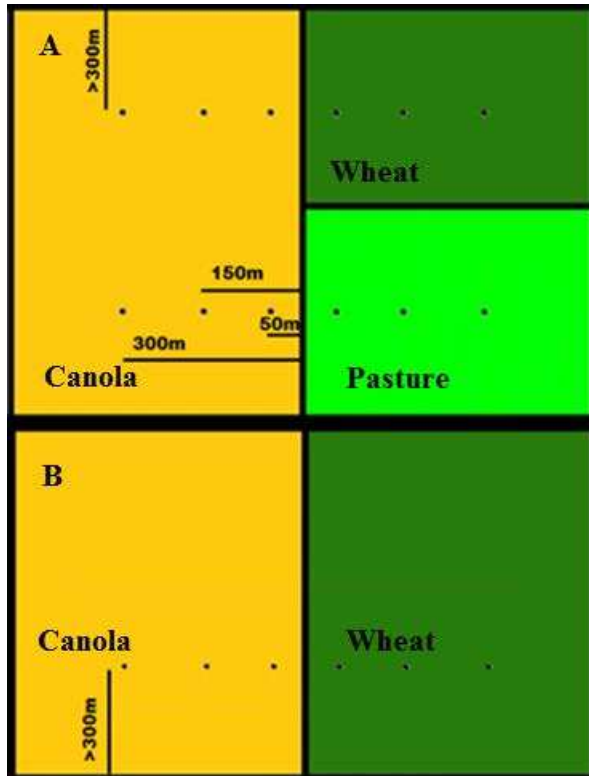
Figure 1: Transects of blue vane traps in areas with adjacent pasture (A) and areas without adjacent pasture (B). Fields differed in size and arrangement, so a hypothetical example is provided.
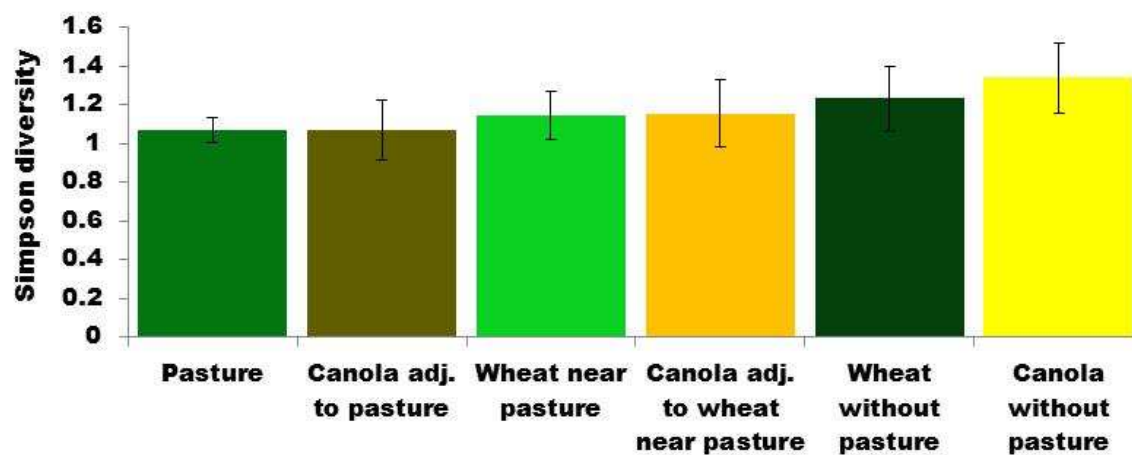
Figure 2: Average Simpson diversity index for each field sampled between 2011 and 2014.
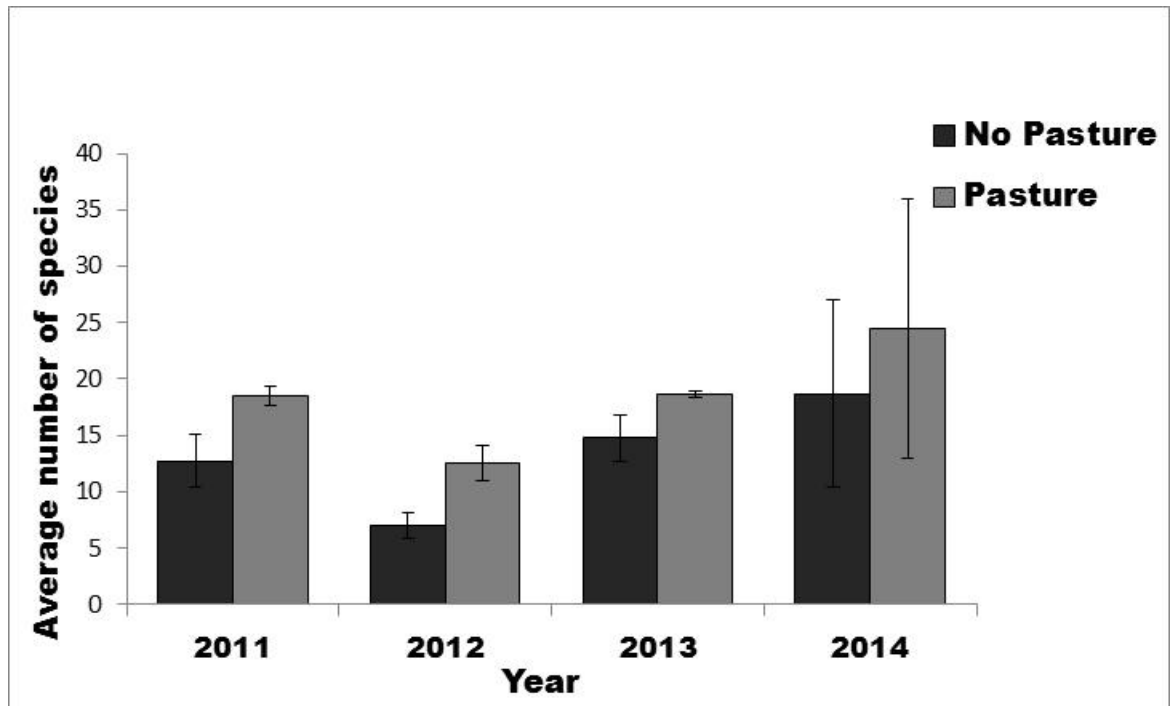
Figure 3: Average number of bee species per transect (± SE) in canola-wheat landscapes with and without pasture during the period of peak canola bloom.
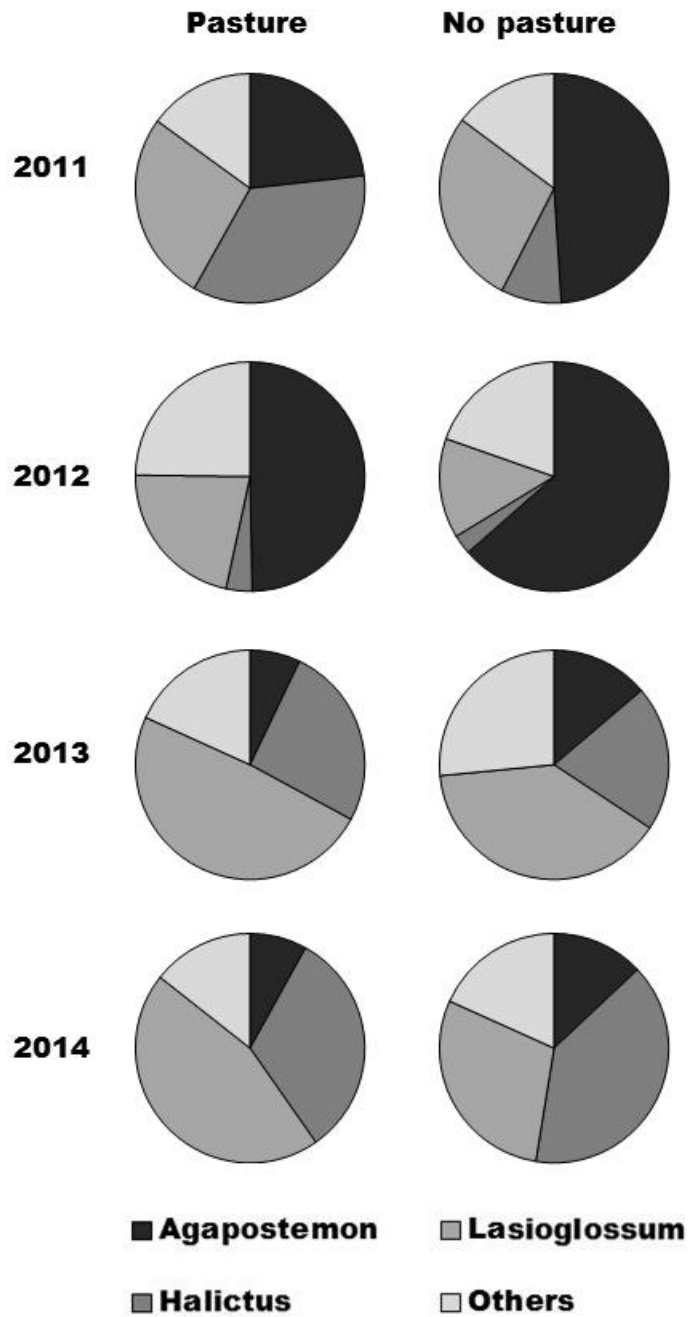
Figure 4: The abundance of the three most abundant morphospecies, each comprising more than 25% of captured bees, varied from year to year as well as between areas with and without pasture. Of the total collected specimens, 63.9% were collected in 2011, 5.5% were collected in 2012, 14.9% were collected in 2013, and 15.7% were collected in 2014.

References

Allred, B. W., S. D. Fuhlendorf, D. M. Engle, and R. D. Elmore. 2011. Ungulate preference for burned patches reveals strength of fire–grazing interaction. Ecology and Evolution **1**:132-144.

Altieri, M. A. 2009. The ecological impacts of large-scale agrofuel monoculture production systems in the Americas. Bulletin of Science, Technology & Society **29**:236-244.

Banda, H., and R. Paxton. 1990. Pollination of greenhouse tomatoes by bees. Pages 194-198 *in* VI International Symposium on Pollination 288.

Bartholomew, C. S., and D. Prowell. 2006. Comparison of bee diversity in upland and wet flatwood longleaf pine savannas in Louisiana (Hymenoptera: Apoidea). Journal of the Kansas Entomological Society **79**:199-206.

Benson, T. J., S. J. Chiavacci, and M. P. Ward. 2013. Patch size and edge proximity are useful predictors of brood parasitism but not nest survival of grassland birds. Ecological Applications **23**:879-887.

Berger, L. A., J. O. Moffett, and D. R. Rummel. 1985. Seasonal cycles of *Agapostemon angelicus* Cockerell relative to hybrid cottonseed production in Texas (Hymenoptera: Halictidae). Journal of the Kansas Entomological Society **58**:1-8.

Bosch, J., and W. Kemp. 2002. Developing and establishing bee species as crop pollinators: the example of Osmia spp.(Hymenoptera: Megachilidae) and fruit trees. Bulletin of Entomological Research **92**:3-16.

Boyles, M., T. F. Peeper, and C. Medlin. 2004. Producing winter hardy canola in Oklahoma. Division of Agricultural Sciences and Natural Resources, Oklahoma State University.

Buchmann, S. L., and G. P. Nabhan. 1997. The Forgotten Pollinators. Island Press. Washington D.C., USA

Cutler, G. C., and C. D. Scott-Dupree. 2014. A field study examining the effects of exposure to neonicotinoid seed-treated corn on commercial bumble bee colonies. Ecotoxicology **23**:1755-1763.

De Oliveira, M. E. D., B. E. Vaughan, and E. J. Rykiel. 2005. Ethanol as fuel: energy, carbon dioxide balances, and ecological footprint. Bioscience **55**:593-602.

Deguines, N., C. Jono, M. Baude, M. Henry, R. Julliard, and C. Fontaine. 2014. Large-scale trade-off between agricultural intensification and crop pollination services. Frontiers in Ecology and the Environment **12**:212-217.

Delbrassinne, S., and P. Rasmont. 1988. Contribution à l'étude de la pollinisation du colza, Brassica napus L. var. oleifera (MOENCH) DELILE, en Belgique. Bulletin des recherches agronomiques de Gembloux **23**:123-152.

Diekotter, T., T. Kadoya, F. Peter, V. Wolters, and F. Jauker. 2010. Oilseed rape crops distort plant-pollinator interactions. Journal of Applied Ecology **47**:209-214.

Dosdall, L. M., and P. G. Mason. 2010. Key pests and parasitoids of oilseed rape or canola in North America and the importance of parasitoids in integrated management. Pages 167-213 Biocontrol-based integrated management of oilseed rape pests. Springer.

Feltham, H., K. Park, and D. Goulson. 2014. Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. Ecotoxicology **23**:317-323.

Franke, T. C., K. D. Kelsey, and T. A. Royer. 2009. Pest management needs assessment for Oklahoma canola producers. Division of Agricultural Sciences and Natural Resources, Oklahoma State University.

Fuhlendorf, S., D. Engle, D. Arnold, and T. Bidwell. 2002. Influence of herbicide application on forb and arthropod communities of North American tallgrass prairies. Agriculture, ecosystems & environment **92**:251-259.

Gathmann, A., and T. Tscharntke. 2002. Foraging ranges of solitary bees. Journal of Animal Ecology **71**:757-764.

Gill, R. J., O. Ramos-Rodriguez, and N. E. Raine. 2012. Combined pesticide exposure severely affects individual-and colony-level traits in bees. Nature **491**:105-108.

Greenleaf, S., N. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. Oecologia **153**:589-596.

Hamilton, R. 2007. Restoring heterogeneity on the Tallgrass Prairie Preserve: applying the fire-grazing interaction model. Pages 163-169 *in* Proceedings of the 23rd Tall Timbers fire ecology conference: fire in grassland and shrubland ecosystems. Tall Timbers Research Station Tallahassee.

Han, W., Z. Yang, L. Di, B. Zhang, and C. Peng. 2014. Enhancing agricultural geospatial data dissemination and applications using geospatial web services. Selected Topics in Applied Earth Observations and Remote Sensing, IEEE Journal of **7**:4539-4547.

Han, W. G., Z. W. Yang, L. P. Di, and R. Mueller. 2012. CropScape: A Web service based application for exploring and disseminating US conterminous geospatial cropland data products for decision support. Computers and Electronics in Agriculture **84**:111-123.

Hendrickx, F., J. P. MAELFAIT, W. Van Wingerden, O. Schweiger, M. Speelmans, S. Aviron, I. Augenstein, R. Billeter, D. Bailey, and R. Bukacek. 2007. How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. Journal of Applied Ecology **44**:340-351.

Hoehn, P., T. Tscharntke, J. M. Tylianakis, and I. Steffan-Dewenter. 2008. Functional group diversity of bee pollinators increases crop yield. Proceedings of the Royal Society B: Biological Sciences **275**:2283-2291.

Hopwood, J. L. 2008. The contribution of roadside grassland restorations to native bee conservation. Biological Conservation **141**:2632-2640.

Huryn, V. M. B. 1997. Ecological impacts of introduced honey bees. The Quarterly Review of Biology **72**:275-297.

Jauker, F., T. Diekötter, F. Schwarzbach, and V. Wolters. 2009. Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat. Landscape Ecology **24**:547-555.

Julier, H. E., and T. a. H. Roulston. 2009. Wild bee abundance and pollination service in cultivated pumpkins: farm management, nesting behavior and landscape effects. Journal of Economic Entomology **102**:563-573.

Kim, J., N. Williams, and C. Kremen. 2006. Effects of cultivation and proximity to natural habitat on ground-nesting native bees in California sunflower fields. Journal of the Kansas Entomological Society **79**:309-320.

Kleijn, D., R. Winfree, I. Bartomeus, L. G. Carvalheiro, M. Henry, R. Isaacs, A.-M. Klein, C. Kremen, L. K. M'Gonigle, and R. Rader. 2015a. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nature Communications **6**.

Kleijn, D., R. Winfree, I. Bartomeus, L. G. Carvalheiro, M. Henry, R. Isaacs, A.-M. Klein, C. Kremen, L. K. M'Gonigle, R. Rader, T. H. Ricketts, N. M. Williams, N. Lee Adamson, J. S. Ascher, A. Baldi, P. Batary, F. Benjamin, J. C. Biesmeijer, E. J. Blitzer, R. Bommarco, M. R. Brand, V. Bretagnolle, L. Button, D. P. Cariveau, R. Chifflet, J. F. Colville, B. N. Danforth, E. Elle, M. P. D. Garratt, F. Herzog, A. Holzschuh, B. G. Howlett, F. Jauker, S. Jha, E. Knop, K. M. Krewenka, V. Le Feon, Y. Mandelik, E. A. May, M. G. Park, G. Pisanty, M. Reemer, V. Riedinger, O. Rollin, M. Rundlof, H. S. Sardinas, J. Scheper, A. R. Sciligo, H. G. Smith, I. Steffan-Dewenter, R. Thorp, T. Tscharntke, J. Verhulst, B. F. Viana, B. E. Vaissiere, R. Veldtman, C. Westphal, and S. G. Potts. 2015b. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nature Communications **6**.

Klein, A.-M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences **274**:303-313.

Kremen, C., N. M. Williams, R. L. Bugg, J. P. Fay, and R. W. Thorp. 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. Ecology Letters **7**:1109-1119.

Kremen, C., N. M. Williams, and R. W. Thorp. 2002. Crop pollination from native bees at risk from agricultural intensification. Proceedings of the National Academy of Sciences **99**:16812-16816.

Laycock, I., and J. E. Cresswell. 2013. Repression and recuperation of brood production in Bombus terrestris bumble bees exposed to a pulse of the neonicotinoid pesticide imidacloprid. PLoS ONE **8**:e79872.

Laycock, I., K. M. Lenthall, A. T. Barratt, and J. E. Cresswell. 2012. Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (Bombus terrestris). Ecotoxicology **21**:1937-1945.

Le Féon, V., F. Burel, R. Chifflet, M. Henry, A. Ricroch, B. E. Vaissière, and J. Baudry. 2013. Solitary bee abundance and species richness in dynamic agricultural landscapes. Agriculture, ecosystems & environment **166**:94-101.

Mandelik, Y., R. Winfree, T. Neeson, and C. Kremen. 2012. Complementary habitat use by wild bees in agro-natural landscapes. Ecological Applications **22**:1535-1546.

McCollum III, F. T., R. L. Gillen, B. R. Karges, and M. E. Hodges. 1999. Stocker cattle response to grazing management in tallgrass prairie. Journal of Range Management:120-126.

Michener, C. D. 2000. The bees of the world. JHU Press.

Moffat, C., J. G. Pacheco, S. Sharp, A. J. Samson, K. A. Bollan, J. Huang, S. T. Buckland, and C. N. Connolly. 2015. Chronic exposure to neonicotinoids

increases neuronal vulnerability to mitochondrial dysfunction in the bumblebee (Bombus terrestris). The FASEB Journal:fj. 14-267179.

Morandin, L. A., and M. L. Winston. 2005. Wild bee abundance and seed production in conventional, organic and genetically modified canola. Ecological Applications **15**:871-881.

Morandin, L. A., M. L. Winston, V. A. Abbott, and M. T. Franklin. 2007. Can pastureland increase wild bee abundance in agriculturally intense areas? Basic and Applied Ecology **8**:117-124.

Moron, D., M. Lenda, P. Skorka, H. Szentgyorgyi, J. Settele, and M. Woyciechowski. 2009. Wild pollinator communities are negatively affected by invasion of alien goldenrods in grassland landscapes. Biological Conservation **142**:1322-1332.

