EVALUATING PERENNIAL AND ANNUAL COMPANION PLANTINGS FOR POLLINATOR ENHANCEMENT OF YIELD IN SMALL-SCALE VEGETABLE PRODUCTION

A Dissertation

by

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ABSTRACT

Pollination is a key component to obtain proper yield and fruit set in numerous vegetable crops, with the honey bee, *Apis mellifera*, being their primary pollinator. Honey bee populations in the United States have experienced dramatic declines, exhibiting a loss of 59% of colonies from 1947 to 2005. Likewise, several native bee species have exhibited sustained declines over the past century. We hypothesized that the placement of pollinator-attracting plants near vegetable crops would increase the yield and quality of vegetable crops by attracting a greater frequency and diversity of pollinators. Cucumbers (*Cucumis sativus*) and habanero pepper (*Capsicum chinense*) growers have observed increased crop yield by placing bees in close proximity to vegetable crops. However, adding bees may not typically be feasible for small-scale farmers. Limited studies have demonstrated the potential of pollinator-attracting plants to be used as a lure to enhance the visitation of pollinators to adjacent food crops species. This study evaluated the potential of adding pollinator-attracting plants in close proximity to cucumber and habanero plants. Two treatment groups of pollinator-attracting plants were evaluated: perennial companion plantings and interplanted annual companion plants. The perennial treatment group consisted of *Phyla nodiflora*, *Borrichia frutescens*, *Salvia* 'Henry Duelberg', and *Eysenhardtia texana*. The annual treatment group consisted of *Cosmos bipinnatus*, *Zinnia* × *marylandica*, *Borago officinalis* and *Ocimum basilicum*. Yield and fruit quality, in addition to frequency and diversity of pollinator visitations were recorded and analyzed using analysis of variance tests. Significant differences in yield were found among treatment groups with greater yields observed in companion planting treatments, particularly with annual pollinator-attracting species, when compared to control treatments. However,

significant differences in fruit quality or size were not found among treatment groups. Significant differences in frequency and diversity of pollinators visiting perennial and annual treatment groups were found among treatment groups with companion planting treatments attracting more pollinators when compared to control treatments. Individual pollinator-attracting plants varied in overall effectiveness and groups of pollinators attracted. *Phyla nodiflora*, *B. officinalis*, and *O. basilicum* were particularly effective for attracting pollinators, whereas *Z.* × *marylandica* was very ineffective. Economic sustainability of the system was measured by determining whether investments in pollinator-attracting plants are justified economically in terms of crop yield. Data from our proof-of-concept experiments suggests growers interested in the addition of pollinator-attracting companion plantings should utilize annual pollinator attractants, as they provide an immediate return on investment with a low risk of failure caused by being re-planted each crop cycle and greater flexibility relative to crop selection.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Professor Michael A. Arnold and Professor Larry Stein of the Department of Horticultural Sciences, Professor Juliana Rangel of the Department of Entomology, and Professor Marco Palma of the Department of Agricultural Economics.

All work for the dissertation was completed by the student under the advisement of Professor Michael A. Arnold of the Department of Horticultural Sciences, with field assistance from student workers and insect identification from Dr. Adrian Fischer.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Background and Rationale

With decreasing honey bee (*Apis mellifera* L., Hymenoptera: Apidae) populations becoming a nationwide concern with numerous consequences, including inadequate pollination of several crop species (Kevan and Phillips, 2001; Klein et al., 2007; Gallai et al., 2009; Potts et al., 2010; Garibaldi et al., 2011; Calderone, 2012), it is important to investigate crop production systems that incorporate companion plantings that attract honey bees and other pollinators to agricultural crops. While a diverse plant community is important to support and maintain pollinator populations, these conditions are lacking from many heavily managed farm landscapes (Williams et al., 2010; Winfree et al., 2011). Therefore, increasing biodiversity with the addition of available pollinator-attracting plant species planted adjacent to crop species could result in a greater quantity and more diverse population of pollinators to visit nearby crop species. The purpose of this dissertation is to begin the evaluation of vegetable production systems that feature companion plantings known to attract pollinators and to measure crop yield differences associated with different companion planting groups. Pollinator-attracting companion plants were chosen from annual and perennial plant species to compare the two groups in terms of differences in adjacent crop yield, visitation and diversity of pollinators, and cost of setup and maintenance. The selected perennial and annual pollinator-attracting companion plant species were known to attract pollinators and were easily managed in a typical commercial vegetable production system where impacts on yield, pollinator visitation, and pollinator diversity could be assessed. The first objective of this study was to evaluate the impact of pollinator-attracting perennial plants on vegetable crop yields and quality, compared to pollinator-attracting annual plants and plots lacking pollinator-attracting plants. The second objective of this study was to determine the visitation rate and flower constancy of honey bees and other pollinators that visited the selected perennial ornamental plants and annual ornamental plants, while simultaneously determining if the attracted insects were also effective pollinators of adjacent crops. The third objective of this study was to perform an economic analysis of vegetable crop yield and quality in plots that had perennial pollinator-attracting companion plant species, compared to those that had annual pollinator-attracting companion plants or those that had conventional vegetable plantings lacking pollinator-attracting companion plants. The economic analysis incorporated costs associated with the initial setup of perennial and annual pollinator-attracting plants and their long-term maintenance, in addition to changes in crop yields, and thus provides a cost-benefit analysis of the use of enhanced perennial ornamental plants and annual ornamental plants on crop yields and quality.

While the placement of companion plantings adjacent to crops is known to increase insect diversity, further research is needed to determine if crop yield can also be reliably increased with the use of companion plants (Haaland et al., 2011). Selection of proper companion plants for a particular crop that meet the requirements of the particular growing conditions of the region needs to be investigated more extensively to maximize pollination and crop yield (Quinn, 2017). Recent research has suggested that the planting of floral restoration plant varieties attracts pollinators and helps ‘export’ them into adjacent crop fields (Morandin and Kremen, 2013). Across heterogeneous landscapes, pollinator abundance and diversity typically correlate with floral diversity and density (Potts et al., 2003). Julier and Roulston (2009) found that wild pollinators alone were sufficient to pollinate pumpkin crops when the farm was not located in an intensively farmed region. Given this information, a new question arose: could adding pollinator-attracting plant species, thereby transforming a given area into a more heterogeneous landscape, attract sufficient pollinators to successfully pollinate a crop without the addition of bee hives? Previous studies have investigated this area, but none has directly quantified differences in crop yield. Barbir et al. (2015) tested multiple herbaceous plants, including *Borago officinalis* L., to find suitable plant species in Spain for their ability to attract pollinators for use in agro-ecosystems. Carreck and Williams (2002) found that the addition of annual flowering plants, including *Borago officinalis*, to non-cropped areas of field production provided nectar and pollen sources to pollinators, even during the offseason. Thom et al. (2016) found that the addition of oilseed crops, including borage, to current soybean [*Glycine max* (L.) Merr.] and maize (*Zea mays* L.) crops provided a supplemental nectar resource for pollinators and a high-value crop for farmers. The goals of this study were to identify individual pollinator-attracting plant companion species from two main plant categories, annuals and perennials, and to determine their impact on the yield and quality of vegetable crops from two different families.

Perennial Companion Plant Species

Perennial companion plantings provide many advantages when compared to annual companion plant species. First, perennial plantings need to be planted only once and then only need to be maintained in the field. Second, utilizing perennial companion plantings to attract honey bees and other pollinators provides a permanent, maintainable source of pollinator attraction. Third, perennial plantings have the potential to provide nutrition for pollinators or nesting sites when crops are not present, potentially enhancing future crops by maintaining healthy populations of pollinators throughout the year. Using this information, the perennial companion plantings to be used in this study will be established in rows adjacent to where vegetable crops are grown. The four regionally well-adapted perennial pollinator-attracting companion plant species proposed for use are *Phyla nodiflora*, *Borrichia frutescens*, *Salvia farinacea* 'Henry Duelberg', and *Eysenhardtia texana*.

Botanical Descriptions

***Phyla nodiflora* (L.) Greene (Verbenaceae)**

Phyla nodiflora is a perennial mat-forming herb in the neotropical genus *Phyla* Lour. In the Americas, *P. nodiflora* occurs from lower North America to northern South America (Gross et al., 2017). It also occurs in Australia and other tropical and sub-tropical regions (Gross et al., 2017). The Phaon crescent butterfly (*Phyciodes phaon* Edwards.), which is present over much of the southeastern United States, utilizes *P. nodiflora* as a host plant for females to lay eggs on the undersides of the plant's leaves (Emel and Kenny, 1997; Genc, 2003; Minno and Minno, 1999). Even though *P. nodiflora* is well known to attract pollinators (Minno and Minno, 1999; Emel and Kenny, 1997; Genc, 2003), research involving its specific interactions with each pollinator species is lacking. In this study, *P. nodiflora* will be included as a ground cover and living mulch in the perennial companion plantings treatment group, as it forms low growing, dense mats that suppress weed species.

***Borrichia frutescens* (L.) DC. (Asteraceae)**

Borrichia frutescens is a branched erect shrub that spreads by rhizomes, grows 0.5 m to 1 m tall, suckers densely to form colonies, and is common in coastal and inland areas with poor drainage and high salt accumulation (Correll and Johnston, 1970). *B. frutescens* is native from Texas to Maryland with some discontinuous populations in west Texas (USDA Plants Database, 2015). Bees and butterflies are the primary pollinators of *B. frutescens*, but beetles and wasps are also known to contribute as pollinators of this plant (Antlfinger, 1982; Crespo et al., 2002; Lonard et al., 2015).

Salvia farinacea **Benth. 'Henry Duelberg' (Lamiaceae)**

Salvia farinacea 'Henry Duelberg', a Texas native herbaceous perennial, has dark blue flowers and grows 0.75 m to 1 m tall and blooms from May until frost (Texas A&M AgriLife Research, 2018). As a Texas Superstar® cultivar, it is highly recommended for planting in Texas because it is tolerant to heat and requires little maintenance (Texas A&M AgriLife Research, 2018). Previous experiments in an open field demonstrated that bumblebees *(Bombus impatiens* Cresson, Hymenoptera: Apidae) preferred *S. farinacea* to other flowering species for pollination (Lázaro and Totland, 2010a)

Eysenhardtia texana **Kunth. (Fabaceae)**

Eysenhardtia texana is a Texas native deciduous to semi-evergreen shrub, which grows from 1 to 4 m tall on calcareous soils of central, and west Texas and south into Mexico (Correll and Johnston, 1970). *E. texana* is known to attract bees and butterflies and is a larval host for the dogface butterfly (Damude, N. & K.C. Bender, 1999). Drought tolerance and adaptability to varied soil pH and light exposures are other desirable attributes of *E. texana* (Arnold, 2008).

Annual Companion Plant Species

Although perennial plants may provide a more permanent source of attraction for pollinators, establishment duration and maintenance, in addition to substituting crop species space for perennial companion plants, may prove to be economically non-viable for growers if costs cannot be negated with increased yield. Annual plantings could be inter-planted with crop species to provide an immediate source of pollinator attraction, therefore eliminating the need for establishment duration, additional space, and continued maintenance. Annual companion plantings would need to be replanted each cropping cycle compared to only an initial planting of the perennial companion plant species. Therefore, annual companion plant species were selected with preference to annuals that are well-known pollinator-attracting plants that grow well in the region. The four annual pollinator-attracting companion plant species selected for this study were *Borago officinalis, Cosmos bipinnatus*, *Ocimum basilicum*, and *Zinnia* x *marylandica*.

***Borago officinalis* L. (Boraginaceae)**

Borage (*Borago officinalis*), an annual herbaceous plant native to Africa, Europe, and Asia (Beaubaire and Simon, 1987), is usually present in the natural pastures of Mediterranean areas (Fedele et al., 1993; Licitra et al., 1997). Borage is cultivated throughout the world and is used for medicinal purposes, as well as for preparing beverages and salads (Bianco et al., 1998; Branca, 2001). *B. officinalis* has been used to provide an additional nectar source for pollinators and as a lure to attract bees to agro-ecosystems (Barbir et al., 2015; Carreck and Williams, 2002; Thom et al., 2016). Borage is primarily a cool or transition season annual in our region (Arnold, 2008) and thus can provide pollinator-attracting services somewhat earlier or later in the growing season than the other annuals used in this study.

