Spring 5-5-2023

Browntail Moth (Euproctis chrysorrhoea) Integrated Pest Management Program: Evaluation of Monitoring Traps and Biopesticides

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Recommended Citation

Crosby, Sadia, "Browntail Moth (Euproctis chrysorrhoea) Integrated Pest Management Program: Evaluation of Monitoring Traps and Biopesticides" (2023). *Electronic Theses and Dissertations*. 3778.
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BROWNTAIL MOTH (*EUPROCTIS CHRYSORRHOEA*) INTEGRATED PEST MANAGEMENT PROGRAM: EVALUATION OF MONITORING TRAPS AND BIOPESTICIDES

By

Sadia Jane Crosby

B.S. Roger Williams University, 2017

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Entomology)

The Graduate School

The University of Maine

May 2023

Advisory Committee:

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BROWNTAIL MOTH (*EUPROCTIS CHRYSORRHOEA*) INTEGRATED PEST MANAGEMENT PROGRAM: EVALUATION OF MONITORING TRAPS AND BIOPESTICIDES

By Sadia Jane Crosby

Thesis Advisors: Dr. Angela Mech & Dr. Philip Fanning

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
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May 2023

Browntail Moth (BTM; *Euproctis chrysorrhoea*) is a non-native species currently outbreaking in Maine. BTM are polyphagous folivores and feed on a variety of woody plant species, including many economically and ecologically important ornamental and forest trees such as oaks and apples. Human health is also a concern as BTM larvae produce urticating hairs that can cause severe dermatitis in people. New monitoring and management programs are urgently needed, with the current population densities reaching a 100-year high.

The Maine Forest Service monitors BTM populations by visually assessing winter nest densities and defoliation patterns, both time and labor intensive. This research investigated the optimization of BTM sex pheromone monitoring traps in field trials during the adult flight period in 2021 and 2022. Trials in 2021 tested lure purity and two trap types, bucket style and delta style sticky traps. Results indicate that male moths were more attracted to lures with > 95% purity and bucket-style traps. Trials in 2022 tested additional trap styles and color variations. Results from 2022 indicate that white traps were significantly more attractive than green or multicolored traps. The Pherocon 1C trap caught the most male BTM; however, it was not

significantly different from the other white traps, indicating that any white trap could be recommended for use in future long-term monitoring programs for BTM.

In addition to monitoring evaluations, management strategies were tested for the control of BTM. Current control is the responsibility of municipalities and landowners, and broad-spectrum insecticides are commonly used due to the limitations of alternative control methods. Trials developing methods and testing the efficacy of more targeted biopesticide were conducted to determine if they were effective at reducing BTM populations. Initial trials observed BTM behavior in bioassay studies. Differences were found in the amount eaten and mass of larvae depending on the number of larvae present in bioassay cups (10, 25, 50 larvae, or the whole winter nest), which indicates that the amount of larvae present can impact lab experiment results.

Treatment bioassay trials testing the efficacy of different commercially available *Bacillus thuringiensis* (Bt) products were conducted in 2021 and 2022 on pre-diapause larvae. Survival and defoliation rates were determined for the various Bt treatments, both alone and including the use of spider peptides, which have the potential to increase the longevity and compound efficacy of Bt treatments. Results indicate that Bt products significantly reduce the amount eaten by larvae from control (water) treatments. Deliver (Bt *kurstaki*) used with peptide products was not significantly different from the current industry standard biopesticide product, Entrust (spinosad), a broad-spectrum insecticide. Peptide treatments alone did not significantly reduce the amount eaten from control treatments, but there were inconsistencies in the results of Basin and further testing is needed. The results of this research provide evidence supporting the adoption of new monitoring approaches and the potential use of less broad-spectrum biopesticides to manage BTM.

DEDICATION

I dedicate this thesis to the citizens of Maine who have been affected by BTM. I hope this work will be used to further our understanding of this pest and bring us closer to long-term management solutions. I feel so fortunate to live and work in this amazing state, surrounded by some of the most hard-working, creative, resilient, and stubborn people I have ever known. It is with these characteristics that I believe we will find effective solutions that limit future outbreaks of BTM from ever getting this bad again without compromising other organisms and ecosystems that make Maine so special. Thank you for your enthusiasm and support for this research.

In loving memory of my father, Captain Angus McGregor Crosby (September 9th, 1966 – November 14th, 2021), who taught me from a young age to respect and love the natural world. His many hobbies and skills inspired my own interests, including fishing, hunting, and becoming licensed arborists together. I could always count on him for a different perspective, a witty response, or a skiff ride when you just needed to feel the power. His incredible work ethic, humor, and fierce love for his friends and family will not be forgotten. Be young, have fun.

ACKNOWLEDGEMENTS

I would like to thank my advisors, Dr. Angela Mech and Dr. Philip Fanning, for their guidance and support throughout my graduate career at the University of Maine. I would also like to thank Dr. Allison Gardner and Dr. William Livingston for their participation on my advisory committee. Thank you to Monica Mulligan, Cameron Robbins, Sierra Croney, James Stewart, Devin Rowe, Ruby McClung, Rachel Jalbert, and Judith Collins for their help with my projects, even when it likely resulted in a BTM rash. I would also like to thank Allison Kanoti, Thomas Schmeelk, and Jeffrey Harriman from the Maine Department of Conservation, Agriculture, and Forestry for their help with sampling and general assistance. Thank you to Keith Kanoti and Jack Houtz from the University of Maine for additional help and resources. Many thanks to the Georgetown Working League and the Mitchell Institute for their support. Thanks to Trécé Inc. and Vestaron Corp. for the supplies and products that made my projects possible. I would also like to thank the University of Maine and the United States Department of Agriculture, Forest Service's Emerging Pest Program for providing funding for this research, as well as the landowners that gave me permission and encouragement along the way.

Over the last three years, I have been supported through difficult times and victories by many amazing friends, including Megan Schierer, Brittany Schappach, Elizabeth Anderson, Bridget Quilty, Miranda Rayner, Aislyn Keyes, Megan Leach, Austin Black, Melissa Simon, and of course my first friend in Orono, Benjamin Johnson, as well as many others. A special thanks to Marsh Island Brewing, Hawkes Tree Service, Irving coffee, and the plow truck drivers of I-95 and 295. Lastly, I would like to thank my mum (Lisa Crosby), sisters (Lauren and Mollie), the rest of my family, and the community of Georgetown Island, Maine, for their love and support along this journey.

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CHAPTER 1:

INTRODUCTION AND LITERATURE REVIEW OF BROWNTAIL MOTH (*EUPROCTIS CHRYSORRHOEA*)

Forest Insect Pests

Insect herbivory has shaped how modern and historical forest ecosystems have evolved. Most insect species have minor impacts on their tree hosts, but some species are characterized by having explosive population growth under the right circumstances that can have profound ecological and economic implications (Logan et al., 2003). Non-native insect species that become established in new areas may lack natural enemies and can exploit resources in forest systems. Defoliators and wood-boring beetles such as spongy moth (formerly known as gypsy moth; *Lymantria dispar* L.; Lepidoptera: Erebidae) and emerald ash borer (*Agrilus planipennis* Fairmaire; Coleoptera: Buprestidae), respectively, are among some of the most destructive invasive forest species in North America (Lovett et al., 2016). Invasive insects account for a loss of forestry-related goods and services worth an estimated $21 billion annually, worldwide (Bradshaw et al., 2016). Approximately 455 non-native insect species were established in U.S. forests by 2011 (Aukema et al., 2010). This figure is greater today, as introductions have continued since then, at a rate of 2.5 new tree-feeding species annually (Aukema et al., 2010). For example, spotted lanternfly (*Lycorma delicatula* White; Hemiptera: Fulgoridae) was first detected in 2014, and the elm zigzag sawfly (*Aproceros leucopoda* Takeuchi; Hymenoptera: Argidae) was detected in 2020 (Urban & Leach, 2022; Martel et al., 2021). In addition, increasing winter temperatures are resulting in the northern expansion of insect pest species such as hemlock woolly adelgid (*Adelges tsugae* Annand; Hemiptera: Adelgidae) and southern pine beetle (*Dendroctonus frontalis* Zimmermann; Coleoptera: Curculionidae) (Dukes et al., 2009; Dodds et al. 2018). The extent and severity of damage to trees by forest insects are determined by several factors, including the type of damage the insect causes (e.g., defoliation, sap depletion, phloem girdling), dispersal and reproductive potential, host specificity, and host tree characteristics (Lovett et al., 2016). An estimated 334 million ha, or 63% of U.S. forestlands, are at risk for basal mortality due to insect pests, with the Northeast harboring the greatest insect density (Krist et al., 2015).

Small fluctuations in densities are common in stable insect populations, and although they may have an equilibrium position, they are seldom static temporally or spatially. Insect outbreaks are a biological phenomenon that naturally occurs and may be cyclical for select species. The processes responsible for the occasional eruptive population dynamics are typically species-specific and poorly understood. However, favorable environmental conditions such as weather patterns and temperature variations, and food web exchanges including the reduction of natural predators and food availability, are all thought to be contributing factors (Liebhold & Bentz, 2011; Myers & Cory, 2013). The endemic population enters a building phase when these processes are triggered and, if left unchecked, can lead to an outbreak until it is disrupted or diminished and begins to decline back down to previous population densities (Myers & Cory, 2013). The time between peak outbreak populations has been determined in many common forest lepidopteran species. For example, the larch budworm (*Zeiraphera diniana* Guenée; Lepidoptera: Tortricidae) has only a single generation each year and is known to have population outbreaks occurring every 8 to 10 years, with the decline phase usually occurring over two generations (Dormont et al., 2006). Administering control efforts while a pest population is building or before it reaches peak density is more successful and uses fewer resources than when a population is already at outbreak levels. Outbreaking pest populations can be challenging to manage and persist until resources are depleted, environmental conditions become unfavorable, or control efforts are used (Myers, 1998). Patterns in the population cycles found through monitoring can determine peak population densities and can assist in predicting future outbreaks.

Study Organism: Browntail Moth

A current outbreaking pest of concern in Maine is browntail moth (BTM) *Euproctis chrysorrhoea* L. (Lepidoptera: Erebidae). The species is native to Eurasia and was first detected in North America in 1897 in Somerville, Massachusetts (Fernald & Kirkland, 1903). A shipment of roses to a florist shop from France or Holland in 1890 is suspected of containing BTM, leading to the accidental introduction (Fernald & Kirkland, 1903). However, it quickly spread in a continuous range from eastern Connecticut northward into New Brunswick, Canada, and throughout most of New England causing complete defoliation of hardwood trees in June and July and public distress with swarms of caterpillars of both BTM and spongy moth in neighborhoods (Burgess & Crossman, 1929). Cultural and chemical control efforts consisting of manual web removal, tree removal, and coating trees in pesticides were attempted, but found only effective in areas where they were extensively used.

BTM are polyphagous folivores and feed on a variety of woody plant species, including many economically and ecologically important ornamental and forest trees such as oaks and apples (Schaefer, 1974). The larvae are efficient defoliators and can denude mature trees during the primary spring feeding months of April to late June. The early instar larvae also cause damage from late August through September (Fig. 1) as they feed and prepare their winter webs, but this feeding is less damaging due to the age of the leaves and approaching fall leaf abscission (Burgess & Crossman, 1929). Larvae in September can molt up to three times while feeding and preparing the winter web (Schaefer, 1974). Winter webs consist of partially skeletonized or whole leaves, tightly wound together in larval silk on the tips of branches that several hundred larvae will communally share while undergoing winter diapause (Schaefer, 1974). Post-diapause larvae become active mid-late April and leave winter webs to feed as buds begin to open. Larvae will molt up to four more times as they feed gregariously and completely skeletonize foliage. Pupation often occurs in late June, in nests made of loosely gathered leaves and silk, and adult moths will then emerge in July (Schaefer, 1974). Mated females will lay eggs on the underside of host plant leaves in August, hatching approximately two weeks later and completing the cycle (Fig. 1).

Human health is an additional concern with this species. BTM is in the family Erebidae, subfamily Lymantriinae, which are commonly referred to as tussock moths. This group of moths are known to cause dermal irritation in humans (Howard, 1899). BTM larvae produce urticating hairs that can cause severe dermatitis to people who come into contact with them (Blair, 1979). Nearly 70% of people experience reactions to contact with BTM hairs (de Jong et al., 1975). Some severe cases can last several weeks and cause heightened respiratory distress to those susceptible. Because these microscopic hairs go airborne with each molt, people do not need to contact the caterpillars to be affected by their hairs directly. The toxin that causes the reaction does not readily break-down in the environment and can persist for several years, leading to increased exposure opportunities for people who are outdoors.

Browntail Moth History in North America

Since their discovery in the U.S. shortly before the 20th century, BTM and spongy moth were declared public nuisances in Massachusetts in 1905. The two closely related species share the subfamily Lymantriinae and research for foreign biological control agents that would target both were pursued. Parasitoids from Europe were released throughout New England with the hope of establishment and control of both BTM and spongy moth (Burgess & Crossman, 1929). There were seven species that established and were considered effective enemies of BTM (Clausen, 1956). The parasitoids preferred BTM as a target because they overwinter as larvae and greater population reductions were seen in this species (Elkinton et al., 2006). Harsh weather conditions consisting of a series of extremely cold winters was also speculated as a factor in their decline. By 1922, there were only a few small populations remaining in eastern Massachusetts and southern Maine (Schaefer, 1974).

For the past century, periodic outbreaks of BTM have occurred throughout New England with management taking the form of winter web removal and pesticide applications (Burgess, 1936; Schaefer, 1974). An outbreak of BTM in 1989 persisted through 1996, when aerial winter web estimates found activity in over 12 km of Midcoast, Maine (Bradbury, 1999). The Maine Forest Service conducted several control projects in affected areas, including manual removal of winter webs, and ground and aerial spray applications of the broad-spectrum insect growth regulator (IGR), diflubenzuron, and microbial pesticide (Bt) *Bacillus thuringiensis* Berliner (Bacillale: Bacillaceae). Results were determined cost-ineffective or limited in large-scale control of BTM, and fluctuations in the BTM population continued, including slight distribution shifts inland (Bradbury, 1999; Dubois et al., 2001). A notable outbreak occurred between 2001-2004 where 4,300 ha were defoliated in 2003 at the peak of the outbreak. Eventually, this resolved to a reduced defoliation of less than 2,000 ha annually, until the most recent outbreak began in 2016. In 2021, the Maine Forest Service observed BTM winter webs in over 80,000 ha of the state (MFS Dashboard, 2022).

In its native range, BTM has experienced outbreaks from southern Spain to eastern Romania and north to England (Marques et al., 2014). The earliest documented outbreaks of BTM occurred in France in 1730 and England in 1782, where swarms of caterpillars seemed to overwhelm oak and fruit trees completely and caused large areas of defoliation (Rennie, 1869). Occasional outbreaks have been observed over time causing damage to orchard and shade trees, disruptions in public health with cases of rashes and respiratory distress, as well as widespread fear in some regions (Lidwell-Durnin, 2022). The cause for the decline in BTM populations after outbreaks is not definitive; however, several key factors have been identified such as introduction of parasitoids, temperature effects on overwintering survival, and weather conditions during important life events. Presence of parasitoids, specifically *Compsilura concinnata* Meigen (Diptera: Tachinidae), which was one of the biological control agents released in New England in 1906, has been shown to reduce BTM populations and alternatively, an absence of *C. concinnata* is where higher densities were found (Elkinton et al., 2006). Overwintering survival and climate effects have also been explored and determined that higher winter mortality due to low temperatures may slow the spread of BTM, but was not the primary cause of population decline (Elkinton et al., 2008). Changes in weather conditions during important life events such as pre-diapause larval feeding has been suggested as a key factor (Klapwijk, et al., 2013; Boyd et al., 2021). Weather patterns during life events can also be used to predict the frequency of population cycles. Favorable fall weather, when pre-diapause larvae are able to feed longer and enter winter webs in stronger fitness and maturity, can result in increasing populations (Boyd et al., 2021). Microsporidia of the entomophthoralean fungi *Entomophaga aulicae* has been attributed to causing mortality in BTM populations across Europe. *E. aulicae* is distributed globally and has been identified as inducing mortality in Serbia after warm temperatures and rainy weather conditions provided favorable habitat for germination and infection of BTM larvae (Tabakovic-Tosic et al., 2018). When observing spring precipitation patterns in Maine during pre- and post- outbreak events of BTM, Boyd et al. (2021), did not see a marked change. Understanding how population densities fluctuate from year to year may hold the answer to what is causing, or working in tandem to cause, outbreaking BTM populations to crash. Monitoring programs are the best way to detect long-term and rapid fluctuations in pest populations, but these need to be set up prior to outbreaks if they are to be used to trace population densities and dispersal.