Paton, P. W. C. 1994. The effect of edge on avian nest success: how strong is the evidence? El efecto de borde sobre el éxito en la nidificación: ¿Cuan firme es la evidencia? Conservation Biology **8**:17-26.

Pimentel, D., A. Marklein, M. A. Toth, M. N. Karpoff, G. S. Paul, R. McCormack, J. Kyriazis, and T. Krueger. 2009. Food versus biofuels: environmental and economic costs. Human Ecology **37**:1-12.

Plowright, R., and T. Laverty. 1984. The ecology and sociobiology of bumble bees. Annual Review of Entomology **29**:175-199.

Pohorecka, K., P. Skubida, A. Miszczak, P. Semkiw, P. Sikorski, K. Zagibajło, D. Teper, Z. Kołtowski, M. Skubida, and D. Zdańska. 2012. Residues of neonicotinoid insecticides in bee collected plant materials from oilseed rape crops and their effect on bee colonies. Journal of Apicultural Science **56**:115-134.

Potts, S., and P. Willmer. 1997. Abiotic and biotic factors influencing nest-site selection by Halictus rubicundus, a ground-nesting halictine bee. Ecological Entomology **22**:319-328.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution **25**:345-353.

Raymer, P. L. 2002. Canola: an emerging oilseed crop. Trends in new crops and new uses **1**:122-126.

Roubik, D. W., and H. Wolda. 2001. Do competing honey bees matter? Dynamics and abundance of native bees before and after honey bee invasion. Population Ecology **43**:53-62.

Royer, T. A., K. L. Giles, and N. C. Elliot. 2004. Common insect and mite pests of small grains. Division of Agricultural Sciences and Natural Resources, Oklahoma State University.

Stephen, W. P., and S. Rao. 2005. Unscented color traps for non-*Apis* bees (Hymenoptera: Apiformes). Journal of the Kansas Entomological Society **78**:373-380.

Stephen, W. P., and S. Rao. 2007. Sampling native bees in proximity to a highly competitive food resource (Hymenoptera: Apiformes). Journal of the Kansas Entomological Society **80**:369-376.

Thies, C., and T. Tscharntke. 1999. Landscape structure and biological control in agroecosystems. Science **285**:893-895.

Tilman, D., and P. M. Kareiva. 1997. Spatial ecology: the role of space in population dynamics and interspecific interactions. Princeton University Press.

Travis, P. D., and J. B. Robb. 2009. Wheat. Encyclopedia of Oklahoma History and Culture. Oklahoma Historical Society www.okhistory.org.

Tscharntke, T., I. Steffan-Dewenter, A. Kruess, and C. Thies. 2002. Contribution of small habitat framents to conservation of insect communities of grassland-cropland landscapes. Ecological Applications **12**:354-363.

USDA NASS 2007-2015. Oklahoma June Reports. http://www.nass.usda.gov/Quick_Stats/ (accessed Sept 30, 2015).

Vinson, S. B., and G. W. Frankie. 1977. Nests of *Centris aethyctera* (Hymenoptera: Apoidea: Anthophoridae) in the dry forest of Costa Rica. Journal of the Kansas Entomological Society **50**:301-311.

Whitehorn, P. R., S. O'Connor, F. L. Wackers, and D. Goulson. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science **336**:351-352.

Williams, N. M., E. E. Crone, H. R. T'ai, R. L. Minckley, L. Packer, and S. G. Potts. 2010. Ecological and life-history traits predict bee species responses to environmental disturbances. Biological Conservation **143**:2280-2291.

Williams, P. H. 1986. Environmental change and the distributions of British bumble bees (Bombus Latr.). Bee world **67**:50-61.

Wood, T. J., J. M. Holland, W. O. H. Hughes, and D. Goulson. 2015. Targeted agri-environment schemes significantly improve the population size of common farmland bumblebee species. Molecular Ecology:n/a-n/a.

Woods, T., J. Jonas, and C. Ferguson. 2012. The invasive Lespedeza cuneata attracts more insect pollinators than native congeners in tallgrass prairie with variable impacts. Biological Invasions **14**:1045-1059.

CHAPTER III

SPRING BLOOMING CROP COVER OF *BRASSICA NAPUS* CORRELATES WITH EARLY SUMMER BEE ABUNDANCE AND HIGHER SEED SET OF *CUCURBITA FOETIDISSIMA*

Abstract

Wild bees provide free pollination services and increase fruit/seed set for many agricultural crops, even in the presence of managed honey bee colonies. Many studies have focused on the distribution and abundance of bees in agricultural fields during crop bloom, but few studies have investigated bee abundance, diversity, and richness post crop bloom, during which time bees continue to forage in the landscape. Nearby land cover types may influence post crop bloom bee communities, as well as native flowering plant reproductive success. Pasture and semi-natural lands are known to increase abundance and species richness of bees in surrounding agricultural fields, and are a common land use type in the South Central United States. Canola (*Brassica napus*) has recently been introduced to Oklahoma as an early spring flowering crop, which is known to be attractive to pollinators, but which is grown in the wheat production region where other floral resources may be scarce. We sampled bees in canola production sites with and

without pasture in June and August using blue vane traps. We also quantified annual land cover within 1.5 km of each sampling location to evaluate the relationship between bee abundance and richness with eight land cover types. Additionally, we collected mature fruits of *Cucurbita foetidissima*, a late spring blooming native plant, from roadsides near canola with and without pasture, and in areas lacking both canola and pastures to evaluate if reproductive success was affected by landscape composition. Bees were more abundant in June, after canola was harvested. Seed set of *C. foetidissima* was highest in areas where canola had been produced. Sites with pastures did not strongly vary in bee communities or seeds per fruit of *C. foetidissima* compared to sites without adjacent pasture. Our landscape analysis showed no significant correlations of bee abundance and species richness with land cover types in June or August except for fallow lands. Based on bee abundance data and seed set data, wild plants in areas with canola production may have higher reproductive success compared to areas without canola production.

Introduction

Wild pollinator communities provide free ecosystem services and are important to the global economy as they significantly increase fruit and seed set for some crops which require pollination (Sahli and Conner 2007, Hoehn et al. 2008) and can be more effective than honey bees (*Apis mellifera*) in 35 of the 41 studied entomophylous crop systems (Garibaldi et al. 2013). However, agricultural and land-use intensification can negatively affect pollinator communities and biodiversity, but the exact mechanisms leading to these negative responses are not completely understood (Vitousek et al. 1997, Kleijn et al. 2009) and some driving factors may be unknown. Possible factors contributing to pollinator decline include competition with non-native pollinators for floral resources (Huryn 1997, Roubik and Wolda 2001), insecticides (Gill et al. 2012), and nest and habitat loss (reviewed in (Potts et al. 2010).

Habitat loss is common in agriculture when semi-natural areas are converted to arable land. This conversion can contribute to local declines in overall biodiversity including native bees due to destruction of nesting sites (e.g., undisturbed ground, woody plants) via plowing (Julier and Roulston 2009) and removal of floral resources. Although certain practices can harm bee species, agricultural areas can also provide suitable habitat for some pollinator species (Pimentel et al. 1992, Morandin et al. 2007, Carré et al. 2009, Flick et al. 2012, Gonthier et al. 2014). Data for multiple guilds (syrphid flies, wasps, butterflies, and bees) suggest natural and semi-natural areas, such as pastures and roadsides, provide resources which permit long-term persistence of pollinator communities in agricultural areas (Williams and Kremen 2007, Ricketts et al. 2008, Garibaldi et al. 2011). In the South Central United States, large crop monocultures

dominate with cattle producing pastures, roads and roadsides commonly occurring in the landscape. Cultivated fields can provide resources for pollinators when crops are flowering (Knight et al. 2009, Holzschuh et al. 2011, Baños-Picón et al. 2013), but may not provide resources at other times of the year. These patterns can cause a local saturation in resources during specific time periods (Morandin et al. 2007, Holzschuh et al. 2011) attracting organisms into the fields, and emigration as resources decrease (Mesa et al. 2013).

Wild pollinators foraging on flowering crops can increase crop production in some settings (Kremen et al. 2002, Kremen et al. 2004, Garibaldi et al. 2013); however, flowering crops can alter plant-pollinator interactions (Diekotter et al. 2010) including pollinator service dilution for native flora (Holzschuh et al. 2011). Pollination service dilution causes a decrease in seed set of simultaneously blooming wild flowers (Holzschuh et al. 2011), but it is not known if the seed set of plants which bloom after crops senesce is affected. Blooming crops concentrate pollinators into surrounding areas (Le Féon et al. 2013) and this may potentially enhance pollination services for surrounding flora when the crop stops blooming.

Conversely, mass flowering crops may attract pollinators which are not effective for some native plants to an area. For example, bumblebees will resort to nectar robbing (Diekotter et al. 2010) if they cannot find sufficient forage, an action which does not pollinate flowers and can decrease the reproductive success of those plants. In addition to attracting pollinators to forage, mass flowering crops may also attract emerging bees to nest near these agricultural fields, which will only have nectar resources for a short period of time. Nest sites are selected based on a variety of biotic and abiotic factors

43

including floral resource availability, plant community structure, and nesting resources (Potts and Willmer 1997, Gathmann and Tscharntke 2002). It is unknown if solitary bees can relocate and build new nests as resources change but due to lack of parental care of young, this movement may be possible (Berger et al. 1985). However, species that do provide parental care to young, such as bumblebees, will stay with their nest and starve when sufficient forage is not available (Goulson et al. 2008).

Canola (*Brassica napus*) has early spring and summer blooming varieties, which add highly concentrated floral resources into the landscape for pollinator communities (Hoyle et al. 2007) and is used for biofuel and cooking oil (Raymer 2002). In Oklahoma, winter canola has been in production since 2008 (USDA NASS 2008). It is rotated with winter wheat, which has been the dominant crop in this region for many decades (Boyles et al. 2004, Travis and Robb 2009). The agricultural landscape also has seminatural areas as well cattle producing pastures, both of which are known to increase native bee abundance and richness by providing potential nesting and forage areas throughout the year (Morandin et al. 2007, Julier and Roulston 2009), but these patterns have not been investigated in the South Central United States.

Production of canola will likely continue to increase to meet both biofuel and cooking oil demands, but it is unclear how the introduction of canola may impact the spatial and temporal distribution of wild bees once it is harvested. Spatial concentration of bees has been positively correlated with canola and other *Brassica* crop peak bloom times (Morandin and Winston 2005, Hoyle et al. 2007, Knight et al. 2009, Le Féon et al. 2013, Mesa et al. 2013), with mass emigration observed during senescence of *Brassica* crops (Mesa et al. 2013) but it is not known if bees stay in the surrounding landscape or

migrate to areas with sufficient forage. It is also unknown whether the presence of pastures affects the abundance and richness of bees in the area, once canola is harvested. Bees leaving canola fields and entering the surrounding landscape can affect pollinator communities (Diekotter et al. 2010) as well as plant-pollinator interactions (Levin and Anderson 1970, Diekotter et al. 2010, Gardiner et al. 2010, Holzschuh et al. 2011), which may also be affected by the presence natural areas.

As canola production increases in the South Central United States, it is important to understand the effects canola production will have on wild bee and wild plant communities, as well as the availability of pollination services. To elucidate the effects of pasture on abundance, richness and diversity of bee species, which may provide pollination services as canola crops senesce, we trapped bees in June and August in the South Central United States in field margins of canola production sites with and without adjacent pasture from 2012 to 2014. Since we could not control for land cover, we performed correlations between the percent of land cover types around canola producing areas and bee abundance and richness. We also sampled a native entomophylous plant (*Cucurbita foetidissima*) to test if seed production per fruit differed in areas with and without pasture next to canola fields as well as in field margins of non-nectar producing cereal crops without pasture or canola.

Materials and methods

Study Sites

Four sites were selected each year in 2012-2014 (n=12) which contained fields that produced canola and wheat in the spring of the sampled year. The majority of sites

were different from previous years due to crop rotation of winter wheat and winter canola. Sites were also sampled during canola bloom (McCoshum et al. in prep). Of the four sites selected each year, two sites had pastures and two sites lacked pastures adjacent to the canola fields.

Bee sampling

Estimates of bee community composition were obtained by placing transects of three blue vane traps for 48 hours at the interface of the canola fields and wheat fields with traps at 0m, 100m and 200m. Sites with pastures had an additional three blue vane traps placed at the interface of the pasture and senesced canola field. We utilized blue vane trapes because they are more effective than pan traps in capturing large bodied bees (Stephen and Rao 2005), and we used these traps during the spring when canola was in bloom due to their success in the presence of a high reward floral resource (Roulston et al. 2007, Baum et al. 2011). Blue vane traps consist of a plastic container (15 cm diameter × 15 cm high) secured under two cross vanes (3 mm thick) composed of blue polypropylene sheets (24 cm × 13 cm) (SpringStar™ LLC, www.springstar.net). These traps are visually attractive to bees and collect living small to large bodied bees, while excluding most honey bees (*A. mellifera)* due to honey bee foraging behavior (Stephen and Rao 2005, 2007).

Utilization of blue vane traps without killing agents allowed us to release living specimens that were easily identifiable such as *Anthophora* spp. and bumblebees (*Bombus* spp.). However, many specimens perished while in the traps due to the summer heat, even when traps were checked daily. Our sampling intensity was based on previously published research with blue vane traps sampling bees in sunflower fields,

which are high density floral resource areas with three traps set per field (Stephen and Rao 2007). Trapping only occurred when rain was not forecasted, winds were below 48kph, and daily high temperatures were above 10°C. Sites with and without pastures were sampled during the same days to avoid confounding weather and trapping effort (Bartholomew and Prowell 2006).

Landscape analysis

Using data from Cropscape: USDA (Han et al. 2012, Han et al. 2014) for the same year our sampling was conducted, we extracted data for land cover within a 1.5 km radius; a distance which was chosen because most bees forage within 1 km from their nest and the transects extended 0.1 km in both directions from the center trap point. Furthermore, we assumed bees may travel further to find nesting sites when they emerge and nest location selection is influenced by floral resource availability (canola). Species which emerged when canola was still producing flowers and selected nest sites near canola fields should influence bee abundance and richness at our sites in June. We kept this buffer size for comparison of August data. Data were categorized into 8 land cover types: canola (recall it blooms before most other crops and native plants); non-nectar producing crops which included winter wheat, corn, sorghum, etc. (these crops do not provide nectar resources for the bees); grass or pasture (these areas may contain both floral and nesting resources); wetland which included open water (these areas can provide mud, reeds, and a variety of resources for bees); fallow or barren land (these areas differ from year to year and likely will not provide many resources); developed land which included homesteads and gardens, fracking platforms, and roads (which may provide nesting areas for ground nesting bees and floral resources on roadsides and homesteads);

47

forests which comprised deciduous and evergreen forest (areas providing nesting resources for xylophylous bees); and other flowering crops such as soybeans and alfalfa which bloom in later summer months (these areas can provide nectar resources after canola senesces). These data were then converted to percent cover for the 1.5 km area around each site.

Seed set study

To test if pollination services for native plants increase after canola senescence, fruits from *C. foetidissima*: Cucurbitaceae were collected in August of 2013 and 2014 from plants growing within roadside margins. We selected plants based on female flowers being produced which are only produced when plants are able to sustain fruit production, and marked in June with pink marking tape. Fruits from marked flowers were collected in August, from roadsides in 3 different types of sites: roadsides without canola production or pastures within 1.6km; roadsides with canola production and no pastures present within 1.6km, and; roadsides with canola production and pastures present within 1.6km. The distance of 1.6km was chosen because it is the normal grid spacing of roads in our sampling area and was larger than the 1.5km radius we used for landscape analyses. One to five fruits were collected from sites (max of 3 fruits acquired from each plant) (N=62 fruits), dried and seeds were counted. We chose to use *C. foetidissima* because these plants are gynodioecious, with female, male, and hermaphrodite plants, all of which require insect pollination for seed set to occur. Reproductive tissue investment and seed count is not known to differ between female and hermaphrodite plants (Kohn 1989) so any differences in seed set should be due to pollination services. Furthermore, these plants bloom in late June, are common on roadsides, and are utilized by various bee

species. When present fruits were collected from sites where we sampled bees, however, not all sites had *C. foetidissima* so collections were also made from areas which were not sampled for bees using the same criteria as site selection described above.

Analyses

Bee species were grouped into morphospecies and keyed to genus. Species level identifications were made when possible, and a synoptic collection was deposited at the USDA Bee Biology and Systematics Laboratory in Logan, Utah. Abundance, richness, and Simpson Diversity, were calculated and compared between sites with and without adjacent pasture using an ANOVA. Sørensen's similarity coefficient was calculated for each year, comparing all sites with adjacent pasture and all sites without adjacent pasture. Due to sampling effort differing between sites with and without pasture, bee abundances were standardized as the number of bees collected per transect for each landscape type (with and without pasture).

To test if adjacent pasture affected species presence, we used common morphospecies, which we defined as those found in both landscape types (areas with and without adjacent pasture) and comprised more than one percent of our data. We compared the frequency at which common morphospecies occurred per transect for sites with pasture vs without pasture using the z-score (VassarStats 2015). We also grouped morphospecies based on their common nesting habit (hive, ground, or twig) and used a Chi-square test to compare the abundance of each morphospecies with the same nesting habit between sites with and without pasture. Additionally, we analyzed adult parasitic species and bumblebees by landscape. Since parasitic species are only visiting traps to

feed, and are not foraging and feeding at similar rates as non-parasitic species, and bumblebees of the same species could potentially be of the same hive, we transformed the data to present or absent by site to test the effects of adjacent pasture.

T-tests were run using VassarStats to compare overall bee richness and abundance in canola landscapes with adjoining pasture to canola landscapes without adjoining pasture for each year. June and August abundances were also compared using a paired t-test (VassarStats 2015). Due to bee foraging distances, we also tested for correlation between types of land cover and bee abundance and richness for both June and August. To test how land cover from each site may have affected our data, we ran a correlation matrix and a Spearman's rank correlation in VassarStats to test the correlation each land cover type had with bee richness and abundance per transect.

We ran t-tests in VassarStats to compare seeds per fruit in canola producing areas with and without adjacent pasture. We also ran an ANOVA in VassarStats to test the difference in seeds per fruit between sites of canola without pasture, canola with pasture, and cereal crops without canola or pastures within 1.6 km.

Results

We collected a total of 2,428 bees and identified 53 morphospecies from our June samples and 2,016 bees and 62 morphospecies from our August samples, between 2012 and 2014. We collected 120 ± 31.5 bees per transect in June at sites with pastures and 165 ± 76.5 bees per transect at sites without pastures ($t = 0.91$, $df = 10$, $p = 0.390$). In August transects at sites with pastures averaged 104 ± 24.7 bees per transect compared to 128 ± 68 bees at sites without pastures ($t = -0.43$, $df = 10$, $p = 0.676$). Our June data had

16 common morphospecies (morphospecies comprising more than 1% of our data and found at more than 1 site) and August data had 18 common morphospecies. Six morphospecies were found at 100% of our sites with pasture, but only one morphospecies (*Agapostemon* type 1) was found at all sites without pasture in June (Table 1). Four morphospecies were collected at all of our sites with adjacent pasture in August, and only one morphospecies (*Lasioglossum*) was collected at all sites without adjacent pasture (Table 2). Seven morphospecies from June and thirteen morphospecies from August were represented by only one specimen. Abundance overall significantly decreased from June to August (t = 1.99, df = 22, p = 0.035). All three *Agapostemon* morphospecies present in June and August were less abundant in our August samples. Similarly, *Eucera, Halictus, Lasioglossum* type 1*, Xenoglossa* types 2 and 3 all decreased in abundance from June to August. *Svastra* type 1 and 2 increased in abundance from June to August (Figure 1) as did *Bombus pensylvanicus*.

