Development of a behaviorally-based pest management strategy for *Eucosma giganteana*

by

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Abstract

Eucosma giganteana (Riley) (Lepidoptera: Tortricidae) is a specialist pest of plants within the genus *Silphium*. *Silphium integrifolium* Michx. is a perennial oilseed crop native to the prairies of North America. Because of its perenniality, it is touted as a more sustainable alternative to the sunflower systems; however, *E. giganteana* is a major limiting factor to the domestication and commercialization of this crop. Despite the yearly damage from *E. giganteana*, there is currently no consistent pest management strategy, rather, the timing of treatment and the treatment type have been highly variable. To have a consistent idea of when to potentially apply a pest management strategy, a growing degree day model can be used. Using this model, the emergence and phenological events of the insect can be accurately predicted in the field and adults can be targeted before they can lay their eggs, thereby effectively disrupting their lifecycle and reducing the density of the larvae that infest flower heads. Timing is only part of what makes a pest control method effective, you also need to know how much a pest population needs to be reduced for a plant to continue to successfully produce. This is known as a damage or economic threshold. Even when a successful pest management program is in place, monitoring pest populations through trapping can provide insight on the current pest populations. Lastly, knowledge of the pest's response to chemical cues within its environment can help inform what types of pest management will be effective against the pest. My project aimed to address these different aspects of developing a pest management strategy for *E. giganteana*. Firstly, I address my first objective: to review and synthesize the state of mating disruption of Lepidoptera (Ch. 1). Then, I cover my second objective: to first link trap captures of *E. giganteana* to plant vigor and/or pest phenology and growing degree days; and then to develop a trap-based threshold or growing degree day model for *E. giganteana* to guide its mangement

using insecticides (Ch. 2). Finally, I go over the studies pertaining to my third objective: to explore the physiological response of *E. giganteana* to common attractants and *S. integrifolium* (Ch. 4); to elucidate the flight capacity of *E. giganteana* and its behavioral response to attractants (Ch. 4); to examine the field response of *E. giganteana* to increasing concentrations of (*E*)-8-dodecenyl acetate (Ch. 3); and to determine headspace emissions from conspecific moths and *S. integrifolium* (Ch. 4). By using Ethovision software to quantify the distance moved in millimeters of *E. giganteana* larvae that were subjected to different acclimation and test temperatures, I determined that their lower activity threshold was approximately 17 °C. The lower activity threshold coupled with localized weather data was used to create a growing degree day model. Field trapping of *E. giganteana* in 2023 and 2024 as well as previously collected data from 2019 and 2020 were used to determine the degree day measures for specific adult *E. giganteana* phenological events. With the 2019 data functioning as the predicted degree day measurements and the following three years functioning as the actual degree day measurements for the same phenological events, I found that the degree day model did successful predict when these events would occur. Unfortunately, I was unable to determine the number of *E. giganteana* larvae required to cause significant damage to *S. integrifolium* root crowns, and thereby form a damage threshold. Using a previously documented pheromone attractant, (*E*)-8-dodecenyl acetate, at different concentrations to trap adult *E. giganteana*, I found that a low concentration was highly effective at monitoring *E. giganteana* presence when used on its own. However, a 100-fold higher concentration of this attractant will mask the effect of the low concentration. Through examining the antennal responses of both sexes of adult *E. giganteana* to common semiochemicals within their environment, I found that the sex has an effect on how strongly the moth reacts to the stimulus. I also found that both male and female moths responded to (*E*)-8-

dodecenyl acetate and female *E. giganteana* headspace which indicates that the females may autodetect their pheromone based on their antennal response. I did not find a difference in the flight propensity and distance flown of adult *E. giganteana* individuals when in the presence of different concentrations of (*E*)-8-dodecenyl acetate and the opposite sex moth. Similarly, I did not find a significant difference in the headspace composition collected from males, females, or a 50/50 mix of adult *E. giganteana* moths. This research is expected to help us better understand the behavior of *E. gigantena* and guide the timing of pest management within *S. integrifolium* fields.

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Dedication

To the little kid who wanted to show the world the beauty in the little creatures all around them and to the many people that encouraged and supported them along the way.

1 A Review of Mating Disruption in Lepidoperan Pests

1.1 Objective 1: To review and synthesize the state of mating disruption of Lepidoptera.

1.2 Abstract

Agricultural pests are of large concern to our food production and storage across the world. Many of these major agricultural pests are moths belonging to the order Lepidoptera. There has been increasing concern over insecticide residues, insecticide resistance, and environmental damage from common insecticides used in controlling these pests. Mating disruption has been proposed and implemented in many places as a more sustainable, effective, and non-toxic pest management strategy. However, there are many different options and considerations that should be taken into account when deciding to use a mating disruption strategy. Each strategy uses different dispensers, formulations, and densities to create effective pest control. Evaluation of mating disruption also has multiple different methods for assessing efficacy including male attraction, mating success, progeny development, and crop damage. Despite the many options for mating disruption throughout different types of pests and environments, there are also a few downsides that need to be considered such as cost. Mating disruption can be an environmentally conscious and successful pest management strategy when applied correctly. The main purpose of this review is to provide an overview on mating disruption in lepidopteran insects, utilities of pheromone dispensers, efficacy and evaluation of mating disruption, and main downsides related to the use of mating disruption in in pest management programs.

Key words: mating disruption, moths, behaviorally-based management, integrated pest management, pheromone dispensers, mechanisms

1.3 Introduction

As insecticide resistance and environmental damage continue to be problems in agriculture, integrated pest management (IPM) strategies, which are more selective and effective against insect pests than broad spectrum insecticides, are being brought to the forefront. IPM includes cultural, chemical, biological, and physical control methods (Chen et al. 2014). IPM strategies attempt to work with the understanding of the pest's biology and only use pesticides as a last resort (Amarasekare and Shearer 2017; Morrison et al. 2021; Stern 1959). Increasingly, there has been introduced legislation and public concern surrounding the usage of insecticides especially regarding their environmental damage, non-target effects, possible harm to workers, and the residues left on food products (Kong et al. 2014; Kovanvi et al. 2005; Lo Verde et al. 2020). In addition, there has been documented resistance of many pests to common insecticides as well as cross resistance to insecticides that contain related compounds or attack the same target sites (Dhanyakumar et al. 2020; Machucha-Mesa et al. 2023; Morrison et al. 2021; Nayak et al. 2020). As a result, IPM practices have pushed for behaviorally-based pest management strategies that take into account the insect's behavior and chemical cues to manipulate pest populations for the protection of the commodity (Morrison et al. 2021). Such strategies include attract and kill, mass trapping, mating disruption, and more (Cardé 2007; Morrison et al. 2021). Mating disruption (MD) is highly specific to the target insect and most commonly utilizes the insect's female-produced sex pheromone to which generally only males respond (Ferracini et al. 2021; Kong et al. 2014; Lance et al. 2016), though there may be some cases of female autodetection (Holdcraft et al. 2016). In this review, we will provide an overview on Lepidoptera MD, encompassing origin and mechanisms; types of dispensers, both passive and active; considerations with dispenser placement including location, density, and environmental conditions; different formulation types; how MD efficacy is evaluated; the challenges with MD; and gaps within the current research.

1.4 Mating Disruption Overview

1.4.1 Origins of mating disruption

Mating disruption became a strategy for pest control after the advent of pheromone identification. Insect pheromone identification for many pest species did not begin until the late 1960s; however, scientists quickly created synthetic sex pheromones and put them into practice as early MD tactics in the 1970s and 1980s (Cardé et al. 2007; Miller and Gut 2015). Consequently, the use of MD rapidly became a widespread pest control tactic across the globe. These tactics have been elucidated in facilities against stored product pests, in the field against crop pests, in greenhouses, in large scale forest applications for invasive species, and even in urban areas (Athanassiou et al. 2016; Bohnenblust et al. 2011; Brokerhoff et al. 2012; Burgio et al. 2020; Ferracini et al. 2021; Lance et al. 2016; Morrison et al. 2021; Soopayaet al. 2015; Trematerra et al. 2019; Witzgall et al. 2010). Lepidopteran pests often create a challenge for management, especially with field and greenhouse crops. Most lepidopteran adults are non-feeding, rather their injurious life stage are the larvae. In many cases, their larvae are internal feeders, so the window to effectively manage the larvae lies between when they hatch from eggs and before they bore into the fruit or stem of the plant (Bohnenblust et al. 2011; Chen et al. 2014; Cocco et al. 2012; Lykouressis et al. 2005; Sreenivas et al. 2021). Insecticides are not usually effective against the eggs, and it is difficult to effectively target the larvae while they feed internally (Nakanishi et al. 2013). This creates a challenge for pest management. Like many other insect orders, Lepidopteran insects can experience multiple generations within a growing season (Ricciardi et al. 2024). If insecticides are used to manage them, then many treatments would be needed through the growing season to be effective (Pluciennik 2013). This can make pest management difficult and expensive. In contrast, MD tactics need only to be applied once or a few times during the growing season (Pluciennik 2013). They also exclusively target the adults, and therefore, do not encounter the issue of internally feeding larvae. For different lepidopteran pest species that MD tactics are deployed against, the action mechanisms of MD can vary widely.

1.4.2 Mechanisms of mating disruption

When a MD tactic is deployed, pheromone dispensers generally saturate the environment with a synthetic pheromone blend mimicking that of a calling female, thereby inhibiting male pests from finding females and decreasing the mating success (Kong et al. 2014). If MD is effective, it will decrease future pest populations, by reducing the progeny of the current population, and breaking the pest's life cycle (Cocco et al. 2012; Ferracini et al. 2021). The mechanisms of MD are often divided into competitive and non-competitive (Miller and Gut 2015). In competitive MD, the male moths fall prey to false trail following, where the plumes of pheromone being released by the dispensers attract males towards the dispensers (Kong et al. 2014; Miller and Gut 2015). There is then competition between the pheromone being released by the calling females and that being released by the dispensers. The amount of sex pheromone dispensers in an area usually outweighs that of calling females, so the male moths are more likely to locate the pheromone plume produced by the dispensers and follow that trail (Kong et al. 2014; Miller and Gut 2015). By following this trail, they are unable to locate female moths and successfully mate. However, competitive MD is highly dependent on the population density of the pest species. Higher pest population densities ultimately decrease the effectiveness of the

MD (Miller and Gut 2015). With a higher population, the odds that a male comes across a calling female is greatly increased regardless of the quantity of synthetic pheromone released in the environment. In non-competitive MD, male moths experience sensory impairment where they are overwhelmed by the additional pheromone application (Cardé 2007). This sensory impairment can lead to habitation in many cases (Miller and Gut 2015). Non-competitive MD is less common as it requires a higher saturation of pheromone into the environment which can be difficult to maintain over time. There are occasionally other terms for MD mechanisms including camouflage, allochrony, masking, and induced allopatry; however, these terms usually describe sub-groups under either competitive or non-competitive MD (Miller and Gut 2015). Although there are similar mechanisms for MD, the way a MD tactic is deployed can be highly variable and specialized for the specific target pest.

1.5 Pheromone Dispensers

There are many different types of pheromone dispensers available to administer MD to a given environment. One point of confusion within current MD literature is the use of "dispenser" as a common term for how pheromone application is being administered for MD. Not all literature specifies the type or brand of dispenser used, which can make replication of experiments difficult. Pheromone dispensers fall into two categories, either active or passive dispensers. The largest diversity in dispensers can be found within the passive dispensers.

1.5.1 Passive dispensers

Most passive dispensers are hand-applied like polyethylene tubes, wax, mesoporous tablets, polyvinyl carbonate, plastic ampoules, tab dispensers, dual-capillary tubes, poly-mix, cylindric silicon dispensers, impregnated halloysite beads, and impregnated rubber septa (Gavara et al. 2022; Kim et al. 2024; Kong et al. 2014; Ricciardi et al. 2024; Savoldelli et al. 2023; Trematerra et al. 2019; Vacas et al. 2011; Wijayaratne and Burks 2020). Both polyethylene tubes and microencapsulated dispensers are often referred to as reservoir type dispensers since they are composed of a pheromone formula suspended inside a polymer matrix (Ortiz et al. 2021). Polyethylene tubes, also sometimes referred to as rope dispensers, plastic polymer tubes, or twist ties, use a reservoir surrounded by a semipermeable membrane to allow for a slow dissipation of the pheromone held within (Ricciardi et al. 2021). These dispensers can be made in multiple shapes, sizes, and lengths to suit their pheromone content and the area they are being used within. When purchased, they are preloaded with the synthetic pheromone designed for a certain pest or multiple pests. They are relatively low cost to manufacture compared to other MD dispensers, but they have a high labor cost to apply them because of the density required for them to disperse enough pheromone for MD (De Lame et al. 2010; Miller and Gut 2015). The labor cost can be further exacerbated in orchards or forests where additional time and equipment is required to attach the dispensers amongst the canopy of the trees (Miller and Gut 2015). Unfortunately, they also decay fairly quickly in the field through environmental conditions and UV degradation. Lo Verde et al. (2020) found that the pheromone release rate was highest when first applied, then declined relatively steadily for the next 50 days, but after 72 days it began a steep decline. Because of this, additional field applications of the polyethylene tubes may be required for effective MD. Regardless of all of this, polyethylene tubes are still one of the most commonly used dispensers for MD.

Impregnated rubber septa are not as commonly used for MD strategies; however, they are often used to bait monitoring traps. They are quite expensive to manage because of their cost to purchase, labor cost to apply, and the speed at which they decay when exposed in the field (Kong et al. 2014). Similar to the polyethylene tubes, they require a high density to emit enough pheromone for MD. Like the impregnated rubber septa, there have not been many studies using mesoporous dispensers, but unlike the previous dispenser types, they can be easily adjusted to accommodate different pheromone quantities which makes them more versatile. Because of their small size, generally only about a centimeter in diameter, they are easily deployed in greenhouse settings (Hasan et al. 2023)**.**

Tab dispensers work a little differently than most other dispensers. They are a powdery pellet containing the pest's sex pheromone. These dispensers are held within a structure that allows the pest to land upon it. Because of its powdery makeup, when a moth lands on it or wing fans adjacent, the powder spreads through the air and onto the insect. The moth is now covered in synthetic pheromone, acting as an additional pheromone source. They also cause more confusion for the males when doused in pheromone (Hasan et al. 2023). These dispensers are only effective in indoor facilities since the material would be easily degraded if exposed to wind and rain.

Wax dispensers can be either hand- or machine-applied. Regardless, they are made by mixing the sex pheromone with wax and occasionally other ingredients like water and vitamin E (De Lame et al. 2010; Kong et al. 2014). The wax component can be paraffin or microcrystalline (Cardé 2007; De Lame et al. 2010; Kong et al. 2014; Stelinski et al. 2007). SPLAT (specialized pheromone and lure application technology) is a commonly used wax based pheromone dispenser system that has been used to manage many lepidopteran pests including the pink bollworm (*Pectinphra gossypiella* (Saunders)), the spongy moth (*Lymantrai dispar* Linnaeus), and the light brown apple moth (*Epiphyas postvittana* (Walker)) (Onufrieva et al. 2019; Soopayaet al. 2015; Sreenivas et al. 2021; Stelinski et al. 2007). Depending on the wax formulation, it can be hand-applied; with makeshift applicators, spoons, syringes, plastic squirt bottles, caulking guns, or be sprayed directly onto the crops (De Lame et al. 2010; Onufrieva et al. 2019; Soopayaet al. 2015; Sreenivas et al. 2021). The most common hand-applied formulations for wax dispensers are paste-like and supports itself when applied to an object like a tree or fence, however it can also be made into a solid wax disk that can be hung in tree canopies or alongside the commodity, but this is much less common (Soopayaet al. 2015; De Lame et al. 2010). Lastly, it can be made thinner into a formulation that can be sprayed out as small droplets either by aircraft, air-blast sprayers, or a specialized tractor attachment (Miller and Gut 2015; Stelinski et al. 2007). With the hand-applied formulations, the size and shape of the wax droplet can be adjusted to add or decrease pheromone concentration within each wax matrix (Svensson et al. 2017). Another positive to wax dispensers is that the majority of them are biodegradable, furthering their accolades as an environmentally safe option (Stelinski et al. 2007). Similar to other passive dispensers, wax dispensers can experience UV degradation, but unlike the hand-applied dispensers, the sprayable wax formulation has the additional problem of being washed away when it rains (Kong et al. 2014; Miller and Gut 2015; Sreenivas et al. 2021; Stelinski et al. 2007). Because of these factors, multiple applications are often required to maintain a continuous rate of MD across a growing season.

Other machine applied dispensers include flakes, microencapsulated formulas, and fibers (Miller and Gut 2015). These dispensers are applied by air-blast sprayers or by aircraft (Cardé 2007; Régnière et al. 2019; Stelinski et al. 2007). Both of these application techniques are commonly used for other crop management applications like pesticides and herbicides. To aid in cost effectiveness, machine applied MD strategies can be mixed with or applied in synchrony with these other management sprays (Kong et al. 2014; Stelinski et al. 2007). Microencapsulated formulas are made of microscopic polymer capsules containing small doses of pheromone

(Miller and Gut 2015). Flakes, sometimes called laminate flakes or microflakes, are small pieces of plastic imbued with pheromone and coated in a sticking agent to allow adherence to the crop being protected (Cardé 2007; Mori and Evenden 2015; Régnière et al. 2019). Microflakes and microencapsulated formulas have been used for management of many pests including the spruce budworm (*Choristoneura fumiferana* (Clemens)), *E. postvittana*, and the navel orangeworm (*Amyelois transitella* (Walker)) (Bohnenblust et al. 2011; Brokerhoff et al. 2012; Haviland et al. 2021; Régnière et al. 2019). Unfortunately, with all these machine-applied MD dispensers, their pheromone release is short lived, requiring multiple applications throughout the pest's generations to provide adequate control (Miller and Gut 2015). They also have difficulty adhering to the foliage they are applied to and can be washed away with rainstorms (Bohnenblust et al. 2011; Miller and Gut 2015).

1.5.2 Active dispensers

Puffers, also known as high-emission dispensers, aerosol dispensers, misters, micro sprayers, or atomizers, are helpful for long-term dispensing capabilities as they are active dispensers (Higbee et al. 2008; McGhee et al. 2016; Mori and Evenden 2015). Puffers are made by diluting a synthetic pheromone with an inert carrier and putting the blend under pressure in a canister (Cardé 2007). A plastic cabinet that contains this pressurized canister, an actuator, a battery, and a timer complete the "puffer" (Mori and Evenden 2015; Vacas et al. 2016). They can be specially programmed to release a spray of pheromone at a specific interval or during a particular time of day to align with the pest's natural circadian rhythm (Higbee et al. 2017). If programmed correctly, puffers can apply sufficient MD without wasting valuable active ingredients. Metered Semiochemical Timed Release System (MSTRS) is a type of puffer which sprays a membrane pad that is held out in front of the sprayer (Baker et al. 2016). The pheromone saturated pad then slowly disperses the pheromone into the surrounding environment. One of the positives of puffers is that unlike most other MD dispensers, they do not expose their active ingredients to the elements which protects them from evaporation and UV degradation, thus saving expensive material and increasing their lifespan (Cardé 2007; Vacas et al. 2016). Although they can sometimes be more expensive to purchase, they are less labor intensive when applied in the field (Higbee et al. 2008). The cabinet portion is also reusable and can be restocked throughout the growing season to allow sufficient pheromone application (Baker et al. 2016; Cardé 2007; Mori and Evenden 2015). Unfortunately, by virtue of being mechanized, unlike the hand-applied dispensers, they run the risk of experiencing mechanical malfunctions. If not caught quickly, the mechanical issues can result in ineffective pheromone release. Repairing the dispensers can require additional knowledge, time, and money (Lance et al. 2016; Vacas et al. 2016).

Each dispenser type has its own advantages and drawbacks ranging from the manufacturing cost, ease of application, and overall effectiveness against a given pest. In the more than fifty years since MD has been employed as a pest control strategy against Lepidoptera, there have been many new innovations and discoveries. Because of their ease in manufacturing and their documented efficacy against many pests, polyethylene tubes are still one of the most commonly used types of dispensers (Table 1.1). As more research has been conducted, the popularity of other dispensers like puffers and wax has increased over time, largely because of their additional sustainable elements (Figure 1.1). Dispensers alone are not what determine the efficacy of a MD strategy. There are many other factors, such as the surrounding environment, deployment density and the pheromone formulation, can affect efficacy.

Table 1.1 Common pheromone dispenser types for mating disruption in Lepidoptera. Data were gathered from a literature search using keywords of "Lepidoptera" and "Mating Disruption". Review papers were not included resulting in 54 papers published between 2010 and 2024 that met our criteria. Occurrences of dispenser type were only recorded if they were used in three or more studies .

Common Dispenser Types		
Common name	Trade names	Application occurrence
Micro-Flakes	Disrupt Micro-Flakes	4
Microencapsulated formula	Cidetrak® CM DA-MEC, Checkmate® CM-F	5
Wax	SPLAT	9
Puffer	Suterra puffer, Isomate Mist, Checkmate puffer, MSTRS	17
Polyethylene tube	Isonet® reservoir dispenser, Shin-Etsu rope dispenser, Checkmate OFM	27

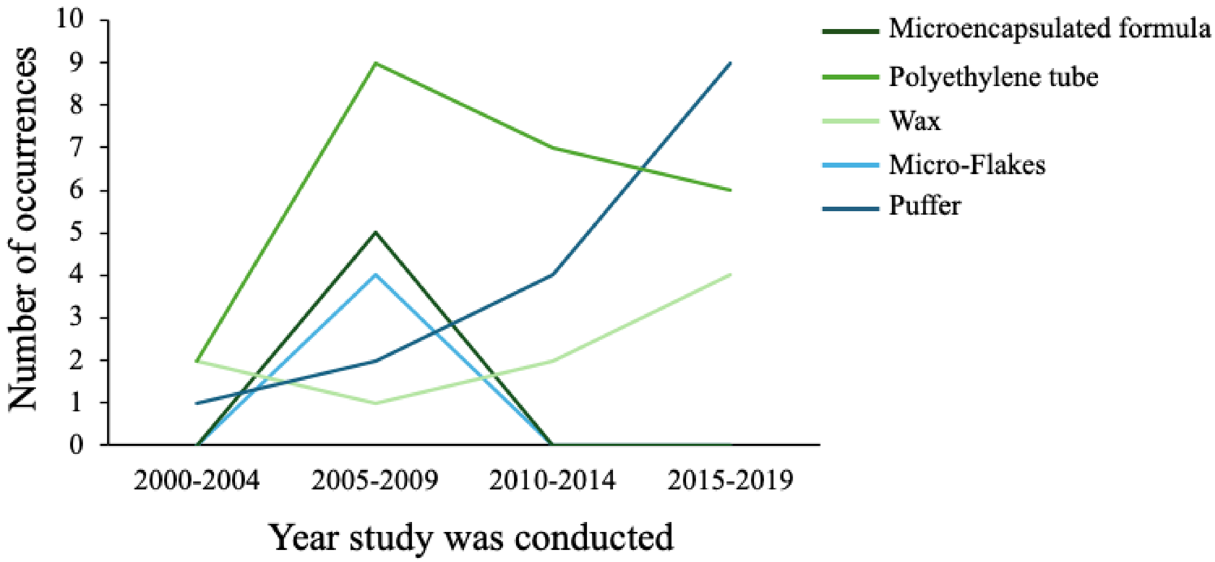

Figure 1.1 Common pheromone dispenser types usage over time based on data collected from 54 papers published after 2010. The year of the experiment was not included for three of the papers that used polyethylene tubes and one of the papers that used a puffer.

1.6 Dispenser Placement

1.6.1 Density

Along with the type of dispenser used, the deployment density is an important consideration. Good area coverage is crucial especially as many pest moth species are highly mobile (Cardé 2007). Hand-applied dispensers usually dispense pheromone at a continuous low level requiring them to be placed at a higher density to achieve efficient MD coverage (Kong et al. 2014). To achieve this coverage, most hand-applied dispensers should be applied at a rate of 250 to 1000 dispensers per hectare depending on the environmental conditions and the amount of pheromone contained within the dispenser (De Lame et al. 2010; Clavijo McCormick et al. 2012; Curtiss et al. 2023; Epstein et al. 2011). Machine deployed dispensers like microencapsulated formulas and micro flakes end up being deployed at a much higher density of a million or more sources per hectare, but they are easier to apply to reach these densities than the hand-applied dispensers and they release a smaller amount of pheromone from each source (Cardé 2007; Miller and Gut 2015). Puffers are deployed at a much smaller density of only around 1 to 3 dispensers per hectare because they release a higher pheromone content from each of their sources than either the hand or machine applied dispensers (Curtiss et al. 2023). In addition to the density the dispensers are applied at, the placement of the dispensers within a MD treated area is also crucial (Ferracini et al. 2021).

1.6.2 Placement

In most cases, the pheromone dispensers should be placed off the ground, within the flight path of the pest. The flight path is usually located near the top of the crop or commodity which can be many meters high when in forested or orchard environments (Epstein et al. 2011; McGhee et al. 2016). Depending on the dispenser type, they can be placed directly onto the commodity, onto a permanent structure nearby, or onto a placed structure alongside the commodity (Hasan et al. 2023; Hegazi et al. 2010; Wijayaratne and Burks 2020). In fields, bamboo poles are often used to hold dispensers at a consistent height as they are inexpensive and do not alter the surrounding habitat (Wu et al. 2012**.** Hoshi et al. (2016) found that polyethylene tube dispensers placed at 1.5 m of height into trees supplied sufficient MD up through the canopy of the trees in the orchard at a tested height of 3 m. However, the height of the trap is not the only consideration. The density of pheromone dispensers only takes into account the number throughout the area, but not their specific placement within that given area. Whether the dispensers are placed towards the center of an area or along the perimeter of the area can affect the application of pheromone. Dispensers that are clustered close to one another tend to provide more effective pest control whereas dispensers that are not as clustered, usually those along the perimeter of the treated field, do not provide as sufficient MD (Lo et al. 2013; Lo Verde et al. 2020; Porcel et al. 2014; Steyn et al. 2024; Tollerup et al. 2012). This could be due both to the decreased pheromone density in those areas with fewer dispensers or because of possible immigrating gravid females from other fields that are not treated with MD (Gordon et al. 2005; Higbee et al. 2017; Svensson et al. 2017). A couple of studies have taken this into account and have placed additional dispensers along the perimeter of their fields within the MD treatments. This not only helps heighten the pheromone emission at the edges of the field, but it also helps prevent the wind from carrying large amounts of the pheromone away from the treated area (Lo Verde et al. 2020; Porcel et al. 2014). Despite the additional dispensers, Porcel et al. (2014) caught significantly more moths with their pheromone baited traps along the perimeter of the field indicating that there may not have been sufficient pheromone coverage or there were more moths located around the perimeter due to moths immigrating from other areas. On the other hand, Steyn et al. (2024) only tracked the recapture of marked sterile moths in their pheromone baited traps to not be swayed by immigrating moths from other locations. With this, they found a non-significant difference between trap captures in the center of the field and those on the perimeter indicating that they did have sufficient pheromone coverage even at the perimeter. Height and placement of pheromone dispensers can be controlled to help maximize the efficacy of MD, but environmental conditions cannot be completely controlled and can negatively impact the dispensers.

1.6.3 Environmental considerations

Environmental factors, including precipitation, temperature, wind speed, UV exposure, and relative humidity, all have impacts on pheromone release rate from passive pheromone dispensers (Gavara et al. 2022; Miller and Gut 2015). Using active dispensers can mitigate some of these effects as well as strategic placement of passive dispensers. Strategic placement can include placement away from direct sunlight where they would experience more rapid UV degradation and pheromone evaporation. In Higbee et al. (2008), the authors found that their hand-applied dispensers decayed at different rates depending on the side of the tree they were placed. With this knowledge they can slow down decay in future years by strategic placement of their dispensers. A lot of the conversation about dispenser placement has been centered on effective pheromone deployment through saturating an area and not wasting the valuable chemical compounds, but the pheromone formulation is also unique to the pest being treated with MD.

1.7 Formulations

Pheromone dispensers are the conduit in which pheromone formulations are dispersed into an area. The pheromones being deployed are synthetic versions of the pest's sex pheromone. Moth pheromones occasionally contain a single compound, but they often contain two or more components (Cardé 2007; Laing et al. 2020) (Table 1.2). The synthetic version does not have to be completely identical to the actual sex pheromone and often only contains one or two of the major components. A more complete pheromone formulation containing multiple compounds to be as close to the natural sex pheromone is more expensive to produce but may be more effective than using just one or two of the major components of the sex pheromone (Clavijo McCormick et al. 2012; Dhanyakumar et al. 2020). Gordon et al. (2005) experimented with their MD formula to compare multiple compounds to a single compound. They found that although it is more expensive to manufacture synthetic pheromones with multiple chemical compounds, it is often more effective compared to a singular compound. Higbee et al. (2017) also found that the MD treatments for *A. transitella* which previously used a single compound, an aldehyde, were not shown to be very attractive to male *A. transitella*. After undergoing more research, an additional five compounds were identified within the sex pheromone gland of *A. transitella*. To find the most effective, albeit maybe not the most inexpensive, formulation, these authors evaluated different combinations of all the six compounds in varying ratios. They found that a more complete formulation was more effective than using aldehyde alone in a MD tactic for *A. transitella*. Similarly, Dhanyakumar et al. (2020) studied how different combinations of the three principal components of the sex pheromone for the legume pod borer (*Maruca vitrata* (Fab.)) yielded different MD efficacy. They found the optimal success by using all three of the primary components of the sex pheromone when it came to decreasing crop damage.