Integrated Pest Management

The increased need for integrated pest management (IPM) for outbreaking pests has led North American forest managers to rely on monitoring systems using semiochemical trap technologies (Ravlin, 1991). Semiochemicals, primarily sex pheromones, are used to monitor insect populations as well as interrupt insect behaviors that can reduce plant host damage (e.g., mating disruption) (Bjostad et al., 1993). Insect sensitivity, even at low population densities, high specificity to the target species, and low manual labor requirements are some of the advantages of using pheromone-baited traps for monitoring forest pests. Traps baited with a sex pheromone have been used for tracking a number of insect pests including the spongy moth, vine mealybug (*Planococcus ficus* Ben-Dov; Hemiptera: Pseudococcidae), and pink bollworm (*Pectinophora gossypiella* Saunders; Lepidoptera: Gelechiidae) (Suckling et al., 2014). In addition, early detection of new pest populations, or shifts in population densities, can help focus control efforts and catch insect pest populations prior to outbreak levels when control efforts may not be as feasible or effective.

Currently, the Maine Forest Service conducts seasonal aerial defoliation and winter web surveys for BTM. Count estimates of winter webs in areas provide us with useful information about the population density of BTM, but contain limitations including, observations primarily focused along major roadways and large time and labor investments by the surveyors. Monitoring through sex pheromones would alleviate the burden of many of the common setbacks with visual surveys, however, preliminary tests are needed to determine the appropriate trap variables such as trap type, pheromone concentration, purity, etc. Factors involved in trap effectiveness can vary including, pheromone plume release rate, rate of target species engagement and capture/escape in the trap, and overall capacity of the trap. Trap effectiveness for Lepidoptera can be assessed using a comparison study, for example, deploying at least two trap types and comparing the total number of males caught in each (Elkinton & Childs, 1983). Trap type can play a major role in trap effectiveness for monitoring and management programs (Bouwer et al., 2017; Wilson et al., 2020). The sex pheromone of female BTM has been isolated and synthesized; however, a statewide monitoring effort for long-term use has never been developed (Burgess & Baker, 1938; Leonhardt et al., 1991; Khrimian et al., 2008).

To reduce the potentially devastating impacts of insect pests, forest managers in the US will take action when monitoring efforts indicate that a population is reaching or growing beyond normal endemic levels. In some cases, a single individual insect is enough to warrant control, such as for spongy moth (Leatherman et al., 1995). Common IPM tactics used to control forest insect pests before they outbreak are silvicultural, biological, mechanical, and/or chemical control methods (Stern et al., 1959; Liebhold et al., 1996). For example, formulations of Bt, a microbial biopesticide, have been successful in aerial applications to control many lepidopteran forest pests, including douglas fir tussock moth (*Orgyia pseudotsugata* McDunnough;

Lepidoptera: Erebidae), spongy moth, and eastern spruce budworm (*Choristoneura fumiferana* Clemens; Lepidoptera: Tortricidae), when their populations were found to be above threshold levels (Dubois et al., 2001). Bt strains contain insecticidal crystal proteins (Cry) that can be toxic for specific insect orders (Bravo et al., 1998). Cry proteins bind to receptors in the midgut of larvae after ingestion, which form holes in the gut that eventually result in larval death (Dubois et al., 2001). Bt variants kurstaki (Btk) and aizawai (Bta) are commonly used to control Lepidoptera and can be effective for large population densities, making them a favorable choice over broad-spectrum insecticides, which can have negative effects on non-target insects such as pollinators and natural enemies (Plata-Rueda et al., 2020).

Bt resistance in insect pest species has been observed in systems that rely on heavy usage of Bt, such as modern agriculture (Gould, 1998; Sparks et al., 2021). New insecticide sources have been explored to reduce the potential for resistance. Spider venom peptides are a more recent development and have been shown to have rapid and debilitating effects on insect nervous systems (King & Hardy, 2013). Spider venom peptides may be used as stand-alone biopesticides or enhance the longevity and efficacy of treatments when included as an adjuvant (Fanning et al., 2018). When Bt is ingested and the Cry proteins are activated, the damage caused in the midgut enables the neurotransmitters of the peptide to bind onto and depolarize nerve cells, leaving them unable to respond or transmit new electrical signals, causing paralysis to the insect (Wilson, 2015). Spear-Lep (GS-omega/kappa-Hxtx-Hvla) and Basin (U1-AGTX-Ta1b-QA) are peptide products developed by Vestron® that the US Environmental Protection Agency has approved for use. The effects and use of peptides in the control of forest pests are understudied, however, previous research using Bt as a solo control agent for BTM was not overly successful (Schaefer, 1974). Bioinsecticides and adjuvants with new modes of action and resistance management are needed.

Thesis Objectives

This research will examine two components that will aid in developing a BTM IPM program - monitoring and controlling. Specifically, I will: 1) investigate the optimization of BTM sex pheromone monitoring lures and traps, and 2) determine the efficacy of commercially available Bt products on controlling BTM populations. Results from the first component will be used to help guide the further development of a long-term, statewide BTM monitoring program. Successful monitoring lures and traps will aid surveying efforts and be useful in documenting the BTM population fluctuations over the coming years as it eventually declines from the current outbreak and starts the cycle over again. Patterns found through population monitoring can help detect future BTM population outbreaks. The second component results can be used to make recommendations regarding the control of BTM. The effectiveness of current, commercially available Bt products on BTM is understudied, and the results of this component have the potential to increase the acceptance of biopesticides as a control agent for BTM over more traditional, broad-spectrum insecticides.

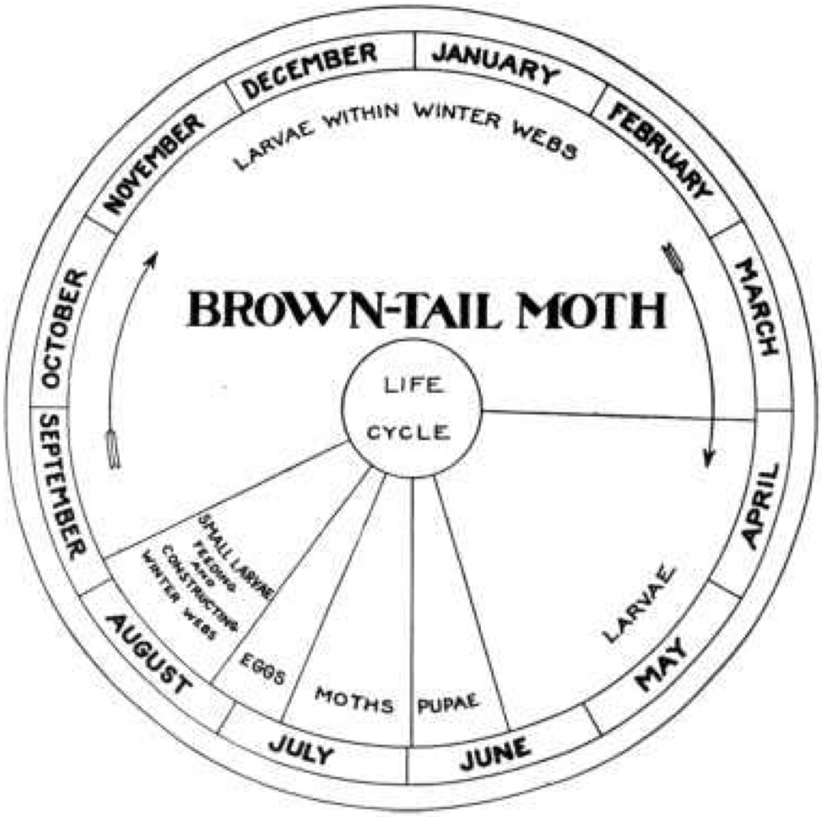

Figure 1. Life cycle of the browntail moth (Burgess & Crossman, 1929).

CHAPTER 2:

COMPARATIVE EVALUATION OF SEX PHEROMONE BAITED MONITORING TRAPS FOR BROWNTAIL MOTH (*EUPROCTIS CHRYSORRHOEA)*

Abstract

Browntail moth (BTM, *Euproctis chrysorrhoea*) is a non-native insect pest that was first detected in the United States in 1897. The initial expansion of BTM covered most of New England and areas of New Brunswick, Canada, until that population receded to small populations in Maine and Massachusetts. Throughout the last century, there have been several BTM population growth events, with the most recent outbreak in Maine currently ongoing. BTM is a polyphagous folivore that utilizes a variety of host plants, such as oak and *Rosaceae* species of ecological importance for forest habitats, as well as economically significant for timber and orchard industries. Human health is another concern of this pest, as the microscopic urticating hairs of the BTM can cause dermatitis and breathing issues for people. Understanding BTM population trends can potentially reduce future outbreaks by determining a critical population threshold that will ultimately lead to a damaging outbreak if it is not managed. Monitoring is key for detecting low-level BTM populations and thresholds, allowing for the deployment of control efforts. Current monitoring strategies are performed by the Maine Forest Service (MFS) through winter nest counts, and aerial defoliation surveys in the early summer and fall. The labor and time needed to perform these tasks is exhaustive and alternative methods for state-wide monitoring of BTM could greatly reduce these demands. Sex pheromone-baited monitoring traps have been successfully used for monitoring a number of Lepidopteran species. The female sex pheromone for BTM was identified and synthesized in 1991, and can be used as a lure to attract and catch adult males during their flight period, which can be counted to determine population trends across large areas. This research investigated the optimization of BTM sex pheromone monitoring traps in field trials during the adult flight period in 2021 and 2022. Trials in 2021 tested lure purity and two trap types, bucket style and delta style sticky traps. Results indicate that male moths were more attracted to lures with > 95% purity and bucket-style traps. Trials in 2022 tested additional trap styles and color variations, and found that white traps were significantly more attractive than green or multicolored traps. The Pherocon® 1C trap caught the most male BTM. However, it was not significantly different than the other white traps, indicating that any white trap could be recommended for use in future long-term monitoring programs for BTM.

Introduction

Semiochemicals are organic compounds produced by organisms that play a vital role in how they communicate with members of the same species and other organisms in their surrounding environment. Of all the senses insects use, they are most dependent on their sense of smell and use it to alter behavior and physiology depending on what they interpret (Brezolin et al., 2018). Insects have olfactory receptors that allow them to interpret chemical messages. Chemosensory neurons inside of sensilla, or sensory hairs, are distributed around the insect's body but mainly on their antennae (Witzgall et al., 2010). Odorant-binding proteins (OBPs) help bind to odoriferous molecules and can help transport them to the chemosensory neurons where they can be detected (Brezolin et al., 2018). OBPs are classified in two categories; general OBPs (interspecific, not species specific) and pheromone-binding proteins (intraspecific, species specific). Prominent antennae in many male species of Lepidoptera helps to optimize their odorant detection, specifically from females who may call to them during mating season using sex pheromones (Cardé & Haynes, 2004). Semiochemicals are further divided into two groups: allelochemicals that interact to mediate communication between individuals of different species (interspecific interactions) and pheromones that interact to mediate communication between individuals of the same species (intraspecific interactions) (Cardé & Haynes, 2004; Brezolin et al., 2018).

Chemical communication in insects can be used for numerous purposes including finding and attracting mates, identifying oviposition sites, locating prey or food, aggregating individuals, and defense (Brezolin et al., 2018). Researchers began exploring these forms of communication in the 1950s just as the concept of integrated pest management (IPM) began taking shape (Stern et al., 1959; Witzgall et al., 2010). The IPM paradigm was introduced as a means of reducing pesticide use and to explore alternatives including biological, cultural, physical and reduced-risk chemical options such as biorationals. The demand for these novel approaches to insect management became increasingly evident as the environmental effects of broad-spectrum insecticide use from chemicals such as DDT were becoming apparent (Hendrichs et al., 2007; Witzgall et al., 2010). The first discovery of silk moth sex pheromones was made in 1959 and opened the door for possibilities using semiochemicals to control and monitor insects (Butenandt et al., 1959; Karlson & Lüscher, 1959; Wright, 1964).

Insect semiochemicals, including pheromones and related attractants for economically important pest species, have been well studied and documented in a collective database that contains hundreds of chemicals and semiochemicals (El-Sayed, 2023). There are many benefits to using semiochemicals to monitor and manage insect pests. Their natural origin makes them considerably more environmentally friendly than synthetic and broad-spectrum insecticides

(Witzgall et al., 2010; Brezolin et al., 2018). Insect pheromones have evolved to be volatile and degrade quickly in the environment. If the semiochemicals remained active for longer periods, there is a risk of it being used as a cue for natural enemies to track and find the emitter (Brezolin et al, 2018). Many of these molecules can be degraded by UV light and are thermally unstable, causing low persistence, which is favorable in the environment (Brezolin et al., 2018). Semiochemicals can also be species-specific which reduces harmful effects to nontarget organisms including humans (El-Shafie & Faleiro, 2017). Depending on the carbon chain, they can have different molecular weights, but generally, they can be used to communicate over very large distances where alternative communication, such as touch, would be lost; and they are active at low concentrations so expensive purity development and production is unnecessary because a little goes a long way (El-Shafie & Faleiro, 2017).

Semiochemicals have been used for different methods of controlling insect pest species through mass trapping, attract & kill, repellent, and disruption tactics (El-Shafie & Faleiro, 2017). In mass trapping and attract & kill efforts, target-specific traps are baited with a semiochemical that is designed to draw in the species (El-Sayed et al., 2009). For example, when mass trapping mountain pine beetle (*Dendroctonus ponderosae* Hopkins; Coleoptera: Curculionidae), the traps may contain the chemical released by stressed coniferous trees, their preferred host, or an aggregation pheromone that signals a good host has been found or a mate is nearby (El-Shafie & Faleiro, 2017). The individual beetles are drawn to the trap based on the lure and once inside the trap, is exposed to a killing agent (insecticide, sterilant, pathogen, or physical barrier). Mass trapping is used to try and reduce the population by eliminating as many adults as possible (El-Sayed et al., 2006). Attract and kill traps are used for less severe insect pests where the goal is to keep the population from growing further (El-Sayed et al., 2009). Repellents are used to reduce populations by inhibiting target insects from finding, feeding, or ovipositing on their preferred host (El-Shafie & Faleiro, 2017). It essentially masks the host chemicals or terpenes with a less favorable chemical that the insect's olfactory receptors detect instead. This can make it difficult for insect pests to find favorable hosts, reducing their overall fitness. Alternatively, disruption tactics use the favorable host chemicals to overload an area with that scent and make it difficult for the pest to locate the true host. A disruption for mountain pine beetles may take the form of placing multiple lures of pinene terpene (a liquid extract of conifers that gives evergreens their smell) in an area with a mix of their preferred host trees present (El-Shafie & Faleiro, 2017). There is so much pinene terpene in the air that the insects struggle to find their preferred conifer trees.