Community similarity between sites with adjacent pasture compared to sites without adjacent pasture in June varied from year to year. Sites were least similar in 2012 (47.06%), and most similar in 2014 (88.24%), with 2013 having a similarity between the two years (64.23%). Similarity for sites with adjacent pasture and sites without adjacent pasture in August followed similar patterns as June sites. Sites were least similar in 2012 (26.65%) and most similar in 2014 (98.62%), with 2013 having intermediate similarity (52.83%). Adjacent pasture did not significantly increase the likelihood of any morphospecies being present at our transects in June or August except for *B. griseocolis* and *Lasioglossum 2* in June.

Bumblebees and cleptoparasites

51

Bombus auricomus, *B. pensylvanicus,* and *B. griseocollis* were only found in areas with pastures during June (Table 1), but *B. pensylvanicus* was only represented by one specimen. In August, *B pensylvanicus* was the most abundant, found at 83% of our sites and *B. auricomus* ($Z = 2.309$, $n = 12$, $p = 0.02$) and *B. griseocollis* ($Z = 2.309$, $n = 12$, $p = 0.02$) were only represented by one specimen each (Table 1). Parasitic species (*Triepeolus remigatus, Xeromelecta californica* and *Triepeolus* sp.) were found at sites with and without pastures in June ($Z = 0.586$, $n = 12$, $p = 0.56$). *Anthidium porterae, Coelioxys* sp., and *Triepeolus remigatus* were the only parasitic species found in August. No species were captured at the same location as another parasitic species, and no species was present at more than one site. Cumulatively, parasitic species did not occur more often in areas with or without pasture ($Z = 1.155$, $n = 12$, $p = 0.25$).

Landscape analysis

Abundance of bees in June was most correlated with non-nectar producing crops ($r_s = -0.494$, $n = 12$, $p = 0.102$) and richness was most correlated with wetlands ($r_s = -0.512$, $n = 12$, $p = 0.090$) neither of which were statistically significant. Similarly, August bee abundance was most correlated with non-nectar producing crops ($r_s = -0.487$, $n = 12$, $p = 0.101$) and richness was most correlated with fallow land ($r_s = 0.6774$, $n = 12$, $p = 0.016$) with only the fallow correlation showing statistical significance.

Seed count

Mature fruits of *C. foetidissima* were collected in August in areas without canola production and in areas with canola production with and without pasture. No difference was found between the number of seeds produced per fruit in areas producing canola with

and without adjacent pasture (t = -1.28, df = 38, p = 0.21). Seeds per fruit averaged 249 ± 9.1 for areas with pasture and 233.8 ± 7.5 for areas without adjacent pasture. Areas without canola or pasture had significantly reduced seeds per fruit ($F_{2,58}$ = 20.039, p < 0.01), with an average of 165.5 ± 12.7 seeds per fruit (Figure 2).

Discussion

Total bee abundance was significantly higher during June, soon after canola sets seed and ceases to produce flowers, compared to August with eight of the ten morphospecies present in both trapping periods decreasing in abundance (Figure 2). The higher abundance of bees during June is potentially due to the influence of canola attracting bees into these areas (Le Féon et al. 2013, Mesa et al. 2013), but canola land cover within 1.5km did not have significant correlation with our data. The decline in August may reflect reduced floral resource availability from the absence of canola during the emergence of fall bee species. Another possible explanation is that August bee community is smaller overall compared to June, but further research is needed into seasonal bee communities in this region to elucidate this possibility.

Bees are known to be attracted to areas where nectar producing crops are blooming (Diekotter et al. 2010, Holzschuh et al. 2011, Mesa et al. 2013) but our data did not suggest this was occurring at our sites in June or August. Fallow land cover was the only land cover in our analyses which had a significant correlation with bee abundance or richness, and only during August for bee richness. All of our sites had less than 1% of fallow land cover at all sites except for one where it was 4.82%. Fallow field margins have been shown to positively correlate with bee abundance (Williams and Kremen

2007) so it is possible our results have biological importance. Further research is needed to explain how August bee communities differ from June bee communities to further understand this correlation.

Annual differences in similarity of bee communities were also observed between our sites with and without pasture with sites being least similar in 2012 for both June and August and most similar in 2014. 2012 was the second year of a 3 year drought which may have influenced the differences in similarity as well as abundance (Frankie et al. 1998). We captured the lowest number of bees in June of 2012 and the most in 2014; but the same pattern was not observed in August as bees were most abundant in August of 2012 and least abundant in August of 2013. Positive correlation of August bee richness with developed areas and forests may be due to these areas potentially having water or floral resources during the drought periods. Further research is needed to understand the effects of drought on bee communities in agricultural areas, as well as the effects each land cover may have in both drought and non-drought years.

Seed counts from *C. foetidissima* suggest pollination services are greater in areas with canola production compared to areas without canola production in early summer when this species starts to bloom. We did not collect fruits which were pollinated in August, although they may have been present, and we did not sample for bees in areas where canola was not produced so we cannot speculate as to whether or not overall bee abundance or richness affects seed set in *C. foetidissima*. However, canola producing areas did have increased seed set which requires pollination (Kohn 1989), so it is possible that bees which were previously attracted to and foraging on canola increased seed set. If canola fields are used in agricultural management plans, our data suggest seed set of wild,

entomophylous plants will be higher near canola fields. Furthermore, our data suggest *C. foetidissima* in areas near cereal crops, has lower reproductive success than plants near canola production. Further studies should investigate long term bee community data as well as seed set of domestic and other wild entomophylous plants in areas with only cereal crop productions, especially in cases of multiple successive years (Le Féon et al. 2013) compared to areas with crops providing early spring floral resources (Riedinger et al. 2015).

Areas with pasture did not have more bees per transect in June or August compared to areas without pasture, which contradicts findings of many studies which suggest bee abundance and richness in agricultural fields are positively correlated with the presence of seminatural areas (Morandin et al. 2007, Carré et al. 2009, Julier and Roulston 2009). Our results may be different than previous studies because our study focused on post bloom bee communities, whereas other studies focused on summer blooming crops while crops were in bloom. However, our trapping effort in areas with pasture was twice as intense (traps in both canola and pasture), which may have diluted the number of bees per transect. It is also possible that competition between traps and flowers for bees, which has been documented with pan traps and bees (Baum and Wallen 2011) could have occurred. Areas with pasture may have had an higher abundance of floral resources (Morandin et al. 2007, Ricketts et al. 2008, Carré et al. 2009) or diversity of floral resources, which affects forage flight patterns (Osborne et al. 2008). Therefore, bees may have been more likely to be captured in areas without pasture if floral resources were less abundant or diverse in these areas.

Bumblebee species were caught mostly at sites with pasture, which aligns with previous research (Carvell et al. 2007, Heard et al. 2007) and in June these were the only sites where we captured bumblebees. However, in August *B. pensylvanicus* was captured in areas without pasture, even though they were not previously captured at those sites in June. This could be due to gynes foraging further from nests in August to accommodate colony growth, or due to more direct flight patterns over harvested fields to reach available floral resources (Osborne et al. 2008, Knight et al. 2009). We also captured males and queens of *B. pensylvanicus* and *B. auricomus* in August, which suggests bumblebee colonies are at least successful in areas where canola is produced which may be beneficial since bumblebees throughout North America, including the Midwest, are declining (Goulson et al. 2008, Grixti et al. 2009, Cameron et al. 2011). However, we did not measure nest size or success, or evaluate other factors, such as insecticide exposure, which may influence the success of bumblebee colonies (Gill et al. 2012, Whitehorn et al. 2012, Rundlof et al. 2015). Further research is needed on queen production and nest success to address how canola production is affecting bumblebee populations in the South Central United States.

Agricultural planning, which is currently not common in our study region, can be implemented to benefit native bee communities by providing consistent floral resources throughout the year (Mandelik et al. 2012). The decrease in bee abundance from June to August supports previous research illustrating wild bees are attracted to nectar producing crops (Diekotter et al. 2010, Holzschuh et al. 2011, Mesa et al. 2013) and suggest canola could be beneficial if integrated into summer entomophylous crop production by being planted adjacent to summer blooming crops. Additional research is needed however to

evaluate the impacts of these crops, and production practices, on wild bee reproduction and persistence in areas where nectar producing crops are grown in consecutive years. Nectar producing crops are clearly attractive to pollinators and add floral resources to the landscape, however bees may be affected by: insecticide applications, including sub-lethal exposure (Laycock et al. 2012, Feltham et al. 2014, Rundlof et al. 2015); no-till practices (Carr et al. 2012) that reduce ground disturbance and may benefit ground nesting bees; additional water availability from irrigation (Qadir and Oster 2004) which may affect plant communities and soils; or other variables common in agricultural landscapes.

Tables and Figures

Table 1: The bumblebee species and most common morphospecies captured in June, in transects between canola and wheat production sites, with and without pasture. The percent of transects in which a morphospecies occurred as well as its overall abundance in similar sites and total data. z-scores and p-values are also reported.

Species	% transects pasture	Specimens pasture	% transects no pasture	Specimens no pasture	z-score	p-value	% total data
Bombus auricomus	33.33	2	0	0	-1.55	0.12	0.08
Bombus griseocollis	50.00	4	0	0	2.00	0.0455*	0.16
Bombus pensylvanicus	16.67	2	0	0	-1.04	0.30	0.08
Agapostemon	100	327	100	419	0.00	1.00	30.72
Agapostemon	66.67	90	66.67	174	0.00	1.00	10.87
Agapostemon	100	23	83.33	13	-1.04	0.30	1.48
Diadasia	66.67	64	50.00	35	0.59	0.56	4.08
Eucera	66.67	26	50.00	14	0.59	0.56	1.65
Halictus ligatus	83.33	75	83.33	20	0.00	1.00	3.91
Halictus parallelus	83.33	25	50.00	11	1.23	0.22	1.48
Lasioglossum 1	100	191	83.33	105	-1.04	0.30	12.19
Lasioglossum 2	83.33	14	16.67	14	2.31	0.0209*	0.95
Melisodes 1	100	133	66.67	38	1.55	0.12	7.04
Melisodes 2	83.33	26	50.00	8	1.23	0.22	1.40
Svastra 1	100	62	66.67	14	1.55	0.12	3.13
Svastra 2	50.00	28	50.00	13	0.00	1.00	1.69
Triepeolus remigatus	66.67	22	16.67	13	1.76	0.08	1.44
Xenoglossa 1	83.33	42	66.67	6	0.67	0.50	1.98
Xenoglossa 2	100	88	83.33	20	-1.04	0.30	4.45
Xenoglossa strenua 3	66.67	91	83.33	24	-0.67	0.50	4.74

Table 2: The bumblebee species and most common morphospecies captured in August, in transects between canola and wheat production sites, with and without pasture. The percent of transects in which a morphospecies occurred as well as its overall abundance in similar sites and total data.

AUGUST	% transects p	Abundance	% transects n	Abundance	Z-score	p-value 2-tail	% total data
Bombus pensylvanicus	83.33	9	83.33	7	0	1	1.18
Bombus auricomus	16.67	1	0.00	0	1.04	0.30	0.04
Bombus griseocollis	16.67	1	0.00	0	1.04	0.30	0.04
Agapostemon 1	100.00	40	83.33	175	-1.04	0.30	15.40
Agapostemon 2	66.67	4	50.00	13	0.59	0.56	1.24
Agapostemon 3	66.67	9	66.67	18	0	1	1.88
Anthophora 1	83.33	9	33.33	8	1.23	0.22	1.19
Eucera	83.33	11	83.33	16	0	1	1.96
Eucerini 1	66.67	48	66.67	37	0	1	6.06
Eucerini 2	66.67	114	66.67	102	0	1	15.53
Halictus	50.00	4	50.00	15	0	1	1.40
Lasioglossum 1	100.00	47	100.00	84	0	1	9.44
Megachile 1	16.67	2	50.00	14	-1.23	0.22	1.10
Mellisodes 3	66.67	14	16.67	1	1.76	0.79	1.08
Svastra 1	83.33	62	50.00	50	1.23	0.22	8.03
Svastra 2	83.33	30	83.33	24	0	1	3.83
Svastra 3	100.00	60	83.33	50	-1.04	0.30	7.91
Xenoglossa 2	66.67	8	66.67	16	0	1	1.73
Xenoglossa 3	100.00	35	83.33	13	-1.04	0.30	3.40
Xenoglossa 4	83.33	24	50.00	52	1.23	0.22	5.44

59

Table 3: Correlation coefficients and their Spearman's rank correlation coefficients and two-tail p-values for the eight land cover types 1.5 km from the center of our transects in June and August for the abundance and richness of bees in areas where winter canola had been planted earlier that year. The average percent land cover is also given.

| June		Non-nectar producing	Grass/ pasture	Canola	Nectar producing	Developed/ open space	Fallow	Forest	Wetland
Abundance	Rs	-0.4939	0.1611	0.4729	-0.1366	0.1786	0.0624	0.1576	0.1159
	p-value	0.102	0.614	0.12	0.669	0.581	0.845	0.628	0.719
Richness	Rs	-0.1954	-0.0142	0.2061	-0.0213	0.2807	-0.0506	0.0959	-0.512
	p-value	0.543	0.96	0.518	0.946	0.379	0.876	0.77	0.0895
August		Non-nectar producing	Grass/ pasture	Canola	Nectar producing	Developed/ open space	Fallow	Forest	Wetland
Abundance	Rs	-0.4869	0.3398	0.1786	0.3783	0.056	0.6454	0.3853	0.1496
	p-value	0.101	0.281	0.581	0.226	0.861	0.235	0.216	0.642
Richness	Rs	-0.3047	0.1856	0.0685	0.5744	-0.1121	0.6774	0.5184	-0.0224
	p-value	0.336	0.562	0.607	0.51	0.726	0.016	0.084	0.946
Percent ± SE		56.71 ± 3.15	23.73 ± 2.27	12.01 ± 1.94	2.02 ± 0.32	3.77 ± 0.24	0.61 ± 0.39	1.10 ± 0.36	0.04 ± 0.02
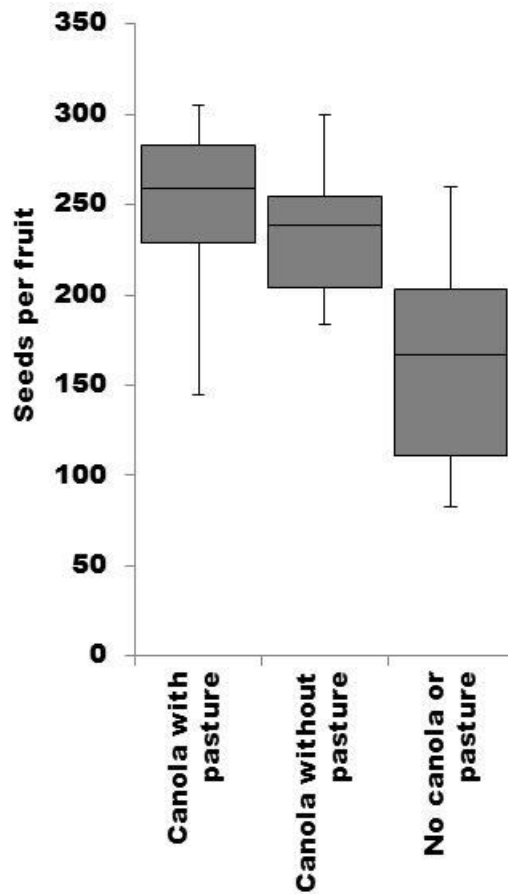
Figure 1: Box-plots for the number of seeds per fruit of *C. foetidissima* collected in August of 2013 and 2014. Fruits were collected from canola producing areas with and without pasture, as well as areas which did not have canola production or pasture within 1.6 km. A significant decrease in seeds per fruit was found in areas without pasture or canola production.
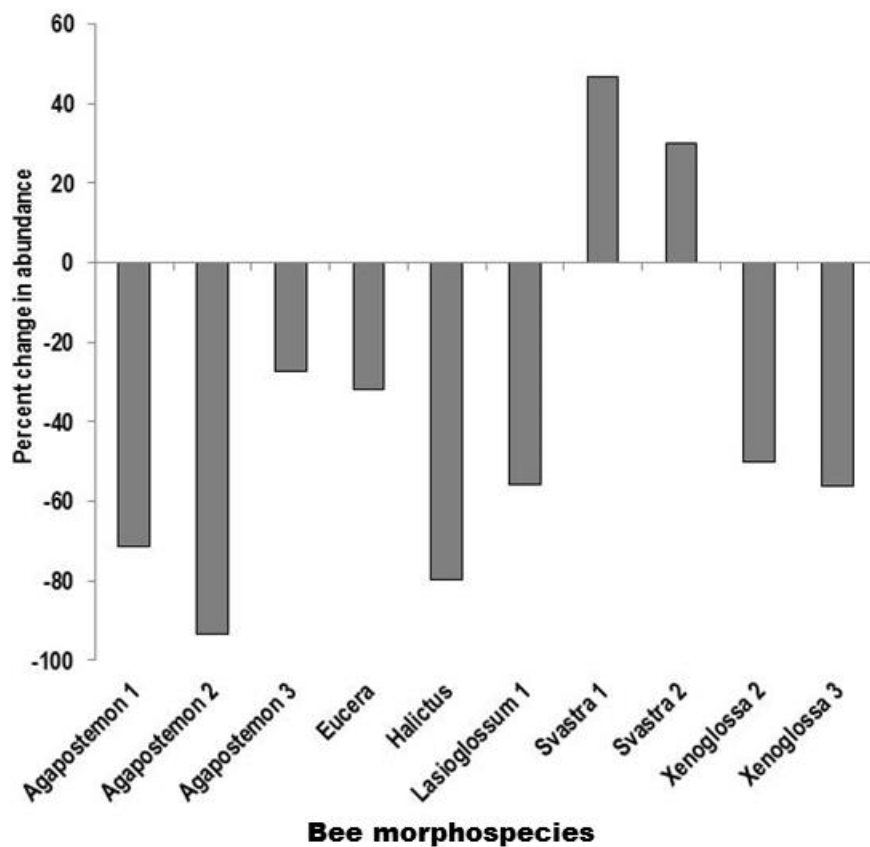
Figure 2: The percent change in abundance of common morphospecies captured in both June and August. Total abundance significantly decreased from June to August, with eight of the ten morphospecies also declining in abundance.

References

Baños-Picón, L., F. Torres, J. Tormos, S. F. Gayubo, and J. D. Asís. 2013. Comparison of two Mediterranean crop systems: Polycrop favours trap-nesting solitary bees over monocrop. Basic and Applied Ecology **14**:255-262.

Bartholomew, C. S., and D. Prowell. 2006. Comparison of bee diversity in upland and wet flatwood longleaf pine savannas in Louisiana (Hymenoptera: Apoidea). Journal of the Kansas Entomological Society **79**:199-206.

Baum, K. A., W. L. Rubink, R. N. Coulson, and V. M. Bryant Jr. 2011. Diurnal patterns of pollen collection by feral honey bee colonies in southern Texas, USA. Palynology **35**:85-93.

Baum, K. A., and K. E. Wallen. 2011. Potential bias in pan trapping as a function of floral abundance. Journal of the Kansas Entomological Society **84**:155-159.

Berger, L. A., J. O. Moffett, and D. R. Rummel. 1985. Seasonal cycles of *Agapostemon angelicus* Cockerell relative to hybrid cottonseed production in Texas (Hymenoptera: Halictidae). Journal of the Kansas Entomological Society **58**:1-8.

Boyles, M., T. F. Peeper, and C. Medlin. 2004. Producing winter hardy canola in Oklahoma. Division of Agricultural Sciences and Natural Resources, Oklahoma State University.