In some cases, pheromone blends can be made to target multiple different pest species. This is usually done when the pest species share one or more component to their sex pheromone blend (Table 1.3). Indeed, this strategy has been popularized for managing stored product lepidopteran pests and has been experimented with for field pests as well. For example, Lo et al. (2013) found that combination dispensers for the codling moth (*Cydia pomonella* L.) and *E. postvittana* were equally as effective as deploying separate dispensers for each moth species. The use of dispensers and pheromone blends that can target multiple species makes MD more cost effective for many growers and facility managers. Unfortunately, not all pheromone blend combinations are effective. Liang et al. (2020) found that simultaneous release of pheromone blends for the rice stem borer (*Chilo suppressalis* (Walker)) and rice leaf roller (*Cnaphalocrocis medinalis* Gueńee) successfully worked for *C. medinalis* but appeared to suppress the efficacy for *C. suppressalis*. Because this initial study found that there was not even efficacy between the two species to the combined pheromone blend, it is not advised to use MD for these two species at the same time.

On top of synthetic sex pheromones, host plant compounds can be used in conjunction to create a more effective MD tactic. Kovanci (2015) found that combining pear ester with codlemone increased the efficacy of MD against *C. pomonella*. One possible reason for this outcome is that the pheromone component provides confusion for the male moths when finding a mate and the pear ester provides confusion for the female moths as they try to find a suitable location to lay their eggs (Kovanci 2015). Host plant compounds in combination with synthetic sex pheromones may be used to increase the efficacy of pheromone baited monitoring traps for certain insect pest species in areas that are undergoing MD (Burks et al. 2016; Burks 2017; Burks et al. 2020).

Table 1.2 53 compounds have been used for mating disruption (MD) by 33 lepidopteran species across 11 families.

		Compounds used by species	
Family	**Species**	**Compound(s)**	**Citations**
Coleophoridae	*Coleophora deauratella*	(*Z*)-7-dodecenyl acetate & (*Z*)-5-dodecenyl acetate	Mori and Evenden 2015
Cossidae	*Cossus insularis*	(*E*)-3-tetradecenyl acetate & (Z)-3-tetradecenyl acetate	Nakanishi et al. 2013
Cossidae	*Zeuzera pyrina*	(*E*,*Z*)-2,13-octadecenyl acetate & (*E*,*Z*)-3,13-octadecenyl acetate	Hegazi et al. 2010
Crambidae	*Chilo suppressalis*	(*Z*)-11-hexadecenal, (*Z*)-13-octadecenal, & (*Z*)-9-hexadecenal,	Chen et al. 2014; Liang et al. 2020; Vacas et al. 2015
Crambidae	*Conogethes punctiferalis*	(*E*)-10-hexadecenal & (*Z*)-10-hexadecenal	Kim et al. 2024
Crambidae	*Diatraea saccharalis*	(*Z*,*E*)-9,11-hexadecadienal	Dam et al. 2024
Crambidae	*Maruca vitrata*	(*E*,*E*)-10,12-hexadecadienal, (*E*,*E*)-10,12-hexadecadienol, & (*E*)-10-hexadecenal	Dhanyakumar et al. 2020
Gelechiidae	*Pectinophora gossypiella*	(*Z*,*Z*)-7,11-hexadecadienyl acetate & (*Z*,*E*)-7,11-hexadecadienyl acetate	Lance et al. 2015; Lykouressis et al. 2005

Gelechiidae	*Tecia solanivora*	(*E*)-3-dodecenyl acetate, (*Z*)-3-dodecenyl acetate, & dodecyl acetate	Clavijo et al. 2012
Gelechiidae	*Tuta absoluta*	(*E,Z,Z*)-3,8,11-tetradecatrienyl acetate & (*E,Z*)-3,8-tetradecadienyl acetate	Cocco et al. 2013; Vacas et al. 2011
Noctuidae	*Helicoverpa armigera*	(*Z*)-11-hexadecenal & (*Z*)-9-hexadecenal	Burgio et al. 2020
Noctuidae	*Sesamia nonagrioides*	(*Z*)-11-hexadecenyl acetate, (*Z*)-11-hexadecenol, (*Z*)-11-hexadecenal, & dodecyl acetate	Albajes et al. 2002
Pyralidae	*Amyelois transitella*	(*Z,Z*)-11,13-hexadecadienal, (*Z,Z*)-11,13-hexadecadienol, (*Z,Z,Z,Z,Z*)-3,6,9,12,15-tricosapentaene, & (*E,Z*)-11,13-hexadecadienol	Burks 2017; Burks et al. 2016; Haviland et al. 2021; Higbee et al. 2017; Higbee and Burks 2008
Pyralidae	*Cnaphalocrocis medinalis*	(*Z*)-11-octadecenal, (*Z*)-13-octadecenal, (*Z*)-11-octadecenol, & (*Z*)-13-octadecenol	Liang et al. 2020
Pyralidae	*Cryptoblabes gnidiella*	(*Z*)-11-hexadecenal, (*Z*)-13-octadecenal, & Tetradecyl acetate	Ricciardi et al. 2024
Pyralidae	*Dioryctria abietella*	(*Z,Z,Z,Z,Z*)-3,6,9,12,15-pentacosapentaene & (*Z,E*)-9,11-tetradecadienyl acetate	Svensson et al. 2018
Pyralidae	*Ephestia kuehniella*	(*Z,E*)-9,12-tetradecadienyl acetate	Athanassiou et al. 2016; Trematerra and Savoldelli 2013
Pyralidae	*Plodia interpunctella*	(*Z,E*)-9,12-tetradecadienyl acetate & (*Z*)-9-tetradecenyl acetate	Burks et al. 2011; Burks and Kuenen 2012; Hasan et al. 2023; Wijayaratne and Burks 2020
Sesiidae	*Cossus insularis*	(*E*)-3-tetradecenyl acetate & (*Z*)-3-tetradecenyl acetate	Hoshi et al. 2016
Sesiidae	*Synanthedon exitiosa*	(*Z,Z*)-3,13-octadecadienol acetate	Frank et al. 2020
Sesiidae	*Synanthedon pictipes*	(*Z,Z*)-3,13-octadecadienol acetate	Frank et al. 2020
Thaumetopoeidae	*Thaumetopoea pityocampa*	(*Z*)-13-hexadecen-11-ynyl acetate	Trematerra et al. 2019
Tineidae	*Nemapogon granellus*	(*Z,Z*)-3,13-octadeca dienyl acetate	Savoldelli et al. 2023
Tortricidae	*Cydia fagiglandana*	(*E,E*)-8,10-dodecadienyl acetate & (*E,E*)-8,10-dodecadienol	Ferracini et al. 2021
Tortricidae	*Cydia pomonella*	(*E,E*)-8,10 dodecadienol, dodecanol, & tetradecanol	Bohnenblust et al. 2011; Epstein et al. 2011; Kovanci 2015; McGhee et al. 2016; Porcel et al. 2015; Pluciennik 2013; Tollerup et al. 2012
Tortricidae	*Cydia splendana*	(*E,E*)-8,10-dodecadienyl acetate & (*E,E*)-8,10-dodecadienol	Ferracini et al. 2021
Tortricidae	*Cydia strobilella*	(*E,E*)-8,10-dodecadienyl acetate & (*E,Z*)-8,10-dodecadienyl acetate	Svensson et al. 2018
Tortricidae	*Epiphyas postvittana*	(*E*)-11-tetradecenyl acetate, (*Z*)-11-tetradecenyl acetate, & (*E,E*)-9,11-tetradecadienyl acetate	Brockerhoff et al. 2012; Soopaya et al. 2015; Suckling et al. 2016
Tortricidae	*Grapholita funebrana*	(*Z*)-8-dodecenyl acetate, (*E*)-8-dodecenyl acetate, & (*Z*)-8-dodecenol	Lo Verde et al. 2020

Tortricidae	*Grapholita molesta*	(*Z*)-8-dodecenyl acetate, (*E*)-8-dodecenyl acetate, & (*Z*)-8-dodecenol	Bohnenblust et al. 2011; De Lame et al. 2010; Kovanci 2015; Stelinski et al. 2007; Tollerup et al. 2010; Trimble 2001; Yang et al. 2003
Tortricidae	*Lobesia botrana*	(*E*,*Z*)-7,9-dodecadienyl acetate, (*E*)-9-dodecenylacetate, & tetradecyl acetate	Gordon et al. 2005; Harari et al. 2015; Louis and Schirra 2001; Ricciardi et al. 2024
Tortricidae	*Proeulia auraria*	(*E*)-11-tetradecenyl acetate & (*E*)-11-tetradecenol	Flores et al. 2021
Tortricidae	*Spilonota ocellana*	(*Z*)-8-tetradecenyl acetate	Porcel et al. 2015
Yponomeutidae	*Prays oleae*	(*Z*)-7-tetradecenal	Ortiz et al. 2021

Table 1.3 Compounds that have been used in mating disruption (MD) for more than one species.

	Common Compounds	
Compound Name	Number of species used for	Species
(*Z*)-11-hexadecenal	4	*Chilo suppressalis, Sesamia nonagrioides, Cryptoblabes gnidiella,* & *Helicoverpa armigera*
(*Z*)-13-octadecenal	3	*Cnaphalocrocis medinalis, Chilo suppressalis,* & *Cryptoblabes gnidiella*
(*E*,*E*)-8,10-dodecadien-1-ol	2	*Cydia fagiglandana* & *Cydia pomonella*
(*E*)-10-hexadecenal	2	*Conogethes punctiferalis* & *Maruca vitrata*
(*E*)-11-tetradecenyl acetate	2	*Epiphyas postvittana* & *Proeulia auraria*
(*E*)-8-dodecenyl acetate	2	*Grapholita molesta* & *Grapholita funebrana*
(*Z*,*E*)-9,12-tetradecadienyl acetate	2	*Ephestia kuehniella* & *Plodia interpunctella*
(*Z*,*Z*)-3, 13-octadecadienyl acetate	2	*Nemapogon granellus* & *Synanthedon exitiosa*
(*Z*)-8-dodecen-1-ol	2	*Grapholita molesta* & *Grapholita funebrana*
(*Z*)-8-dodecenyl acetate	2	*Grapholita molesta* & *Grapholita funebrana*
(*Z*)-9-hexadecenal	2	*Chilo suppressalis* & *Helicoverpa armigera*
dodecyl acetate	2	*Sesamia nonagrioides* & *Tecia solanivora*
tetradecyl acetate	2	*Lobesia botrana* & *Cryptoblabes gnidiella*

1.8 Efficacy

Regardless of the dispenser type, placement, and pheromone formulation being deployed, MD strategies are only going to be used if they are effective. To determine their efficacy, MD treated areas are most commonly compared to control areas where the pest and crop are present, but no pest control method is being applied, or compared to an area that is treated using the grower's conventional strategies. Unfortunately, because MD does not directly kill insects, it may take a few seasons for a significant change in the pest population density to be seen. For example, Hegazi et al. (2010) saw no significant change in the larval activity of the leopard moth (*Zeuzera pyrina* (L.)) within the first year; however, in the following two years, they saw increasingly significant reductions. Monitoring the pest's population while using a MD tactic is important to accurately evaluate how well it's working (Cardé 2007). The efficacy of MD treatments can be determined in multiple ways. Some of the common ways are using tethered unmated females, female baited traps, mating cages, lure baited traps, monitoring trap shutdown, and by examining crop damage (Hoshi et al. 2016).

1.8.1 Lure baited traps

Lure or pheromone baited traps may not be an accurate way to determine if a MD tactic is effective in all cases (Lo Verde et al. 2020; Porcel et al. 2014). Nonetheless, they can be used in conjunction with other efficacy verification strategies to form a complete picture of how the MD strategy is performing (Vacas et al. 2011). If using pheromone/lure baited traps to examine the efficacy of MD, the goal is often to see a complete trap shutdown. That is when there is a 95%+ reduction in captures within the MD treated area compared to an untreated area (Porcel et al. 2014). The type of trap used is dependent on the environment where the MD tactic is being deployed in. Delta style wing traps are most commonly used, but funnel traps can also be used (Hegazi et al. 2010). Porcel et al. (2014) found high trap shutdown of 99.6% and 99.2% for one of their species examined, the large fruit tree tortrix (*Archips podana* (Scopoli)). However, this trap shutdown did not equate to a lack of larvae within the crops. This showed that use of trap monitoring for *A. podana* was not an accurate way alone to verify MD success. Multiple studies have found a significant decrease in moths captured within baited traps in the MD treatment but did not find a related decrease in fruit injury (Lo Verde et al. 2020; McGhee et al. 2015). To couple with baited traps, sterile, lab-reared, moths can be marked and released into the MD and control areas. With this, there is a known density of the moths released that is equal in all treatments. This eliminates moth catches related to differing population densities, immigrating individuals, and other factors between the treatment and the control (Curtiss et al. 2023; Gordon et al. 2005; Higbee et al. 2017; Onufrieva et al. 2019; Steyn et al. 2024).

Not all monitoring traps used for MD verification are baited. In many stored product facilities, water traps are laid out for multiple days. These traps attract both male and female moths. Because there is only water in the trap, the specimens that are caught are easy to remove and sex. For the female moths captured, they can also be dissected to confirm if successful mating has occurred (Savoldelli et al. 2023; Trematerra and Savoldelli 2013). Using these traps can be used then to both monitor pest density and to confirm if MD is effective. Determining if mating has occurred in the presence of a MD treatment is often a good method of evaluation.

1.8.2 Mating occurrence

Although pheromone-baited traps can give an indication of how male moths are responding to the influx of additional sex pheromone into their environment, they do not actually indicate where mating is or is not occurring in the environment (Mori and Evenden 2012). When testing if mating has occurred within MD treatment or control treatments, tethered females, sentinel females, or mating cages are often set up. In the case of tethered females, larvae of the pest species are usually reared in a lab until adulthood to confirm their mating status. These unmated moths then have a length of cotton, monofiber nylon, or polyester thread tied to the base of one of their wings (Epstein et al. 2011; Régnière et al. 2019). The other end of the thread is anchored to a pole placed amongst the crops at a similar height to where the top of the crop is, to a branch of one of the crop producing trees, or to a mesh wire stage anchored to the crop (Epstein et al. 2011; Nakanishi et al. 2013; Régnière et al. 2019; Stelinski et al. 2007). Multiple or singular female moths are anchored to the same pole or branch (Epstein et al. 2011). For mating cages, both unmated male and female moths are placed in a cage that they cannot escape from. Usually, an even pairing of males and female moths are used ranging from five pairs to ten pairs (Briand et al. 2011; Dhanyakumar et al. 2020). This cage is anchored within the treatment or control areas and can have the pheromone dispenser directly placed inside of it (Briand et al. 2011). For the sentinel females, lab reared female moths are partially immobilized usually through clipping their wings so they cannot fly away. To further prevent them from crawling away, they are placed in a cup that has the sides coated with Fluon® (Burks et al. 2011). Because the cup is open to the environment, males are able to access the females. To examine mating with tethered females, sentinel females, or mating cages, the females are removed after 24 h or less and dissected in a lab for the presence of spermatophore (Hoshi et al. 2016; Nakanishi et al. 2013; Stelinski et al. 2007). Finding spermatophore indicates that the female moth was successfully mated.

1.8.3 Female baited traps

Female baited traps can also be used to monitor male response to MD, mating occurrence, or both. Common versions of female baited traps are single or double entry only traps which are made of small containers or cups where one to two of the ends have been replaced with a funnel directed towards the center cavity of the container. A lab-reared unmated female is placed in the center cavity of the trap and placed in the MD treated area or control. After a specific amount of time has elapsed, the number of males trapped are counted and the female is dissected for the presence of spermatophore (Régnière et al. 2019; Soopayaet et al. 2015). Soopayaet et al. (2015) examined MD using manually applied SPLAT as an effective strategy for urban control of *E. postvittana*. Using entry only traps baited with a single sentinel female, they confirmed that mating had significantly decreased within the area treated with SPLAT MD.

Porcel et al. (2014) used a different female baited trap. In their study, they encased three trees within an orchard with netting. Within this netted enclosure, they set up a sticky trap containing 48 h unmated female safely held in a metal tea ball. Finally, they released 10-30 unmated male moths into the netted enclosure and nine days later counted the number of males recovered in the sticky trap. This strategy does not verify if successful mating has occurred, but does indicate how well male moths can locate calling females in the presence of MD. There has been some controversy surrounding the efficacy of using these female-based strategies for evaluating MD. Briand et al. (2011) argued that tethered females might not be the most accurate way to evaluate successful mating. Their specific concerns are that females may not be able to accurately perform their courtship behaviors and have the chance of being picked off by predators. Rather than evaluating mating in the moment, progeny can also be examined to evaluate if mating has occurred.

1.8.4 Egg, larval, or pupal presence

Since the primary goal of using MD is to lower successful mating of the pest insect, a good way to determine if the MD strategy is successful, is to examine the progeny production at different life stages after the strategy is deployed. For many stored product pest moths, oviposition sites can be set out to attract female moths to lay their eggs in (Burks et al. 2011; Hasan et al. 2023). These sites can be compared between MD treatments and a control to see if there has been decreased progeny production. Similarly, pupation sites can be set out to give wandering larvae a safe space to pupate. These sites are usually made of rolled corrugated cardboard (Burks et al. 2011; Pluciennik 2013). Crops themselves can be directly examined for eggs, larvae, or pupal exuviae (Hegazi et al. 2010; Mori and Evenden 2015; Nakanishi et al. 2013; Ortiz et al. 2021). For example, Régnière et al. (2019) examined egg density from *C. fumiferana* in MD treatments as well as in the control. Unfortunately, although they had seen a decrease in male captures in pheromone traps and female mating both with tethered females and entry traps, there was no subsequent decrease in eggs laid. This shows that for *C. fumiferana*, egg density is a more effective indicator of MD success. Closely related with the presence of progeny, is visible damage to the crops which also serves as a reliable indicator for the efficacy of MD treatments.

1.8.5 Crop damage

At the end of the day, growers will only use a MD tactic if they can see an economic advantage to using it. In most cases, an economic advantage is considered when MD decreases the amount of damaged product beyond what their standard pest control methods do (Burgio et al. 2020; Lo et al. 2013; Ortiz et al. 2021; Pluciennik 2013; Régnière et al. 2019; Ricciardi et al. 2024; Svensson et al. 2017; Vacas et al. 2016). Lo Verde et al. (2020) found that although there was a decrease in fruit injury throughout their orchards and cultivar treatments under MD, the decrease was not economically significant. There are positive experiments that show an economically effective crop damage decrease under MD like in Gavara et al. (2022) where they found that both of their tested dispenser types, polyethylene tubes and plastic ampoules, reduced crop damage in grape vineyards to under 5%. In addition, Tollerup et al. (2012) found such a dramatic reduction in pest abundance and damage within peach and apple orchards across two years. As a result, no treatment was needed during a third year. On the other hand, the pest's generation time can affect how quickly results can be seen under MD. In a study conducted by Nakanishi et al. (2013), the lifecycle of the carpenter moth (*Cossus insularis* Staudinger) is unknown, but previous lab studies have shown that the pest may complete their life cycle within two years. This could be a contributing factor to why they only saw a significant decrease in pest damage to the trees in the third year of their study. It can be difficult to convince growers or facilities to use something new that may take a while to be effective, but when MD works, there can be a dramatic decrease in damage.

1.9 Disadvantages

When MD is effective, it can be a sustainable pest control method. However, there is still a lot of troubleshooting that needs to be done to eliminate some of the difficulties with MD tactics. Firstly, MD can take a few growing seasons before a decrease in pest populations and crop damage is noticed. Many growers cannot afford to use a product that takes multiple growing seasons before the investment starts to be returned (Kong et al. 2014). One of the many positive aspects of using MD is that it is non-toxic; however, this does mean that it does not completely eliminate pest populations. Thus, the application of MD should be paired with a secondary strategy to cause lethality within the pest populations (Cardé 2007; Lo Verde et al. 2020). The need for additional control tactics can also decrease the accessibility and affordability of MD for many growers (Lo Verde et al. 2020). Similarly, multiple studies have found that MD is not effective if the pest population is too large (Lo Verde et al. 2020; Miller and Gut 2015; Pluciennik 2013; Trematerra and Savoldelli 2013). This limits the efficacy and versatility of MD. If using pheromones for the MD tactic, pheromone-based trapping for recording or monitoring pest phenology throughout the season will not be highly effective (Burks et al. 2020; Knight et al. 2017). Without accurate pest monitoring, it can be difficult to discern when additional pest control treatments are needed. Because MD is species specific, either combination MD treatments are needed, or alternative treatments are required to handle additional pests (Wu et al. 2012). Requiring too many different products can become cost prohibitive especially for smaller growers and facility owners. Many of these drawbacks including the affordability of MD are currently working on being addressed worldwide. In future years, there will hopefully be fewer downsides to implementing MD.

1.10 Conclusions

Mating disruption has evolved so much since its creation over 50 years ago. It is not a one size fits all pest control tactic rather it is customizable and tailored to fit specific needs. There are many pests across the worldwide that are being managed in large part by MD and still more where an effective MD plan is underway. Pheromone synthesis and dispenser production is becoming less cost prohibitive making MD an increasingly accessible pest management tactic. Future research will continue to improve MD by filling current gaps in knowledge including female Lepidoptera response to MD, long-term impacts of MD on pest population sizes, and easier application methods. All of this will contribute to creating fewer downsides to MD.
As the world continues to strive to create a more sustainable future, pest control that protects people, the environment, beneficial insects, and our food sources has become crucial. Hopefully we will continue to see MD grow in popularity and accessibility within the next fifty years.

1.11 References

Albajes R, Konstantopoulou M, Etchepare O, Eizaguirre M, Frérot B, Sans A, Krokos F, Améline A, and Mazomenos B. 2002. Mating disruption of the corn borer Sesamia nonagrioides (Lepidoptera: Noctuidae) using sprayable formulations of pheromone. Crop Protection. 21(3):217–225. https://doi.org/10.1016/S0261-2194(01)00088-6.

Amarasekare K and Shearer P. 2017. Stability of *Cacopsylla pyricola* (Hemiptera: Psyllidae) populations in pacific northwest pear orchards managed with long-term mating disruption for *Cydia pomonella* (Lepidoptera: Tortricidae). Insects. 8(4):105. https://doi.org/10.3390/insects8040105.

Athanassiou CG, Kavallieratos NG, Sciarretta A, and Trematerra P. 2016. Mating disruption of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) in a storage facility: Spatio-temporal distribution changed after long-term application. Journal of Stored Products Research. 67:1–12. https://doi.org/10.1016/j.jspr.2016.01.001.

Baker TC, Myrick AJ, and Park KC. 2016. Optimizing the point-source emission rates and geometries of pheromone mating disruption mega-dispensers. Journal of Chem Ecology. 42(9):896–907. https://doi.org/10.1007/s10886-016-0769-9.

Benelli, Lucchi and Thomson, et al. 2019. Sex pheromone aerosol devices for mating disruption: challenges for a brighter future. Insects. 10(10):308. https://doi.org/10.3390/insects10100308.

Bohnenblust E, Hull LA, and Krawczyk G. 2011. A comparison of various mating disruption technologies for control of two internally feeding Lepidoptera in apples: Mating disruption of two moth species. Entomologia Experimentalis et Applicata. 138(3):202–211. https://doi.org/10.1111/j.1570-7458.2010.01089.x.

Briand F, Guerin PM, Charmillot P-J, and Kehrli P. 2012. Small cages with insect couples provide a simple method for a preliminary assessment of mating disruption. The Scientific World Journal. 2012:1–8. https://doi.org/10.1100/2012/960468.

Brockerhoff EG, Suckling DM, Kimberley M, Richardson B, Cocker G, Gous S, Kerr JL, Cowan DM, Lance DR, Strand T, Zhang A, and Niedz RP. 2012. Aerial application of pheromones for mating disruption of an invasive moth as a potential eradication tool. Niedz RP, editor. PLoS ONE. 7(8):e43767. https://doi.org/10.1371/journal.pone.0043767.

Burgio G, Ravaglia F, Maini S, Bazzocchi GG, Masetti A, and Lanzoni A. 2020. Mating disruption of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on processing tomato: first applications in northern Italy. Insects. 11(4):206. https://doi.org/10.3390/insects11040206.

Burks CS. 2017. Combination phenyl propionate/pheromone traps for monitoring navel orangeworm (Lepidoptera: Pyralidae) in almonds in the vicinity of mating disruption. Journal of Economic Entomology. 110(2):438–446. https://doi.org/10.1093/jee/tow318.

Burks CS, Higbee BS, and Beck JJ. 2020. Traps and attractants for monitoring navel orangeworm (Lepidoptera: Pyralidae) in the presence of mating disruption. Lee J, editor. Journal of Economic Entomology. 113(3):1270–1278. https://doi.org/10.1093/jee/toz363.

Burks CS, Kuenen LPS 'Bas', and Daane KM. 2016. Phenyl propionate and sex pheromone for monitoring navel orangeworm (Lepidoptera: Pyralidae) in the presence of mating disruption. Journal of Economic Entomology. 109(2):958–961. https://doi.org/10.1093/jee/tov339.

Burks CS, McLaughlin JR, Miller JR, and Brandl DG. 2011. Mating disruption for control of

Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) in dried beans. Journal of Stored Products Research. 47(3):216–221. https://doi.org/10.1016/j.jspr.2011.03.001.

Cardé RT. 2007. Using pheromones to disrupt mating of moth pests. In: Kogan M, Jepson P, editors. Perspectives in Ecological Theory and Integrated Pest Management. 1st ed. Cambridge University Press. p. 122–169. https://doi.org/10.1017/CBO9780511752353.006.

Chen R-Z, Klein MG, Sheng C-F, Li QY, Li Y, Li LB, and Hung X. 2014. Mating disruption or mass trapping, compared with chemical insecticides, for suppression of *Chilo suppressalis* (Lepidoptera: Crambidae) in northeastern China. Journal of Economic Entomology. 107(5):1828–1838. https://doi.org/10.1603/EC14148.

Clavijo McCormick AL, Karlsson M, Bosa Ochoa CF, Bengtsson M, Zuluaga MV, Fukumoto T, Oehlschlager C, Cotes Prado AM, and Witzgall P. 2012. Mating disruption of Guatemalan potato moth *Tecia solanivora* by attractive and non-attractive pheromone Blends. Journal of Chemical Ecology. 38(1):63–70. https://doi.org/10.1007/s10886-011-0051-0.

Cocco A, Deliperi S, and Delrio G. 2013. Control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in greenhouse tomato crops using the mating disruption technique: Control of *Tuta absoluta* by mating disruption technique. Journal of Applied Entomology. 137(1–2):16–28. https://doi.org/10.1111/j.1439-0418.2012.01735.x.

Curtiss RT, Nottingham L, Adams CG, and Gut LJ. 2023. Estimating plume reach and trapping radii for male and female *Cydia pomonella* (Lepidoptera: Tortricidae) captured in pheromone–kairomone baited traps in Washington State apple orchards under mating disruption. Rodriguez-Saona C, editor. Journal of Economic Entomology. 116(5):1592–1603. https://doi.org/10.1093/jee/toad167.

Dam MI, Ding B, Svensson GP, Wang HL, Melo DJ, Lassance JM, Zarbin PH, and Löfstedt C. 2024. Sex pheromone biosynthesis in the sugarcane borer *Diatraea saccharalis*: paving the way for biotechnological production. Pest Management Science. 80(3):996–1007. https://doi.org/10.1002/ps.7830.

De Lame FM, Epstein D, Gut LJ, Goldfarb H, and Miller JR. 2010. Effect of varying dispenser point source density on mating disruption of *Grapholita molesta* (Lepidoptera: Tortricidae). Journal of Economic Entomology. 103(4):1299–1305. https://doi.org/10.1603/EC09239.

Dhanyakumar O, Srinivasan R, Mohan M, Venkatesan T, Murali Mohan K, Nagesha N, and Sotelo-Cardona P. 2020. Effect of pheromone-mediated mating disruption on pest population density of *Maruca vitrata* (Fabricius) (Crambidae: Lepidoptera). Insects. 11(9):558. https://doi.org/10.3390/insects11090558.

Epstein DL, Stelinski LL, Miller JR, Grieshop MJ, and Gut LJ. 2011. Effects of reservoir dispenser height on efficacy of mating disruption of codling moth (Lepidoptera: Tortricidae) in apple. Pest Management Science. 67(8):975–979. https://doi.org/10.1002/ps.2141.

Ferracini C, Pogolotti C, Rama F, Lentini G, Siatta V, Mereghetti P, Mancardi P, and Alma A. 2021. Pheromone-mediated mating disruption as management option for *Cydia* spp. in chestnut orchard. Insects. 12(10):905. https://doi.org/10.3390/insects12100905.