Pheromones are the most widely used of all the semiochemicals used for insect monitoring and management. By definition, they are species-specific and, due to the evolution of specific mate identification, even incomplete or synthetic pheromone blends can successfully match their target species (Cardé & Haynes, 2004). This specificity enables pheromones to be effective at reaching their target with extremely small amounts. Female codling moths (*Cydia pomonella* L.; Lepidoptera: Tortricidae) calling for male mates, release their sex pheromone at a rate of a few ng/h. In lures used for monitoring traps they release 10-100 times more pheromone than a calling female, and in mating disruption applications they may use up to 10,000 times more (Witzgall et al., 2018). Mating disruption techniques using sex-pheromones of insect pests has been widely used and a highly successful form of control.

Pheromones for mating disruption have been identified for several insect orders including Diptera and Coleoptera but the predominant order is Lepidoptera (El-Sayed, 2023). In most Lepidoptera, the female calls to the male by releasing her pheromones into the air and the males follow the pheromone plume using their olfactory receptors until they find her and mate (Cardé & Haynes, 2004). Mating disruption hinders this communication by permeating the sex pheromone in huge quantities that makes it very difficult for the searching adult male to pick out the pheromone plume coming from an actual female, thus preventing mating (Adams et al., 2017). Spongy moth (formerly known as gypsy moth; *Lymantria dispar dispar* L.; Lepidoptera: Erebidae), is an example of one of the largest applications of mating disruptions for an invasive forest pest in the Eastern United States (Witzgall et al., 2010). In areas where high spongy moth populations are detected, control efforts are used including the application of insecticides or more commonly, mating disruption. The sex pheromone for spongy moth was identified in 1970 by Bierl et al. (1970) but prior to this discovery researchers would use virgin females or just their last two abdominal segments as a lure for traps (Holbrook et al., 1960). Early field testing applications for mating inhibition used hydrophobic paper treated with the pheromone and distributed it by aircrafts (Stevens & Beroza, 1972). Modern technology has produced plastic laminated flakes and paraffin wax formulations that are active for >10 weeks, providing sufficient coverage for the entire flight window of adult moths (Onufrieva et al., 2015). Mating disruption has been proven to be effective at low to moderate-density populations of spongy moth, however in high-density populations, the abundance of female moths are overwhelmingly abundant and easy for males to find regardless of false lures. In high-density populations it is recommended other IPM practices be used and additional control methods such as the use of bioinsecticides are recommended (Boukouvala et al., 2022).

Although pheromones have limitations for mating disruption, use in monitoring traps has been an essential component of the “Slow the Spread” project started in 1992 for spongy moth (Mayo et al., 2003). The project was designed to monitor populations on the fringe or outer front of the established population zone, and when they are detected these isolated populations are targeted for treatment (Boukouvala et al., 2022). This allows managers to slow the movement of established spongy moth populations by focusing on low-density populations using highly efficient and intensive monitoring (Sharov et al., 1997). Successful pheromone monitoring must be able to trap the target pest even at emergent or low-density populations (Cardé & Haynes, 2004; El-Sayed et al., 2006). If detected early, these isolated populations can be eradicated, thus reducing their spread. Extensive research to develop and improve trap and lure efficiency as well as catch probabilities and sample area estimates have been done to standardize them for accurate population density estimates (Bierl et al, 1970; David et al., 1983; Tcheslavskaia et al., 2005; Onufrieva et al., 2020). The current threshold for the standard monitoring traps, "milk cartons" made of reusable cardboard and plastic that contains a killing agent, to elucidate control for spongy moth is 10 male moths per trap (Sharov et al., 1997).

The body of work surrounding the use of pheromones for monitoring and control of spongy moth is vast and has beneficial implications for similar invasive forest pest insects such as the browntail moth [BTM, *Euproctis chrysorrhoea* L. (Lepidoptera: Erebidae)]. BTM is a univoltine species that can cause widespread damage to deciduous trees (Fernald & Kirkland, 1903). In its native Eurasian range, the polyphagous defoliator feeds on a variety of hosts but prefers oak and *Rosaceae* species (Schaefer, 1974). BTM was first detected in North America in 1897 in Somerville, Massachusetts (Fernald & Kirkland, 1903). The accidental introduction at a floral shop/nursery allowed BTM to quickly spread throughout New England and cause widespread defoliation in the early 1900s. In mid-April through mid-June, larvae will cease diapause and emerge from winter nests built the previous fall and begin feeding on newly budding leaves. They feed gregariously for the first few weeks and skeletonize the leaves of their hosts until they pupate mid to late-June. Adults emerge weeks later in early-July through mid-August. BTM actively seek mates at night and are inactive during the day (Schaefer, 1974). Successful females will lay eggs on the underside of host tree leaves that will hatch in September and first instar larvae will emerge and begin feeding as they build their communal winter nests. The defoliation by young larvae in the fall is physiologically less harmful as most deciduous trees have been photosynthesizing through spring and summer to gain their associated seasonal carbon needs and are prepared to shed these leaves during the winter (Asaro & Chamberlin, 2015). Repeated years of heavy defoliation in the spring however, can lead to branch dieback and even mortality to some trees. This threatens the timber and fruit orchard industries that are susceptible to BTM damage and rely on their trees' health and fruit production.

Besides the economically important hosts that BTM can threaten, it is also a human health pest. BTM larvae have microscopic urticating hairs that can be disbursed through contact when they are disturbed and can also go airborne when they molt or feel threatened (Schaefer, 1974; Blair, 1979). These hairs contain a toxin that can cause severe dermatitis in people and does not break down readily in the environment, increasing exposure risk. The most common symptom is dermal rashes however, severe cases have led to respiratory distress (Blair, 1979). Reducing BTM populations is the most effective measure to lower risk of exposure to their toxic hairs and the damaging defoliation they cause to economically important tree species.

Like other cyclical outbreaking pests such as spruce budworm (*Choristoneura fumiferana* Clemens; Lepidoptera: Tortricidae), BTM populations outbreak periodically and can be extremely difficult to manage when in these high population densities (Schaefer 1974; Myers, 1988; Régnière et al., 2019). Monitoring efforts are key for detecting BTM populations when they are low enough to observe their presence as well as take action when the populations begin to grow or reach a critical threshold of individuals that warrants control efforts be deployed (Witzgall et al., 2010). Many orchard and forest pests, including spongy moth, codling moth, and spruce budworm, have specific threshold limits in place that require control action be taken to reduce growing populations when a number of individual insects are observed in processed branches or caught in traps (Sharov et al., 1997; Adams et al., 2017; Régnière et al., 2019). BTM however, does not currently have a threshold limit set because semiochemical traps have not been used for monitoring populations. The Maine Forest Service (MFS) currently monitors for BTM by visually assessing BTM winter nests along major roads throughout Maine from December and April. The winter webs of BTM are covered in a bright white silk produced by the larvae that keeps the nest hydrophobic and insulated in the harsh Maine winters and also strongly anchors it to the tree branch (Schaefer, 1974). In the winter when most deciduous trees have lost their leaves, these winter nests are easily visible against a contrasting blue sky. This method of monitoring is time and labor intensive as one person must drive the vehicle while the other makes visual assessments of the tree canopy. The surveyors take information about host tree(s), distribution patterns of nests (single, patchy, continuous), and the average number of nests per tree (Maine Forest Service, 2022). Routes are selected based on the areas known to have previous BTM populations and major roads are driven to best cover the area. Only surveying along major roads and making assessments from a moving vehicle where nests can't always be seen, leaves the information incomplete. The MFS also surveys BTM defoliation using a fixed-wing aircraft. Surveyors fly over areas with medium-high BTM densities and survey the damage when larvae are actively feeding or just after, usually in the early summer (June-July) and again in the fall (September). Due to limited resources, these surveys often underestimate the impacted areas. The data is used to help predict the intensity of BTM populations over a broad area and the information is posted on the MFS's "Browntail Moth (BTM) Dashboard" (Maine Forest Service, 2022). Although this is a valuable service and monitoring resource to have for the current BTM outbreak, it is cost and labor intensive, and has limitations. Using pheromones baited traps for BTM could provide a cost-effective approach for monitoring statewide BTM populations, especially at low population densities when emergent detections or building populations could be controlled more effectively, prior to reaching outbreak densities.

The main component of the sex pheromone for BTM was isolated using a combination of gas chromatography, microreactions, and mass spectrometry and was identified in 1991 as (7Z,13Z,16Z,19Z)-docosatetraenyl isobutyrate (Leonhardt et al., 1991). The extracts used in the identification came from ovipositor tips from adult female BTM. The synthesis was later published in 2008 and has since been used by various manufacturers to develop synthetic pheromone lures for BTM (Khrimian et al., 2008). Pheromone baited traps have further aided research in optimizing load and isomeric purity rates in lures for BTM (Khrimian et al., 2008), detecting phenological shifts in adult flight as a response to host plant diet (Frago et al., 2019), and changes in population trends across multiple years (Mujezinović et al., 2017). Using pheromone traps to develop monitoring techniques for BTM has been limited and further investigation is needed. We believe that there is great potential in developing effective trap and lure combinations that are successful at trapping BTM even at low density populations that would be essential for large-scale or Maine state-wide population monitoring efforts.

This research explores the effectiveness of two different BTM pheromone lure purities and six trap types. We hypothesized that male BTM would be more drawn to a high purity lure (> 95%) than a lower purity lure and that traps with bright, reflective colors would be the most attractive to them during their evening flight. Traps that provided high airflow capabilities (open traps) were predicted to be more effective at drawing in male BTM by dispensing the largest and most directionally-even pheromone plumes. Results from this research will help describe the most successful trap-lure combination by comparative trials over two BTM flight seasons. The most effective trap-lure combination will be recommended for future research in BTM monitoring and potentially mating disruption research for BTM. Results will also be used to determine if the use of pheromone traps as an IPM strategy is feasible for BTM and encourage the potential development of a large-scale monitoring program for BTM.

Methods

Trapping Protocol

The female BTM sex pheromone was synthesized, loaded into rubber septa and made available for research (Trécé, Inc. Adair, OK). Lures were pinned to the center of delta traps or placed in the lure basket of bucket-style traps. The lures were rated for potency up to eight weeks, however, to ensure there was high potency throughout the flight season, lures were replaced after four weeks of use. All traps were placed at least 125 feet apart to reduce the likelihood that the pheromone plumes would overlap and confuse male moths. Traps were hung from tree branches between 4-6 feet above the ground. When traps were checked, they were emptied and the number of male BTM per trap were recorded. Delta traps with sticky liners were replaced with new ones, unless they were empty of specimens, and the original lure was placed in the new trap. Bucket traps (Trécé, Inc. Adair, OK) were equipped with a Vaportape II® insecticidal strip (Hercon Environmental, Emigsville, PA) as the killing agent when insects entered the cup.

Trapping Trial 1 (2021)

The first monitoring trial compared lures with two different purities [high purity (> 95%) and low purity (< 95%)] in two different trap types (green delta and green bucket traps; Fig. 2 A, B) for a total of four lure-trap pairs that tested if BTM had a selection preference.

Figure 2. Trap types used for comparison of adult male browntail moth captures: A) green delta trap, B) green bucket trap, C) multicolored bucket trap, D) white delta trap, E) white modified delta trap, and F) white 1C trap.

Traps were set up at a total of 15 sites in Maine (Fig. 3). These sites were selected from areas with established BTM populations ranging from trace to high densities of winter nests that were assessed visually in the winter of 2020/21 by the MFS. A variety of BTM densities were chosen to distinguish if the lures and traps would be effective in all population levels, which is important for monitoring emergent and building populations. Additional site information was collected as potential covariates to determine whether they may influence trap catches. These included a description of the site (forested vs. open), proximity to major water bodies, proximity to light sources, the percent of hardwood composition, and current defoliation (healthy, partial, or full).

All four lure-trap combinations were randomly distributed at each corner of a site polygon. Traps were set the first week of July 2021, when supplies were received, and kept up while the adult moths were actively flying. Traps were collected weekly to record the male BTM activity, as well as all bycatch, which was also collected for later identification. Traps were observed until two consecutive weeks of zero BTM catches, at which point the traps were taken down.

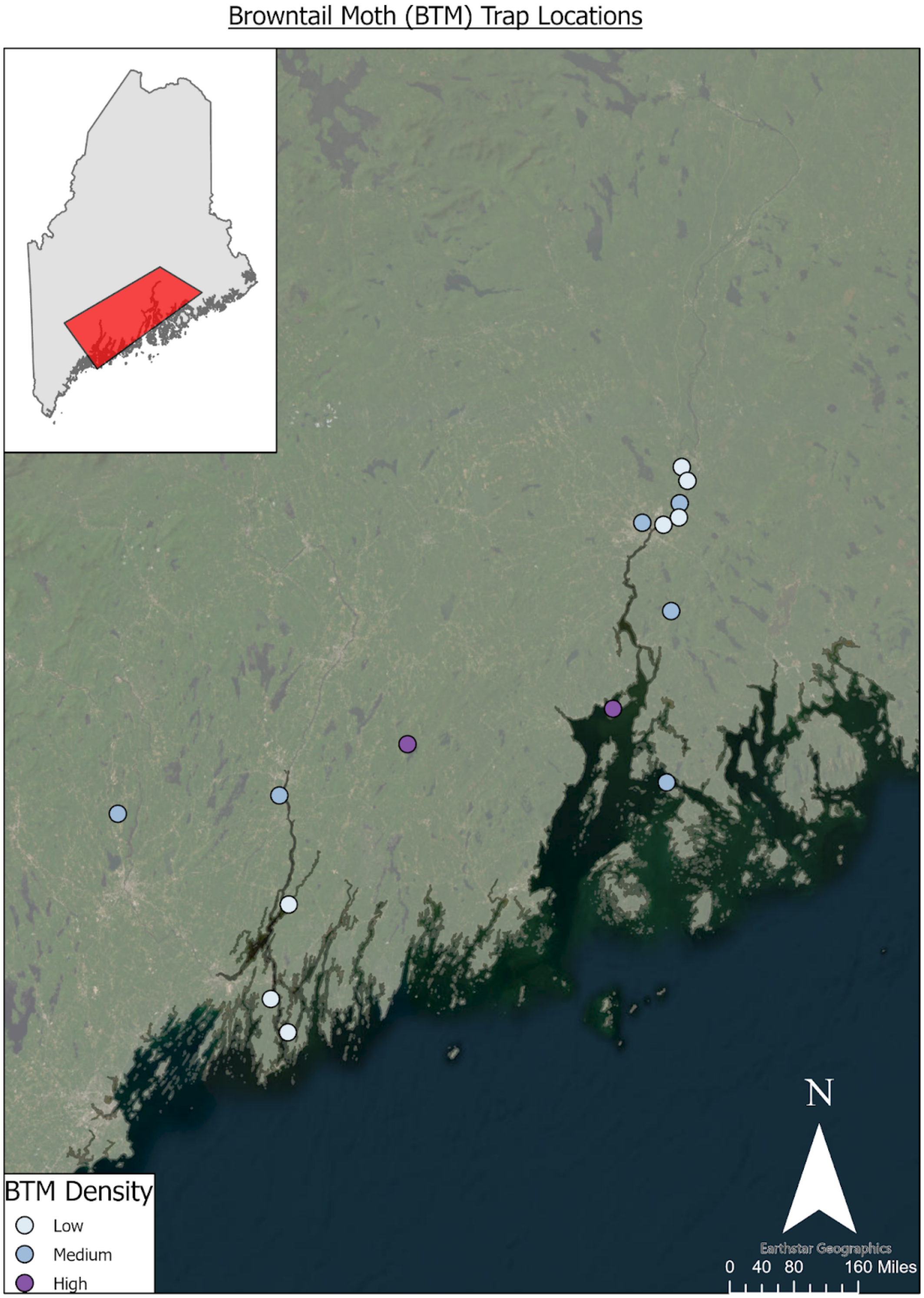

Figure 3. Location of 15 sites (dots) used in 2021 browntail moth trap and lure study in Maine. Sites encompassed multiple moth densities, from low (light blue) to high (purple) as assigned by site evaluator.

