Measuring factors affecting honey bee attraction to soybeans using nectar and bioacoustics monitoring

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

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2022

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Abstract

Soybean (*Glycine max*) is an important agricultural crop around the world, and previous studies suggest that honey bees (*Apis mellifera*) can be a component for optimizing soybean production through pollination. Determining when bees are present in soybean fields is critical for assessing bee pollination activity and for identifying periods when bees are absent so that bee-toxic pesticides may be applied. There are currently several methods for detecting pollinator presence and activity, but these existing methods have substantial limitations, including the bias of pan trappings against large bees and the limited duration of observation possible using manual netting or observation. The challenges of bee detection prevent growers from fully implementing honey bee pollination into their decision-making within integrated pest and pollinator management (IPPM) frameworks. This research consists of two studies aimed at determining the effects of soybean varietal differences and environmental conditions on the attractiveness of soybean blooms to foraging honey bees, with the goal of assessing honey bee activity in soybean fields and developing better methodologies for detecting and predicting honey bee activity.

The first study aimed to develop a new method for detecting honey bees in soybean fields using bioacoustics monitoring. Microphones were placed in soybean fields to record the audible wingbeats of foraging honey bees. Foraging activity was then identified using the wingbeat frequency of honey bees (234±13.9 Hz) through a combination of algorithmic and manual approaches. A total of 243 bees were detected over ten days of recording in four soybean fields. Bee activity was significantly greater in blooming fields than in non-blooming fields. Temperature had no significant effect on bee activity, but bee activity differed significantly between soybean varieties, suggesting that soybean attractiveness to honey bees is heavily dependent on varietal characteristics. Refinement of bioacoustics methods, particularly through incorporation of machine learning, could provide a practical tool for measuring activity of honey bees and other flying insects in soybeans as well as other crops and ecosystems.

The second study assessed the effects of day-night temperature cycles on soybean flower characteristics related to honey bee attractiveness. Using controlled lab experiments, flower and nectar production of five soybean varieties were monitored under four day-night temperature regimens representative of summer weather patterns in Ohio. Flower opening differed significantly between soybean varieties, temperature regimens, and hours after onset of the daytime phase. Flower opening generally increased with time and temperature. There were no significant differences in nectar sugar concentrations, but nectar volume differed significantly between soybean varieties and temperature regimens. Nectar volume did not differ significantly between hours after onset of the daytime phase.

Acknowledgments

I would like to thank my advisor for all his encouragement, academic support, and computer programming expertise over these two years of research and study. I would also like to thank Chia Hua-Lin for providing invaluable information about soybean biology and varieties, Sreelakshmi Suresh for developing the first iteration of our lab's protocol for measuring soybean nectar, Dylan Ricke and Ellis Meyer for helping to keep the soybeans watered and happy, Mike Sword for mapping the soybean fields of Apple Creek, and Jim Hacker for setting up the growth chamber and having such incredible patience with the technical difficulties of the new research building. Finally, I would like to give a warm thank you to Jihan Mourabiti, Grace Sward, and Valerie Anderson for their moral support when things got difficult. This wouldn't have been possible without you.

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Chapter 1. Introduction

Honey Bee Pollination and Soybean Production

Soybean (*Glycine max*) is a growing agricultural crop around the world and has become the number one agricultural export of the United States. In 2020, 17.6% of agricultural export value in the United States was attributed to soybeans (USDA FAS 2021). The United States exported $25.7 billion worth of soybeans grown for oil, animal feed, industrial products, and human consumption (USDA FAS 2021), an increase of $7.0 billion from the previous year (USDA FAS 2020). In 2021, soybeans covered 87.6 million acres of agricultural land in the United States, an increase of 5% from the previous year (NASS, USDA 2021). Soybeans are also a major crop in Ohio. Of the 9.1 million acres of principal crops planted in Ohio during 2021, 4.8 million acres (52%) were soybeans. This accounted for 5% of the nation’s annual soybean production (NASS, USDA 2021).

The growing soybean industry is faced with the continued need to optimize resource input and maximize yield output, and many studies suggest that honey bees (*Apis mellifera*) can be a component for optimizing soybean production through pollination (Table 1). Soybeans are self-fertile angiosperms that often exhibit cleistogamy, meaning that self-fertilization occurs within closed flowers (Benitez et al 2010). Because of this, early publications purported that soybeans are not attractive pollinator plants and are rarely visited by honey bees (Lent 1934, Milum 1940). However, more recent studies have shown that honey bees frequently forage in soybean fields, and cross-pollination by pollinators contributes additional benefits to soybean fruiting. Soybean yield is positively correlated with honey bee visitation, with yield increases ranging from 5.7% to 81% across studies, likely due to differences in experimental methods, soybean varieties, and variables such as time and location. (Blettler et al 2018, Chiari et al 2005a, Erickson 1975a, Erickson et al 1978, Esquivel et al 2021, Issa et al 1984, Jaycox 1970, Juliano 1976, Kettle and Taylor 1979, Levenson et al 2022, Milfont et al 2013, Monasterolo et al 2015, Santos et al 1993, Toledo et al 2011, Vila 1992). Chiari et al (2005a) demonstrated that pollination by honey bees and pollination by wild pollinators both increased soybean yield. Milfont et al (2013) demonstrated a yield increase of 11% when open-pollinated soybeans were supplemented with nearby honey bee colonies. Soybean yield also may benefit from the presence of nearby pollinator habitat (Levenson et al 2022). Together, these studies suggest that optimal soybean production can be achieved through the combined pollination activity of wild pollinators and managed honey bees.

Publication	Cultivar/Variety	Comparison		Results
Milum 1940	n/a	Caged soybeans	Uncaged soybeans	No effect
Jaycox 1970	Clark	Caged soybeans	Caged soybeans with bees	0.69% hybridization with bees, none without bees
		Caged soybeans	Uncaged soybeans	Unspecified yield increase; 0.21-0.47% hybridization in uncaged soybeans, none in caged
	n/a	Caged soybeans with honey bees	Uncaged soybeans with colonies near field	15.5% hybridization with cages, 11.6% hybridization without cages
Erickson 1975a	Chippewa 64	Caged soybeans	Caged soybeans with honey bees	No effect
		Caged soybeans, insecticide-treated	Caged soybeans with honey bees	No effect
	Corsoy	Caged soybeans	Caged soybeans with honey bees	13.8% yield increase
		Caged soybeans, insecticide-treated	Caged soybeans with honey bees	14.8% yield increase
	Hark	Caged soybeans	Caged soybeans with honey bees	7% yield increase (non-significant)
		Caged soybeans, insecticide-treated	Caged soybeans with honey bees	5.7% yield increase (non-significant)
		Caged soybeans	Caged soybeans with honey bees	16.4% yield increase
		Caged soybeans, insecticide-treated	Caged soybeans with honey bees	11.8% yield increase
Juliano 1976	Santa Rosa	Caged soybeans	Uncaged soybeans	39.58% yield increase, 37.95% pod increase, 40.13% pod weight increase
Abrams et al 1978	Bonus, Calland, & Cutler 71	Uncaged soybeans, insecticide-treated	Uncaged soybeans with colony in field	No effect on yield, up to 19.55% hybridization increase
Erickson et al 1978	Pickett	Caged soybeans	Caged soybeans with honey bees	21.6% yield increase, 20.4% pod increase, 18.6% seed abortion decrease
Kettle & Taylor 1979	Forrest	Caged soybeans	Caged soybeans with honey bees	20% yield increase
Sheppard et al 1979	Amsoy & Williams	Uncaged soybeans with colonies near field		No correlation between yield and proximity to apiary

Table 1. Effects of pollination on soybean production across previous studies.