***Cosmos bipinnatus* Cav. (Asteraceae)**

Cosmos bipinnatus is an Central American herbaceous annual that grows up to 2 m tall (Crowe, 1954). *C. bipinnatus* grows and blooms rapidly and is easily cultivated in soils of varied pH and fertility (Arnold, 2008). *Apis mellifera* and *Bombus bellicosus* Smith (Hymenoptera: Apidae) have shown preference for specific color morphs (particularly the pink morph) of *C. bipinnatus* (Malerba and Nattero, 2012).

***Ocimum basilicum* L. (Lamiaceae)**

Ocimum basilicum is an herbaceous annual that grows to approximately 50 to 60 cm (Pereira et al., 2015). *O. basilicum* has been found to attract more than 55 different species of bees in Brazil, a tropical country where basil flowers year round (Muniz et al., 2013). *O. basilicum* has been used in other pollinator companion planting studies, including one in which it was found to increase yield and pollination of *Capsicum annuum* L. (Pereira et al., 2015).

***Zinnia × marylandica* D.M. Spooner, Stimart, & T. Boyle (Asteraceae)**

The genus *Zinnia* L. contains 11 species of annual or perennial herbs or small shrubs native largely to Mexico (McVaugh, 1984; Torres, 1963). *Zinnia × marylandica*, a hybrid species developed at the University of Wisconsin-Madison, is an annual herb with stems 35 to 55 cm tall, 0.7 to 1.3 cm in diameter, and highly branched at the base resulting from crosses between *Zinnia angustifolia* H.B.K. var. *angustifolia* and *Zinnia violacea* Cav. (Spooner et al., 1991). This disease resistant species is better adapted to our regionally hot humid summers than *Z. violacea* (Arnold, 2008), and flowers prolifically at an early age, thus making it a potentially useful as a pollinator-attracting plant.

Crop Species

The two crop species were selected from two families, *Cucurbitaceae* Juss. and *Solanaceae* Juss., as both families contain prominent crop species known to have an increase in yield when a greater number of pollinators is present (McGregor, 1976; Stanghellini et al., 1997). *Cucumis sativus* L., and *Capsicum chinense* L. were selected for this study because both species grow well in this region of Texas and are documented to have an increase in yield with the addition of pollinators (McGregor, 1976; Stanghellini et al., 1997).

***Cucumis sativus L.* (Cucurbitaceae)**

Cucumber (*Cucumis sativus* L.) is an herbaceous plant that forms a creeping vine with small yellow dioecious flowers. Production of cucumbers is generally divided into two types, slicing cucumbers and pickling cucumbers (Reference?). Pickling cucumbers have thin skins, and are shorter than slicing varieties, as they are generally used for pickling or processing. Slicing cucumbers have thick skins, are longer than pickling varieties, and are generally consumed raw. Slicing cucumbers were selected in this study for their increased resistance to handling damage. The slicing variety 'Marketmore' was used in this study because it is a variety commonly grown by small-scale farmers and home gardeners, which is the target size of production system to be evaluated in this study. Cucumbers are typically planted when soil temperatures reach 60^{o}F (15.6^{o}C), with field preparation largely dependent on soil fertility (Schultheis, 2000). Slicing cucumbers in commercial production are generally placed in rows 3 to 4 feet apart with plants spaced 9 to 12 inches from each other in double rows (Kemble et al., 1998). For this study, it was proposed to keep a 5-feet distance between rows and a 12-inch distance between plants, as increased distance between plants is recommended for hand harvesting of cucumbers (Kemble et al., 1998). Harvesting methods for cucumber vary depending on the production size (Schultheis, 2000). Given the proposed production scale of this study, a hand harvesting method was proposed with a three-week harvest period. Pollination is critical for proper cucumber production, as cucumbers with improper pollination result in aborted or disfigured fruit (McGregor 1976; Stanghellini et al. 1997). Studies have been conducted on the effects of yield as a result of an increase in pollinators, particularly honey bees, near cucumber crops (Azmi et al., 2017; Quinn et al., 2017; Shuler et al., 2005). However, significant differences in yield have not been found by some researchers (Quinn et al., 2017).

***Capsicum chinense Jacq.* 'TAM Mild Habanero' (Solanaceae)**

Habanero, *Capsicum chinense,* is a shrub-like species of pepper native to South America that grows 50 to 75 cm in height (D'Arcy and Esbaugh, 1974). Habanero is a warm season crop that is typically planted in rows placed approximately 5 feet from each other with 12- to 18-inch spacing, and planted in double rows (Kemble et al., 1998). Harvesting methods for habanero vary depending on production size (Kemble et al., 1998). Given the proposed production scale of this study, a hand harvesting method is proposed with a three-week harvest period. *C. chinense* is self-pollinating and therefore does not require pollination for proper fruit formation. However, increases in fruit set and quality with respect to seed production have been found with the addition of pollinators (Cauich et al., 2006). *C. chinense* 'TAM Mild Habanero' was selected for this study due to seed availability and its ability to grow in various regions of Texas (Crosby et al., 2005).

Frequency and Diversity of Pollinators

Approximately 35% of the world's staple food crops and 75% of world's major crops require pollination services (Kevan and Imperatriz-Fonseca, 2002; Klein et al., 2007; Nabhan and Buchmann, 1997b), with the majority of the pollination being performed by bees (Klein et al., 2007). In 2014, the United States White House acknowledged the integral nature of insect pollinators and national food security, indicating that pollinators were estimated to contribute $24 billion annually to our economy in 2014, with $15 billion attributed to honey bee pollination services and another $9 billion to native bees and other pollinators (Pollinator Health Task Force, 2014). Additional economic contributions of honey bees and native pollinators to the U.S.

economy are associated with the commercial insect pollination industry through employment of commercial beekeepers and apiculture experts, constituting a cross-country migratory service vital to several fruit, vegetable, and nut crops estimated to be valued at $655.6 million in 2012 (Bond et al., 2014; Perez and Plattner, 2014). Models looking at the potential loss of fruit and crop production in the event of total or partial pollinator disappearance have estimated that the production of these crops could drop below the current consumption level at the world scale (Gallai et al., 2009). Furthermore, poor pollination has been associated with the reduction in yield and quality of some fruit and vegetable crops (Angbanyere and Baidoo, 2014; Dag et al., 2007; Garibaldi et al., 2014). Because the need for crop pollination has increased globally, native bee-pollinated crops are increasingly being supplemented with the addition of honey bees to provide sufficient pollination services in many crops (Morse, 1991; Goodwin et al., 2011; Rucker et al., 2012). Therefore, there is a critical need worldwide in general, and in the U.S. in particular, to develop management protocols that will enhance the number of bee species available to provide pollination services for the increasing quantity of fruit and vegetable crops consumed by growing human populations.

Honey bees have been shown to increase crop productivity in vegetables, pulses, oilseeds, fruits, nuts and forage crops (Abrol, 1991; Dulta and Verma, 1987; Gupta et al., 2000; Partap, 2000; Partap et al., 2000; Abrol, 2012). In addition, honey bee pollination has been found to reduce fruit drop in a number of horticultural crops (Dulta and Verma, 1987; Partap, 2000; Partap et al., 2000). For example, asparagus (*Aparagus* L.), carrots (*Daucus* L.), onion (*Allium cepa* L.), turnips (*Brassica rapa* var. *rapa*), and cabbage (*Brassica oleracea* var. *capitata* L.) have significant increase in seed yield as a result of honey bee pollination (Deodikar and Suryanarayana, 1972, 1977; Woyke, 1981). Furthermore, honey bee pollination has been reported to enhance seed quality and production in vegetable crops such as cauliflower (*Brassica oleracea* L.), radish (*Raphanus sativus* L.), cabbage, mustard (*Brassica juncea* L.) and lettuce (*Lactuca sativa* L.) (Abrol, 1991; Partap and Verma, 1992, 1994; Verma and Partap, 1993, 1994).

European honey bees are experiencing a loss of 59% of colonies between 1947 and 2005 in the United States, and a loss of 25% of colonies between 1985 and 2005 in Europe (National Research Council, 2007; van Engelsdorp et al., 2008). The most recent survey of honey bee colony losses in the U.S. reported an average colony loss rate of 42% during the winter of 2014-2015, creating concerns that extend among the general public on the state of our managed honey bee population (Steinhauer et al., 2015). In addition to existing threats to colony health such as pests, pathogens, and parasites, other drivers of honey bee declines include rapid habitat loss, intensive fertilizer and pesticide application in agricultural fields visited by honey bees, and limited food supplies (Mullin et al., 2010; Paxton et al., 2015; USDA, 2012). Likewise, a number of native bee species have exhibited sustained declines over the past century (Bartomeus et al., 2013), with most declines being tightly linked to reductions in local and landscape-level nesting and food availability, often as a result of agricultural and urban intensification (Winfree et al., 2009), as well as habitat loss and fragmentation (Kremen et al., 2002; Rathcke and Jules, 1993; Steffan-Dewenter and Westphal, 2008; Tscharntke et al., 2005).

Approximately 16,325 species of bees belonging to 425 genera and 7 families have been described but have been largely understudied as potential pollinators of agricultural crops (Michener, 2000). Bumble bees, *Bombus* (Latreille, 1804) are already being used in the production of greenhouse tomatoes and bees in the genera *Nomia* (Latreille, 1804) and *Osmia* (Panzer, 1806) for orchard pollination (Abrol, 2012). Bees in the genus *Megachile* (Latreille,

1802) are used as a pollinator for alfalfa (*Medicago sativa* L.) and even outperform the European honey bee in this capacity (Abrol, 2012; Richard, 1987). Social stingless bees are used to pollinate coffee (*Coffea* L.) and other crops (Abrol, 2012) in tropical and subtropical regions and are now being investigated for greenhouse pollination of *Cucumis sativus* and *Capsicum chinense* (Azmi et al., 2017; Palma et al., 2008; Sawatthum et al., 2017). Wild pollinators have outpreformed managed bees in several studies using a number of crops, including cucurbits (Artz and Nault, 2011; Blaauw and Isaacs, 2014; Gajc-Wolska et al., 2011; Garibaldi et al., 2013; Holzschuh et al., 2012). One objective of this dissertation is to document the diversity and frequency of wild pollinators that visit agricultural crops and to evaluate which species may be contributing to crop pollination and yield.

A pollinator sampling technique that utilizes observation combined with netting for determining pollinator diversity and frequency of visitation is proposed in this study, as it is been successfully used in previous pollinator studies to determine which pollinator species may be contributing to crop pollination (Ritchie et al., 2016). Limitations of this sampling technique include observer biases and limited time of sampling, which excluded an examination of potential nocturnal pollinators (Ritchie et al., 2016). To minimize variation, observations of pollinators were performed by one observer only.

Economic Analysis

With decreasing honey bee (*Apis mellifera* L., Hymenoptera: Apidae) populations becoming a nationwide concern with numerous consequences, including inadequate pollination of several crop species (Calderone, 2012; Gallai et al., 2009; Kevan and Phillips, 2001), it is important to investigate crop production systems that incorporate companion plantings that attract honey bees and other pollinators to agricultural crops. Even though a diverse plant community is important to support and maintain pollinator populations, these conditions are lacking from many heavily managed farm landscapes as well as urban settings (Williams et al., 2010; Winfree et al., 2011).

Therefore, increasing biodiversity with the addition of available pollinator-attracting plant species adjacent to crop species could result in a greater quantity and more diverse population of pollinators to nearby crop species. The addition of companion plantings to crops have been known to increase insect diversity (Haaland et al., 2011). However, further research is needed to determine if crop yield can also increase reliably with the adition of companion plantings. Furthermore, it is necessary to determine if a subsequent increase in yield is economically viable for a grower to reach a break-even point on incurred costs of companion plantings. Measuring the full economic value of pollination as a ecosystem service is a complex process and a relatively new concept that is still being developed (Hanley et al., 2015). However, using yield differences in a production setting gives researchers a tangible measuring tool to evaluate the major economic potential of added pollinator services.

The purpose of this study was to conduct an economic analysis of a vegetable production system that features companion plantings known to attract pollinators and to measure yield differences associated with different pollinator-attracting companion planting groups, and tocompare them to the increased production cost of the companion planting groups when compared to conventional small-scale vegetable production systems. Pollinator-attracting companion plant species were chosen from annual and perennial plant species in order to compare the two groups to each other in an economic analysis comparing adjacent crop yield and cost of setup and maintenance.