Trapping Trial 2 (2022)

Based on the results of trial one, new trap designs were tested in 2022 along with the top capturing trap from 2021 (green bucket trap). In total, five trap types (Fig. 2 B-F) were used to determine if there was a selection preference for adult male BTM. The high purity lure was used in all traps for this trial. Traps without a lure were also included as negative controls to determine if the traps alone captured BTM adults.

Five linear transects containing five baited and five negative controls for a total of 10 traps were placed in Searsport, Maine on June 8th, 2022, to catch the entire flight period of adult moths. A single site was selected to reduce confounding factors of different site locations and to have the same BTM density making the selection. Traps were checked biweekly and the amount of BTM adults captured was recorded. Traps were rotated randomly at each check. Evidence of dead BTM larvae were observed shortly after the start of the trapping began, indicating presence of an unforeseen epizootic event in Searsport. With the limited flight period in mind, we made the decision to disband four of the five transects in Searsport and relocate them to areas where healthy BTM populations seemed likely. Subsequently, only the one remaining transect of the original five setup in Searsport was used in the data analysis for this trial. With permission, two transects were set up in Orono, Maine on July 18th, 2022, and one was set up in Orrington, Maine on July 19th, 2022. Due to limited resources, these transects did not include negative controls and each transect had five of the baited trap types. These traps were checked either weekly or biweekly until they were removed on August 11th, 2022.

Data Analysis

Analyses were conducted using R studio (v4.1.2; R Core Team, 2021). Trap catches were summed for all dates and the total amount of BTM caught per lure and trap combination was calculated. A zero-inflated Poisson regression was used to determine if there was a difference in the number caught between the different lure and trap combinations using the PSCL package (Zeileis et al., 2008; Jackman, 2020). If significant differences were found, then post-hoc pairwise comparisons were conducted using the emmeans package with a Tukey adjustment (Lenth, 2022).

Site characteristics were evaluated using only green bucket-high purity lure trap catches and without the Searsport site, which was determined to be a substantial outlier. Each site variable was modeled individually using a zero-inflated poisson model, and a Wald χ^2 test was used to determine whether they were a potential driver of BTM catches. Analyses were conducted using JMP Pro (v16; SAS Institute Inc., 2022).

Results

Trapping Trial 1 (2021)

All trap types and lures used in the 2021 trapping trial successfully caught male BTM (Fig. 4). The green delta trap with the low purity lure caught the least amount of moths during the flight season and had an average of less than one moth caught per site. The green delta trap with the high purity lure and the green bucket trap with the low purity lure also caught less than one male moth on average per site and were not significantly different ($p = 1.00$). The green bucket trap with the high purity lure was the most successful trap-lure combination and caught an average of two adults per site, which was significantly more than the other trap-lure combinations (all *p*-values < 0.05; Fig. 4). The number of individual specimens caught as accidental bycatch in each trap type was also recorded; the green delta trap caught 1,918 individuals and the green bucket trap caught 718 individuals. Of the 2,636 specimens collected, 769 belonged to the taxonomic order, Lepidoptera. Many of these specimens were unidentified microlepidoptera, however there were nine individuals identified as belonging to the Erebidae family, eight of which were identified as spongy moths.

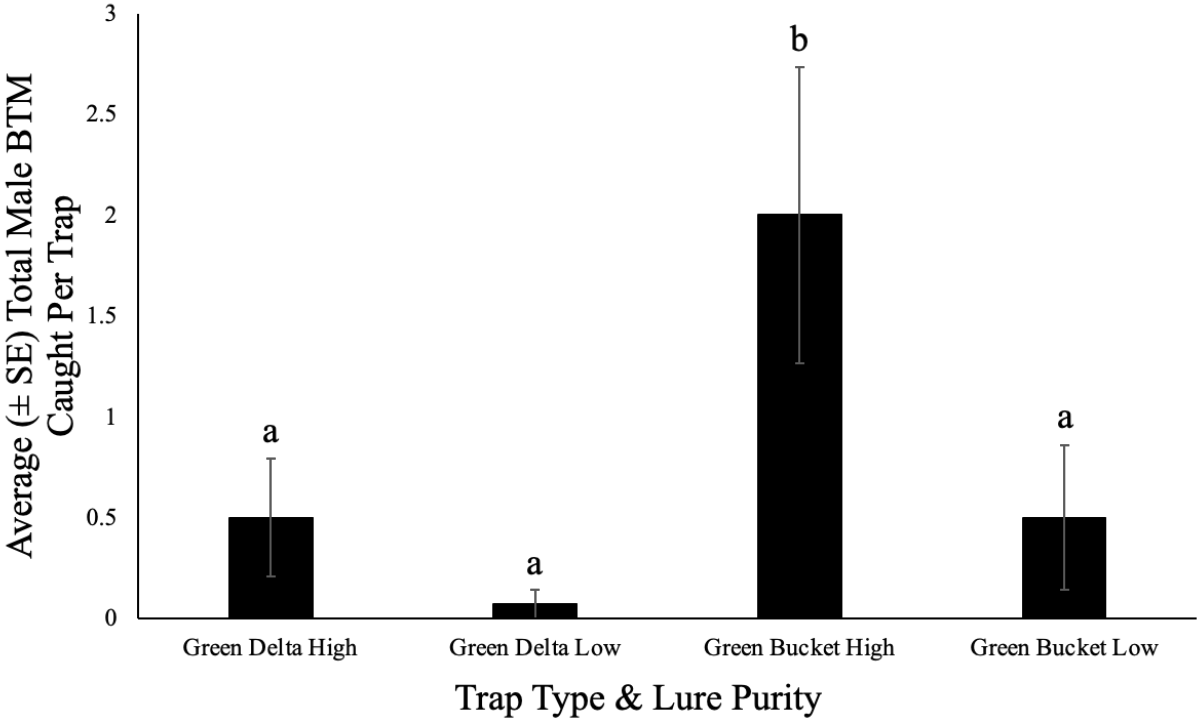

Figure 4. The average (± SE) total male browntail moth caught in each trap type at 15 sites across Maine in 2021. The lure purity used is indicated by "High" or "Low", indicating the high purity pheromone (> 95%) or the low purity lure (< 95%).

The site data collected at the 15 sites was analyzed to determine if they influenced overall catch amounts (Table 1). MFS density was found to be a significant driver of the number of male moths caught (*p* = 0.02). The MFS's high density yielded the largest number of moths with an average of 3.5 male moths per site. There was a significant difference found between the highest and lowest MFS densities (*p* = 0.01). Sites that had high BTM densities caught 71% more males than sites with trace densities. Results found that none of the other site factors were found to have a significant effect on the number of male BTM caught (all *p*-values > 0.05; Table 2).

Table 1. Site variable information collected for the 14 sites used in the browntail moth 2021 trap and lure study conducted in Maine.

Site name	Total male BTM caught	Site descriptor (landscape)	Proximity to nearest light (feet)	Proximity to nearest water body (feet)	Tree defoliation in site area	Hardwood trees in site area (%)	Maine Forest Service BTM density
U. Maine	2	Open	≤ 100	> 100	Healthy	26-50	Trace
Bangor	8	Open	> 100	> 100	Healthy	51-75	Low
Eddington	0	Edge	> 100	≤ 100	Partial	51-75	Trace
Bucksport	0	Edge	> 100	> 100	Partial	51-75	Low
Little Deer Isle	7	Edge	≤ 100	> 100	Partial	26-50	High
Brewer	1	Edge	≤ 100	> 100	Healthy	51-75	Low
Palermo	0	Edge	> 100	> 100	Healthy	76-100	High
Dresden	1	Edge	> 100	≤ 100	Healthy	51-75	Moderate
Turner	3	Open	> 100	> 100	Healthy	76-100	Low
Old Town	1	Edge	> 100	> 100	Healthy	26-50	Trace
Hallowell	0	Edge	> 100	> 100	Partial	51-75	Low
Georgetown	0	Edge	≤ 100	> 100	Healthy	51-75	Moderate
Phippsburg	0	Edge	> 100	> 100	Healthy	26-50	Moderate
Orono	5	Edge	≤ 100	≤ 100	Healthy	51-75	Low

Table 2. Model outputs for the six different site variables collected for the sites (Table 1) used in the browntail moth 2021 trap and lure study conducted in Maine.

Model	Wald χ^2	DF	*p*-value
Site descriptor	1.33	1,12	0.25
Proximity to nearest light	0.01	1,12	0.95
Proximity to nearest water body	0.18	1,12	0.67
Tree defoliation in site area	3.84	1,12	0.05
Hardwood trees in site area	0.16	2,11	0.92
Maine Forest Service BTM density	9.55	3,10	0.02*

*Denotes significant difference at the $\alpha = 0.05$ level

Trapping Trial 2 (2022)

Negative control traps set in Searsport without lures for the five trap styles used in the 2022 survey were unsuccessful at catching any male BTM. Four of the five experimental traps set with high purity lures caught male BTM, the multicolored bucket style trap was unsuccessful at catching any moths (Fig. 5). The green bucket style trap caught, on average, just less than two moths per trap and was the least effective of the four trap styles that caught BTM (all $p < 0.05$). The white modified delta trap caught the most BTM per trap with eight and a half moths, however, it was not significantly more than the amount caught by the white delta ($p = 0.53$) and the white 1C style traps ($p = 0.99$).

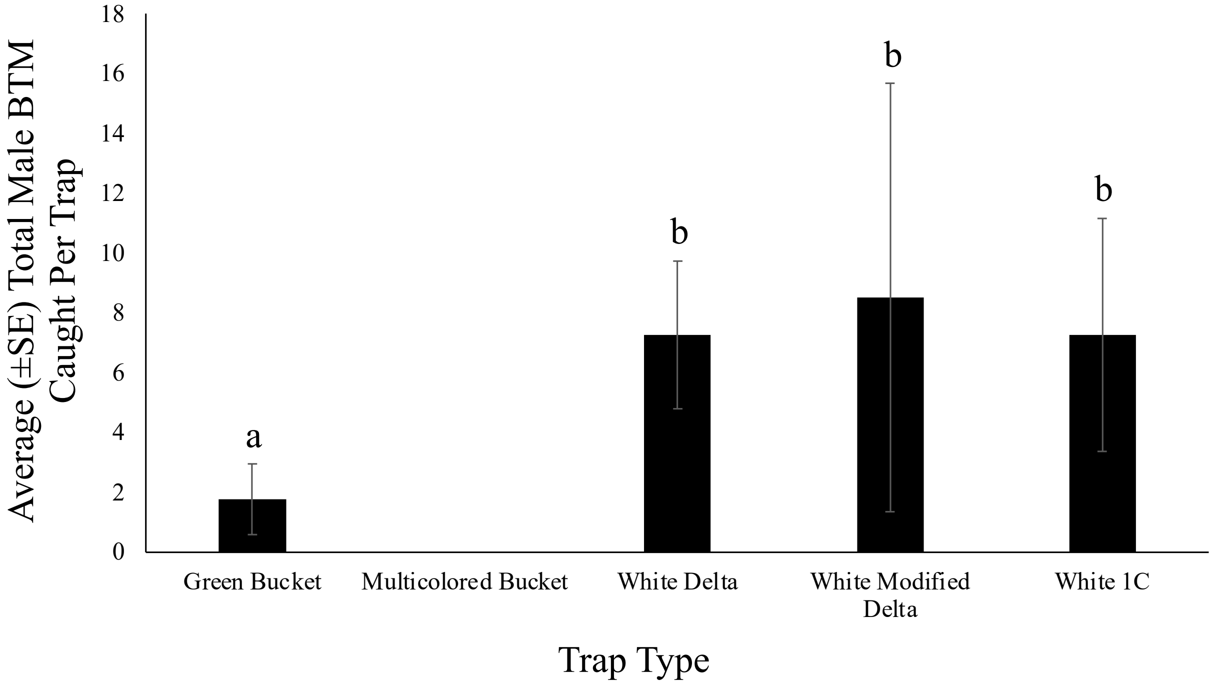

Figure 5. The average (± SE) total male browntail moth caught in each trap type tested at four sites in Maine in 2022.

Total BTM catches for each trap type at the three sites sampled were variable in their preference (Fig. 5). At the Searsport site, the 1C trap caught the most BTM (58.8%) followed by the green bucket trap (29.4%). In Orono, the white delta was very successful and caught 75% of the males at that site. In Orrington, just less than half of the 62 moths caught (48%) were in the white modified delta. Interestingly, the trap that caught the most BTM was different at all three sites (Fig. 6).

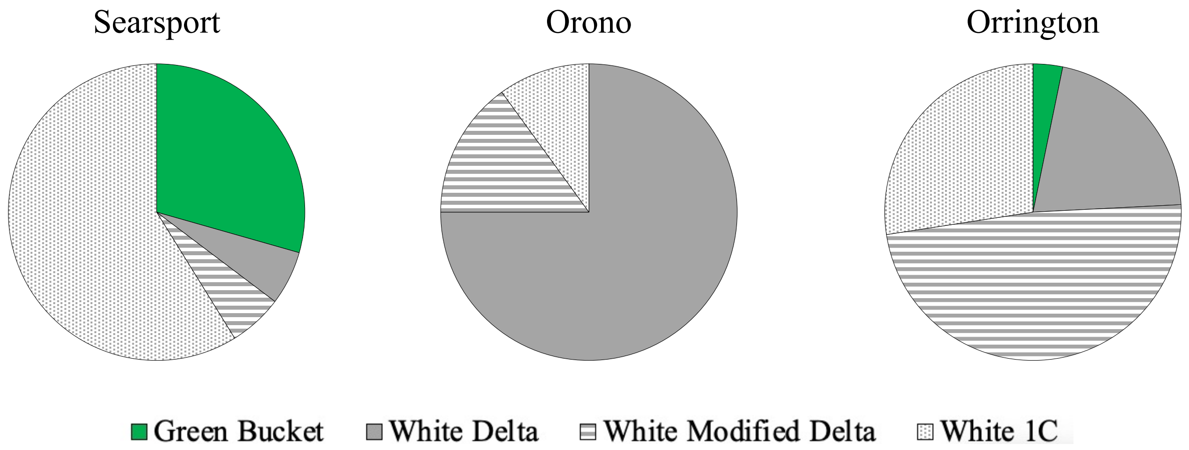

Figure 6. Proportion of male browntail moth catches for each trap type tested at the three sites used in 2022 in Maine.

Discussion

Monitoring traps that can effectively catch the target species at all population densities is essential for successful monitoring programs. Monitoring traps are often the first indicator of an emerging pest population and must be sensitive even at low densities to detect establishing populations (Liebold & Tobin, 2008). Resource managers use catch data to make informed decisions about pest populations, including determining if management practices need to be exercised (Witzgall et al., 2010). There are currently no traps being used to monitor BTM populations in the United States, however, this work demonstrates that BTM can be successfully caught in the field using pheromone-baited traps at varying densities and could therefore be incorporated into an IPM program.