Flores MF, Bergmann J, Ballesteros C, Arrazito D, and Curkovic T. 2021. Development of monitoring and mating disruption against the Chilean leafroller *Proeulia auraria* (Lepidoptera: Tortricidae) in orchards. Insects. 12(7):625. https://doi.org/10.3390/insects12070625.

Frank DL, Starcher S, and Chandran RS. 2020. Comparison of mating disruption and insecticide application for control of peachtree borer and lesser peachtree borer (Lepidoptera: Sesiidae) in peach. Insects. 11(10):658. https://doi.org/10.3390/insects11100658.

Gavara A, Navarro-Llopis V, Primo J, and Vacas S. 2022. Influence of weather conditions on *Lobesia botrana* (Lepidoptera: Tortricidae) mating disruption dispensers' emission rates and efficacy. Crop Protection. 155:105926. https://doi.org/10.1016/j.cropro.2022.105926.

Gavara A, Vacas S, Navarro I, Primo J, and Navarro-Llopis V. 2020. Airborne pheromone quantification in treated vineyards with different mating disruption dispensers against *Lobesia botrana*. Insects. 11(5):289. https://doi.org/10.3390/insects11050289.

Gordon D, Zahavi T, Anshelevich L, Harel M, Ovadia S, Dunkelblum E, and Harari AR. 2005. Mating disruption of *Lobesia botrana* (Lepidoptera: Tortricidae): effect of pheromone formulations and concentrations. Journal of Economic Entomology. 98(1):135–142. https://doi.org/10.1093/jee/98.1.135.

Harari AR, Zahavi T, and Steinitz H. 2015. Female detection of the synthetic sex pheromone contributes to the efficacy of mating disruption of the European grapevine moth, *Lobesia botrana*: Synthetic pheromone affects female moth fitness. Pest Management Science. 71(2):316–322. https://doi.org/10.1002/ps.3830.

Hasan MM, Mahroof RM, Aikins MJ, Athanassiou C, and Phillips TW. 2023. Pheromone-based auto-confusion for mating disruption of *Plodia interpunctella* (Lepidoptera: Pyralidae) in structures with raw and processed grain products. Journal of Stored Products Research. 104:102201. https://doi.org/10.1016/j.jspr.2023.102201.

Haviland DR, Rijal JP, Rill SM, Higbee BS Burks CS, and Gordon CA. 2021. Management of navel orangeworm (Lepidoptera: Pyralidae) using four commercial mating disruption systems in California almonds. Lee J, editor. Journal of Economic Entomology. 114(1):238–247. https://doi.org/10.1093/jee/toaa297.

Hegazi EM, Khafagi WE, Konstantopoulou MA, Schlyter F, Raptopoulos D, Schweil S, El-Rahman SA, Atwa A, Ali SE, and Tawfik H. 2010. Suppression of leopard moth (Lepidoptera: Cossidae) populations in olive trees in Egypt through mating disruption. Journal of Economic Entomology 103(5):1621–1627. https://doi.org/10.1603/EC09435.

Higbee BS, Burks CS, and Cardé RT. 2017. Mating disruption of the navel orangeworm (Lepidoptera: Pyralidae) using widely spaced, aerosol dispensers: is the pheromone blend the most efficacious disruptant? Journal of Economic Entomology. 110(5):2056–2061. https://doi.org/10.1093/jee/tox185.

Higbee BS and Burks CS. 2008. Effects of mating disruption treatments on navel orangeworm (Lepidoptera: Pyralidae) sexual communication and damage in almonds and pistachios. Journal of Economic Entomology. 101(5):1633–1642. https://doi.org/10.1093/jee/101.5.1633.

Holdcraft R, Rodriguez-Saona C, and Stelinski L. 2016. Pheromone autodetection: evidence and implications. Insects. 7(2):17. https://doi.org/10.3390/insects7020017.

Horner RM, Lo PL, Rogers DJ, Walker JTS, and Suckling DM. 2020. Combined effects of mating disruption, insecticides, and the sterile insect technique on *Cydia pomonella* in New Zealand. Insects. 11(12):837. https://doi.org/10.3390/insects11120837.

Hoshi H, Takabe M, and Nakamuta K. 2016. Mating disruption of a carpenter moth, *Cossus insularis* (Lepidoptera: Cossidae) in apple orchards with synthetic sex pheromone, and registration of the pheromone as an agrochemical. Journal of Chemical Ecology. 42(7):606–611. https://doi.org/10.1007/s10886-016-0723-x.

Kim J, Jung S, and Kim YU. 2024. Pheromone-based mating disruption of *Conogethes punctiferalis* (Lepidoptera: Crambidae) in chestnut orchards. Insects. 15(6):445. https://doi.org/10.3390/insects15060445.

Knight AL, El-Sayed AM, Judd GJR, and Basoalto E. 2017. Development of 2-phenylethanol plus acetic acid lures to monitor obliquebanded leafroller (Lepidoptera: Tortricidae) under mating disruption. Journal of Applied Entomology. 141(9):729–739. https://doi.org/10.1111/jen.12393.

Kong WN, Li J, Fan RJ, Li SC, and Ma RY. 2014. Sex-pheromone-mediated mating disruption technology for the oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae): overview and prospects. Psyche: A Journal of Entomology. 2014:1–8. https://doi.org/10.1155/2014/253924.

Kovanci OB. 2015. Co-application of microencapsulated pear ester and codlemone for mating disruption of *Cydia pomonella*. Journal of Pest Science. 88(2):311–319. https://doi.org/10.1007/s10340-014-0619-x.

Kovanci OB, Schal C, Walgenbach JF, and Kennedy GG. 2005. Comparison of mating disruption with pesticides for management of oriental fruit moth (Lepidoptera: Tortricidae) in North Carolina apple orchards. Journal of Economic Entomology. 98(4):1248–1258. https://doi.org/10.1603/0022-0493-98.4.1248.

Lance DR, Leonard DS, Mastro VC, and Walters ML. 2016. Mating disruption as a suppression tactic in programs targeting regulated lepidopteran pests in US. Journal of Chemical Ecology. 42(7):590–605. https://doi.org/10.1007/s10886-016-0732-9.

Liang Y, Luo M, Fu X, Zheng LX, and Wei HY. 2020. Mating disruption of *Chilo suppressalis* from sex pheromone of another pyralid rice pest *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). Ziesmann J, editor. Journal of Insect Science. 20(3):19. https://doi.org/10.1093/jisesa/ieaa050.

Lo PL, Walker JTS, Horner RM, and Hedderley DI. 2013. Development of multiple species mating disruption to control codling moth and leafrollers (Lepidoptera Tortricidae). New Zealand Plant Protection. 66:264–269. https://doi.org/10.30843/nzpp.2013.66.5646.

Lo Verde G, Guarino S, Barone S, and Rizzo R. 2020. Can mating disruption be a possible route to control plum fruit moth in Mediterranean environments? Insects. 11(9):589. https://doi.org/10.3390/insects11090589

Louis F and Schirra K-J. 2001. Mating disruption of *Lobesia botrana* (Lepidoptera: Tortricidae) in vineyards with very high population densities. IOBC wprs Bulletin. 24(2):75–79.

Lykouressis D, Perdikis D, Samartzis D, Fantinou A, and Toutouzas S. 2005. Management of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) by mating disruption in cotton fields. Crop Protection. 24(2):177–183. https://doi.org/10.1016/j.cropro.2004.07.007.

Machuca-Mesa LM, Turchen LM, and Guedes RNC. 2024. Phosphine resistance among stored product insect pests: A global meta-analysis-based perspective. Journal of Pest Science. 97(3):1485–1498. https://doi.org/10.1007/s10340-023-01713-6.

McGhee PS, Miller JR, Thomson DR, and Gut LJ. 2016. Optimizing aerosol dispensers for mating disruption of codling moth, *Cydia pomonella* L. Journal of Chemical Ecology. 42(7):612–616. https://doi.org/10.1007/s10886-016-0724-9.

Miller JR and Gut LJ. 2015. Mating disruption for the 21st century: matching technology with mechanism. Environmental Entomology. 44(3):427–453. https://doi.org/10.1093/ee/nvv052.

Mori BA and Evenden ML. 2013. When mating disruption does not disrupt mating: fitness consequences of delayed mating in moths. Entomologia Experimentalis et Applicata. 146(1):50–65. https://doi.org/10.1111/j.1570-7458.2012.01309.x.

Mori BA and Evenden ML. 2015. Challenges of mating disruption using aerosol-emitting pheromone puffers in red clover seed production fields to control *Coleophora deauratella* (Lepidoptera: Coleophoridae). Environmental Entomology. 44(1):34–43. https://doi.org/10.1093/ee/nvu001.

Morrison WR, Scully ED, and Campbell JF. 2021. Towards developing areawide semiochemical-mediated, behaviorally-based integrated pest management programs for stored product insects. Pest Management Science. 77(6):2667–2682. https://doi.org/10.1002/ps.6289.

Nakanishi T, Nakamuta K, Mochizuki F, and Fukumoto T. 2013. Mating disruption of the carpenter moth, *Cossus insularis* (Staudinger) (Lepidoptera: Cossidae) with synthetic sex pheromone in Japanese pear orchards. Journal of Asia-Pacific Entomology. 16(3):251–255. https://doi.org/10.1016/j.aspen.2013.03.002.

Nayak MK, Daglish GJ, Phillips TW, and Ebert PR. 2020. Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. Annual Review of Entomology. 65(1):333–350. https://doi.org/10.1146/annurev-ento-011019-025047.

Onufrieva KS, Hickman AD, Leonard DS, and Tobin PC. 2019. Ground application of mating disruption against the gypsy moth (Lepidoptera: Erebidae). Journal of Applied Entomology. 143(10):1154–1160. https://doi.org/10.1111/jen.12696.

Ortiz A, Porras A, Marti J, Tudela A, Rodríguez-González A, and Sambado P 2021. Mating disruption of the olive moth *Prays oleae* (Bernard) in olive groves using aerosol dispensers. Insects. 12(12):1113. https://doi.org/10.3390/insects12121113.

Płuciennik Z. 2013. The control of codling moth (*Cydia Pomonella* L.) population using mating disruption method. Journal of Horticultural Research. 21(1):65–70. https://doi.org/10.2478/johr-2013-0009.

Porcel M, Sjöberg P, Swiergiel W, Dinwiddie R, Rämert B, and Tasin M. 2015. Mating disruption of *Spilonota ocellana* and other apple orchard tortricids using a multispecies reservoir dispenser. Pest Management Science. 71(4):562–570. https://doi.org/10.1002/ps.3844.

Régnière J, Delisle J, Dupont A, and Trudel R. 2019. The impact of moth migration on apparent fecundity overwhelms mating disruption as a method to manage spruce budworm populations. Forests. 10(9):775. https://doi.org/10.3390/f10090775.

Ricciardi R, De Fazi L, D’Anna G, Savino S, and Ladurner E. 2024. Simultaneous mating disruption of two moth pests of the vineyard (*Lobesia botrana* and *Cryptoblabes gnidiella*) through a biodegradable sex pheromone dispenser. Environmental Science and Pollution Research. 31(31):43865–43873. https://doi.org/10.1007/s11356-024-33980-w.

Ricciardi R, Di Giovanni F, Cosci F, Ladurner E, Savino F, Iodice A, Benelli G, and Lucchi A. 2021. Mating disruption for managing the honeydew moth, *Cryptoblabes gnidiella* (Millière), in Mediterranean vineyards. Insects. 12(5):390. https://doi.org/10.3390/insects12050390.

Savoldelli S, Jucker C, Lupi D, Malabusini S, Peri E, and Guarino S. 2023. Pheromone-mediated mating disruption of the European grain moth *Nemapogon granellus* in ham factories. Journal of Stored Products Research. 102:102117. https://doi.org/10.1016/j.jspr.2023.102117.

Soopaya R, Woods B, Lacey I, Verdi A, Mafra-Neto A, and Suckling DM. 2015. Feasibility of mating disruption for agricultural pest eradication in an urban environment: light brown apple moth (Lepidoptera: Tortricidae) in Perth. Journal of Economic Entomology. 108(4):1930–1935. https://doi.org/10.1093/jee/tov142.

Sreenivas A G, Markandeya G, Harischandra N R, Usha R, Hanchinal SG, and Badariprasad PR. 2021. SPLAT-PBW: An eco-friendly, cost-effective mating disruption tool for the management of pink bollworm on cotton. Crop Protection. 149:105784. https://doi.org/10.1016/j.cropro.2021.105784.

Stelinski LL, Miller JR, Ledebuhr R, Siegert P, and Gut LG. 2007. Season-long mating disruption of *Grapholita molesta* (Lepidoptera: Tortricidae) by one machine application of pheromone in wax drops (SPLAT-OFM). Journal of Pest Science. 80(2):109–117. https://doi.org/10.1007/s10340-007-0162-0.

Stern VM, Smith RF, Van Den Bosch R, and Hagen KS. 1959. The integration of chemical and biological control of the spotted alfalfa aphid: The integrated control concept. Hilgardia. 29(2):81–101. https://doi.org/10.3733/hilg.v29n02p081.

Steyn VM, Malan AP, and Addison P. 2024. Experimental quantification of mating disruption for false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae), in stone fruit and table grape cultivation. Crop Protection. 182:106737. https://doi.org/10.1016/j.cropro.2024.106737.

Suckling DM, Baker G, Salehi L, and Woods B. 2016. Is the combination of insecticide and mating disruption synergistic or additive in lightbrown apple moth, *Epiphyas postvittana*? Dickens JC, editor. PLoS ONE. 11(8):e0160710. https://doi.org/10.1371/journal.pone.0160710.

Svensson GP, Wang H-L, Jirle EV, Rosenberg O, Liblikas I, Chong JM, Löfstedt C, and Anderbrant O. 2018. Challenges of pheromone-based mating disruption of *Cydia strobilella* and *Dioryctria abietella* in spruce seed orchards. Journal of Pest Science. 91(2):639–650. https://doi.org/10.1007/s10340-017-0929-x.

Tollerup KE, Rucker A, and Shearer PW. 2012. Whole-farm mating disruption to manage Grapholita molesta (Lepidoptera: Tortricidae) in diversified New Jersey orchards. Journal of Economic. Entomology. 105(5):1712–1718. https://doi.org/10.1603/EC11419.

Trematerra P, Colacci M, Athanassiou CG, Kavallieratos NG, Rumbos CI, Boukouvala MC, Nikolaidou AJ, Kontodimas DC, Benavent-Fernández E, and Gálvez-Settier S. 2019. Evaluation of mating disruption for the control of *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) in suburban recreational areas in Italy and Greece.

Sullivan B, editor. Journal of Economic Entomology. 112(5):2229–2235. https://doi.org/10.1093/jee/toz129.

Trematerra P and Savoldelli S. 2013. The use of water traps and presence of spermatophores to evaluate mating disruption in the almond moth, *Ephestia cautella*, during exposure to synthetic sex pheromone. Journal of Pest Science. 86(2):227–233. https://doi.org/10.1007/s10340-012-0477-3.

Trimble RM, Pree DJ, and Carter NJ. 2001. Integrated control of oriental fruit moth (Lepidoptera: Tortricidae) in peach orchards using insecticide and mating disruption. Journal of Economic Entomology. 94(2):476–485. https://doi.org/10.1603/0022-0493-94.2.476.

Vacas S, Alfaro C, Primo J, and Navarro-Llopis V. 2011. Studies on the development of a mating disruption system to control the tomato leafminer, *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae). Pest Management Science. 67(11):1473–1480. https://doi.org/10.1002/ps.2202.

Vacas S, Navarro I, Primo J, and Navarro-Llopis V. 2016. Mating disruption to control the striped rice stem borer: Pheromone blend, dispensing technology and number of releasing points. Journal of Asia-Pacific Entomology. 19(2):253–259. https://doi.org/10.1016/j.aspen.2016.02.001.

Wijayaratne LKW and Burks CS. 2020. Persistence of mating suppression of the indian meal moth *Plodia Interpunctella* in the presence and absence of commercial mating disruption dispensers. Insects. 11(10):701. https://doi.org/10.3390/insects11100701.

Witzgall P, Kirsch P, and Cork A. 2010. Sex pheromones and their impact on pest management. Journal of Chemical Ecology. 36(1):80–100. https://doi.org/10.1007/s10886-009-9737-y.

Wu Q-J, Zhang S-F, and Yao J-L, et al. 2012. Management of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) by mating disruption: *Management of DBM by mating disruption*. Insect Science. 19(6):643–648. https://doi.org/10.1111/j.1744-7917.2011.01497.x.

Yang CY, Han KS, Jung JK, Boo KS, and Yiem MS. 2003. Control of the oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) by mating disruption with sex pheromone in pear orchards. Journal of Asia-Pacific Entomology. 6(1):97–104. https://doi.org/10.1016/S1226-8615(08)60174-0.

2 Developing a Growing Degree Day Model to Guide Integrated Pest Management of *Eucosma giganteana*, a Pest of a Novel Perennial Oilseed Crop

2.1 Objective 2A: Link trap captures of *E. giganteana* to plant vigor and/or pest pheneology and growing degree days.

2.2 Objective 2B: Develop a trap-based threshold or growing degree day model for *E. giganteana* to guide its magagement using insecticides.

2.3 Abstract

Eucosma giganteana, or the giant Eucosma moth, is a specialist pest on *Silphium* spp. including *Silphium integrifolium*. This pest is currently one of the major limiting factors in the development and commercialization of *S. integrifolium* as a more sustainable oilseed alternative. One of the factors making *E. giganteana* difficult to manage is the lack of knowledge about when pest management tactics should be implemented for maximum effect. To aid with proper timing, our objectives were to determine a lower activity threshold, then use it to develop a growing degree day model to estimate important phenological events in the life history of adult *E. giganteana* in the field. In addition, we found a good fit between the actual phenological events for *E. giganteana* from 2020, 2023, and 2024 and the predicted phenological events from trapping data collected in 2019 in Salina KS. The lower activity threshold was determined to be 17°C using a series of environmental chamber experiments with overwintering *E. giganteana* larvae. Furthermore, we found a significant correlation between predicted growing degree days for phenological events in 2019 and the actual degree day measurements for those events in subsequent years. Finally, the model was able to accurately predict adult *E. giganteana* emergence in the field during 2024. It is expected that the model will provide accurate predictions for the coming years, which allows for improved timing of pest management of *E. giganteana* to be implemented.

Key words: giant Eucosma moth, *Silphium integrifolium*, weather data, thermal ecology, growing degree day model, phenological event

2.4 Introduction

Rosinweed, *Silphium integrifolium* Michx., is a novel perennial oilseed crop native to the prairies of North America currently under domestication by the Land Institute in Salina, KS (Vilela et al. 2018). It is a more sustainable alternative to other oilseed crops like canola and sunflower due to its perenniality and its drought tolerance (Price et al. 2022). As we continue to seek out more sustainable agricultural practices, crops like *S. integrifolium* are becoming increasingly important. However, interfering with its ongoing domestication is a specialist pest on *Silphium* spp., *Eucosma giganteana* (Riley) (Lepidoptera: Tortricidae), or the giant Eucosma moth (Vilela et al. 2020). This moth is univoltine and its larvae are internal feeders on the florets and root crowns of *S. integrifolium* (Johnson and Martens 2019, Murrell et al. 2023, Vilela et al. 2018). When feeding on the florets of *S. integrifolium*, *E. giganteana* larvae cause up to 85% more damage on seedset compared to un-infested florets (Prasifika et al. 2017). In areas with high *E. giganteana* populations, the larvae can infest upwards of 100% of the *Silphium* florets (Johnson and Boe 2011). There is currently no set timing for pest control methods nor a set pest control method for addressing *E. giganteana* infestations. Rather, broad-spectrum insecticides, like permethrin, or whole field mowing are used for management once an infestation has been noticed (Murrell et al. 2023). Finally, prior work has established (*E*)-8-dodecenyl acetate as a successful monitoring attractant for *E. giganteana* when combined with a clear sticky card (Ruiz et al. 2022).

Growing degree day (GDD) models have been used to accurately time pest management for many lepidopteran crop pests including; the grape berry moth (*Paralobesia vetiana* Clemens) (Lepidoptera: Tortricidae), potato tubeworm (*Phthorimaea operculella* Zeller) (Lepidoptera: Gelechiidae), western bean cutworm (*Striacosta albicosta* (Smith)) (Lepidoptera: Noctuidae), pink bollworm (*Pectinophora gossypiella* (Saunders)) (Lepidoptera: Gelechiidae) and codling moth (*Cydia pomonella* (L.)) (Lepidoptera: Tortricidae) (Chen et al. 2015; Hanson et al. 2015; Sporleder et al. 2004). GDD models are especially effective for insect pests as they are poikilothermic, relying on external heat to develop (Chen et al. 2015; Sridhar and Reddy 2013). Using a GDD model to time pest management strategies helps decrease insecticide usage as crops need only be treated once an insect emergence is near, or during peak flight to maximize effectiveness of insecticides.

Degree day models utilize the insect's lower developmental threshold (LDT) and the surrounding environmental temperature to track developmental or phenological milestones for the insect for which they are tailored. Different GDD measurements can be made for the different phenological events by the life stages of an insect species. With a GDD model, growers are able to use the environmental data from their own specific area to more accurately time pest management (Morrison et al. 2014). The GDD accumulation within the model is calculated by the accumulation of daily average temperature above the insect's LDT and below the upper developmental threshold (UDT), if applicable (Chen et al. 2015; Morrison et al. 2014). The UDT is not always utilized and can be difficult to determine for an insect species. Unfortunately, it has yet to be made possible to keep *E. giganteana* in colony currently, despite a lot of effort invested in developing an artificial diet (Murrell et al., unpublished data). This means that it is not possible to assess the lower developmental threshold in the laboratory; however, it is possible to evaluate the lower activity threshold for the species as a surrogate for the LDT. The lower activity threshold, is above the lower walking threshold and is most similar to a lower trap threshold for walking insects. We define it as the temperature in which the insect significantly increases its movement showing that the individual insect is active at the given temperature.

Finally, GDD models require a biofix date, or a date after which degree-days start accumulating in the year, which around the Great Plains tends to be between January 1 or March 1 (Dupuy et al. 2017; Knutson and Muegge 2010; Murray 2020). Beyond GDD models, damage thresholds have also been used to time pest management of multiple agricultural pests. However, this tactic allows for the pest to be present and cause damage before eventually being treated (Leybourne et al. 2024; Thiel et al. 2024). The aims of this study were to: (1) determine the lower activity threshold of *E. giganteana*; (2) develop the GDD model and determine the GDD measurements for key milestones in adult *E. giganteana*; (3) validate the model with historical trap capture data; and (4) assess if a damage threshold can be created based on larval *E. giganteana* infestation in *S. integrifolium* root crowns.

Materials and Methods

2.4.1 Lower activity threshold study

Eucosma giganteana has not yet been successfully lab reared, because of this, we examined the larval lower activity threshold instead of an LDT. To evaluate the lower activity threshold, *E. giganteana* larvae were collected starting in the first week of April to May 21, 2023 at the Land Institute, Salina, KS (38.768402, -97.567081). The larvae were collected from outdoor potted *S. integrifolium* plants. The first three to four inches of soil within each pot was removed and sifted through a screen to remove all loose soil. The remaining debris was examined by hand and any lepidopteran hibernacula were removed and placed into a screw-top container that had been altered to contain a mesh bottom to allow airflow. This process was repeated for all potted *S. integrifolium* plants until each plant's soil had been sifted. All collected hibernacula were transported to the USDA-ARS Center for Grain and Animal Health Research in an insulated ice chest. If they were unable to be transported the same day they were collected, they were instead kept in a refrigerator at 4.4°C. A total of 97 *E. giganteana* larvae were collected across 10 dates (e.g., 2 April, 7 April, 8 April, 12 April, 14 April, 3 May, 4 May, 17 May, 19 May, 21 May).

Once in the lab all hibernacula were opened and the larvae were counted, and any non-*E. giganteana* larvae were removed and frozen out. Larvae were then sorted into Petri dishes (100 × 15 mm diameter: height) and labeled according to when they had been collected, the date they were entering the environmental chamber (Percival Scientific Inc., Perry, IA, USA), and the temperature (7, 12.5, 18, 27.5, and 30 °C). The Petri dishes with larvae were placed in a larger container (300 × 150 × 100 mm length:width:height) containing potting soil to mimic their natural environment, insulation, and humidity. The soil-filled container was watered once it dried out to mimic the usual environment the larvae were in as well as to provide additional moisture. Starting on May 11, *E. giganteana* larvae in chambers were routinely checked on, newly constructed hibernacula were counted, and dead larvae were documented and removed. No further larvae were placed in the 30°C after May 16th as they had all rapidly died.

To determine their lower activity threshold (LAT), two *E. giganteana* larvae were removed from their initial environmental chamber at a time (e.g., 7, 12.5, 18, or 27.5 °C) and placed in a separate environmental chamber with a different temperature as a common garden experiment. This new temperature intended to mimic a change in temperature that the larvae would experience during the spring in their natural environment. In the chamber, the larvae's movements were recorded for 30 minutes in a smaller Petri dish (35 × 15 mm diameter: height) using a Dino-Lite camera (AF4135ZTE, Dino-Lite, VA, USA) attached to a Dino-Lite stand (RK-06A Dino-Lite, VA, USA) using a fully rotating clip before being returned to its chamber of origin. Video was streamed live to a nearby laptop and captured with DinoCapture 2.0 (v.1.5.48.A, AnMo Electronics, New Taipei City, Taiwan). Each larva was only used once at each temperature. The selection of larvae for a given temperature was randomized. The temperature of the common chamber included 5, 6, 8, 9, 10, 11, 14, 17, 20℃. There was a total of n = 5–18 replicates per common chamber temperature. Video files were uploaded manually into Ethovision software (v.16.0, Noldus Inc., Leesburg, VA, USA), which was then used to track and quantify the movement of each larva in the recordings (n= 233 total recordings, 6,990 minutes). The total distance moved (cm) and velocity (cm/s) was recorded.

2.4.2 Weather data and GDD Model

Weather data was provided by The Land Institute through a weather station positioned on its property (38.80000, -97.60000). Shielded air temperature was measured using a Vantage Pro2 Plus weather station (Davis Instruments, Hayward, CA, USA) that fed its data to WeatherLink. The station has been in continuous operation for more than 10 years. This weather station provided readings of the high and low temperature every 30 minutes. GDD were calculated based on the Baskerville-Emin method (Baskerville and Emin, 1969). Briefly, the temperature diurnal time course in a 24-h period is approximated by a sine wave using the high and low temperature readings from the weather station, and area above the lower activity threshold (from the study above), but below the daily maximum approximated by the sine wave is integrated for the resulting GDD. The biofix date for the GDD was set to the 60th day of the year (e.g., March 1st for most years).

2.4.3 Phenological Data

There were two sources of phenological data for the GDD model. One was from historical trap capture data from 2019 and 2020 at The Land Institute (Ruiz et al. 2022). Another was from this study, which was conducted in six fields, three each in 2023 and 2024. All fields were located on The Land Institute's property in Salina, Kansas (Table 2.1 and Table 2.2). This trapping data was used to pair key milestones of adult *E. giganteana* development to GDDs. The key milestones we examined were the beginning of flight, peak flight, middle of flight, and end of flight. Data from 2019 was used to develop predictions, while data in the three other years (e.g., 2020, 2023, 2024) were used to validate the model.

2.4.4 Phenological Data from 2023

Field trapping was done according to the methodology described by Ruiz et al. 2022. The fields were located in North-Central Kansas at the Land Institute (Table 2.1). No pesticides were applied to these fields during this experiment. Starting the first week of June, six transects were set out, two in each *Silphium integrifolium* field. Each transect contained seven 30.4 cm × 30.4 cm clear sticky card traps (Alpha Scents, Canby, OR, USA) folded in half and affixed to the top of a 1.27 cm diameter, 91.4 cm PVC pole that was hammered into the ground about 80 cm. The cards were affixed using a 271-cm-long sticky card ring holder (Olson Products Inc., Medina, OH, USA) that was bent to a 90° angle the placed inside the PVC pipe. Two large binder clips were also used to anchor the sticky card to its card holder (Figure 2.1).

The sticky traps in each transect were spaced 10 m apart around the perimeter of the field. For each transect, three of the seven traps were baited with a control of 50 µl of acetone inside a LDPE 3-mL dropping bottle (Wheaton, DWK Life Sciences, Millville, NJ, USA). The remaining four traps were baited with 50 µl of diluted (*E*)-8-dodecenyl acetate, (Alfa Chemistry, Ronkonkoma, NY, USA). The low concentration of (*E*)-8-dodecenyl acetate was made by diluting 5.75 µl of (*E*)-8-dodecenyl acetate in 5 ml of acetone. A doubled concentration of (*E*)-8-dodecenyl acetate was made by diluting 11.5 µl in 5 ml of acetone. In all cases, the baited dropping bottle was placed in the top of the PVC pipe by the base of the sticky card (Figure 2.1). The sticky cards were collected and replaced biweekly until the first *E. giganteana* individual was caught, at which time it was changed to weekly. The lures and control bottles were replaced biweekly, and the treatment positions were rotated weekly.