The economic analysis of this study was evaluated by measuring three separate effects including: (1) added costs of production incurred by the use of additional materials, cultural practices, and/or irrigation treatments; (2) added income resulting from increased levels of production; and (3) income that may be lost when substituting crop space for perennial companion plantings in the production system. A "with" and "without" comparison, which tries to identify and value the costs and benefits that arise with a proposed project and then compares them with a situation without the project as be used to conduct the economic analysis in this study (Gittinger, 1982). The difference between the "with" proposed project and the "without" proposed is the incremental net benefit arising from the project investment (Gittinger, 1982).

Once the economic analysis was conducted, projected yield thresholds that reach a break-even point were determined for each crop, with fluctuations in crop price taken into consideration. Projected yield thresholds were used to determine the ratio of crop rows to perennial rows needed for the proposed system to be economically viable. Annual and perennial pollinator-attracting companion plantings were compared to one another in terms of initial and continued cost of operating each system.

CHAPTER II

CROP YIELD RESPONSE TO COMPANION PLANTINGS

With decreasing honey bee (*Apis mellifera* L., Hymenoptera: Apidae) populations becoming a nationwide concern with numerous consequences, including inadequate pollination of several crop species (Calderone, 2012; Gallai et al., 2009; Kevan and Phillips, 2001), it is important to investigate crop production systems that incorporate companion plantings that attract honey bees and other pollinators to agricultural crops. Even though a diverse plant community is important to support and maintain pollinator populations, these conditions are lacking from many heavily managed farm and urban landscapes (Williams et al., 2010; Winfree et al., 2011). Therefore, increasing biodiversity with the addition of pollinator-attracting plant species adjacent to crop species could result in a greater quantity and more diverse population of pollinators for nearby crop species. The purpose of these experiments was to begin the evaluation of vegetable production systems that feature companion plantings known to attract pollinators, and to measure yield differences associated with different companion planting groups. Pollinator-attracting companion plant species were chosen from annual and perennial plant species to compare the two groups in terms of differences in adjacent crop yield, visitation and diversity of pollinators, and cost of setup and maintenance. Perennial and annual pollinator-attracting companion plant species were selected that were known to attract pollinators and be easily managed in a typical commercial vegetable production system where impacts on yield, pollinator visitation, and pollinator diversity could be assessed. Even though the addition of companion plantings to crops has been known to increase insect diversity, further research is needed to determine if crop yield can also be reliably increased (Haaland et al., 2011). Selection of proper companion plants for a particular crop being grown that meet the requirements of the particular growing conditions of the region need to be investigated more extensively to maximize pollination and crop yield (Quinn, 2017). In order to support more abundant and diverse pollinator populations and simultaneously improve crop yields, recent research has suggested the planting of floral restoration plant varieties to attract pollinators and help ‘export’ them into adjacent crop fields (Morandin and Kremen, 2013).

Across heterogeneous landscapes, pollinator abundance, and pollinator diversity typically correlate with floral diversity and density (Potts et al., 2003). Julier and Roulston (2009) found that, provided the farm was not located in an intensively farmed region, wild pollinators alone were sufficient to pollinate pumpkin (*Cucurbita pepo* L.) crops. Therefore, the question arose: would adding pollinator-attracting plant species, thereby transforming a given area into a more heterogeneous landscape, attract sufficient pollinators to successfully pollinate a crop without the addition of imported bee hives? Previous studies have investigated similar questions but none have directly quantified differences in yield. Barbir et al. (2015) tested multiple herbaceous plants, including *Borago officinalis* L., to find suitable plant species in Spain for their ability to attract pollinators in agro-ecosystems. Carreck and Williams (2002) found that the addition of annual flowering plants (including *B. officinalis*) to non-cropped areas of field production provided nectar and pollen sources to pollinators, even during the offseason. Thom et al. (2016) found that the addition of oilseed crops, including borage, to soybean [*Glycine max* (L.) Merr.] and maize (*Zea mays* L.) crops provided a supplemental nectar resource for pollinators and a high-value crop for farmers. One goal of this study was to identify individual pollinator-attracting plant companion species from two main plant categories, annual and perennial, and determine their impact on vegetable crop yield.

Perennial companion plantings provide many advantages when compared to annual companion plant species, largely because perennial plantings need to be planted only once and then they only need to be maintained in the field. Utilizing perennial companion plantings to attract honey bees and other pollinators provides a permanent, maintainable source of pollinator attraction. Perennial plantings have the potential to provide nutrition for pollinators or nesting sites when crops are not present, potentially enhancing future crops by maintaining healthy populations of pollinators throughout the year. Perennial companion plantings were established in rows adjacent to where vegetable crops were grown. The four regionally well-adapted perennial pollinator-attracting companion plant species used in this study were *Phyla nodiflora* (L.) Greene, *Borrichia frutescens* (L.) DC., *Salvia farinacea* Benth. 'Henry Duelberg', and *Eysenhardtia texana* Kunth.

Although perennial plants may provide a more permanent source of attraction for pollinators, establishment duration and maintenance in addition to substituting crop species space for the placement of perennial companion plants could prove to be economically non-viable for growers if the costs cannot be negated with increased crop yield. Annual plantings can be inter-planted with crop species and would provide an immediate source of pollinator attraction, therefore eliminating the need for establishment duration, additional space, and continued maintenance. However, annual companion plantings would need to be replanted each cropping cycle compared to only one initial planting of the perennial companion plant species. Annual companion plant species were selected with preference to annuals that are well-known pollinator attractors that grow well in this region of central Texas. The four annual pollinator-attracting companion plant species selected were *Borago officinalis* L., *Cosmos bipinnatus* Cav., *Ocimum basilicum* L., and *Zinnia* x *marylandica* D.M. Spooner, Stimart, & T. Boyle. The objective of this study was to evaluate the impact of pollinator-attracting perennial plants, compared to both pollinator-attracting annual plants and plots lacking pollinator-attracting plants, on cucumber, *Cucumis sativus* L., and habanero, *Capsicum chinense Jacq.*, crop yields and quality.

Materials and Methods

The greenhouse production portion of this experiment was conducted at the Texas A&M University Horticultural Teaching, Education, and Research Center (HortTREC) of College Station, TX (30.52°N, 96.43°W) in the fall 2016 and fall and spring of 2017. Minimum and maximum temperatures were recorded (Table 1). Greenhouses were equipped with automated shade cloths that provided 44% shade when light sensors exceeded 750 µmol.

Table 1. Cucumber and habanero planting dates, harvesting periods, maximum/minimum air temperature, and total rainfall.

	Planting dates	Harvesting periods	Temperature Max. (°C)	Min. (°C)	Rainfall (mm)
Cucumber					
	20 Sept. 2016	2 Nov. – 19 Nov. 2016	35.6	3.3	74.42
	28 March 2017	18 May – 8 Jun. 2017	35.6	4.4	190.75
	18 Sept. 2017	1 Nov. – 22 Nov. 2017	35.0	8.3	112.27
Habanero					
	23 Sept. 2016	7 Nov. – 28 Nov. 2016	35.6	3.9	74.17
	30 Mar. 2017	22 May – 12 Jun. 2017	35.6	4.4	205.74

Annual Plant Species

The annual pollinator-attracting companion plant species *Borago officinalis, Cosmos bipinnatus*, *Ocimum basilicum*, and *Zinnia* x *marylandica* were germinated from seed in greenhouses during fall of 2016, and summer and fall of 2017. Annual seeds were hand planted into 0.16 L cells (606 cell flats, T.O. Plastics., Clearwater, MN) filled with Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.) and hand watered as needed. Liquid fertilization (300 mg/L of N from Peters Professional 20N-8.74P-16.6K, Scotts Co., Marysville, OH) was provided on a weekly basis.

Perennial Plant Species

The perennial plant species *Phyla nodiflora*, *Borrichia frutescens*, and *Salvia farinacea* 'Henry Duelberg' were propagated clonally from stock plants maintained in a gravel bottom nursery in College Station, TX (30° 37' 24.24" N, - 97° 22' 0.17" W) in February 2016. *Eysenhardtia texana* plants were purchased in #2 containers (6.3 L) from Barton Creek Nursery, Austin, TX, and planted directly in the field on 15 March 2016.

Borrichia frutescens and *P. nodiflora* were propagated by tip cuttings, 4-6 cm long, with basal ends of cuttings dipped in talc based indole-3-butyric acid at a concentration of 1 g/kg (Hormodin® 1, OHP, Inc., Mainland, PA). Cuttings were placed in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, OH) filled with coarse perlite (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.). Intermitted mist was applied at 16-min intervals of 15 s each using reverse osmosis water set to a light sensor programed to sunrise and sunset. Rooted cuttings were potted in 0.47 L black plastic pots (Dillen Products, Middlefield, OH) containing Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.) and hand watered. Fertigation (300 mg/L of N, Peters Professional 20N-8.74P-16.6K, Scotts Co., Marysville, OH) was applied on a weekly basis.

Salvia farinacea 'Henry Duelberg' was propagated by stem cuttings and potted into 2.5 L black injected molded pots (Dillen Products, Middlefield, OH) containing Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.) and hand watered. Plants were fertigated (300 mg/L of N, Peters Professional 20N-8.74P-16.6K, Scotts Co., Marysville, OH) on a weekly basis.

Vegetable Crop Species

Cucumber, *Cucumis sativus* ‘Marketmore’, was propagated in greenhouses at the HortTREC of College Station, TX, on 28 July 2016, 7 March 2017, and 1 August 2017. Plants were hand seeded into 0.16 L cells (606 cell flats, T.O. Plastics., Clearwater, MN) filled with Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.) and hand watered. Plants were fertigated (300 mg/L of N, Peters Professional 20N-8.74P-16.6K, Scotts Co., Marysville, OH) on a weekly basis.

Habanero, *Capsicum chinense* ‘TAM Mild Habanero’, was propagated in greenhouses at the HortTREC on 5 August 2016, 10 February 2017, and 3 August 2017. Plants were hand seeded into 0.52 L black plastic pots (T.O. Plastics., Clearwater, MN) filled with Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.) and hand watered. Fertigation (300 mg/L of N, Peters Professional 20N-8.74P-16.6K, Scotts Co., Marysville, OH) was applied weekly.

Field Experiment

Experimental Design

The field portion of this study took place at the HortTREC field plots in fall 2016 and fall and spring 2017 (Table 1). The experiment was conducted on a 1.2 hectare (3 acre) field site in which nine 12.2 m (40 ft.) long by 7.6 m (25 ft.) wide plots were established with three replicates of each of three treatments: 1) perennial pollinator-attracting companion plants; 2) annual pollinator-attracting companion plants; 3) no pollinator-attracting companion plants (control plots). Plots were separated on the site as distant from each other as possible to minimize overlap of the pollinator treatment effects. Three 12.2 m (40 ft.) long by 1.5 m (5 ft.) wide rows were created within each of the nine plots. Three plots were randomly assigned to either a pollinator-attracting treatment or the control treatment. In each plot, the first 6.1 m (20 ft) of a row was allocated to cucumbers and the second 6.1 m (20 ft) was allocated to peppers.

Field Preparation

Plots were established on clay soil with 8.2 pH in Burleson County, Texas. A USDA soil survey classifies the soil as Burleson clay, described as “clayey, very deep, nearly level to gently sloping, moderately well drained soils that are slightly alkaline” (USDA, 2014). Irrigation water was provided from an on-site well water with 6.9 pH, and 1074 mg/L of total dissolved salts. Prior to each planting field plots were fertilized with a 17N-7.4P-14.1K fertilizer (Brazos Best Fortify, Producers Cooperative Association, Bryan, TX) at a rate of 0.90 kg N / 93 m^2 (2lbs N / 1,000 sq ft).