Trap color can be a strong attractant that draws insects to traps. Many insects use color to locate hosts, such as flowers or other plant materials, and the vibrance and hue of the color can affect how the insects respond (Epsky et al., 2004). The color preference of monitoring traps for major forest and orchard pests has been determined for a number of species. Emerald ash borer

(*Agrilus planipennis* Fairmaire; Coleoptera: Buprestidae), an invasive wood-boring beetle, is attracted to purple traps (Poland et al., 2019), some ambrosia beetles (subfamily Scolytinae) are attracted to green traps (Abbasi et al., 2007), and olive leaf moths (*Palpita unionalis* Hübner; Lepidoptera: Pyralidae), a major pest of olive nurseries, are attracted to white traps (Athanassiou et al., 2004). For moths that are typically active during the night, actual color is less important than the contrast of colors, or the trap color and the background color. Light is another visual cue that can affect trap attractiveness. Insects use the light of the moon to help align themselves so that they can travel in straight lines by maintaining a constant angle to the light (Epsky et al. 2004). Traveling at a constant angle also directs the insect towards the source and artificial lights can produce the same effect. Many insect traps take advantage of this relationship and use lights to lure-in insects, such as using blacklights or mercury bulbs (Band et al., 2014; White et al., 2016). Light colored traps or traps that can reflect moonlight may produce a similar effect. In our experimental trials using green, multicolored, and white traps, we saw a significant trend for higher catches in white traps (Fig. 5). Male BTM are highly sensitive to light so this trend is likely due to the contrast of these traps with the dark background and their reflective properties (Reiff, 1909). The green traps blend into the dark of their background or the tree limb they were hanging from, potentially making it more difficult for moths to locate. Multicolored bucket traps were also tested, which consisted of three colors; green top, yellow mid-section, and white cup (bottom) (Fig. 2 C). Although the total surface area of these traps were approximately half white, they did not catch any moths, unlike the green bucket traps, which suggests that color was not the only factor driving BTM catches. Traps without lures were used as negative controls to provide evidence that traps were not effective at trapping BTM males on their own. Our results suggest that the pheromone lures are the driving factor in trap effectiveness.

Although trap styles were not the most predominant driver in trap catches of BTM, they are still an important consideration. Besides the color and contrast of the traps, their structure and the design of how their lure plume is released out of the traps varied. The bucket style traps are made of a rigid plastic with a basket that hangs down from the lid which holds the lure (Fig. 2 B-C). Between the lure and the bucket is an opening that allows the pheromone plume to release at essentially 360 degrees. Research suggests that larger trap openings or traps with a smaller baffle surrounding the opening, catch more moths because there is less surface area for moths to rest on, leading them into the trap (Frewin et al., 2022). This can also lead to greater bycatch amounts with less constrictive openings. Ultimately, the plume shape and structure emitted from traps is highly influential in total trap catches (Lewis & Macauley, 1976). The 1C trap also allows for the plume to flow out at 360 degrees and is designed to maintain a gap between the top and bottom portions of the trap. The delta style traps are triangular prism-shaped with an entrance at either end, limiting the size of the trap opening and causing the plume to flow out of each end of the trap. The green Delta trap had the smallest openings of 3.9 cm^2 each and the white delta and white modified delta had larger openings of 42 cm^2 (Fig. 2 A, D, E). The white modified delta trap had additional rectangular windows cut out along each side of the trap approximately 102 cm^2 each that allows for additional plume dispersal and larger entrances to the trap. We anticipated that traps with larger openings and wide plume dispersal capabilities would be the most attractive and easiest for moths to enter such as the bucket and 1C style traps. Our results suggest however, that this was less of a factor for trap catches because the bucket traps did not perform as well as other styles, even though they had a 360-degree plume flow.

Trap material and cost is another consideration to have when selecting the style to use for large-scale monitoring efforts. The bucket traps are made from rigid plastic and designed to be reused for many seasons (Fig. 2 B-C). After several years of use, the higher initial cost may eventually be more cost effective than single-use traps. A downside of the bucket trap style is that a killing agent such as the pesticide strip we used, must also be present, which can lead to further costs. The white delta and white modified delta are also partly reusable and made from a corrugated plastic that protects the disposable sticky sheet inside that catches the moths. The 1C trap is made from a thick waxed paper that makes it durable in the elements and is also partially reusable, the top of the trap stays, and the sticky bottom must be replaced. Sticky sheets in all the traps must be replaced throughout the season, but unlike the green delta traps which must be fully replaced, some of the parts are reusable for multiple years, reducing costs.

Higher pheromone purity takes longer to synthesize and is generally more expensive to produce, so determining if there is a difference in selection preference is important when considering the cost of a long-term monitoring program. Pheromone lure development for spongy moth pheromone (disparlure) found that racemic or equal parts of the (+ & -) enantiomers in the pheromone compound was just as effective at catching moths and less costly to produce than the previous product using pure (+) enantiomers of dispalure (Miller & Gut, 2015). We compared two purities of the BTM pheromone lure, a high purity (> 95%) and a low purity (< 95%). Traps containing the high purity lure caught significantly more moths than the low purity lure did, indicating that BTM are more attracted to the higher purity. At this stage, > 95% purity is the highest our team could synthesize (Trece pers. comm.). Eventually, there may be tools or synthesis methods that allow us to reach even higher purity concentrations such as, > 99%, which could be even more competitive than adult females and improve attractiveness to male BTM. Understanding the preference is important but it also means that monitoring for BTM using the most effective traps may come at a higher financial cost. Communicating and working with companies that are synthesizing and producing these pheromones will be important for monitoring programs to find a means of mass producing these lures at a cost effective price point that managers can meet.

Alongside purity development, release rate and lifetime of the lures should also be explored. During the 2021 field season, materials were delayed in transit which did not allow for the traps to be set until after the start of the BTM flight season. Consequently, the lures used in these traps were only used for the five weeks remaining in the flight window. Trials in 2022 replaced lures after four weeks of use at the Searsport site and new lures were placed at the other two sites when the new traps were set the following week, so the longest a lure was in use was four weeks. For Lepidoptera, most monitoring programs use lures that are active for the whole flight season. Spongy moth adults are typically active for six weeks, so the lure is designed to be active for ten weeks to sufficiently catch the whole flight season including a two-week buffer at the beginning and end of the flight season (Onufrieva et al., 2015). This is an advantage of this type of monitoring because once the traps are set, the lures do not have to be changed during the season, saving time and resources. The lures used for monitoring BTM were designed to be effective for 8 weeks to match their total flight period, however, due to logistics of setting traps and maintaining consistency, the longest a lure was used was 5 weeks (Trece pers. comm.) Although lures begin to degrade as soon as they are exposed to moderate temperatures, the lures we used within the length of this trial should have been effective. Further studies should address the release rate and lifetime of these lures to ensure that they are sufficiently releasing the pheromone at a constant and effective rate for the whole BTM flight period.

Due to the fact that it was an extreme outlier (it caught 238% more males than the next highest site), Searsport was eliminated from our site characteristic analysis. Comparison of models with and without the site were found to consistently be different except for MFS density, which was still a significant driver after removing Searsport (Table 2). Density estimates from the MFS Dashboard came from visual assessment of winter nests in the general area that the site was located in (Maine Forest Service, 2022). The density of nests is a good indicator for what to expect in terms of emergence of larvae in the spring. Using the presence of winter nests in an area to predict BTM density proved to be an effective predictor of the number of adult males caught at the site. This monitoring tactic, used currently by the MFS, can be used to corroborate trap catches and in the future, be used to develop threshold models. Although the defoliation at the site seems likely to be a good indicator of the number of male catches, it is hard to tease apart because of the lack of quantified groups used to describe it. This was qualitative data taken at the initial trap setup - repeated estimates during return visits to the site and determining a more accurate description of the defoliation might have yielded different results. Heavy defoliation in the spring would most likely be correlated with an abundance of mature BTM larvae that would be active adult moths in the summer.

Besides MFS density and the marginally significant defoliation site characteristics, none of the other factors were major drivers in male moth catches (Table 2). We were surprised to see that proximity to light was not a major predictor based on our knowledge and observations of BTM attraction to lights (Reiff, 1909). Our study may have had limited results due to the range of lights present at our sites and low sample sizes (Table 1). For example, only three of our sites had traps placed at or within 50 feet of light sources and ten of the sites were at least 100 feet from lights, some of which were several hundred feet away. These large distances may not have fully captured how lights affected catches, whereas all three of the sites at or within 50 feet of a light caught BTM. The percent of hardwood trees in the site area was also not a primary driver of trap catches. BTM are polyphagous folivores that feed on a variety of hardwood tree species, such as oak, apple, and cherry trees (Schaefer, 1974). We had expected areas that had limited hosts for larvae would not support high densities of BTM, and further limit the amount of adults collected. Understanding observed trends or site characteristics that can influence catches may be important for future risk assessments and understanding why some sites may harbor greater densities of adults. Including more sites in future monitoring studies with a greater diversity of site characteristics and more trapping data can help draw further conclusions about site characteristics and determine if there are consistent drivers that may help predict adult BTM trap catches.

Spatial arrangement of traps is another important consideration to be made. Traps were only checked and rotated weekly or biweekly so not every trap had an even amount of time at each location or was placed at every location of the site, leaving room for possible confounding effects. Due to the short flight window of BTM, typically eight weeks between June-August (Frago et al., 2019), we recommend that future studies rotate traps more frequently, such as every few days, to ensure all traps spend the same amount of time at each position of the site, including throughout the flight period so as to account for any early/late flight window preferences that could affect trap catches.

The low trap catches in the first year of our study were an inadvertent effect of delayed equipment, however it had consequences in terms of our low catch numbers that season. The low values do not provide us with an overabundance of data to work with and limits the reach of our conclusions. Moving forward, it is essential that traps be placed prior to the initial emergence of BTM adults to ensure a full capture of the flight period and to provide ample catch data to analyze. Low moth catches were less of a problem in the second season, however, the small sample size was. Originally, the five transects in Searsport should have provided sufficient replication, however an unforeseen epizootic event caused us to abandon four of these transects and deploy three new transects in additional areas, halfway through the flight window. Replication is important to help tease apart discrete preferences, such as the more preferential trap to be used in large-scale monitoring efforts. Future studies should plan for the unexpected and include many replicates to ensure that sample size will not be a limiting factor for the research.

Despite the low sample size, we were able to obtain some interesting results from the four transects we had at the three sites in year two. Although main conclusions can be drawn about the white color preference of traps, there was no clear evidence of a single trap being the most effective. At all three of our sites, the traps with the highest amounts of catches were different (Fig. 6). This may be due to some site characteristic that we did not sufficiently analyze or observe. All three sites vary in their landscape type; the Orono site is a mostly developed area with several ornamental trees present and many artificial lights, the Searsport site is heavily wooded and located near the Gulf of Maine, and the Orrington site is an open grass recreation area with many trees experiencing BTM damage for the first time during this outbreak. We have preliminary evidence from our results that previous density determinations using winter nests can help predict future trap catches, however there are still many variables and models that we have not considered that could play a role in trap catches. Several factors to consider in future studies are rainfall/drought conditions, tree age in the immediate site area, presence of biocontrol agents, insecticide use history, and competition from other native and non-native defoliators, such as spongy moth.

We tested a variety of different trap styles and colors, and moving forward, there is still an assortment that could be explored for BTM monitoring traps. The high purity lure was the most effective for BTM catches and should be used as the primary lure until further synthesis and purity development can be tested. Traps were effective at catching BTM in all density categories, demonstrating that it could be utilized in long term monitoring efforts where trace populations of emerging BTM are beginning to establish. Previous winter nest density from the MFS is a primary driver of monitoring trap catches, however further research is needed to determine and understand the major factors and drivers of trap catches. Future research surrounding BTM pheromone lure plume structure and release rates would also benefit the cause. In conclusion, although current monitoring strategies for BTM in Maine are able to detect the density and spread of this forest pest, monitoring with the use of pheromone lure traps is a potential system that would allow us to detect BTM populations more efficiently even at low population densities where other strategies may be hindered.

CHAPTER 3:

EFFICACY OF BIOPESTICIDE APPLICATIONS IN BIOASSAYS OF BROWNTAIL MOTH (*EUPROCTIS CHRYSORRHOEA*) ACROSS TWO YEARS IN MAINE

Abstract

Browntail Moth (BTM, *Euproctis chrysorrhoea*), is a non-native species currently outbreaking in Maine. BTM are polyphagous folivores and feed on a variety of woody plant species, including many economically and ecologically important ornamental and forest trees. Human health is also a concern as BTM larvae produce urticating hairs that can cause breathing issues and severe dermatitis to people. After its introduction into the United States in the late 1800s, BTM was partially controlled by a number of factors including manually removing and destroying winter nests that larvae communally overwinter in. The challenges and labor costs associated with removing these nests from tree canopies eventually made this method prohibitive, and broad-spectrum pesticide use became the primary tactic for control of outbreaking populations of BTM. Many of these pesticide products, such as DDT, have been banned for use due to prolonged toxicity in the environment. The integrated pest management movement has promoted the use of biological-based pesticides, derived from natural ingredients such as microbes, to provide more targeted control of pests with limited environmental effects. Current management for BTM relies heavily on broad-spectrum insecticides, including biopesticides, but alternative, more targeted products that can effectively control BTM while limiting the environmental impact of their applications, should be evaluated. Trials developing methods and testing the efficacy of more targeted biopesticide products for BTM were conducted to determine if they were as effective as insecticides currently used for control. Initial trials observing BTM behavior in bioassay studies found that there were differences in the amount eaten and mass of larvae depending on the number of larvae present in bioassay cups, indicating that the amount of larvae present per cup can affect behavior and therefore results. Treatment bioassay trials testing the efficacy of different commercially available *Bacillus thuringiensis* (Bt) products were conducted in 2021 and 2022 on pre-diapause larvae. Survival and defoliation rates were determined for the various Bt treatments, both alone and with spider peptides, which have the potential to increase longevity and compound efficacy of Bt treatments. Results indicate that Bt products significantly reduce the amount larvae eat compared to control (water) treatments. Deliver (Bt *kurstaki*) used with peptide products was not significantly different from the current industry standard biopesticide product, Entrust (spinosad), a broad-spectrum insecticide. Peptide treatments alone did not significantly reduce the amount eaten from control treatments, but there were inconsistencies in the results of Basin, and further testing is needed. This research provides evidence that Bt products may be effective for BTM control, reducing the need for broad-spectrum biopesticides to manage BTM.

Introduction

The browntail moth [BTM, *Euproctis chrysorrhoea* L. (Lepidoptera: Erebidae)] is an invasive insect pest currently experiencing population outbreaks in the northeastern U.S. state of Maine. It was introduced from Europe, first detected in Massachusetts, U.S., in the late 1800s, and spread throughout New England. The BTM larvae is a polyphagous folivore, with a feeding preference on oak and *Rosaceae* species such as apples (Schaefer, 1974). Heavily infested areas can observe complete defoliation of these trees in the spring when post-diapause larvae emerge from their overwintering nests and bronzing, another form of defoliation, by the newly hatched pre-diapause larvae in the fall. Repeated years of damage is a concern to forest and orchard managers as tree health declines. The human health hazard caused by the urticating hairs of the larvae is also a concern to community members inside the range of BTM. The hairs contain a toxin that can cause severe dermatitis or respiratory distress to some humans and the toxin does not break down quickly, so it can remain viable in the environment for years (Blair, 1979). These hairs can be freely released by larvae through molting or when they feel threatened, releasing these hairs airborne, so direct contact is not necessary to experience the effects of exposure. Reducing BTM populations is the best strategy for reducing long-term exposure to toxic hairs of this pest.