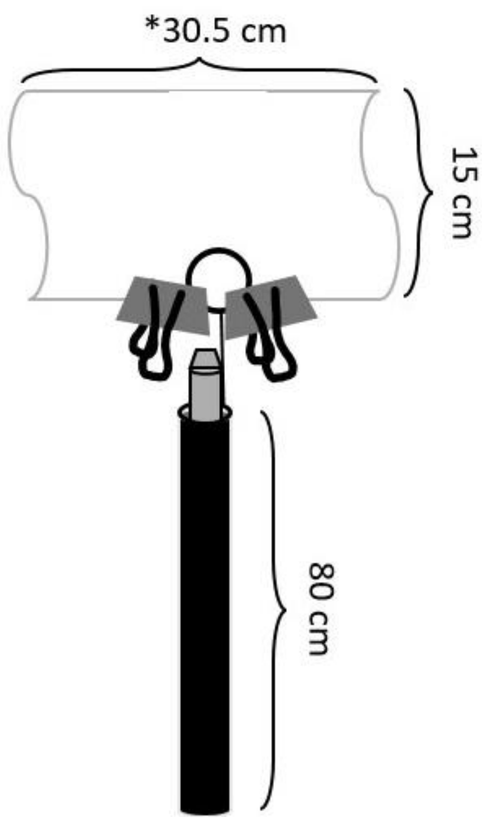

Figure 2.1 Schematic of sticky card assembly in the field including the PVC pipe, dropping bottle lure, sticky card ring holder, sticky card, and two binder clips.

Not pictured is the wire cage. The sticky card is folded in half from top to bottom creating an oval shaped tunnel down the center before being attached to the sticky card ring holder. *The width of the sticky cards was 30.5 cm in 2023 and 21.5 cm in 2024.

When collected, the sticky cards were held in a 7.6 L (=2 gal) labeled Ziploc® bag for transport back to USDA-ARS. All collected sticky traps were placed in a freezer for approximately 24 hours prior to counting. The total number of *E. giganteana* per trap and their distance from the lure in millimeters was recorded. In addition, the number of nontarget Lepidoptera were recorded on each trap. Individual *E. giganteana* and non-target Lepidoptera were only counted if more than half of the specimen was remaining on the sticky trap at the time of counting.

Table 2.1 Summary of field sites used during field trapping of *E. giganteana* in 2023 at the Land Institute in Salina, KS.

Field ID	County	Latitude	Longitude	Area (ha)	Insecticide application?
A	Saline	38.771031	-97.569505	0.226	No
B1	Saline	38.772959	-97.592470	0.152	No
B2	Saline	38.76969	-97.59314	0.152	No

2.4.5 Phenological Data from 2024

Field trapping in 2024 was conducted similarly to that in 2023 with the following modifications. Three different fields located at The Land Institute were used (Table 2.2). The pesticides (methoxyfenozide and chlorantraniliprole) were applied once during the season directly to one of the fields and adjacent to one of the other fields. Three transects were set out in each of the three fields used. Each transect contained four traps for a total of 36 traps. The traps were assembled similarly as in 2023, however hand-made sticky cards were used instead of manufactured ones, because of a noticeable decrease in efficacy in capturing *E. giganteana*. These sticky cards were made of a laminated 21.6 × 27.9 cm (=8.5 by 11 in) piece of white cardstock paper (Astrobright, Neenah, WI, USA) coated on both sides with TAD® all-weather (Trécé Adhesives Division, Adair, OK, USA). The sticky sides were covered with wax paper for transport. In the field, the sticky cards had a chicken wire cage placed over the sticky card to try to prevent the capture of birds and other nontargets on the traps. In each transect, one of the traps was baited with a control of 50 µl of acetone inside a 3-mL dropping bottle, one with 50 µl of (*E*)-8-dodecenyl acetate at a low concentration (5.75 µl of (*E*)-8-dodecenyl acetate in 5 ml of acetone), a medium dose (78.5 µl of (*E*)-8-dodecenyl acetate in 5 ml of acetone), and at a much higher concentration (580.4 µl of (*E*)-8-dodecenyl acetate in 5 ml of acetone). The traps were replaced weekly, and the baited dropping bottles were replaced biweekly at which point the position of the treatment was rotated weekly. The total number of *E. giganteana* per trap and the number of nontarget Lepidoptera was recorded on each trap. For both years, captures are averaged across baited treatments to generalize phenological events for GDD model.

Table 2.2 Summary of field sites used during the field trapping of *E. giganteana* in 2024 at the Land Institute in Salina, KS.

Field ID	**County**	**Latitude**	**Longitude**	**Area (ha)**	**Insecticide application?**
B3	Saline	38.772315	-97.592594	0.168	Yes
C	Saline	38.769622	-97.598576	0.557	No
D	Saline	38.698001	-97.575080	0.141	Adjacent

2.4.6 Damage threshold

Silphium integrifolium root crows were dug up at the beginning of April from a site managed by The Land Institute in Colby, KS. All *S. integrifolium* plants from this site were half siblings and had no prior record of *E. giganteana* damage. The dead stalks from the *S. integrifolium* plants in prior years were used to guide where to dig. More than one hundred root crowns were retrieved and transported back to The Land Institute in Salina, KS. The root crowns were placed in pots (15.8 cm in diameter × 20.5 cm height) and allowed time to establish themselves and sprout approximately three weeks. Once they were sprouted and rootbound within the pot, they were re-potted into the cloth pots (3 gallon) that would be used for the experiment. The plants were subsequently brought outside to continue maturing in the cages (68.58 cm x 68.58 cm x 182.88 cm length:width:height) for the experiment starting in June to prevent other pests from interfering with the plants. There were 20 cages total, each containing four plants, for a total of 80 plants. The cages were arranged in a grid over irrigation channels.

The *E. giganteana* larvae were collected in the middle of August by pulling them out of damaged *S. integrifolium* flower heads at The Land Institute. Each flower head was torn open and any *E. giganteana* larvae found within were removed and placed on diet cups. They remained on the diet cups for two weeks before being placed in the *S. integrifolium* pots. *Eucosma giganteana* diet was made according to a pre-existing recipe designed by Edy Chérémond at the Land Institute. The diet consisted of 76.6 g *S. integrifolium* flower heads collected fresh from the field, 20 g agar, 30 g wheat germ, 11.7 g sucrose, 10 g salts Wessons, 15 g vitamins, 2 g sorbic acid, 2 g MethylParaben, 11.7 g casein, 0.37 g aureomycin, and 850 L water (600 L boiling to dissolve agar and 250 L room temperature to cool the mixture) The diet mixture was placed into individual cups and allowed to dry for about a week before larvae were added to the cups. A total of 4–7 larvae were added to each cup.

Cages were broken up into five treatments, with varying densities of *E. giganteana* larvae: control (e.g. zero larvae), 10, 20, 30, and 40 larvae per cage. The number of larvae were distributed among the four plants within the cage (0, 2–3, 5, 7–8, 10 larvae per pot) and labeled accordingly. A metal mesh top was constructed to cover the pots, and to prevent the larva from escaping once they were placed inside. There were 16 replicates of each larval density. Cages were left out until the first week of December 2023 to allow the larvae time to first descend into the root crowns and then exit the root crows to prepare for overwintering. The pots containing the root crowns and the larvae were held in a cold stage room at 4°C until it was warm enough outside to sift through the samples. During April and May of 2024, the soil surrounding the root crown of each potted plant was sifted through using 27-gauge hardware cloth to locate any remaining larvae and empty hibernacula. The numbers found of larvae and hibernacula were recorded and the root crown was washed of all remaining soil and placed in a labeled bag.

Prior to being X-rayed, each root crown was weighed and then cut into slices smaller than 10 × 10 cm and 2 cm thick. Each slice was placed in the bottom half of a 10 × 10 cm square Petri dish. Each slice was then x-rayed using a Faxitron radiography system (Faxitron X-ray Corp., Marlborough, MA, USA) on two of its sides to determine if there was tunneling by larvae. After X-raying was complete, the best image from each slice was imported into ImageJ v.1.53 (Wayne Rasband, National Institutes of Health, USA), and the two-dimensional area of the root crown slice was taken as well as the number and two-dimensional area of the tunnels. Using this information, we were able to determine the proportion of the area of each root crown that was tunneled.

2.4.7 Statistical Analysis

A general linear model (GLM) was used to analyze the distance moved and velocity of larval movement. Temperature in the common chamber was used as an independent, fixed, explanatory variable along with rearing chamber temperature and their interaction. Residuals were inspected to ensure conformity to normality and homogeneity in variances, which was not fulfilled, so a log-transformation was used. Upon a significant model result, multiple comparisons were employed using Tukey HSD. For this and all other tests, $\alpha = 0.05$. All analyses were conducted in R Software (R Core Team, 2024).

To validate the GDD model, the predicted GDD from 2019 were regressed against the actual GDD for each of the phenological events in each of the subsequent years. In addition, a repeated measures ANOVA was performed on trap captures in 2023 and 2024 using GDD as an explanatory variable. A first order autoregressive variance-covariance matrix was used to model the temporal autocorrelation. Field was included as a random effect. The years were analyzed separately.

For the X-ray data, an ordered-logistic regression was performed on the proportion tunneled. larval density was used as a fixed, explanatory variable. Upon a significant result, Tukey HSD was used for multiple comparisons at $\alpha = 0.05$.

2.5 Results

2.5.1 Lower activity threshold

We found that the common test temperature had a significant effect on the distance moved and velocity of larval movement ($p < 0.001$) (Table 2.3). Acclimation temperature was not significant in either distance moved or velocity of the test individual (Table 2.3). However, the interaction between the acclimation temperature and the test temperature were significant in both cases

(Table 2.3). In all acclimation temperatures except for 12.5 ℃, in which it was second, the individual larvae moved the most at 17 ℃. Because of this, 17 ℃ was chosen as the lower activity threshold to use in the GDD model in place of the LDT.

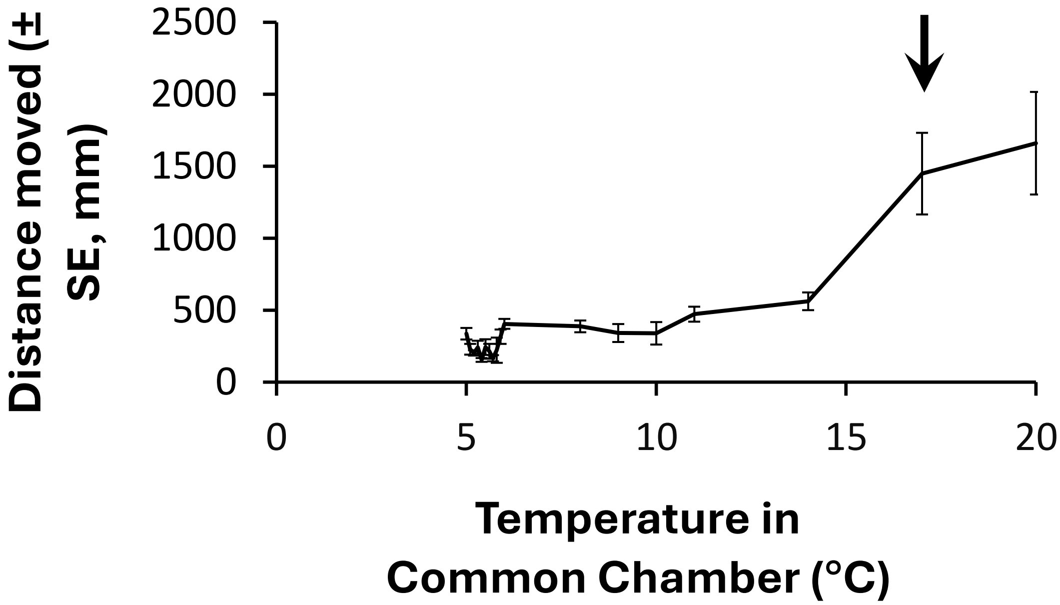

Figure 2.2 Distance moved (± SE) in millimeters by overwintering *E. giganteana* larvae emerging in the spring when subjected to the same temperature in a common chamber after being acclimated to temperatures first at 7, 12.5, 18, 27.5, and 30℃. Black arrow points out the lower activity threshold for the GDD model.

Table 2.3 Statistical summary from the GLM performed on the velocity and distance moved by *E. giganteana* larvae according to test temperature, acclimation temperature, and their interaction.

	Velocity				Distance moved		
Factor	**df**[1]	*F*	*P*		**df**[1]	*F*	*P*
Test temp.	1	105.9	0.001		1	126.0	0.001
Acc. temp.	1	2.65	0.105		1	0.95	0.33
Interaction	1	7.08	0.008		1	6.37	0.012

[1] Residual df = 229 for both models.

2.5.2 Trap captures

We observed similar patterns in trap captures for most years typically with a unimodal curve reflecting *E. giganteana*'s univoltine life history (Figure 2.3). The single year that was bimodal arouse as an artefact of the manufacturer switching glue formulation on sticky cards used in 2023. Though we do not directly compare abundance across years, it appears like *E. giganteana* pressure has been much less pronounced after 2019.

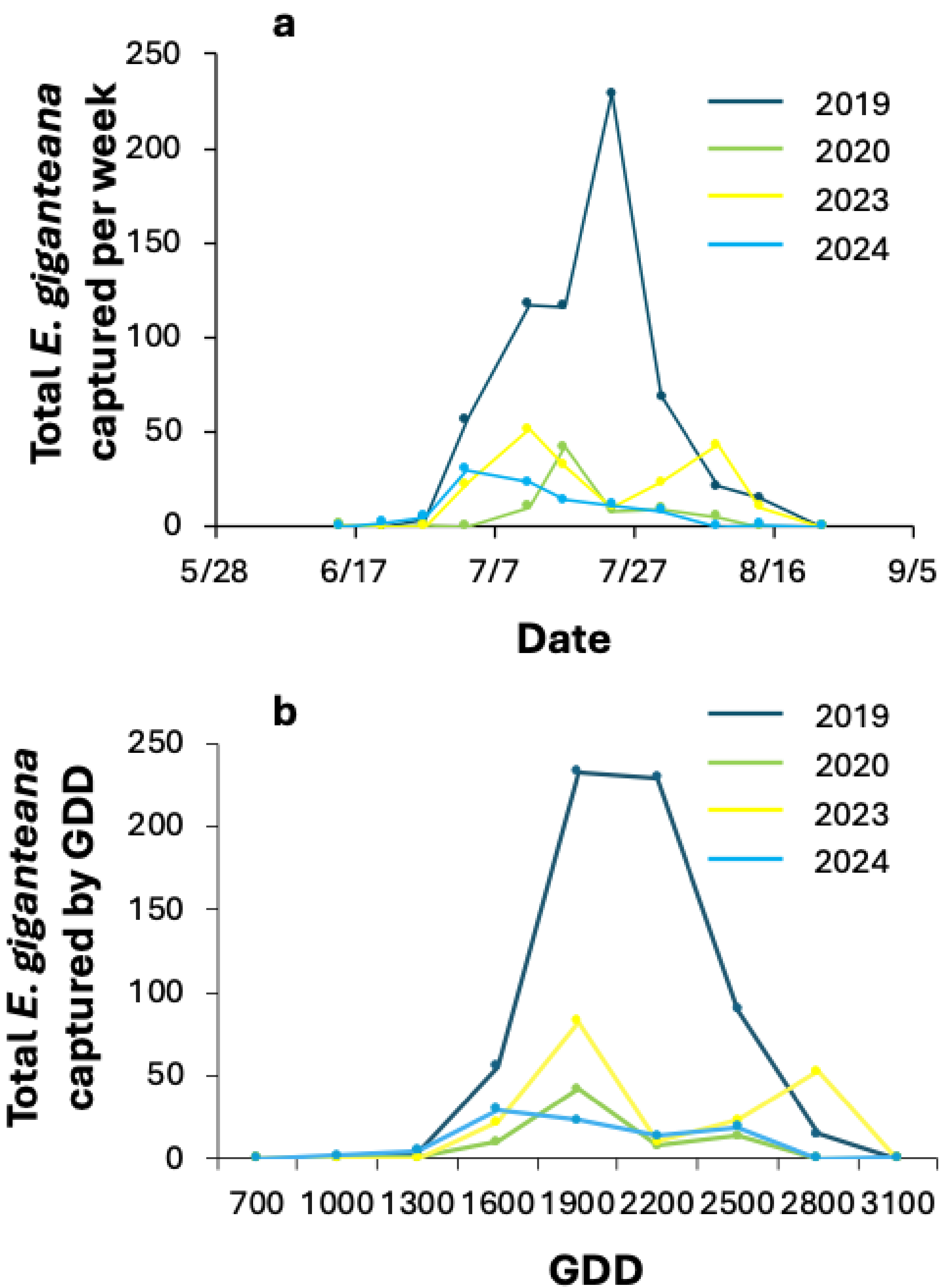
a
Total E. giganteana captured per week
250
200
150
100
50
0
5/28
6/17
7/7
7/27
8/16
9/5
Date
2019
2020
2023
2024
b
Total E. giganteana captured by GDD
250
200
150
100
50
0
700
1000
1300
1600
1900
2200
2500
2800
3100
GDD
2019
2020
2023
2024

Figure 2.3 Total weekly trap captures throughout the growing season of 2019, 2020, 2023, and 2024 by date (a) and by GDD (b).

2.5.3 Growing degree days

Degree day accumulations followed a similar trend in all four years, 2019. 2020, 2023, and 2024 (Figure 2.4). Each year resulted in increasing GDD measurements with 2024 having the highest GDD accumulation by September. We observed that 2020 and 2023 showed the most similar GDD accumulation pattern. The trapping data in 2023 and 2024 was combined with previous trapping data from 2019 and 2020. Through this, we were able to pair GDD measurements with actual *E. giganteana* phenological events (Table 3). The average ± SE beginning of *E. giganteana* flight was 1608 ± 87 GDD, while the average peak flight was 1872 ± 127. The middle of flight for *E. giganteana* was 2336 ± 100 GDD and the end of flight was 2947 ± 64.

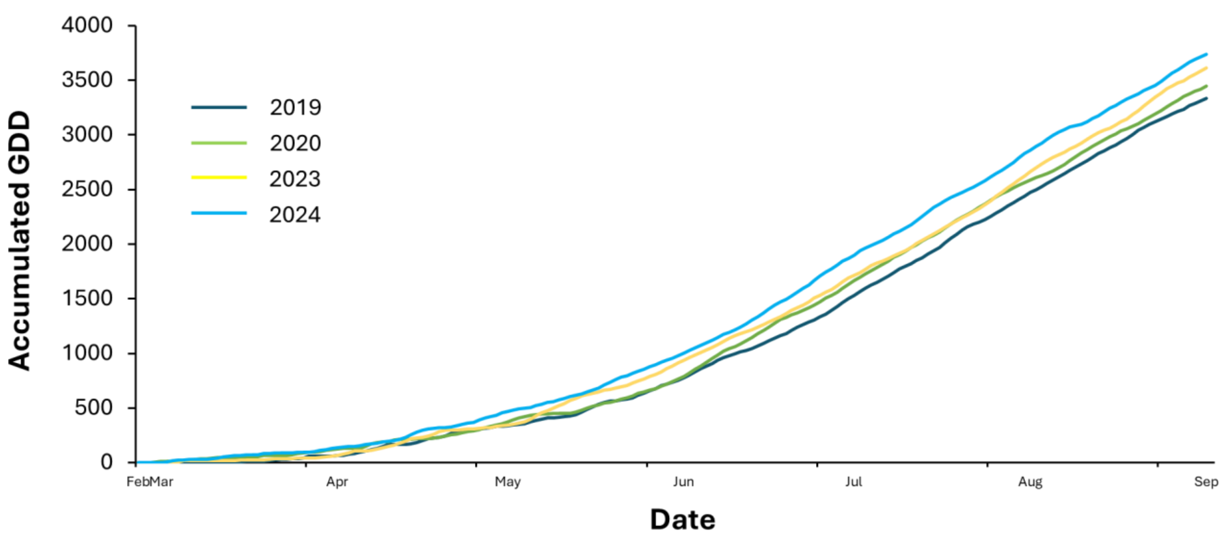

Figure 2.4 Accumulated GDD in 2019, 2020, 2023, and 2024 using a biofix date of Mar 1, 17°C as a LDT, the Baskerville-Emin method of calculation, and terminating at the end of the phenological period for *E. giganteana*.

Table 2.4 GDD measurements from 2019, 2020, 2023, and 2024 for the beginning of *E. giganteana* flight, peak flight, middle of flight, and the end of flight. Based on a GDD model with a biofix of 1 Mar, 17°C as an LDT, and that used the Baskerville-Emin method of calculation. The GDD for the beginning of flight was calculated by the GDD on the day the sticky card was taken down. The middle of flight was when half of the flight period had elapsed. The GDD for the end of flight was calculated by the GDD on the day the trap was placed that yielded zero *E. giganteana* trap captures during that week.

	Phenological Events			
Year	**Emergence**	**Peak**	**Middle**	**End**
2019[1]	1432	2090	2225	2854
2020	1595	2030	2115	N/A[2]
2023	1847	1847	2550	3092
2024	1561	1523	2453	2894

[1] Predicted GDD for phenological events for *E. giganteana* were based on 2019 data.

[2] Field trapping in 2020 was terminated prior to the end of *E. giganteana* flight.

2.5.4 GDD Model Validation

Validation of the GDD model was done by conducting a linear regression of the predicted GDD measurements for events from the 2019 data to the actual GDD measurements in 2020, 2023, and 2024 (Figure 2.5). The relationship between the predicted GDD in 2019 and the actual GDD in subsequent significantly explained 73.3% of the variation in the data for *E. giganteana*'s flight ($R^2 = 0.733$; $P < 0.001$).

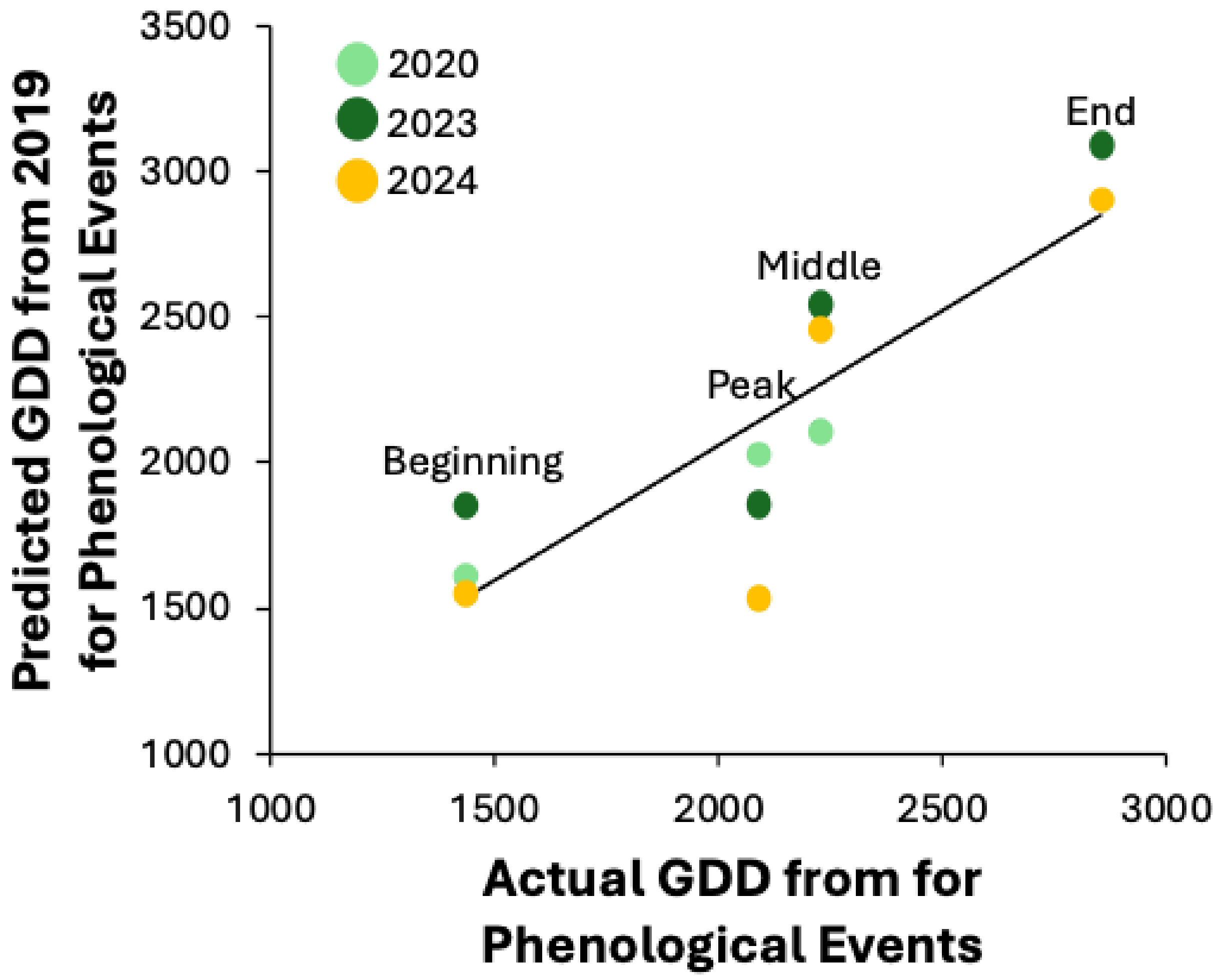

Figure 2.5 Linear regression of the predicted GDD from 2019 plotted against the actual GDD for phenological events of *E. giganteana* for 2020, 2023, and 2024.The calculated line is y = 0.9245x + 208.07. Multiple R^2 value equals 0.733. Standard error of 295.4 on 9 degrees of freedom.

2.5.5 Root Crown Damage

There was no significant difference in either the number of tunnels found (χ^2 = 4.92; df = 4; P = 0.29) within root crowns or the proportion of the root crown area that was tunneled (F = 0.09; df = 1, 78 P = 0.77) compared to the treatment of *E. giganteana* larval density infesting the root crowns.

Although the number of tunnels and the average proportions of the root crown area tunneled were not statistically different between the control with no larva and the different treatments with 2-10 larvae, there were numerically more tunnels and a higher proportion of tunneled area in all the infested treatments compared to the control treatment (0 larvae)(Figure 2.6 and Figure 2.7).

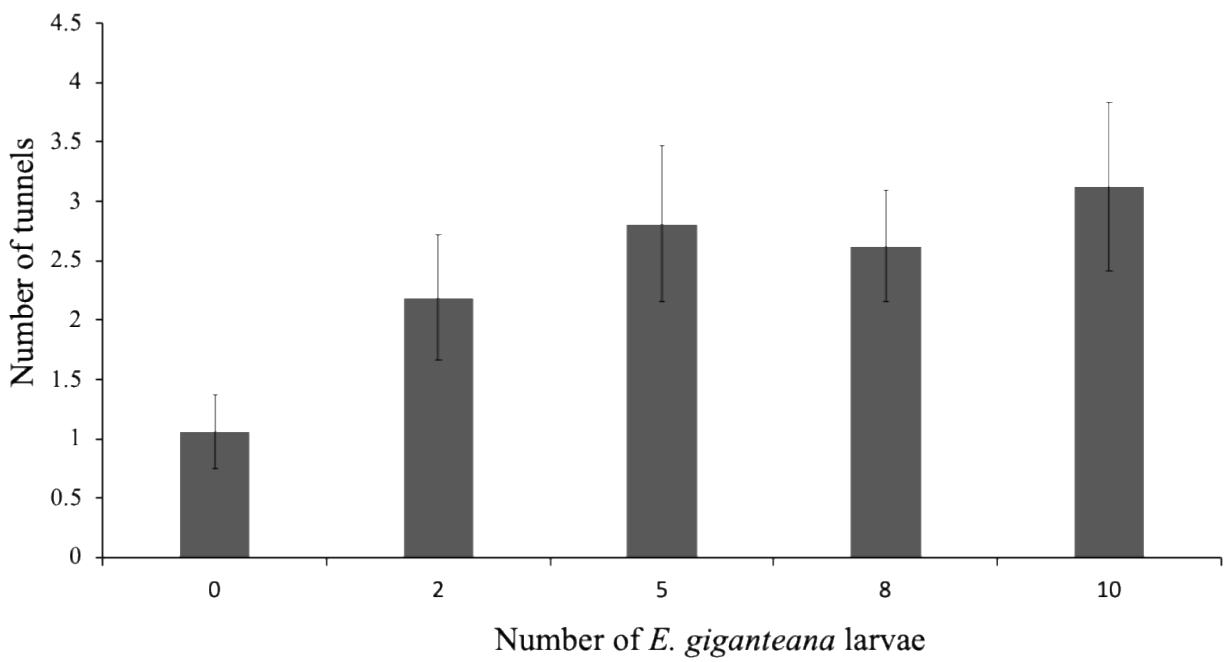

Figure 2.6 Average number (± SE) of tunnels found in the root crows by treatment of *E. giganteana*. There were no significant differences among densities, so letters were omitted from the graph (Tukey HSD, α = 0.05).