Perennial Species

The perennial pollinator-attracting companion planting treatments were planted within a 12.2 m (40 ft.) long by 1.5 m (5 ft.) wide row containing four perennial pollinator-attracting companion species, and adjacent to this row were two 1.5 m (5 ft.) wide rows in which vegetable crop species were grown as described for the control plots. Spacing between rows was 1.5 m (5 ft). Perennial companion plant species field rows were planted at 45.7 cm (18 inch) spacings, alternating three plants across a single row. Plants were mulched with 5 cm (2 in.) of composted, shredded, pine bark mulch (Ohio Mulch, Columbus, OH) and were dual drip-line irrigated (T-Tape Model 505, Deere and Company, Moline, IL) as needed, to maintain turgidity. Weed management was conducted initially on a weekly basis by hand-removal until establishment of *Phyla nodiflora* as living mulch occurred, at which point weed management was conducted in the perennial companion plant rows only as needed.

Annual Species

The annual pollinator-attracting companion planting treatment was planted with three 12.2 m (40 ft.) long by 1.5 m (5 ft.) wide row containing vegetable crop species inter-planted with annual companion plants (Figure 1). Annual companion plant species rows were inter-planted with vegetable crop species at 30.5 to 45.7 cm (12 to 18 inch) spacings. Vegetable crops were planted as described for the control plots. Spacing between rows was 1.5 m (5 ft). Plants were planted on black plastic mulch and were dual drip-line irrigated (T-Tape Model 505, Deere and Company, Moline, IL) as needed to maintain turgidity.

Figure 1. Annual pollinator-attracting companion plantings.

Conventional Plots Lacking Companion Plants (Control Plots)

The remaining three plots included crop species without any companion plants, which were used as controls. Vegetable crop rows were 12.2 m (40 ft.) in length with each row containing 6.1 m (20 ft.) of Cucumber, *Cucumis sativus*, and 6.1 m (20 ft.) of habanero, *Capsicum chinense*. *C. sativus* field row sections were planted in double rows at 30.5 cm (12 inch) spacings and *C. chinense* field row sections were planted in double rows at 45.7 cm (18 inch) spacings. Spacing between rows was 1.5 m (5 ft). Plants were planted on black plastic mulch and dual drip-line irrigated (T-Tape Model 505, Deere and Company, Moline, IL) as needed to maintain turgidity.

Cucumber Yield

Cucumber yield data were collected in a three-week harvest period during the fall of 2016 and spring and fall of 2017 (Table 1). Harvest time periods were initiated when a majority of plants contained mature fruit. All cucumbers with length greater than 12.5 cm were harvested by hand and categorized by grade according to their diameter and length in accordance with the USDA grades for cucumbers (USDA, 2007). Total yield was recorded for each plot and marketable yield was obtained by summing the fresh weight of cucumbers from all grades and the remaining ones were classified as culls.

Habanero Yield

Habanero yield data were collected during a three-week harvest period during the fall of 2016 and the spring of 2017 (Table 1). An unexpected early frost prevented a complete harvest of habanero in fall 2017, thus those data were omitted from the analysis. Harvesting time periods were initiated when a majority of plants contained mature fruit. Habaneros were harvested by hand and categorized by grade in accordance with the USDA grades of peppers

(USDA, 2007). Total yield and marketable yield for each plot was obtained by summing the fresh weight of habaneros from all grades along with the remaining classified as culls.

Statistical Analysis

Cucumber and habanero yield data were analyzed for each harvest with a randomized complete block design using a normal distribution with pollinator-attracting treatment as the main effect. Raw yield consisted of measuring yields from each plot without making any adjustment for the space utilized for perennial pollinator attractants. Annual and control treatments represented yields from 83.6 m^2 (900 ft^2), whereas perennial treatments represented yields from 55.7 m^2 (600 ft^2) due to space required for the perennial pollinator attractants. In our model it was reasonable to assume that pollinator effects would extend to both sides of a pollinator-attracting row. Thus, adjusted yield consisted of yields compared on assumed perennial row effect of two adjacent rows to each side rather than a single side of the perennial pollinator-attracting companion plants, as tested. These values assume that one in five rows was used as perennial pollinator attractants. Raw yields were recorded on a total kilogram basis initially and subsequently converted to a kilogram-per-hectare basis for ease of comparison to small-scale commercial production systems. Whenever main effects were statistically significant (α=0.05), Tukey least-square means tests were performed (JMP 13, SAS Institute, Cary, NC).

Results and Discussion

Cucumber Yield

Blocking effects were not statistically significant for any of the raw or adjusted yield data ($P \leq 0.05$). Therefore, Tables 2, 3 and 4 present only the main effects of pollinator-attracting treatments. Statistical differences ($P \leq 0.05$) in culls (Figure 2) or individual fruit mean weight among treatment groups were not found in Fall 2016, Spring 2017, or Fall 2017 harvests (Table

2). This lack of difference in cull rates or in mean fruit size indicates that there was no impact of pollinator-attracting companion plants on measures of fruit quality in cucumbers. However, yield data indicated a different story. In fall of 2016, there was a significant difference ($P < 0.05$) among pollinator-attracting companion plant treatments in cucumber raw total and marketable yields (Table 2), and in both raw and adjusted marketable yields on a per-hectare basis (Tables 3 and 4). Raw yield of cucumber was significantly greater in the annual treatment followed by control and perennial treatments, respectively (Tables 2 and 3). Adjusted yield, accounting for lost production area in perennial companion plantings for fall of 2016, indicated significant differences ($P < 0.05$) in yield between annual companion plant treatments and the control and perennial companion plant treatments (Table 4). However, no significant difference in adjusted yield was found between the control and the perennial pollinator companion plant treatment in fall 2016 (Table 4).

In spring 2017, there was no significant difference ($P < 0.05$) in yield among pollinator-attracting companion plant treatments in either cucumber raw total or adjusted yield (Table 2), nor among raw or adjusted marketable yields (Tables 3 and 4). In fall of 2017, there was a significant difference ($P < 0.05$) in yield among pollinator-attracting companion plant treatments in cucumber raw total and raw and adjusted marketable yields (Tables 2, 3 and 4). Annual companion plant treatments had greater raw yield of cucumbers in fall 2017 compared to the control and perennial companion plant treatments, which did not differ statistically from each other (Tables 2 and 3). Adjusted marketable yield data indicated significant differences ($P < 0.05$) in yield between annual companion plant treatments and the control and perennial companion plant treatments. However, adjusted marketable yield data for fall 2017 indicated no significant difference between the control and the perennial companion plant treatment (Table 4).

Cucumber yield in fall of 2016, regardless of adjustment, was significantly greater ($P < 0.05$) in the annual companion plant treatment, and is likely attributable to the increased numbers of pollinators visiting cucumber flowers from adjacent flowering annual companion species. The lack of significant increase in yield from the perennial companion plant treatment may be due to the longer establishment period required for perennial plant species to reach optimum flowering potential.

No significant differences in yield were found among treatments in spring 2016, regardless of adjustment, which may be attributed to pollinator abundance already being at a sufficient level across the landscape for adequate pollination without the need for pollinator-attracting companion plants. For instance, Quinn et al. (2017) reported cucumber yield to be largely unaffected by the addition of pollinator-attracting species. However, their data were only taken during spring harvests, which may explain the concurrence with spring results herein, but differences compared with results herein from fall harvest yields.

In Fall of 2017 the cucumber raw yield was again greatest in the annual companion plant treatments, however the perennial companion plant treatments now statistically performed at the same level as the control treatment, regardless of yield adjustment. This suggests that despite giving up approximately a third of the production space to perennial plants, the increase in yield was sufficient to offset the loss of production area for the fall 2017 crop. Given yield adjustment, which assumes an effect of two rows to the adjacent sides of the perennial plantings or approximately a fifth of the production, the perennial and control pollinator-attracting companion plant treatments performed statistically at the same level, but with a greater absolute numerical value. This suggests that it may be possible for perennial plot yields to surpass controls even with a decreasing proportion of the production space allocated to perennial companion plants. Alternatively, the increasing pollinator-attracting ability of perennial plantings as they mature might also increase yields relative to control plantings, as was seen with the annual plantings during fall crops.

Future research will need to determine the maximum row distance at which perennial plantings have a significant impact on yield to further compare perennial planting treatments and control treatments. Additional replications of seasonal harvests and establishment of perennial plantings should be examined to determine if the impact on yield of pollinator-attracting companion plants is consistently a seasonal effect as suggested herein for cucumbers, and to what extent establishment phases of perennial companion plantings can be expected to impact yield. Likewise, it may be possible to select seasonally superior combinations of annual or perennial companion plantings targeted to coincide with specific crop flowering cycles, or to more efficiently attract pollinators that are most effective for pollination of specific crops.

Figure 2. Cucumber culls

Table 2. Total yield (Total), marketable yield (Mkt.), cull percentage (Cull), and mean individual fruit weight (Inv.) of pollinator-attracting annual or perennial companion planting treatments on cucumber and habanero in comparison to control plots without companion plants during Fall 2016, Spring 2017, and Fall 2017.

	Fall 2016				Spring 2017				Fall 2017			
Treatment	**Total (Kg)**	**Mkt. (Kg)**	**Cull (%)**	**Inv. (g)**	**Total (Kg)**	**Mkt. (Kg)**	**Cull (%)**	**Inv. (g)**	**Total (Kg)**	**Mkt. (Kg)**	**Cull (%)**	**Inv. (g)**
Cucumber												
Perennial	36.2 c[z]	27.2 c	24.8 a	175.9 a	62.0 a	47.5 a	23.4 a	173.3 a	51.4 b	38.9 b	24.4 a	177.4 a
Annual	89.6 a	66.8 a	25.4 a	178.7 a	74.9 a	58.3 a	22.3 a	181.8 a	92.1 a	71.2 a	22.7 a	172.6 a
Control	59.0 b	45.9 b	22.3 a	182.4 a	79.2 a	58.9 a	25.7 a	175.4 a	61.5 b	47.1 b	23.4 a	175.1 a
Sig.	*	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	*	N.S.	N.S.
Habanero												
Perennial	10.2 c	9.5 c	6.2 a	5.8 a	17.7 b	16.9 b	4.2 a	5.3 a	---	---	---	---
Annual	18.8 a	17.9 a	4.8 a	5.2 a	23.9 a	22.5 a	5.9 a	5.8 a	---	---	---	---
Control	15.0 b	14.1 b	5.9 a	5.4 a	17.1 b	16.3 b	4.8 a	5.5 a	---	---	---	---
Sig.	*	*	N.S.	N.S.	*	*	N.S.	N.S.	---	---	---	---

[z] N.S. * Nonsignificant or significant F test at $P < 0.05$, respectively. Treatments with the same letters within a species and column are not significantly different (Tukey's HSD, α=0.05).

Habanero Yield

Habanero yields were generally consistent with the results seen for cucumbers in response to pollinator-attracting companion plants (Tables 2, 3, and 4). As with our cucumber crops, yield measures were significantly affected ($P < 0.05$), whereas no effects attributable to pollinator-attracting companion plants were seen for the fruit quality measures of cull percentages or mean fruit mass (Table 2). In fall of 2016, there was a significant difference ($P < 0.05$) in yield among pollinator-attracting companion plant treatments in habanero raw total yield (Table 2), and in raw and adjusted marketable yield (Tables 3 and 4). In fall 2016 a significant difference in raw yield was present between annual companion planting treatments and the control and perennial treatments, with the annual plantings treatment ranking greatest, followed by the control and perennial treatments, respectively. When adjustments to yield for space occupied by perennial plantings were factored into the analysis, no differences were found among perennial and control treatments for the fall 2016 habanero crop (Table 4).

In spring of 2017, there was a significant difference ($P < 0.05$) in yield among pollinator-attracting companion plant treatments in habanero raw total yield, as well as raw and adjusted marketable yield (Tables 2, 3, and 4). Annual companion plant treatment raw yields were greater than the control and perennial companion plant treatments. However, no significant difference in yield was found between the raw total yield or raw or adjusted marketable yields between the control and the perennial pollinator companion plant treatments (Tables 2, 3, and 4). This suggests that increased yields due to inclusion of perennial plants were sufficient to offset the allocation of approximately a third of the space to perennial plants (Table 2, and 3). Adjusted yield data for spring of 2017 indicated that the perennial plantings were intermediate between the annual plot yields and control plots and did not differ statically from the controls (Table 4).

Habanero yields suggest that the pollinator-attracting companion plant species impact yields in a shorter time duration when compared to cucumbers. In addition, it appears that pollinator-attracting companion plant species can influence the spring habanero yield, whereas they did not influence cucumber yield. We suggest that additional replications of seasonal harvests should be performed to confirm the pollinator-attracting companion plant species' impact on seasonal habanero yield.