Attempts to control BTM in the early 1900s used manual winter nest removal as the primary method. These nests are built communally by early instar larvae to protect them from the elements and predation over the winter months. In 1907, over 1.2 million winter nests were collected and destroyed by burning (Hitchings, 1908). This method continued to be recommended and was effective for small localized growing populations of BTM that emerged in Maine over the next 100 years, especially in coastal areas where environmental factors challenge and reduce overall tree height so nests can be reached. The challenges of BTM web removal became apparent on tall trees as many nests are anchored to the top outer branches. For example, some oak trees can reach up to 25 meters in height, making manual removal from the ground nearly impossible. During BTM population outbreaks, the associated labor costs to hire licensed and insured arborists with bucket trucks or climbing equipment necessary for the task and hazards involved with web removal make this method cost-ineffective. In such cases, insecticides may be needed for effective control (Schaefer, 1974). In both the native range of BTM in Europe and the invasive range in northeastern North America, insecticides have historically been used to control BTM. Historically insecticides used included lead arsenate, chlordane, and Dichlorodiphenyltrichloroethane (DDT), which have all been deregistered for use as insecticides in the U.S. due to environmental hazards such as, groundwater contamination, insufficient degradation or half-life, and mild to severe toxicity to numerous organisms, including mammals (Schaefer, 1974; Groden et al., 2022). Carbaryl, a carbamate, was used as an insecticide replacement for BTM after DDT was banned because it is not as persistent in the environment and detoxifies quickly in vertebrates (EPA, 2008). However, the United States of America Environmental Protection Agency (EPA) has restricted the use of Carbaryl because it has high mammalian toxicity and is likely a carcinogen to humans (EPA, 2008).

Integrated pest management (IPM) was formally recognized by the US National Academy of Science in 1969 and has been the predominant paradigm used in pest management systems in the United States since (Hendrichs et al., 2007). IPM was originally introduced to curb chemical pesticide use and reliance by including additional control strategies for pest management so that pesticides were an option, among others, instead of the only option for controlling pests. Chemical formulations of pesticides have been used to control insect pests for centuries. However, despite their short-term effectiveness at controlling these species, they can also cause a suite of concerning effects, including negative impacts on non-target organisms, environmental contamination through leaching, drift and run-off, residues in food and clean water, and bioaccumulation of chemicals in food chains. These effects have led modern day insect pest managers to seek alternative biorational or "reduced risk" insecticides. Biorationals are defined by Hara (2000) as natural or synthetic products that effectively control insect pests with low toxicity to non-target organisms and are non-degrading or polluting to the environment. Insect growth regulators (IGRs) and biopesticides are examples of biorationals.

Juvenile insects must undergo a molting process to shed their rigid exoskeleton every time they grow, and this process is initiated and managed by specific chemicals inside the insect. Many of these chemicals have been identified and replicated or imitated as a means to control insect pests by disrupting their molting process and preventing target species from maturing fully. The IGR product, Dimilin (diflubenzuron), is recommended for use in Europe and has shown effective control of BTM populations (Percival, 2016). In field applications conducted by Bradbury (1999) in coastal Maine, it was also considered a successful treatment for BTM. The Maine Board of Pesticide Control currently regulates the use of IGRs and limits their usage within 250 feet of water bodies where drift or runoff could increase potential contamination (Maine DACF BPC, 2022). Some coastal towns have banned the usage outright due to its potential threat to economically important commercial marine fishing species, specifically lobster (*Homarus gammarus* Dana; Decapoda: Nephropidae).

Biopesticides are pesticides derived from natural materials such as animals, plants, minerals, and microbes (EPA, 2022). Microbial insecticides such as spinosad and *Bacillus thuringiensis* (Bt) Berliner (Bacillale: Bacillaceae) have also been evaluated and applied for BTM control. Entrust SC (spinosad) is an Organic Materials Review Institute (OMRI) certified product that is available for organic uses. Many of the host species BTM prefers are orchard trees, and the areas of Maine currently experiencing BTM outbreaks correspond with large numbers of organic farmers (Groden et al., 2022). Spinosad is currently used widely in Maine by commercial pesticide applicators for BTM control. The Maine Board of Pesticide Control only limits it to foliar applications within 50 feet of water bodies. However, spinosad is a broad-spectrum contact-kill insecticide that can be lethal to beneficial pollinators and natural enemies that utilize the areas treated. Spinosad is an effective treatment option against BTM however, its broad-spectrum activity and non-target effects warrant a need for a Lepidopteran-specific option.

Bt has a more targeted specificity against some insect orders. Variants kurstaki (Btk) and aizawai (Bta) are used to control Lepidoptera pest species and can be effective at large populations for similar forest pest species such as spongy moth (formerly known as gypsy moth; *Lymantria dispar dispar* L.; Lepidoptera: Erebidae) and Douglas-fir tussock moth (*Orgyia pseudotsugata* McDunnough; Lepidoptera: Erebidae) (Plata-Rueda et al., 2020). The different strains of Bt contain crystal proteins (Cry) that have insecticidal properties and toxicity (Bravo et al., 1998). These Cry proteins are activated when the Bt is ingested and the midgut is damaged. Cry proteins bind to receptors in the midgut of insect larvae after ingestion, which form holes in the gut that eventually result in larval death (Dubois et al., 2001). Bt must be ingested by the insect, making it even more specific than contact insecticides, however the initial ingestion may not be enough. A feeding cycle has been observed where larvae feed, gut damage causes cessation of feeding, feeding continues with gut recovery, and a lethal dose of Bt is then consumed, killing the larvae (van Frankenhuyzen and Nystrom, 1987; Groden et al., 2022).

Modern agriculture heavily relies on the use of Bt insecticides for crop pest control, and resistance has been observed for some insect species, resulting in a need for the development of new biopesticides (Gould, 1998; Sparks et al., 2021). Many spider venoms have high levels of disulfide insecticidal peptides, which can interact with a vast range of ion channels and receptors in the insect nervous system. Peptides are being produced as insecticides due to their broad pest species specificity, low toxicity to non-target species, cheap and ease of formulation, and persist in the environment long enough to be effective but not long enough for resistance development to be a threat (Windley et al., 2012). Spiders inject venom directly into the body of their prey, so initially, the lack of oral or topical activity made peptides less suitable for insecticide development. However, it has since been proven that spider venom peptides are orally active (King, 2019). Research and utilization of spider venom peptides have shown rapid and debilitating effects on insect nervous systems as stand-alone biopesticides or can enhance the efficacy of treatments when included as a synergist (King & Hardy, 2013; Sun et al., 2016; Fanning et al., 2018).

Btk has been explored for BTM control with mixed results (USDA-APHIS, 1985; Bradbury, 1995; Bradbury, 1999; Dubois et al., 2001, Groden et al., 2022). Field trials using aerial applications of Btk conducted by Bradbury (1995) in coastal Maine during the spring of 1992 and 1993 had little success due to cool and wet weather conditions and equipment limitations. Additional laboratory work found that better control of BTM could be achieved with more targeted applications of the product. Laboratory trials using oak saplings sprayed with Btk found limited improvements in defoliation and mortality once a particular dosage threshold was used (USDA-APHIS, 1985). In other words, a higher dosage of the product did not improve the results beyond a certain dosage amount. More recently, Groden et al. (2022) conducted laboratory bioassays with post-diapause or "overwintered" larvae and found that larvae fed Btk (Foray48B, Valent BioSciences, Libertyville, IN) treated leaves for 24 hours had a median time to death that was half of that observed for the controls. After seven days, 12% of the larvae fed Btk remained alive compared to controls which had 91% of larvae survive. In field trials using foliar applications, they also found that Btk caused a reduction in feeding and host defoliation but recommended that two applications may be needed for adequate mortality. There are many Btk products available besides Foray 48b, so without a full comparison, there may be other Btk products more effective at controlling BTM larvae. Groden et al. (2022) also tested exclusively post-diapause larvae so it is unknown how this would compare if applied to pre-diapause BTM larvae. In addition, the inclusion of peptides in tandem with these treatments could potentially increase their overall efficacy.

This study tests the effectiveness of eight commercially available Bt biopesticide products on early instar (pre-diapause) BTM larvae with and without spider venom peptides. It is hypothesized that Bt products, when used with peptides, would increase overall mortality and decrease the total amount and rate of consumption than the products alone. Results from this study will help determine the effectiveness of Bt biopesticides on BTM larvae and indicate the best products through a series of comparative trials. The most effective products will be recommended for future research and current control efforts by commercial applicators. Results will ultimately be used to make recommendations for the most effective Bt insecticide bioassay treatments and determine whether the use of peptides increases the product's overall efficacy. The results can also be used to determine if Bt is effective on early instar larvae and if that treatment window should be considered for future BTM control research.

Methods

Bioassay Protocol

Bioassays were designed to observe larval behavior with minimal disturbance while maintaining safety from toxic hairs. A single bioassay consisted of a clear 946 mL Uline® deli cup and lid, with a Royal Imports® three inch water pick inserted down through the bottom of the deli cup using a soldering tool so that the water pick sat firmly in the bottom of the cup without room for small larvae to escape. Water picks were filled with tap water, and lids were pressed down firmly to the bottom of the cup so that larvae could not crawl under the water pick cap for risk of drowning. In addition, dental wicking was placed in the bottom of each cup to absorb any additional moisture that could be a source of control mortality or impact their behavior. Cups were kept upright for the duration of the trial.

Before treating, leaves were chosen with minimal blemishes so as not to be confused with feeding. Leaves were trimmed so that at least one inch of stem remained and any additional leaves or fruit were removed. Remaining branches were placed in a five-gallon bucket of water and kept at 40°F if replacement leaves were needed. Leaves were randomly assigned to a treatment and then scanned using an Epson® scanner to later determine the surface area of leaves. Due to some leaves overlapping during the scanning process, images were printed, trimmed, and weighed on a digital scale. The mass was converted to surface area using the masses associated with paper of known surface areas. Squares of 1, 3, 5, and 10 cm^2 were cut and weighed to determine the average mass per cm^2 conversion formula.

All Bt trials used a total of 200 mL of mixed product that was made one hour prior to application at a rate of 15 gal/acre at the highest recommended mix rate on each product label (Table 1). The application rate was selected to represent that of a ground application for ornamental trees closely. Application of treatments were made using 8 oz plastic hand spray bottles with two sprays on each side of the leaf cluster. Leaves were air dried and then placed inside the waterpik of correspondingly labeled bioassay cups. Individual larvae and/or winter nests were painted directly onto the leaves or placed inside the cups and then capped. Bioassays were kept at room temperature (approximately 20°C) for the duration of the experiment. If bioassays observed > 90% defoliation, new leaves were scanned, treated, dried and introduced into the cup. The total mass consumed included the sum of the original leaf plus all the added replacement leaves.

Bioassays used newly formed BTM overwintering nests collected in mid-August. The larvae from the winter nests used in the bioassays varied in development and were a mixture of 1st-2nd instars. The overwintering larvae live in galleries inside the nests except for when they leave to feed or continue with construction, making the total amount of BTM larvae in each winter nest undetermined. Whole nests were used to reduce potential changes in behavior that occur when larvae are separated from each other. Changes in behavior including reduced feeding have been observed historically when larvae are disturbed and separated (Lau, 1996). Excess plant materials were removed from around the nest to ensure that the only available food source was the treated leaves. Nests were stored at room temperature in deli cups overnight until used the following morning in bioassays.

To determine the number of larvae per nest, the contents were soaked in a 2.5-gallon caustic solution of 1% NaOH for 3 hrs at a temperature of 155°F. The solution dissolved the nest silk so that they could be more easily counted than by dissecting the nests by hand. Differences in exoskeletons and larvae were more apparent as well. Larvae were tallied per nest and used to calculate the per larva variable(s).

Behavioral Assay

Fifty BTM overwintering nests were collected from four cherry trees on August 16th, 2022, on the University of Maine campus in Orono, Maine. There were four behavioral treatments; a whole overwintering nest, 50, 25, and 10 larvae, observed with five replicates for a total of 20 bioassays. Bioassay treatments with different numbers of larvae were separated from the same original overwintering nest to reduce the confounding effects of different overwintering nests and introduced to the bioassay cups. Observations were made by two researchers every 24 hrs who determined: active feeding status (yes or no), a qualitative assessment of the number of dead larvae (none, some, or all), and percent of total leaf area defoliated. A total of 7 assessments were made after which bioassays were frozen. Wet weights were taken from 8-10 larvae in each bioassay using a microbalance (Sartorius, MCA3.6P-2S00-M).

Treatment Trial 1

BTM overwintering nests were collected from a single crabapple tree on August 16th, 2021, from Mountainside Cemetery in Bangor, Maine. Cherry tree branches were collected from Old Town, Maine, to use as food for the larvae on the same day. Four Bt products (each individual, with Spear-Lep, and with Basin), Entrust, Spear-Lep, Basin, and a control sprayed with tap water were used in this experiment (Table 3). Entrust (spinosad) was used as a comparable reference to a commonly used treatment for BTM currently by commercial applicators. The average volume applied was 2.84 mL (± 0.25 mL). A total of 16 treatments with six replicates were used for a total of 96 bioassays. Winter nests were placed in bioassays and observed in the same order. Dental wicking was not used in the bioassays in this trial because they were unavailable during the setup.

Observations were made by 1-2 researchers every 24 hrs who determined: active feeding status (yes or no), a qualitative assessment of the number of dead larvae (none, some, or all), percent of total leaf area defoliated, and presence of exoskeletons (an indicator of growth and molting). A total of nine assessments were made after which bioassay cups were frozen until the number of larvae per nest could be counted. Larval counts in this trial were done manually by two researchers. The larval totals per nest were used to standardize the amount of foliage eaten by calculating a per larva consumption amount per bioassay.

Treatment Trial 2

BTM overwintering nests were collected on August 16th, 2022, from the University of Maine, Orono campus in Orono, Maine. A total of 100 nests were collected; fifty from five crabapple trees and 50 from four cherry trees. Apple tree branches were collected from a private residence in Orono, Maine, to use as food for the larvae on the same day. To ensure there wasn't bias from host trees, hosts were split so replicates 1-3 received an overwintering nest from a crabapple host and replicates 4-6 received an overwintering nest from a cherry host. Leaf clusters containing 4-5 leaves attached to a single stem were clipped for each bioassay cup. Four Bt products (each individual and with Basin), Basin, and a control sprayed with tap water were used in this experiment (Table 3). A total of ten treatments with six replicates were used for a total of 60 bioassays. The average volume applied was 2.86 mL (± 0.39 mL). Observations were made by two researchers every 24 hrs who determined: active feeding status (yes or no), a qualitative assessment of the number of dead larvae (none, some, or all), and percent of total leaf area defoliated. A total of seven assessments were made after which bioassay cups were frozen until the number of larvae per nest could be counted.

Table 3. Information for products used in 2021 and 2022 browntail moth laboratory bioassays. Trial 1 refers to treatments on early instar larvae in 2021, and Trial 2 on early instar larvae in 2022. Application rates for both trials used 15 gal/acre. Btk = *bacillus thuringiensis kurstaki*; Bta = *bacillus thuringiensis aizawai*.