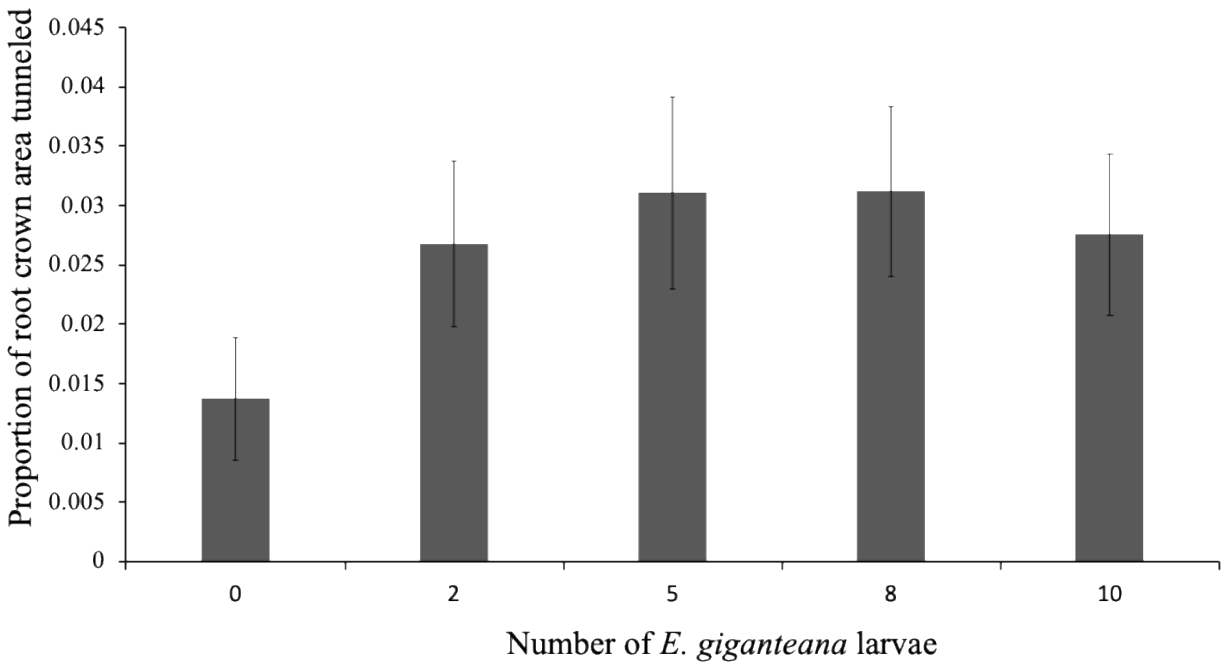

Figure 2.7 Average proportion (± SE) of root crown area tunneled by treatment of *E. giganteana*. There were no significant differences among densities, so the letters were omitted from the graph (Tukey HSD, α = 0.05)**.**

2.6 Discussion

This is a novel study developing and validating a growing degree day model for *E. giganteana* in *S. integrifolium* fields. Trap captures were similar throughout 2019, 2020, 2023, and 2024 with some variation due to different fields used, different kinds of sticky cards used, and the yearly variation in insect numbers. In 2024, the GDD model accurately predicted the beginning of flight for *E. giganteana*, even though it was two weeks ahead of schedule from prior years. This was confirmed by checking the fields on a daily basis around the predicted emergence (Murrell, personal communication). Using the GDD model, we were able to provide updates during the growing season in 2024 about when the estimated *E. giganteana* emergence would begin. This allowed *E. giganteana* to be noticed earlier and subsequently treated than if the GDD model had not been used (Murrell, personal communication). In fact, the regression model showed the GDD model is relatively effective at accurately predicting when *E. giganteana* phenological events will occur based on GDD measurements. However, it may under predict the peak *E. giganteana* flight. Overall, this will be an enormously helpful tool for those trying to establish *S. integrifolium* plantings on the Great Plains who would like a support tool to help manage *E. giganteana* infestations.

We determined that the lower activity threshold was 17°C for overwintering *E. giganteana*, which served as a suitable surrogate for the LDT. This alternative approach was necessary given the fact that the species cannot be successfully reared indoors. In this case, it was successful in developing a GDD model that could accurately predict the phenological events for *E. giganteana* moths. Other GDD models, for example for European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) have investigated the base temperature in the model as the threshold temperature intending to equal the highest daily mean temperature before growth or activity of the organism commences (Baker et al. 1984). Given how late in the season that *E. giganteana* emerges (e.g., late June), it makes sense that the lower activity threshold is relatively high compared to other organisms that emerge earlier in the spring (e.g., Morrison et al. 2014).

Although we varied the different densities for *E. giganteana* larva over realistic values from the field, it was interesting to see that there was very little variation in regard to tunneling with any of the infested root crowns. Since this was the first study of its kind with *S. integrifolium* and *E. giganteana* we had used root crowns from already established plants rather than growing new plants from seed, which can take up to two years for *S. integrifolium* to flower. However, this allowed the root crowns to have prior pest damage that we were not able to differentiate from the damage done by the current larval infestation in the study. Similarly, there were some arthropods including isopods and beetle larvae that were able to make it inside the cages and into the pots that may have been able to contribute to root crown damage. Nonetheless, we did find that damage was numerically elevated with the addition of *E. giganteana*. Future work can be done to evaluate how *E. giganteana* larval damage to *S. integrifolium* root crowns effects biomass production.

A next step will be to pair the GDD model with an insecticide application or other management action to understand if it can be strategically used in conjunction with the model to reduce pest infestation at a key point in their life history, while reducing the amount of inputs into growing *S. integrifolium*. For example, Finnish growers readily following GDD models for key vegetable pests were able to target insecticide applications to peak populations of those pests in three vegetable crops (Räsänen et al. 2023). Ideally, insecticide applications for *E. giganteana* would be targeted for peak flight (e.g., 1872 ± 127 GDD) so only one insecticide application needs to be applied in the system. Prior work has suggested that permethrin may be effective against *E. giganteana* (Vilela et al. 2020) and may be one option to use. Murrell et al. (2023) also found permethrin, cyfluthrin, chlorantraniliprole, and methoxyfenozide are effective at inducing *E. giganteana* mortality and/or reducing infestation. In Kansas, there is also the MesoNet network of weather stations, through which local fields can access up-to-date weather information for the GDD model. Together, a GDD model for *E. giganteana*, along with weather information will help guide management for this pernicious pest of *S. integrifolium*.

It is likely that this GDD model will accurately predict *E. giganteana* populations at similar latitudes in North America to Salina, KS, since this is where it was developed. However, it is unknown if the GDD model for *E. giganteana* will function properly for populations much more extreme northward or southward. Future work should examine if it can be used for other populations in *S. integrifolium* fields in Nebraska, Missouri, and South Dakota (Prasifka et al. 2017, Reinert et al. 2019). Climate change is a looming issue for much of the agricultural supply chain (Gerken and Morrison, 2022), with some species predicted to expand their ranges (Harman et al. 2024). While it is still unknown if climate change will affect the reliability of GDD models (Murray 2020), it is possible that it can gradually shift the actual GDD at which phenological events occur. However, we did not see major variation from our predicted GDD measurements in 2024, the warmest year, and one in which there was increasing temperatures caused by El Niño (Varotsos et al. 2024). Overall, our GDD model for *E. giganteana* is a novel contribution to the literature that will help improve integrated pest management for a pest in this unique agroecosystem.

2.7 References

Baker DG, Sharratt BS, Chiang HC, Zandlo JA, and Ruschy DL. 1984. Base temperature selection for the prediction of european corn borer instars by the growing degree day method. Agricultural and Forest Meteorology. 32(1):55–60. https://doi.org/10.1016/0168-1923(84)90028-5.

Baskerville GL and Emin P. 1969. Rapid estimation of heat accumulation from maximum and minimum temperatures. Ecology. 50(3):514–517. https://doi.org/10.2307/1933912.

Beasley CA and Adams CJ. 1996. Field-based, degree-day model for pink bollworm (Lepidoptera: Gelechiidae) development. Journal of Economic Entomology. 89(4):881–890. https://doi.org/10.1093/jee/89.4.881.

Chen S, Fleischer SJ, Saunders MC, Thomas MB, and Hull JJ. 2015. The influence of diurnal temperature variation on degree-day accumulation and insect life history. PLoS ONE. 10(3):e0120772. https://doi.org/10.1371/journal.pone.0120772.

Dupuy MM, Powell JA, and Ramirez RA. 2017. Developing a degree-day model to predict billbug (Coleoptera: Curculionidae) seasonal activity in Utah and Idaho turfgrass. Journal of Economic Entomology. 110(5):2180–2189. https://doi.org/10.1093/jee/tox210.

Gerken AR and Morrison WR. 2022. Pest management in the postharvest agricultural supply chain under climate change. Frontiers in Agronomy. 4:918845. https://doi.org/10.3389/fagro.2022.918845.

Hanson AA, Moon RD, Wright RJ, Hunt TE, and Hutchinson WD. 2015. Degree-day prediction models for the flight phenology of western bean cutworm (Lepidoptera: Noctuidae) assessed with the concordance correlation coefficient. Journal of Economic Entomology. 108(4):1728–1738. https://doi.org/10.1093/jee/tov110.

Harman RR, Morrison WR, Ludwick D, Gerken AR, and Campbell J. 2024. Predicted range expansion of *Prostephanus truncatus* (Coleoptera: Bostrichidae) under projected climate change scenarios. Campbell J, editor. Journal of Economic Entomology. 117(4):1686–1700. https://doi.org/10.1093/jee/toae085.

Johnson PJ, and Boe A. 2011. Three interesting insects and the cause of reduced vigor of cup plant (Silphium perfoliatum) in agronomic plantings. Proceedings of the South Dakota Academy of Science. 90.

Johnson PJ, Boe A, and Martens AP. 2019. *Eucosma giganteana* (Riley) and *Silphium perfoliatum* L., morphological variation in an insect-plant association in eastern South Dakota. Proceedings of the South Dakota Academy of Science, 98:91–104.

Jones VP, Hilton R, Brunner JF, Bentley WJ, Alston DG, Barrett B, Van Steenwyk RA, Hull LA, Walgenbach JF, Coates WW, and Smith TJ. 2013. Predicting the emergence of the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), on a degree-day scale in North America: codling moth emergence in North America. Pest Management Science 69(12):1393–1398. https://doi.org/10.1002/ps.3519.

Knutson AE and Muegge MA. 2010. A degree-day model initiated by pheromone trap captures for managing pecan nut casebearer (Lepidoptera: Pyralidae) in pecans. Journal of Economic Entomology. 103(3):735–743. https://doi.org/10.1603/EC09319.

Leybourne DJ, Storer KE, Marshall A, Musa N, Telling S, Abel L, White S, Ellis S, Yang P, and Berry PM. 2024. Thresholds and prediction models to support the sustainable management of herbivorous insects in wheat. A review. Agronomy for Sustainable Development. 44(3):29. https://doi.org/10.1007/s13593-024-00965-5.

Morrison WR and Andresen J, Szendrei Z. 2014. The development of the asparagus miner

(*Ophiomyia simplex* Loew; Diptera: Agromyzidae) in temperate zones: a degree-day model. Pest Management Science. 70(7):1105–1113. https://doi.org/10.1002/ps.3654.

Murray M. 2020 Mar. Using degree days to time treatments for insect pests. Utah State University Extension and Utah Plant Pest Diagnostic Laboratory:1–5.

Murrell EG, Zio KR, Chérémond NE, and Van Tassel DL. 2023. Assessing effective mechanical and chemical strategies for managing *Eucosma giganteana* (Lepidoptera: Tortricidae) in the perennial oilseed crop, *Silphium integrifolium* (Asteraceae: Heliantheae). Journal of Insect Science. 23(6):4. https://doi.org/10.1093/jisesa/iead102.

Prasifka JR, Mallinger RE, Hulke BS, Larson SR, and Van Tassel DL. 2017. Plant–herbivore and plant–pollinator interactions of the developing perennial oilseed crop, *Silphium integrifolium*. Environmental Entomology 46(6):1339–1345. https://doi.org/10.1093/ee/nvx134.

Price JH, Van Tassel DL, Picasso VD, and Smith KP. 2022. Assessing phenotypic diversity in silflower (*Silphium integrifolium* Michx.) to identify traits of interest for domestication selection. Crop Science 62(4):1443–1460. https://doi.org/10.1002/csc2.20748.

Räsänen K, Kaseva J, Aaltonen M, and Vänninen I. 2023. Temporal dynamics of pesticide use in three field vegetable crops with respect to sowing date and degree days. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science. 73(1):213–225. https://doi.org/10.1080/09064710.2023.2275793.

Reinert S, Van Tassel DL, Schlautman B, Kane NC, and Hulke BS. 2019. Assessment of the biogeographical variation of seed size and seed oil traits in wild *Silphium integrifolium* Michx. genotypes. Plant Genetic Resources 17(5):427–436. https://doi.org/10.1017/S1479262119000248.

Ruiz K, Bruce A, Chérémond N, Stratton C, Murrell E, Gillette S, and Morrison WR. 2022. Field trapping and flight capacity of *Eucosma giganteana* (Riley) (Lepidoptera: Tortricidae) in response to behaviorally active congeneric semiochemicals in novel silflower agroecosystems. Insects 13(4):350. https://doi.org/10.3390/insects13040350.

Sporleder M, Kroschel J, Quispe MRG, and Lagnaoui A. 2004. A temperature-based simulation model for the potato tuberworm, *Phthorimaea operculella* Zeller (Lepidoptera; Gelechiidae). Environmental Entomology 33(3):477–486. https://doi.org/10.1603/0046-225X-33.3.477.

Sridhar V and Reddy PVR. 2013. Use of degree days and plant phenology: A reliable tool for predicting insect pest activity under climate change conditions. In: Singh HCP, Rao NKS, Shivashankar KS, editors. Climate-Resilient Horticulture: Adaptation and Mitigation Strategies. India: Springer India. p. 287–294. https://doi.org/10.1007/978-81-322-0974-4_25.

Thiel L, Mergenthaler M, Wutke M, and Haberlah-Korr V. 2024. Use of insect pest thresholds in oilseed rape and cereals: is it worth it? Pest Management Science. 80(5):2353–2361. https://doi.org/10.1002/ps.7647.

Varotsos C, Sarlis NV, Mazei Y, Saldaev D, and Efstathiou M. 2024. A composite tool for forecasting El Niño: the case of the 2023–2024 event. Forecasting. 6(1):187–203. https://doi.org/10.3390/forecast6010011.

Vilela A, González-Paleo L, Turner K, Peterson K, Ravetta D, Crews T, and Van Tassel DL. 2018. Progress and bottlenecks in the early domestication of the perennial oilseed *Silphium integrifolium*, a sunflower substitute. Sustainability 10(3):638. https://doi.org/10.3390/su10030638

3 Concentration Response in Trapping of *Eucosma giganteana* to a Known Attractant, (*E*)-8-dodecenyl acetate

3.1 Objective 3C: Examine the field response of *E. giganteana* to increasing concentrations of (*E*)-8-dodecenyl acetate

3.2 Abstract

Silphium integrifolium, a perennial oilseed crop native to North American prairies, is currently under domestication to be a more sustainable alternative to sunflower and canola oil. However, one major limiting factor to its domestication is its specialist pest, *Eucosma giganteana* (Lepidoptera: Tortricidae (Riley). The larvae of these moths drastically reduce seed set and biomass production. Previous work was conducted to determine an element of their sex pheromone ((*E*)-8-dodecenyl acetate) to use for monitoring the pest's population. While it was known that this pheromone compound attracts *E. giganteana*, it is unknown if there is a related concentration effect to trap captures. In this study, our objective was to evaluate *E. giganteana* trap captures in response to increasing concentrations of a known attractant, (*E*)-8-dodecenyl acetate. During trapping in 2023, the low and double concentration of (*E*)-8-dodecenyl acetate captured 4- and 6-fold more *E. giganteana* compared with the control, respectively. However, captures were equivalent between the low and doubled concentration. In 2024, *E. giganteana* captures were 3- and 6-fold higher in the medium and high concentration baited traps, respectively, compared to the low concentration and controls. The linear or exponential concentrations did not significantly affect captures of nontarget Lepidoptera in 2023 or 2024, suggesting (*E*)-8-dodecenyl acetate is specific in attraction to *E. giganteana*. This knowledge can

help with the development of a more effective monitoring program for *E. giganteana* or possibly a behaviorally-based pest management program in the future.

Key words: giant Eucosma moth, sex pheromone, concentration effect, pheromone trapping, (*E*)-8-dodecenyl acetate

3.3 Introduction

Eucosma giganteana (Riley) (Lepidoptera: Tortricidae) is a univoltine, specialist pest, on *Silphium* spp. The adult moths begin their flight in the early summer around the timing of *Silphium* blooming. Adult female *E. giganteana* lay their eggs on the inflorescences of *Silphium* and shortly after, the young larvae hatch out and bore into the flower heads to feed, decreasing seed set (Prasifka et al. 2017). The larvae feed there for a few weeks before descending into the soil and to the root crown to finish feeding (Murrell et al. 2023). The insect overwinters as larvae in a hibernaculum. One of its main host plants, *Silphium integrifolium*, is a perennial oilseed crop native to North American prairies and it is currently under development as a more drought tolerant replacement to sunflower and canola (Price et al. 2022; Turner et al. 2018; Van Tassel et al. 2017; Vilela et al. 2020). However, *E. giganteana* is a major limiting factor to the growth and seed production of *S. integrifolium*, causing an upwards of 85% seed loss in infested flower heads (Johnson and Boe 2011; Johnson et al. 2019; Vilela et al. 2018).

Pest population monitoring is an important aspect of any integrated pest management strategy (Miller et al. 2015). Successful monitoring generally provides insight into when the pest is present and the size of the pest population and can often be linked to a threshold. We recently found that a growing degree model could reliably predict when phenological stages of *E. giganteana* emerge in the field based on trapping data (Chapter 2). Long-term monitoring programs often provide a way to ground truth the results from predictive models and can be useful for growers.

Historically, sex pheromones have been historically used for monitoring and management of other tortricid pests in agricultural environments (Frerot et al. 1979; Hung et al. 2001; Knight et al. 2017). In recent years, prior work has identified the compound (*E*)-8-dodecenyl acetate as a general attractant for *E. giganteana* adults during flight (Ruiz et al. 2022). When combined with a clear sticky card, low concentrations of (*E*)-8-dodecenyl acetate have been found to be an effective lure for monitoring *E. giganteana*. However, there is little known about its prospects for use in mating disruption, or other behaviorally-based management approaches (Morrison et al. 2016). Generally, to be effective in a behaviorally-based approach, it is helpful if pest individuals exhibit concentration-dependent increases in attraction (e.g., Morrison et al. 2016). There has been much interest in developing a behaviorally-based management program for *E. giganteana* in order to reduce the need for insecticide inputs. Thus, our aims were to examine the effects of different dosages of (*E*)-8-dodecenyl acetate on *E. giganteana* attraction over the course of a season using a 1) a linear set of concentrations, or 2) an exponential set of concentrations in 2023 and 2024.

3.4 Methods

3.4.1 Trapping in 2023 with a linear set of concentrations of (*E*)-8-dodecenyl acetate

Field trapping was done according to the methodology described by Ruiz et al. (2022). The fields were located in North-Central Kansas at the Land Institute (Table 3.1). No pesticides were applied to these fields during the experiment in 2023. Starting the first week of June, six transects were set out, two in each *Silphium integrifolium* field. Each transect contained seven 30.4 cm x 30.4 cm sticky card traps (Alpha Scents, Canby, OR, USA) affixed to the top of a 1.27 cm diameter, three foot in length PVC pole that was hammered into the ground until sturdy. The cards were affixed using a 271 cm long sticky card ring holder (Olson Products Inc., Medina, OH, USA) that was bent to a 90° angle inside the PVC pipe. Two large binder clips were also used to anchor the sticky card to its card holder.

The sticky traps in each transect were spaced 10 meters apart around the perimeter of the field. Within each transect, traps were baited with a linear increase in concentrations in 2023 either a control (50 µl of acetone), a low concentration (50 µl of a solution made by mixing 5.75 µl of (*E*)-8-dodecenyl acetate in 5 ml of acetone), or a doubled concentration (11.5 µl of (*E*)-8-dodecenyl acetate diluted in 5 ml of acetone) of (*E*)-8-dodecenyl acetate (Alfa Chemistry, Ronkonkoma, NY, USA). These yielded a total concentration of 0.11% and 0.23% (*E*)-8-dodecenyl acetate for the low and doubled concentrations respectively. All lures were added to a 3-ml LDPE dropping bottle (Wheaton, DWK Life Sciences, Millville, NJ, USA). The clear sticky card traps were collected and replaced biweekly until the first *Eucosma giganteana* individual was caught at which time it was changed to weekly. The lures and control bottles were replaced biweekly and their position in the field rotated at each change. Each lure was in each position twice over the course of the season.

When collected, the sticky cards were held in a 7.6 L (=2 gal) labeled Ziploc® bag and transported back to USDA-ARS. All collected sticky traps were placed in a freezer for approximately twenty-four hours. The total number of *E. giganteana* per trap and their placement on the sticky card in relation to the lure in millimeters was recorded. In addition, the number of nontarget lepidopterans were recorded on each trap. Individual *E. giganteana* and non-target Lepidoptera were only counted if more than half of the specimen was remaining on the sticky trap at the time of counting.

Table 3.1 Summary of field sites used for trapping *E. giganteana* in 2023 and 2024 in Salina, KS.

Field ID	County	Latitude	Longitude	Area (ha)	Insecticide application?

A	Saline	38.771	-97.570	0.226	No
B1	Saline	38.773	-97.592	0.152	No
B2	Saline	38.770	-97.593	0.152	No
B3	Saline	38.772	-97.593	0.168	Yes
C	Saline	38.770	-97.599	0.557	No
D	Saline	38.698	-97.575	0.141	Adjacent

3.4.2 Trapping in 2024 with an exponential set of concentrations of (*E*)-8-dodecenyl acetate

Field trapping in 2024 was conducted similarly to that in 2023 with the following modifications. Three different fields located at the Land Institute were used (Table 3.1). Pesticides were applied once to one of the fields and adjacent to one of the others. Three transects were set out in each of the three fields. Each transect contained four traps for a total of 36 traps. The traps were assembled similarly to those used in 2023, but a hand-made sticky card was used instead of a manufactured one to improve captures. These sticky cards were made of a laminated 21.6 × 27.9 cm (=8.5 by 11 in) piece of white cardstock paper (Astrobright, Neenah, WI, USA) coated on both sides with TAD® all-weather (Trécé Adhesives Division, Adair, OK, USA). The sticky sides were covered with wax paper for travel. Additionally, the sticky cards had a chicken wire cage placed over them to try to prevent the capture of birds on the traps. Traps in 2024 were baited with an exponential set of concentrations of (*E*)-8-dodecenyl acetate. In each transect, there was a solvent only control (50 µl of acetone), a low concentration equivalent to the 2023 treatment (50 µl of a solution made of 5.75 µl of (*E*)-8-dodecenyl acetate diluted in 5 ml of acetone), a medium concentration (50 µl of a solution made of 78.5 µl of (*E*)-8-dodecenyl acetate diluted in 5 ml of acetone), and a high concentration (50 µl of a solution made of 580.4 µl of (*E*)-8-dodecenyl acetate diluted in 5 ml of acetone). The concentration of (*E*)-8-dodecenyl acetate for the low, medium, and high concentrations were 0.11%, 1.55%, and 8.77% respectively. The traps were replaced weekly, and the lures were replaced biweekly, as well as rotated positions in the transect. Each lure was in each position twice over the course of the season.

3.4.3 Analysis

Repeated-measures ANOVA was performed using *E. giganteana* or nontarget Lepidoptera weekly captures as the response variable. Separate models were run for each year because of differences in the experimental design including fields, the number of traps, sticky cards, and pest population among other differences. Field was coded as a random variable. Treatment type (control, low, and doubled concentration in 2023 or control, low, medium, and high in 2024) was used as a fixed, explanatory variable. A variance-covariance matrix employed a first order autoregressive structure. Residuals were inspected to ensure normality and homogeneity of variances was satisfied, and when there were deviations, data was log-transformed. Upon a significant result from the model, Tukey HSD was used for pairwise comparisons. R software (R Core Team, 2024) was used for all analyses with $\alpha = 0.05$.

3.5 Results

3.5.1 *E. giganteana* captures in 2023 and 2024

The model confirmed that that the two years, 2023 and 2024, were statistically different from each other and thus needed to be analyzed separately (Table 3.2). Looking at the weekly captures of *E. giganteana*, the 2024 high concentration of (*E*)-8-dodecenyl acetate consitantly captures more *E. giganteana* than the other concentrations (Figure 3.1). There was also a clear peak in trap captures across treatments around the week of July 7th in both years.

Table 3.2 Statistical summary of repeated measures ANOVA analyzing *E. giganteana* captures through model comparison to baseline model with only an intercept.

	E. giganteana			Non-target Lepidoptera		
Model	**df**	**Log Likelihood**	***P***	**df**	**Log Likelihood**	***P***
Treatment	8	32.3	<0.001	8	8.56	0.07
Year	5	4.34	0.04	5	97.8	<0.001
Year + treatment	9	35.8	< 0.001	9	99.9	<0.001

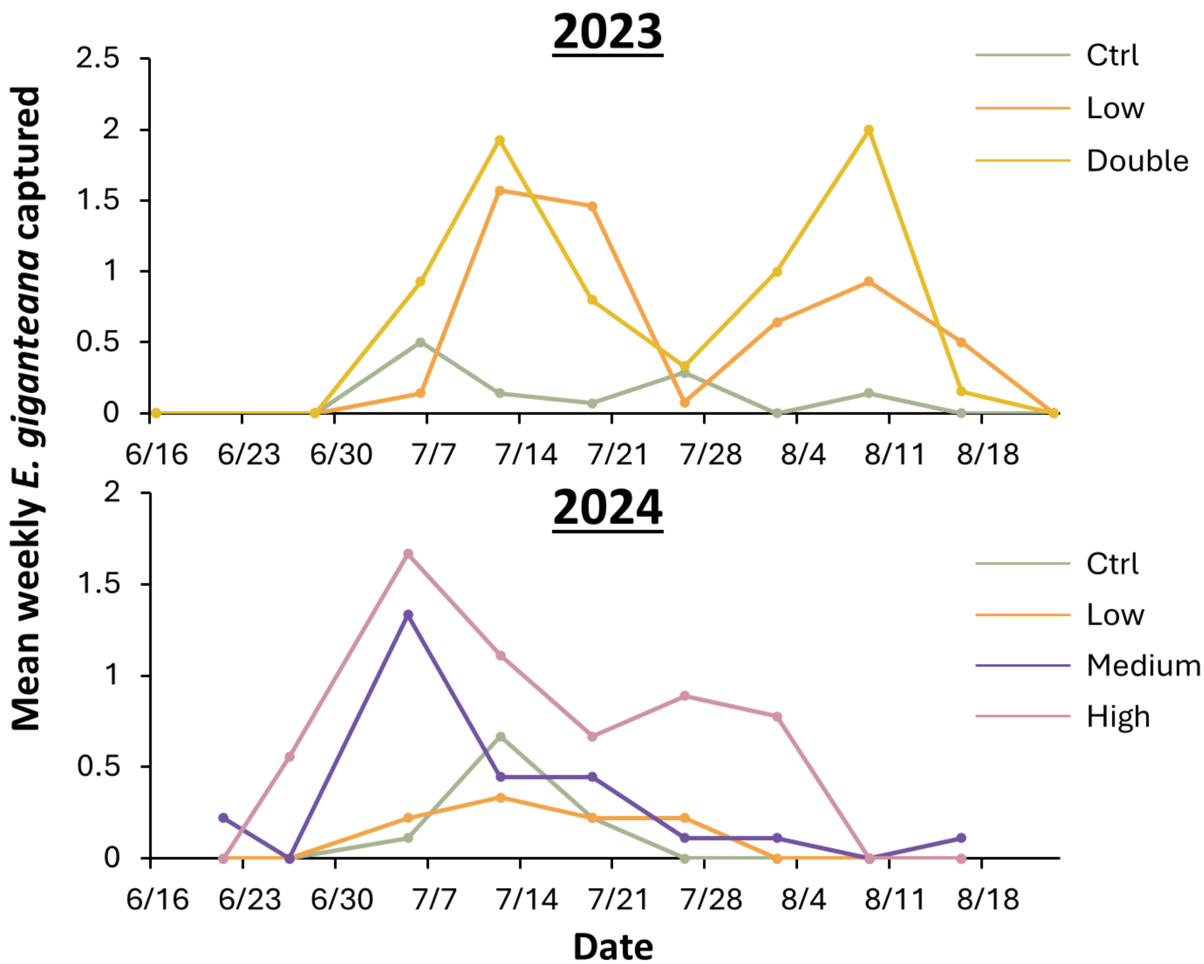

Figure 3.1 Weekly captures of *E. giganteana* (± 1 SE) conducted in 2023 (top panel) and 2024 (bottom panel) on clear sticky cards baited with (E)-8-dodecenyl acetate from June through August.

