SOLIDAGO ALTISSIMA AND TERPENES: RELATIONSHIP WITH INSECT
POLLINATORS AND PREFERENCES

A Thesis
by
FAITH LAUREL WEAVER

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ABSTRACT

SOLIDAGO ALTISSIMA AND TERPENES: RELATIONSHIPS

WITH INSECT POLLINATORS AND PREFERENCES

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The interaction between plants and insect pollinators plays an important role contributing to the biodiversity of terrestrial ecosystems. Flowers produce chemicals resulting in olfactory and gustation ques that largely contribute to these interactions by attracting the most beneficial pollinators. *Solidago altissima* is an often-dominant old-field plant species that supports a large insect community and contains substantial intraspecific genetic variation resulting in different genotypes that may contain different concentrations of terpenes in the flowers. Though a previous study identified insect pollinator abundance differences among *S. altissima* genotypes due in part to terpenes, it is not clear which compounds are responsible for these different insect taxa responses, and therefore more study is needed concerning preferences for particular compounds.

My thesis research examined differences in floral terpenes in *S. altissima* in an old-field containing different genotypes. My objective was to investigate if terpene variation resulted in significant relationships with pollinator abundances for several insect taxonomic groups. An established field dominated by *S. altissima* was used to quantify insect pollinator visitation in nine plots established at least 35 meters apart to increase the

likelihood that they were separate genotypes. Pollinator surveys were conducted in each plot four times for 10 minutes over the span of two weeks during peak blooming season. Pollinator abundance was visually assessed and insects placed into taxonomic groups in the field. At the end of the surveys, two inflorescence samples were taken from each plot and analyzed for terpenes using gas chromatography. Compounds were identified using analytical standards and quantified using an internal standard. Linear regressions were used to examine possible relationships between pollinator abundance and floral terpenes. Partial Least Squares Regression was also used to examine effects of terpene combinations on pollinators. I found there were differences in both pollinator abundance and terpene concentrations between the plots and that there were significant relationships among some terpenes and pollinator taxonomic groups. Honeybees had a strong significant relationship to camphor and linalool concentrations and a moderate significance to total terpene concentrations. When examined in combination, the total pollinator abundance increased with increasing terpene concentrations. My data suggest that pollinators are using terpenes at some phytochemical level to evaluate the *S. altissima* plant they choose to visit.

I also used individual terpenes that are abundant in *S. altissima* to determine if a bee pollinator (*Bombus sp.*) had preferences for α-pinene, β-pinene, trans-Caryophyllene, and p-cymene over a terpene-free sucrose solution using a simple two-choice bioassay. I found for α-pinene that the bumblebee made an informed decision by choosing the terpene over sugar water alone. My data demonstrate that terpenes play a potentially important role in pollinator choice of *S. altissima* plants in old-fields, and that the preference for specific compounds is evident in some cases. To better understand terpene

preferences, more studies are needed examining more individual compounds and

combinations of compounds preferred by insect pollinators in this system.

ACKNOWLEDGMENTS

DEDICATION

I would like to dedicate this thesis to my friends and family, who gave me the best support, patience, and love throughout this entire process. I especially appreciate my significant other, Alex Gustafson, who showed me the greatest encouragement and taught me to cherish the little moments in life.

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Thesis followed Environmental Entomology Journal Format

INTRODUCTION

Plant-Pollinator Interactions

Pollinating insects play a major role in the biodiversity of natural terrestrial ecosystems (Barber and Gorden 2015) since angiosperms depend on biotic interactions with insect pollinators in order to sexually reproduce, showing great diversity in traits like scent, color and nectar production (Borghi et al. 2017, Emel et al. 2017, Giron-Calva et al. 2017, Zhang et al. 2020). Insect pollinator abundance and distribution depend on the preference of specific plant traits and they may use olfactory cues to find flowers (Emel et al. 2017, Jakobs and Muller 2018, Rosati et al. 2018, 2019, Koski 2020, Pyzza et al. 2020), where the specific signal plants emit provide the right message for pollinator receivers (Koski 2020). Though olfactory cues are important for pollinators, other factors such as environment, temperature, ozone pollution, moisture, soil nutrients, and sun exposure may play a large role in how plants and insects interact (Farré-Armengol 2020, Koski 2020, Vanderplanck et al. 2021). A particular focus in my study is the contribution of phytochemicals (olfaction contribution) in attracting pollinators to flowers in an old-field plant species.

It is important for many angiosperm species to attract a large abundance and diversity of insects (Fenesi et al. 2015). Angiosperms are the most diverse group of vascular plants, consisting of over 295,000 species in 416 families, with some species representing important human food crops (Junker and Parachnowitsch 2015, Christenhusz and Byng 2016, Rosati et al. 2018, 2019). Insect pollinators are essential not only for the health of ecosystems but importantly in food production where they are

responsible for approximately 35% of crop production by pollinating nearly 75% of crop species (Powney et al. 2019). Approximately 88% of all angiosperms are pollinated by insects with a large portion of them being pollinated by bees alone, making insect pollinators essential for ecosystem services (Barber and Gorden 2015).

In order to entice beneficial insect pollinators, flowers produce pollen, nectar, and oils that contain necessary nourishment like nitrogen, proteins, lipids, amino acids, carbohydrates, and sugars that insects require (Borghi et al. 2017, Prasifka et al. 2018, Barragán-Fonseca et al. 2020, Van der Kooi et al. 2021). Flowers advertise what reward they are offering and communicate it accurately depending on floral age, pollen/ nectar availability, and maturity of reproductive parts so that pollinators learn to associate these plants with good resources and continually seek them out (Dudareva and Pichersky 2000). Alternately, flowers that falsely advertise their nectar components and cause pollinators to not receive an adequate reward will result in no repeated visits (Dudareva and Pichersky 2000). Clearly, the insect pollinator-flower interaction can be complex.

Flower Chemistry and Insect Pollinators

Insect pollinators use certain cues like taste, smell, and sight to choose specific plant species flowers. For taste and smell, phytochemicals play a large role in which flowers they choose (Benvenuti et al. 2020), resulting in flower chemistry having a large influence on pollinator fidelity, diversity, and abundance (Jakobs and Muller 2018). Because they contribute largely to the attraction of pollinating insects to flowers (Zhang et al. 2020), floral scents are of great ecological importance. Plants may produce a floral scent that consist of volatile organic compounds (VOCs), composed of many classes of

compounds that can differ among species (Borghi et al. 2017, Boncan et al. 2020).
Studies have shown that pollinators prefer a combination of VOCs (Burkle and Runyon
2016, 2017) and that the emissions of VOCs by the flowers act as cues for pollinators to
identify the quality of nectar provided by the flowers as a reward for their services
(Benvenuti et al. 2020, Vanderplanck et al. 2021). Floral VOC richness, composition, and
emission rates can differ among species (Farré-Armengol 2020). In a study by
Rachersberger et al. (2019) examining the importance of visual and olfactory cues of
apple flowers (*Malus domestica*), they found that olfaction was more favorable to
honeybees, *Apis mellifera*. For insects such as hawkmoths, which are nighttime
pollinators, olfactory signals were clearly more important than visual cues
(Rachersberger et al. 2019). Given this, understanding the contribution of flower
chemicals that may be encountered by insects is important.

The chemical compounds that attract insects may target generalist or specialist
pollinators (Junker and Parachnowitsch 2015, Benvenuti et al. 2020, Farré-Armengol
2020). Some plant species have developed private channels of floral scents that are
composed of species-specific blends of chemical compounds to entice particular
pollinating insect species (Okamoto and Su 2021). This means that plants only need to
make distinctive compositions of a few common compounds to attract these pollinators
(Okamoto and Su 2021). Floral scent may also change and evolve under selective
pressures of pollinating insects (Burkle and Runyon 2016, 2017, Okamoto and Su 2021),
due to factors like herbivory, which alter the floral bouquet and possibly reduce pollinator
abundance (Kessler et al. 2011, Erb 2018, Alonso et al. 2019).

After pollination, flowers can change their VOCs which alters how pollinators interact with them (Barragán-Fonseca et al. 2019). Biotic interactions like herbivores and pathogens will affect the VOCs that plants emit which impact the interactions between them and pollinators and possibly reduce fitness (Giron-Clava et al. 2017, Alonso et al. 2019, Wise 2020). Floral fragrances can also be changed in order to attract more general pollinators if flowers have not yet been pollinated (Dudareva and Pichersky 2000). Plants may also emit the same or similar signals and cues in order to avoid wasting pollen or receiving pollen from heterospecifics (Junker and Parachnowitsch 2015). It seems clear that not only are chemical signals from flowers important for pollinators but also that their production is a complex process that may be expected to vary among species.

Insect pollinators in flight have to choose multiple plant species that differ in many traits and process sensory modalities to make decisions on which flowers to visit (Chittka and Raine 2006). Insects have a highly sensitive sense of smell because they have specialized olfactory sensory neurons (OSNs) that are located in a sensory organ called sensilla that sit on the exoskeleton of the insect, especially the antennae (Pyzza et al. 2020, Blande 2021, Cassau and Krieger 2021). These sensilla are used to detect odorants from food and predators, pheromones for mate attraction, and choosing oviposition sites (Blande 2021, Cassau and Krieger 2021). Identifying the attractive cues that pollinators prefer in plant species could potentially optimize crop production by improving the efficiency of detection of these olfactory cues (Rachersberger et al. 2019).

Terpenes

        Plants contain over 200,000 secondary metabolites, such as alkaloids and terpenes, that act in roles from deterring herbivory to attracting pollinators (Holopainen et al. 2018, Boncan et al. 2020, Baldwin 2021). Terpenes are the largest and most diverse class of the secondary metabolites and the most common floral volatile, with complex functions in plants involving interactions with other organisms (Johnson et al. 2007, Heath et al. 2014, Borghi et al. 2017, Yip et al. 2018, Boncan et al. 2020, Farré-Armengol et al. 2020). In addition to terpenes, other commonly found compounds in floral volatiles are benzenoids, fatty acid derivatives, nitrogen-containing compounds, amino acid derivatives, and sulphur-containing compounds (Farré-Armengol et al. 2020). Terpenes are derived from 5-C isoprene units and can be classified into groups by the number of carbon atoms they contain, including mono-, homo-, and sesquiterpenes (Tholl et al. 2011, Boncan et al. 2020). Common terpenes found in floral scents are monoterpenes such as linalool, limonene, and myrcene as well sesquiterpenes like farnesene, nerolidol, and caryophyllene (Dudareva and Pichersky 2000).

        The terpenoid bouquet that some plant species produce can potentially increase the abundance of pollinators while deterring the number of herbivores (Johnson et al. 2007, Erb 2018, Yip et al. 2018, Wise 2020). Plants can also adjust and manipulate the composition of terpene compounds in order to emit specific signals (Dudareva and Pichersky 2000). In a study by Plata-Rueda et al (2018), it was found that cinnamon and clove oils contain many toxic terpenoids like caryophyllene, α-pinene, α-humulene, and α-phellandrene that were repellent towards the grain-eating insect *Sitophilus granaries* (Plata-Rueda et al. 2018, Boncan et al. 2020). Though often clearly acting as deterrents,

with respect to pollinators, terpenes may serve as indicators of floral stages and pollination status (Boncan et al. 2020). Chen and Song (2008) found that the wasp pollinator *Ceratosolen solmsi marchali* uses the terpenes linalool, limonene, and d β-pinene to identify the specific life stages of the plant species *Ficus hispida* (Chen and Song 2008, Boncan et al. 2020). To understand the relationship between plants and pollinators, chemically mediated studies that focus on floral VOCs need to be completed.