Conclusion

Similar responses were observed with both cucumber and habanero crops in response to pollinator-attracting companion plantings in a general sense. Fruit quality measures in terms of cull percentages and mean fruit masses for cucumber and habanero pepper were not affected by the presence of pollinator-attracting companion plantings. Annual companion plantings were more effective at enhancing yield of adjacent crops species than perennial companion species under the tested conditions and time frames. The lack of differences in several yield measures between perennial companion plant plots and control plots despite the allocation of one in three or one in five rows to the perennial plantings suggests that there is a potential for yields in perennial companion plots to surpass those of control plots if the effective distance between rows occupied by perennial plantings is increased. The apparent seasonality of responses to pollinator-attracting companion plantings suggest that it may be feasible to improve responses even further by refining the fit of annual and perennial companion plant bloom cycles to be maximized with that of targeted crops species. Our results also suggest a time lag for perennial plants to reach maximum effectiveness (Figures 3, and 4). Benefits associated with the additional pollination and ecosystem services resulting from the year-round presence of the perennial plantings on other crops during the year or long-term pollinator populations were not determined in this study, but also represent potential tangible benefits of perennial companion plantings. The goal of this study was to investigate from a horticulture perspective (not one focused in entomology) whether crop yield can be reliably increased by the addition of pollinator-attracting crops (Haaland et al., 2011). Similar cross disciplinary approaches that bring plant and pollinator scientists together may be needed to accurately investigate how plants that attract pollinators increase the yield of vegetable crops.

Figure 3. Installation of perennial companion plantings.

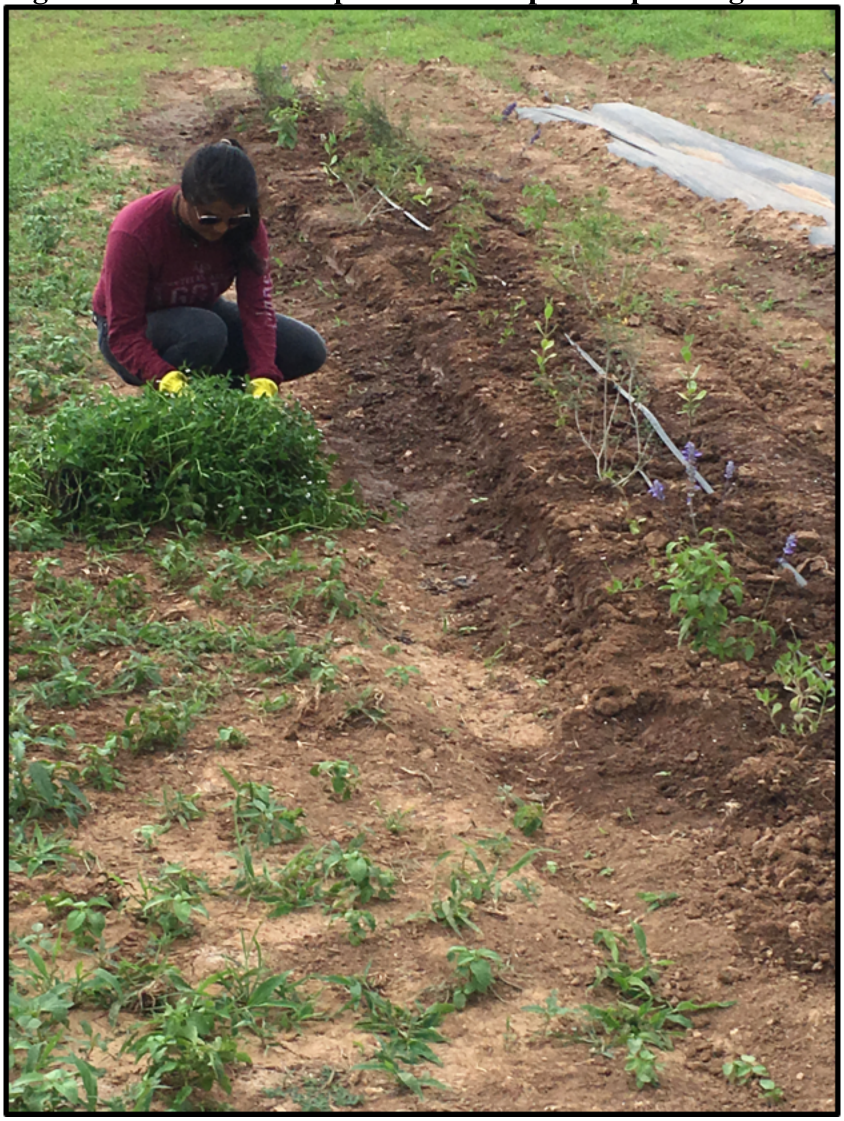

Figure 4. Perennial companion plantings after one year of establishment.

Table 3. Effects of pollinator-attracting annual or perennial companion planting treatments on cucumber and habanero pepper total raw yield per plot converted to Kg/ha in comparison to control plots without companion plants.

	Raw Yield		
Treatment	**Fall 2016** (Kg/ha)	**Spring 2017** (Kg/ha)	**Fall 2017** (Kg/ha)
Cucumber			
Perennial	7793 c[z]	13351 a	11073 b
Annual	19278 a	16135 a	19829 a
Control	12705 b	17056 a	13239 b
Significance	*	N.S.	*
Habanero			
Perennial	2185 c	3810 b	---
Annual	4038 a	5153 a	---
Control	3231 b	3687 b	---
Significance	*	*	

[z] N.S. * Nonsignificant or significant F test at $P < 0.05$, respectively. Treatments with the same letters within a species and column are not significantly different (Tukey's HSD, α=0.05).

Table 4. Effects of pollinator-attracting annual or perennial companion planting treatments on cucumber and habanero adjusted total yield per plot to Kg/ha in comparison to control plots without companion plants.

	Yield		
Treatment	**Fall 2016** (Kg/ha)	**Spring 2017** (Kg/ha)	**Fall 2017** (Kg/ha)
Cucumber			
Perennial	8654 b[z]	14746 a	12303 b
Annual	19278 a	16135 a	19829 a
Control	12705 b	17056 a	13239 b
Significance	*	N.S.	*
Habanero			
Perennial	2427 b	4230 b	---
Annual	4038 a	5153 a	---
Control	3231 b	3687 b	---
Significance	*	*	---

[z] N.S. * Nonsignificant or significant F test at $P < 0.05$, respectively. Treatments with the same letters within a species and column are not significantly different (Tukey's HSD, α=0.05).

CHAPTER III

FREQUENCY AND DIVERSITY OF POLLINATORS

Human sustenance is directly dependent on natural processes known as ecosystem services, which are not typically accounted for in the valuation of global economic markets (Hanley et al., 2015). The degradation of ecosystem services can lead to the inability of agricultural settings to meet the growing and increasingly resource-consuming human population (Roberts, 2011; Tilman et al., 2001). Pollination of agricultural crops by insects is a type of ecosystem service that is highly vulnerable to degradation (Klein et al., 2007), given the rapid decline of key pollinators in terms of diversity and abundance in many agro-ecosystems (Potts et al., 2010; Garibaldi et al., 2011). Increased pollinator diversity and abundance, and therefore visitation to flowering crops, directly increases the proportion of flowers that develop into mature fruits or seeds, therefore increasing crop yield (e.g., Klein et al., 2003).

Pollinator richness has been shown to increase overall crop pollination in many species (Cardinale et al., 2012). Worldwide studies looking at the importance of pollinators in various agricultural landscapes have shown that nearly 90 of the leading global food crops are dependent to some degree on animal pollination (Klein et al., 2007). While pollination is essential for 13 of the most consumed food crops in the world, agricultural intensification has undoubtedly jeopardized the population of native bee communities and their stabilizing effects on pollination services in changing landscapes (Klein et al., 2007). Across the globe, the economic value of fruits, vegetables, nuts, oil crops, and spices dependent on insect pollination has been estimated to be greater than $200 billion annually (Gallai et al., 2009).

In the United States, the conservation of pollination services is a national priority. For instance, the value of insect pollinated crops was estimated at $15.12 billion in 2009, with honey bee (*Apis mellifera*) pollination services accounting for $11.68 billion of this total (Morse and Calderone 2000, Calderone, 2012). More recently, the U.S. White House acknowledged the integral nature of insect pollinators and national food security, indicating that pollinators contributed approximately $24 billion annually to our economy in 2014, $15 billion of which were attributed to honey bee pollination services and another $9 billion to native bees and other pollinators (Pollinator Health Task Force, 2014). Additional economic contributions of honey bees and native pollinators to the U.S. economy are associated with the commercial insect pollination industry through employment of commercial beekeepers and apiculture experts, constituting a cross-country migratory service that is vital to several fruit, vegetable, and nut crops, estimated to be valued at $655.6 million in 2012 (Bond et al., 2014; Perez and Plattner, 2014). Models looking at the potential loss of fruit and crop production in the event of total or partial pollinator disappearance will lead production of these crops to below current levels (Gallai et al., 2009). Furthermore, poor pollination has been associated with the reduction in yield and quality of some fruit and vegetable crops (Angbanyere and Baidoo, 2014; Dag et al., 2007; Garibaldi et al., 2014). Because the need for crop pollination has increased globally, native bee-pollinated crops are increasingly being supplemented by the addition of honey bee colonies to provide sufficient pollination services in many crops (Morse, 1991; Goodwin et al., 2011; Rucker et al., 2012). Therefore, there is a critical need worldwide in general, and in the U.S. in particular, to develop management protocols that will enhance the number of insect pollinator species available to provide pollination services for the increasing quantities of fruit and vegetable crops consumed by growing human populations.

With decreasing honey bee populations becoming a nationwide concern with numerous consequences, including inadequate pollination of several crop species (Calderone, 2012; Gallai et al., 2009; Kevan and Phillips, 2001), it is important to investigate crop production systems that incorporate companion plantings that attract honey bees and other pollinators to agricultural crops. While a diverse plant community is important to support and maintain pollinator populations, these conditions are lacking from many heavily managed farm landscapes (Williams et al., 2010; Winfree et al., 2011).

Wild pollinators have outpreformed managed bees in several studies for a number of crops including cucurbits (Artz and Nault, 2011; Blaauw and Isaacs, 2014; Gajc-Wolska et al., 2011; Garibaldi et al., 2013; Holzschuh et al., 2012). Therefore, increasing biodiversity with the addition of available pollinator-attracting plant species planted adjacent to crop species could result in a greater quantity and more diverse population of wild pollinators visiting nearby crop species.

The purpose of this study was to begin the evaluation of vegetable production systems that feature companion plantings known to attract pollinators and to measure yield differences associated with different companion planting groups. The objective of this chapter was to determine the frequency and diversity of pollinators on the proof-of-concept study described in chapter two in which the primary goal was to evaluate the impact of pollinator-attracting perennial plants on cucumber, *Cucumis sativus* L., and habanero, *Capsicum chinense Jacq.*, crop yields and quality, compared to both pollinator-attracting annual plants and plots lacking pollinator-attracting plants. Perennial companion plantings were established in rows adjacent to where vegetable crops were grown. The four regionally well-adapted perennial pollinator-attracting companion plant species used were *Phyla nodiflora nodiflora* (L.) Greene, *Borrichia frutescens* (L.) DC., *Salvia farinacea* Benth. 'Henry Duelberg', and *Eysenhardtia texana* Kunth.

Annual plantings were inter-planted with crop species and replanted at each cropping cycle compared to only an initial planting of the perennial companion plant species. Annual companion plant species were selected with preference to annuals that are well-known pollinator attractors that grow well in the region. The four annual pollinator-attracting companion plant species selected were *Borago officinalis* L., *Cosmos bipinnatus* Cav., *Ocimum basilicum* L., and *Zinnia* x *marylandica* D.M. Spooner, Stimart, & T. Boyle.

To determine which pollinator species may be contributing to crop pollination, a pollinator sampling technique that utilizes observation, based on the methods described by Ritchie et al., (2015) for determining pollinator diversity and frequency of visitation, was conducted as it has been successfully used in previous pollinator studies. Limitations of this sampling technique include variation in observers and limited time of sampling, which would exclude an examination of potential nocturnal pollinators (Ritchie et al., 2015). To minimize variation, observations of pollinators were limited to only one observer.