Publication	Cultivar/Variety	Comparison		Results
Pinzauti & Frediani 1980	Hei-iee-jia & Grangeneuve	Caged soybeans	Uncaged soybeans with colony near field	pods/plant increase, seeds/plant increase, seeds/pod increase
Hallman & Edwards 1981	Crawford, Essex, & Forrest	Caged soybeans	Caged soybeans with honey bees	No effect
		Caged soybeans	Uncaged soybeans	No effect
Issa et al 1984	IAC-3	Caged soybeans	Uncaged soybeans	9% yield increase (non-significant), more pods with 2 or 3 grains
	IAC-5115	Caged soybeans	Uncaged soybeans	81% yield increase, more pods with 2 or 3 grains
Chiang and Kiang 1987	Cayuga, Evans, & Mandarin	Uncaged soybeans		Hybridization positively correlated with number of honey bees observed
Vila 1992	Primavera	Caged soybeans	Caged soybeans with honey bees	Unspecified seed weight increase
		Uncaged soybeans	Caged soybeans with honey bees	No effect
Sim & Choi 1993	Dankyeongkong	Caged soybeans	Caged soybeans with honey bees	5.6% fruiting rate increase, 60% hybridization increase
	Danyeobkong	Caged soybeans	Caged soybeans with honey bees	15% hybridization increase
	Hwangkeumkong	Caged soybeans	Caged soybeans with honey bees	15% hybridization increase
	Milyangkong	Caged soybeans	Caged soybeans with honey bees	12% hybridization increase
	Muhankong	Caged soybeans	Caged soybeans with honey bees	11% hybridization increase
	Paldalkong	Caged soybeans	Caged soybeans with honey bees	7.1% fruiting rate increase, 36% hybridization increase
Moreti et al 1998	IAC-14	Caged soybeans	Uncaged soybeans	15.66% seeds/pod increase (non-significant), 58.58% pod increase, 82.31% seed increase, more pods with 3 or 4 seeds
Chiari et al 2005a	BRS-133	Uncaged soybeans	Caged soybeans with honey bees	No effect
		Caged soybeans	Caged soybeans with honey bees	50.64% yield increase, 61.38% pod increase
		Caged soybeans	Uncaged soybeans	57.73% yield increase
Chiari et al 2005b	BRS-133	Caged soybeans	Caged soybeans with honey bees	30.25% flower abortion decrease
		Caged soybeans	Uncaged soybeans	28.96% flower abortion decrease

Table 1 Continued (1)

Publication	Cultivar/Variety	Comparison		Results
Toledo et al 2011	Mon Soy 3329	Caged soybeans	Uncaged soybeans	43.68% seed weight/plant increase, 50.42% seed weight/grain increase, more pods with 1 seed, less pods with 3 seeds
Milfont et al 2013	BRS Carnaúba	Caged soybeans	Uncaged soybeans	6.34% yield increase
		Caged soybeans	Uncaged soybeans with colonies in field	18.09% yield increase, pod increase, more pods with 1 or 3 seeds
		Uncaged soybeans	Uncaged soybeans with colonies in field	11.04% yield increase, more pods with 3 seeds
Santos et al 2013	A6411RG	Caged soybeans	Uncaged soybeans 200 m from apiary	5% yield increase (non-significant)
		Caged soybeans	Uncaged soybeans 500 m from apiary	25% yield increase
Tchuenguem and Dounia 2014	n/a	Caged soybeans	Uncaged soybeans	5.86% fruiting rate increase, 31.29% seeds/pod increase, 22.85% normal seed increase
		Caged soybeans	Soybeans pollinated exclusively by *Apis mellifera adansonii*	13.06% fruiting rate increase, 36.30% seeds/pod increase, 30.93% normal seed increase
BeaudelaineKengni et al 2015	n/a	Caged soybeans	Uncaged soybeans with colonies near field, inoculated with *Bradyrhizobium*	32.16% fruiting rate increase, 32.87% seeds/pod increase, 73.26% normal seed increase
		Caged soybeans	Soybeans pollinated exclusively by *Apis mellifera adansonii*	35.87% fruiting rate increase, 73.09% seeds/pod increase, 31.1% normal seed increase
Monasterolo et al 2015	ALM 3830	Caged soybeans	Caged soybeans	20% flower abortion decrease, seed weight increase, pod weight increase, seed abortion decrease
Blettler et al 2018	Nidera A 4990 RG	Caged soybeans	Uncaged soybeans with colonies in field	18% yield increase, 12% seed per unit area increase, 3.5% seed weight decrease
		Caged soybeans	Uncaged soybeans with colonies in field	3.5% seed weight decrease

Table 1 Continued (2)

Publication	Cultivar/Variety	Comparison		Results
Cunningham-Minnick et al 2019	Steyer® 835BCU77	Caged soybeans	Uncaged soybeans	23.1% seed increase, 24.4% pod increase, seed mass decrease, less pods with 2 seeds, more pods with 3 or 4 seeds
Blanco et al 2020	Pioneer® P29A25X	Caged soybeans	Uncaged soybeans with colonies near field	No effect
Levenson et al 2022	n/a	Uncaged soybeans	Uncaged soybeans near pollinator habitat	6.25% seed weight increase, 2.50% seeds/pod decrease

Table 1 Continued (3)

Although direct comparisons cannot be made across previous studies due to differences in experimental methods, soybean varieties, and environmental variables, the consensus is that pollination by honey bees and wild pollinators increases soybean yield. Early estimates of the economic benefit that honey bee pollination provides to soybeans were $4.94 to $7.41 per hectare (Reichelderfer and Caron 1979). More recent estimates suggest that honey bee pollination could be contributing $59.70 to $110.50 of profits per hectare, representing a contribution of $6.1-17.4 billion to the global economy (Milfont et al 2013).

Honey bee pollination can have multiple effects on soybean fruiting that may lead to increased yields. Pollination has been shown to increase hybridization by up to 60%, which may be useful for production of more robust soybean varieties (Abrams et al 1978, Chiang and Kiang 1987, Cutler 1934, Jaycox 1970, Sim and Choi 1993). Several studies demonstrated an increase in the number of pods by 20% to 61% (Chiari et al 2005a, Cunningham-Minnick et al 2019, Erickson et al 1978, Juliano 1976, Moreti et al 1998). Moreti et al (1998) and Cunningham-Minnick et al (2019) found an 82% and 23% increase in the number of seeds, respectively, while Blettler et al (2018) found a 12% increase in the number of seeds produced per unit area. Juliano (1976) and Toledo et al (2011) both demonstrated an increase in pod weight. Tchuenguem and Dounia (2014) calculated a 5.9% increase in fruiting rate and found a 23% increase in healthy seeds for open-pollinated plants. They also reported a 13% increase in fruiting rate and a 31% increase in healthy seeds for plants pollinated exclusively by the African honey bee (*Apis* *mellifera adansonii*). BeaudelaineKengni et al (2015) report similar findings, with a 32% increase in fruiting rate and a 73% increase in healthy seeds in open-pollinated plants, and a 36% increase in fruiting rate and a 31% increase in healthy seeds in plants pollinated exclusively by *A. mellifera adansonii*. Sim and Choi (1993) also found a significant fruiting rate increase of 5.6% and 7.1% in two soybean varieties, Dankyeongkong and Paldalkong, respectively. In a study by Erickson et al (1978), empty pods due to seed abortion were 19% lower in plants pollinated by bees, and yield was positively correlated with proximity to an apiary. Monasterolo et al (2015) found a decrease in both seed abortion and flower abortion, and Chiari et al (2005b) found that flower abortion was reduced by 29-30%. BeaudelaineKengni et al (2015) reported that soybean yield increased when pollinated by *A. mellifera adansonii* or inoculated with a *Bradyrhizobium* microsymbiont in the soil, and together there was a synergistic effect on yield, possibly because the plants inoculated with *Bradyrhizobium* produced more flowers for bees to pollinate.

Honey bee pollination also results in an increase in seeds per pod across studies. Tchuenguem and Dounia (2014) and BeaudelaineKengni et al (2015) found a 31% and 33% increase in seeds per pod, respectively, in open-pollinated plants, as well as a 36% and 73% increase in seeds per pod, respectively, in plants pollinated exclusively by *A. mellifera adansonii*. Several studies found that honey bee pollination resulted in more pods producing 3 or 4 seeds compared to 1 or 2 seeds (Moreti et al 1998, Milfont et al 2013), and Issa et al (1984) found that pollination caused more pods to produce 2 or 3 seeds than 0 or 1 seeds regardless of soybean variety. Pinzauti & Frediani (1980) found significant increases in pods per plant, seeds per plant, and seeds per pod with honey bee pollination.