Preference Studies

As previously mentioned, pollinators may use visual and olfactory cues simultaneously to make informed decisions about flower choice (Borghi et al. 2017, Barragán-Fonseca et al. 2019, Pyzza et al. 2020, Okamoto and Su 2021). These decisions are based on preferences of certain plant floral traits like nectar or pollen aimed to entice different pollinator taxa (Melendez-Ackerman et al. 1997, Urbanowicz et al. 2020). As previously presented, floral scent VOCs are highly variable in plant species and act as attractants to many pollinators (Burkle and Runyon 2016, 2017). Flower olfactory cues can also be altered by many factors like plant species, location, and composition of compounds (Borghi et al. 2017), making definitive conclusions about insect choice difficult. Preference studies are useful in identifying which floral traits pollinators are exploiting when they are making a choice of flowers (Barragán-Fonseca et al. 2019, 2020).

Studies that look at plant-pollinator interactions involving choices with a focus on chemical ecology typically use two-choice bioassays that provide the pollinators with only two options, making it easier to identify preferences (Barragán-Fonseca et al. 2020).

When performing preference studies involving floral cues and pollinators, the behavioral response of the insect is often determined using a y-tube olfactometer, which will give the pollinators two olfactory cues simultaneously, forcing them to make one choice (Park et al. 2019). Behavioral responses of the pollinator can consist of attempted antennal contact, hovering over the sample for a period of time, or landing and tasting the sample (Park et al. 2019). Barragán-Fonseca et al. (2020) performed behavioral bioassays to look at the importance of visual and olfactory cues in two plant species from the family Brassicaceae. The two species, *Brassica nigra* and *Raphanus sativus* (Barragán-Fonseca et al. 2020) are dependent on the pollinators *Episyrphus balteatus* (syrphid fly) and *Pieris brassicae* (butterfly) for sexual reproduction. They tested if olfactory or visual cues were used independently or in combination. They found that the two pollinating species exploited floral cues in different ways and a combination of cues was preferred. When these cues were tested against each other at longer distances, visual cues were more important.

Though preference studies can be insightful, they can be hard to perform in a natural environment because of the multitude of abiotic and biotic factors involved. An additional concern is the chemicals used when performing preference studies, where it was shown that bees may develop a preference for synthetic volatiles after they obtain a reward from artificial flowers containing these compounds (Zhang et al. 2020).

Solidago altissima

Solidago altissima (tall goldenrod) is a wide-spread and often dominant old-field herbaceous plant species in North America found growing on the side of roadways and in

abandoned fields (Bernhard and Dolt 1994, Walck et al. 1999, Halverson et al. 2008, Hafdahl 2013, Sakata et al. 2018). *Solidago altissima* reproduces clonally through rhizomes causing noticeable clusters of patches in fields (Meyer and Schmid 1999). All goldenrod species are self-incompatible and depend on insect pollination for sexual reproduction due to their pollen being too heavy for wind dispersal (Gross and Werner 1983, Bernhard and Dolt 1994, Hafdahl 2013, Ustinova and Lysenkov 2020). With *S. altissima* being self-incompatible and an obligate outcrosser, it recognizes and rejects pollen from other genotypes (Walck et al. 1999, Hafdahl 2013). Above-ground shoots may range between 30 to 280 centimeters in height and emerge in early spring, with flowering in the months of August-November (Cain 1990, Meyer and Schmid 1999, Weber 1999, Hafdahl 2013, Ustinova and Lysenkov 2020). Inflorescences form pyramidal panicles on top of the shoot in many clusters of branches where it can hold up to 1200 flower heads consisting of around 10 fertile female ray florets and three to seven disc flowers that surround the corolla. The plant can produce up to 200,000 seeds (achenes) per shoot (Bernhard and Dolt 1994, Meyer and Schmid 1999, Weber 1999, Genung et al. 2012, Ustinova and Lysenkov 2020). Seeds can be dispersed by wind allowing colonization of new locations (Hafdahl 2013).

Wildflowers like goldenrod make a large contribution to plant-pollinator networks and have a large environmental impact on insect biodiversity (Benvenuti et al. 2020). Understanding the relationships between wildflower communities and associated pollinating insects is important for conservation because we would have the knowledge to manage beneficial plant species and provide pollinators with adequate foraging resources (Urbanowicz et al. 2020). Many insects like hoverflies, honeybees, muscoid flies, and

wasps are frequent visitors to goldenrod species (Ustinova and Lysenkov 2020). These pollinators are probably making informed decisions on these species based on floral abundance and certain plant traits. For example, honeybees could have a preference for specific floral traits because of coevolving with certain plant species in their native areas (Urbanowicz et al. 2020). For tall goldenrod, the floral traits that pollinators are using are still largely unknown, making it a great model system to use (Takafuji et al. 2020).

Intraspecific Genetic Variation

Plant intraspecific genetic variation can affect insect diversity and hence plays a key role in ecosystem functioning (Hersch-Green et al. 2011, Ehlers et al. 2016, Xu et al. 2020). Numerous investigations have demonstrated large genetic variation in *S. altissima*, affecting insect communities (Crutsinger et al. 2006, 2008), pollinators (Johnson 2006, Genung et al. 2010, Burkle et al. 2013), and phytochemistry (Williams and Avakian 2015). This species is known for its large abundance of herbivores and diversity of pollinators (Richardson and Hanks 2011, Wise 2020), and the genetic variation in *S. altissima* contributes to how floral visitors interact with the species (Burkle et al. 2013). In addition to the direct effects of intraspecific genetic variation, an individual of one type of genotype may affect the visitations of neighboring plants or individuals (Genung et al. 2012, Ehlers et al. 2016). This could have implications in large old fields with numerous genotypes.

Though a previous study identified the importance of flower terpenes in *S. altissima* on the abundance of insect pollinators (Ragsdale 2016), it specifically set out to compare genotype variation on a landscape scale and not the plant-pollinator relationship

in a single old field. Because of previous findings showing substantial intraspecific variation in *S. altissima* (see above) different flower terpenes could be identified by more investigation and related to a different suite of pollinators than the previous *S. altissima*-pollinator investigations. In addition, to date, no attempt had been made to examine the pollinator preference for specific terpene compounds identified in tall goldenrod flowers. My study makes an important contribution to further understanding the role plant chemistry plays for insects in this important foundation species.

My study had five objectives, which were to determine if:

- potential differences in flower terpenes among genotypes in an old-field relate to insect pollinator abundance
- there are differences among pollinator taxa in the response to terpenes
- pollinator responses to individual terpenes differ from those of terpene combinations
- in laboratory choice trials, Bumblebees (*Bombus* sp.) prefer terpene flowers over those with sugar alone, and
- in field choice trials, pollinators prefer some combinations of terpenes over sugar water alone.

MATERIALS AND METHODS

2020 Field Season

Experimental Design

To examine possible relationships between insect pollinators and terpenes in *S. altissima*, I used a mature old-field located on Tom Jackson Road in Watauga County, Boone NC to set up plots for pollinator observation and collection, as well as flower sampling for terpenes. The field site was dominated by *S. altissima*, though other herbaceous plants such as *Rubus* and *Asclepias* spp. were sporadically present. In July 2020 nine 1 m^2 plots were established at least 35 meters apart from each other to increase the probability that they represented different genotypes. To account for the slight relief in the field, plots were distributed so that there were three at the highest (plots 7-9), middle (4-6), and lowest elevations in the field.

Pollinator Surveys

Pollinators were quantified in each plot during the months of September and the beginning of October (9/16/2020 - 10/02/2020). Observations started when at least 70% of *S. altissima* plants within each plot were in full bloom. Plots were observed four times for 10 minutes on different days, with observations done only on sunny days between the hours of 11 AM and 3 PM and with a minimum temperature of 18.3 °C (65 °F) to ensure optimal conditions for pollinators. Visiting pollinators were counted if they landed on any flower within the plot. The plots at the top of the field bloomed earlier in the season than the plots near the bottom, therefore plots 7-9 had to be observed before plots 1-6 in order

to guarantee that they did not go to seed before trials were done. Individual plots were only observed once a day and only four to six plots were observed each day. The number of pollinator visits (expressed as abundance) were visually assessed during each observation trial and unknown species (see below) were initially placed into the taxonomic categories Coleoptera, Hymenoptera, Diptera, Bees, and other. More specific identifications of honeybee (*Apis mellifera*) and bumblebee (*Bombus sp.*) were made in the field.

Terpene Samples and Analysis

Flower samples for terpene quantification were taken in each plot after the four observation trials. Two samples of inflorescences were cut from three randomly chosen ramets within each plot for a total of 18 flower samples in the field. Samples were kept in a temperature-controlled flammable storage refrigerator until they could be processed, which was within two days of the collection. Inflorescences were removed using tweezers and weighed to roughly 1.2 grams per sample, then placed into a glass culture tube with 20 mL of HPLC-grade pentane. Samples were returned to the refrigerator and stored for a minimum of one month.

The flower samples were prepared for gas chromatography following the protocols of Williams and Avakian (2015) and Ragsdale (2016). Each sample was ground with a Polytron homogenizer for 1 minute and filtered into a glass tube. The filtered sample was evaporated with nitrogen gas to 1 mL. A 1 μL sample was injected into a Shimadzu 14A gas chromatograph with a flame ionization detector (FID) set on the program from Williams and Avakian (2015): injector temperature 250°C; detector

temperature 275°C; start temperature 80°C, held for 2 minutes with a temperature increase to 280°C (10°C/minute) and final hold of 2 minutes, for a total run time of 24 minutes. Each sample was run in duplicate and averaged. The retention time of analytical standards (Sigma-Aldrich) were used to identify terpenes. Some compounds (e.g., germacrene D) with no standard available were identified with high confidence based on previous experiments in the Williams laboratory. The internal standard (IS) tri-decane (Sigma-Aldrich) was used to calculate compound concentration (Williams and Avakian 2015, Ragsdale 2016).

2021 Experiments

Laboratory Preference Studies

Though relationships between flower terpenes and insect variation in the field could be made using statistical techniques, this did not allow a determination if pollinators were actually recognizing terpene compounds. To examine if a native pollinator (bumblebee/*Bombus sp.*) preferred wicks containing sugar water and a terpene over sugar water alone, preference studies were conducted in the laboratory in July and August 2021. Four terpenes were chosen based on their concentration and response to pollinators in *S. altissima* flowers from a previous study (Ragsdale, 2016):α-pinene, β-pinene, trans-Caryophyllene, and p-cymene.

For preference trials, both sucrose and terpene solutions were made. Fresh sucrose solution was made every few days, while terpene solutions were previously made and kept in a temperature-controlled flammable storage refrigerator. Terpene samples were made to a concentration of 1 mM which is thought to be an ecologically relevant

concentration (Andrew Bellemer, personal communication). Because terpenes are lipophilic, ethanol was used to dissolve the compounds.