Cameron, S. A., J. D. Lozier, J. P. Strange, J. B. Koch, N. Cordes, L. F. Solter, and T. L. Griswold. 2011. Patterns of widespread decline in North American bumble bees. Proceedings of the National Academy of Sciences **108**:662-667.

Carr, P. M., P. Mäder, N. G. Creamer, and J. S. Beeby. 2012. Editorial: Overview and comparison of conservation tillage practices and organic farming in Europe and North America. Renewable Agriculture and Food Systems **27**:2-6.

Carré, G., P. Roche, R. Chifflet, N. Morison, R. Bommarco, J. Harrison-Cripps, K. Krewenka, S. G. Potts, S. P. M. Roberts, G. Rodet, J. Settele, I. Steffan-Dewenter, H. Szentgyörgyi, T. Tscheulin, C. Westphal, M. Woyciechowski, and B. E. Vaissière. 2009. Landscape context and habitat type as drivers of bee diversity in European annual crops. Agriculture, Ecosystems & Environment **133**:40-47.

Carvell, C., W. R. Meek, R. F. Pywell, D. Goulson, and M. Nowakowski. 2007. Comparing the efficacy of agri-environment schemes to enhance bumble bee abundance and diversity on arable field margins. Journal of Applied Ecology **44**:29-40.

Diekotter, T., T. Kadoya, F. Peter, V. Wolters, and F. Jauker. 2010. Oilseed rape crops distort plant-pollinator interactions. Journal of Applied Ecology **47**:209-214.

Feltham, H., K. Park, and D. Goulson. 2014. Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. Ecotoxicology **23**:317-323.

Flick, T., S. Feagan, and L. Fahrig. 2012. Effects of landscape structure on butterfly species richness and abundance in agricultural landscapes in eastern Ontario, Canada. Agriculture, Ecosystems & Environment **156**:123-133.

Frankie, G. W., R. W. Thorp, L. E. Newstrom-Lloyd, M. A. Rizzardi, J. F. Barthell, T. L. Griswold, J.-Y. Kim, and S. Kappagoda. 1998. Monitoring solitary bees in modified wildland habitats: implications for bee ecology and conservation. Environmental Entomology **27**:1137-1148.

Gardiner, M., J. Tuell, R. Isaacs, J. Gibbs, J. Ascher, and D. Landis. 2010. Implications of three biofuel crops for beneficial arthropods in agricultural landscapes. BioEnergy Research **3**:6-19.

Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, C. Kremen, L. G. Carvalheiro, L. D. Harder, and O. Afik. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science **339**:1608-1611.

Garibaldi, L. A., I. Steffan-Dewenter, C. Kremen, J. M. Morales, R. Bommarco, S. A. Cunningham, L. G. Carvalheiro, N. P. Chacoff, J. H. Dudenhoeffer, and S. S. Greenleaf. 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. Ecology Letters **14**:1062-1072.

Gathmann, A., and T. Tscharntke. 2002. Foraging ranges of solitary bees. Journal of Animal Ecology **71**:757-764.

Gill, R. J., O. Ramos-Rodriguez, and N. E. Raine. 2012. Combined pesticide exposure severely affects individual-and colony-level traits in bees. Nature **491**:105-108.

Gonthier, D. J., K. K. Ennis, S. Farinas, H.-Y. Hsieh, A. L. Iverson, P. Batáry, J. Rudolphi, T. Tscharntke, B. J. Cardinale, and I. Perfecto. 2014. Biodiversity conservation in agriculture requires a multi-scale approach. Proceedings of the Royal Society of London B: Biological Sciences **281**.

Goulson, D., G. C. Lye, and B. Darvill. 2008. Decline and conservation of bumble bees. Annu. Rev. Entomol. **53**:191-208.

Grixti, J. C., L. T. Wong, S. A. Cameron, and C. Favret. 2009. Decline of bumble bees (Bombus) in the North American Midwest. Biological Conservation **142**:75-84.

Han, W., Z. Yang, L. Di, B. Zhang, and C. Peng. 2014. Enhancing agricultural geospatial data dissemination and applications using geospatial web services. Selected Topics in Applied Earth Observations and Remote Sensing, IEEE Journal of **7**:4539-4547.

Han, W. G., Z. W. Yang, L. P. Di, and R. Mueller. 2012. CropScape: A Web service based application for exploring and disseminating US conterminous geospatial cropland data products for decision support. Computers and Electronics in Agriculture **84**:111-123.

Heard, M. S., C. Carvell, N. L. Carreck, P. Rothery, J. L. Osborne, and A. F. G. Bourke. 2007. Landscape context not patch size determines bumble-bee density on flower mixtures sown for agri-environment schemes. Biology Letters **3**:638-641.

Hoehn, P., T. Tscharntke, J. M. Tylianakis, and I. Steffan-Dewenter. 2008. Functional group diversity of bee pollinators increases crop yield. Proceedings of the Royal Society B: Biological Sciences **275**:2283-2291.

Holzschuh, A., C. F. Dormann, T. Tscharntke, and I. Steffan-Dewenter. 2011. Expansion of mass-flowering crops leads to transient pollinator dilution and reduced wild plant pollination. Proceedings of the Royal Society B: Biological Sciences **278**:3444-3451.

Hoyle, M., K. Hayter, and J. E. Cresswell. 2007. Effect of pollinator abundance on self-fertilization and gene flow: application to GM canola. Ecological Applications **17**:2123-2135.

Huryn, V. M. B. 1997. Ecological impacts of introduced honey bees. The Quarterly Review of Biology **72**:275-297.

Julier, H. E., and T. a. H. Roulston. 2009. Wild bee abundance and pollination service in cultivated pumpkins: farm management, nesting behavior and landscape effects. Journal of Economic Entomology **102**:563-573.

Kleijn, D., F. Kohler, A. Báldi, P. Batáry, E. D. Concepción, Y. Clough, M. Díaz, D. Gabriel, A. Holzschuh, E. Knop, A. Kovács, E. J. P. Marshall, T. Tscharntke, and J. Verhulst. 2009. On the relationship between farmland biodiversity and land-use intensity in Europe. Proceedings of the Royal Society of London B: Biological Sciences **276**:903-909.

Knight, M. E., J. L. Osborne, R. A. Sanderson, R. J. Hale, A. P. Martin, and D. Goulson. 2009. Bumblebee nest density and the scale of available forage in arable landscapes. Insect Conservation and Diversity **2**:116-124.

Kohn, J. R. 1989. Sex ratio, seed production, biomass allocation, and the cost of male function in Cucurbita foetidissima HBK (Cucurbitaceae). Evolution:1424-1434.

Kremen, C., N. M. Williams, R. L. Bugg, J. P. Fay, and R. W. Thorp. 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. Ecology Letters **7**:1109-1119.

Kremen, C., N. M. Williams, and R. W. Thorp. 2002. Crop pollination from native bees at risk from agricultural intensification. Proceedings of the National Academy of Sciences **99**:16812-16816.

Laycock, I., K. M. Lenthall, A. T. Barratt, and J. E. Cresswell. 2012. Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (Bombus terrestris). Ecotoxicology **21**:1937-1945.

Le Féon, V., F. Burel, R. Chifflet, M. Henry, A. Ricroch, B. E. Vaissière, and J. Baudry. 2013. Solitary bee abundance and species richness in dynamic agricultural landscapes. Agriculture, Ecosystems & Environment **166**:94-101.

Levin, D. A., and W. W. Anderson. 1970. Competition for pollinators between simultaneously flowering species. The American Naturalist **104**:455-467.

Mesa, L. A., B. G. Howlett, J. E. Grant, and R. K. Didham. 2013. Changes in the relative abundance and movement of insect pollinators during the flowering cycle of Brassica rapa crops: implications for gene flow. Journal of Insect Science **13**:13.

Morandin, L. A., and M. L. Winston. 2005. Wild bee abundance and seed production in conventional, organic and genetically modified canola. Ecological Applications **15**:871-881.

Morandin, L. A., M. L. Winston, V. A. Abbott, and M. T. Franklin. 2007. Can pastureland increase wild bee abundance in agriculturally intense areas? Basic and Applied Ecology **8**:117-124.

Osborne, J. L., A. P. Martin, N. L. Carreck, J. L. Swain, M. E. Knight, D. Goulson, R. J. Hale, and R. A. Sanderson. 2008. Bumblebee flight distances in relation to the forage landscape. Journal of Animal Ecology **77**:406-415.

Pimentel, D., U. Stachow, D. A. Takacs, H. W. Brubaker, A. R. Dumas, J. J. Meaney, J. A. S. O'Neil, D. E. Onsi, and D. B. Corzilius. 1992. Conserving biological diversity in agricultural/forestry systems: most biological diversity exists in human-managed ecosystems. Bioscience **42**:354-362.

Potts, S., and P. Willmer. 1997. Abiotic and biotic factors influencing nest-site selection by Halictus rubicundus, a ground-nesting halictine bee. Ecological Entomology **22**:319-328.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution **25**:345-353.

Qadir, M., and J. Oster. 2004. Crop and irrigation management strategies for saline-sodic soils and waters aimed at environmentally sustainable agriculture. Science of the Total Environment **323**:1-19.

Raymer, P. L. 2002. Canola: an emerging oilseed crop. Trends in new crops and new uses **1**:122-126.

Ricketts, T. H., J. Regetz, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, A. Bogdanski, B. Gemmill-Herren, S. S. Greenleaf, A. M. Klein, M. M. Mayfield, L. A. Morandin, A. Ochieng', and B. F. Viana. 2008. Landscape effects on crop pollination services: are there general patterns? Ecology Letters **11**:499-515.

Riedinger, V., O. Mitesser, T. Hovestadt, I. Steffan-Dewenter, and A. Holzschuh. 2015. Annual dynamics of wild bee densities: attractiveness and productivity effects of oilseed rape. Ecology **96**:1351-1360.

Roubik, D. W., and H. Wolda. 2001. Do competing honey bees matter? Dynamics and abundance of native bees before and after honey bee invasion. Population Ecology **43**:53-62.

Roulston, T. a. H., S. A. Smith, and A. L. Brewster. 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**:179-181.

Rundlof, M., G. K. S. Andersson, R. Bommarco, I. Fries, V. Hederstrom, L. Herbertsson, O. Jonsson, B. K. Klatt, T. R. Pedersen, J. Yourstone, and H. G. Smith. 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature **521**:77-80.

Sahli, H. F., and J. K. Conner. 2007. Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, Raphanus raphanistrum (Brassicaceae). American Journal of Botany **94**:203-209.

Stephen, W. P., and S. Rao. 2005. Unscented color traps for non-*Apis* bees (Hymenoptera: Apiformes). Journal of the Kansas Entomological Society **78**:373-380.

Stephen, W. P., and S. Rao. 2007. Sampling native bees in proximity to a highly competitive food resource (Hymenoptera: Apiformes). Journal of the Kansas Entomological Society **80**:369-376.

Travis, P. D., and J. B. Robb. 2009. Wheat. Encyclopedia of Oklahoma History and Culture. Oklahoma Historical Society www.okhistory.org.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of earth's ecosystems. Science **277**:494-499.

Whitehorn, P. R., S. O'Connor, F. L. Wackers, and D. Goulson. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science **336**:351-352.

Williams, N. M., and C. Kremen. 2007. Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. Ecological Applications **17**:910-921.

CHAPTER IV

EARLY SPRING BUTTERFLY RICHNESS WITHIN AREAS PRODUCING

BRASSICA NAPUS INCREASES WITH PERCENT OF GRASS/ PASTURE

Abstract

Human dominated land use covers more than 20% of the world, and mainly

consists of agriculture, which reduces landscape heterogeneity, biodiversity, and

community structure. Invertebrate pollinators are sensitive to land use patterns and are in

decline globally in part due to habitat fragmentation and loss. However, some agricultural

management practices which include pastures and semi-natural areas within agricultural

landscapes have been shown to increase pollinator diversity as well as landscape

heterogeneity and plant diversity. Butterflies are generally considered poor crop

pollinators, but some species are important native plant pollinators. The South Central

United States has historically been intensively cultivated to produce non-nectar producing

cereal crops and has intermittent pastures and semi natural areas. Both migratory and

non-migratory butterfly species are active in early spring in this area, but data on the

butterfly communities are lacking. In 2008 Oklahoma wheat producers began rotating

winter wheat crops with winter canola (*Brassica napus*), a nectar producing, early-spring

67

blooming crop which may benefit pollinators, but the effects on pollinator assemblages is unknown. We sampled butterfly communities with yellow sticky traps from 2011 to 2013 in wheat and canola fields with and without adjacent pasture to evaluate if butterfly communities differed in the presence or absence of pasture. Using 30 m resolution land cover data we tested for correlations between land cover and butterfly abundance and richness. Sites with canola, wheat and pasture did not have significantly different butterfly communities compared to areas of canola and wheat without pasture. Yearly populations varied over the course of the study, which may reflect drought conditions and/or spring temperatures. We also found four species of migratory butterfly species active during canola bloom in the South Central United States, which may enable long distance cross-pollination of canola. Our study suggests butterfly communities in this area are fairly similar and yearly assemblages may be more sensitive to temperature and drought than land cover type.

Introduction

More than 20% of the world's land has been converted from its natural state into human dominated use, with various biomes being affected at different rates (Hoekstra et al. 2005). These lands are mostly converted to agricultural lands (McLaughlin and Mineau 1995, Hoekstra et al. 2005) and with advances in technology and pest management, agricultural lands have become more simplified (Persson et al. 2010). Simplification of agricultural areas reduces landscape heterogeneity (Fahrig et al. 2011), threatens biodiversity (Foley et al. 2005) and alters overall community structure (Cronin and Reeve 2005, Haddad et al. 2009, Diekotter et al. 2010). Plant community structure can be changed by local extirpations, changes in plant dominance or addition of non-native species, and these changes can affect the surrounding pollinator community (Tepedino et al. 2008, Haddad et al. 2009, Moron et al. 2009, Woods et al. 2012). These changes strongly affect plant pollination and reproductive success, creating a feedback loop which influences plant community structure as well as food security (Moron et al. 2009, Holzschuh et al. 2011).

Declines in pollinators have been documented over the past 30 years (Potts et al. 2010), although proper management has successfully slowed pollinator declines in some areas (Donald and Evans 2006, Carvalheiro et al. 2013). Additionally, management practices in agricultural settings can enhance local biodiversity and ecosystem services provided by pollinators and natural predators of crop pests (Bosch et al. 2000, Landis et al. 2000, Esther Julier and Roulston 2009, Dosdall and Mason 2010). However for most species, including pollinators, definitive assessment of populations is extremely difficult due to insufficient long term data (National Research Council 2007); therefore it is

important to document spatial and temporal patterns in species abundance and richness. Moreover, studies which document occurrences assist in identifying range changes (Hill et al. 2001, Grixti et al. 2009, Cameron et al. 2011) and introductions into areas (Lange and Cap 1986, Davis and Peña 1990, McCoshum et al. 2012). These data are extremely important for creating effective agricultural management plans.

Long term studies on butterfly populations in agricultural settings have mainly focused on pest species of perennial crops and are lacking for annual crops and non-pest butterfly species (Gilbert and Singer 1975). Butterfly assemblages are good indicators for landscape heterogeneity because assemblages will change with disturbance (Kremen 1992) and are used to conditionally assess habitat for other species (Landres et al. 1988). Furthermore, many natural predators of crop pests use resources within the surrounding landscape, including hosts for overwintering (Pfannenstiel et al. 2010) and many non-pest Lepidopteran species can host important natural enemies of crop pests (Takagi 1985, Stapel et al. 1997, Pfannenstiel et al. 2010). Most butterflies (Hesperiidae and Papilionoidea) are considered poor pollinators due to their long legs and lack of pollen carrying structures (Sahli and Conner 2007); however, they are important for various native plants (Cruden and Hermann-Parker 1979, Jennersten 1984, Borges et al. 2003). Furthermore, some butterfly species, such as *Pieris rapae,* are more effective at increasing seed set in *Brassica raphnus* than some bee species (Sahli and Conner 2007), although this has not been evaluated for *B. napus*.

Within North America, grasslands have been reduced by more than 50% due to agricultural conversion (Hoekstra et al. 2005) especially within the Great Plains with most states having a 99% reduction (Samson and Knopf 1994). The South Central United

States has intense agricultural production, mainly producing wheat and other non-nectar producing crops for more than nine decades (Travis and Robb 2009). Consecutive years of wheat production in an area have been shown to negatively affect bee communities (Le Féon et al. 2013) and may similarly affect butterfly communities. However, the recent addition of winter canola to the South Central United States could positively affect butterfly species active in the early spring. Pastures, seminatural areas, and even organic farming are known to positively affect butterfly abundance and richness (Rundlöf and Smith 2006), as they increase landscape heterogeneity which is positively correlated with butterfly abundance and richness (Flick et al. 2012). Similarly, pastures in agricultural areas of the South Central United States could increase butterfly abundance and richness by supporting host and/or nectar plants. Alternatively, butterfly communities in heavily cultivated areas could experience homogenization, regardless of the presence or absence of grasslands.

Canola is an early spring flowering crop that produces an indeterminate raceme of flowers. Each flower produces relatively large amounts of nectar (0.2µl - 6µl/flower, with an average of 2µl/flower), depending on the variety (Pierre et al. 1999). Flowering occurs in early spring, prior to most native plants (Berger et al. 1985, Bosch et al. 2000), and typically lasts approximately 3 to 5 weeks, making it attractive and potentially beneficial to adult butterflies. Oklahoma is the primary producer of canola within the South Central US, where winter canola is planted in rotation with winter wheat. In the last 7 years, production of canola in Oklahoma has increased from 0 to 270,000 acres (109,265 hectares) per year (USDA NASS 2008-2015), but how butterfly communities are affected by this increase is not yet known. Furthermore, within the canola-wheat landscapes of

Oklahoma, semi-natural grassland areas (often mowed), roadsides and pasture are common which increases overall landscape heterogeneity.

Although canola production has the potential to benefit adult butterflies by providing nectar resources, canola is susceptible to several crop pests including aphids (Dosdall and Mason 2010), and insecticide application is the primary method used to control aphid infestations. Each year, 90% of fields are sprayed with insecticide, some more than once (Franke et al. 2009) and applications often occur around the time of flowering to control cabbage aphids that infest canola during bloom and seed-pod development (Boyles et al. 2004, Royer et al. 2004) and can significantly reduce crop production. Due to the potential positive and negative effects canola production may have on butterfly communities, it is important to evaluate how butterflies may be affected to develop appropriate agricultural management strategies.

Using passive capture techniques in fields of canola, wheat, and pasture, we sampled butterfly communities in Oklahoma to assess: the influence of adjacent pastures as well as land cover on butterfly abundance and richness near and within canola fields; whether overall butterfly abundance differed between years as canola production increased; and the temporal pattern of butterfly presence in canola producing areas in the South Central United States (especially as it relates to the arrival of migratory butterflies). Collectively these data will inform agricultural management strategies in this region that are relevant to butterfly communities.

Materials and methods

Non-scented, yellow sticky traps were placed in winter canola fields, winter wheat fields, and pastures in 8 sites throughout the canola producing region in north-central Oklahoma in April and early May of 2011, 2012, and 2013 when canola began to bloom. Each field had 17 trap points (Figure 1) with two sticky traps per point, one facing the field margin, and one facing away from the field margin. Five trap points parallel to the field margin, were located 50 m into the field, with the center three traps spaced 40 m apart, and the two end traps spaced 20 m apart. A grid of twelve traps in a 3 x 4 grid or equally spaced trap points 40 m apart was placed 60 m further into the field than the first row, with the furthest traps located 230 m from the margin. Sticky traps were set up when canola fields began to bloom and replaced 6 times on days 2, 4, 6, 8, and 12 after initial placement, and taken down on day 18, which was during peak bloom. This study was designed to investigate the movement of aphid predating insects, and the butterflies were by-catch. Although the study was not designed to sample butterflies, their activity is likely highest near the margins compared to the center of the fields based on daily movement estimates (Scott 1975, Davis et al. 2007), so this design should reflect butterfly assemblages in the area.