During trapping in 2023, the low (0.11%) and double concentration (0.23%) of (*E*)-8-dodecenyl acetate captured significantly more *E. giganteana* than the control (Table 3.2, Figure 3.3). In particular, those concentrations captured 4- and 6-fold more individuals than those in the control, respectively. However, there was no significant difference between the low and double concentration of (*E*)-8-dodecenyl acetate. In 2024, *E. giganteana* captures were also significantly affected by the concentrations (Table 3.2, Figure 3.3). In particular, there was 3- and 6-fold higher capture of *E. giganteana* in the medium (1.55%) and high (8.77%) baited traps, respectively, compared to the low concentration and unbaited controls. The low concentration captured a similar number of *E. giganteana* as the unbaited control in 2024 (Figure 3.3). Despite the exponential increase in concentration among the four treatments, there was not an exponential proportional increase in captures among concentrations.

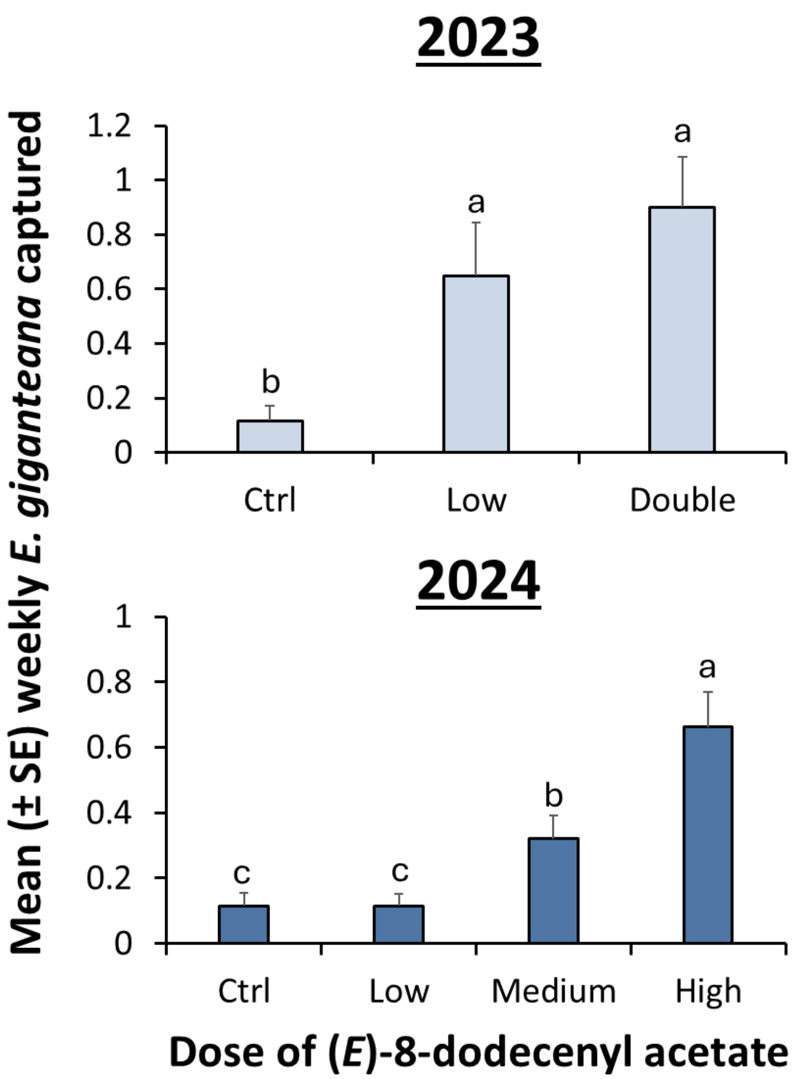

Figure 3.2 Season-long weekly mean captures (± SE) of *E. giganteana* for trapping conducted with (*E*)-8-dodecenyl acetate in 2023 (top panel) and 2024 (bottom panel) from June through August by concentration. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

In 2023, we also measured the distance between the lure placement at the base of the sticky trap and where *E. giganteana* were captured on the trap. We did not find a significant difference between treatments regarding where *E. giganteana* individuals were captured on the trap (df = 7; Log likelihood = 1.51; $P = 0.68$) (Figure 3.4).

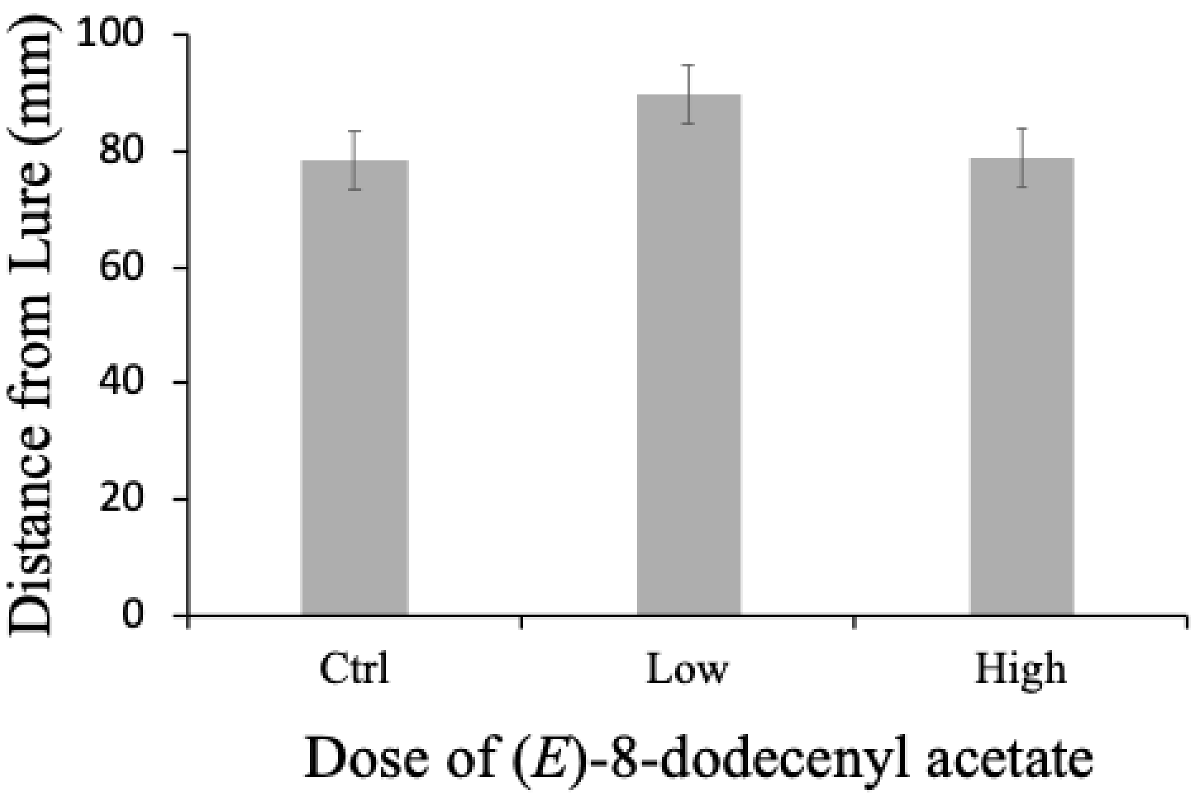

Figure 3.3 Distance (± SE) from the lure (mm) that *E. giganteana* individuals were stuck on the sticky card. There were no significant differences among treatments (Tukey HSD, α = 0.05)**.**

3.5.2 Nontarget lepidopteran captures in 2023 and 2024.

The concentrations did not significantly affect captures of nontarget lepidopterans in 2023 or 2024 (Table 3.2; Figure 3.4; Figure 3.5). Importantly, the presence of (*E*)-8-dodecenyl acetate did not appear to affect nontarget lepidopterans in either year (Figure 3.5). Captures of nontarget lepidopterans were reduced by 77% in 2024 compared to 2023 (Figure 4).

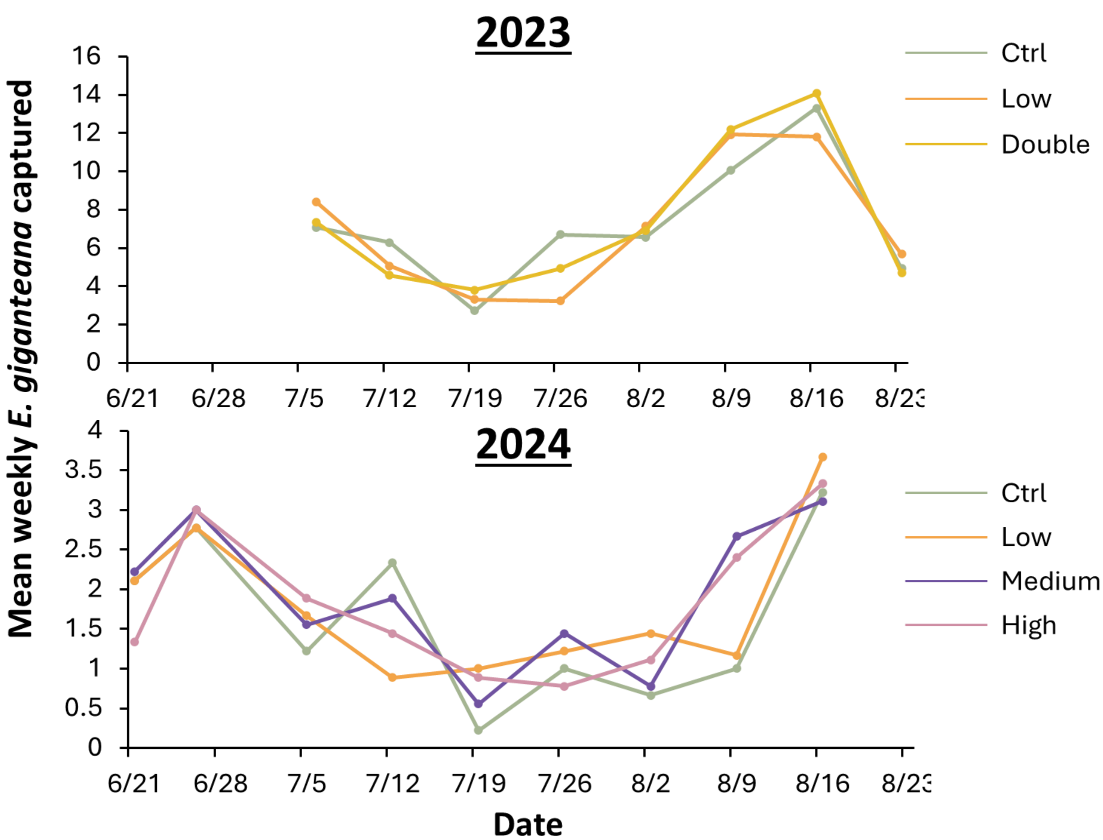

Figure 3.4 Weekly captures of nontarget lepidopterans in 2023 (top panel) and 2024 (bottom panel) on clear sticky cards baited with (*E*)-8-dodecenyl acetate from June through August at the Land Institute in Salina, KS.

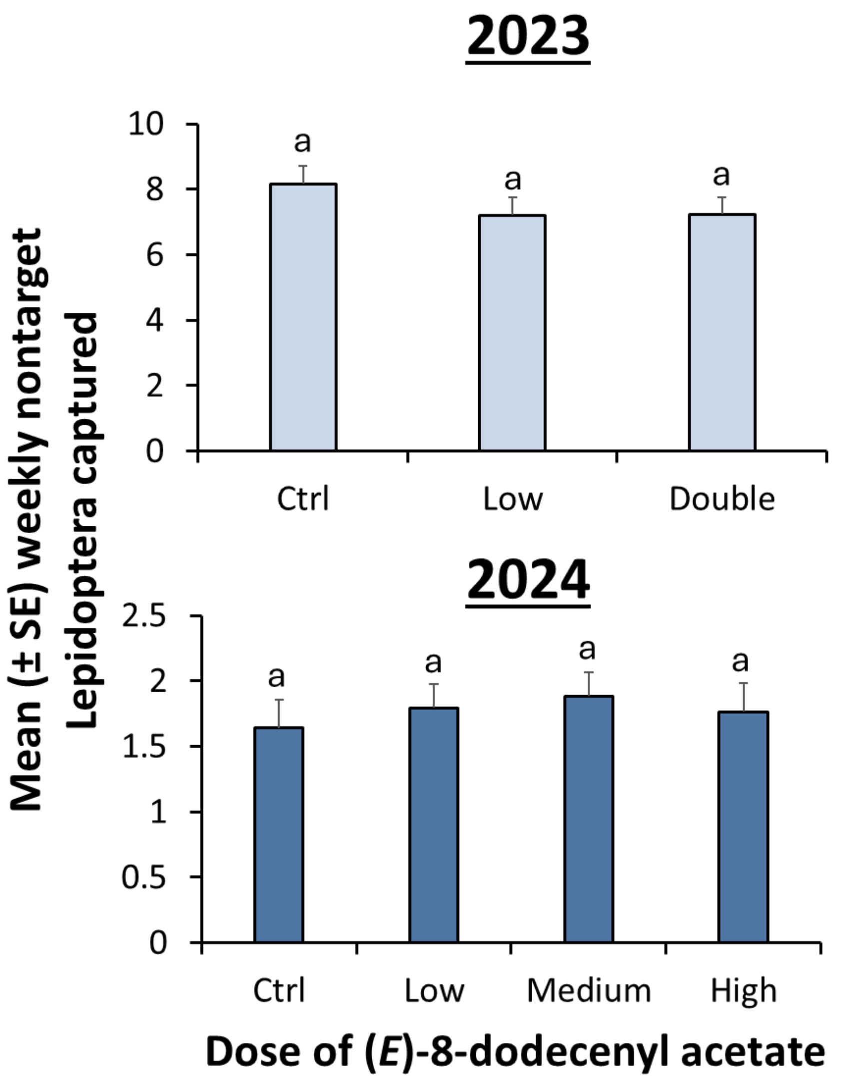

Figure 3.5 Season-long weekly mean captures (± SE) of *E. giganteana* for trapping conducted with (*E*)-8-dodecenyl acetate in 2023 (top panel) and 2024 (bottom panel) from June through

August by concentration the Land Institute in Salina, KS. Bars with the same letters indicate no significant difference between treatments (Tukey HSD, $\alpha = 0.05$).

3.6 Discussion

This study, evaluating different concentrations of (*E*)-dodecenyl acetate in the field on captures of *E. giganteana* and nontarget Lepidoptera has major implications for the future of *E. giganteana* research. We have demonstrated that there was a concentration-dependent response by *E. giganteana*. This is an important first step in demonstrating the value of this compound in behaviorally-based management programs for this pest (e.g., Morrison et al. 2016), such as attract-and-kill and mating disruption. Semiochemical-mediated behaviorally-based strategies typically function best when used against intermediate or small sized populations of pests, which is the size of population we have observed in the field for *E. giganteana*. Prior work found that among multiple compounds identified from related species in the genus *Eucosma*, only (*E*)-8-dodecenyl acetate was found to elicit a response in *E. giganteana* (Ruiz et al. 2022). It is possible that the actual pheromone for this species is structurally similar to (*E*)-8-dodecenyl acetate. Further work should be done to determine additional compounds that are attractive to *E. giganteana* to create a more complete pheromone blend. Moreover, follow-up experiments should evaluate whether deploying high concentrations of (*E*)-8-dodecenyl acetate in an area-wide fashion can lead to sustained decreases in *E. giganteana* activity and damage.

Importantly, we did not see a significant difference between nontarget lepidopteran captures between the control and any of the baited treatments in either year. This further confirms the specificity of this attractant to *E. giganteana*. It should be noted that (*E*)-8-dodecenyl acetate is a component in other tortricid sex pheromone blends including for the plume fruit moth (*Grapholita funebrana* Treitschke) and the oriental fruit moth (*Grapholita molesta* (Busck)) (Lo Verde et al. 2020; Tollerup et al. 2012; Trimble et al. 2001). While this compound could hypothetically lead to more nontarget captures in areas with higher populations of these species, we did not observe an increase in lepidopteran captures in our study using *Silphium integrifolium* fields in Kansas.

A low dosage of (*E*)-8-dodecenyl acetate should be suitable for monitoring *E. giganteana* with clear sticky cards, but its effectiveness may decrease towards the end of the season. In addition, when higher concentrations of (*E*)-8-dodecenyl acetate are used in the same field, it is possible that a low concentrations may not be sufficient to track populations of *E. giganteana*, as we observed in the field in 2024. However, higher concentrations of (*E*)-8-dodecenyl acetate should be examined for possible usage in a mating disruption strategy because trap shutdown is a characteristic of effective mating disruption protocols (Chapter 1). While we did not assess this specifically, the high concentrations of (*E*)-8-dodecenyl acetate may also be effective at preventing male moths from locating a mate or successfully producing offspring in a mating disruption strategy. Mating disruption using synthetic pheromone blends has been highly effective in managing other tortricid moths in agriculture (Amarasekare and Shearer 2017; Bohnenblust et al. 2011; Flores et al. 2021; Knight et al. 2017;).

Changes in the commercial formulation of the sticky cards used in 2023 led to the decline in trap captures around July 26, resulting in an unusual bivoltine pattern of captures instead of the usual univoltine pattern (Ruiz et al. 2022). The dip in captures around July 29th during the 2024 season, could be explained by a heat wave that the area experienced. The 2024 trends were mirrored by the UV light captures conducted for ongoing *E. giganteana* monitoring (Chérémond personal communication), indicating it was not specific to the sticky cards. In the future, it may be useful to consider multiple modalities (e.g., light and pheromone) to trap *E. giganteana*, as some species have shown increased sensitivity to traps with more than one modality. For example, adding an LED light to pheromone-baited traps increased tortricid moth captures by 2-12-fold in pome fruit (Knight et al. 2023). Follow-up experimentation should be conducted examining the relationship between trap captures with UV light and the attractant as used in this study. Overall, this study serves as a suitable starting point for additional work on behaviorally-based approaches to managing *E. giganteana*.

3.7 References

Amarasekare K and Shearer P. 2017. Stability of *Cacopsylla pyricola* (Hemiptera: Psyllidae) populations in pacific northwest pear orchards managed with long-term mating disruption for *Cydia pomonella* (Lepidoptera: Tortricidae). Insects. 8(4):105. https://doi.org/10.3390/insects8040105.

Bohnenblust E, Hull LA, and Krawczyk G. 2011. A comparison of various mating disruption technologies for control of two internally feeding Lepidoptera in apples: Mating disruption of two moth species. Entomologia Experimentalis et Applicata. 138(3):202–211. https://doi.org/10.1111/j.1570-7458.2010.01089.x.

Flores MF, Bergmann J, Ballesteros C, Arrazito D, and Curkovic T. 2021. Development of monitoring and mating disruption against the chilean leafroller *Proeulia auraria* (Lepidoptera: Tortricidae) in Orchards. Insects. 12(7):625. https://doi.org/10.3390/insects12070625.

Frerot B, Priesner E, and Gallois M. 1979. A Sex attractant for the green budworm moth, *Hedya nubiferana*. Zeitschrift für Naturforschung C. 34(12):1248–1252. https://doi.org/10.1515/znc-1979-1229.

Hung CC, Hwang JS, Hung MD, Yen YP, and Hou RF. 2001. Isolation, identification and field tests of the sex pheromone of the carambola fruit borer, *Eucosma notanthes*. Journal of Chemical Ecology. 27(9):1855–1866. https://doi.org/10.1023/A:1010464810443.

Johnson PJ, Boe A. 2011. Three interesting insects and the cause of reduced vigor of cup plant (*Silphium perfoliatum*) in agronomic plantings. Proceedings of the South Dakota Academy of Science. 90.

Johnson PJ, Boe A, and Martens AP. 2019. *Eucosma giganteana* (Riley) and *Sliphium* *perfoliatum* L., morphological variation in an insect-plant association in eastern South Dakota. Proceedings of the South Dakota Academy of Science. 98:91–104.

Knight AL, El-Sayed AM, Judd GJR, and Basoalto E. 2017. Development of 2-phenylethanol plus acetic acid lures to monitor obliquebanded leafroller (Lepidoptera: Tortricidae) under mating disruption. Journal of Applied Entomology. 141(9):729–739. https://doi.org/10.1111/jen.12393.

Knight AL, Preti M, Basoalto E, and Fuentes-Contreres E. 2023. Increasing catches of adult moth pests (Lepidoptera: Tortricidae) in pome fruit with low-intensity LED lights added to sex pheromone/kairomone lure-baited traps. Journal of Applied Entomology. 147(9):843–856. https://doi.org/10.1111/jen.13176.

Lo Verde G, Guarino S, Barone S, and Rizzo R. 2020. Can mating disruption be a possible route to control plum fruit moth in Mediterranean environments? Insects. 11(9):589. https://doi.org/10.3390/insects11090589.

Miller JR, Adams CG, Weston PA, and Schenker JH. 2015. Trapping of small organisms moving randomly: principles and applications to pest monitoring and management. Cham: Springer International Publishing (SpringerBriefs in Ecology). https://doi.org/10.1007/978-3-319-12994-5.

Morrison WR, Lee D-H, Short BD, Khrimian A, and Leskey TC. 2016. Establishing the behavioral basis for an attract-and-kill strategy to manage the invasive *Halyomorpha halys* in apple orchards. Journal of Pest Science. 89(1):81–96. https://doi.org/10.1007/s10340-015-0679-6.

Murrell EG, Zio KR, Chérémond NE, and Van Tassel DL. 2023. Assessing effective mechanical and chemical strategies for managing *Eucosma giganteana* (Lepidoptera: Tortricidae) in the perennial oilseed crop, *Silphium integrifolium* (Asteraceae: Heliantheae). Del Pozo-Valdivia A, editor. Journal of Insect Science. 23(6):4. https://doi.org/10.1093/jisesa/iead102.

Prasifka JR, Mallinger RE, Hulke BS, Larson SR, and Van Tassel DL. 2017. Plant–herbivore and plant–pollinator interactions of the developing perennial oilseed crop, *Silphium integrifolium*. Environmental Entomology. 46(6):1339–1345. https://doi.org/10.1093/ee/nvx134.

Price JH, Van Tassel DL, Picasso VD, and Smith KP. 2022. Assessing phenotypic diversity in silflower (*Silphium integrifolium* Michx.) to identify traits of interest for domestication selection. Crop Science. 62(4):1443–1460. https://doi.org/10.1002/csc2.20748.

Ruiz K, Bruce A, Chérémond N, Stratton C, Murrel E, Gillette S, and Morrison WR. 2022. Field trapping and flight capacity of *Eucosma giganteana* (Riley) (Lepidoptera: Tortricidae) in response to behaviorally active congeneric semiochemicals in novel silflower agroecosystems. Insects. 13(4):350. https://doi.org/10.3390/insects13040350.

Tollerup KE, Rucker A, and Shearer PW. 2012. Whole-farm mating disruption to manage *Grapholita molesta* (Lepidoptera: Tortricidae) in diversified New Jersey orchards. Journal of Economic Entomology. 105(5):1712–1718. https://doi.org/10.1603/EC11419.

Trimble RM, Pree DJ, and Carter NJ. 2001. Integrated control of oriental fruit moth (Lepidoptera: Tortricidae) in peach orchards using insecticide and mating disruption. Journal of Economic Entomology. 94(2):476–485. https://doi.org/10.1603/0022-0493-94.2.476.

Turner M, Ravetta D, and Van Tassel D. 2018. Effect of *Puccinia silphii* on yield components and leaf physiology in *Silphium integrifolium*: lessons for the domestication of a perennial oilseed crop. Sustainability. 10(3):696. https://doi.org/10.3390/su10030696.

Van Tassel DL, Albrecht KA, Bever JD, Boe AA, Brandvain Y, Crews TE, Gansberger M, Gerstberger P, González-Paleo L, Hulke BS, Kane NC, Johnson PJ, Pestsova EG, Piccaso Risso VD, Prasifka JR, Ravetta DA, Schlautman B, Sheaffer CC, Smith KP, Speranza PR, Turner MK, Vilela AE, Von Gehren P, and Wever C. 2017. Accelerating *Silphium* domestication: an opportunity to develop new crop ideotypes and breeding strategies informed by multiple disciplines. Crop Science. 57(3):1274–1284. https://doi.org/10.2135/cropsci2016.10.0834.

Vilela AE, González-Paleo L, Ravetta DA, Murrell E, and Van Tassel DL. 2020. Balancing forage production, seed yield, and pest management in the perennial sunflower *Silphium integrifolium* (Asteraceae). Agronomy. 10(10):1471. https://doi.org/10.3390/agronomy10101471.

Vilela A, González-Paleo L, Turner K, Peterson K, Ravetta D, Crews TE, and Van Tassel DL. 2018. Progress and Bottlenecks in the Early Domestication of the Perennial Oilseed *Silphium integrifolium*, a Sunflower Substitute. Sustainability. 10(3):638. https://doi.org/10.3390/su10030638

4 Behavioral and Physiological Response of *Eucosma giganteana* to Semiochemicals from Conspecifics and *Silphium integrifolium*

4.1 Objective 3A: Explore the physiological response of *E. giganteana* to common attractants and *S. integrifolium*.

4.2 Objective 3B: Elucidate the flight capacity of *E. giganteana* and its behavioral response to attractants.

4.3 Objective 3D: Determine headspace emmisions from conspecific moths and *S. integrifolium*.

4.4 Abstract

Eucosma giganteana is a specialist pest on *Silphium* spp. and is a major limiting factor to the commercialization of *Silphium integrifolium*, a sunflower alternative. Previous work has revealed (*E*)-8-dodecenyl acetate as a potential pheromone compound attractive to *E. giganteana*. Understanding the antennal and flight response of *E. giganteana* to (*E*)-8-dodecenyl acetate will impact future pest management using this attractant. Our aims were to quantify male and female *E. giganteana* responses to semiochemicals from conspecifics and its host plant, *Silphium integrifolium* by 1) characterizing headspace emissions from conspecifics and *S. integrifolium*, 2) examining male and female *E. giganteana* flight response on a flight mill, and 3) assessing the physiological response of *E. giganteana* using electroantennography (EAG). We found that *S. integrifolium* headspace emissions were significantly different from those from *E. giganteana* conspecifics and the blank control. In addition, antennal response was significantly greater for female *E. giganteana* to headspace from *S. integrifolium*. Importantly, we confirm the tendency for arrestment properties of (*E*)-dodecenyl acetate on the flight behavior of *E.*

giganteana males and found *E. giganteana* have an equal propensity to fly at both the beginning and ending of their summer flight period. On average, male and female *E. giganteana* flew 12–13 km in a 24 h period. In the presence of semiochemical stimuli, this response varied from 0.001–44.6 km for female moths exposed to no stimuli or nearby male moths, whereas the response ranged from 0.002–42.9 km for male moths exposed to a high concentration of (*E*)-8-dodecenyl acetate and nearby female moths. By characterizing the compounds emitted by *E. giganteana* and evaluating their physiological and flight responses, this study helps to lay the foundation for developing a behaviorally-based management tactic for *E. giganteana*.

Keywords: giant Eucosma moth, Lepidoptera, *Silphium integrifolium*, Tortricidae, flight mill, electroantennogram, headspace

4.5 Introduction

Silphium integrifolium Michx is a native perennial oilseed crop currently under domestication and commercialization at the Land Institute in Salina, KS (Van Tassel et al. 2017). It is a highly drought-tolerant crop, making it a great candidate for being resilient as we continue to see climate change on the Great Plains (Price et al. 2022; Reinart et al. 2019; Vilela et al. 2020). The major limiting factor of *S. integrifolium* domestication is its specialist pest, *Eucosma giganteana* (Riley) (Lepidoptera: Tortricidae) (Vilela et al. 2018). These moths are univoltine and the adults only fly for about a month. The larvae are internal feeders on *S. integrifolium*'s flower heads and root crowns, reducing the plant's biomass and seed set drastically (Johnson et al. 2019; Prasifka et al. 2017). Because of their internal feeding, there is limited time to treat the larvae, making pest management difficult. Scribner et al. (Chapter 2) recently developed a growing degree model to better guide management and was able to accurately predict phenological events for the pest. Typically, *E. giganteana* infestations are treated with broad-spectrum insecticides once their presence has been documented (Murrell et al. 2023). Past work by Ruiz et al. (2022) found that (*E*)-8-dodecenyl acetate worked as an attractant to *E. giganteana* in the field. However, little is known about the chemical ecology of this or other semiochemicals *E. giganteana* may encounter within its environment.