Trade name (abbreviated)	Active ingredient (strain)	Concentration of active ingredient (%)	Company	Maximum field rate (oz/acre)	Mix rate (ml/g per 100ml $H_2 0$)	Trial #
Control	-				1.000	1 & 2
Basin®	U1-AGTX-Ta1b-QA	8.5	Vestaron	7.97	0.398	1 & 2
Spear-Lep®†	GS-omega/kappa-Hxtx-Hv1a	2	Vestaron	32	1.667	1
Deliver®	Btk (SA-12)	85	Certis	24	1.199	1 & 2
Foray®†	Btk (ABTS-351)	12.65	Valent	30.4	4.311	1 & 2
Javelin®	Btk (SA-11)	7.5	Certis	20	0.999	1 & 2
Entrust SC®†	Spinosad	80	Corteva Agriscience	12	0.625	1
Leprotec®†	Btk (EVB-113-19)	14.49	Vestaron	32	1.667	2
Agree®	Bta (GC-91)	50	Certis	32	1.598	1
Xentari®	Bta (ABTS-1857)	54	Valent	2.4	0.120	1

†Liquid formulation used

Data Analysis

All relationships between treatments and the amount of leaf consumed or larval mass per BTM larva in bioassays were explored using linear regression models. Data were log-transformed (x +1) to meet assumptions of normality and homoscedasticity, and then models evaluated whether the response variables were a function of the treatments applied. Post hoc pairwise comparisons of treatments were used to check for significant differences in means between the different products (emmeans package; Lenth, 2022). All analyses were run using RStudio statistical software (v4.1.2; R Core Team, 2021).

Results

Behavioral Assay

Overall, BTM larval behavior appears to be influenced by the density of larvae in the shared bioassay cup. The average wet mass per larva was found to have a significant quadratic relationship with the behavioral assay treatment (10, 25, or 50 larvae or whole nest) [$F_{(2,17)} = 9.01$; $p = 0.002$; Fig. 7a]. The highest average mass per larva was found in the cups that contained 50 BTM larvae, with larvae weighing less if they were in cups with fewer or more than this number. The 50 larvae/cup treatment was also found to have the least variability in average larval masses between replicates (Fig. 7a). When only 10 larvae were placed in a cup, their mass was, on average, 45% smaller than when 50 larvae were placed in a cup, indicating a potential negative behavioral change when just a few larvae are separated from the rest of the communal group.

Results also showed an effect of larval density per cup on the amount eaten per larva. There was a significant increase in the amount eaten per larva as the number of larvae per cup increased [$F_{(1,18)} = 5.30$; $p = 0.03$; Fig. 8]. BTM larvae in cups with more than 140 fellow larvae (i.e., whole nests) were found to have eaten 25% more than those in cups with only 10 larvae. Again, the 50 larvae/cup treatment was found to have the least variability in the average amount eaten between replicates (Fig. 8). The was a significant positive linear relationship between mass of larvae and the amount eaten by larvae [$F_{(1,18)} = 10.91$; $p = 0.004$; Fig. 7b], indicating that the number of larvae per cup is most likely indirectly affecting larval mass by influencing the amount eaten.

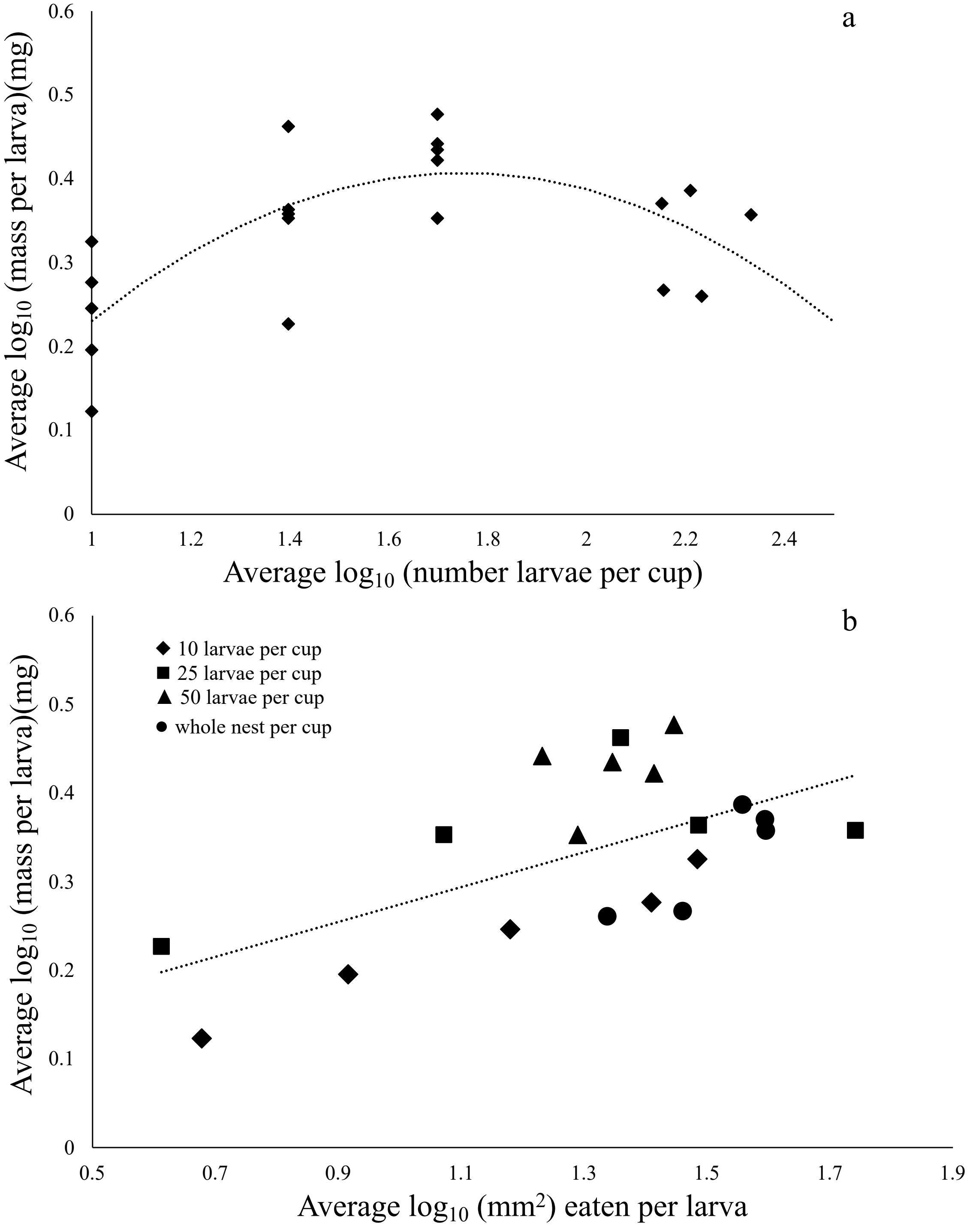

Figure 7. Relationship between the average $\log_{10}$ browntail moth wet mass (mg) per larva per cup and (a) the average $\log_{10}$ number of total larvae per cup, and (b) the average $\log_{10}$ amount of foliage eaten (mm^2) in each bioassay cup (n = 5 replicates) following seven days of feeding. Points represent (a) observed average mass per larva per cup and (b) the average $\log_{10}$ amount of foliage eaten (mm^2) per larva per cup. The dashed lines represent the fitted quadratic (a) and linear (b) models.

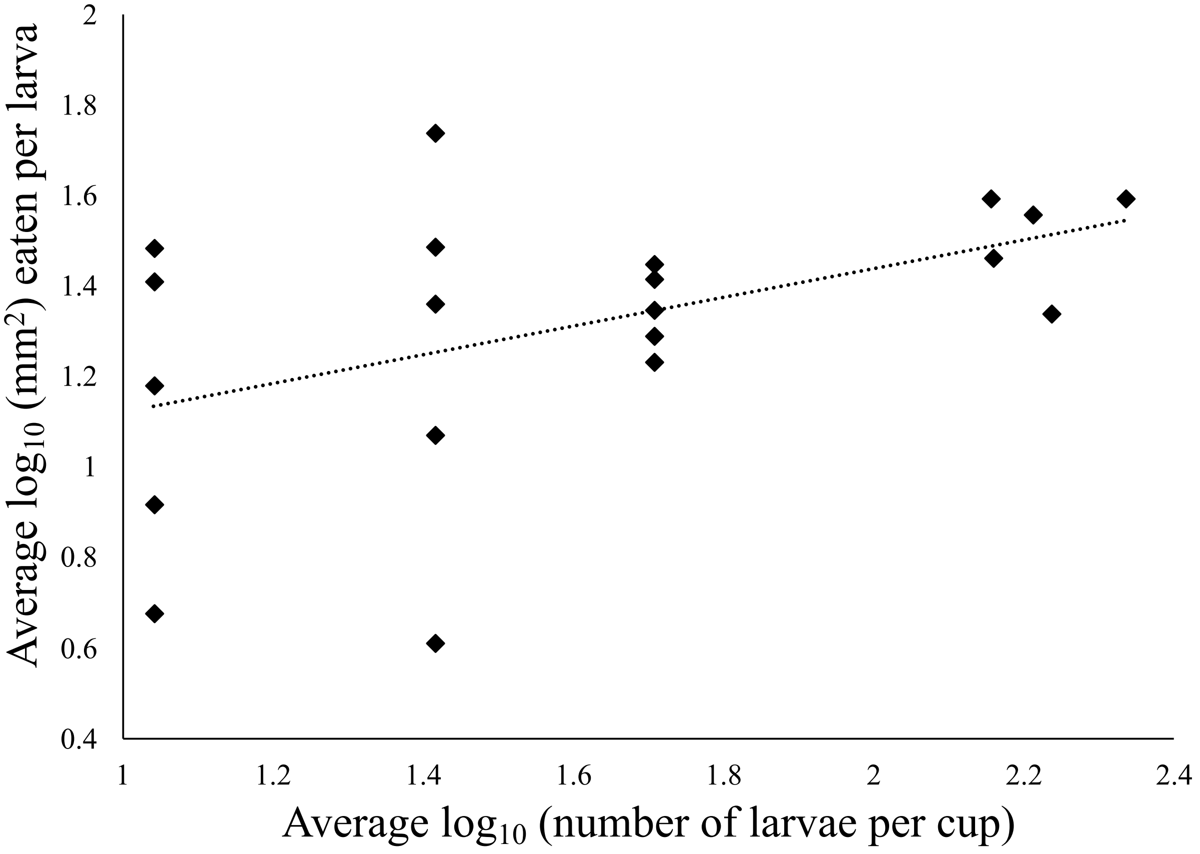

Figure 8. Relationship between the average $\log_{10}$ amount of foliage eaten (mm^2) per larva per cup and the average $\log_{10}$ number of total larvae in each bioassay cup (n = 5 replicates) following seven days of feeding. Points represent the observed average amount of foliage eaten per larva per cup and the dashed line represents the fitted linear model.

Treatment Trial 1

Bt treatments were found to significantly reduce the amount of plant material eaten per BTM larva [$F_{(15,80)} = 22.28$, $p < 0.001$; Fig. 9]. The average eaten per larva was not significantly different between the control (water-treated) and the Spear-Lep peptide product ($p = 0.29$) (Fig. 9). The peptide product, Basin, significantly reduced the amount eaten from the control ($p = 0.03$) and was not significantly different from the Bt treatment, Xentari ($p = 0.06$). All biopesticide products, including Bt and spinosad (Entrust), were found to have reduced the average amount eaten by at least 31% (Xentari) compared to water treated leaves (Fig. 9). Entrust reduced the amount eaten per larvae by 89% compared to the control, and was not found to be significantly different from Btk products Deliver with Basin (*p* = 0.22) and Deliver with Spear-Lep (*p* = 0.10) (Fig. 9).

When evaluating active feeding within the treatment cups, all cups (*n* = 6; 100%) within the control treatment maintained active feeding every day of the trial. Peptide alone-treated cups all had active feeding until day seven (Basin) or eight (Spear-Lep), whereas 100% of the Bt product cups experienced feeding cessation between 6-9 days. Entrust eliminated all feeding within the first 24 hours (Fig. 10). The time it took for half of the Bt treated cups to no longer experience feeding was between 4-7 days, compared to the controls which took nine days (Basin) or was not reached by the end of the experiment (water and Spear-Lep). When assessing larval mortality, all Entrust treatment bioassay cups observed no larval movement by day one (i.e., moribund) (Fig. 11). All six Javelin with Spear-Lep cups (100%) had moribund larvae on day two with other Bt product cups reaching 100% between three and six (Xentari with Spear-Lep) days after treatment application (Fig. 11).

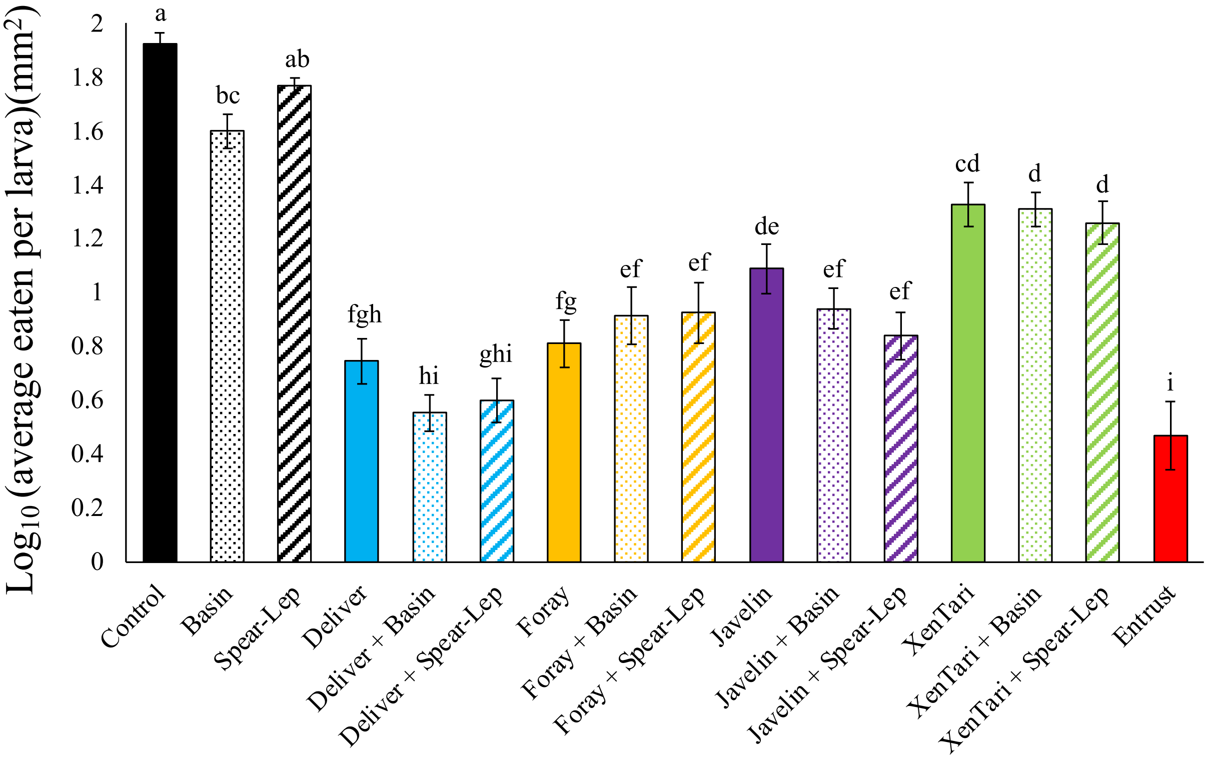

Figure 9. Average (± SE) amount of foliage eaten (Log_{10} mm²) by browntail moth larvae with different treatments in bioassay experiments ($n = 6$ replicates). Different letters indicate significant differences at the $\alpha = 0.05$ level.