Soybean Bloom Characteristics and Attractiveness to Honey Bees

Some of the earliest studies on the relationship between honey bee pollination and soybean yield reported that yield benefits differ by variety, likely due to variation in bloom characteristics and their resulting attractiveness to honey bees (Erickson 1975a, Issa et al 1984). Differences between soybean varieties include flower color (ranging from white to violet), growth habit (determinate or indeterminate), maturity group (time from planting to maturity), frequency of cleistogamy, flower size, flower fragrance, number of flowers, nectar volume, and nectar sugar concentration (Benitez et al 2010, Erickson 1975b, Stowe and Vann 2022). In addition to phenotypic variation across soybean varieties, soybeans produce nectar of varying quality and quantity depending on environmental factors, including day-night temperature cycles (Robacker et al 1983), time of day (Blettler et al 2016, Severson and Erickson 1984), soil macronutrients (Robacker et al 1983), and soil microbiota (BeaudelaineKengni et al 2015). Flower production, flower opening, and nectar production increase with temperature until reaching a critical point, after which attractive characteristics remain unchanged or begin to decrease (Robacker et al 1983). However, the effect of temperature may be inconsistent, as Severson and Erickson (1984) found little to no impact of temperature on nectar production. Cleistogamy in some varieties increases at colder temperatures, although the temperature range that induces this increase differs across varieties (Erickson 1975b). High levels of nitrogen in the soil increase flower production and nectar secretion, while high levels of phosphorus in the soil cause a reduction in these same variables (Robacker et al 1983).

Variations in soybean bloom characteristics are correlated with attractiveness to honey bees. Sugar concentrations above 25% and low rates of cleistogamy are generally most attractive to honey bees (Erickson 1975b), whereas flower color has little impact on honey bee foraging (Chiang and Kiang 1987, Jaycox 1970, Mason 1979, Severson and Erickson 1984). Attractiveness also changes over time, with most studies observing peak honey bee activity in soybeans around midday (Toledo et al 2011**,** BeaudelaineKengni et al 2015, Blettler et al 2016, Chiari et al 2005a, Issa et al 1984, Jaycox 1970, Santos et al 1993). This is likely due to changes in floral nectar quality and quantity throughout the day, with sugar concentration increasing and nectar volume decreasing as the day progresses (Severson and Erickson 1984). Honey bee foraging also peaks in the middle of the month-long blooming period when flower production is at its maximum (Blettler et al 2016).

Dynamic variables such as time of day and day-night temperature cycles influence both honey bee foraging activity and the attractiveness of certain soybean bloom characteristics to honey bees. Therefore, these variables have the potential to be used as predictors of honey bee activity. Better characterization of these factors would allow soybean varieties to be bred for pollinator attractiveness to optimize yields, allow soybean growers to better implement honey bee pollination into their integrated pest and pollinator management (IPPM) frameworks, and allow pesticide applicators to make more informed decisions about use of pesticides in relation to soybean attractiveness and honey bee activity to protect honey bee health and pollination services.

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Chapter 2. Bioacoustics monitoring as a novel method to detect foraging honey bees

Introduction

It has been demonstrated across multiple studies that honey bees (*Apis mellifera*) frequently forage in soybeans (*Glycine max*) and that pollination by honey bees can benefit soybean yields (Blettler et al 2018, Chiari et al 2005, Erickson 1975a, Erickson et al 1978, Esquivel et al 2021, Issa et al 1984, Jaycox 1970, Juliano 1976, Kettle and Taylor 1979, Levenson et al 2022, Milfont et al 2013, Monasterolo et al 2015, Santos et al 1993, Toledo et al 2011, Vila 1992). Milfont et al (2013) estimated that honey bee pollination could be contributing $59.70 to $110.50 of soybean yield per hectare, representing a potential contribution of $6.1-17.4 billion to the global economy from bee pollination of soybean. However, because soybeans are capable of self-pollination – and possibly as a consequence of early studies purporting that honey bees are poor pollinators of soybeans (Lent 1934, Milum 1940) – honey bees are often disregarded as a factor of production, which opens the door for potentially bee-toxic insecticides to be applied during soybean bloom (Jaycox 1970, Milfont et al 2013).

Many soybean insecticides applied during bloom are highly toxic to bees and carry cautionary language to protect bees on the pesticide label. While there have been reports that pyrethroid insecticides repel bees from areas where they have been applied (Rieth and Levin 1988), the pyrethroids cypermethrin and lambda-cyhalothrin were not found to deter honey bee foraging when applied to blooming soybeans (Fagúndez et al 2016). Pesticides are picked up by foraging honey bees and cause lethal and sub-lethal effects in the colony, creating a major risk for foraging colonies and reducing long-term viability of pollination and its associated benefits (Reichelderfer and Caron 1979, Santos et al 2013). This both raises legal concerns and prevents growers from fully implementing honey bee pollination into integrated pest and pollinator management (IPPM) frameworks. The conditions for pesticide application in soybeans must be reassessed within the context of honey bee activity to support the long-term sustainability of soybean production.

In order to assess how environmental variables and soybean varieties can predict honey bee foraging in soybeans, we must first be able to detect foraging honey bees across time. There are several existing methods for detecting pollinator activity, mainly through the use of pan traps, visual observation, and manual collection (Portman et al 2020). Pan traps are brightly colored bowls filled with soapy water that are used as a passive sampling method. Pollinators are attracted to the bright colors, then become trapped in the liquid. Manual collection methods include targeted netting and sweep netting, and visual observation provides a nonlethal method of insect detection. Pan traps, visual observation, and netting have all previously been used to assess honey bee activity in soybean fields, with visual observation being the most common method (Table 2).

Publication	Methods Used
Abrams et al 1978	visual observation
Sheppard et al 1979	visual observation
Issa et al 1984	visual observation
Chiang and Kiang 1987	visual observation
Toledo et al 2011	visual observation
Milfont et al 2013	sweep netting
Santos et al 2013	visual observation
Tchuenguem and Dounia 2014	visual observation and targeted netting
BeaudelaineKengni et al 2015	visual observation and targeted netting
Blettler et al 2018	sweep netting
Cunningham-Minnick et al 2019	pan traps
St. Clair et al 2020	pan traps
Levenson et al 2022	visual observation and targeted netting

Table 2. Summary of sampling methods used in previous studies of bee activity in soybeans.

Existing methods for bee detection have drawbacks that make them ineffective for accurate large-scale detection of honey bees in crops. Pan traps tend to favor smaller bees, such as those belonging to the family *Halictidae*, over larger bees such as honey bees (Portman et al 2020). Visual observation and netting techniques are time and labor intensive, and these methods are more susceptible to collector bias (Portman et al 2020, Westphal et al 2008). In addition, detection methods such as trapping are fatal to the target organisms. A novel method is necessary for accurate bee detection, particularly in large cropping systems like soybean.

Bioacoustics is a branch of science that focuses on sound production by living organisms, and bioacoustics monitoring is a relatively new method for detection and identification of species through audio recording and analysis. Deep learning approaches to bioacoustics monitoring are currently being developed for detection and identification of mosquitoes that vector malaria (Hassall et al 2021, Khalighifar et al 2022, Kim et al 2021, Kiskin et al 2020a, Kiskin et al 2020b, Vasconcelos et al 2019, Vasconcelos et al 2020). Bioacoustics monitoring has also been combined with machine learning to automate analysis of birdcall recordings and increase bird monitoring efficiency through the use of the deep neural network BirdNET (Kahl et al 2021, Toenies and Rich 2021, Wood et al 2021). A study by Zhang et al (2017) proposed the use of wingbeat spectrum imaging for insect identification using convolutional neural networks. Similar methods could be used for in-field detection and identification of any insect with a distinct and detectable wingbeat, and honey bees fulfill these criteria by producing an audible wingbeat frequency of 234±13.9 Hz (Clark et al 2017).

The goal of this study was to develop an effective and efficient bioacoustics method for detecting honey bee activity in soybean fields. To accomplish this, we made audio recordings in soybean fields around the blooming period and used a combination of automated and manual techniques to identify honey bee activity.