Butterflies were identified to the lowest possible taxon using Butterflies of North America (Brock et al. 2003). Due to damage to specimens from struggling against the glue and bird predation, which was evident by missing bodies of butterflies, some specimens were only identifiable to family or genus. Butterflies in Lycaeniidae and Hesperiidae, except for *Pyrgus communis* were identified to family due to color distortions and wing damage caused by the sticky traps. *Vanessa cardui*, *V. virginiensis*,

Colias eurytheme and *C. philodice* were identified to species when possible but we grouped species in each genus for analysis due to the number of specimens that were not identifiable beyond genus and because the two *Colias* species can hybridize.

Analyses

We calculated diversity using the Shannon-Weiner Index for each field (canola, wheat and pasture) and performed a T-test comparing canola fields and wheat fields in areas without pasture, and comparing canola fields and wheat fields in areas with pasture. Fields were then grouped in order to compare sites with pasture and sites without pasture. Due to differences in trapping effort between site types (34 trap points in areas without pasture and 68 trap point in areas with pasture), we standardized our site data by trapping effort to number of butterflies caught per two fields because sites without pasture had canola and wheat fields (butterflies per 34 traps) to calculate diversity, richness, and abundance at each site, and then utilized T-tests to compare each measure between sites with and without pasture. Similarity was calculated using Sørensen's coefficient for sites with and without pasture as well.

Landscape analysis

To test for land cover effects, we used data from CropScape (Han 2014, USDA NASS 2014) and extracted land cover type for 1.5 km radius from the center of our transects. We selected 1.5 km because distance of daily butterfly movement is known to be higher in agricultural settings compared to natural areas (Loos et al. 2015). *Colias eurytheme* were documented moving 1.12 km in a mark release recapture study (Watt et al. 1979) and our trap points extended 0.27 km into crops. We rounded up to 1.5 km to

74

capture these movements. Data were categorized into 8 land cover types: canola (recall it blooms before most other crops and native plants); non-nectar producing crops which included winter wheat, corn, sorghum, etc. (these crops do not provide nectar resources for the butterflies); grass or pasture (these areas may contain both floral and larval host plant resources); wetland which included open water (these areas can provide areas for puddling, where butterflies congregate to collect minerals and salts); fallow or barren land (these areas differ from year to year and likely will not provide many resources except potentially from annual plants); developed land which included homesteads and gardens, fracking platforms, and roads (which may provide ground for puddling and potentially unmanaged plant buffers with floral resources on roadsides and homesteads); forests which comprised deciduous and evergreen forest (areas potentially providing more nectar resources and overwintering areas) and other flowering crops such as soybeans and alfalfa which bloom in later summer months (these areas can provide nectar resources and potentially host plants after canola is harvested). These data were then converted to percent cover for each of our sites. We ran a Spearman's rank coefficient to test the correlation of butterfly abundance and richness with land cover type.

Results

Our traps collected 1,871 identifiable butterflies from 5 families: Hesperiidae, Lycaeniidae, Nymphalidae, Pieridae, and Papilionidae. The most abundant species were *C. eurytheme* and *C. philodice*. Due to damage and hybridization species specific identification were difficult for ~20% of the *Colias* specimens. Cumulatively, *Colias* spp. comprised 53.72% of the collected butterflies. *Vanessa* spp. were the second most abundant group comprising 18.8% of our data, and we were unable to make

75

identifications for ~45% of the *Vanessa* butterflies. *Pontia protodice* accounted for 9% of our data followed by *Danaus plexippus* at 8.5%. These percentages varied between years, and some less common species only occurred in a single year (Figure 2).

Abundance, diversity, and similarity differed from year to year, with no consistent patterns between sites with pastures compared to sites without pastures. Sites with pastures had higher abundance of butterflies in 2011, but sites without pastures had almost double the number of butterflies than areas without pasture in 2012 and 2013. Overall, 2012 had the highest abundance and richness of butterflies compared to the other years. Species richness was higher at sites with pastures in 2011 and 2012, but lower in 2013. Similarity of sites had small changes between years, ranging between 0.75 and 0.8 (Table 1) with the most similarity seen in 2012. No statistical differences were found between areas with adjacent pasture vs areas without adjacent pasture when all years were grouped and compared for abundance (t = -0.67, df = 17 p = 0.51), richness (t = 1.27, df = 17, p = 0.22), or diversity (t = 1.01, df = 17, p = 0.33).

Landscape analysis

Landscape analyses showed our sites with and without adjacent pasture had large variations in the amount of grass/pasture land cover within 1.5 km, with only three sites containing less than 10% grass/pasture cover at this larger spatial scale. The three most common land cover types, non-nectar producing crops, grass/pasture and canola, did not have any significant correlation with butterfly abundance ($r_s < 0.21$, p > 0.41) or richness ($r_s < 0.28$, p > 0.28) (Table 2). Developed/open space was the only land cover type with a significant positive correlation with butterfly richness ($r_s = 0.70$, p = 0.002) (Table 2).

Temporal patterns

Four species of migratory butterflies were captured on our traps: *Danaus plexippus, Vanessa atalanta, V. cardui,* and *V. virginiensis. Danaus plexippus* was encountered in all 3 years and was captured first on April 7, 2011, 2 days after our first traps were placed; April 5, 2012, 1 day after our first traps were setup (half of the sites were setup on April 4, 2012 which did not collect any monarchs); and April 24, 2013. *Vanessa atalanta* was only captured in 2012 starting on the first day we placed traps in fields (April 4, 2012), and throughout our trapping period with a total of 22 identifiable specimens. Identifiable specimens of *V. cardui* were not very abundant in 2011, with only 3 specimens collected starting on April 17, 2011. Similarly in 2013, only 3 individuals were captured starting on April 19, 2013. In 2012, we captured 93 *V. cardui* starting on April 4, 2012, the first day our traps were placed. *Vanessa virginiensis* was captured in all 3 years as well, with 3 specimens collected in 2011 starting on April 18, 2011, 58 collected in 2012 starting on April 6, 2012, and 2 collected in 2013 starting April 27, 2013.

Discussion

The most common butterflies collected were *Colias* (*C. philodice* and *C. eurytheme*), both of which can be crop pests of alfalfa. Butterfly abundance was not higher in pastures adjacent to canola fields, but these sites tended to have higher species richness. Our landscape analysis showed a positive correlation of butterfly richness with developed space, which included private gardens and homesteads. It is possible these areas provided a wider variety of host plants and nectar resources as well as

77

overwintering areas. No significant correlations were found between butterfly richness and any other land cover category, which is not consistent with previous research (Hill et al. 2001, Rundlöf and Smith 2006, Winfree et al. 2011, Loos et al. 2015). However our study focuses on early spring butterfly communities in the South Central United States, where other studies have focused on annual communites, or mid to late summer communities.

Due to the differences in butterfly abundance between years, it is likely that variables such as drought, seasonal temperature or overwintering sites influence abundance more than land cover in the study region. The tendency for higher species richness in areas with development is likely due to host plant diversity in these areas compared to other landscape types (Öckinger and Smith 2006, Williams and Kremen 2007) Further research should look into plant community differences in pastures compared to unmanaged areas which may have similar host plant species for butterflies. For example, some management practices for pastures increase forage species for cattle by promoting graminoid growth via broadleaf herbicide application (e.g., Lochmiller et al. 1991, Hamilton 2007), which decreases heterogeneity within the pastures. Prescribed fire, which was implemented at some sites, can increase plant biodiversity (Fuhlendorf and Engle 2001) or decrease it depending on frequency (Blair et al. 2008). It is unclear which pastures within our study utilized prescribed fire or how frequently burning occurred, so we did not include burns in our analysis, but further research should investigate burning and burn frequency in addition to grass/pasture coverage with butterfly abundance and richness.

Abundance of the crop pests is interesting and warrants further consideration. Canola is a host plant to *Pieris rapae*, the cabbage white, (Scott 1986, Graves and Shapiro 2003) which we captured few specimens of; however, *C. eurytheme* and *C. philodice* were the most abundant species we collected. Both of these *Colias* species can develop on alfalfa (*Medicago sativa*), which is produced in the South Central United States. It is not clear if the availability of canola (and associated nectar) in the landscape influences the distribution and abundance of these crop pests at larger spatial scales; however, this warrants further research. Furthermore, four species of parasitoids have been collected in Oklahoma from *C. eurytheme* (Soteres et al. 1984) and canola may alter their distribution, abundance, and subsequent parasitism of *C. eurytheme* in the landscape as well. Similarly, canola may attract butterflies to areas where host plant abundance is low leading to competition among larvae, or where host plant abundance is high and both situations may benefit parasitoid communities. Natural predators of crop pests are known to benefit from pastures and seminatural areas, so although we did not find butterfly abundance to be correlated with pastures or grass/ pasture (Kruess 2003, Cronin and Reeve 2005, Haddad et al. 2009, Dosdall and Mason 2010), natural enemies of these species may be more abundant in these areas.

Other flowering crops in the area, mainly alfalfa and soybeans, bloom after our butterflies were collected so they did not provide floral resources while our traps were deployed. However, these crops were also produced in previous years which may have influenced butterfly assemblages at our sites because diversity of floral resources has been shown to increase pollinator abundance (Ries et al. 2001, Davis et al. 2007) and butterflies may have overwintered near fields of soybean or alfalfa, which would not be

captured in our analyses. Similarly, wetlands and forested areas generally create areas

which are not disturbed during crop production, so these areas may provide floral

resources and host plants the previous year as well as areas for butterflies to overwinter,

but both of these land covers were generally between 0 and 1% of the site land cover.

Developed land cover had a moderately positive influence on both species abundance and

number of species, but this land cover type did not exceed 6.5% at any site, and it is

possible a different pattern would be observed if developed land comprised a greater

percent of the landscape since butterflies can be negatively affected by intense

development (Van Dyck et al. 2009). Further analyses for effects of crops from the

previous year, and environmental patterns are warranted as well as species level analysis

for common species.

We did not formally survey roadsides for our study but plants in the Asteraceae,

Convolvulaceae, Cucurbitaceae, Poaceae and Solanaceae, were common along roadsides

near our study sites. Roadside buffers differed between sites with larger buffers existing

next to paved roads and smaller buffers next to dirt roads. Buffers next to paved roads

were generally mowed, with a narrow unmanaged strip next to agricultural fields, and

buffers along dirt roads were generally left unmanaged. These differences in both buffer

size and management are known to affect biodiversity (Ries et al. 2001, Hopwood 2008,

Ernoult et al. 2013), and likely affected community similarity, diversity, abundance and

richness around canola fields.

The differences we observed between years for similarity, abundance and richness

may also be explained by climatic differences between the years. April of 2011 was dry

with a total of 2.67 cm of rain and a daily high temperature average of 23.89 °C. April of

2012 had more rain with 6.91 cm in total and a daily high temperature average of 23.33 °C. April was much cooler in 2013 with a daily high temperature average of 17.22 °C and 4.01 cm of precipitation (NOAA 2015). The unseasonably cold weather in 2013 delayed spring migration of *D. plexippus* into the area (JourneyNorth 2013, McCoshum and Baum 2014) and likely had a similar effect on the other migrating species. Since temperature affects when butterflies emerge (Sparks and Yates 1997) in spring as well as migrate, it is plausible most of the differences we observed between years can be attributed to spring temperatures and this may explain the low abundance of butterflies in 2013. Even with these yearly differences, our data show that canola does bloom during the period of spring migration for four of our study species, which may provide an important nectar source in the area during that time and may also enable long distance cross-pollination or plant pathogen dispersal (Roy 1993). Furthermore, canola blooms when spring butterfly species are active, which may benefit these populations in agricultural settings as well.

Data show that butterfly pollinated plants are declining in areas consisting of smaller patches (Hendrix and Kyhl 2000), which also supports the data showing their pollinators are also in decline (Biesmeijer et al. 2006, Carvalheiro et al. 2013). In the past, agricultural fields with annual crops often supported wild plants (e.g., milkweeds; (Hartzler 2010, Pleasants and Oberhauser 2013) in addition to the crop species. With the development of widely used herbicide resistant crops, weed diversity has significantly decreased (Young et al. 2013). Weed loss from agricultural fields has been suggested to harm species that are dependent upon those plants for all or part of their life cycles (e.g., monarch butterflies are declining due to milkweed loss from agricultural fields (Pleasants

and Oberhauser 2013). Our study is one of the first to investigate butterfly abundance and richness in Oklahoma agricultural areas. Although we found positive correlation with butterfly richness and grass/ pasture land cover, butterfly communities were fairly similar in areas of canola production with and without pasture, which suggests butterfly pollination services for canola should also be fairly similar. The similarity of butterfly assemblages in areas with and without pasture may be due to the reduction of wild host and nectar plants. Further research into the distribution of these wild plants in this region may provide better data for the butterfly community structure.

Canola is often treated with insecticides around the time of flowering to reduce aphid pests, and our study did not investigate the effects of insecticide exposure. Non-lethal exposure to insecticides has been linked to problems within bumble bees, honey bees, and caterpillars including lower reproductive success and decreased foraging capability (Longley and Sotherton 1997, Goulson et al. 2002, Dai et al. 2010, Feltham et al. 2014). Most studies have focused on caterpillar exposure to sub-lethal effects, which led to smaller adults (Longley and Sotherton 1997, Hoang et al. 2011); no studies have investigated the effects of sub-lethal insecticide exposure on adult butterflies. Our data also showed a high prevalence of two species that can be pests to alfalfa production, and further research should investigate if canola influences the abundance of these crop pests or whether alfalfa fields near canola production sites are more likely to have more caterpillar damage than areas without canola production..

Agriculture management plans will also have to consider butterfly communities structure including migratory and non-migratory species. In general, butterflies make daily movements of between 200 and 600 m and non-migratory butterflies have dispersal

movements from 1 to 2.9 km (Scott 1975, Davis et al. 2007) generally making habitat or risk assessment easy because host plants and nectar resources need to be within this range. Migratory species, however, can move much further on a daily basis overwintering in southern regions and moving north during the spring and summer (Drake and Gatehouse 1995, Stefanescu et al. 2013) which is an important aspect when considering spread of plant pathogens that use pollinators as vectors (Real et al. 1992, Roy 1993, Shykoff and Bucheli 1995) and cross-pollination (Hayter and Cresswell 2006). Floral and anther rusts affect mainly non-cultivated plants and affect reproductive success (Real et al. 1992, Shykoff and Bucheli 1995) and may be of concern to land managers. Cross-pollination is mainly a concern for seed contamination by genetically modified organisms (GMOs); regulations are quickly changing around the world concerning GMO labeling and production (Anderson et al. 2001, Vigani and Olper 2015). Gene dispersal via cross-pollination is strictly dependent upon pollen movement and migratory butterflies may contribute to long range movement of pollen.

Our study shows that the early spring butterfly community in the South Central United States has four migratory butterfly species: the well-studied monarch butterfly (*Danaus plexippus*) (Urquhart and Urquhart 1978, Flockhart et al. 2013, McCoshum and Baum 2014) which feeds on non-crop plants and three species in the *Vanessa* genus: the painted-lady butterfly (*Vanessa cardui*); the red admiral (*V. atalanta*); and the American lady (*V. virginiensis*). Few studies have investigated the migration *Vanessa* species in North America (Abbott 1951, Stefanescu 2001, Stefanescu et al. 2013), but it is clear that these species overlap with canola bloom, which may facilitate long-range cross-pollination of canola. Further details on migrating butterflies will be important as

regulations of GMO's change. Further research is also needed to elucidate: whether temperature and precipitation have effects on the community structure and how migratory species are affected by these variables; whether canola puts butterflies at risk of insecticide exposure; and how land cover affects long term annual populations and migrations.

Canola production in the South Central United States will likely continue to increase due to biofuel and cooking oil demands (Raymer 2002). This early nectar resource may also be a valuable integration into crop rotation and land management policy to support pollinator communities. Our study shows percent of grass/ pasture area cover positively correlates with butterfly richness and that butterfly communities are similar in areas of canola production with and without pasture in the South Central United States, which suggests butterfly pollination services for canola should also be similar. Butterflies are generally not considered important pollinators (Cruden and Hermann-Parker 1979, Jennersten 1988, Hendrix 2000, Borges et al. 2003), but some species can increase seed set at or above the rate of small solitary bees in some settings (Sahli and Conner 2007). Populations fluctuated between years, and did not steadily increase or decrease from year to year in our study, so the short-term effects of canola on butterfly populations are not clear, but longer-term studies may clarify canola's effects on the butterfly community.

Tables and Figures

Table 1: Data for Shannon-Weiner diversity index (H'), butterfly richness and abundance for sites with and without pasture, and Sørensen's similarity (QS) comparing sites with and without pasture for each year.

	Measure	2011	2012	2013
	H'	1.51	1.92	1.53
Pasture	Richness	11	12	4
	Abundance	114	256	17
	H'	1.80	2.25	1.35
No Pasture	Richness	7	8	4
	Abundance	84	733	63
Similarity	QS	0.78	0.80	0.75

Table 2: Spearman's rank order coefficients and p-values for land cover and butterfly richness and abundance and the average percent of each land cover across all sites.