An important component of behavioral response to semiochemicals is how it may affect flight behavior. Flight capacity in the laboratory is a key measure, and important for understanding the dispersal capabilities of both field and stored product pests (Abshire et al. 2024; Jones et al. 2015; Naranjo 2019). Both static tethering and flight mills have been used in the past to measure dispersive capabilities of numerous insect species (Naranjo 2019). Critically, pheromones, kairomones, and food cues have been shown to influence the flight distance and velocity of individuals (Abshire et al. 2024). Dispersal capabilities for multiple other tortrix species have been examined including the oriental fruit moth (*Cydia molesta* Busck) and the codling moth (*Cydia pomonella* L.) (Hughes et al. 2004; Matveev et al. 2017; Schumacher et al. 1997). In prior work, Ruiz et al. (2022) examined the flight capacity of *E. giganteana* by attractants found in congener species, including (*E*)-8-dodecenyl acetate, but only tested a low concentration. The presence of (*E*)-8-dodecenyl acetate had arresting properties and decreasing flight distance by *E. giganteana* on the flight mill by 78 to 80% compared to controls without the stimulus. However, it is unknown whether higher concentrations of this compound elicit more extreme behavioral effects.

There has been little work performed on characterizing *S. integrifolium* headspace or the behavioral response by *E. giganteana* to its emissions. In fact, the only prior work found terpenoids were upregulated in *S. integrifolium* plants when monocropped with wheat and when herbivory was induced (Fyie et al. 2024). In the same study, carboxylic acids and organooxygen compounds were reduced in sweet clover when intercropped with S. *integrifolium* but increased under herbivory. However, no work has been done to elucidate the response of *E. giganteana* to emissions from *S. integrifolium*. By contrast, much more work has been done on the related oilseed crop, sunflower, which has found that alpha-pinene made up a significant portion of the volatiles emitted for most sunflower taxa, and most of the volatiles emitted were monoterpenoids with a significant share of sesquiterpenoids (Anandappa et al. 2023).

One method to quickly determine whether pests can detect volatiles from complex blends is to use gas chromatography coupled with electroantennography detection (GC-EAD) or electroantennography (EAG). EAG is a technique that uses insect antennae as a biosensor for identifying physiologically active compounds (Brezolin et al. 2018). While the first EAG was performed in 1957 to identify the pheromone of *Bombyx mori*, it has since been used to evaluate the physiological response of many species to semiochemicals, including many tortricid species (Casado et al. 2006; Fu et al. 2022; McNair et al. 1999). As such, it is a highly useful tool for answering questions about what a particular species can detect in its environment.

Together, understanding the headspace emitted by conspecifics and their host plant, as well as the behavioral and physiological responses by *E. giganteana* to those semiochemicals will help in laying the foundation for a behaviorally-based management strategy for this species (Morrison et al. 2021). These strategies include mating disruption and attract-and-kill and seem ideally suited for a pest such as *E. giganteana* with low to intermediate population size. Thus, in this study, we quantified male and female *E. giganteana* reaction to semiochemicals from conspecifics and its host species, *Silphium integrifolium* through 1) characterizing headspace emissions, 2) examining male and female *E. giganteana* flight capacity on a tethered flight mill, and 3) assessing the physiological response of *E. giganteana* of both sexes using EAG.

4.6 Methods

4.6.1 *Eucosma giganteana* and *Silphium integrifolium* collections from the field

Eucosma giganteana cannot yet be reared successfully in the laboratory, thus we sourced all specimens from the field. Adult *E. giganteana* individuals were carefully captured by hand in one of the fields planted to *Silphium integrifolium* at the Land Institute (38.769622, -97.598576) between 22:00 and 24:00 five times a week from June to August 2024. Moths were immediately sexed and individually placed in small deli cups with appropriate labels. They were brought back to the USDA-ARS Center for Grain and Animal Health (39.1955486, -96.5987334) for the experiments described below. Once in the lab but prior to use in experiments, moths were kept in a quiet environment at approximately 23 ± 0.1℃ and 16:8 L:D photoperiod. Importantly, no lures were used to capture insects to avoid biasing the results of the experiments below. *Silphium integrifolium* flower heads were cut 1 cm below the flower and brought back on a weekly basis during the same timeframe and stored at 4°C until needed for experiments. Flower heads were never more than 4 days old prior to use.

4.6.2 Headspace Characterization

Headspace was collected from the following treatments: 10 *E. giganteana* male moths only, 10 *E. giganteana* female moths only, an even mix of male and female moths (5:5), flower cuttings of *S. integrifolium*, and a blank control. For the *E. giganteana* treatments, only alive, healthy adult moths that were collected within five days were used. For the *S. integrifolium* collections, approximately 25 grams of flower heads cut the same week as collections were used.

For each treatment, *E. giganteana* or *S. integrifolium* were placed in a clean 100-mL beaker. To prevent moth escapees, a metal mesh top was constructed and affixed to the opening of the beaker. The beaker was then placed in one of eight 500-mL glass headspace collection containers with a PTFE septum and lid. A Pora-Pak Q volatile collection trap (VCT) was inserted in the output end. The VCT consisted of an angled drip-tip collection point borosilicate glass tube with a mesh (Stainless Steel #316 screen), packed with 20 mg of PoraPak-Q™ chemical absorbent held in place with a borosilicate glass wool plug, and followed by a PTFE Teflon™ compression seal. A PTFE tube spanned from the flow meter (CADS-4CPP, Clean Air Delivery System, Sigma Scientific, LLC, Micanopy, FL, USA) to the input end of the headspace container at a flow rate of 1 L/min. Prior to that, the air was scrubbed with an activated carbon filter and was pumped in using the central air pump for the center. Samples ran for 24 h. Each volatile collection trap was collected and eluted with 150 µl of dichloromethane in a fume hood into a 2-mL GC vial containing a 250 µl glass insert with polymer feet. The solvent was gently pushed through the volatile collection trap with N_2 gas. At the end of collecting all the samples, 1 µl of an internal standard, tetradecane (190.5 ng), was added to each of the samples. The samples were then all capped with a magnetic screw top lid and secured with PTFE tape before being placed in a freezer at -20 °C until they could be run. All headspace samples were collected within 5 weeks. After each replication, the headspace collection containers were all washed with methanol and then hexane. VCTs were rinsed in triplicate with dichloromethane. A total of at least n = 5 replicates were tested for each treatment.

4.6.3 Gas Chromatography Coupled with Mass Spectrometry

All headspace collection sample extracts were run on an Agilent 7890B gas chromatograph (GC) equipped with an Agilent Durabond HP-5 column (30 m length, 0.250 mm diameter and 0.25 µm film thickness) with He as the carrier gas at a constant 1.2 mL/min flow and 40 cm/s velocity. The GC was coupled with a single-quadrupole Agilent 5997B mass spectrometer (MS). The compounds were separated by auto-injecting 1 µl of each sample under splitless into the GC–MS at room temperature (approximately 23 °C). The flow rate was 18 ml/min. The GC program consisted of 40 °C for 1 min followed by 10 °C/min increases to 300 °C and then held for 26.5 min. After a solvent delay of 3 min, mass ranges between 50 and 550 atomic mass units were scanned. Compounds were tentatively identified by comparison of spectral data with those from the NIST 14 library and by GC retention index. The samples were normalized according to the following formula: $(Pk_{sam} – Pk_{min})/(Pk_{max} – Pk_{min})$, where Pk_{sam} is the peak area from the sample, Pk_{min} is the global minimum peak area, and Pk_{max} is the global max peak area.

4.6.4 Electroantennography of *E. giganteana*

All electroantennogram (EAG) recordings of *E. giganteana* were taken from 19:00 to 23:00 which corresponded to the peak activity period of *E. giganteana* based on prior literature (Ruiz et al. 2022). Prior to recordings, the machine and software were powered on and given 30 min to warm up. Only field-captured moths within three days were used for the recordings. The moths were sexed prior to recordings and knocked down in a freezer for 1–2 min. The moth's antenna was then ablated, alternating between left and right antennae for each recording, at the pedicle as close to the scape as possible. Then, using electroactive gel (Spectra 360 Electrode gel, Parker Laboratories INC., Fairfield, NJ, USA), the ablated antennae was placed on an antenna holder (Syntech GmbH, Buchenbach, Germany) and covered with gel to ensure connection and prevent drying out. The antennal holder was then slotted into the EAG probe (EAG Combi Probe, Syntech GmbH, Buchenbach, Germany). The software was opened to evaluate the EAG trace and ensure the antenna was connected correctly. The recording was started once the line showing the antennal baseline had stabilized. Subsequently, the antenna was given an additional minute or two to ensure the baseline remained stable. A flow meter (CS-55, Syntech GmbH, Buchenbach, Germany) was triggered to puff air at a rate of 0.3 µl s^{-1} through a pasteur pipette and across the antennae. Once the flow meter was triggered, there was a brief delay before the first puff, then a 1 min delay before the second puff.

The treatments included a control of blank air, neat concentration of (*E*)-8-dodecenyl acetate (Alfa Chemistry, Ronkonkoma, NY, USA), neat dichloromethane solvent control, diluted concentration of (*E*)-8-dodecenyl acetate (e.g., consisting of 11.5 µl of neat (*E*)-8-dodecenyl acetate diluted with 5 ml of dichloromethane), 150 µl dichloromethane + 1 µl tetradecane (190.5

ng μl^{-1}) control, female *E. giganteana* headspace (see above), *S. integrifolium* headspace (see above), and neat acetone solvent control. For each treatment, 6 µl of the treatment was pipetted into the center of a small filter paper (2.5 cm diameter), which was then folded and placed into the top of a pasteur pipette. The treatments were presented in the order given above to each antenna for standardization. Apart from the blank control which was puffed four times at the beginning of each recording and twice at the conclusion of each recording, all other treatments were only puffed twice. The pasteur pipettes containing the treatments were replaced after an hour to ensure the antennae was supplied with a fresh odor source. There was a total of n = 15 different individuals for female moths and n = 17 different individuals for male moths for each treatment.

4.6.5 Flight Capacity of *E. giganteana* in Response to Semiochemicals

Only moths captured within 1–3 days of the start of flight mill recording were used. Ten to 12 moths were prepared at a time for the eight flight mills. The 89-mL (=3 oz) solo cups containing these moths were placed in an ice bath to partially knock them down. Individual moths were then removed, and their scales were gently brushed off their pronotum using a small silicone brush to allow better adherence of the glue. A small dollop of T-7000 glue was applied to the blunt end of a size 2 entomology pin (Bioquip, Rancho Dominguez, CA, USA) and this was pressed against the pronotum for 1 min to allow time for the glue to dry. The pins with moths were inserted into an elevated piece of styrofoam until the moths were ready to be placed on the flight mill. Eight moths were run simultaneously on their own flight mill, with a preference for moths that were more active (after Attisano et al. 2015). Moths that could not properly beat their wings due to the glue or wing placement were not used on the flight mills.

The tip of the pin of each moth was inserted into the arms of the flight mill (19-gauge center pivot) which were weightlessly supported between two neodymium magnets and positioned to fly counterclockwise. Each moth was encouraged to fly by gently blowing on its abdomen to make sure the arm with the moth on it could pass through the sensor. The rotations of the moth were recorded through the breaking of an infrared beam produced by an IR sensor (OPB800Q, Optek Technology, Texas USA) mounted on the side of the cell. Once all moths were set up and vetted, the automated software (WINDAQ, DI- 149, Ohio, USA) was started, and flight was logged. Moths were given the opportunity to fly in a 24-h period. After the end, the moths were removed from the flight mill arms, their condition was noted, they were weighed on a microbalance (QUINTIX2102-1S, Sartorius AG, Göttingen, Germany), and then they were frozen. The moths were weighed to evaluate whether size affects flight capacity. For each round of the flight mill, each sex was run independently (blocked) to determine if flight capacity was altered in the presence of the other sex. An additional block included both sexes being run, which consisted of four male and four female moths running simultaneously. The semiochemical treatments included a low concentration of (*E*)-8-dodecenyl acetate (made with 11.5 µl of (*E*)-8-dodecenyl acetate diluted with 5 ml of acetone) and a high concentration of (*E*)-8-dodecenyl acetate (made with 580.4 µl of (*E*)-8-dodecenyl acetate diluted with 5 ml of acetone). For all semiochemical treatments, a total of 25 µl was pipetted onto a piece of filter paper (10 cm diameter) and placed in a Petri dish (100 × 15 mm diameter: height). The Petri dish was left uncovered in front of and centered adjacent to the flight mill. In total, there were two blocks of eight moths used for each of the treatments, so n = 16 behavioral replicates for each treatment combination of sex and semiochemical.

4.6.6 Analysis

To analyze the headspace collected from the odor treatments, raw chromatograms were deconvoluted in MassHunter Unknowns Analysis (Agilent Technologies, Inc., Santa Clara, CA, USA) after GC-MS. Peaks with a height less than 0.3% the height of the tallest peak were excluded from the exported peak table. Compounds were aligned across samples by obtaining the underlying m/z ratio data from public repositories and flexibly aligning compounds based on structural matches and retention time, and normalized emission rates were calculated using the R package *uafR* (Stratton et al. 2023). Volatile compounds appearing in fewer than two samples were discarded, as these represent transient background volatiles in the general vicinity of headspace collection but are not informative of differences among the treatments. Additionally, siloxane and phthalate peaks were removed as these represent common contaminants in GC-MS analysis. All other volatiles exceeding a 70% match to the reference library were retained in the analysis. Pairwise Bray-Curtis similarities were calculated between headspace samples, and non-metric multi-dimensional scaling (NMDS) was used to visualize the differences in volatile emissions among treatments. A total of n = 1,000 permutations were used for the ordination procedure. Stress values for the NMDS procedure were less than 0.13, indicating that good interpretation was possible. The package ggplot2 was used for plotting the NMDS (Wickham 2016). Analysis of similarity (ANOSIM) was applied to test whether the volatile profiles varied among treatments using the R package *vegan* (Oksanen et al. 2024). A total of n = 1,000 permutations were performed for the statistical test. Following the global ANOSIM, pairwise ANOSIMs were performed using the *veganEx* package with a Holm-Bonferroni correction to the *P*-value (Zhonghui 2021). R Software (R Core Team, 2024) was used for all analyses with $\alpha = 0.05$, except where noted.

Before the EAG data could be analyzed, the depolarizations following each stimulus puff were quantified for each moth's recordings using the software GC-EAD .1.2.5 (Syntech, Kirchzarten, Germany). Within each recording, the average depolarization for each blank in each replicate across blank puffs was subtracted from all the other stimulus puffs to remove the baseline *E. giganteana* reaction. Similarly, the DCM and/or the DCM plus tetradecane depolarizations were subtracted from all treatments that included DCM and/or DCM plus tetradecane in the solutions. This value left us the actual depolarization for only the treatments of interest. Each sex was analyzed separately using a general linear model with depolarization potential as the response variable. The fixed, explanatory variable consisted of our semiochemical treatments. Upon a significant result from the model, we used Tukey HSD for multiple comparisons.

Before analysis, flight mill data was first vetted to eliminate all flights that were clockwise, indicating that the moth was drifting and not flying, as well as those with no recorded directionality, which indicated that the moth's flag was stuck in the sensor. Then, distance flown was calculated based on the circumference of the circle made with the flight mill arm (58.975 cm) and the instantaneous velocity was calculated by dividing that set distance by the time it took to complete the rotation. Each sex was analyzed separately using a general linear model with flight distance or velocity as the response variable. The fixed, explanatory variable consisted of semiochemical treatments. Upon a significant result from the model, Tukey HSD was used for multiple comparisons.

4.7 Results

There were significant differences between the headspace composition of the different treatments with the *S. integrifolium* headspace composition differing from all other treatments (Table 4.1; Figure 4.1). Headspace from *S. integrifolium* had 2-, 1.8-, 2.3-, and 1.6-fold more compounds than the control, female, male, and mixed cohorts of *E. giganteana* (Table 4.2). None of the *E. giganteana treatments* differed significantly from each other or the control (Table 4.1). Camphene and 1,4-difluoro-1,3-butadiyne was relatively enriched in *S. integrifolium* headspace compared to other treatments. Headspace collected from male *E. giganteana* was fairly clustered together despite not differing from any other treatments aside from *S. integrifolium* headspace (Figure 4.1). Carbonic chloride fluoride was relatively enriched in headspace from female *E. giganteana* compared to headspace from male *E. giganteana*, mixed *E. giganteana*, and *S. integrifolium* by 43-, 8.6-, and 2.3-fold (Table 4.2).

There was a significant difference in the mean depolarization due to the semiochemical treatment for both the male and the female *E. giganteana* (Table 4.3). Depending on whether it was the first or second puff impacted the behavioral response of male *E. giganteana*, with a greater overall response to the first puff (Table 4.3). Female moths were significantly more responsive to *S. integrifolium* headspace by 2.7- and 2.6-fold than *E. giganteana* female only headspace, respectively (Figure 4.2). In addition, there was also an elevated response by female *E. giganteana* to (*E*)-8-dodecenyl acetate by 3.4-fold. In the second puff, female *E. giganteana* was significantly more responsive to (*E*)-8-dodecenyl acetate (Figure 4.2). For male moths, there was an elevated response to acetone, and pure and diluted (*E*)-8-dodecenyl acetate (Figure 4.3).

There were no significant differences by date in the average distance flown for either sex of *E. giganteana* nor in the semiochemical treatment to which the individual moths were exposed (Table 4.4). However, with both sexes, we saw a numerical increase in the average distance flown when the moths were in the presence of the opposite sex (Figure 4.4; Figure 4.5). Similarly, there was no significant difference in the average velocity of flight for either sex of *E. giganteana* across the control and semiochemical treatments (Figure 4.6; Figure 4.7).

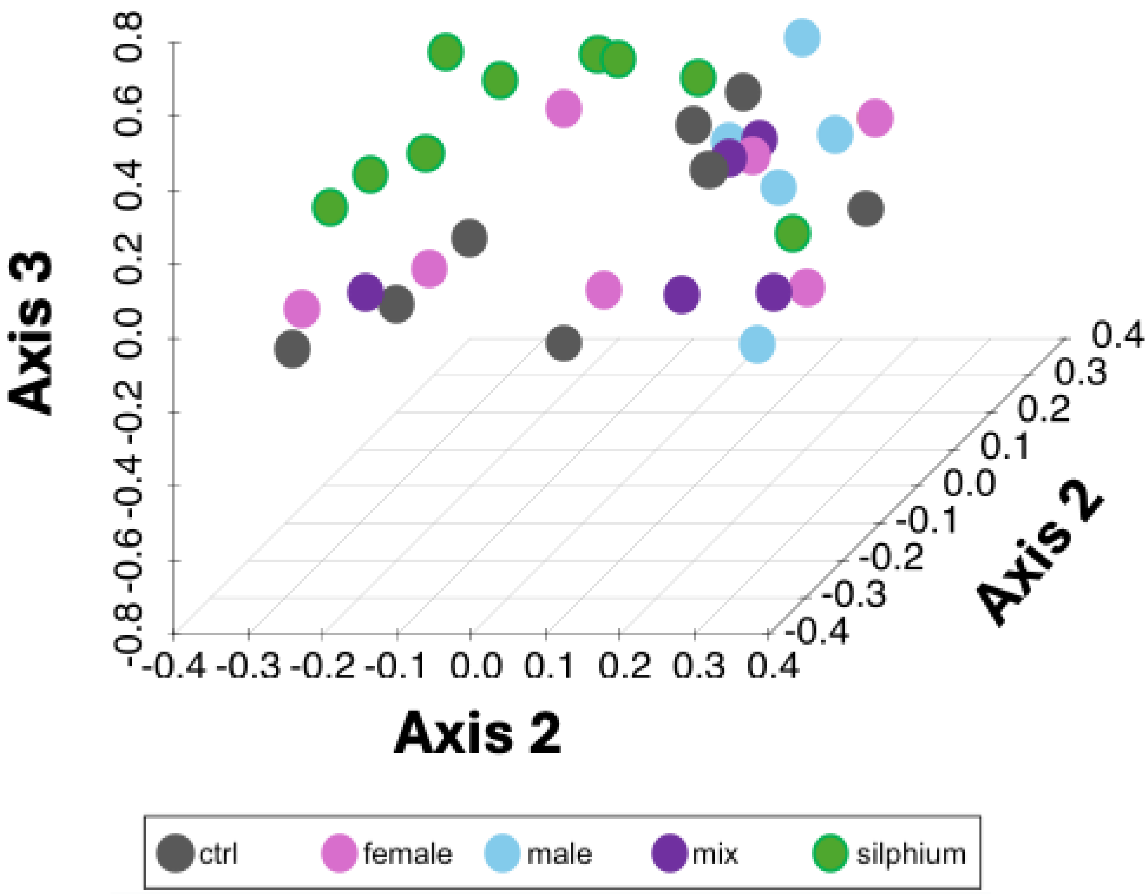

Figure 4.1 Headspace collected visualized through non-metric dimensional scaling (NMDS) based on a pairwise distance matrix calculated from a Bray-Curtis index for samples from E. giganteana conspecifics, flower heads of S. integrifolium, or a blank control. There was a total of at least n = 5 replicates per treatment, and stress < 0.13. Lines represent convex hulls.

Table 4.1 Statistical results of overall and pairwise ANOSIM tests in evaluating differences among E. giganteana adults and S. integrifolium flower heads with bolded lines indicating a significant result.

Comparison		***R***	***P***
Overall			
	All	**0.202**	**0.0020**
Pairwise			
	Ctrl vs. Female	0.0601	0.22
	Ctrl vs. Male	-0.0474	0.62
	Ctrl vs. Mix	-0.00526	0.46
	Ctrl vs. *Silphium*	**0.260**	**0.005**
	Female vs Male	0.00829	0.49
	Female vs. Mix	-0.13	0.18
	Female vs. *Silphium*	**0486**	**0.002**
	Male vs. Mix	-0.056	0.67
	Male vs. *Silphium*	**0.498**	**0.007**
	Mix vs. *Silphium*	**0.529**	0.001

Table 4.2 Tentatively identified normalized headspace emissions from cohorts of field-collected adult *E. giganteana* moths and *S. integrifolium* flower heads over a 24 hour period in the laboratory.

Compound[1]	RT[2]	Control		Female		Male		Mixed		*S. integrifolium*	
		Mean ± SE	%Total	Mean ± SE	%Total	Mean ± SE	%Total	Mean ± SE	%Total	Mean ± SE	%Total
2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	8.45	0.0001 ± 0.0001	0.04	0.005 ± 0.004	0.42	0.0029 ± 0.0027	25.9	- ± -	-	- ± -	-
N-Fluoroiminomalonic nitrile	22.1	0.0008 ± 0.0008	0.33	- ± -	-	0.0002 ± 0.0002	1.82	0.0002 ± 0.0002	0.38	0.0001 ± 0.0001	0.02
(1R)-2,6,6-Trimethylbicyclo[311]hept-2-ene	4.41	0.0003 ± 0.0002	0.12	0.0008 ± 0.0005	0.74	0.0004 ± 0.0004	3.46	0.0176 ± 0.0121	35.3	0.0186 ± 0.0078	3.97
1,3-Diphenyl-4H-1,2,4-triazoline-5-thione	25.5	0.008 ± 0.0008	0.33	0.0015 ± 0.001	1.34	- ± -	-	- ± -	-	- ± -	-
Bicyclo[221]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)	9.27	0.0027 ± 0.0015	1.11	- ± -	-	0.0032 ± 0.002	28.5	- ± -	-	0.0005 ± 0.0005	0.1
2-Methyl-5,5-diphenyl-4-(methylthio)imidazole	26.0	0.0035 ± 0.0035	1.42	0.0064 ± 0.0044	5.71	0.0039 ± 0.0039	34.2	- ± -	-	- ± -	-
Tricyclo[2210(2,6)]heptane, 1,3,3-trimethyl	4.3	0.0727 ± 0.0390	29.4	0.0772 ± 0.0488	69.0	- ± -	-	- ± -	-	0.1114 ± 0.0656	23.7
L-Valine, N-(2-furoyl)-, undecyl ester	25.1	0.1667 ± 0.1667	67.5	0.0138 ± 0.009	12.3	- ± -	-	0.0048 ± 0.0041	9.53	0.0048 ± 0.0042	1.03

4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative	24.8	- ± -	-	0.0002 ± 0.0002	0.15	- ± -	-	- ± -	-	- ± -	-
1H-1,2,3-Triazole-4-carboxaldehyde	21.0	- ± -	-	0.0007 ± 0.0005	0.67	- ± -	-	- ± -	-	- ± -	-
Carbonic chloride fluoride	8.01	- ± -	-	0.0109 ± 0.0102	9.71	0.0003 ± 0.0003	2.12	0.0013 ± 0.0006	2.52	0.0002 ± 0.0002	0.05
gamma-Terpinene*	4.3	- ± -	-	- ± -	-	- ± -	-	- ± -	-	0.0037 ± 0.0021	0.78
Camphene*	4.63	- ± -	-	- ± -	-	- ± -	-	0.0170 ± 0.0027	34.1	0.1997 ± 0.0762	42.6
beta-Phellandrene*	4.91	- ± -	-	- ± -	-	- ± -	-	0.0027 ± 0.0027	5.37	0.0356 ± 0.0119	7.58
Bicyclo[310]hex-2-ene, 4-methyl-1-(1-methylethyl)		- ± -	-	- ± -	-	- ± -	-	- ± -	-	0.0112 ± 0.0055	2.38
beta-Myrcene*		- ± -	-	- ± -	-	- ± -	-	0.0026 ± 0.0017	5.21	0.003 ± 0.0017	0.63
D-Limonene*		- ± -	-	- ± -	-	- ± -	-	- ± -	-	0.0094 ± 0.0045	2.00
1,3-Butadiyne, 1,4-difluoro-		- ± -	-	- ± -	-	- ± -	-	0.0031 ± 0.0031	6.28	0.0629 ± 0.0354	12.4
Succinic acid, 2,2,3,3,4,4,4-heptafluorobutyl 2-methylhex-3-yl ester		- ± -	-	- ± -	-	- ± -	-	- ± -	-	0.0079 ± 0.0029	1.68
1H-Imidazole, 5-iodo-2-methyl-4-nitro-		- ± -	-	- ± -	-	- ± -	-	0.0002 ± 0.0002	0.32	0.0001 ± 0.0001	0.03

Benzenamine, 2-iodo-	- ± -	-	- ± -	-	0.0004 ± 0.0004	3.86	0.0005 ± 0.0005	1.01	0.0003 ± 0.0003	0.07
Total Mean Emissions	0.0309 ± 0.0265	100	0.0124 ± 0.0083	100	0.0016 ± 0.0014	100	0.005 ± 0.0037	100	0.0293 ± 0.0135	100

[1]Tentatively identified compounds compared to the NIST14 library

[2]Retention time on HP-5 column

*Confirmed with commercial standards purchased from Sigma-Aldrich

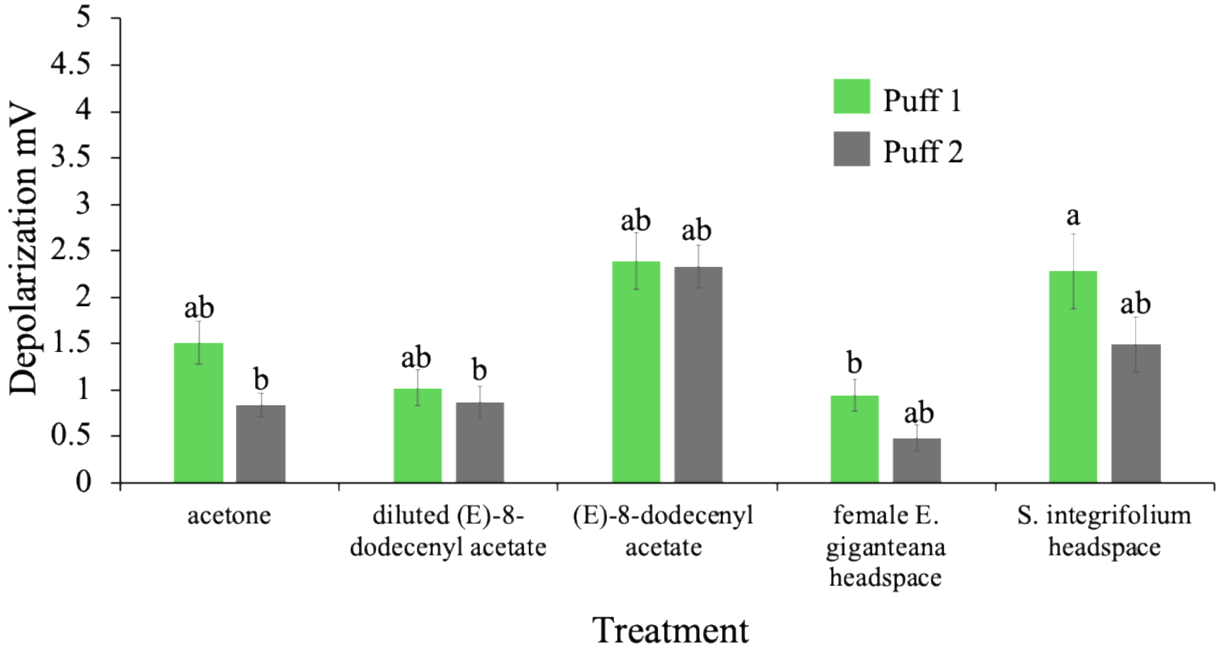

Figure 4.2 Female *E. giganteana* antennal depolarization via EAG across the seven semiochemical treatments on the first stimulus puff and second stimulus puff. Bars sharing the same letters are not significantly different among the treatment in the same stimulus puff (i.e. Puff 1 or Puff 2) (Tukey HSD, $\alpha = 0.05$).