Methods

Study Area

This study was completed in four soybean fields near Apple Creek, Ohio (Figure 1). Fields A and B were planted with the soybean variety Synergy 9720, field C was planted with Synergy 9727, and field D was planted with Synergy 9723 (Table 3). Data was collected over ten days on July 21-24, July 26-28, and August 5-7, 2021, except for field B which yielded no data on August 7 due to a microphone malfunction. For this study, a field was considered to be in bloom during growth stages R2 and R3, and audio was recorded during stage R3. Fields A, B, and D were in bloom July 21-24 and July 26-28, while only field C was in bloom August 5-7. All four fields were located in predominantly agricultural areas and were surrounded by corn fields, wheat fields, alfalfa fields, additional soybean fields, deciduous forestland, and major and minor roads. An apiary with approximately ten colonies was located 25 meters east of field B, but the locations of other managed apiaries or feral colonies established in wooded areas were not known.

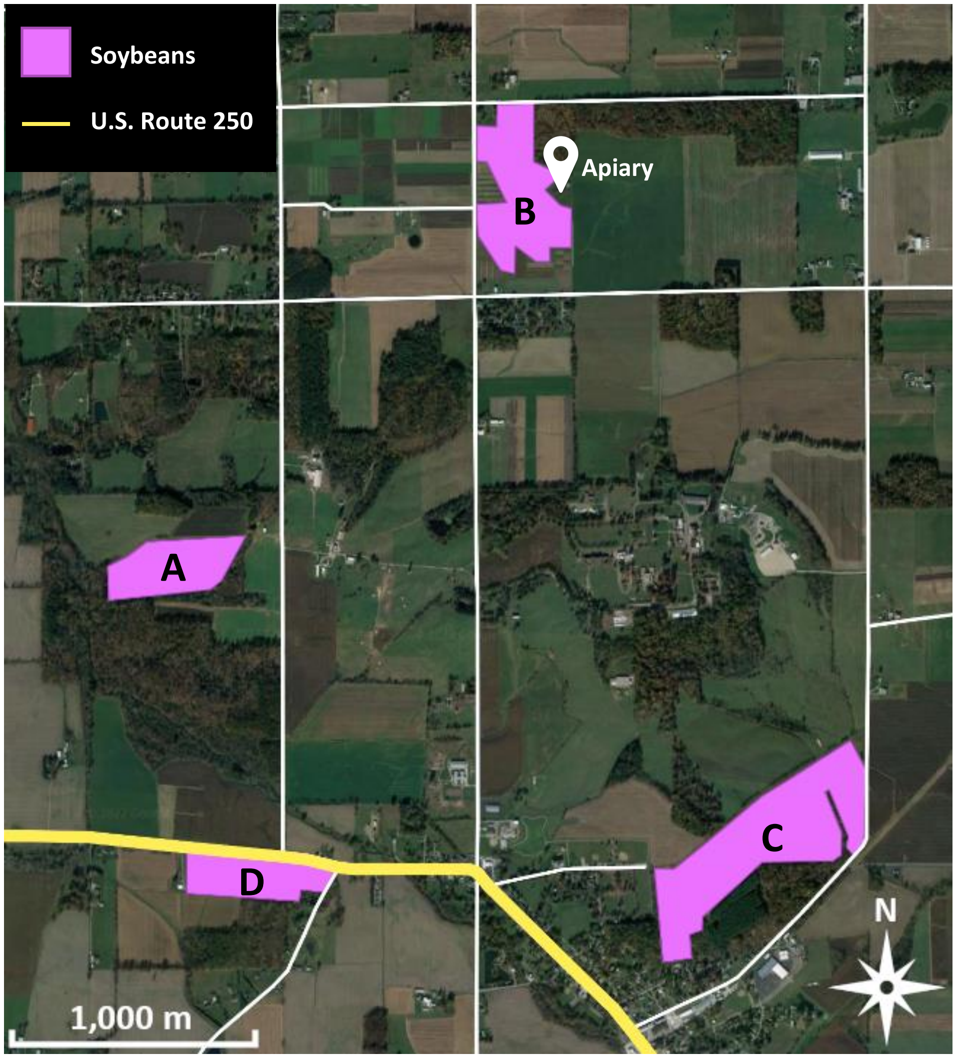

Figure 1. Map of study fields designated A through D. Modified from Google Earth Pro 7.3 (2022).

Field	Acreage	Variety	Maturity Group	Planting Date	Surroundings
A	29 acres	Synergy 9720	2.0	May 20	corn, soybeans, forestland
B	46 acres	Synergy 9720	2.0	May 21	alfalfa, corn, soybeans, forestland, minor roads, apiary
C	80 acres	Synergy 9727	2.7	June 15	corn, wheat, soybeans, forestland, minor roads
D	25 acres	Synergy 9723	2.3	May 25	corn, wheat, soybeans, forestland, minor roads, U.S. Route 250

Table 3. Characteristics of soybean fields within study area.

Data Collection

Audio recorders (Sony model ICD-PX370) were attached to 0.635 x 91.44 cm (0.25 x 36 inch) square wooden stakes and protected from wind and rain with high-density foam and 3D-printed white plastic rain covers (https://www.thingiverse.com/thing:5380356, Figure 2). The recorders were programmed to record audio at the highest microphone sensitivity setting and a bitrate of 48 kbps (mono). A recorder was placed in each field 35 meters from an easily accessible field edge. The recorders were left to continuously record environmental audio for three-day periods, then retrieved for battery replacement. The location of each recorder was marked with a colored flag on top of a stake to aid in retrieval. In blooming fields, the recorders were placed at the same height as the highest blooming nodes beneath the leaf canopy. If the field was not in bloom, the recorders were placed at the same height as the highest non-blooming nodes beneath the leaf canopy. Each recorders' radius of detection for bee wingbeats was approximately one meter.

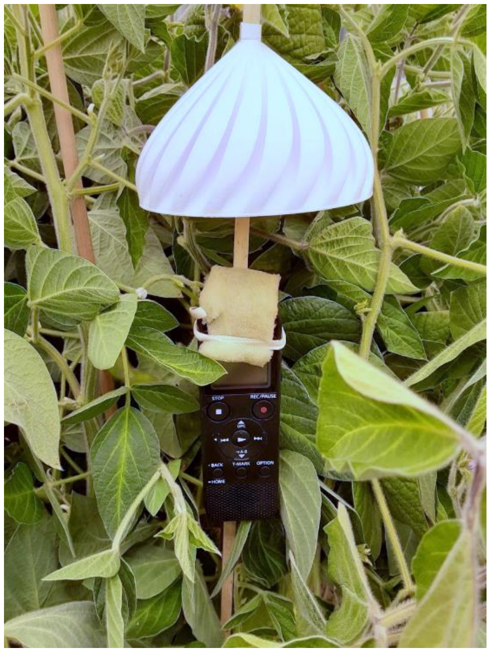

Figure 2. Audio recorder in soybeans.

Audio Processing

Audio files were downloaded from audio recorders in MP3 format, downsampled to 16kHz and converted to WAV files using FFMPEG (Tomar 2006), then split into one-hour segments using the AudioSegment module in the pydub library (v.0.25.1, Robert et al 2018) using Python (v.3.9.7). Hours of audio recorded before sunrise or after sunset were removed from analysis. Potential bee detections were identified based on a range of audio frequencies corresponding with the second harmonic of the honey bee wingbeat frequency (370-570 Hz) with a duration of 1 sec. and a threshold of 0.0001 using the "find_rois_cwt" function in the scikit-maad soundscape analysis package in Python (Ulloa et al 2021). Possible bee detections were output as a CSV file for manual audio assessment.

Manual Audio Assessment

Automated honey bee detections were loaded as labels overlayed on a spectrogram of one hour audio sequences and the source of each detected sound was manually identified using Audacity® (v.3.1.3, Audacity Team 2022). Audio assessment was completed by listening to the areas of interest and identifying the source of each detection event in a manually curated label file (Table 4). Visual assessment of spectrograms in the areas of interest augmented audio assessment (Figure 3). The spectrograms were adjusted to show recorded frequencies in the 100-600 Hz range, which included the target frequency for honey bee wingbeats (234±13.9 Hz) and its second harmonic (468±27.8 Hz). Gain was set to 35 dB and spectrograms were displayed using Mel scaling to more clearly distinguish target frequencies from background noise. A total of 403 hours of audio recordings were manually assessed.