Before each preference trial, three or four bumblebees were collected from nearby flowers on the Appalachian State University Campus in Boone, NC, and placed in a cooler between the hours of 11 AM- 4 PM. Chilled bees were placed in separate holding boxes until ready to use for preference trials. Bees were without food source and starved for two hours. For an individual trial, one bee at a time was placed into a clear plastic arena (50 cm X 40 cm) containing two artificial flowers made to resemble *Solidago* flowers. Each bee was trained by giving them one small sucrose-soaked cotton swab for fifteen seconds. This initial sugar-containing swab provided energy, as well as taught the bee to recognize the swabs as a reward. Each individual bee always drank from the training swab before the start of the trial. After training, two freshly soaked (50% sucrose solution) large wicks were placed into the box, one with and one without a droplet of diluted terpene sample. Since the terpenes were dissolved in ethanol each sugar-only wick also had a drop of ethanol added. The wicks were placed on the artificial *Solidago* flowers in the arena on stands to give them height. One drop (using a Pasteur pipette) of terpene solution was placed on a swab previously soaked in the sucrose solution. The two artificial flowers in the arena were identical with the exception of a small piece of yellow tape to indicate the flower containing the terpene sample. Flowers were placed approximately 15 cm apart in the box, in opposite corners. Sample placements were randomly chosen using a random choice generator before being placed in the left or right side of the box between each trial to reduce bias. If a bee made contact with a flower/wick sample behavior (example, land, hover, taste, etc.) was recorded. Each

observation period for the trial was 10 minutes. Bees were used only once in a trial and any bee that did not exhibit a behavior within an hour after starting a trial was discarded.

Field Preference Studies

To expand preference to more than one terpene and examine it in a natural setting, the Tom Jackson Road field site was used in the month of September 2021 for terpene preference studies. A wooden stake was used to hold four samples using tongue depressors evenly spaced onto the top of the stake. An artificial *Solidago* flower was placed in the middle of the four tongue depressors. The terpenes α-pinene, β-pinene, and trans-Caryophyllene were used in the field trials. In addition to the terpene wicks, one wick had only sugar water and ethanol.

Stakes with the four samples were placed randomly in the field and left overnight for the insects to familiarize themselves with the samples. The next morning between the hours of 11 AM and 4 PM all wicks were replaced. Wicks were observed for 10 minutes and insect visitations were recorded for each sample visited. Individual insects were counted and placed into groups similar to the 2020 field work. A visitation was recorded if an individual landed on the wick and was visually seen to drink from the sample. A total of 17 trials were completed at four locations across the field used. Unfortunately, near the end of the trials yellow jackets (*Vespula* sp.) had learned that the wicks gave sugar rewards and became too aggressive to continue the trials.

Statistical Analyses

For the pollinator field observation trails in 2020, possible relationships between pollinator abundance and terpenes in flowers were made using linear regression (JMP Pro 13). Each pollinator taxonomic category (also Total Pollinators and Dominant Pollinators (honey + bumblebee) were the dependent variables and individual terpene compounds the independent variables. In each analysis, the presence of outliers was determined using Cooks D Influence (JMP, Cary, NC), which serves as an appropriate analysis for my study (see Staton et al. 2019). p values ≤ 0.05 were reported as significant, while $p \leq 0.1$ was considered marginally significant in my analyses. In order to analyze possible effects of multiple terpenes simultaneously affecting pollinator abundance, I used a Partial Least Squares Regression (PLSR, JMP Pro 13.0). This analysis has been used in ecological research to examine relationships between multiple predictor variables and insect data (see Couture et al. 2013, Williams and Avakian 2015), including pollinators (Ragsdale 2016). This analysis is especially relevant where predictor variables are collinear (Wold 1984). To develop a model, it is important that the proper number of latent variables is added to the model to achieve the best fit for the data but not to overfit (Cox and Gaudard 2013). Using all terpenes and each pollinator measure, I found two taxa, All Pollinators and Bumblebees (*Bombus* sp.) where a single factor model could be validated. To identify individual terpenes not contributing to the model, I used a Variable Importance Projection (VIP) and a cut-off value of 0.8, similar to previous studies examining plant phytochemical-insect relationships (Couture et al. 2013). A linear regression of observed versus predicted values examines potential relationships between terpenes and pollinator abundance.

Insect preference experiments were analyzed using a proportions test. In the 2021 laboratory experiment, the bees were presented with two options, one being sucrose only and the other being one of four terpenes. The compare proportions test was used to assess whether there was a significance between the pollinator choice and the terpenes samples. The two options were "Choose" for the terpene sample and "No Choose" for the sucrose sample. A population proportions calculator was used (Proportion calculator) to look at the sample size (10), the number of successes (choosing terpenes), and expected proportion (null hypothesis: 0.5); performing a not equal test ($\neq$) due to sample size. The proportions test was used because we were interested in seeing if there was a significant negative or positive response from the pollinators and the different terpene samples. A chi-square test was used in the 2021 field trials, the chi-square matrix had four choices; sugar only or three terpenes.

RESULTS

Fall 2020 Field Data

Overall, total pollinator abundance was more similar in the lower and middle plots compared to the highest (i.e., plots 7-9), where the number of flowers was comparatively less, possibly explaining in part why the pollinator abundance was lower (See appendix 1). Figure 1 shows the variation in average pollinator abundance per plot across the field.
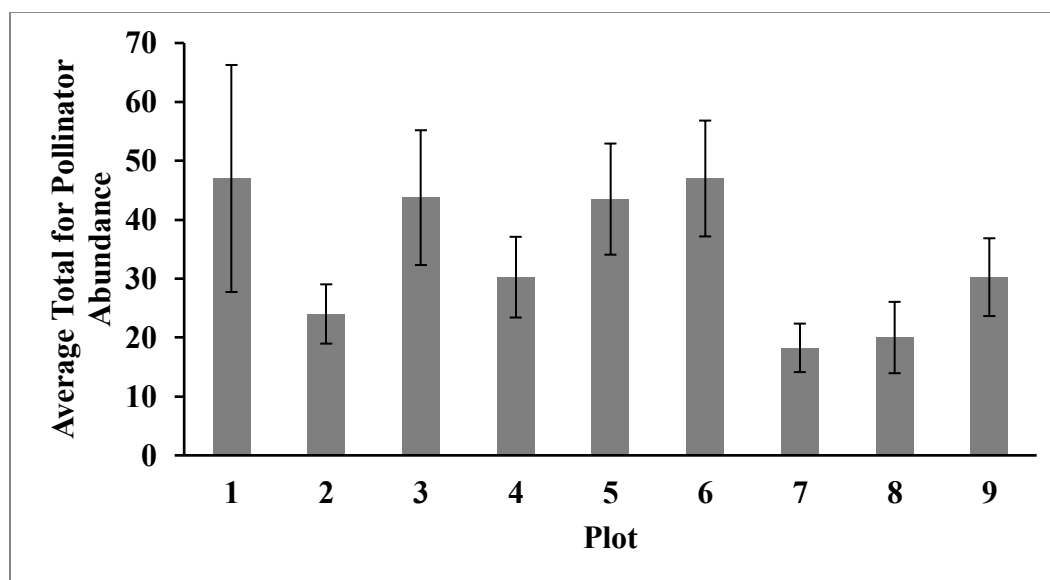
Fig. 1. Average total pollinator abundance per plot.

Bumblebees (*Bombus sp.)* and Honeybees (*Apis mellifera*) abundances are found in Figures 2 and 3 (see appendix 2). There was considerable variation among plots and bumblebee abundance was considerably lower than that of honeybees.