Materials and Methods

Pollinator Sampling

Pollinator sampling was conducted with observations starting as plants initiated flowering, and occurred on a weekly basis by one researcher between 10:00 AM and 3:30 PM until crops were harvested. Observations were conducted in 30-minute intervals by walking parallel to crop rows with plots and recording the number and order of pollinators observed on cucumber flowers, habanero flowers, and flowers of the annual and perennial pollinator-attracting companion plantings.

Statistical Analysis

Arthropod abundance by taxonomic groups was analyzed with a randomized complete block design using a normal distribution with pollinator-attracting treatment as the main effect. Where main effect was significant ($\alpha = 0.05$), Tukey least-square means tests were performed to compare individual treatment means (JMP 13, SAS Institute, Cary, NC).

Results and Discussion

Frequency of Pollinators on Crop Species and Companion Plant Groups

We found were significant differences ($P < 0.05$) in the frequency of pollinators observed on pollinator-attracting companion plant species in fall 2016, spring 2017, and fall 2017 (Table 5). In the fall of 2016, athe frequency of pollinators visiting both cucumber and habanero flowers was significantly greater($P < 0.05$) in the annual treatment compared to those on perennial or control treatments, while no significant difference was found between crop plants in control and perennial treatments (Table 5). Concurrently, the frequency of pollinators visiting the flowers of pollinator-attracting companion plants indicated a greater significant frequency of visitation on annual companion plantings when compared to perennial companion plantings (Table 5). In the spring of 2017, the frequency of pollinators visiting both cucumber and habanero flowers was significantly greater in the annual and perennial companion planting treatments when compared to the control treatment, while frequency of pollinators visiting the flowers of pollinator-attracting companion plants indicated significantly greater ($P < 0.05$) visitation in perennial companion plantings when compared to annual companion plantings (Table 5). In the fall of 2017, the frequency of pollinators visiting cucumber flowers was significantly greater in the annual and perennial companion planting treatments when compared to the control treatment, and,as seen in spring and fall 2017, the frequency of pollinators visiting the flowers of pollinator-attracting companion plants was greater in perennial companion plantings compared to annual companion plantings (Table 5). Habanero data for fall 2017 were unavailable due to plant damage caused by early frost.

The change in visitation frequency from greater visitation on annual companion plants and crops in annual companion planting treatments in fall of 2016, to similar levels of visitation on both crops in annual and perennial companion plantings in spring and fall of 2017, were likely a result of the substantial increase in pollinator visitation on perennial companion plants as the plants became more established in the field plots. It is common for herbaceous perennial plants to flower more limitedly during the first growing season after transplant from containers to a landscape setting, and to flourish more abundantly in subsequent years (Arnold, 2008).

Table 5. Frequency of pollinators visiting annual and perennial companion planting treatment plots, by crop species and companion species in comparison to pollinators visiting control plot crop species without companion plants.

	Frequency of pollinator visitation		
Treatment	**Fall 2016 (Mean # of insects)^{y}**	**Spring 2017 (Mean # of insects)^{y}**	**Fall 2017 (Mean # of insects)^{y}**
Cucumber			
Perennial	12.31 b^{z}	42.73 a	45.32 a
Annual	30.46 a	34.67 a	36.25 a
Control	10.83 b	11.26 b	10.36 b
Significance	*	*	*
Habanero			
Perennial	5.05 b	23.43 a	---
Annual	17.18 a	16.95 a	---
Control	4.88 b	5.44 b	---
Significance	*	*	---
Companion Species			
Perennial	18.76 b	90.93 a	93.72 a
Annual	70.39 a	68.66 b	72.31 b
Significance	*	*	*

^{y}Mean number of pollinators observed per plot.

^{z} N.S., * indicates nonsignificant or significant F test at $P < 0.05$, respectively. Treatments within a column and treatment category with the same letters are not significantly different (Tukey's HSD, α=0.05).

Diversity of Pollinators on Crop Species and Companion Plant Groups

In the fall of 2016 and spring of 2017, significantly more ($P < 0.05$) honey bees, non-honey bees, and pollinators in the order Lepidoptera and Diptera were observed on cucumber flowers on annual plantings compared to the control and perennial companion plant treatments, which did not differ statistically from each other (Table 6). Habanero flowers were only frequented by honey bees and significantly more($P < 0.05$) honey bees were observed on annual plantings when compared to perennial plantings or control treatments, which did not differ statistically from each other in fall 2016 or spring 2017 (Table 6). Significantly more honey bees, non-honey bees, and pollinators in the order Diptera and Lepidoptera were observed on flowers of the annual companion planting treatments when compared to perennial companion planting treatments in fall 2016 (Table 6).

In fall 2017, significantly more($P < 0.05$) honey bees, non-honey bees, and pollinators in the order Diptera and Lepidoptera were observed on cucumber flowers that were planted near perennial and annual pollinator-attracting plantings, which did not statistically differ from each other, when compared to control treatments (Table 6). Habanero data were unavailable for fall of 2017 due to plant damage caused by early frost. Significantly more honey bees, non-honey bees and pollinators in the orders Diptera and Lepidoptera were observed on flowers of the perennial companion planting treatments when compared to annual companion planting treatments in the spring and fall 2017 (Table 6).

The change in diversity of visitation from greater diversity of pollinators on annual companion plants and crops in annual companion planting treatments in fall 2016 to similar levels of diversity on both crops in annual and perennial companion plantings in spring and fall 2017 are likely a result of the substantial increase in pollinator visitation on perennial companion plantings as the plants became more established in the field plots. This mirrored a shift in greater diversity of pollinators on annual companion plants in fall 2016 to greater diversity on the more established perennial companion plants in spring and fall 2017. Herbaceous perennial plants commonly flower on a more limited basis during the first season after transplant from containers to a landscape setting, compared to their increased flowering pattern in subsequent years (Arnold, 2008).

The lack of diversity of pollinators observed on habanero flowers could be a result of the relatively small size and attractiveness of habanero flowers compared to the greater size and attractiveness of cucumber flowers, as changes in flower size, type, and color have been known to influence pollination (Hannan, 1981).

Table 6. Diversity of pollinators visiting annual and perennial companion planting treatments, by crop species and companion species groups in comparison to pollinators visiting control plot crop species without companion plants. The numbers provided represent the mean number of insects found per season on each type of plant every X unit of time

	Diversity of pollinators		
Treatment	**Fall 2016 (Mean # of insects)[y]**	**Spring 2017 (Mean # of insects)[y]**	**Fall 2017 (Mean # of insects)[y]**
Cucumber			
Honey bee			
Perennial	9.23 b[z]	32.05 a	33.99 a
Annual	22.82 a	26.00 a	27.19 a
Control	8.12 b	8.44 b	7.77 b
Significance	*	*	
Non-honey bee			
Perennial	1.11 b	3.85 a	4.08 a
Annual	2.74 a	3.12 a	3.26 a
Control	0.97 b	1.01 b	0.93 b
Significance	*	*	
Diptera			
Perennial	0.62 b	2.14 a	2.27 a
Annual	1.52 a	1.73 a	1.81 a
Control	0.54 b	0.56 b	0.52 b
Significance	*	*	*
Lepidoptera			
Perennial	1.35 b	4.70 a	4.99 a
Annual	3.35 a	3.81 a	3.99 a
Control	1.19 b	1.24 b	1.14 b
Significance	*	*	*
Habanero			
Honey bee			
Perennial	3.79 b	17.57 a	---
Annual	12.89 a	12.71 a	---
Control	3.66 b	4.08 b	---
Significance	*	*	---
Non-honey bee			
Perennial	---	---	---
Annual	---	---	---
Control	---	---	---
Significance	---	---	---
Diptera			
Perennial	---	---	---
Annual	---	---	---
Control	---	---	---
Significance	---	---	---
Lepidoptera			
Perennial	---	---	---
Annual	---	---	---
Control	---	---	---
Significance	---	---	---
Companion Species			
Honey bee			
Perennial	14.07 b	68.20 a	70.29 a
Annual	52.79 a	51.49 b	54.23 b
Significance	*	*	*
Non-honey bee			
Perennial	1.69 b	8.18 a	8.43 a
Annual	6.34 a	6.18 b	6.51 b
Significance	*	*	*
Diptera			
Perennial	0.94 b	4.55 a	4.69 a
Annual	3.52 a	3.43 b	3.62 b
Significance	*	*	*
Lepidoptera			
Perennial	2.06 b	10.00 a	10.31 a
Annual	7.74 a	7.55 b	7.95 b
Significance	*	*	*

[y]Mean number of pollinators observed per plot.
[z] N.S. or * indicate nonsignificant or significant F test at $P < 0.05$, respectively. Treatments with the same letters within a crop or companion species and pollinator group in a column are not significantly different (Tukey's HSD, α=0.05).

Frequency of Pollinator Visitation on Individual Companion Plant Species

There was a significant difference ($P < 0.05$) among the frequency of pollinators observed on individual plant species within the annual pollinator-attracting companion plant treatment in fall 2016, and spring and fall 2017 (Table 7). In all three cropping cycles, the frequency of pollinators visiting both *Ocimum basilicum* and *Borago officinalis* flowers was significantly greater($P < 0.05$) compared to pollinators visiting *Cosmos bipinnatus* flowers, while no significant difference was found between pollinators visiting *O. basilicum* and *B. officinalis* flowers (Table 7). In addition the mean number of insects on a given species was fairly consistent across the three crop cycles for all species of annuals, but not so for perennial species (Table 7). *Zinnia* x *marylandica* flowers were only frequented by pollinators twice during the entire study and therefore were excluded from data analysis. Lack of pollinators visiting *Z.* x *marylandica* may likely be because the *Z.* x *marylandica* seed stock used contained double flower varieties, and double flowering varieties of several flowers have been reported to be less attractive to pollinators, which is thought to be attributable to reduced nectar production of double varieties (Corbet et al., 2001).

There was a significant difference ($P < 0.05$) among the frequency of pollinators observed on individual plant species within the perennial pollinator-attracting companion plant treatment in fall 2016, and spring and fall 2017 (Table 7). During all three cropping cycles, the frequency of pollinators visiting *Phyla nodiflora* flowers was significantly greater ($P < 0.05$) than the frequency of pollinators visiting *Salvia farinacea* flowers. No significant difference was found between the frequency of pollinators visiting *Borrichia frutescens* and and *Eysenhardtia texana* flowers, however, and this frequency was significantly lower than the frequency of pollinators observed visiting *S. farinacea* and *P. nodiflora* flowers (Table 7). However, for each species of perennial companion plant, the number of pollinators increased approximately three to four-fold from the first growing season after transplant in fall 2016, to the second growing season after transplant in spring and fall 2017 (Table 7). This different pattern of responses between annual and perennial species in frequency of visitation is likely attributable to the slower maturity and establishment of the perennial companion plants compared to the annual species.

Conclusion

In conclusion, one year after establishment, perennial pollinator-attracting plant treatments attracted a similar number of pollinators to cucumber flowers compared to annual pollinator-attracting treatments. Likewise, perennial plant treatments attracted a similar number of honey bees to habanero flowers than annual plant treatments. In addition, after one year of establishment, the perennial pollinator-attracting treatment was statistically greater ($P < 0.05$) at attracting pollinators, averaged across all pollinator types. Future recommendations would include adding additional methods for sampling pollinators, such as sticky traps and netting, in addition to observation. The potential benefits of adding other sampling protocols include the investigation of nocturnal pollinator visitation and pollinators that are difficult to observe in field conditions. In addition, preliminary pollinator sampling is recommended for future investigation of pollinator-attracting companion plantings to more accurately determine any pre-existing pollinator populations and subsequently obtain more precise data on the impact of companion plantings on pollinator populations, particularly across time. The conflicting anecdotal reputation of *Zinnia spp.* as strong attractors of Lepidoptera was contradictory with our observations on *Z.* x *marylandica* (Table 7). It would be interesting to compare the impacts of flower morphology and color of various horticultural cultivars on their supposed pollinator-attracting abilities. In addition, given the highly effective nature of *P. nodiflora* in attracting pollinators (Table 7), additional potential applications for its use and refinement may be fruitful.