Label	Description
bee	Wingbeats of honey bees. Other insect wingbeats were excluded.
insect	Wingbeats of all non-honey bee insects.
combine	Combines and other agricultural equipment.
goose	Canadian geese (*Branta canadensis*) calls.
human	Manual setup and takedown of microphones at the start and end of each recording increment.
traffic	Vehicles, excluding agricultural equipment. Ground traffic was not distinguished from air traffic.
other	All sounds not falling into one of the above categories. Includes wind, rain, and minor construction.

Table 4. Labels used to categorize audio detections.

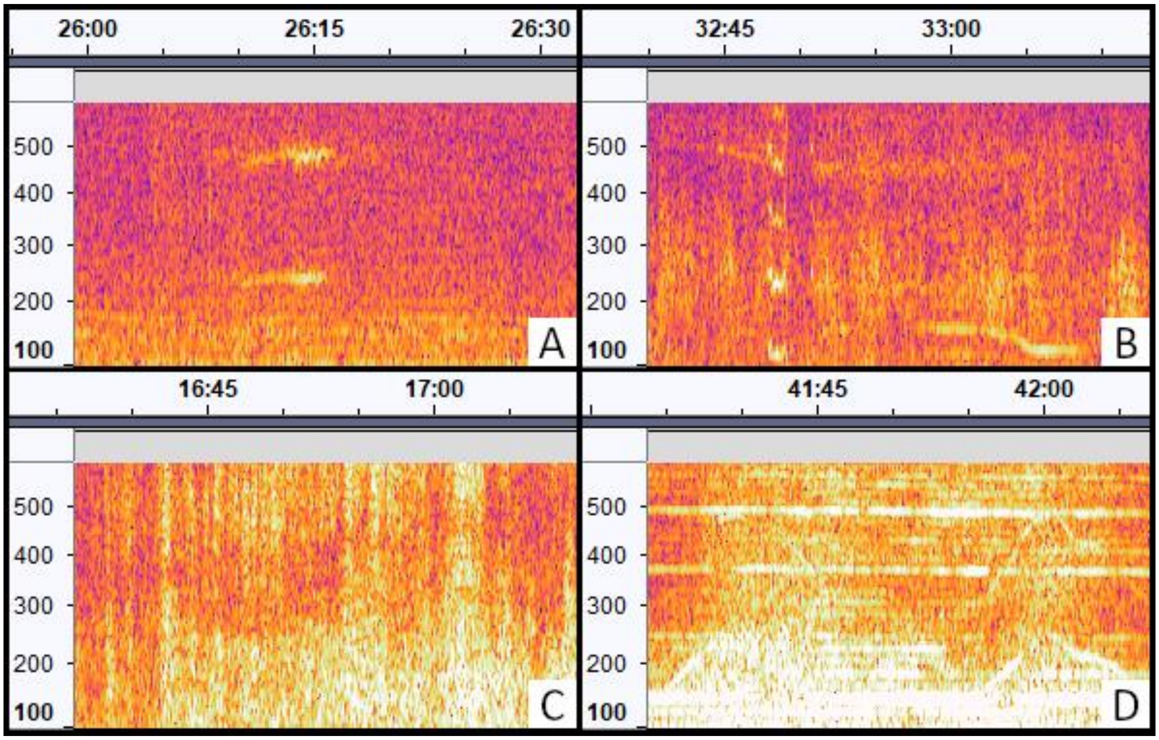

Figure 3. Spectrograms of potential bee activity. The x-axis shows time (mm:ss) and the y-axis shows frequency (Hz). Screenshots modified from Audacity® (Audacity Team 2022). **A)** Honey bee wingbeats (230-250 Hz) with second harmonic (460-500 Hz). **B)** Non-honey bee insect wingbeats (105-125 Hz) with second, third, and fourth harmonics. **C)** Air traffic. **D)** Agricultural equipment operating in a neighboring field.

Statistical Analysis

Due to non-normal distribution, a Kruskal-Wallis one-way analysis of variance and Dunn's test were used to determine significant differences in the number of bee detections between soybean varieties, between fields, and between blooming and non-blooming soybeans. Weather data for the ten recordings dates were obtained from the Ohio State University CFAES Weather System Wooster Station (OSU CFAES 2022), and the correlation between average daily temperature and the number of bee detections was determined using a linear regression analysis and Pearson's correlation coefficient.

All statistical analyses were completed using R statistical software (v.4.0.3, R Core Team 2022) and visualized with the ggplot2 package (Wickham 2016).

Results

The automated audio assessment identified a total of 11,638 potential bee detections over the ten days of recording (Table 5). Of those detections, 10,307 were produced by vehicles, 130 were produced by insects other than honey bees, and 243 were produced by honey bees. Most bee activity occurred between 10 AM and 5 PM Eastern Daylight Time, with the greatest activity occurring between 1 PM and 4 PM (Figure 4). Field A had the least amount of recorded bee activity with only 34 bee detections. Fields B and C yielded 52 and 45 bee detections, respectively. Field D yielded 112 bee detections, more than twice the bee activity of any other field. July 21 was the most active day for bees in all three fields blooming at that time (Figure 5). Bee activity was significantly greater in blooming fields than in non-blooming fields ($p = 0.001$, $N = 39$, $df = 1$). Bee activity also differed significantly between soybean varieties ($p = 0.004$, $N = 39$, $df = 2$), with less activity in variety 9720 than in varieties 9723 and 9727, and between fields ($p = 0.010$, $N = 39$, $df = 3$), with less bee activity in fields A and B than in fields C and D. There was no significant correlation between bee detections and average daily temperature ($p = 0.107$, $N = 24$, $df = 22$).

	Sound Source						
Field	bee	insect	farm machinery	goose	human	traffic	other
A	34	4	23	0	30	2354	116
B	45	13	28	0	19	2101	323
C	52	68	844	7	7	2177	202
D	112	45	109	18	13	2671	223
TOTAL	243	130	1004	25	69	9303	864

Table 5. Summary of detection events in each field, identified using the scikit-maad (Ulloa et al 2021) package in Python followed by manual assessment.

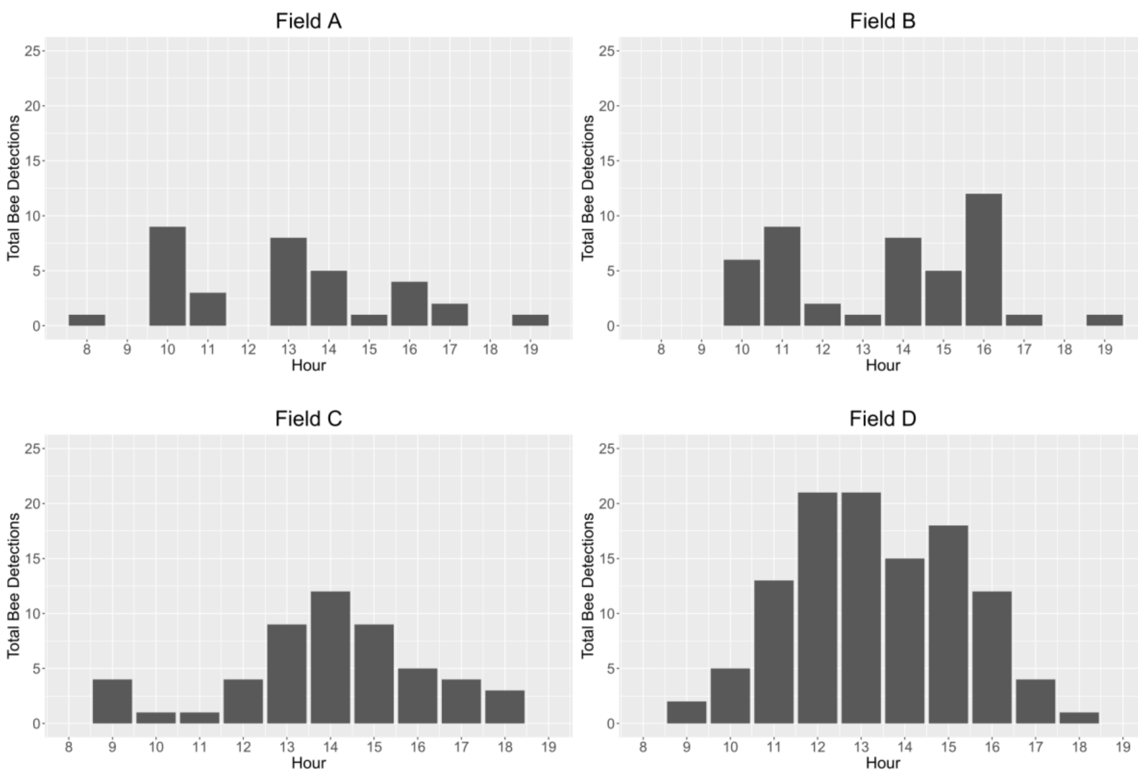

Figure 4. Bee detections per hour across the four study fields pooled over the 10 days of recording (July 21-24, July 26-28, and August 5-7, 2021).