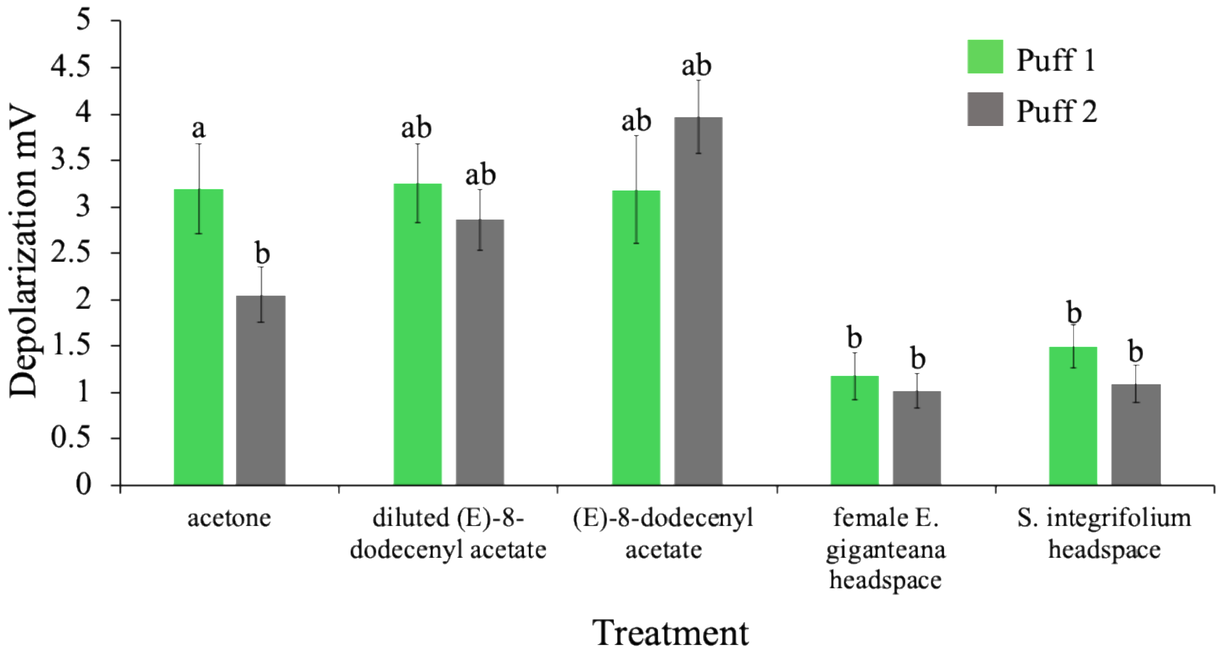

Figure 4.3 Male *E. giganteana* antennal depolarization via EAG across the seven semiochemical treatments on first stimulus puff and second stimulus puff. Bars sharing the same letters are not significantly different among the treatment in the same stimulus puff (i.e. Puff 1 or Puff 2) (Tukey HSD, α = 0.05).

Table 4.3 Summary of ANOVA results for *E. giganteana* antennal depolarization via EAG by sex and stimulus puff sequence as well as the interaction between the two.

	Female[1]			Male[2]		
Factor	**df**	***F***	**P**	**df**	**F**	**P**
Treatment	6	3.94	0.001	6	6.58	< 0.001
Stimulus puff	1	2.61	0.107	1	7.37	0.007
Interaction	6	0.95	0.463	6	1.81	0.098

[1] Female model residual df = 166.

[2] Male model residual df = 195.

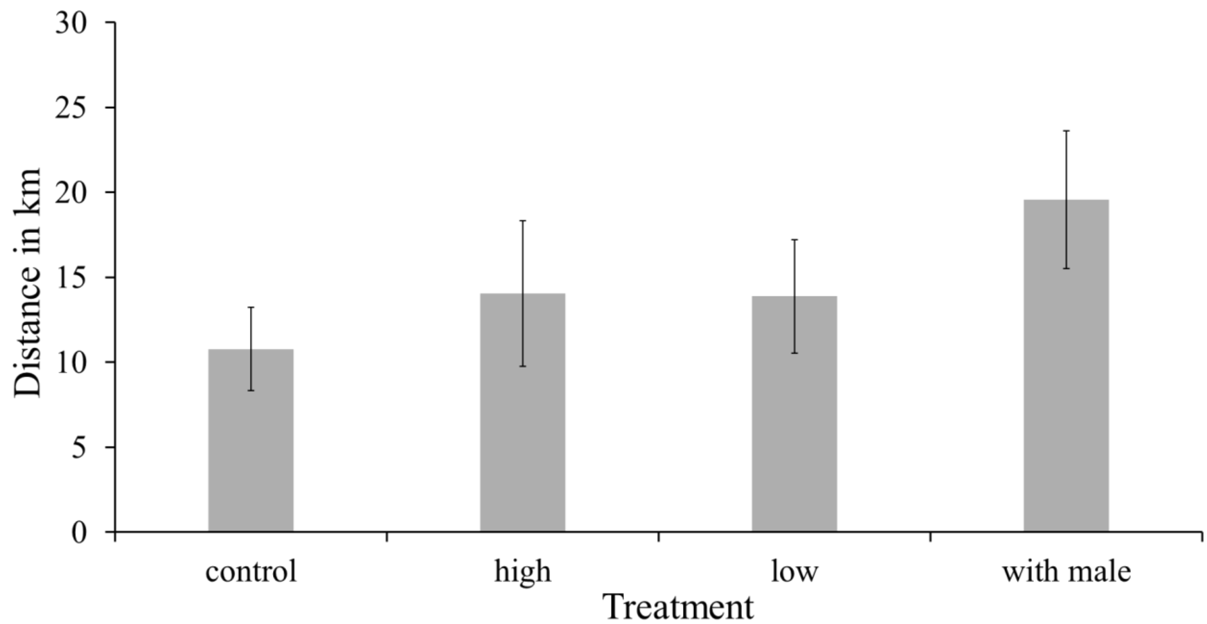

Figure 4.4 Field-collected female *E. giganteana* distance flown in a 24-h period in response to different semiochemical treatments on an automated flight mill in the laboratory.

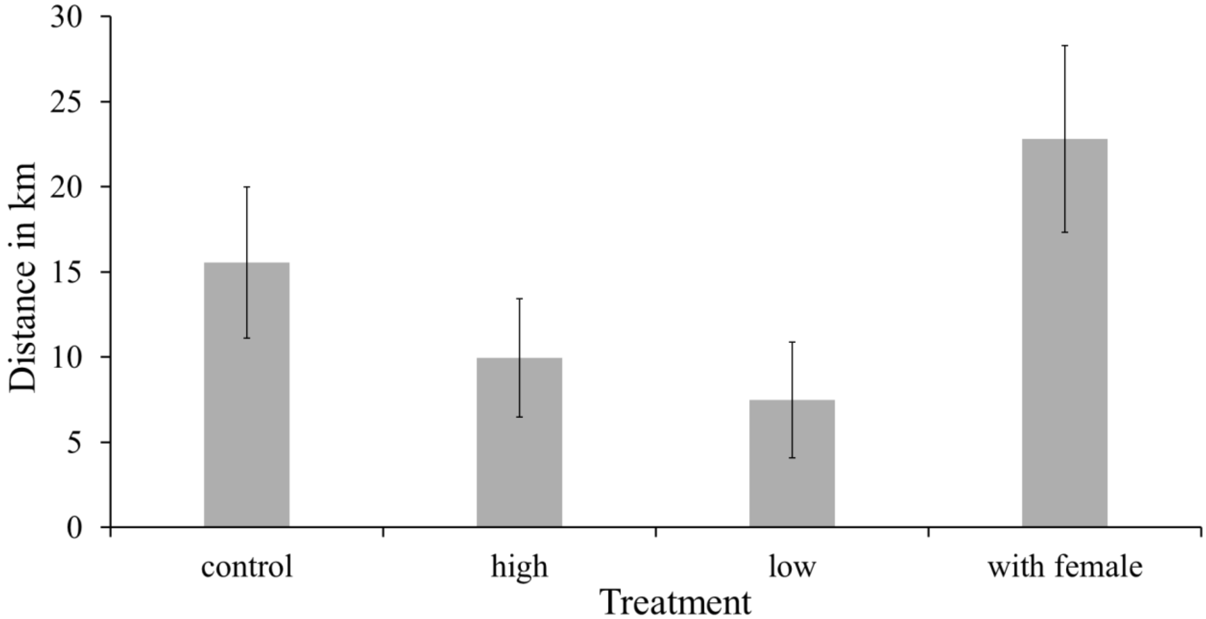

Figure 4.5 Field-collected male *E. giganteana* distance flown in a 24-h period in response to different semiochemical treatments on an automated flight mill in the laboratory.

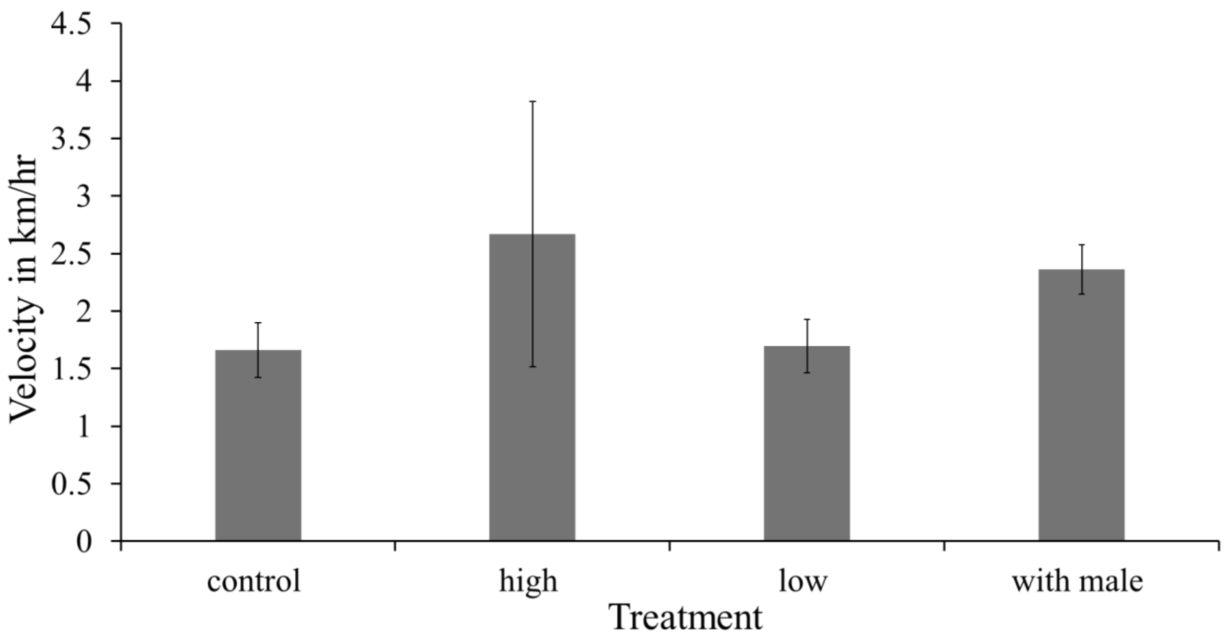

Figure 4.6 Field collected female *E. giganteana* average velocity in km/hr within a 24-h period determined from the average velocity of the individual moths in response to different semiochemical treatments on an automated flight mill in the laboratory.

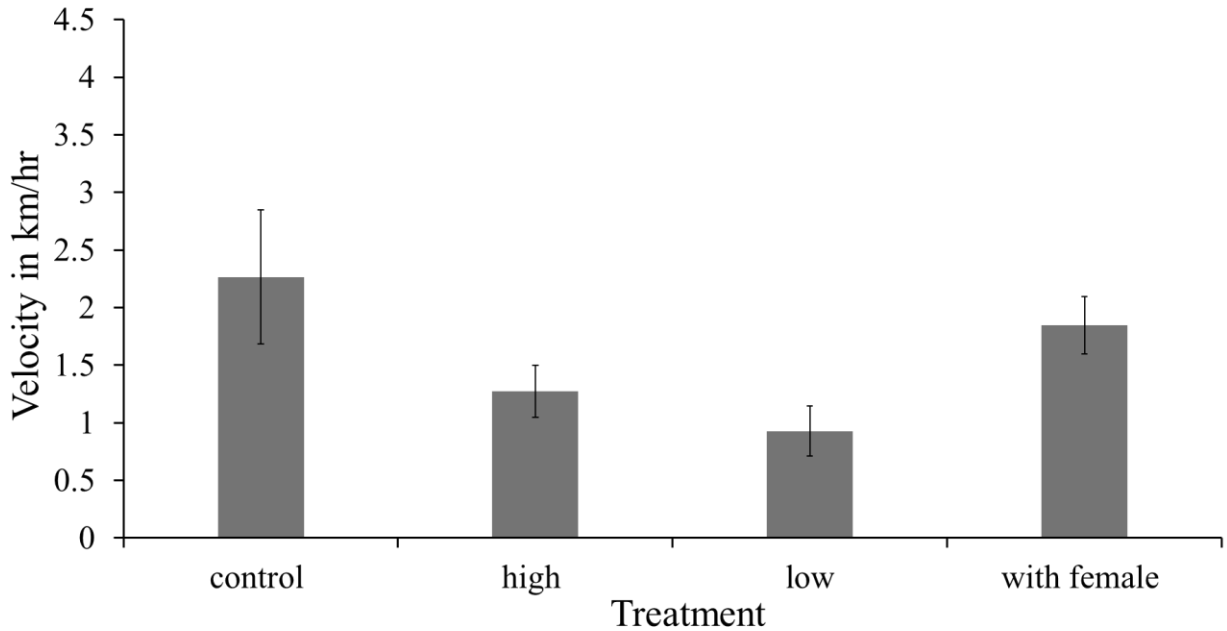

Figure 4.7 Field-collected male *E. giganteana* average velocity in km/hr within a 24-h period determined from the average velocity of the individual moths in response to different semiochemical treatments on an automated flight mill in the laboratory.

Table 4.4 Summary of ANOVA results on *E. giganteana* flight capacity by sex and date.

		Female			Male	
	df	F value	p value	df	F value	p value
Treatment	3	2.19	0.1	3	0.41	0.75
Treatment residuals	51			46		

Date	7	1.88	0.09	7	0.46	0.86
Date residuals	47			42		

4.8 Discussion

This is the first in-depth investigation of the chemical ecology of *E. giganteana*. We have demonstrated that volatile bouquets from *E. giganteana* and its host crop are significantly different. In addition, we have determined that *E. giganteana* females exhibit enhanced responsiveness to *S. integrifolium*, while males do not. Both sexes show elevated response to (*E*)-8-dodecenyl acetate through the EAG. While the EAG can confirm that a species perceives a volatile blend, it is limited in having any bearing on the behavioral response (Brezolin et al. 2018; Lu et al. 2024). However, prior research has shown that (*E*)-8-dodecenyl acetate is attractive in the field and laboratory (Ruiz et al. 2022; Chapter 3). It may be that there is a synergistic effect of presenting both *S. integrifolium* volatiles and (*E*)-8-dodecenyl acetate together, as they were evaluated in the field prior and there was attraction observed to clear sticky cards (Chapter 3). However, we did not specifically address this issue in the current study. Our results showed that both sexes do have a strong response to (*E*)-8-dodecenyl acetate which may indicate that the attractant works for both sexes. By contrast, female *E. giganteana* headspace alone elicited only a small depolarization using EAG. This information could be especially helpful for future management strategies as most pheromone-based lures primarily attract male moths (Giri et al. 2023). Related to that, although it was not a strong response, there was a response of female moths to the headspace collected from other female *E. giganteana* individuals, indicating that female *E. giganteana* may autodetect their pheromone. Autodetection has been confirmed in multiple noctuid species as well as another Tortricid moth, the European grapevine moth (*Lobesia botrana* (Denis and Shiffermüller)) (Holdcraft et al. 2016; Koutsompeli et al. 2024). Gerken et al. (2022) showed that Indianmeal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) autodetect their own pheromone, but this depended on strain, suggesting there is behavioral plasticity. That study further found that one strain increased the duration of calling behavior while the other decreased calling when exposed to pheromone lures (Gerken et al. 2022). Future work should be done examining if female *E. giganteana* alter their pheromone calling strategies when in the presence of other female *E. giganteana* pheromone or (*E*)-8-dodecenyl acetate as this could determine how effective strategies such as mass trapping and mating disruption may be on both sexes of *E. giganteana* (Chen et al. 2014; Knight et al. 2017; Witzgall et al. 2010).

The flight mill results followed a similar pattern as those between the control and (*E*)-8-dodecenyl acetate that the authors of Ruiz et al. (2022) reported on. In particular, there was an arrestment tendency in the presence of (*E*)-8-dodecenyl acetate on the flight distance of male *E. giganteana*. Ruiz et al. (2022) also found evidence of arrestment behavior in *E. giganteana* on the flight mill and showed a greater arrestment effect for male moths. It was interesting to note that there was no significant difference between the dates that the recordings were taken as this indicates that the moths active towards the end of the season are equally as eager to fly as those in the beginning of the season. The variability of using field caught specimens could have contributed to the lack of significance, including variation in age and mating status of moths since their history was unknown. Previous studies on other tortricid species have found that age and mating status have an effect on the moth's distance flown and propensity to fly (Schumacher et al. 1997; Su et al. 2022). In the future, moths reared from pupae should be tested, so that flight capacity in response to age and mating status can be evaluated.

We found *S. integrifolium* headspace was strongly separated from the other headspace treatments. Overall, *S. integrifolium* headspace was much more complex and contained a wider variety in classes of compounds. It primarily consisted of 10 compounds that were relatively unique, with those in highest abundance identified as camphene, 1,4-difluoro-1,3-butadiyne, and beta-phellandrene. Interestingly, camphene was also enriched in the headspace of mixed sex *E. giganteana*, suggesting moths may produce or sequester camphene and use it in intraspecific communication. This hypothesis was not explicitly tested but should be followed up on in future years. Overall, the lack of differences seen between the other semiochemical treatments, may be the result of using a density of *E. giganteana* that was too low in headspace collection jars. A higher density may be required, as some experiments require 40–100 individuals (Stanly et al. 2018; Zagrobelny et al. 2015). Future studies could be done using increasing numbers of *E. giganteana* individuals used during headspace collection to see if a higher density would yield different results. A higher density of individuals could also have increased the antennal response of the headspace during the EAG experiment. Overall, these results help us better understand how *E. giganteana* reacts to different semiochemical stimuli and lays a foundation for the development of behaviorally-based management tactics.

Specimens used in this research are deposited as voucher number 269 in the KSU Museum of Entomological and Prairie Arthropod Research.

4.9 References

Abshire J, Harman R, Bruce A, Gillette S, Maille JM, Ranabhat S, Scully ED, Zhu KY, Gerken AR, and Morrison WR. 2024. Flight capacity and behavior of *Ephestia kuehniella* (Lepidoptera: Pyralidae) in response to kairomonal and pheromonal stimuli. Environmental Entomology. 53(4):567–576. https://doi.org/10.1093/ee/nvae039.

Anandappa JA, Stanford HE, Marek LF, Goolsby EW, and Mason CM. 2023. Bioprospecting for improved floral fragrance in wild sunflowers. Helia. 46(79):169–186. https://doi.org/10.1515/helia-2023-0008.

Attisano A, Murphy JT, Vickers A, and Moore PJ. 2015. A simple flight mill for the study of tethered flight in insects. Journal of Visualized Experiments. (106):53377. https://doi.org/10.3791/53377.

Brezolin AN, Martinazzo J, Muenchen DK, De Cezaro AM, Rigo AA, Steffens C, Steffens J, Blassioli-Moraes MC, and Borges M. 2018. Tools for detecting insect semiochemicals: a review. Analytical and Bioanalytical Chemistry. 410(17):4091–4108. https://doi.org/10.1007/s00216-018-1118-3.

Casado D, Gemeno C, Avilla J, and Riba M. 2006. Day-night and phenological variation of apple tree volatiles and electroantennogram responses in *Cydia pomonella* (Lepidoptera: Tortricidae). Environmental Entomology. 35(2):258–267. https://doi.org/10.1603/0046-225X-35.2.258.

Chen R-Z, Klein MG, Sheng C-F, Li Q-Y, Li Y, Li L-B, and Hung X. 2014. Mating disruption or mass trapping, compared with chemical insecticides, for suppression of *Chilo suppressalis* (Lepidoptera: Crambidae) in northeastern China. Journal of Economic Entomology. 107(5):1828–1838. https://doi.org/10.1603/EC14148.

Fu N, Magsi FH, Zhao Y, Cai X, Li Z, Bian L, Xiu C, Chan Z, and Luo Z. 2022. Identification and field evaluation of sex pheromone components and its antagonist produced by a major tea pest, *Archips strojny* (Lepidoptera: Tortricidae). Insects. 13(11):1056. https://doi.org/10.3390/insects13111056.

Gerken AR, Dryer D, Abts SR, Campbell JF, and Stelinski L. 2022. Behavioral response of unmated female *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) to synthetic sex pheromone lure. Environmental Entomology. 51(6):1200–1209. https://doi.org/10.1093/ee/nvac087.

Giri AP, Short BD, and Piñero JC. 2023. Male and female tortricid moth response to non-pheromonal semiochemicals. Insects. 14(11):884. https://doi.org/10.3390/insects14110884.

Holdcraft R, Rodriguez-Saona C, and Stelinski L. 2016. Pheromone autodetection: evidence and implications. Insects. 7(2):17. https://doi.org/10.3390/insects7020017.

Hughes J, Hern A, and Dorn S. 2004. Preimaginal environment influences adult flight in *Cydia molesta* (Lepidoptera: Tortricidae). Environmental Entomology. 33(5):1155–1162. https://doi.org/10.1603/0046-225X-33.5.1155.

Johnson PJ, Boe A, and Martens AP. 2019. *Eucosma giganteana* (Riley) and *Sliphium perfoliatum* L., morphological variation in an insect-plant association in Eastern South Dakota. Proceedings of the South Dakota Academy of Science. 98:91–104.

Jones HBC, Lim KS, Bell JR, Hill JK, and Chapman JW. 2016. Quantifying interspecific variation in dispersal ability of noctuid moths using an advanced tethered flight technique. Ecology and Evolution. 6(1):181–190. https://doi.org/10.1002/ece3.1861.

Knight AL, El-Sayed AM, Judd GJR, and Basoalto E. 2017. Development of 2-phenylethanol plus acetic acid lures to monitor obliquebanded leafroller (Lepidoptera: Tortricidae) under mating disruption. Journal of Applied Entomology. 141(9):729–739. https://doi.org/10.1111/jen.12393.

Koutsoumpeli E, Manassakis G, Betsi P-C, Raptopoulos D, and Konstantopoulou M. 2024. Sex pheromone autodetection by *Lobesia botrana* females (Lepidoptera: Tortricidae). Crop Protection. 178:106580. https://doi.org/10.1016/j.cropro.2024.106580.

Lu H, Wu Q, Yang Y-Q, Li Q-K, Huang Y-Z, Li Y-C, Wan X-C, and Gou F. 2024. (*Z* , *Z* , *Z*)-3,6,9-Nonadecadiene, a potential inhibitor of sex pheromone of grey tea geometrid (Lepidoptera: Geometridae): electroantennogram test, wind tunnel, and in silico study. Journal of Economic Entomology. 117(3):793–799. https://doi.org/10.1093/jee/toae033.

Matveev E, Kwon JJ, Judd GJR, and Evenden ML. 2017. The effect of cold storage of mass-reared codling moths (Lepidoptera: Tortricidae) on subsequent flight capacity. Canadian Entomologist. 149(3):391–398. https://doi.org/10.4039/tce.2017.1.

McNair C, Gries G, and Gries R. 1999. Sex pheromone components of *Enarmonia formosana* (Lepidoptera: Tortricidae). Canadian Entomologist. 131(1):85–92. https://doi.org/10.4039/Ent13185-1.

Murrell EG, Zio KR, Chérémond NE, and Van Tassel DL. 2023. Assessing effective mechanical and chemical strategies for managing *Eucosma giganteana* (Lepidoptera: Tortricidae) in the perennial oilseed crop, *Silphium integrifolium* (Asteraceae: Heliantheae). Journal of Insect Science. 23(6):4. https://doi.org/10.1093/jisesa/iead102.

Naranjo SE. 2019. Assessing insect flight behavior in the laboratory: A primer on flight mill methodology and what can be learned. Annals of the Entomological Society of America. 112(3):182–199. https://doi.org/10.1093/aesa/say041.

Oksanen J, Simpson G, Blanchet F, et al. 2024. vegan: Community Ecology Package. Available from https://CRAN.R-project.org/package=vegan.

Prasifka JR, Mallinger RE, Hulke BS, Larson SR, and Van Tassel DL. 2017. Plant–herbivore and plant–pollinator interactions of the developing perennial oilseed crop, *Silphium integrifolium*. Environmental Entomology. 46(6):1339–1345. https://doi.org/10.1093/ee/nvx134.

Price JH, Van Tassel DL, Picasso VD, and Smith KP. 2022. Assessing phenotypic diversity in silflower (*Silphium integrifolium* Michx.) to identify traits of interest for domestication selection. Crop Science. 62(4):1443–1460. https://doi.org/10.1002/csc2.20748.

Reinert S, Van Tassel DL, Schlautman B, Kane NC, and Hulke BS. 2019. Assessment of the biogeographical variation of seed size and seed oil traits in wild *Silphium integrifolium* Michx. genotypes. Plant Genetic Resources. 17(5):427–436. https://doi.org/10.1017/S1479262119000248.

Ruiz K, Bruce A, Chérémond N, Stratton C, Murrell E, Gillette S, and Morrison WR. 2022. Field trapping and flight capacity of *Eucosma giganteana* (Riley) (Lepidoptera: Tortricidae) in response to behaviorally active congeneric semiochemicals in novel silflower agroecosystems. Insects. 13(4):350. https://doi.org/10.3390/insects13040350.

Schumacher P, Weyeneth A, Weber DC, and Dorn S. 1997. Long flights in *Cydia pomonella* L. (Lepidoptera: Tortricidae) measured by a flight mill: influence of sex, mated status and age. Physiological Entomology. 22(2):149–160. https://doi.org/10.1111/j.1365-3032.1997.tb01152.x.

Stanley J, Chandrasekaran S, Preetha G, and Subaharan K. 2018. Evidence of male pheromone in *Conogethes punctiferalis* (Lepidoptera: Pyralidae). Journal of Entomological Science. 53(4):455–466. https://doi.org/10.18474/JES17-98.1.

Su S, Zhang X, Zhang J, Huang B, Jian C, Peng X, Vreysen MJB, and Chen M. 2022. Flight performance, fecundity, and ovary development of *Grapholita molesta* (Lepidoptera: Torticidae) at different ages. Insects. 13(9):837. https://doi.org/10.3390/insects13090837.

Van Tassel DL, Albrecht KA, Bever JD, Boe AA, Brandvain Y, Crews TE, Gansberger M, Gerstberger P, González-Paleo L, Hulke BS, Kane NC, Johnson PJ, Pestsova EG, Piccaso Risso VD, Prasifka JR, Ravetta DA, Schlautman B, Sheaffer CC, Smith KP, Speranza PR, Turner MK, Vilela AE, Von Gehren P, and Wever C. 2017. Accelerating *Silphium* domestication: an opportunity to develop new crop ideotypes and breeding strategies informed by multiple disciplines. Crop Science. 57(3):1274–1284. https://doi.org/10.2135/cropsci2016.10.0834.

Vilela AE, González-Paleo L, Ravetta DA, Murrell E, and Van Tassel DL. 2020. Balancing forage production, seed yield, and pest management in the perennial sunflower *Silphium integrifolium* (Asteraceae). Agronomy. 10(10):1471. https://doi.org/10.3390/agronomy10101471.

Vilela A, González-Paleo L, Turner K, Peterson K, Ravetta D, Crews TE, and Van Tassel DL. 2018. Progress and bottlenecks in the early domestication of the perennial oilseed *Silphium integrifolium*, a sunflower substitute. Sustainability. 10(3):638. https://doi.org/10.3390/su10030638.

Wickham H. 2016. Elegant graphics for data analysis. Springer-Verlag New York.

Witzgall P, Kirsch P, and Cork A. 2010. Sex pheromones and their impact on pest management.

Journal of Chemical Ecology. 36(1):80–100. https://doi.org/10.1007/s10886-009-9737-y.

Zagrobelny M, Simonsen HT, Olsen CE, Bak S, and Møller BL. 2015. Volatiles from the burnet moth *Zygaena filipendulae* (Lepidoptera) and associated flowers, and their involvement in mating communication. Physiological Entomology. 40(4):284–295. https://doi.org/10.1111/phen.12113.

Zhonghui G. 2021. veganEx R Package. Github Repository. Available from https://rdrr.io/github/ZhonghuiGai/veganEx/.