Butterflies		Non-nectar producing	Grass/ pasture	Canola	Developed/ open space	Nectar producing	Fallow	Forest	Wetland
Richness	rs	-0.22	0.28	0.05	0.70	-0.09	-0.27	0.02	0.08
	p-value	0.39	0.28	0.85	0.002*	0.72	0.30	0.95	0.77
Abundance	rs	-0.07	0.21	-0.21	-0.05	0.30	0.02	0.16	-0.28
	p-value	0.78	0.41	0.41	0.85	0.25	0.94	0.53	0.28
Percent ± SE		62.66 ± 2.35	18.41 ± 2.08	9.83 ± 1.15	3.47 ± 0.21	3.34 ± 0.91	1.65 ± 0.61	0.56 ± 0.25	0.07 ± 0.03

Figure 1: Layout of yellow sticky trap points in a single field. Five traps were placed parallel to the margin located 50 m into the agricultural field (canola, wheat, or pasture). Traps were spaced along a 120m transect at 0 m, 20 m, 60 m, 100 m, and 120 m. Twelve additional traps points were equally spaced 40 m apart in a 3 x 4 grid within the field, located 60 m from the first five traps with the furthest traps placed 230 m into the agricultural field.
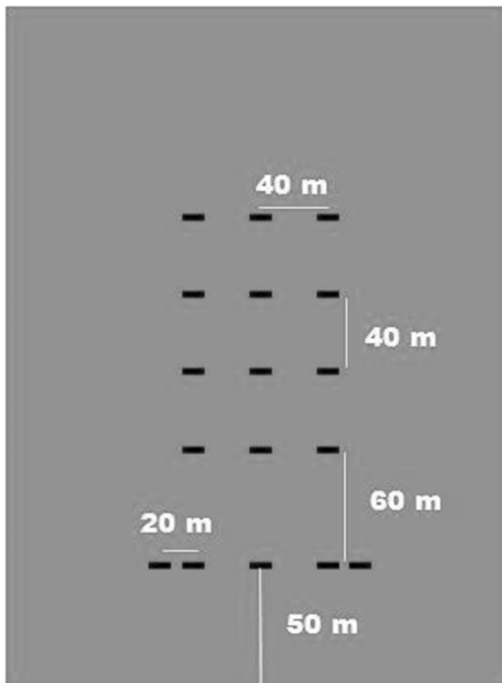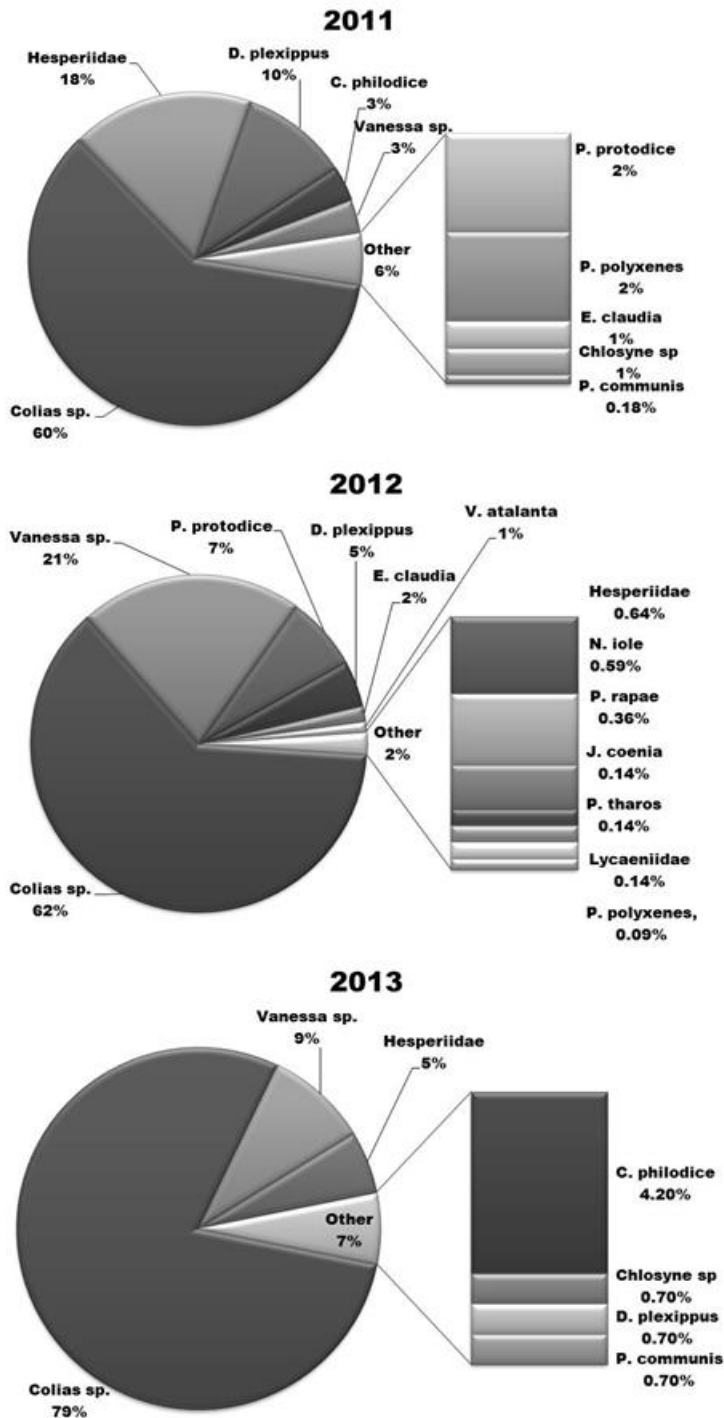
Figure 2: Yearly butterfly assemblages for all sites and the percent composition of each taxa. The "Other" category for each year includes the least abundant butterfly species.

References

Abbott, C. H. 1951. A quantitative study of the migration of the painted lady butterfly, *Vanessa cardui* L. Ecology:155-171.

Abergel, E. 2015. Corporate Crops: Biotechnology, Agriculture and the Struggle for Control, by Gabriela Pechlaner. Journal of Agrarian Change **15**:148-151.

Anderson, K., B. Hoekman, and A. Strutt. 2001. Agriculture and the WTO: Next steps. Review of International Economics **9**:192-214.

Berger, L. A., J. O. Moffett, and D. R. Rummel. 1985. Seasonal cycles of *Agapostemon angelicus* Cockerell relative to hybrid cottonseed production in Texas (Hymenoptera: Halictidae). Journal of the Kansas Entomological Society **58**:1-8.

Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemüller, M. Edwards, T. Peeters, A. P. Schaffers, S. G. Potts, R. Kleukers, C. D. Thomas, J. Settele, and W. E. Kunin. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science **313**:351-354.

Blair, J. M., M. D. Smith, and S. L. Collins. 2008. Influence of grazing and fire frequency on small☐scale plant community structure and resource variability in native tallgrass prairie. Oikos **117**:859-866.

Borges, R., V. Gowda, and M. Zacharias. 2003. Butterfly pollination and high-contrast visual signals in a low-density distylous plant. Oecologia **136**:571-573.

Bosch, J., W. P. Kemp, and S. S. Peterson. 2000. Management of *Osmia lignaria* (Hymenoptera: Megachilidae) Populations for Almond Pollination: Methods to Advance Bee Emergence. Environmental Entomology **29**:874-883.

Boyles, M., T. F. Peeper, and C. Medlin. 2004. Producing winter hardy canola in Oklahoma. Division of Agricultural Sciences and Natural Resources, Oklahoma State University.

Brock, J. P., J. P. Brock, and K. Kaufman. 2003. Kaufman field guide to butterflies of North America. Houghton Mifflin Harcourt.

Cameron, S. A., J. D. Lozier, J. P. Strange, J. B. Koch, N. Cordes, L. F. Solter, and T. L. Griswold. 2011. Patterns of widespread decline in North American bumble bees. Proceedings of the National Academy of Sciences **108**:662-667.

Carvalheiro, L. G., W. E. Kunin, P. Keil, J. Aguirre-Gutiérrez, W. N. Ellis, R. Fox, Q. Groom, S. Hennekens, W. Van Landuyt, D. Maes, F. Van de Meutter, D. Michez, P. Rasmont, B. Ode, S. G. Potts, M. Reemer, S. P. M. Roberts, J. Schaminée, M. F. WallisDeVries, and J. C. Biesmeijer. 2013. Species richness declines and biotic homogenisation have slowed down for NW-European pollinators and plants. Ecology Letters **16**:870-878.

Council, N. R. 2007. Status of pollinators in North America. *in* Committee on the Status of Pollinators in North America, & National Academies Press. The National Academic Press. USA.

Cronin, J. T., and J. D. Reeve. 2005. Host–parasitoid spatial ecology: a plea for a landscape-level synthesis. Proceedings of the Royal Society B: Biological Sciences **272**:2225-2235.

Cruden, R., and S. M. Hermann-Parker. 1979. Butterfly pollination of Caesalpinia pulcherrima, with observations on a psychophilous syndrome. The Journal of Ecology:155-168.

Dai, P. L., Q. Wang, J.-H. Sun, F. Liu, X. Wang, Y.-Y. Wu, and T. Zhou. 2010. Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee Apis mellifera ligustica. Environmental Toxicology and Chemistry **29**:644-649.

Davis, J., D. Debinski, and B. Danielson. 2007. Local and landscape effects on the butterfly community in fragmented Midwest USA prairie habitats. Landscape Ecology **22**:1341-1354.

Davis, D. R., and Peña, J. E. 1990. Biology and morphology of the banana moth, *Opogona sacchari* (Bojer), and its introduction into Florida (Lepidoptera: Tineidae). Proceedings of the entomological Society of Washington, **92**:593-618.

Diekotter, T., T. Kadoya, F. Peter, V. Wolters, and F. Jauker. 2010. Oilseed rape crops distort plant-pollinator interactions. Journal of Applied Ecology **47**:209-214.

Donald, P. F., and A. D. Evans. 2006. Habitat connectivity and matrix restoration: the wider implications of agri-environment schemes. Journal of Applied Ecology **43**:209-218.

Dosdall, L. M., and P. G. Mason. 2010. Key pests and parasitoids of oilseed rape or canola in North America and the importance of parasitoids in integrated management. Pages 167-213 Biocontrol-based integrated management of oilseed rape pests. Springer.

Drake, V. A., and A. G. Gatehouse. 1995. Insect migration: tracking resources through space and time. Cambridge University Press.

Ernoult, A., A. Vialatte, A. Butet, N. Michel, Y. Rantier, O. Jambon, and F. Burel. 2013. Grassy strips in their landscape context, their role as new habitat for biodiversity. Agriculture, ecosystems & environment **166**:15-27.

Esther Julier, H., and T. A. H. Roulston. 2009. Wild Bee Abundance and Pollination Service in Cultivated Pumpkins: Farm Management, Nesting Behavior and Landscape Effects. Journal of Economic Entomology **102**:563-573.

Fahrig, L., J. Baudry, L. Brotons, F. G. Burel, T. O. Crist, R. J. Fuller, C. Sirami, G. M. Siriwardena, and J. L. Martin. 2011. Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. Ecology Letters **14**:101-112.

Feltham, H., K. Park, and D. Goulson. 2014. Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. Ecotoxicology **23**:317-323.

Flick, T., S. Feagan, and L. Fahrig. 2012. Effects of landscape structure on butterfly species richness and abundance in agricultural landscapes in eastern Ontario, Canada. Agriculture, ecosystems & environment **156**:123-133.

Flockhart, D. T. T., L. I. Wassenaar, T. G. Martin, K. A. Hobson, M. B. Wunder, and D. R. Norris. 2013. Tracking multi-generational colonization of the breeding grounds by monarch butterflies in eastern North America. Proceedings of the Royal Society B: Biological Sciences **280**.

Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, J. H. Helkowski, T. Holloway, E. A. Howard, C. J. Kucharik, C. Monfreda, J. A. Patz, I. C. Prentice, N. Ramankutty, and P. K. Snyder. 2005. Global consequences of land use. Science **309**:570-574.

Franke, T. C., K. D. Kelsey, and T. A. Royer. 2009. Pest management needs assessment for Oklahoma canola producers. Division of Agricultural Sciences and Natural Resources, Oklahoma State University.

Fuhlendorf, S. D., and D. M. Engle. 2001. Restoring heterogeneity on rangelands: ecosystem management based on evolutionary grazing patterns we propose a paradigm that enhances heterogeneity instead of homogeneity to promote biological diversity and wildlife habitat on rangelands grazed by livestock. Bioscience **51**:625-632.

Gilbert, L. E., and M. C. Singer. 1975. Butterfly ecology. Annual Review of Ecology and Systematics:365-397.

Goulson, D. G., W. H. Hughes, L. D. Derwent, and J. S. Stout. 2002. Colony growth of the bumblebee *Bombus terrestris* in improved and conventional agricultural and suburban habitats. Oecologia **130**:267-273.

Graves, S. D., and A. M. Shapiro. 2003. Exotics as host plants of the California butterfly fauna. Biological Conservation **110**:413-433.

Grixti, J. C., L. T. Wong, S. A. Cameron, and C. Favret. 2009. Decline of bumble bees (Bombus) in the North American Midwest. Biological Conservation **142**:75-84.

Haddad, N. M., G. M. Crutsinger, K. Gross, J. Haarstad, J. M. H. Knops, and D. Tilman. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. Ecology Letters **12**:1029-1039.

Hamilton, R. G. 2007. Restoring heterogeneity on the Tallgrass Prairie Preserve: applying the fire-grazing interaction model. In Proceedings of the 23rd Tall Timbers Fire Ecology Conference: Fire in Grassland and Shrubland Ecosystems. Tall Timbers Research Station, Tallahassee FL. 163-169.

Han, W. G., Z. W. Yang, L. P. Di, and R. Mueller. 2012. CropScape: A Web service based application for exploring and disseminating US conterminous geospatial cropland data products for decision support. Computers and Electronics in Agriculture **84**:111-123.

Hartzler, R. G. 2010. Reduction in common milkweed (Asclepias syriaca) occurrence in Iowa cropland from 1999 to 2009. Crop Protection **29**:1542-1544.

Hayter, K. E., and J. E. Cresswell. 2006. The influence of pollinator abundance on the dynamics and efficiency of pollination in agricultural Brassica napus: implications for landscape-scale gene dispersal. Journal of Applied Ecology **43**:1196-1202.

Hendrix, S. D. 2000. Population size and reproduction in Phlox pilosa. Conservation Biology **14**:304-313.

Hill, J. K., Y. C. Collingham, C. D. Thomas, D. S. Blakeley, R. Fox, D. Moss, and B. Huntley. 2001. Impacts of landscape structure on butterfly range expansion. Ecology Letters **4**:313-321.

Hoang, T. C., R. L. Pryor, G. M. Rand, and R. A. Frakes. 2011. Use of butterflies as nontarget insect test species and the acute toxicity and hazard of mosquito control insecticides. Environmental Toxicology and Chemistry **30**:997-1005.

Hoekstra, J. M., T. M. Boucher, T. H. Ricketts, and C. Roberts. 2005. Confronting a biome crisis: global disparities of habitat loss and protection. Ecology Letters **8**:23-29.

Holzschuh, A., C. F. Dormann, T. Tscharntke, and I. Steffan-Dewenter. 2011. Expansion of mass-flowering crops leads to transient pollinator dilution and reduced wild plant pollination. Proceedings of the Royal Society B: Biological Sciences **278**:3444-3451.

Hopwood, J. L. 2008. The contribution of roadside grassland restorations to native bee conservation. Biological Conservation **141**:2632-2640.

Jennersten, O. 1984. Flower visitation and pollination efficiency of some North European butterflies. Oecologia **63**:80-89.

Jennersten, O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology **2**:359-366.

JourneyNorth. 2013. Monarch Butterfly. learner.org.

Kremen, C. 1992. Assessing the indicator properties of species assemblages for natural areas monitoring. Ecological Applications **2**:203-217.

Kruess, A. 2003. Effects of landscape structure and habitat type on a plant—herbivoreparasitoid community. Ecography **26**:283-290.

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology **45**:175-201.

Landres, P. B., J. Verner, and J. W. Thomas. 1988. Ecological uses of vertebrate indicator species: a critique. Conservation Biology **2**:316-328.

Lange, C., and R. Cap. 1986. *Bythotrephes cederstroemi* (Schödler).(Cercopagidae: Cladocera): a new record for Lake Ontario. Journal of Great Lakes Research **12**:142-143.

Le Féon, V., F. Burel, R. Chifflet, M. Henry, A. Ricroch, B. E. Vaissière, and J. Baudry. 2013. Solitary bee abundance and species richness in dynamic agricultural landscapes. Agriculture, ecosystems & environment **166**:94-101.

Lochmiller, R. L., J.F. Boggs, S. T. McMurry, D. M. Leslie Jr, and D. M. Engle. 1991. Response of cottontail rabbit populations to herbicide and fire applications on cross timbers rangeland. Journal of Range Management, **44**:150-155.

Longley, M., and N. Sotherton. 1997. Factors determining the effects of pesticides upon butterflies inhabiting arable farmland. Agriculture, ecosystems & environment **61**:1-12.

Loos, J., M. Kuussaari, J. Ekroos, J. Hanspach, P. Fust, L. Jackson, and J. Fischer. 2015. Changes in butterfly movements along a gradient of land use in farmlands of Transylvania (Romania). Landscape Ecology **30**:625-635.

McCoshum, S. M., and K. A. Baum. 2014. Sex ratios and *Ophryocystis elektroscirrha* infection levels of *Danaus plexippus* during spring migration through Oklahoma, USA. Entomologia Experimentalis et Applicata **153**:266-272.

McCoshum, S. M., M. S. Caterino, and A. R. Cline. 2012. Introduced sap beetles (Coleoptera: Nitidulidae) on Santa Catalina Island, California, USA. The Coleopterists Bulletin **66**:347-350.

McLaughlin, A., and P. Mineau. 1995. The impact of agricultural practices on biodiversity. Agriculture, ecosystems & environment **55**:201-212.

Moron, D., M. Lenda, P. Skorka, H. Szentgyorgyi, J. Settele, and M. Woyciechowski. 2009. Wild pollinator communities are negatively affected by invasion of alien goldenrods in grassland landscapes. Biological Conservation **142**:1322-1332.

Öckinger, E., and H. G. Smith. 2006. Landscape composition and habitat area affects butterfly species richness in semi-natural grasslands. Oecologia **149**:526-534.

Persson, A. S., O. Olsson, M. Rundlöf, and H. G. Smith. 2010. Land use intensity and landscape complexity—Analysis of landscape characteristics in an agricultural region in Southern Sweden. Agriculture, Ecosystems & Environment **136**:169-176.

Pfannenstiel, R., T. Unruh, and J. Brunner. 2010. Overwintering hosts for the exotic leafroller parasitoid, Colpoclypeus florus: Implications for habitat manipulation to augment biological control of leafrollers in pome fruits. Journal of Insect Science **10**:75.

Pierre, J., J. Mesquida, R. Marilleau, M. H. Pham-Delègue, and M. Renard. 1999. Nectar secretion in winter oilseed rape, *Brassica napus*— quantitative and qualitative variability among 71 genotypes. Plant Breeding **118**:471-476.

Pleasants, J. M., and K. S. Oberhauser. 2013. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. Insect Conservation and Diversity **6**:135-144.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution **25**:345-353.

Raymer, P. L. 2002. Canola: an emerging oilseed crop. Trends in new crops and new uses **1**:122-126.

Real, L. A., E. A. Marschall, and B. M. Roche. 1992. Individual behavior and pollination ecology: implications for the spread of sexually transmitted plant diseases. Pages 492-508 Individual-Based Models and Approaches in Ecology. Springer.

Ries, L., D. M. Debinski, and M. L. Wieland. 2001. Conservation value of roadside prairie restoration to butterfly communities. Conservation Biology **15**:401-411.

Roy, B. 1993. Floral mimicry by a plant pathogen.

Royer, T. A., K. L. Giles, and N. C. Elliot. 2004. Common insect and mite pests of small grains. Division of Agricultural Sciences and Natural Resources, Oklahoma State University.

Rundlöf, M., and H. Smith. 2006. The effect of organic farming on butterfly diversity depends on landscape context. Journal of Applied Ecology **43**:1121-1127.

Sahli, H. F., and J. K. Conner. 2007. Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, Raphanus raphanistrum (Brassicaceae). American Journal of Botany **94**:203-209.

Scott, J. A. 1975. Flight patterns among eleven species of diurnal Lepidoptera. Ecology:1367-1377.

Scott, J. A. 1986. The butterflies of North America. A natural history and field guide. Stanford University Press.

Shykoff, J. A., and E. Bucheli. 1995. Pollinator visitation patterns, floral rewards and the probability of transmission of Microbotryum violaceum, a veneral disease of plants. Journal of Ecology:189-198.

Soteres, K., R. Berberet, and R. McNew. 1984. Parasitic insects associated with lepidopterous herbivores on alfalfa in Oklahoma. Environmental Entomology **13**:787-793.

Sparks, T., and T. Yates. 1997. The effect of spring temperature on the appearance dates of British butterflies 1883–1993. Ecography **20**:368-374.

Stapel, J. O., J. R. Ruberson, H. R. Gross, and W. J. Lewis. 1997. Progeny allocation by the parasitoid Lespesia archippivora (Diptera: Tachinidae) in larvae of Spodoptera exigua (Lepidoptera: Noctuidae). Environmental Entomology **26**:265-271.

Stefanescu, C. 2001. The nature of migration in the red admiral butterfly Vanessa atalanta: evidence from the population ecology in its southern range. Ecological Entomology **26**:525-536.

Stefanescu, C., F. Páramo, S. Åkesson, M. Alarcón, A. Ávila, T. Brereton, J. Carnicer, L. F. Cassar, R. Fox, and J. Heliölä. 2013. Multi□generational long□distance migration of insects: studying the painted lady butterfly in the Western Palaearctic. Ecography **36**:474-486.