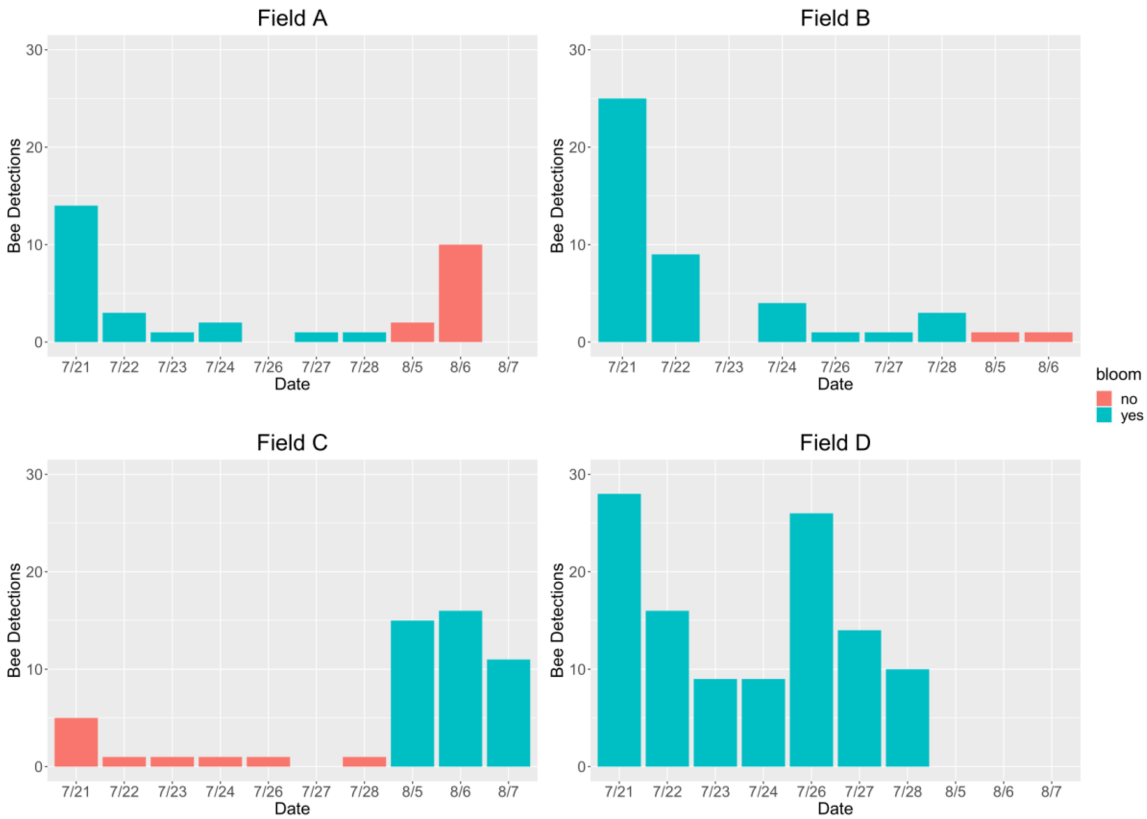

Figure 5. Daily bee detections in each field across all recording dates. No recording was made in field B on August 7.

Discussion

These results demonstrate that bioacoustics monitoring is a viable method for detecting honey bees and other insects in soybean fields. Comparison of detections during bloom and non-bloom showed that there was more bee activity in each field when it was blooming than when it was not blooming. There were significant differences in bee detections between soybean varieties, with less bee activity in both fields that contained variety 9720.

Interestingly, there was no significant correlation between bee detections and average daily temperature even though the findings in Chapter 3 indicate that average daily temperature has a significant effect on nectar volume and cleistogamy in these three soybean varieties. One possible explanation is that there is also more nectar production in other plants such as clovers on days with higher temperatures, so the increased nectar volume and reduced cleistogamy in soybeans causes no significant change in honey bee activity due to competing bloom.

It was noted that bee detections in field A exceeded the expected bee activity for non-blooming fields on August 6, with a total of 10 bee detections. Upon reassessment of the data, it was discovered that 7 of those 10 bee detections occurred in the span of 25 seconds and were likely produced by the same bee. If this is the case, only four bees were detected in field A on this date, which more closely aligns with the number of bee detections on other dates and in other fields during non-bloom. This was the only instance of an individual bee exceeding 3 detections, and most bees were detected only once.

The recorders picked up a wide array of non-bee activity such as traffic, birds, cicadas, and agricultural equipment. By targeting the second harmonic of honey bee wingbeats, the scikit-maad soundscape analysis package was able to exclude cicadas, most birds, and other general noises from detection output. Vehicular noise occupied a similar frequency range to honey bee wingbeats, but the spectrograms produced for vehicular audio were visually distinct from the spectrograms produced for honey bee wingbeats. Despite the high occurrence of traffic detections and the proximity of some fields to major and minor roads, bee activity was still detectable, with only 7 of the 243 manually assessed bee detections overlapping with traffic detections. The only noise source that confounded bee detection was the use of agricultural equipment near fields A and C during periods of July 21, field C during periods of July 22, fields C and D during periods of July 27, fields B and D during periods of July 28, and fields C and D during periods of August 5. Bees could not be detected when agricultural equipment was present due to the sustained duration and intensity of the noise overlapping with bee wingbeat frequencies. However, agricultural equipment was only present for one or two hours on a given day, and enough bees were detected on days when agricultural equipment was present to yield usable data.

This method also proved to be much more time and labor efficient than other methods. It took approximately 8 hours to set up and retrieve the microphones across all recording periods and 34 hours to manually assess the audio data. Altogether, a total of 42 hours was needed to collect and analyze 403 hours of audio recordings. This method also allowed data to be collected in four fields simultaneously, was nonlethal, and was not susceptible to observer bias. Refinement of this method using machine learning for bee detection could further reduce the amount of time and labor required for manual audio analysis.

These results provide a general picture of when honey bees are most active in soybean fields, and conclusions can be made about when to make pesticide applications. Bee activity greatly decreases after 5 PM in blooming fields, and bees are rarely present in non-blooming fields. Based on these data, pesticide applicators can minimize honey bee exposure to harmful insecticides by only spraying soybean fields when bees are not actively foraging a) before and after the blooming period or b) after 5 PM during the blooming period.

A novel methodology such as audio detection could be used to develop a better IPPM framework for soybean growers that takes honey bee activity into account. Tracking honey bee activity in soybeans across the hours of the day has already been proposed as a means for reducing honey bee exposure to harmful pesticides (Blettler et al 2016), but this has not been utilized at a commercial scale due to the practical challenges presented by current honey bee detection methods. Bioacoustics monitoring could provide an efficient, effective, and accessible method for determining when honey bees are present in blooming soybean fields, allowing pesticide applicators to better follow pesticide label guidelines and mitigate pesticide exposure. Research to refine this audio detection technology for future implementation in precision agriculture, including the use of advanced machine learning to assist with data analysis and interpretation, is ongoing.

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Chapter 3. Soybean bloom characteristics across day-night temperature cycles

Introduction

Soybean (*Glycine max*) is a major agricultural crop that has been selectively bred for the production of unique varieties for many decades, with at least 195 common varieties commercially available from 2013-2021 (Stark 2020). Soybean varieties differ in phenotypic characteristics such as flower color (white and violet), growth habit (determinate and indeterminate), maturity group (time from planting to maturity), frequency of cleistogamy, flower size, fragrance, number of blooms, nectar volume, and nectar sugar concentration (Benitez et al 2010, Erickson 1975b, Stowe and Vann 2022). Soybean varieties may also show differences in nectar production and cleistogamy depending on environmental factors such as temperature (Erickson 1975b, Robacker et al 1983), time (Blettler et al 2016, Severson and Erickson 1984), and soil constituents (BeaudelaineKengni et al 2015, Robacker et al 1983).