The average abundance of the six larger taxonomic groups of pollinators by plot is found in Fig. 4. Abundance in each group varied across plots, representing the heterogeneous nature of the field. Insects in Order Hymenoptera (A, D, and F) contributed most to the observed pollinators in my study, with insect insects in Order Coleoptera having the lowest abundance.
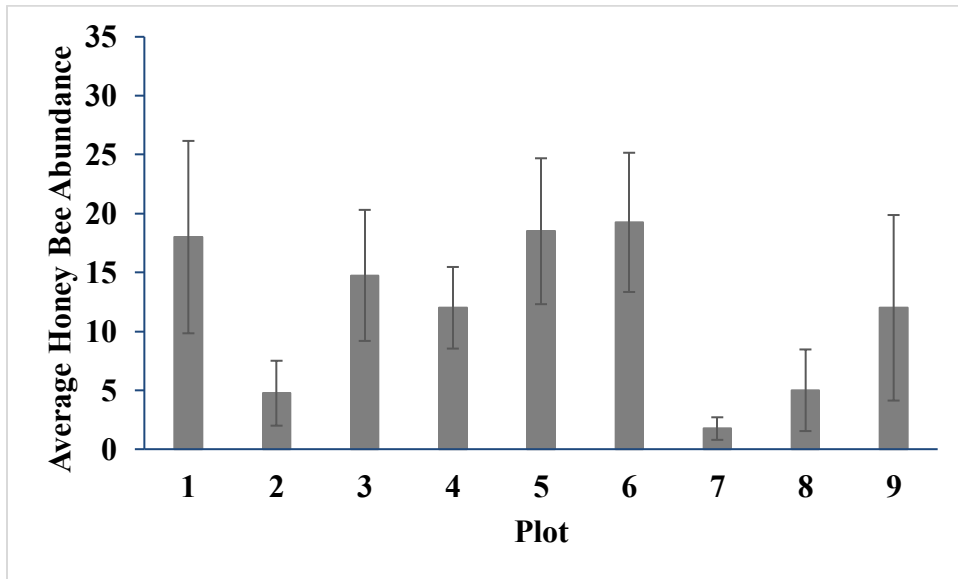
Fig. 2. Average Honeybee abundance per plot.
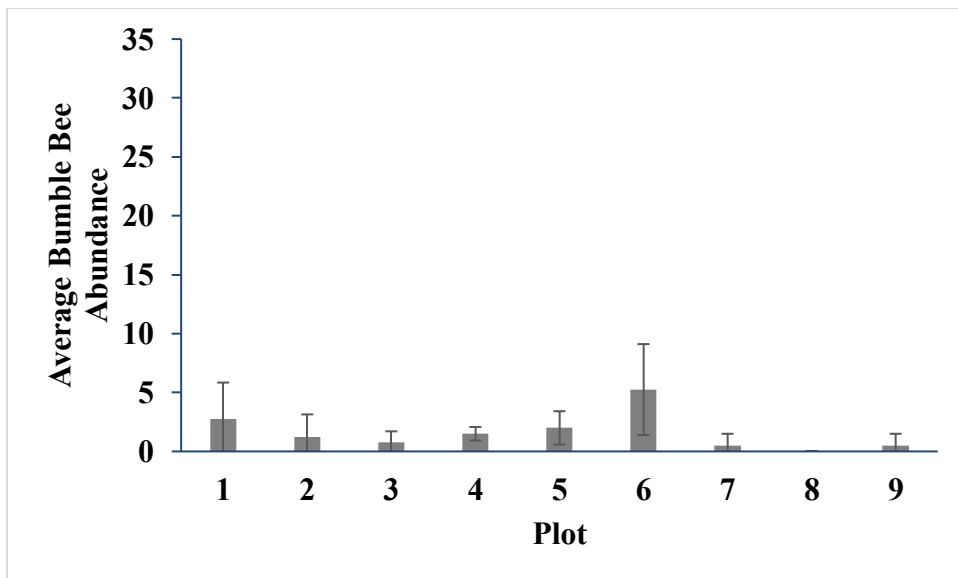
Fig. 3. Average Bumblebee abundance per plot.
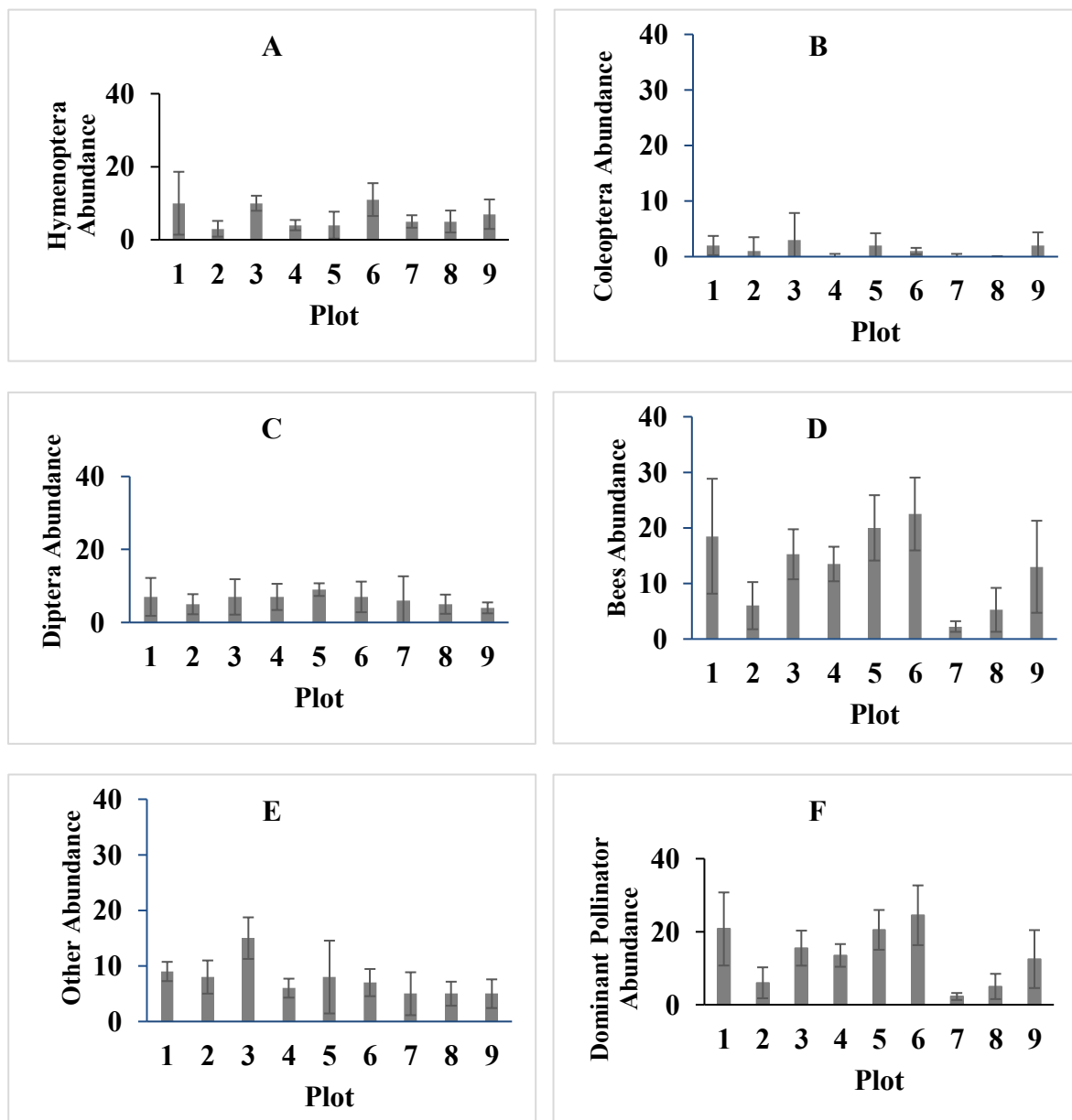
Fig. 4 Average Abundance of (A) Hymenoptera, (B) Coleoptera, (C) Diptera, (D) Bees, (E) Other, and (F) Dominant Pollinators.

The concentration of each terpene compound identified per plot is found in Figures 5 and 6.
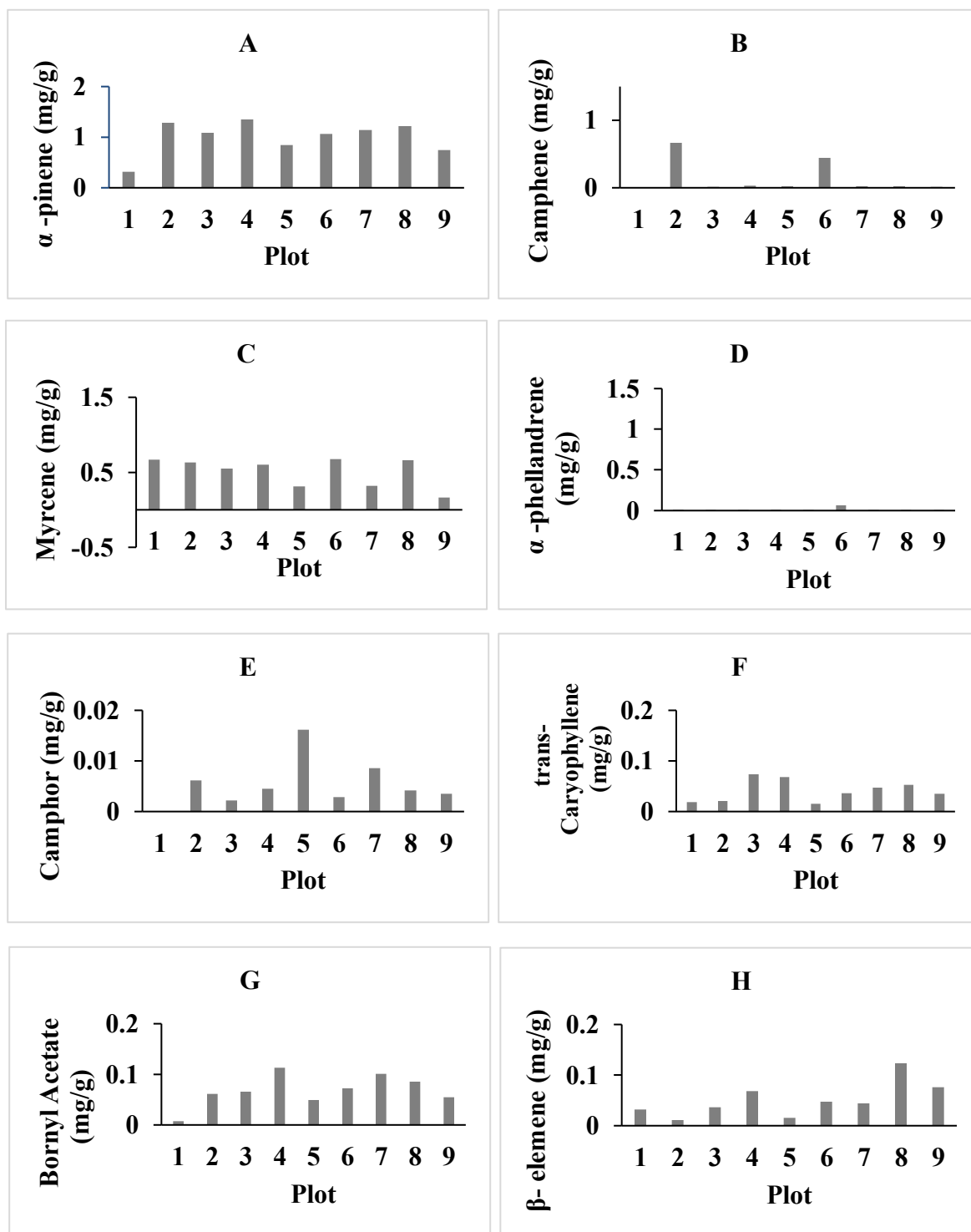
Fig. 5. Concentration of (A) α-pinene, (B) Camphene, (C) Myrcene, (D) α-phellandrene, (E) Camphor, (F) trans-Caryophyllene, (G) Bornyl Acetate, and (H) β-elemene.
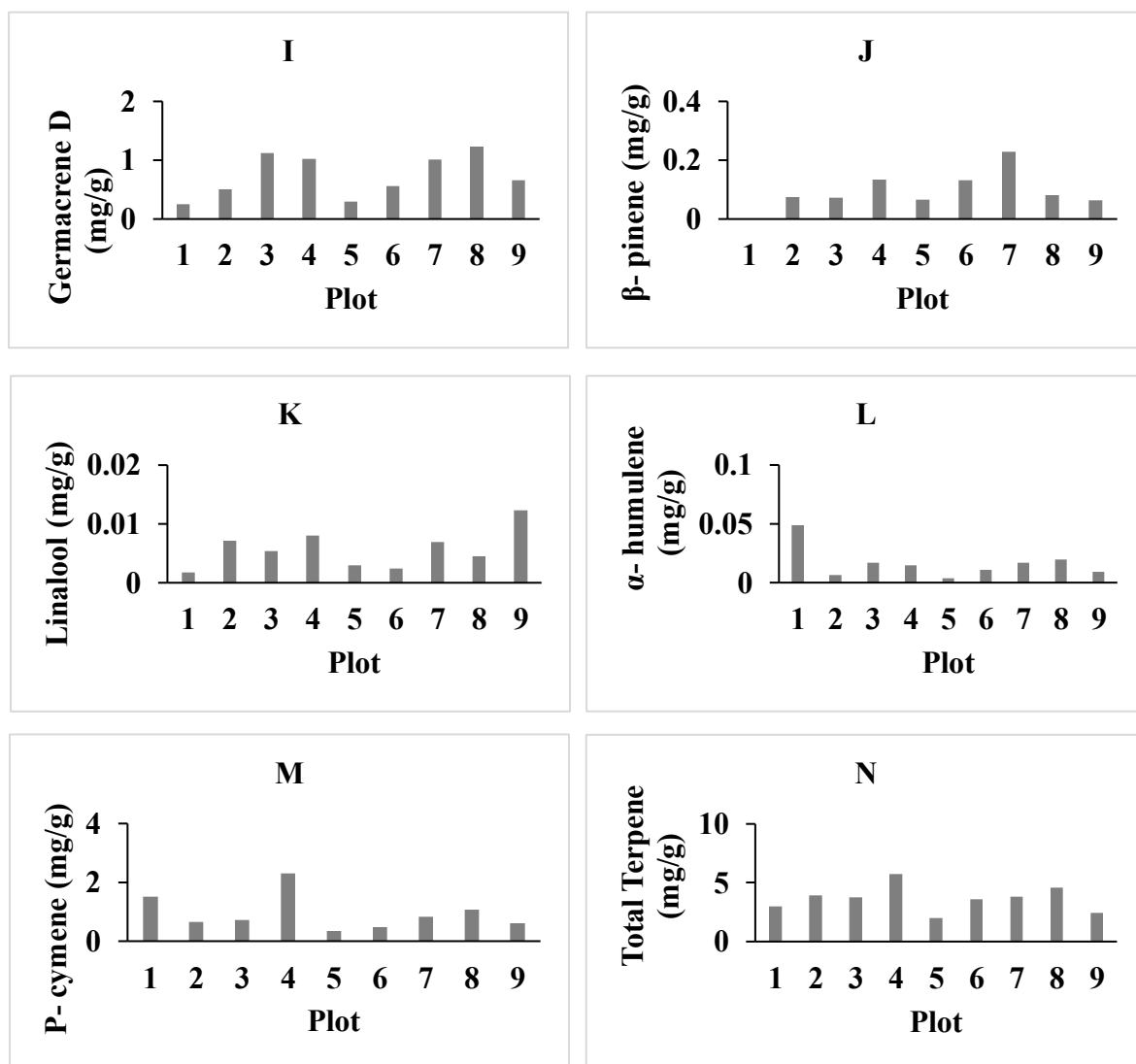
Fig. 6. Concentration of (I) Germacrene D, (J) β-pinene, (K) Linalool, (L) α-humulene, (M) P-cymene, and (N) Total Terpenes.