Table 7. Frequency of pollinators visiting annual and perennial companion planting treatment plots, by individual companion plant species

	Frequency of pollinator visitation		
Treatment	**Fall 2016 (Mean # of insects)^{y}**	**Spring 2017 (Mean # of insects)^{y}**	**Fall 2017 (Mean # of insects)^{y}**
Annual			
Ocimum basilicum	15.38 a^{z}	15.43 a	18.31 a
Borago officinalis	13.55 a	17.51 a	16.13 a
Cosmos bipinnatus	1.52 b	1.73 b	1.81 b
Zinnia x marylandica	---	---	---
Significance	*	*	*
Perennial			
Borrichia frutescens	0.12 c	0.43 c	0.45 c
Eysenhardtia texana	0.18 c	0.64 c	0.68 c
Salvia farinacea	0.92 b	3.20 b	3.40 b
Phyla nodiflora	11.08 a	38.46 a	40.79 a
Significance	*	*	*

^{y}Mean number of pollinators observed per plot.

^{z} N.S., * indicates nonsignificant or significant F test at $P < 0.05$, respectively. Species within a column and annual or perennial treatment category with the same letters are not significantly different (Tukey's HSD, α=0.05).

CHAPTER IV

ECONOMIC ANALYSIS

With decreasing honey bee (*Apis mellifera* L., Hymenoptera: Apidae) populations becoming a nationwide concern with numerous consequences, including inadequate pollination of several crop species (Calderone, 2012; Gallai et al., 2009; Kevan and Phillips, 2001), it is important to investigate crop production systems that incorporate companion plantings that attract honey bees and other pollinators to agricultural crops. While a diverse plant community is important to support and maintain pollinator populations, these conditions are lacking from many heavily managed farm landscapes (Williams et al., 2010; Winfree et al., 2011).

Therefore, increasing biodiversity with the addition of available pollinator-attracting plant species adjacent to crop species could result in a greater quantity and more diverse population of pollinators to nearby crop species. Furthermore, while the addition of companion plantings to crops has been known to increase insect diversity, more research is needed to determine if crop yield can also be reliably increased by the addition of companion plants (Haaland et al., 2011). It is also necessary to determine if a subsequent increase in yield is economically viable for a grower to reach a break-even point on incurred costs of companion plantings. Measuring the full economic value of pollination as an ecosystem service is a complex process and a relatively new concept that is still being developed (Hanley et al., 2015). However, using yield differences in a production setting gives researchers a tangible measuring tool to evaluate a primary economic potential of adding pollinator services to small-scale vegetable production systems.

The purpose of this study was to conduct an economic analysis of a vegetable production system that featured companion plantings known to attract pollinators. We also wanted to measure yield differences associated with selected pollinator-attracting companion planting groups and compare them to the increased production cost of the companion planting groups versus costs of conventional small-scale vegetable production systems. Pollinator-attracting companion plant species were chosen from either annual or perennial plant species to compare the economic benefits and costs of incorporating them versus that of a conventional production system relative to crop yield and cost of setup and maintenance.

The economic analysis of this study was evaluated by measuring three separate effects including: (1) added costs of production incurred by use of additional materials and cultural practices; (2) added income resulting from increased levels of production; and (3) income that may be lost when substituting crop space for perennial companion plantings in the production system. A "with" and "without" comparison, which attempts to identify and value the costs and benefits that arise from the proposed project, and then compares them with the situation as it would be without the project, were used to conduct the economic analysis (Gittinger, 1982). The difference between the "with" proposed project and the "without" proposed was the incremental net benefit arising from the project investment (Gittinger, 1982).

Once the economic analysis was conducted, projected yield thresholds that reach a break-even point with respect to the added costs of companion plantings were determined by crop, with fluctuations in crop price taken in consideration. Projected yield thresholds were used to determine the ratio of crop rows to perennial rows needed for the proposed system to be economically viable. Annual and perennial pollinator-attracting companion plantings were compared to one another in terms of initial and continued cost of operating each system.

Materials and Methods

The objective of this chapter was to conduct an economic analysis on the proof-of-concept study described in chapter two, in which we evaluated the impact of pollinator-attracting perennial plants, compared to both pollinator-attracting annual plants and plots lacking pollinator-attracting plants, on cucumber, *Cucumis sativus* L., and habanero, *Capsicum chinense Jacq.*, crop yields and quality. Perennial companion plantings were established in rows adjacent to where vegetable crops were grown. The four regionally well-adapted perennial pollinator-attracting companion plant species used were *Phyla nodiflora* (L.) Greene, *Borrichia frutescens* (L.) DC., *Salvia farinacea* Benth. 'Henry Duelberg', and *Eysenhardtia texana* Kunth.

Annual plantings were inter-planted with crop species and replanted each cropping cycle compared to only an initial planting of the perennial companion plant species. Annual companion plant species were selected with preference to annuals that are well-known pollinator attractors and which grow well in the region. The four annual pollinator-attracting companion plant species selected were *Borago officinalis* L.*, Cosmos bipinnatus* Cav., *Ocimum basilicum* L., and *Zinnia* x *marylandica* D.M. Spooner, Stimart, & T. Boyle.

Crop yields obtained from the annual pollinator-attracting companion plant species treatment, perennial pollinator-attracting companion plant species treatment, and control treatment lacking companion plantings in the experiments described in chapter two were used to create commercial budgets. Annuals directly interplanted with the crop species and control treatments represented yields from 83.6 m^2 (900 ft^2), whereas perennial treatments represent yield from 55.7 m^2 (600 ft^2), due to space required for the perennial pollinator attractants. Because it was reasonable to assume that pollinator effects would extend to both sides of the pollinator-attracting row, yield estimates consisted of yields compared on assumed perennial row effect of two adjacent rows to each side rather than a single side of the perennial companion plants, as tested. These values assumed that one in five rows were occupied by perennial pollinator attractants.

Commercial budgets were created that used a break-even analysis to accurately determine the added costs of the perennial and annual companion plantings and compared them to the added revenue from increases in yield derived from each companion planting. A break-even analysis determines the sale amount required to cover total costs (Beierlein et al., 2008). Commercial budgets used variable costs for each companion planting treatment were developed. Variable costs were obtained by calculating the production costs for each specific treatment, including manual labor at a rate of $10/hour and wholesale prices of media, fertilizer and planting containers. For perennial plantings, prices of 1-gallon containers of *Borrichia frutescens*, *Salvia farinacea* 'Henry Duelberg', and *Eysenhardtia texana* and 4-inch flats of *Phyla nodiflora* were obtained from a local nursery specializing in native perennial species to create a typical standard cost of perennial plant material. For annual plantings, the prices of seed of *Borago officinalis, Cosmos bipinnatus*, *Ocimum basilicum*, and *Zinnia* x *marylandica* were obtained from two seed companies to create an average standard cost of annual seed. The time value of money was not taken into consideration for commercial budgets, as the time duration of the study (less than two years) was deemed short enough to omit this addition.

Perennial yield and commercial budget data were utilized to project break-even points of potential extended row replacements assuming that the distance threshold effect of perennial treatments extended beyond two adjacent rows to three and four adjacent rows, resulting in a one in seven or one in nine rows in utilization for perennial pollinator attractants.

Results and Discussion

Crop yield was averaged across each harvest for annual companion planting treatments, perennial companion planting treatments, and control treatments (Table 8). Yield differentials between pollinator companion planting treatments and the controls were obtained and then used for price per pound comparisons (Table 8, 9). For all treatments (with the exception of habanero), the addition of perennial companion plantings exhibited a positive yield differential in (Table 8). The negative yield differential of the perennial companion plantings in habanero was likely because the third habanero harvest was damaged by an early frost resulting in only two harvests being averaged, therefore minimizing the increasing effectiveness of the perennial treatment across three harvests, as the perennial treatment data for cucumbers suggests.

Cucumber annual pollinator companion planting treatments resulted in a net loss when cucumbers were priced between \$0.50 to \$1.78 per pound, at which the break-even point of \$1.79 was reached with the cucumber annual planting treatment cost of \$12,594 / ha and exhibited an increasing net profit as cucumbers were priced ranging up to \$3.00 per pound (Table 9). The USDA Agricultural Marketing Service listed cucumbers in the "Local or Organic" section at \$1.89 per pound on 26 June 2018 (USDA, 2018). In addition, GO TEXAN®, a brand sponsored by the Texas Department of Agriculture (2018), issues a monthly farmers market price report and listed slicing cucumber price per pound at \$2.38 for June 2017.

Habanero annual pollinator companion planting treatments had a net loss when habaneros were priced between \$3.00 and \$5.02 per pound, at which the breakeven point of \$5.03 was reached with the habanero annual treatment cost of \$12,594 / ha and exhibited increasing net profit when habaneros were priced ranging up to \$8.00 per pound (Table 9). Habanero data were unavailable from USDA Agricultural Marketing Service reports, but jalapeno pepper, a related crop, was listed at $6.00 per pound (USDA, 2018).

Cucumber perennial pollinator companion planting treatments had a net loss when cucumbers were priced between $0.50 and $1.45 per pound, at which the breakeven point of $1.46 was reached with the cucumber perennial treatment cost of $3,236 / ha, and exhibited increasing net profit as cucumbers were priced ranging up to $3.00 per pound (Table 9). Habanero perennial pollinator companion planting treatments had a net loss regardless of habanero price, as net average yield was greater in the control treatments (Table 9).

Although the perennial pollinator-attracting companion planting treatments reached a breakeven point at a lower price per pound due to lower annual input costs as initial establishment costs are amortized across cropping cycles, annual treatments generated substantially more profit as the price per pound was increased due to greater enhancement of yield compared to perennial companion plantings (Table 9). Breakeven prices per pound were generally within range of average crop price per pound in recent years (USDA, 2018). Small-scale growers may incur a value-added profit by promoting these crop production techniques as beneficial to pollinators and potentially pursue a "pollinator friendly" label marketing the crop in this manner, as it has been found that consumers will select products that are labeled this way willing to pay more per pound (Khachatryan and Rihn, 2017). In addition, growers may be eligible for agricultural tax exemption for providing habitat and resources for pollinators (Xerces Society, 2013).

Results point to advantages and disadvantages of each pollinator-attracting companion planting treatment, with the best option likely being dependent on the circumstances of each particular grower. A main advantage of the annual companion treatment would be the treatment is installed each crop cycle. Even though it may be costly to repeat each harvest, there are fewer chances of any damage to the annual companion treatment over time, as could potentially occur for the perennial treatments during time periods between crops, such as winter damage. Replanting annual pollinator-attracting plants each crop cycle would facilitate rapid changes in the species used permitting a more crop-specific targeted companion planting if the crops species is changed or the same crop is planted in a different season of the year. Perennial pollinator-attracting plantings would lack this flexibility and may instead have certain plant species die out over time that would need to be replaced periodically, incurring additional costs. Perennial plantings would also be at risk of being lost during non-cropping times, for instance due to severe winter temperatures or flooding. Furthermore, irrigation lines would need to be changed periodically with perennial companion plantings incurring additional costs. The main advantage of the perennial companion plantings is they are installed year-round, not only saving costs of replanting the treatment each crop as is required with the annual treatments, but in addition, perennial pollinator plantings provide year-round resources and habitat for pollinators, potentially attracting pollinators to nearby non-target adjacent crops.

Further optimizing the blooming times of companion plants with a desired crop could potentially change yield estimates. Likewise, pairing companion plants that attract optimal pollinators for a given crop could potentially further enhance the effectiveness of the companion plantings. Determining effective pollinators for a specific crop is critical as there is a wide assortment of effectiveness of pollinator types for crops (Haaland et al., 2011; Quinn et al., 2017). For example, wild pollinators have exhibited higher rates of pollination in many crop pollination studies (Artz and Nault, 2011; Gajc-Wolska et al., 2011; Holzschuh et al., 2012; Garibaldi et al., 2013; Blaauw and Isaacs, 2014; Quinn et al., 2017). Further considerations would include testing additional plant species within each treatment to better determine the companion planting species that are best suited to targeted crops and respective pollinators, and to determine the longevity of each particular plant species and infrastructure of perennial planting treatments across crop cycles. The species selected in the proof-of-concept experiments (chapter 2) as pollinator attractants in both annual and perennial companion plants contained a range in plant height from a few centimeters to up to about 1.5 m. It would be useful to know if a mix of heights of pollinator-attracting species is needed or if plant height matters at all. Short growing companion plants may be advantageous if taller crops are used so that there would be less chance of interference with mechanical harvesters.