Soybeans are self-fertile and often exhibit cleistogamy, so pollination is not required for seed production (Benitez et al 2010). However, multiple studies show that soybean yield can benefit from pollination by honey bees (*Apis mellifera*) and wild pollinators (Blettler et al 2018, Chiari et al 2005, Erickson 1975a, Erickson et al 1978, Esquivel et al 2021, Issa et al 1984, Jaycox 1970, Juliano 1976, Kettle and Taylor 1979, Levenson et al 2022, Milfont et al 2013, Monasterolo et al 2015, Santos et al 1993, Toledo et al 2011, Vila 1992). The impact of pollinators on soybean production differs greatly between studies, likely as a result of varietal differences in bloom characteristics and their attractiveness to pollinators, as well as environmental conditions (Erickson 1975a, Issa et al 1984). Nectar sugar concentrations above 25%, high flower number, and low rates of cleistogamy have been shown to be the most important factors in determining attractiveness of soybean blooms to honey bees (Blettler et al 2016, Erickson 1975b). Jaycox (1970) found that the sugar concentration of soybean nectar collected by honey bees ranged from 28% to 60%, with peak foraging activity occurring when sugar concentrations were highest at the end of the blooming period. Jaycox speculated that nectar production was most attractive to honey bees when daytime temperatures were above 80°F, as these warmer temperatures were associated with greater honey production and an earlier start to flowering. Cleistogamy and flower deformities also decrease at higher temperatures in some varieties (Erickson 1975b, Thomas and Raper 1981).

With new soybean varieties constantly under development and appearing on the market, and most studies on floral characteristics having been performed in the 1970's and 1980's on varieties that are no longer available, it is important to investigate how bloom characteristics differ between currently available varieties and under different environmental conditions. The goal of this study was to measure the impact of day-night temperature cycles on three variables that have been shown to correlate with attractiveness to honey bees in soybeans: frequency of cleistogamy, nectar volume, and nectar sugar concentration. To accomplish this, the bloom characteristics of five soybean varieties were monitored under four day-night temperature regimens maintained in a growth chamber to determine the influence of day-night temperature cycles on bloom attractiveness.

Methods

Plant Material

Five soybean varieties grown in Ohio were used for this study: Synergy 9720 (maturity group 2.0), Synergy 9723 (maturity group 2.3), Synergy 9727 (maturity group 2.7), Dyna-Gro S37xS89 (maturity group 3.7), and AGI 9737AE (maturity group 3.7). All varieties exhibited an indeterminate growth habit. The 9720, 9723, and 9727 varieties produced violet flowers, whereas the S37xS89 and 9737AE varieties produced white flowers. All plants were maintained in the same walk-in plant growth chamber (Conviron model BDW80) (Figure 6). Temperature, humidity, and lighting were controlled and monitored throughout the entire study period.

Figure 6. Stage V5 soybeans in growth chamber.

One-gallon plastic nursery pots were filled with clay-loam soil collected from a field near Apple Creek, OH that had grown soybeans in the previous year and was presumably inoculated with *Bradyrhizobia* microsymbionts required for proper root nodule development and nitrogen uptake. Three pots were planted for each variety, and three seeds were planted in each pot. After reaching growth stage V2, the seedlings were thinned to one plant per pot. Young plants were maintained at a daytime temperature of 28°C, a nighttime temperature of 22°C, 70% humidity, and a 16-hour photoperiod. Plants were irrigated daily, unhealthy leaves were regularly trimmed, and weeds were regularly removed from the soil. After reaching stage V2, plants were fertilized weekly with a 12-8-16 NPK water-soluble fertilizer. After reaching stage V7, plants were vertically supported with wooden stakes. After all plants had reached stage R2, growth chamber conditions were cycled between four day-night temperature regimens representing summer weather scenarios in Ohio (Table 6). All temperature regimens were maintained with a 16-hour photoperiod and 70% humidity. The cycle of temperature regimens was repeated three times, and the order of the temperature regimens in each cycle was randomized.

Regimen	Daytime Temperature	Nighttime Temperature	Average Temperature
1	32°C (89.6°F)	20°C (68.0°F)	28°C (82.4°F)
2	28°C (82.4°F)	16°C (60.8°F)	24°C (75.2°F)
3	26°C (78.8°F)	14°C (57.2°F)	22°C (71.6°F)
4	25°C (77°F)	13°C (55.4°F)	21°C (69.8°F)

Table 6. Day-night temperature regimens representing summer weather scenarios.

Data Collection

To determine the optimal time of day to collect nectar, five open flowers were taken from a randomly chosen plant of each variety at 4 hours, 6 hours, and 8 hours after the onset of the daytime phase for each temperature regimen during the first cycle. A flower was considered open if the wing petals were exposed and the standard petal was poised backward (Figure 7). If there were not five or more open flowers on any of the plants of a variety, that variety was omitted from the hour's measurements. Flowers were allowed to rest for five minutes at room temperature prior to extraction to avoid potential discrepancies in liquid density. Nectar was then extracted from each flower nectary using a 0.5 µL microcapillary tube (Drummond Scientific). Volume was calculated from the height of the nectar in the microcapillary tube, and sugar concentration was measured using a digital light refractometer (Sper Scientific model 300003). A minimum of 0.15 µL nectar was needed to obtain a reliable sugar concentration using the refractometer, so nectar from flowers of the same plant was combined for a single measurement per plant. The total number of open and closed flowers on all plants was also measured at 4-, 6-, and 8-hour increments. Cleistogamy was then calculated as the proportion of closed flowers on each plant. All flowers were removed from all plants at the end of each day to ensure that only new flowers would be counted and harvested for nectar extraction and analysis.

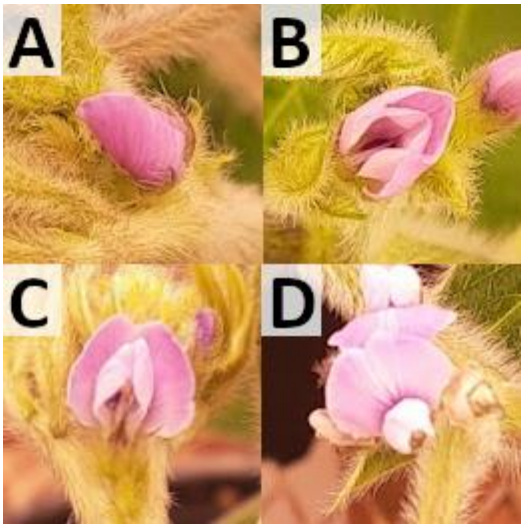

Figure 7. Soybean flowers at various stages of opening. For this study, only flowers at stage D were considered truly open. **A)** Completely closed flower. **B)** Partially opened flower. **C)** Flower with wings exposed but standard still poised forward. **D)** Fully open flower with wings exposed and standard poised backward.

After one complete cycle of the four day-night temperature regimens, the optimal nectar collection time was determined to be 6 hours after the onset of the daytime phase. Nectar was extracted and measured at this time for two more cycles for a total of three replicates. All flowers continued to be removed from all plants at the end of each day to ensure that only new flowers would be harvested for nectar extraction and analysis.

Statistical Analysis

Nectar sugar concentration between soybean varieties and temperature regimens was assessed for statistically significant differences using a two-way analysis of variance and Tukey's test. Nectar sugar concentration at 4, 6, and 8 hours after the onset of the daytime phase was assessed for significant differences using a one-way analysis of variance and Tukey's test. Due to non-normal distribution of the nectar volume and cleistogamy data, a Kruskal-Wallis one-way analysis of variance and Dunn's test were used to determine differences in 1) nectar volume between soybean varieties, 2) nectar volume between temperature regimens, 3) nectar volume between hours after the onset of the daytime phase, 4) cleistogamy between varieties, 5) cleistogamy between temperature regimens, and 6) cleistogamy between hours after the onset of the daytime phase. All statistical analyses were completed using R statistical software (v.4.0.3, R Core Team 2022) and visualized with the ggplot2 package (Wickham 2016).