P-cymene and α-pinene had the highest overall average concentration, contributing to the use of these two terpenes in the Fall 2021 lab and field preference research. Camphene and α-phellandrene had lower concentrations overall in all plots.

For the linear regression results, only significant ($p \leq 0.05$) or marginally significant ($P < 0.1$) relationships are graphically presented.

Table 1. Linear regression (JMP Pro 15) results (F, p, and R^2) for **Hymenoptera** abundance and flower terpenes

Terpene	F	P	R^2
α-pinene	2.327	0.171	0.249
Camphene	1.691	0.241	0.220
β-pinene	0.490	0.506	0.065
Myrcene	0.479	0.511	0.064
α-phellandrene	0.692	0.437	0.103
P-cymene	0.385	0.558	0.060
Linalool	3.883	**0.096**	0.393
Camphor	7.412	**0.035**	0.553
Bornyl Acetate	1.609	0.245	0.187
trans-Caryophyllene	0.076	0.791	0.011
β-elemene	0.010	0.923	0.001
α-humulene	2.206	0.181	0.240
Germacrene D	0.120	0.739	0.017
Total Terpenes	0.453	0.522	0.061

Df with no outlier removed= 1,7 for trans-Caryophyllene, Total Terpene, Myrcene, α-humulene, Germacrene D, Bornyl Acetate, β-pinene, β-elemene, α-pinene.
Df with outlier removed: 1,6 for P-cymene, Camphene, Linalool, Camphor, α-phellandrene.
Values of $P \leq 0.1$ shown in **bold type.**

Hymenoptera is found in Table 1. Two compounds, camphor, and linalool (marginally) were significantly related to insect abundance (Fig. 7. A, B). For each compound, Hymenoptera abundance decreased with increasing terpene concentration.
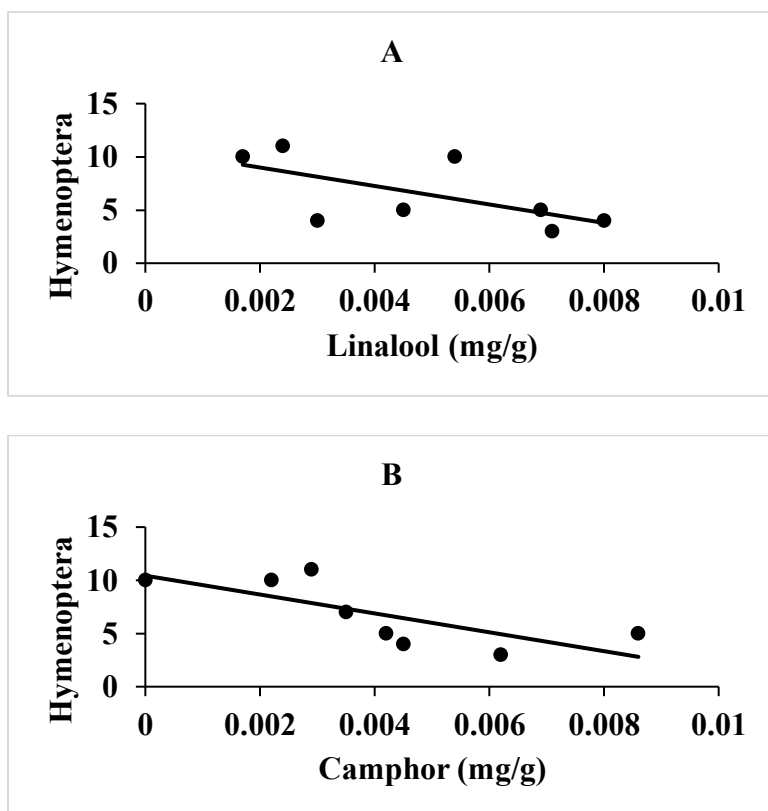
Fig. 7. Significant regressions for Hymenoptera with (A) Linalool (p-value 0.096 and R^2 0.393) and (B) Camphor (p-value 0.035 and R^2 0.553).

Table 2 shows camphor and linalool were significantly related to honeybee abundance (Fig. 8. A, B), while total terpene was moderately related to insect abundance (Fig. 8. C). For all compounds as concentration increased the honeybee abundance decreased.

Bumblebee abundance was unrelated to any terpenes (Table 3).

Table 2. Linear regression (JMP Pro 15) results (F, P, and R^2) for **Honeybee** abundance and flower terpenes

Terpene	F	P	R^2
α-pinene	3.049	0.124	0.303
Camphene	0.499	0.506	0.077
β-pinene	2.439	0.162	0.258
Myrcene	0.051	0.828	0.007
α-phellandrene	0.889	0.377	0.113
P-cymene	0.039	0.848	0.006
Linalool	5.820	**0.052**	0.492
Camphor	17.84	**0.006**	0.748
Bornyl Acetate	2.954	0.129	0.297
trans-Caryophyllene	0.384	0.555	0.052
β-elemene	0.841	0.389	0.107
α-humulene	1.183	0.319	0.165
Germacrene D	3.090	0.123	0.306
Total Terpenes	3.923	**0.095**	0.395

Df with no outlier removed= 1,7 for trans-Caryophyllene, P-cymene, α-phellandrene, germacrene D, Bornyl Acetate, β-pinene, β-elemene, α-pinene.
Df with outlier removed: 1,6 for Total Terpene, Myrcene, Camphene, Linalool, Camphor, α-humulene.
Values of $P \le 0.1$ shown in **bold type.**
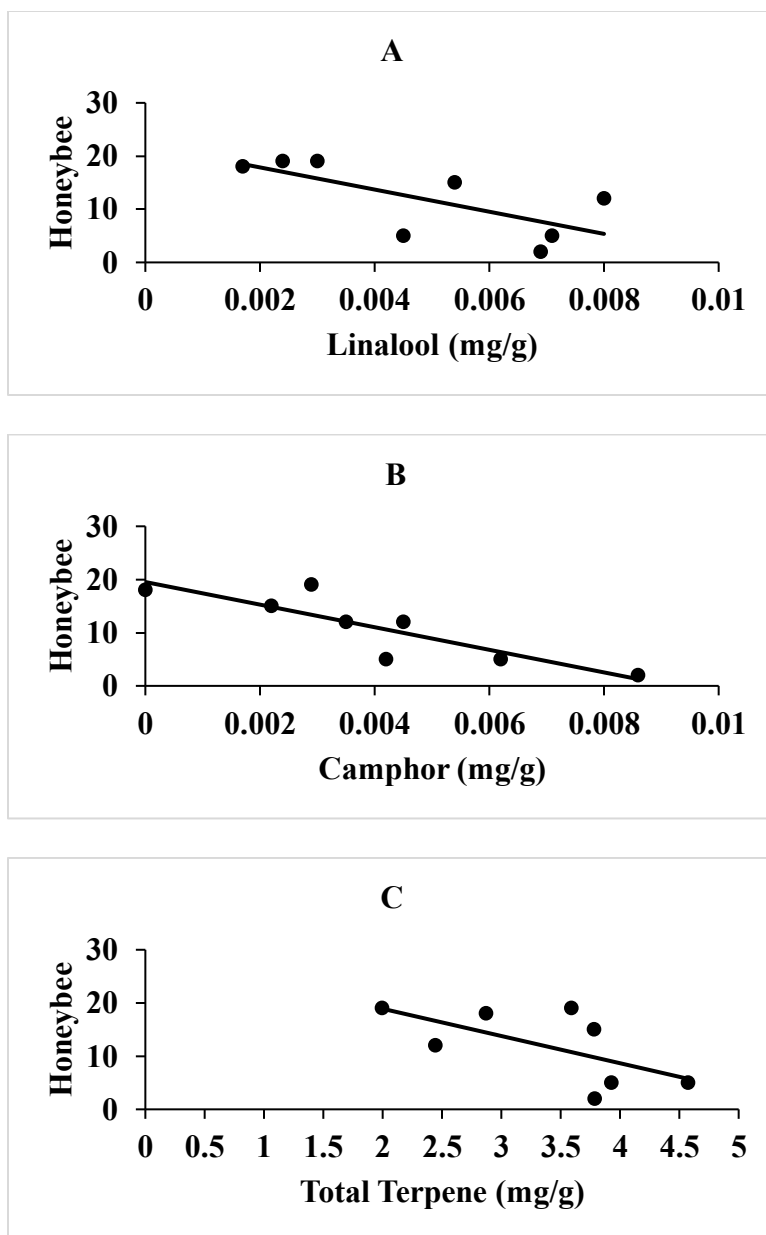
Fig. 8. Significant regressions for Honeybee with (A) Linalool (p-value 0.052 and R^2 0.492), (B) Camphor (p-value 0.006 and R^2 0.748), and (C) Total Terpenes (p-value 0.095 and R^2 0.395).

Table 3. Linear regression (JMP Pro 15) results (F, P, and R^2) for **Bumblebee** abundance and flower terpenes

Terpene	F	P	R^2
α-pinene	0.589	0.472	0.089
Camphene	2.197	0.198	0.305
β-pinene	0.005	0.944	0.001
Myrcene	0.666	0.445	0.099
α-phellandrene	1.041	0.347	0.148
P-cymene	0.002	0.969	>0.001
Linalool	2.561	0.154	0.268
Camphor	1.317	0.295	0.180
Bornyl Acetate	0.417	0.542	0.065
trans-Caryophyllene	0.662	0.443	0.086
β-elemene	0.898	0.375	0.114
α-humulene	1.593	0.254	0.209
Germacrene D	2.836	0.136	0.288
Total Terpenes	0.193	0.673	0.027

Df with no outlier removed= 1,7 for trans-Caryophyllene, Total Terpene, P-cymene, Linalool, Dermacrene D, β-pinene, β-elemene.
Df with outlier removed: 1,6 for Myrcene, Camphene, Camphor, Bornyl Acetate, α-pinene, α-phellandrene, α-humulene.
Values of $P \leq 0.1$ shown in **bold type.**

Table 4 shows that the abundance of Bees was related to linalool (marginally) and camphor (Fig. 9. A, B). As the compound concentration increased insect abundance decreased.