Takagi, M. 1985. The reproductive strategy of the gregarious parasitoid, *Pteromalus puparum* (Hymenoptera: Pteromalidae). Oecologia **68**:1-6.

Tepedino, V. J., B. A. Bradley, and T. L. Griswold. 2008. Might flowers of invasive plants increase native bee carrying capacity? Intimations from Capitol Reef National Park, Utah. Natural Areas Journal **28**:44-50.

Travis, P. D., and J. B. Robb. 2009. Wheat. Encyclopedia of Oklahoma History and Culture. Oklahoma Historical Society www.okhistory.org.

Urquhart, F., and N. Urquhart. 1978. Autumnal migration routes of the eastern population of the monarch butterfly (Danaus p. plexippus L.; Danaidae; Lepidoptera) in North America to the overwintering site in the Neovolcanic Plateau of Mexico. Canadian Journal of Zoology **56**:1759-1764.

Van Dyck, H., A. J. Van Strien, D. Maes, and C. A. Van Swaay. 2009. Declines in common, widespread butterflies in a landscape under intense human use. Conservation Biology **23**:957-965.

Vigani, M., and A. Olper. 2015. Patterns and determinants of GMO regulations: an overview of recent evidence.

Watt, W. B., D. Han, and B. T. Tabashnik. 1979. Population structure of pierid butterflies. II. A" native" population of Colias philodice eriphyle in Colorado. Oecologia:44-52.

Williams, N. M., and C. Kremen. 2007. Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. Ecological Applications **17**:910-921.

Winfree, R., I. Bartomeus, and D. P. Cariveau. 2011. Native pollinators in anthropogenic habitats. Annual Review of Ecology, Evolution, and Systematics **42**:1.

Woods, T., J. Jonas, and C. Ferguson. 2012. The invasive Lespedeza cuneata attracts more insect pollinators than native congeners in tallgrass prairie with variable impacts. Biological Invasions **14**:1045-1059.

Young, B. G., D. J. Gibson, K. L. Gage, J. L. Matthews, D. L. Jordan, M. D. Owen, D. R. Shaw, S. C. Weller, and R. G. Wilson. 2013. Agricultural weeds in glyphosate-resistant cropping systems in the United States. Weed Science **61**:85-97.

APPENDICES

APPENDIX I

Title: Sex ratios and *Ophryocystis elektroscirrha* infection levels of *Danaus plexippus* during spring migration through Oklahoma

Shaun M. McCoshum[1] & Kristen A. Baum[1]

1: 501 Life Science West: Department of Zoology, Oklahoma State University, Stillwater, OK 74078, USA

Key words: Apicomplexa, Lepidoptera, monarch butterfly, OE, parasite, southern Great Plains, trap bias, sticky traps

Abstract

Monarch butterflies (*Danaus plexippus*; Lepidoptera: Nymphalidae) have a multiple brood migration in the spring as they move between their overwintering grounds and summer breeding grounds. In Oklahoma, monarchs produce at least one generation in the spring, which develop and continue the northward migration, leaving Oklahoma without a breeding population during the hot summer months. Female monarchs leave the overwintering grounds prior to males, but it is not clear whether females re-colonize areas along the migration route prior to, or at the same time as males. Studies have documented similar male to female ratios at emergence, but have identified a male biased sex ratio in the field. Both males and females are susceptible to infections from the neo-gregarine parasite, *Ophryocystis elektroscirrha* (OE), which is an obligate spore-forming protist that reduces the flight abilities and life spans of infected individuals. We examine the number of male and female monarchs during the spring migration through Oklahoma and whether sex ratios or OE infection estimates vary with capture technique (active or passive). Our data suggest populations are male biased during the first week of spring migration in Oklahoma, but shift to female biased by the third week in both cool and warm springs. Therefore, males may leave southern areas prior to females or migrate longer distances per day. Active sampling (i.e., netting) did not lead to more males than females being caught compared to passive sampling (i.e., sticky traps). Significantly fewer OE carrying monarchs (with 2 or more spores) were captured via netting than by

sticky traps which may be caused by sticky trap glue affecting tape sampling

effectiveness, but there was no difference in the number of heavily infected individuals

(more than 100 spores). Therefore, data from netted monarchs may underestimate OE

infection rates within populations.

Introduction

North America has three populations of monarch butterflies (*Danaus plexippus*;

Lepidoptera: Nymphalidae): the western population overwinters in CA and breeds in the

western US and Canada; the eastern population breeds east of the Rockies and

overwinters in Mexico; and a year round breeding population occurs in southern Florida

(Brower & Jeansonne, 2004). The eastern population is well known for its long distance

migration from breeding grounds as far north as Canada to overwintering sites in the

Transvolcanic Mountains in Mexico (Urquhart & Urquhart, 1976). Each year during the

spring migration, the eastern monarch population arrives in Oklahoma, produces one to

two generations, and continues moving north (Malcolm et al., 1987; Flockhart et al.,

2013). Re-colonization of eastern North America occurs via movement of successive

broods, with multiple generations being produced between the overwintering grounds and

the most northern summer breeding grounds (Malcolm et al., 1993, Wassenaar & Hobson

1998, Flockhart et al., 2013). Monarchs migrate from the south into Oklahoma during the

spring, produce a generation, and continue moving north, vacating the area during the

hotter summer months (Malcolm et al., 1987) when high temperatures (>29°C) increase larval mortality rates and delay larval development (Zalucki, 1982).

All three North American populations of monarchs are infected with the protozoan parasite, *Ophryocystis elektroscirrha* (OE) (Leong et al., 1997), which is an obligate parasite of the monarch genus, *Danaus* (McLauphlin & Myers, 1970). OE negatively affects larval development and reduces adult longevity (deRoode et al., 2009) and flight ability (Bradley & Altizer, 2005). OE spores are spread by infected adult monarchs, which develop in the cells of the caterpillar until pupation when the parasites move to the hemolymph and hypoderm, before undergoing meiosis and forming spores on the exterior surface of the adult butterfly (McLaughlin & Myers, 1970). These external spores are transferred to eggs and host plants during oviposition, which are then ingested by larvae leading to infection (McLaughlin & Myers, 1970). Female monarchs are not as severely inhibited by similar OE loads as males (Altizer & Oberhauser, 1999) suggesting infected females can travel further than infected males during migration and dispersal, but females are more likely to possess heavier infections than males (de Roode et al., 2008). Long distance migration of monarchs likely removes infected individuals from the population through migratory culling (Bartel et al., 2011), because infected individuals are less likely to survive long distance movements (Altizer et al., 2000). Therefore, the eastern North American monarch population has the lowest number of OE infected individuals for the year as it leaves the overwintering grounds and the proportion

of infected individuals in the population increases over the breeding season until fall migration (Bartel et al., 2011).

Monarchs overwintering in CA and Mexico historically have a male biased sex ratio (Tuskes & Brower, 1978; Sakai, 1991; Frey & Leong, 1993, 1995; Nagano et al., 1993), even though no sex bias has been found during development (Hilburn, 1989; Frey & Leong, 1995). Male bias has increased over the past thirty years in the overwintering sites in Mexico (Davis & Rendón-Salinas, 2010) and numerous studies have documented male biased sex ratios of captured wild adult monarchs (Hilburn, 1989; Frey & Leong, 1993; Borland et al., 2004; Brindza et al., 2008; McCord & Davis, 2010). Knight et al. (1999) tested if male bias in studies was due to males being more readily observed in breeding areas because male monarchs will remain in an area, searching milkweed patches for mates, while females fly in more direct flight paths, entering and leaving areas with milkweed. However, data suggest both males and females have the same likelihood of being captured in breeding areas (Knight et al., 1999; Davis & Rendón-Salinas, 2010), as well as at overwintering sites (Frey & Leong, 1993) so no solid evidence has been found to explain the male bias. No studies to date have evaluated if the active capture technique of netting, chasing and capturing butterflies with a hand-held butterfly net, biases results compared to passive capture techniques, such as sticky traps.

Data may also be biased by monarch behavior which differs between the sexes. Although breeding can occur year round in the eastern population (Oberhauser, 1997),

99

oviposition occurs after monarchs leave Mexico until the fall migration generation emerges. Female monarchs leave the overwintering grounds in Mexico and CA prior to males, sometimes even before mating, and migrate north (Van Hook, 1996). The eastern population of monarchs arrives in the Gulf Coast states of the USA where they begin laying eggs on *Asclepias* spp. (Van Hook, 1996). The monarchs encountered north of the Gulf Coast states in spring are thought to be the offspring of these re-migrants (monarchs which migrated south, overwintered in Mexico, then migrated north) from the overwintering population which reproduced in the southern US (Cockrell et al., 1993; Malcolm et al., 1993; Hobson et al., 1999). Previous studies suggest it is uncommon for re-migrants to travel further north than the Gulf Coast states (Fales, 1984; Brower, 1985). It is unknown whether the female offspring of re-migrants leave the southern USA first, as they do from the overwintering sites, or if both sexes migrate north at equal times and speeds. Based on the migration pattern from the overwintering grounds, we expect females will arrive prior to males or be more abundant than males in the initial spring re-colonization of Oklahoma.

Using monarchs collected in spring 2011-2013 with both active and passive capture techniques, we evaluated if sex ratios or OE infection rates varied over the course of spring migration and reproduction in Oklahoma. We also tested whether sex ratios or OE infection status differed with capture technique.

Materials and methods

Monarchs were collected from April-June using two different methods in two

different land use types. Sticky traps, a passive capture method, were used in agricultural

fields with canola (*Brassica napus*), a high floral resource, wheat fields (*Triticum* spp.),

and pasturelands. In separate pasturelands and open grasslands, we collected monarchs

by actively searching areas with and without larval food resources (i.e., milkweed plants)

using hand-held butterfly nets (netting). To understand re-colonization patterns, data from

the first generation of each year were separated into one week intervals starting from the

first week monarchs were observed with regularity. Due to OE infections differing by

generation (i.e., infection increases over the breeding season), monarch generations were

separated into generations with generation 1 representing the first 3 weeks of samples,

and generation 2 the next 3 weeks. Generation times of approximately three weeks were

used based on the mean adult lifespan of 22.5 days (Oberhauser, 1997).

OE Sampling:

Monarchs were sampled for OE spores using the tape count method (Altizer et al.,

2000). Transparent Scotch^{TM} tape was pressed against the abdomen and placed on white

index cards. Each tape count sample was inspected for OE spores using a dissecting

microscope and ranked on a 0-5 infection scale with 0 = no spores, 1 = 1 spore, 2 = 2-20

spores, 3 = 21-100 spores, 4 = 101-1000 spores, and 5 = 1001+ spores (Altizer et al.,

2000). Monarchs were classified as infected when more than 100 spores were found on

the tape samples (rankings 4 and 5) and classified spore carrying (rankings 2-5) when 2

or more spores were found. We used this spore carrying classification because accuracy of separation between categories is reduced because of the loss of scales and spores incurred from the monarchs' struggle against the glue on the sticky traps. Furthermore, glue from the traps was also present on the tape sample and monarchs on sticky traps could not always be sampled for OE on the ventral abdominal side (if that side was stuck to the sticky trap), so tape samples sometimes had to be taken from the dorsalsurface of the abdomen. Both categories of monarchs (actively and passively captured) were tested separately for differences between capture method and generation.

Wing Wear:

Wing wear scoring was used to estimate the age of the monarchs in the arriving generation of monarchs on a 5 interval scale (excellent condition, light damage, moderate damage, significant damage, and poor condition) (Oberhauser, 2003; McCord & Davis, 2012) This information informs the interpretation of our results by approximating if arriving monarchs were recently emerged or potentially from the Mexico overwintering population. Monarchs from the sticky traps were classified as excellent condition, moderate damage, or poor condition, due to lower accuracy of separation between categories due to the damage and scale loss incurred from the monarchs' struggle against the glue on the sticky traps.

Passive Capture:

As part of a separate study on the movement of aphid predating insects, non-scented, yellow sticky traps were placed in canola fields, winter wheat fields, and pasturelands in 8 sites each year throughout north-central Oklahoma in April and early May of 2011, 2012, and 2013. Canola blooms during the early spring and is utilized by monarchs as a floral resource (personal observation). Winter wheat does not produce nectar, but is an important agricultural commodity in Oklahoma with an average of approximately 2,270,000 hectares planted each year for the last ten years (USDA NASS, 2003-2013). Each field had 17 sticky traps equally spaced in a 300 sq m area. Sticky traps were replaced 6 times on days 2, 4, 6, 8, and 12 after initial placement, and taken down on day 18. Monarch butterflies were a by-catch of the original study. Tape count samples for OE were taken on the exposed side of the abdomen.

Active Capture:

In 2012 and 2013 adult monarchs were captured using hand-held butterfly nets in 4 separate field settings, including roadsides, pasturelands, grasslands, and urban gardens. Most areas contained milkweed, and had varying degrees of floral resources. Each site also had vegetation which was generally less than 0.5m tall to enable pursuit of monarchs. Canola and wheat fields were not used because canola is too dense to run through and wheat fields have plowed soils which make running difficult and we did not want to damage the crops. Adult monarchs were actively pursued and captured mid-flight, or while nectaring, resting or ovipositing on plants. Netting days were dependent

on weather, and varied from 1 h to 11 h with 1 to 4 people netting per day. Netting occurred 3-7 days per week between 0800 and 1900 hours Central Daylight Savings Time.

Capture Bias Studies:

To test for a bias in sampling methods, data from sticky traps and netted monarchs were compared. We defined bias as not obtaining similar representation in our samples for OE infection and for sex ratios. To control for the movement of monarchs through Oklahoma and overlapping generations, we compared monarchs that were captured within the same generation time to reduce the error that could arise from the OE infection rate differing between generations.

Analyses:

We examined how sex ratios of the arriving generation differed between weeks using R package lmer, (N=153). 2011 and 2012 had average temperature springs with monarchs arriving with regularity by April 5. In 2013, unseasonably low temperatures (April was 3.89°C cooler than the 30 year average) (Mesonet, 2013) kept monarchs south (Journey North, 2013), and although monarchs were sporadically encountered from April 15 until May 22, low temperatures on May 22 reached 8.3°C, well below the minimum temperature monarch caterpillars can survive (11.5°C; Zalucki, 1982). Monarchs were not seen again until June 1, 2013. Three models were created to test if sex ratios were explained by arrival time (Bolker et al., 2009). Our models treated each week as a fixed

effect and treated the year and site of collection as random effects. We tested if sex was dependent on week or year or a combination of the two, or if the collection site could explain our observations using the following three models: 1) sex ~ week + (1 | Site), 2) sex ~ week + year (1| Site). 3) sex ~ (1| Site). Sex data were transformed into binomial data; weeks, site, and year were used as categorical data.

Chi-square tests were used to evaluate whether infection rates of OE varied between generations, and whether capture technique biased results for sex ratios or OE infections. Differences of OE infections between years were not compared because infections are known to vary between years within populations (Bartel et al., 2011). We ran two separate Chi-square tests for OE; one comparing OE infected individuals (more than 100 spores), and a second test for spore carrying monarchs (more than 2 spores) to evaluate if OE infections significantly differed between generations. Wing wear for the first generation of monarchs was grouped into excellent condition, moderate damage, or poor condition for 2011 and 2012. Using a Chi-square test we evaluated if changes were observed in the percent of each population comprising each wing wear group.

Results

A significant trend was found between sex ratio and week in our linear mixed effects model (P < 0.05). In all 3 years a similar trend was observed with more males arriving in the first week (> 60%) than in subsequent weeks, with the lowest abundance

of males (< 50%) occurring in the third week. The average male to female ratio was 67:33 the first week, 58:42 the second week, and 45:55 the third week (Figure 1).

OE infection for the first and second 3-week periods (the two generations) did not exhibit a similar trend between years. In 2012 infection decreased between generation 1 and generation 2 (χ^2= 4.44, df= 1, P < 0.05) and in 2013 infection did differ significantly between generations (χ^2= 1.18, df= 1, P > 0.05). Heavy infection rates, individuals with a score of 4 or 5, in the first generation varied between years with a range from 3.4% to 11.1% (Table 1).

Wing wear results showed that more than 10% of the first-week monarchs arriving in Oklahoma in both 2011 and 2012 were in poor condition (e.g., old) and less than 20% were in excellent condition (i.e., young or newly emerged), with the majority of monarchs having moderate damage to their wings (Figure 2). No significant differences were found in the percent of samples in each category between years (χ^2= 0.37, df=3, P > 0.05). Data from 2013 were omitted from this analysis due to the nearly 2 month difference in arrival of monarchs compared to 2011 and 2012.

Capture technique did not show a significant bias for sex (χ^2= 0.06, df= 1, P > 0.05). Netting, or active capture, collected significantly more non-spore carrying individuals (χ^2= 3.84, df= 1, P < 0.05), with higher proportions of individuals captured passively on sticky traps being spore carrying (i.e., having more than 2 spores). Heavy

infections (more than 100 spores) did not significantly differ between trapping methods (χ^2= 0.19, df= 1, P > 0.05).

Discussion

Initial re-colonization of Oklahoma during spring migration comprises a high male abundance which declines over the course of 3 weeks to a slightly female bias. Due to the lack of scent attractiveness of the sticky traps and lack of milkweed in the cropland areas where the sticky traps collected the majority of our first 3 weeks of data in 2011 and 2012 we make the following assumptions: OE infected individuals are as equally likely to be captured on the sticky traps as non-infected individuals; both males and females utilize these areas (crop fields and pasturelands) equally and with similar levels of activity. Sticky traps are more likely to be encountered by more active individuals (Rhainds & Shipp, 2003) and males are known to be more active in areas with milkweed (Knight et al., 1999), so it is possible males are more likely to be captured than females. In our analysis we assume males and females, and OE-infected and uninfected individuals, will have the same activity in the agricultural areas with monocultures of *B. napus* (and an absence of milkweed) because floral resources are abundant. We also expect similar male and female activity levels in *B. napus* fields because males are only known to patrol areas with milkweed. If our assumptions are correct, these data should represent the sex ratios present during spring migration in Oklahoma through agricultural areas. It is possible a bias exists if males and females utilize areas without milkweed

differently during migration, or exhibit different activity levels, but additional research is necessary to evaluate these possibilities.

We predicted females would arrive first in Oklahoma because females leave overwintering sites prior to males (Van Hook, 1996). Our data show that male monarchs arrive first in north-central Oklahoma, but the mechanism behind this sex ratio bias is not clear. Previous studies have shown a male bias in recent overwintering populations in Mexico (Davis & Rendón-Salinas, 2010) but do not fully explain the extent of the observed bias. Furthermore, this pattern occurs in all years, including 2013 when temperatures kept monarchs south until 1-June, at which time all re-colonizing monarchs likely represented the first spring generation (and not over-wintering individuals). Our data suggest monarchs arriving in Oklahoma during average temperature springs are comprised of monarchs both recently and not recently emerged, evidenced by minimal to extensive wing wear, respectively (Figure 2). Assuming the older individuals represent the overwintering population that reaches Oklahoma, this could explain some of the bias seen in average temperature years (i.e., the documented male bias in recent overwintering populations; Davis & Rendón-Salinas, 2010). If the sampled individuals represent subsequent generations (offspring of the overwintering population), then a sex ratio closer to 1:1 would be expected in all three weeks. We also would expect to see different patterns in years with colder spring temperatures that keep monarchs south for longer, since under these conditions re-colonizing monarchs in Oklahoma would likely have few,

to no overwintering monarchs represented and be primarily comprised of the first spring generation of the year. Our data show a male bias in the first week of arrival in both warm and cold springs, followed by a shift to female dominance by the third week, suggesting males move north faster than females.