Results

Cleistogamy

Cleistogamy was analyzed using the Kruskal-Wallis one-way analysis of variance and Dunn's test due to the data being non-normally distributed. Cleistogamy differed significantly between soybean varieties ($p < 0.001$, N = 180, df = 4), temperature regimens ($p < 0.001$, N = 180, df = 3), and hours after the onset of the daytime phase ($p < 0.001$, N = 180, df = 2) (Table 7). Flowers did not exclusively open during morning hours, instead continuing to open as the day progressed up to the final measurement at 8 hours after daytime onset. Cleistogamy was significantly less under the hottest temperature regimen (28°C average temperature), and varieties 9720 and 9723 produced the greatest proportion of open flowers. Variety S37xS89 exhibited high cleistogamy under all temperature regimens except for the hottest regimen and thus did not produce enough open flowers for nectar measurements on eight of the twelve days of data collection.

Bloom Characteristic	Variable	Treatment	Mean ± SE	Significance
Cleistogamy	Hour	4 hours	74.9 ± 3.69 %	a
		6 hours	51.0 ± 4.27 %	b
		8 hours	32.4 ± 4.11 %	c
	Temperature Regimen	21°C average	64.7 ± 5.17 %	a
		22°C average	62.9 ± 4.70 %	a
		24°C average	61.6 V 4.97 %	a
		28°C average	21.9 ± 3.51 %	b
	Variety	9720	29.5 ± 4.87 %	a
		9723	34.5 ± 5.47 %	a
		9727	57.7 ± 5.72 %	b
		9737AE	60.0 ± 5.15 %	b
		S37xS89	82.3 ± 3.90 %	c
Nectar Sugar Concentration	Hour	4 hours	38.3 ± 1.52 %	a
		6 hours	40.8 ± 0.73 %	a
		8 hours	39.3 ± 1.14 %	a
	Temperature Regimen	21°C average	39.8 ± 1.29 %	a
		22°C average	42.2 ± 1.80 %	a
		24°C average	43.1 ± 1.07 %	a
		28°C average	40.0 ± 1.21 %	a
	Variety	9720	42.5 ± 1.16 %	a
		9723	40.3 ± 1.79 %	a
		9727	40.9 ± 1.17 %	a
		9737AE	41.8 ± 1.39 %	a
		S37xS89	40.0 ± 3.03 %	a
Nectar Volume	Hour	4 hours	0.0833 ± 0.0099 µL	a
		6 hours	0.0652 ± 0.0027 µL	a
		8 hours	0.0733 ± 0.0053 µL	a
	Temperature Regimen	21°C average	0.0422 ± 0.0032 µL	a
		22°C average	0.0483 ± 0.0036 µL	a
		24°C average	0.0640 ± 0.0034 µL	b
		28°C average	0.0916 ± 0.0062 µL	c
	Variety	9720	0.0535 ± 0.0042 µL	a
		9723	0.0649 ± 0.0064 µL	a
		9727	0.0584 ± 0.0047 µL	a
		9737AE	0.0759 ± 0.0052 µL	b
		S37xS89	0.0844 ± 0.0128 µL	b

Table 7. Summary of significant differences between means of all measurements. Different lowercase letters indicate significant differences between treatments of that variable ($p < 0.05$).

Sugar Concentration

There was no significant difference in nectar sugar concentration between soybean varieties ($p = 0.833$, $N = 56$, $df = 4$) or temperature regimens ($p = 0.352$, $N = 56$, $df = 3$) (Table 7), nor was there a significant interaction between variety and temperature ($p = 0.944$, $N=56$, $df = 12$). There was also no significant difference in sugar concentration between hours after the onset of the daytime phase ($p = 0.306$, $N = 58$, $df = 2$).

Nectar Volume

Nectar volume was analyzed using the Kruskal-Wallis one-way analysis of variance and Dunn's test due to the data being non-normally distributed. Nectar volume differed significantly between soybean varieties ($p < 0.001$, $N = 240$, $df = 4$) and temperature regimens ($p < 0.001$, $N = 240$, $df = 3$) (Table 7). Soybean varieties did not differ from other varieties in the same maturity group, but maturity group 3 varieties (9737AE and S37xS89) produced more nectar than maturity group 2 varieties (9720, 9723, and 9727). Nectar production generally increased with temperature. Nectar volume did not differ significantly between hours after the onset of the daytime phase ($p = 0.242$, $N = 385$, $df = 2$).

Discussion

These findings reveal clear trends in bloom characteristics across all tested varieties. Nectar volume was greatest, and cleistogamy at 4 hours after the onset of the daytime phase was lowest, at the hottest temperature regimen. This agrees with Jaycox's (1970) observations of earlier flowering and greater nectar production at temperatures higher than 80°F. The average nectar sugar concentration was 40.7%, which is similar to the average nectar sugar concentration of 39.5% reported by Kettle and Taylor (1979). Nectar sugar concentration exceeded the minimum concentration that Jaycox (1970) considered highly attractive to honey bees (25%) across all varieties, times, and temperature regimens, ranging from 29% to 50%. This suggests that nectar volume and cleistogamy are likely to be better predictors of honey bee attractiveness than nectar sugar concentration amongst these five varieties. Cleistogamy and nectar volume both differed between certain varieties and temperature regimens, supporting the claims in previous studies that both varietal differences and temperature are driving honey bee attractiveness in soybeans (Erickson 1975a, Erickson 1975b, Issa et al 1984, Robacker et al 1983). Six hours after the onset of the daytime phase was determined to be the optimal time for nectar collection due to insufficient flower opening at 4 hours. This is analogous to midday in soybean fields, which has been shown in previous studies and in Chapter 2 to be the peak time for honey bee activity (Toledo et al 2011**,** BeaudelaineKengni et al 2015, Blettler et al 2016, Chiari et al 2005, Issa et al 1984, Jaycox 1970, Santos et al 1993).

Severson and Erickson (1984) speculated that soybean attractiveness to honey bees may vary throughout the day due to a decrease in nectar volume and an increase in sugar concentration as the day progresses. However, this study did not find a significant change in nectar sugar concentration or nectar volume as the day progressed. Sheppard et al (1979) speculated that variation in nectar sugar concentration may be caused by highly localized rainfall and soil moisture conditions, so it is possible that nectar sugar concentration is more heavily affected by these factors which were not tested in this study. Variation in soil conditions may have resulted in the varietal differences found in Chapter 2 that were not reflected in the results of this study. In Chapter 2, there was less honey bee activity in soybean fields planted with variety 9720 than in fields planted with varieties 9723 and 9727. In this study, there was no difference in nectar sugar concentration or nectar volume between these three varieties, and cleistogamy was higher in variety 9727. This suggests that varietal differences in soybean attractiveness may be driven by factors other than average daily temperature when grown in field conditions. Future research should investigate the effects of water availability and water retention in the soil on bloom characteristics.

It is possible that honey bee attractiveness is also affected by timing across the entire blooming period. Jaycox (1970) found that honey bees were most active in soybeans of the cultivar ‘Clark’ near the end of the blooming period due to higher nectar sugar concentrations. In contrast, Blettler et al (2016) found that honey bees were most active in soybeans of the variety Nidera A 5009 RG during the middle of the blooming period due to higher total flower production. In this study, soybean varieties of both maturity groups 2 and 3 were planted and measured simultaneously. Because of this, the maturity group 2 varieties reached growth stage R2 approximately one week before the maturity group 3 varieties reached R2 and data collection began. It is possible that this difference in timing within the blooming period contributed to the higher nectar production observed in the maturity group 3 varieties. Future work should investigate the relationship between bloom characteristics and timing across the entire blooming period, taking into account possible differences in vegetative growth and flower physiology between soybean varieties of different maturity groups.

This study used both day and night temperatures to assess how temperature affects soybean bloom characteristics. However, most previous studies used only daytime temperature when assessing soybean bloom response to temperature (Erickson 1975b, Jaycox 1970, Severson and Erickson 1984), with only Robacker et al (1983) examining both daytime and nighttime temperatures. Further research is necessary to determine how soybean bloom characteristics are influenced by day and night temperatures independently compared to day-night temperature cycles.

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