Table 4. Linear regression (JMP Pro 15) results (F, P, and R^2) for **Bees** abundance and flower terpenes

Terpene	F	P	R^2
α-pinene	2.198	0.182	0.239
Camphene	0.733	0.425	0.109
β-pinene	>0.001	0.989	>0.001
Myrcene	0.131	0.728	0.018
α-phellandrene	1.200	0.390	0.147
P-cymene	0.028	0.873	0.004
Linalool	5.143	**0.064**	0.461
Camphor	10.88	**0.016**	0.644
Bornyl Acetate	2.142	0.187	0.234
trans-Caryophyllene	0.415	0.540	0.056
β-elemene	0.773	0.408	0.099
α-humulene	1.461	0.272	0.196
Germacrene D	3.210	0.116	0.314
Total Terpenes	3.268	0.121	0.353

Df with no outlier removed= 1,7 for trans-Caryophyllene, P-cymene, Germacrene D, Bornyl Acetate, β- elemene, α-pinene, Myrcene, α-phellandrene.
Df with outlier removed: 1,6 for Total Terpenes, Camphene, Linalool, Camphor, β-pinene, α-humulene.
Values of $P \leq 0.1$ shown in **bold type.**
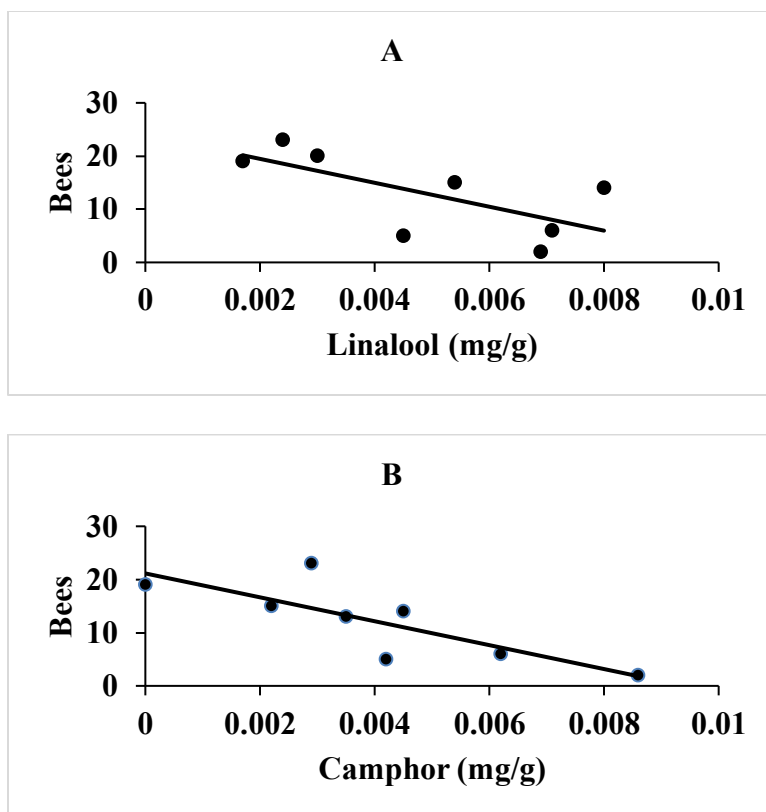
Fig. 9. Significant regressions for Bees with (A) Linalool (p-value 0.064 and R^2 0.461) and (B) Camphor (p-value 0.016 and R^2 0.644).

Diptera abundance was significantly related to linalool (Table 5, Fig. 10), with Dipteran abundance decreasing as linalool concentration increased.

Table 5. Linear regression (JMP Pro 15) results (F, P, and R^2) for **Diptera** abundance and flower terpenes

Terpene	F	P	R^2
α-pinene	0.194	0.673	0.027
Camphene	0.208	0.662	0.029
β-pinene	0.014	0.910	0.002
Myrcene	1.521	0.263	0.202
α-phellandrene	0.505	0.504	0.078
P-cymene	0.009	0.925	0.001
Linalool	5.805	**0.047**	0.453
Camphor	0.909	0.377	0.131
Bornyl Acetate	0.148	0.712	0.021
trans-Caryophyllene	0.057	0.818	0.008
β-elemene	2.321	0.171	0.249
α-humulene	0.178	0.687	0.029
Germacrene D	0.795	0.402	0.102
Total Terpenes	0.623	0.460	0.094

Df with no outlier removed= 1,7 for trans-Caryophyllene, P-cymene, Camphene.
Linalool, Germacrene D, Bornyl Acetate, β-pinene, β-elemene, α-pinene.
Df with outlier removed: 1,6 for Total Terpene, Myrcene, Camphor, α-phellandrene, α-humulene.
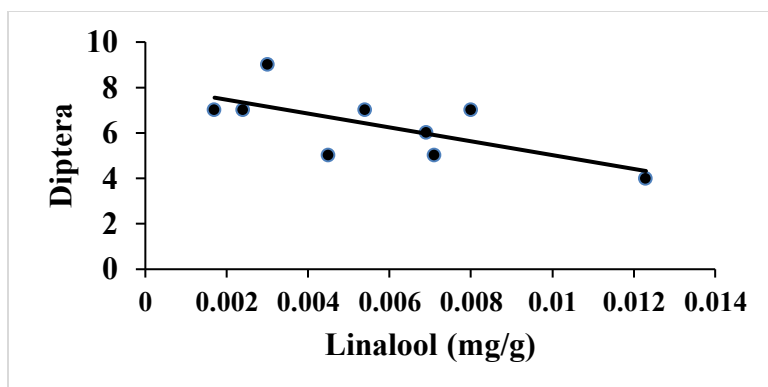Values of $P \leq 0.1$ shown in **bold type.**

Fig. 10. Significant regressions for Diptera with Linalool (p-value 0.047 and R^2 0.453).

Table 6 is data for Dominant Pollinators, which is the combination of honeybees and bumblebees. Linalool and camphor were significantly related to abundance (Fig. 11 A, B). Dominant pollinator abundance decreased as linalool concentration increased.

Table 6. Linear regression (JMP Pro 15) results (F, P, and R^2) for **Dominant Pollinators** abundance and flower terpenes

Terpene	F	P	R^2
α-pinene	2.700	0.143	0.280
Camphene	0.800	0.405	0.118
β-pinene	1.672	0.237	0.193
Myrcene	0.129	0.729	0.018
α-phellandrene	1.348	0.284	0.161
P-cymene	0.031	0.864	0.004
Linalool	6.016	**0.049**	0.501
Camphor	11.46	**0.015**	0.656
Bornyl Acetate	2.532	0.156	0.266
trans-Caryophyllene	0.473	0.514	0.063
β-elemene	0.945	0.363	0.119
α-humulene	1.390	0.283	0.188
Germacrene D	3.479	0.104	0.332
Total Terpenes	1.429	0.271	0.169

Df with no outlier removed= 1,7 for trans-Caryophyllene, Total Terpenes, P-cymene, Myrcene, Germacrene D, Bornyl Acetate, β-pinene, β-elemene, α-pinene, α-phellandrene. Df with outlier removed: 1,6 for Camphene, α-humulene, Linalool, Camphor. Values of $P \leq 0.05$ and $P \leq 0.1$ shown in **bold type.**
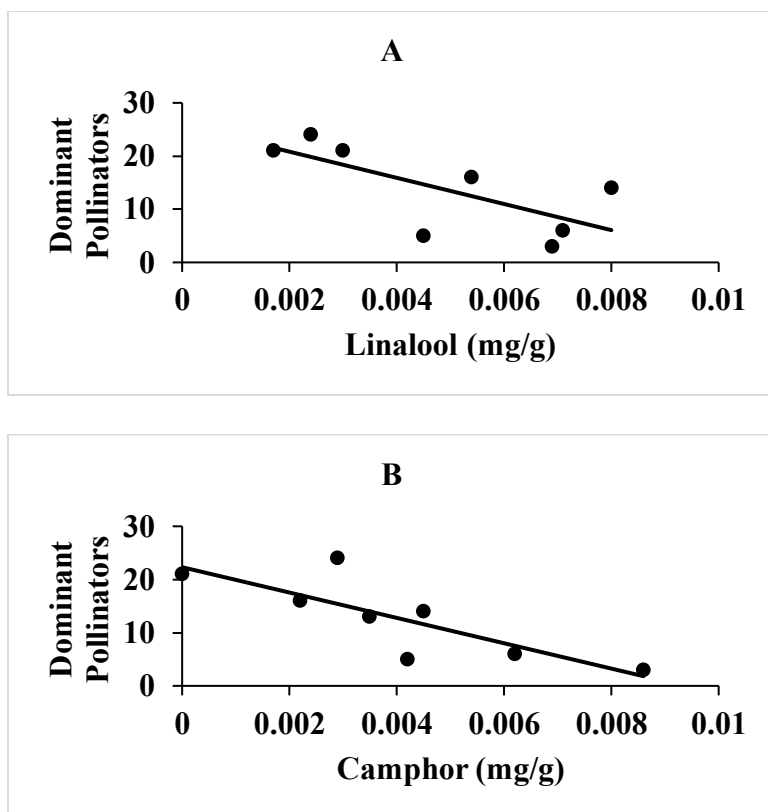
Fig. 11. Significant regressions for Dominant pollinators with (A) Linalool (p-value 0.049 and R^2 0.0.501) and (B) Camphor (p-value 0.015 and R^2 0.656).

Other pollinator abundance was significantly related to trans-Caryophyllene and Germacrene D (Table 7). As seen in Figures 12 A, B, as the terpene concentrations increases Other pollinator abundance decreases.

Table 7. Linear regression (JMP Pro 15) results (F, P, and R^2) for **Others** abundance and flower terpenes

Terpene	F	P	R^2
α-pinene	0.317	0.591	0.043
Camphene	0.015	0.906	0.002
β-pinene	1.764	0.226	0.201
Myrcene	0.044	0.841	0.007
α-phellandrene	0.007	0.937	0.001
P-cymene	0.595	0.466	0.078
Linalool	0.534	0.489	0.071
Camphor	2.024	0.205	0.252
Bornyl Acetate	1.578	0.249	0.184
Trans caryophyllene	19.69	**0.004**	0.766
β-elemene	2.578	0.152	0.269
α-humulene	0.548	0.487	0.084
Germacrene D	63.56	**>0.001**	0.914
Total Terpenes	0.694	0.432	0.090

Df with no outlier removed= 1,7 for Total Terpenes, P-cymene, Camphene, Linalool, Bornyl Acetate, β-pinene, β-elemene, α-pinene, α-phellandrene.
Df with outlier removed: 1,6 for trans-Caryophyllene, Myrcene, α-humulene, germacrene D, Camphor.
Values of $P \leq 0.1$ shown in **bold type.**
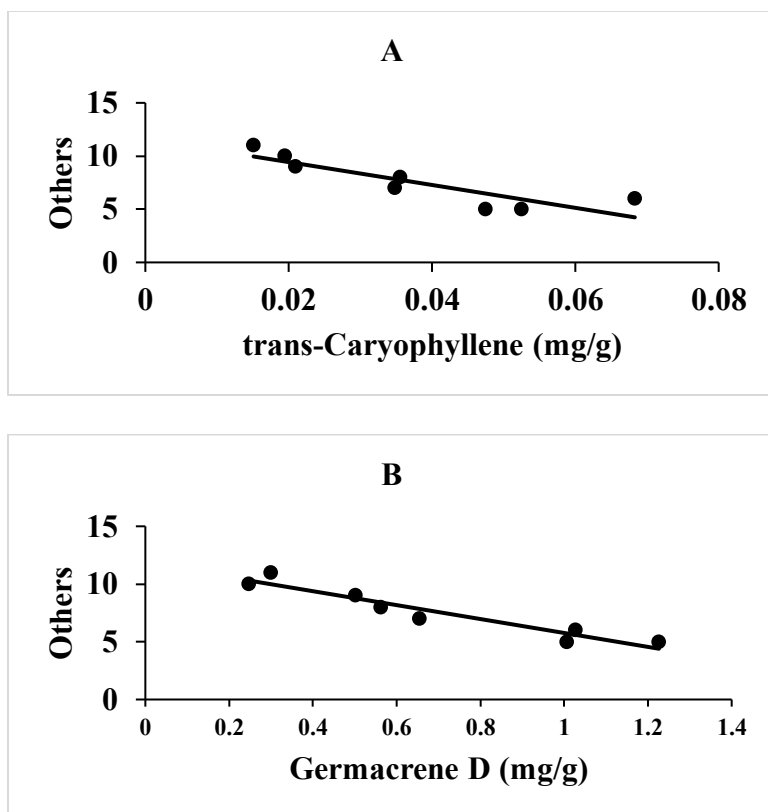
Fig. 12. Significant regressions for Others with (A) trans-Caryophyllene (p-value 0.004 and R^2 0.766) and (B) Germacrene D (p-value >0.001 and R^2 0.914).