Female bias in the third week may be caused by females having longer residence times than males in areas along the spring migration route (Knight et al., 1999) which may lead to females not migrating as far per day as males. Monarchs mate year round, and mating in the spring could also explain the male bias observed in the individuals re-colonizing Oklahoma and the transition to female bias in the population by the third week. Monarchs emerge at a 1:1 ratio (Urquhart, 1960; Frey & Leong, 1995), and as the overwintering monarchs die off the population should return to equal numbers of males and females. The time frame used to estimate the 1:1 male to female ratio reported by Urquhart (1960) and Frey & Leong (1995) is not clear so it is also possible that generations have biased sex ratios over short periods of time. Many insects exhibit protandry where males emerge more often than females in the first few days, followed by more females in subsequent days (Wiklund & Fagerström, 1977) which could explain our observations. However, additional research is needed to clarify sex ratios over different time periods and generations.

Journey North reports first monarch sightings in Texas and other Gulf Coast states after March 15, 2011, and after March 10, 2012. These first sightings represent the first

109

observations of monarchs in the area, and occur when abundances are relatively low. Our data collection began on April 5, 2011, 21 days after the first 2011 sighting reports for the study area and April 6, 2012, 27 days after the first 2012 sightings. Considering monarch development from egg to adult takes a minimum of 23 days, it is plausible that it is more common than initially thought for monarchs to re-migrate further north than the Gulf Coast states. Further research using cardeneloid and/or stable isotope analysis or tracking of marked individuals should be conducted to evaluate this possibility.

Other factors may also contribute to the observed sex ratio bias. Male-male competition within areas with milkweed may lead to some males (those without territories) migrating further north than females each day until males encounter a milkweed patch not patrolled by other males. Males are known to have area-restricted search patterns when searching for mates, whereas females will move in and out of areas in a more linear fashion when searching for host plants to oviposit on (Knight et al., 1999). Females also do not prefer milkweed areas when males are absent (Zalucki & Suzuki, 1986; Knight et al., 1999) which may contribute to males re-colonizing areas first and intercepting migrating females. Another possible contributing factor is the amount of time females spend ovipositing could reduce distance traveled by females per day compared to males once females reach areas suitable for oviposition. Oberhauser (1989) documented that mating multiple times leads to higher egg production, although mating too often can have negative consequences for females (e.g., ruptured bursa, increased

wing damage). Furthermore, Svärd and Wiklund (1988) showed that males will induce and hold females in copulation during the day with sperm transfer and copula termination occurring after dark. This time held in copulation would delay both males and females, but if males are more abundant than females then some males (those not involved in copulations) may be more likely to move further north.

OE infected individuals were found in the first week of capture, but our data did not show a correlation between OE infection and time. 2013 had unseasonably low temperatures in spring, which kept monarchs south, as well as the lowest population numbers recorded prior to 2013 at overwintering sites in Mexico (Journey North 2013), both of which could possibly interfere with understanding correlations in years with warmer temperatures and higher populations. The proportion of monarchs infected with OE tends to increase over the course of the breeding season (Bartel et al., 2011) with non-migratory generations having heavier infections than migratory generations (Altizer et al., 2000) and populations with shorter migration distances (i.e., the population that overwinters in California) having heavier infections than those with longer migrations (Bartel et al., 2011). Non-migratory populations are also known to have higher OE infection levels, such as the Florida and Caribbean populations (Altizer & Oberhauser, 2000). Migratory culling is suggested to occur during fall migration (Bradley & Altizer, 2005), but it is not clear if spring migration also removes OE infected individuals.

Bartel et al. (2011) report data showing the eastern North American population that overwinters in Mexico has lower OE infection on the overwintering grounds compared to other times of the year, and their data also indicate a decrease of OE from the overwintering site to the early spring breeding grounds. Specifically, Bartel et al. (2011) report less than five percent of individuals on average in the early spring (monarchs collected between 15-April and 30-June 2007-2009) had a heavy-OE infection. Our data show heavy OE infection rates ranging from 3.9% to 18.5% between 15-April and 30-June, 2011-2013 (Table 1). Oklahoma has two migratory generations in the spring: the first which moves into Oklahoma from the south, arriving as early as April 5, and a second generation which develops in Oklahoma and migrates north (Malcolm et al., 1987). OE infection rates in Oklahoma and other states with similar migration patterns should be further studied to evaluate infection patterns and if migratory culling also occurs during the multigenerational northern migration.

Overall flight ability of adult monarchs is negatively affected by heavy OE infection (Altizer & Oberhauser, 1999). To date, studies investigating monarch populations have used active pursuit of adult butterflies and found equal catchability between males and females (Knight et al., 1999), but studies have not compared capture techniques for biases. We predicted that OE infected individuals would be more likely to be netted, since heavily infected monarchs have reduced flight ability (Altizer &

Oberhauser, 1999); however we did not find a difference between netting and sticky trap data for OE infected and non-infected monarchs.

Although our data suggest infected individuals are not captured significantly more by netting or sticky traps, sticky traps did capture significantly more spore carrying monarchs (more than 2 spores) than netting. Netted monarchs were either heavily infected (more than 100 spores) or not infected with very few spore carrying monarchs found in these samples. It is unclear why sticky traps would capture more monarchs with spores transferred horizontally (i.e., the presence of few spores may represent horizontal transfer between individuals and not an active infection) since few spores are not expected to affect activity levels of monarchs. It is likely that spore counts were diluted by the presence of glue from the sticky traps being incorporated into the tape count samples and our inability to sample the ventral side of the abdomen for most individuals, both factors which could reduce test accuracy. If dilution of spores occurred, then it is possible that netting surveys underestimate OE infection levels. However, if the glue from sticky traps did not reduce spore density then netting surveys should accurately depict OE infection rates. Additional research is needed to evaluate these possibilities.

Conclusion:

During the first week of spring migration into Oklahoma male monarchs were more abundant than females within the population, but female monarchs were more abundant than males by the third week of migration. This shift in sex ratios suggests

males may leave southern areas prior to females or migrate longer distances per day. We also documented older male and female monarchs migrating into Oklahoma, which combined with the timing of first sightings of monarchs in the Gulf Coast states suggests some of the initial re-colonizing monarchs are from the overwintering generation and not solely the first spring generation from the Gulf Coast states. Neither males nor females were more likely to be captured by active pursuit (netting) compared to passive capture (sticky traps), however sticky traps did capture significantly more OE carrying individuals, suggesting data from netted monarchs may underestimate OE infection rates within populations.

Acknowledgements

 Literature cited

Altizer SM & Oberhauser KS (1999) Effects of the protozoan parasite *Ophryocystis elektroscirrha* on the fitness of monarch butterflies (*Danaus plexippus*). Journal of Invertebrate Pathology 74: 76-88.

Altizer SM, Oberhauser KS & Brower LP (2000) Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. Ecological Entomology 25: 125-39.

Bartel RA, Oberhauser KS, de Roode JC & Altizer SM (2011) Monarch butterfly migration and parasite transmission in eastern North America. Ecology 92: 342-51.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, & White JSS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24: 127-135.

Borland J, Johnson CC, Crumpton III TW, Thomas M, Altizer SM & Oberhauser KS. (2004) Characteristics of fall migratory monarch butterflies, *Danaus plexippus*, in Minnesota and Texas. (ed by KS Oberhauser & MJ Solensky). Monarch Butterfly Biology & Conservation. Cornell University Press, Ithaca, NY USA pp.97-104.

Bradley CA & Altizer S (2005) Parasites hinder monarch butterfly flight: implications for disease spread in migratory hosts. Ecology Letters 8: 290-300.

Brindza L, Brower LP, Davis AK & Van Hook T (2008) Comparative success of monarch butterfly migration to overwintering sites in Mexico from inland and coastal sites in Virginia. Journal of Lepidoterists Society 62: 189-200.

Brower AVZ & Jeansonne MM (2004) Geographical populations and "subspecies" of
New World monarch butterflies (Nymphalidae) share a recent origin and are not
phylogenetically distinct. Annals of the Entomological Society of America 97:
519-23.

Brower LP (1985) New perspectives on the migration biology of the monarch butterfly,
Danaus plexippus L. Migration: mechanisms and adaptive significance. Vol. 27
(ed. by MA Rankin) University of Texas, Austin, Texas, USA, pp. 748-785.

Cockrell BJ, Malcom SB & Brower LP (1993) Time, temperature, and latitudinal
constraints on the annual recolonization of eastern North America by the monarch
butterfly. Biology and Conservation of the Monarch Butterfly Series 38. (ed. by
SB Malcolm & MP Zalucki) Natural History Museum of Los Angeles County,
Science Series, Los Angeles, California, USA, pp. 233-251.

Davis AK & Rendón-Salinas E (2010) Are female monarch butterflies declining in
eastern North America? Evidence of a 30-year change in sex ratios at Mexican
overwintering sites. Biology Letters 6: 45-47.

de Roode JC, Pedersen AB, Hunter MD & Altizer AM (2008) Host plant species affects
virulence in monarch buttefly parasites. Journal of Animal Ecology 77: 120-126.

de Roode CJ, Rarick R & Altizer SM (2009) Strength in numbers: high parasite burdens increase transmission of a protozoan parasite of monarch butterflies (*Danaus plexippus*). Oecologia 161: 67-75.

Fales JH (1984) Spring occurrence of the monarch butterfly in Maryland. Maryland Entomologist 2: 76-79.

Flockhart DTT, Wassenaar LI, Martin TG, Hobson KA, Wunder MB & Norris DR (2013) Tracking multi-generational colonization of the breeding grounds by monarch butterflies in eastern North America. Proceedings of the Royal Society: Biological Sciences 280. doi: 10.1098/rspb.2013.1087

Frey DF & Leong KLH (1993) Can microhabitat selection or differences in 'catchability' explain male-biased sex ratios in overwintering populations of monarch butterflies? Animal Behavior 45: 1025-1027.

Frey DF & Leong KLH (1995) Reply to Nylin, Wickman & Wiklund regarding sex ratios of California overwintering monarch butterflies. Animal Behavior 49: 515-518.

Hilburn DJ (1989) A non-migratory, non-diapausing population of the monarch butterfly, *Danaus plexippus* (Lepidoptera: Danaidae), in Bermuda. The Florida Entomologist 72: 494-499.

Hobson KA, Wassenaar LI & Taylor OR (1999) Stable isotopes (δD and δ13C) are geographic indicators of natal origins of monarch butterflies in eastern North America. Oecologia 120: 397-404.

Journey North (2013) Monarch butterfly migration: first adult sighted. 2011 map (http://www.learner.org/jnorth/maps/monarch_spring2011.html) accessed Oct. 30, 2013: 2012 map (http://www.learner.org/jnorth/maps/monarch_spring2012.html) accessed Oct. 30, 2013.

Knight A, Brower LP & Williams EH (1999) Spring remigration of the monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae) in north-central Florida: estimating population parameters using mark-recapture. Biological Journal of the Linnean Society 68: 531-56.

Leong KLH, Yoshimura MA & Kaya HK (1997) Occurrence of a neogregarine protozoan, *Ophryocystis elektroscirrha* McLaughlin and Myers, in populations of monarch and queen butterflies. Pan-Pacific Entomologist 73: 49-51.

Malcolm SB, Cockrell BJ & Brower LP (1987) Monarch butterfly voltinism: effects of temperature constraints at different latitudes. Oikos 49: 77-82

Malcolm SB, Cockrell BJ & Brower LP (1993) Spring recolonization of eastern North America by the monarch butterfly: successive brood or single sweep migration. Biology and conservation of the monarch butterfly, 38: 253-67.

McCord J & Davis A (2010) Biological observations of monarch butterfly behavior at a

    migratory stopover site: results from a long-term tagging study in coastal South

    Carolina. Journal of Insect Behavior 23: 405-18.

McLaughlin R & Myers J (1970) *Ophryocystis elektroscirrha* sp. n., a neogregarine

    pathogen of the monarch butterfly *Danaus plexippus* (L.) and the Florida queen

    butterfly *D. gilippus* (Berenice Cramer). Journal of Eukaryotic Microbiology 17:

    300-5.

Mesonet 2013. Temperature averages map. Accessed Oct. 30, 2013

    (http://www.mesonet.org/index.php/weather/mesonet_averages_maps)

Nagano CD, Sakai WH, Malcolm SB, Cockrell BJ, Donahue JP & Brower LP (1993)

    Spring migration of monarch butterflies in California. Biology and conservation

    of the monarch butterfly, 217-32.

Oberhauser KS (1989) Effects of spermatophores on male and female monarch butterfly

    reproductive success. Behavioral Ecology and Sociobiology 25: 237-46.

Oberhauser KS (1997) Fecundity, lifespan and egg mass in butterflies: effects of male-

    derived nutrients and female size. Functional Ecology 11: 166-75.

Oberhauser K & Frey D (1999) Coercive mating by overwintering male monarch butterflies. In 1997 North American conference on the monarch butterfly. Montreal, Commission of Environmental Cooperation p. 67.

Oberhauser KS (2003) Monarch lab: wing wear scoring. Accessed Oct. 30, 2013. (http://www.monarchlab.org/lab/research/topics/vitalstats/howtomeasure.aspx)

Rhainds M & Shipp L (2003) Dispersal of adult western flower thrips (Thysanoptera: Thripidae) on Chrysanthemum plants: impact of feeding-induced senescence of inflorescences. Environmental Entomology 32: 1056-1065.

Sakai W (1991) Monarch sabhatical update. The Monarch Newsletter 2: 4-5.

Svärd L & Wiklund C (1988) Prolonged mating in the monarch butterfly *Danaus plexippus* and nightfall as a cue for sperm transfer. Oikos 51: 351-54.

Tuskes P & Broweh L (1978) Overwintering ecology of the monarch butterfly, *Danaus plexippus* (L), in California. Ecological Entomology 3: 141-153.

Urquhart F (1987) The monarch butterfly: international traveler. University of Toronto Press. Toronto, Canada.

Urquhart FA (1976) Found at last-monarchs winter home. National Geographic 150: 161-73.

USDA NASS 2007-2013. Oklahoma June Reports.

　　http://www.nass.usda.gov/Quick_Stats/ (accessed August 9, 2013).

Van Hook T (1996) Monarch butterfly mating ecology at a Mexican overwintering site:

　　Proximate causes of non-random mating. PhD Dissertation, University of Florida,

　　Gainesville, FL.

Wassenaar LI & Hobson KA (1998) Natal origins of migratory monarch butterflies at

　　wintering colonies in Mexico: New isotopic evidence. Proceedings of the

　　National Academy of Sciences 95: 15436-39.

Wiklund C & Fagerström T (1977) Why do males emerge before females? Oecologia 31:

　　153-158.

Zalucki MP (1982) Temperature and rate of development in *Danaus plexippus* L. and *D.*

　　chrysippus L. (Lepidoptera: Nymphalidae). Australian Journal of Entomology 21:

　　241-246.

Zalucki MP & Kitching RL (1984) The dynamics of adult *Danaus plexippus* L.

　　(Danaidae) within patches of its food plant *Asclepias* spp. Journal of the

　　Lepidopterists' Society 38: 209-219.

Zalucki M & Suzuki Y (1987) Milkweed patch quality, adult population structure, and

egg laying in the monarch butterfly. Journal of the Lepidopterists' Society 41: 13-

22.

Tables and Figures

	2011	2012	2013
First generation	11.1% (n = 54)	3.4% (n = 88)	9.3% (n = 22)
15-April - 30-June	18.5% (n = 27)	3.9% (n = 51)	12.5% (n = 32)

Table 1: Percent of population infected with OE (greater than 100 spores; score of 4 or 5) each year. The first generation is defined as the first 3 weeks after regular monarch occurrence, which starts April 5, 2011 and April 6, 2012, and on June 1 for 2013. For comparison to data previously published by Bartel et al. (2011), we also report our data from April 15-June 30 (note: this time period overlaps with the first generation data reported in the first row). Using this longer time frame, OE infection estimates increase in all 3 years.
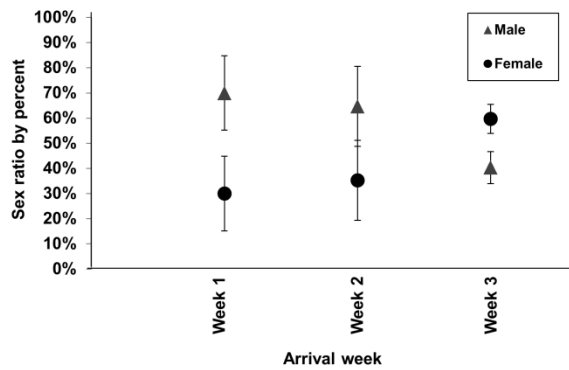
Figures and legends

Figure 1: Average weekly male and female sex ratios from 2011-2013 of the re-colonizing

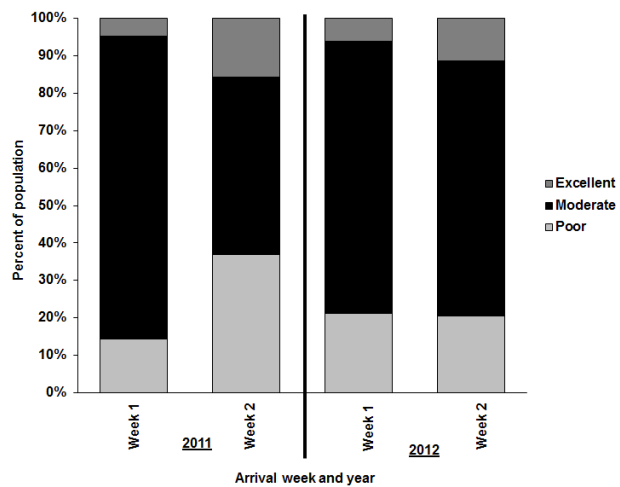generation of monarch butterflies in Oklahoma separated by week.

Figure 2: Wing scores based on wing wear for the first 2 weeks of arriving monarchs in Oklahoma during April 2011 and 2012. More heavily damaged and duller wings (Poor) are considered typical of older adults, whereas undamaged and vibrantly colored wings (Excellent) are considered typical of newly eclosed adults.

VITA

Shaun Michael McCoshum

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS AND ASSOCIATIONS OF THE PRODUCTION OF THE BIOFUEL CROP *BRASSICA NAPUS* ON WILD INVERTEBRATE POLLINATOR COMMUNITIES IN THE SOUTH CENTRAL UNITED STATES

Major Field: Zoology

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Zoology at Oklahoma State University, Stillwater, Oklahoma in December, 2015.

Completed the requirements for the Master of Science in Botany at Miami University, Oxford, Ohio, August, 2009.

Completed the requirements for the Bachelor of Science in Biology at Humboldt State University, Arcata, California, December, 2006.

Publications:

Stenoien CS, **McCoshum SM**, Caldwell W, De Anda A, and KS Oberhauser (2015) New reports that monarch butterflies (Lepidoptera: Nymphalidae, *Danaus plexippus* Linnaeus) are hosts for a pupal parasitoid (Hymenoptera: Chalcidoidae, *Pteromalus cassotis* Walker). Kansas Journal of Entomology (88) 16-26

McCoshum SM, and KA Baum (2014) Sex ratios and *Ophryocystis elektroscirrha* infection levels of *Danaus plexippus* during spring migration through Oklahoma, USA. Entomologia Experimentalis et Applicata (153) 266-272.

McCoshum SM, Caterino MS, and AR Cline (2012) Introduced Sap Beetles (Coleoptera: Nitulidae) on Santa Catalina Island, California, U.S.A. The Coleopterists Bulletin (66) 4:1-4.