Perennial pollinator companion planting treatment projected effectiveness to a distance of three and four adjacent rows resulted in lower price per pound breakeven points for cucumber, $0.91 and $1.19, respectively, resulting in a shorter duration of return on investment if future studies determine the effectiveness of perennial pollinator attractants to be greater than two adjacent rows. However, a recent study found that the effectiveness of pollinator-attracting plantings strips was greatest in the row furthest from the pollinator-attracting strips (Quinn et al., 2017), suggesting a more complex pattern of pollinator distribution surrounding pollinator-attracting plantings.

Conclusion

In conclusion, an economic analysis of data from our proof-of-concept experiments (chapter 2) suggests that growers interested in the addition of pollinator-attracting companion plantings should utilize annual pollinator attractants as they provide an immediate return on investment with a lower risk of failure because they need to be re-planted each crop cycle and because they provide greater flexibility relative to crop selection. However, further research is recommended on the effective distance of perennial plantings in attracting pollinators as their full potential and impact on pollinators has not yet been determined. It should be noted that this concept is proposed for small-scale growers and home gardeners, and not necessarily intended for large commercial conditions. It is likely that as economic values for ecosystem services are more effectively and efficiently obtained (Hanley et al., 2015), the resulting economic analyses will more accurately encompass the entire economic value of each pollinator-attracting cropping system.

Table 8. Pollinator-attracting companion planting treatment crop yield differentials in comparison to a control treatment without companion plants. Yields are based on the results of proof-of-concept experiments from chapter 2.

Treatment	Cucumber Yield (lb/ha)	Habanero Yield (lb/ha)
Annual Pollinator Plantings	31,045	10,131
Control	24,019	7,626
Yield Differential	**7,026**	**2,505**
Perennial Pollinator Plantings	26,237	7,338
Control	24,019	7,626
Yield Differential	**2,218**	**-288**

Table 9. Pollinator-attracting companion planting treatment incremental revenue and cost per hectare price per pound comparison to control treatment without companion plants.

Crop	Treatment	$0.5/lb ($/ha)	$1.00/lb ($/ha)	$1.50/lb ($/ha)	$2.0/lb ($/ha)	$2.50/lb ($/ha)	$3.00/lb ($/ha)
Cucumber	Annual	$15,522	$31,045	$46,567	$62,090	$77,612	$93,135
	Control	$12,009	$24,019	$36,028	$48,038	$60,047	$72,056
	Gross revenue	$3,513	$7,026	$10,539	$14,052	$17,565	$21,078
	Annual Cost	$12,594	$12,594	$12,594	$12,594	$12,594	$12,594
	Profit/Loss	**-$9,081**	**-$5,568**	**-$2,055**	**+$1,458**	**+$4,971**	**+$8,484**
	Perennial	$13,119	$26,237	$39,356	$52,474	$65,593	$78,711
	Control	$12,009	$24,019	$36,028	$48,038	$60,047	$72,056
	Gross revenue	$1,110	$2,218	$3,328	$4,436	$5,546	$6,655
	Perennial Cost	$3,236	$3,236	$3,236	$3,236	$3,236	$3,236
	Profit/Loss	**-$2,126**	**-$1,018**	**+$92**	**+$1,200**	**+$2,310**	**+$3,419**
		$3.00/lb ($/ha)	**$4.00/lb ($/ha)**	**$5.00/lb ($/ha)**	**$6.00/lb ($/ha)**	**$7.00/lb ($/ha)**	**$8.00/lb ($/ha)**
Habanero	Annual	$30,393	$40,524	$50,655	$60,786	$70,917	$81,048
	Control	$22,878	$30,504	$38,130	$45,756	$53,382	$61,008
	Gross revenue	$7,515	$10,023	$12,525	$15,030	$17,535	$20,040
	Annual Cost	$12,594	$12,594	$12,594	$12,594	$12,594	$12,594
	Profit/Loss	**-$5,079**	**-$2,571**	**-$69**	**+$2,436**	**+$4,941**	**+$7,446**
	Perennial	$22,014	$29,352	$36,690	$44,028	$51,366	$58,704
	Control	$22,878	$30,504	$38,130	$45,756	$53,382	$61,008
	Gross revenue	$-864	$-1,152	$-1,440	$-1,731	$-2,016	$-2,304
	Perennial Cost	$3,236	$3,236	$3,236	$3,236	$3,236	$3,236
	Profit/Loss	**-$4,100**	**-$4,388**	**-$4,676**	**-$4,967**	**-$5,252**	**-$5,540**

CHAPTER V

CONCLUSIONS

Cucumber and habanero pepper crops responded similarly to pollinator-attracting companion plantings in regard to fruit yield and quality (Table 2). Fruit quality measures, as measured by cull percentages and mean fruit masses, were not affected by the presence of pollinator-attracting companion plantings near cucumber and habanero pepper plots (Table 2). Annual pollinator-attracting companion plantings were more effective at enhancing yield of adjacent crops species than perennial pollinator-attracting companion species under the conditions and time frames tested (Table 2). The lack of differences in several yield measures between perennial companion plant plots and control plots, despite the allocation of one in three or one in five rows to the perennial plantings, suggests that there is a potential for yields in perennial companion plots to surpass those of control plots if the effective distance between rows occupied by perennial plantings can be increased. The apparent seasonality of responses to pollinator-attracting companion plantings suggests that it may be feasible to improve yield responses even further by refining the fit of annual and perennial companion plant bloom cycles to maximize synchronization of flowering with that of targeted crops species. Our results also suggest a time lag for perennial plants to reach maximum effectiveness (Table 2). Benefits associated with the additional pollination and ecosystem services resulting from the year-round presence of the perennial plantings on other crops in adjacent fields during the year or long-term pollinator populations were not determined in this study, but also represent potential tangible benefits for using perennial pollinator-attracting companion plantings. Haaland et al. (2011) suggested that further research was needed to determine if crop yield could be reliably increased by the addition of pollinator-attracting plants near small-scale vegetable crops. The goal of the current study was to continue to investigate this problem by incorporating a cross disciplinary approach that brought horticulturists, economists, and pollinator scientists together to approach the project from different perspectives.

Results point to advantages and disadvantages of each pollinator-attracting companion planting treatment, with the best option likely being dependent on the circumstances of each particular grower. A main advantage of the annual companion treatment would be that the treatment is installed each crop cycle, which although costly to repeat each harvest, minimized potential damage to the annual companion treatment over time, as could potentially occur for the perennial plants during time periods between crops. For instance, winter damage could be an issue for perennial plants, but likely would not be an issue for the annuals plants. Replanting annual plants during each crop cycle would facilitate rapid changes in the species used, permitting a more crop-specific targeted companion planting if the crops species is changed or the same crop is planted in a different season of the year. Perennial plantings would lack this flexibility. Perennial treatments may potentially have certain plant species die out over time and need to be replaced periodically, incurring additional costs. Perennial plantings would also be at risk of being lost during non-cropping times, including periods with severe winter temperatures or flooding. Furthermore, irrigation lines will need to be changed periodically with perennial]companion plantings, which incurs additional costs, whereas annual plantings would use shared irrigation lines with the crop plants. The main advantage of the perennial companion plantings is they are installed year-round, not only saving cost of replanting the treatment each year as is required with the annual treatments, but in addition, perennial pollinator plantings provide year-round resources and habitat for pollinators and potentially attracting pollinators to nearby non-target adjacent crops. Although not studied in the current experiments, it is possible that perennial pollinator-attracting plantings might also serve as reservoirs of beneficial insects that could aid biological control of pests. This might be future avenue of research.

Further optimizing the blooming times of companion plants with a desired crop could potentially change yield estimates. Likewise, pairing companion plants that attract optimal pollinators for a given crop could potentially further enhance the effectiveness of the companion plantings. Our sampling indicated a high degree of variation among pollinator-attracting plants and the pollinators that each species attracted (Table 6). Determining the effective pollinators for a specific crop is critical as there is a wide assortment of effectiveness of pollinator types for crops (Haaland et al., 2011; Quinn et al., 2017). For example, wild pollinators have exhibited higher rates of pollination in many crop pollination studies compared to reared honeybees (Artz and Nault, 2011; Gajc-Wolska et al., 2011; Holzschuh et al., 2012; Garibaldi et al., 2013; Blaauw and Isaacs, 2014; Quinn et al., 2017). Further considerations would include testing additional plant species within each treatment to better determine the companion planting species that are best suited to targeted crops and respective pollinators and to determine the longevity of each particular plant species and infrastructure of perennial planting treatments across crop cycles. The species selected in the proof-of-concept experiments (Chapter 2) as pollinator attractants in both annual and perennial companion plants contained a range of plant heights from a few centimeters tall to those that were up to about 1.5 m tall. It would be useful to know if a mix of heights of pollinator-attracting species is needed or if plant height matters at all. Low growing companion plants may be advantageous if taller crops are used so that there would be less chance of interference with mechanical harvesters.

With regard to frequency and diversity of pollinator attraction, perennial pollinator-attracting treatments, after one year of establishment, performed at the same level as annual pollinator-attracting treatments in attracting pollinators, regardless of type, to cucumber flowers, and honey bees to habanero flowers (Table 6). In addition, the perennial planting treatment after one year of establishment was statistically better (*P* < 0.05) at attracting pollinators, regardless of pollinator type, to companion planting flowers (Table 6). Future recommendations would include adding additional methods for sampling pollinators, such as sticky traps and netting, in addition to observations. A few potential benefits of increasing the types of sampling methods used would include investigation of nocturnal pollinator visitation and pollinators that are difficult to observe in field conditions. Likewise, additional sampling methods would also allow for better curation and more specific taxonomic identification of pollinators, as well as potential assessment of pollinator nutrition impacts of the plantings. In addition, preliminary pollinator sampling is recommended for future investigation of pollinator-attracting companion plantings to more accurately determine preexisting pollinator populations in a particular area where crops are to be planted, and subsequently obtain more precise data on the impact of companion plantings on pollinator populations, particularly over time.

Break-even economic analysis for annual plantings indicated breakeven points of $1.46 and $5.03 for cucumbers and peppers, respectively, with increasing profits occurring at higher price points (Table 9). Our break-even economic analysis of the addition of pollinator-attracting companion plant treatments (Chapter 4) found that perennial pollinator companion planting treatment projected effectiveness to a distance of three and four adjacent rows resulted in lower price per pound breakeven points for cucumber, at $0.91 and $1.19, respectively. This would lead to a shorter duration of return on investment if future studies determine that the effectiveness of perennial pollinator attractants is greater than two adjacent rows. However, a recent study reported that the effectiveness of pollinator-attracting plantings strips was greatest in the row furthest from the pollinator-attracting strips (Quinn et al., 2017), suggesting that there is a more complex pattern of pollinator distribution surrounding pollinator-attracting plantings.

In conclusion, an economic analysis of data from our proof-of-concept experiments (Chapter 2) suggests that growers interested in the addition of pollinator-attracting companion plantings should utilize annual pollinator attractants, as they provide an immediate return on investment with a lower risk of failure due to being re-planted each crop cycle and from having greater flexibility relative to crop selection. However, further research is recommended on the effective distance of perennial plantings in attracting pollinators as their full potential and impact on pollinators has not yet been determined. Once economic values for ecosystem services are more effectively and efficiently obtained (Hanley et al., 2015), the resulting economic analyses will more accurately encompass the entire economic value of each pollinator-attracting cropping system. Although difficult to quantify, ecosystem services of perennial pollinator plantings were likely undervalued in our assessment model.

Overall the experiments presented here provide a proof-of-concept for the enhancement of yield of cucumber and habanero peppers using interplanted annual and perennial pollinator-attracting plants, and suggest a future potential for benefits of the use of adjacent plantings of perennial companion plantings. Increased frequency of visitation and diversity of pollinators attracted were documented for both crops and pollinator-attracting plants. Finally, economic analysis indicated reasonable breakeven prices for both crops with annual pollinator-attracting plantings and the potential for development of economically viable uses of perennial companion plantings.

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