As seen in Table 8 for All Pollinators, two compounds, linalool and camphor were significantly related to insect abundance (Fig. 13 A, B) and boranyl acetate was marginally related (Fig. 13. C). For each compound the abundance of pollinators declined with increasing terpene concentration. For the sake of representation selected non-significant relationships are presented in Fig A-F.

Table 8. Linear regression (JMP Pro 15) results (F, P, and R^2) for **All Pollinators** abundance and flower terpenes

Terpene	F	P	R^2
α-pinene	3.214	0.116	0.315
Camphene	0.745	0.421	0.110
β-pinene	1.943	0.213	0.245
Myrcene	0.258	0.627	0.035
α-phellandrene	1.306	0.291	0.157
P-cymene	0.116	0.743	0.016
Linalool	6.209	**0.047**	0.508
Camphor	14.36	**0.009**	0.705
Bornyl Acetate	4.042	**0.084**	0.366
trans-Caryophyllene	0.315	0.592	0.043
β-elemene	1.716	0.231	0.197
α-humulene	1.179	0.319	0.164
Germacrene D	2.946	0.130	0.296
Total Terpenes	1.708	0.232	0.196

Df with no outlier removed= 1,7 for trans-Caryophyllene, Total Terpenes, P-cymene, Myrcene, Germacrene D, Bornyl Acetate, β-elemene, α-pinene, α-phellandrene.
Df with outlier removed: 1,6 for Camphene, Linalool, Camphor, β-pinene, α-humulene.
Values of $P \leq 0.1$ shown in **bold type.**
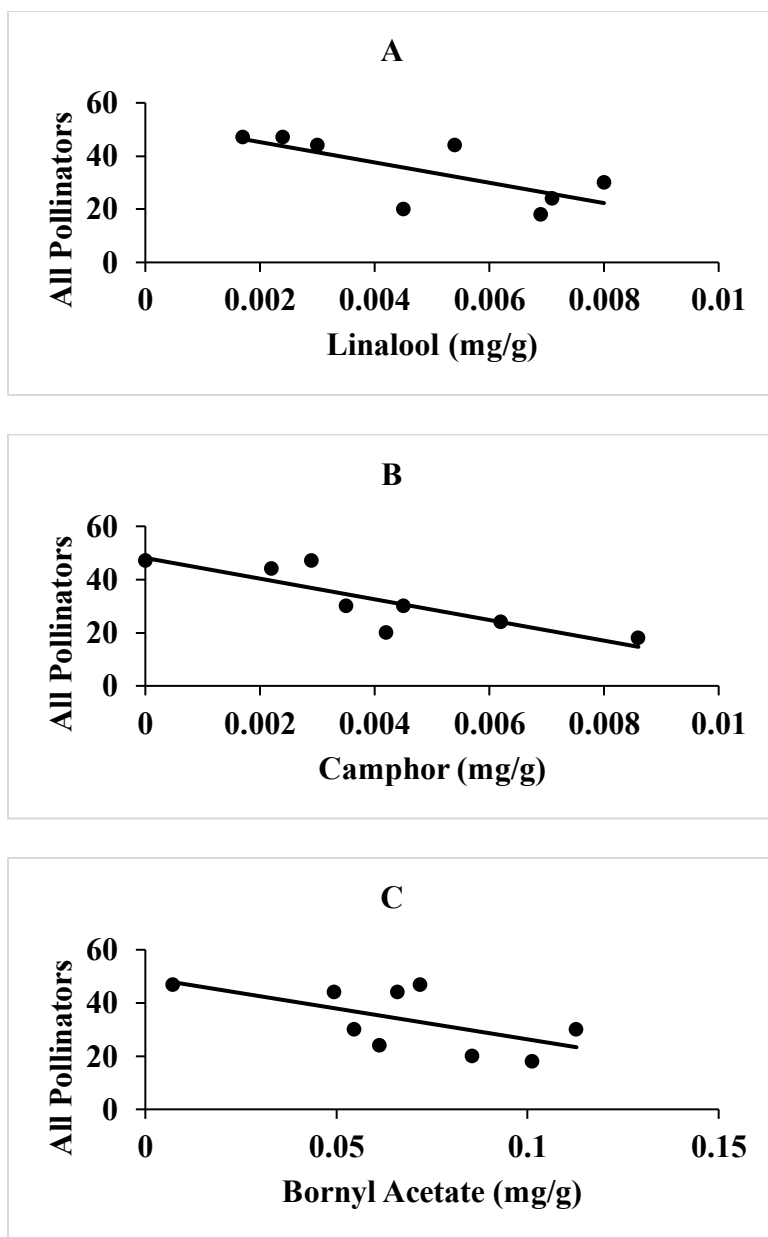
Fig. 13. Significant regressions for All pollinators with (A) Linalool (p-value 0.047 and R^2 0.508), (B) Camphor (p-value 0.009 and R^2 0.705), and (C) Bornyl Acetate (p-value 0.084 and R^2 0.366).

The results of the partial least squares analysis for All Pollinators (Fig. 14 A) and Bumblebees (Fig. 14 B) show that with all terpenes that contribute to the model included the abundance of pollinators increased with increasing concentration of terpenes.
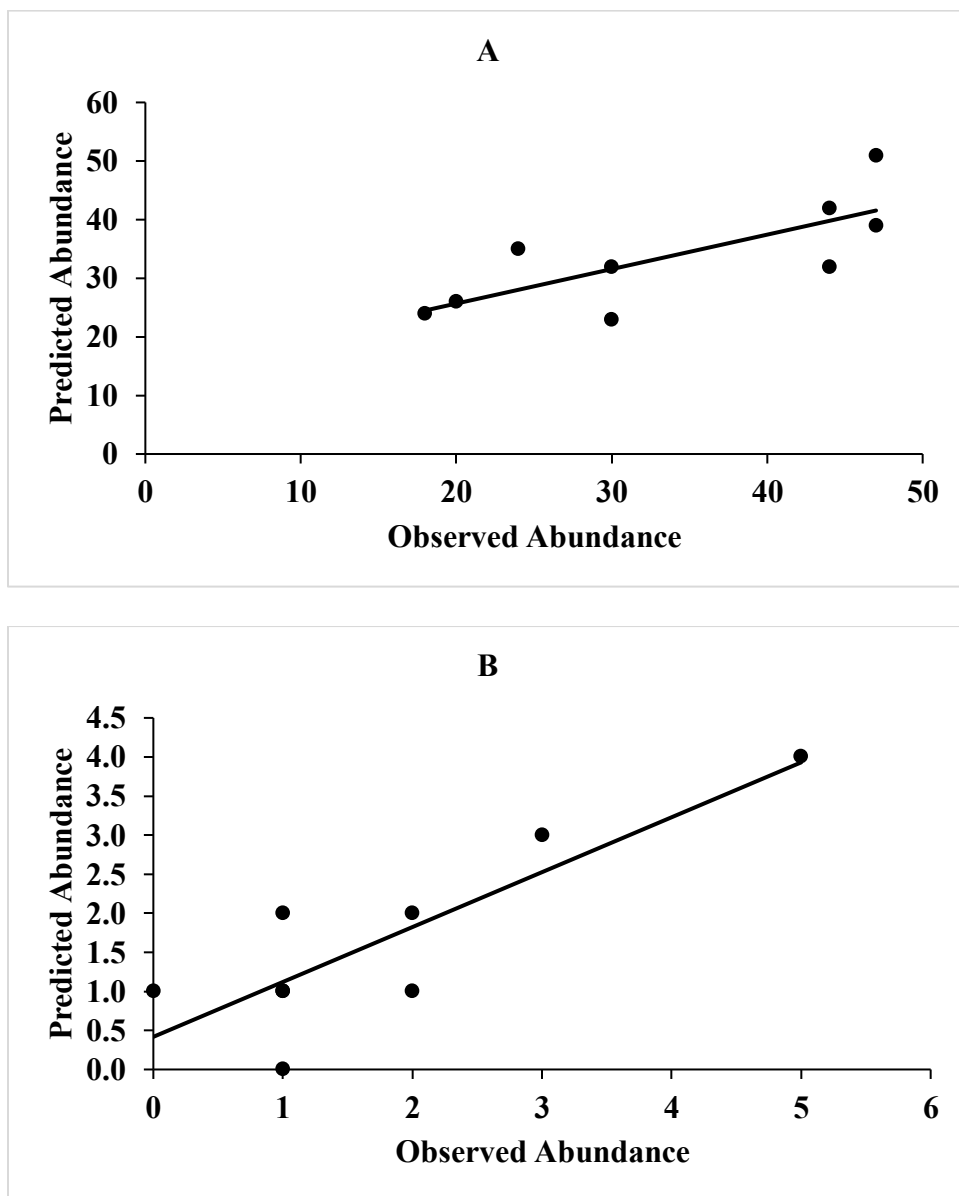
Fig. 14. PLSR Data for (A) All Pollinators and (B) Bumblebees.

Preference Trials 2021

A proportions test of the bumblebee (*Bombus sp.*) two-choice trials between a terpene and sugar alone is shown in Table 9. There was a significant choice demonstrated between β-pinene and sugar alone, with eight of the individuals choosing sugar over the terpene. There was no significant choice between sugar or P-cymene, α-pinene, and trans-Caryophyllene. The non-significance of the not equal proportions tests suggests that more trials would be needed.

Data on preference of terpenes tested in the field with a four-choice system (see Appendix 3). No significant choice was found in honeybees or wasps between terpenes and sugar wicks, with a marginally significant relationship found for yellow jackets.

Table 9. Pollinator preference lab trials

	Choose	No Choose	$\neq$
α-pinene	7	3	0.206
β-pinene	2	8	**0.058**
trans-Caryophyllene	4	6	0.527
P-cymene	3	7	0.206

Note: $p \leq 0.05$ and $p < 0.1$ (significant) presented in **bold text**.

DISCUSSION

To better understand the interaction between plant flower chemistry and insect pollinators, I investigated if there are relationships between flower terpenes and pollinator abundance and if pollinator preferences occur for certain terpenes in *S. altissima.* Although a previous study in the Williams laboratory found pollinator abundance differences among genotypes of *S. altissima* (Ragsdale, 2016), the role of flower terpenes for pollinators in this species was still relatively understudied, and knowledge about the preference for some terpenes were unknown in this plant-pollinator system. In my study, I focused on the possible role of flower terpenes for insect pollinators in a native old-field dominated by *S. altissima*, and if a bee pollinator had preferences for certain terpenes in a laboratory setting.

One major objective of my research was to investigate potential differences in flower terpenes among genotypes in *S. altissima* spread across an old-field. This plant species is well known to contain substantial intraspecific genetic variation that can affect insect species richness (Crutsinger et al. 2006, 2008) and pollinator communities (Genung et al. 2010, 2012, Burkle et al. 2013, 2017). For pollinators, variation could be due to a number of genetic and environmental factors, including soil type and floral phenology (Burkle et al. 2013, 2017). The differences found in various pollinator groups abundance among plots in my study (See appendix 1 and 2) largely support the importance of genetic variation previously seen, though I was unable to statistically analyze for genotype effects. Phytochemical differences between genotypes of *S. altissima* have been identified in previous studies, where terpenes differ among the

genotypes (Johnson et al 2010, Williams and Avakian 2015, Ragsdale 2016, Williams and Howells 2017), though flower chemistry is less explored. Data from my study found substantial variation in flower terpenes across the field (Figure 5) in support of similar findings by Ragsdale (2016).

In one of the few studies examining *Solidago* and pollinator interactions using a common garden approach, Genung et al. (2012) examined genotypic variation in *S. altissima* and *S. gigantea* and how this affected associated pollinator communities. Floral biomass and genotype differences were large contributors to differences in pollinator abundance among neighboring plants. My investigation did not have adjacent plots with which to compare abundance of insects and did not set out to determine nearest-neighbor effects, but instead, focused on terpene variation among genotypes and how this could relate to which *S. altissima* patches pollinators visit. With Total Pollinators considered, four of nine plots had higher overall pollinator abundance (Fig. 1) compared to the other five. When compared to terpene variations among plots (Figs. 5 and 6), and a significant relationship of total pollinator abundance with some terpenes (Table 8), my data support the conclusion that with all pollinator taxa combined terpenes in part explain some observed patterns of insect visitation on *S. altissima* plants.

In one study, floral insect visitor species richness was affected by genetic variation of the Solidago plants (Burkle et al. 2013). Using a common garden approach examining eight genotypes and the floral visitor abundance, they found that flowering time and soil nutrient availability influenced insect richness. An important finding was that *S. altissima* genotypes bloom at different times during the flowering season and this influences the observed community richness of pollinators. I did not observe insects on *S.*

altissima flowers in my study until at least 75% of stems in the patch were in full bloom. Though it was not a specific objective of my study, it is possible that flowering time could have had an effect on pollinator abundance I observed, as some genotypes flowered later in the season than others. One example is Plot 1, where flowers were the last to be observed because the flowering time was toward the end of the blooming season. Because my study was in a native old-field with variation among flowering stems due possibly to both genetic and local environmental effects, differences in pollinator abundances among plots I observed could partially be explained by this, according to Burkle et al. (2013). How such variation would affect terpene production in flowers, however, is unclear and would take further investigation beyond the scope of my study.

Floral fragrances are known to influence plant-pollinator interactions (Wright and Schiest 2009, Emel et al. 2017, Tölke et al. 2020). Though certain compounds in these fragrances are likely used to attract insects for pollination, their identity is still relatively unknown (Tölke et al. 2020). Terpenes are predominantly volatile compounds produced by many flowers (Borg-Karlson et al. 1996, Johnson et al. 2007, Wright and Schiest 2009, Giron-Calva et al 2017, Yip et al. 2018). Since a primary goal of my study was to investigate pollinator responses to different terpenes, both individually and in combination, accurate identification of flower terpenes was key. Many terpenes are found in *Solidago* species, including α-pinene, myrcene, p-cymene, bornyl acetate, and germacrene-D (Kalemba et al. 2001), though the composition in flowers is much less studied. In addition to the compounds previously shown for *Solidago*, I was able to quantify 13 mono and sesquiterpenes, consistent with previous work in the species by the Williams laboratory (see Williams and Avakian 2015, Williams and Howells 2017,

Thomas et al. 2019). My study identified a number of significant relationships between several terpenes in *S. altissima* flowers and pollinator taxonomic groups. The abundance of Hymenoptera, for example (Table 1 and Figure 7), declined with increasing concentrations of camphor and linalool, suggesting that these insects were cueing in on these particular compounds. In a study of the plant Dianthus which are pollinated predominantly by moths, Jurgens et al. (2003) found that linalool was involved in attracting many moth-pollinated species and some bee-pollinated flowers. and can be the main compound in some flower fragrances (Borg-Karlson et al. 1996, Dudareva and Pichersky 2000). Linalool is particularly interesting since it has been found that the pollinator *Bombus impatiens* was influenced by linalool emissions when foraging for food resources (Burdon et al. 2020) and some bee species have even been known to exude linalool in their mandibular glands (Borg-Karlson et al. 1996). Evidence such as this suggests that linalool has a strong biological significance in some pollination systems (Borg-Karlson et al. 1996). In addition to Hymenoptera, I found in my study that insect abundances declined with increasing concentrations of linalool for honeybees (Fig. 8), Diptera (Fig. 10), and dominant pollinators (Fig. 11). It seems clear that for several taxonomic groups of pollinators in my study linalool especially played an important role in insect visitation to goldenrod flowers, though more focused studies are needed to specifically elucidate which concentrations are attractants vs. deterrents. Pollinators like the honeybee and bumblebee were a particular focus of this study, with the former considerably more abundant (Fig. 2 and 3), which is largely in agreement with previous studies (Gross and Werner 1983) identifying them as one of the major pollinators of *Solidago*. While the abundance of honeybees was related to linalool and camphor (Table

2), none of the terpenes I identified affected bumblebee abundances (Table 3). This suggests different cues are used by these pollinators and it is currently not known, from either an ecological or physiological standpoint, why honeybees and bumblebees should respond so differently.

Flowers are composed of many combinations and ratios of chemical compounds (Williams and Dodson 1972, Filella et al. 2011, Benvenuti et al. 2020) that can contain both attractants and repellents towards insect visitors, and distinguishing between those compounds is important for understanding how plant-pollinator interactions work (Williams and Dodson 1972, Boncan et al. 2020). Pollinators can have preferences for pure chemical compounds and altering those compounds slightly by adding additional compounds can reduce pollinator attraction (Williams and Dodson 1972). These authors found that visitation by Euglossine bee species was greatly reduced when α-pinene was added to the combination of floral fragrances. I did not examine foliar airborne fragrances but rather, used internal terpene concentrations as a surrogate for what would be volatilizing from the flowers. If, however, internal and airborne amounts are not highly correlated, then further studies would have to be done, such as headspace analyses of volatile terpenes to determine what pollinators are experiencing in the atmosphere as they approach *Solidago* flowers. In my study, I did not find that α-pinene was related to pollinator abundances, although, in my feeding trials, bumblebees did prefer this compound over sucrose. But in the field, this compound would not occur in isolation from other terpenes, and one of the objectives of my research was to determine if combinations of compounds affected pollinator preferences and abundances. I was able to do this using a Partial Least Squares statistical approach. In isolation, linalool was

negatively correlated with pollinator abundance, but for all pollinators and bumblebees, there was a strong positive relationship when a combination of compounds were used in the predictive model (Figs. 13 and 14). This is somewhat supportive of Williams and Dodson (1972) and illustrates the complex nature of flower chemistry for pollinator attraction.

Though a regression relationship between terpenes and insect abundance is insightful and suggestive, this analysis does not actually demonstrate a causal preference for individual terpenes. Another objective of my study was to perform laboratory choice trials to investigate whether bumblebees prefer "flowers" spiked with particular terpenes over those with sugar alone. As seen in Table 9, bumblebees prefer α-pinene to a "flower" where it is absent, suggesting this terpene may play a role in choosing which plants to visit. My findings stand in contrast to other studies that have found α-pinene to act as a bee repellent (Williams and Dodson 1972, Fernandes et al. 2019). My data from additional feeding trials showed that bumblebees preferred a sucrose solution over the terpenes β-pinene and P-cymene, suggesting that these compounds may not influence whether bumbles pollinate *S. altissima* flowers.

Though I did not test the role of a visual cue as others have done (see Barragán-Fonseca et al. 2020), the use of the same artificial *Solidago* "flower" in all trials suggests this was not a major factor in my insect choice results.

In conclusion, I found that some flower terpenes in *S. altissima* may play a role in determining the abundance of insect pollinators but that it varies depending on the insect taxonomic group. My data also found that a number of pollinator groups responded to linalool, a terpene known previously as important in plant-pollinator systems. I also

found a robust relationship between terpenes and pollinator abundance when all terpenes were analyzed in combination, suggesting that insects likely use a combination of chemicals to make flower visitation decisions. My investigation sets a framework for further studies to test more individual terpenes, and their combinations, to elucidate the importance of terpene preference for pollinators in the *S. altissima* plant system.

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APPENDICES

Appendix 1

Total Pollinator abundance by observation trial

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8	Plot 9
	Low Elevation			Middle Elevation			High Elevation		
Trial 1	31	23	44	33	44	35	22	14	23
Trial 2	42	31	43	20	30	43	21	21	33
Trial 3	75	23	58	34	50	54	13	17	27
Trial 4	40	19	30	34	50	56	17	28	38
Total	188	96	175	121	174	188	73	80	121

Appendix 2

Abundance of honeybee and bumblebee by observation trial

Honeybees

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8	Plot 9
Trial 1	6	2	11	13	19	12	1	2	1
Trial 2	24	8	9	7	15	25	1	4	16
Trial 3	22	3	19	15	13	23	2	4	19
Trial 4	20	6	20	13	27	17	3	10	12
Total	72	19	59	48	74	77	7	20	48

Bumblebee

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8	Plot 9
Trial 1	0	1	2	2	1	1	0	0	0
Trial 2	3	4	1	2	4	3	2	0	0
Trial 3	1	0	0	1	2	9	0	0	0
Trial 4	7	0	0	1	1	8	0	0	2
Total	11	5	3	6	8	21	2	0	2

Appendix 3

Terpene preference field trials

	Total # of Visits	P	X^2
Honeybees	23	0.667	1.565
Wasps	28	0.859	0.762
Yellow Jackets	308	**0.066**	7.203

Note: $p \leq 0.05$ and $p < 0.1$ (significant) presented in **bold text**.

VITA

Faith Laurel Weaver was born in 1997 in Gainesville, Florida to mother Sonjia and father Brian Weaver. She attended Appalachian State University and received a Bachelor of Science in Biology with a concentration in Cellular/Molecular Biology in 2019. Faith began the Biology Graduate program in August of 2019 at Appalachian State University.