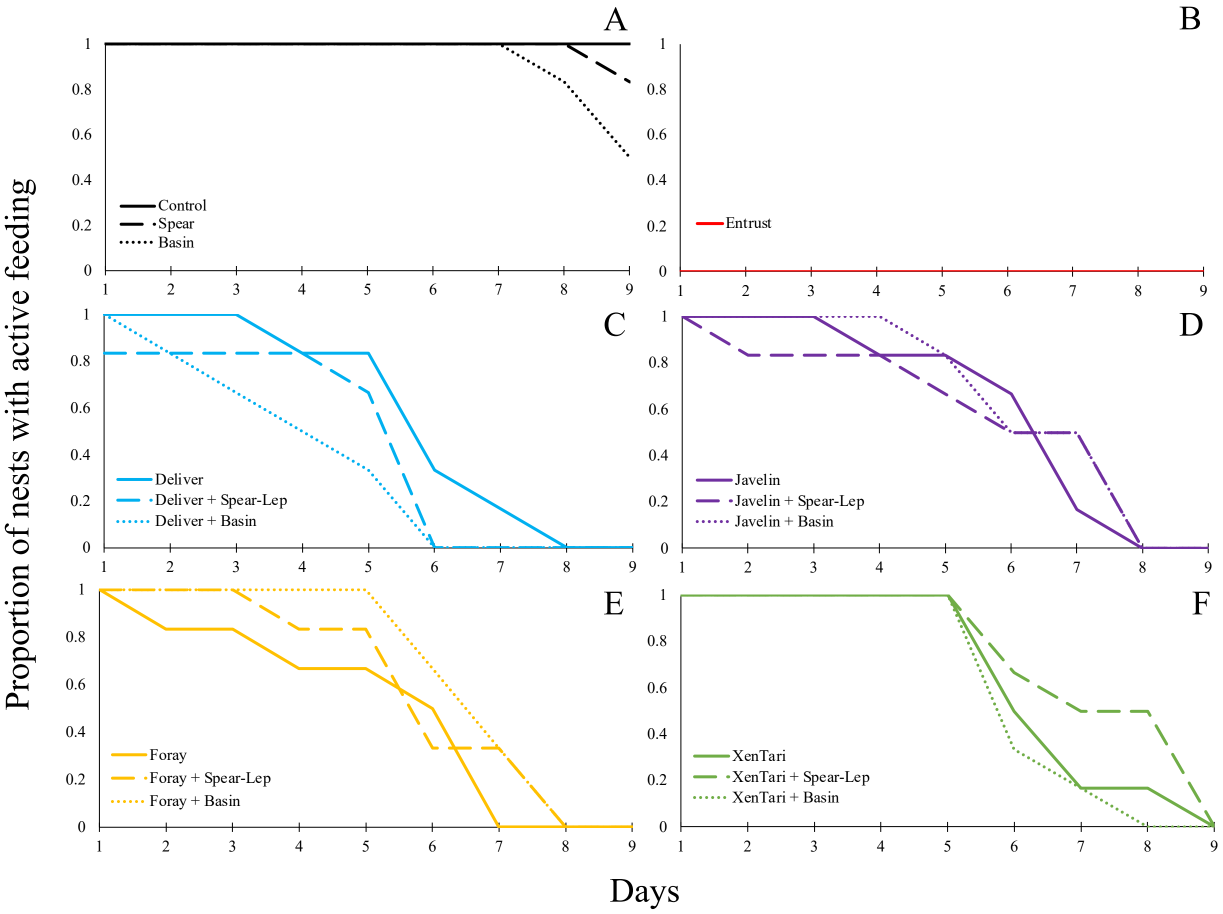

Figure 10. Proportion of browntail moth nest bioassay cups per treatment (n = 6 replicates) with actively feeding larvae over time.

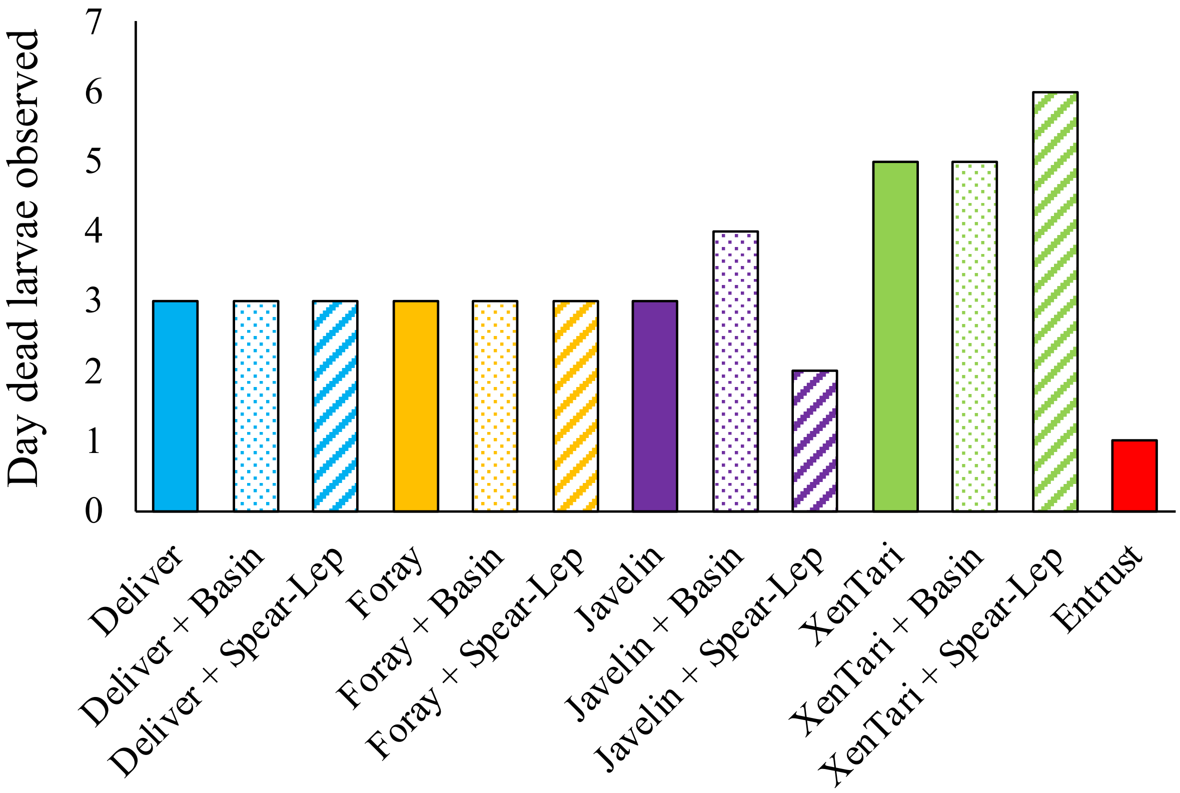

Figure 11. The first day after the treatment was applied that some browntail moth larval mortality was observed in all six of the bioassay cups per treatment.

Treatment Trial 2

Bt treatments were found to significantly reduce the amount of plant material eaten per BTM larva [$F_{(9,50)} = 8.25$, $p < 0.001$; Fig. 12]. The average eaten per larva was not significantly different between the control (water-treated) and Basin alone ($p = 0.38$) (Fig. 12). All Bt products were found to have reduced the average amount eaten by at least 58% (Leprotec with Basin) compared to water treated leaves. Bt products did not have significant differences in the amount eaten per larva (all p-values > 0.05).

When evaluating active feeding within the treatment cups, all cups (n = 6; 100%) within the control (water) treatment maintained active feeding until day three and Basin alone treated cups all had active feeding until day two (Fig. 14). Javelin experienced feeding cessation on day six and Agree alone, Agree with Basin, and Deliver with Basin ceased feeding on day seven. On day seven, 67% of cups with control treatments maintained active feeding and Basin maintained feeding in 20% of cups. Remaining Bt treatments maintained active feeding in 20-50% (Foray) of bioassay cups (Fig. 14). The time it took for half of the Bt treated cups to no longer experience feeding was between 1-5 days, compared to controls which took six days (Basin) or did not reach feeding cessation by the end of the experiment (water). When assessing larval mortality, 100% of Bt treated bioassay cups observed moribund larvae by day 3, whereas 100% of Basin observed moribundity by day 6.

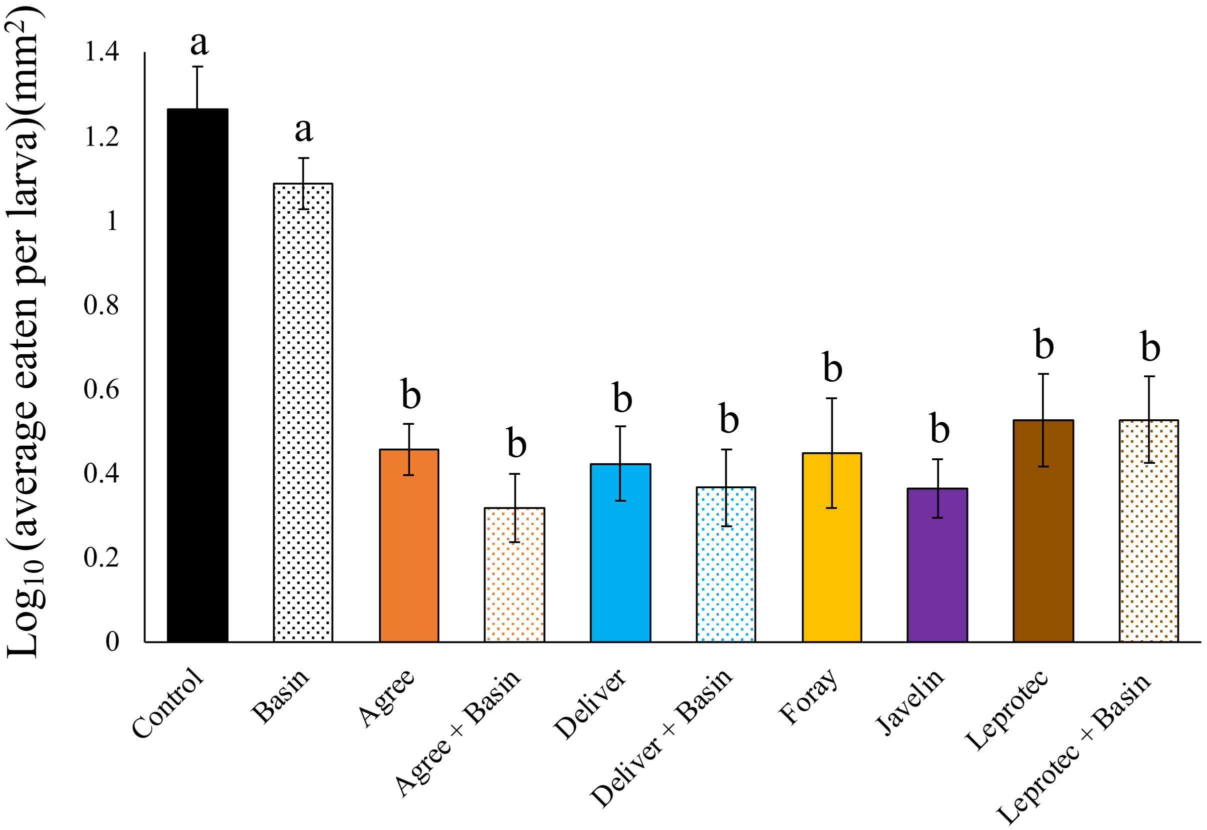

Figure 12. Average (± SE) amount of foliage eaten (Log_{10} mm^2) by browntail moth larvae per treatment in seven-day bioassay experiments (n = 6 replicates). Different letters indicate significant differences at the α = 0.05 level.

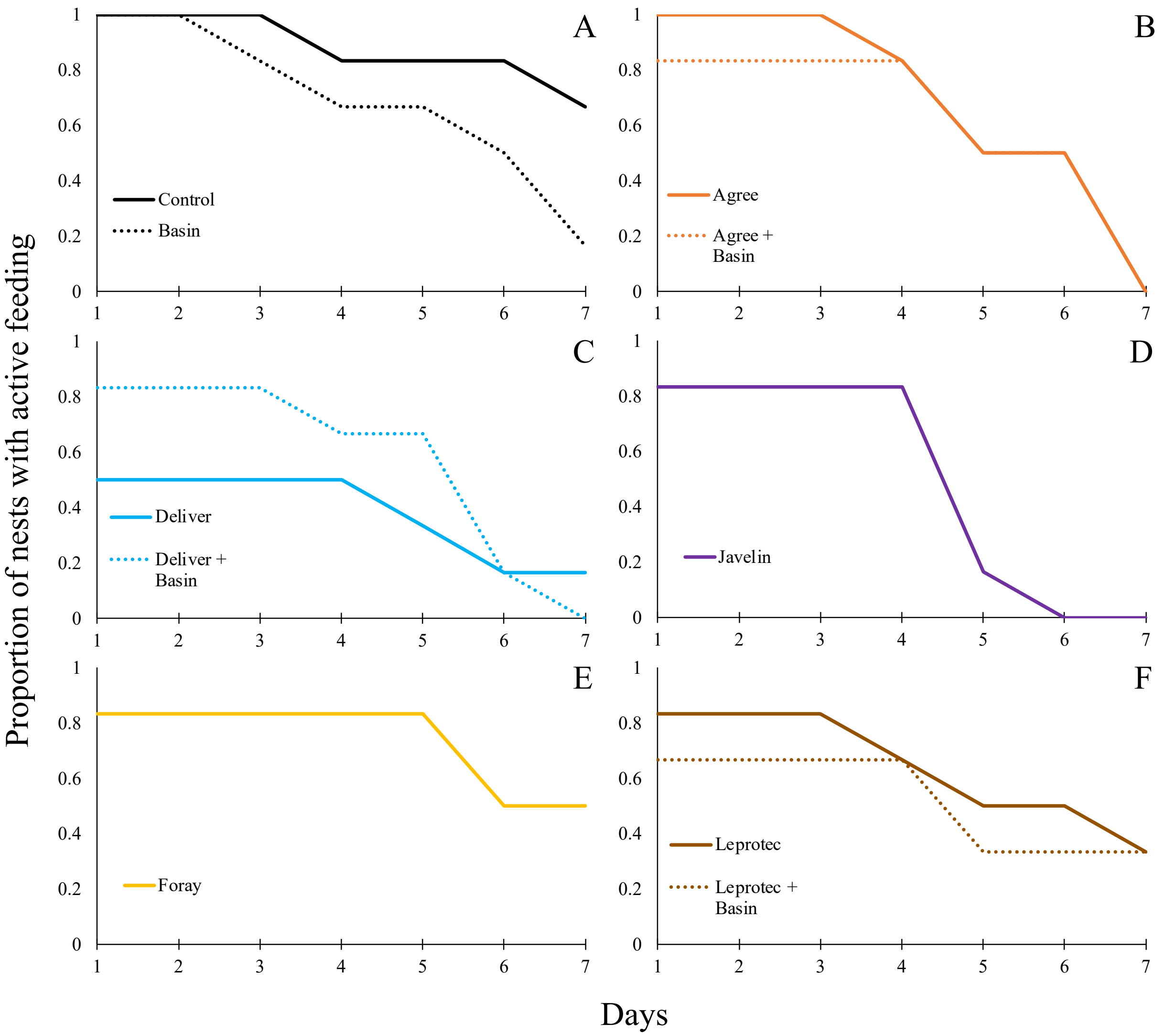

Figure 13. Proportion of browntail moth nest bioassay cups per treatment (n = 6 replicates) with actively feeding larvae over time.

Amounts eaten per larva for both trials in 2021 and 2022 were compared and were significantly higher in 2021 than 2022 for the control [$F_{(11,60)} = 37.17$; $p = <0.001$] and Basin ($p = 0.003$) treatments (Fig. 14). This increasing trend continued with all the Btk treatments used in both years, however, Javelin was the only treatment that was significantly greater in 2021 than 2022 ($p = <0.001$; Fig. 14). Deliver, Deliver with Basin, and Foray were not significantly different between 2021 and 2022.

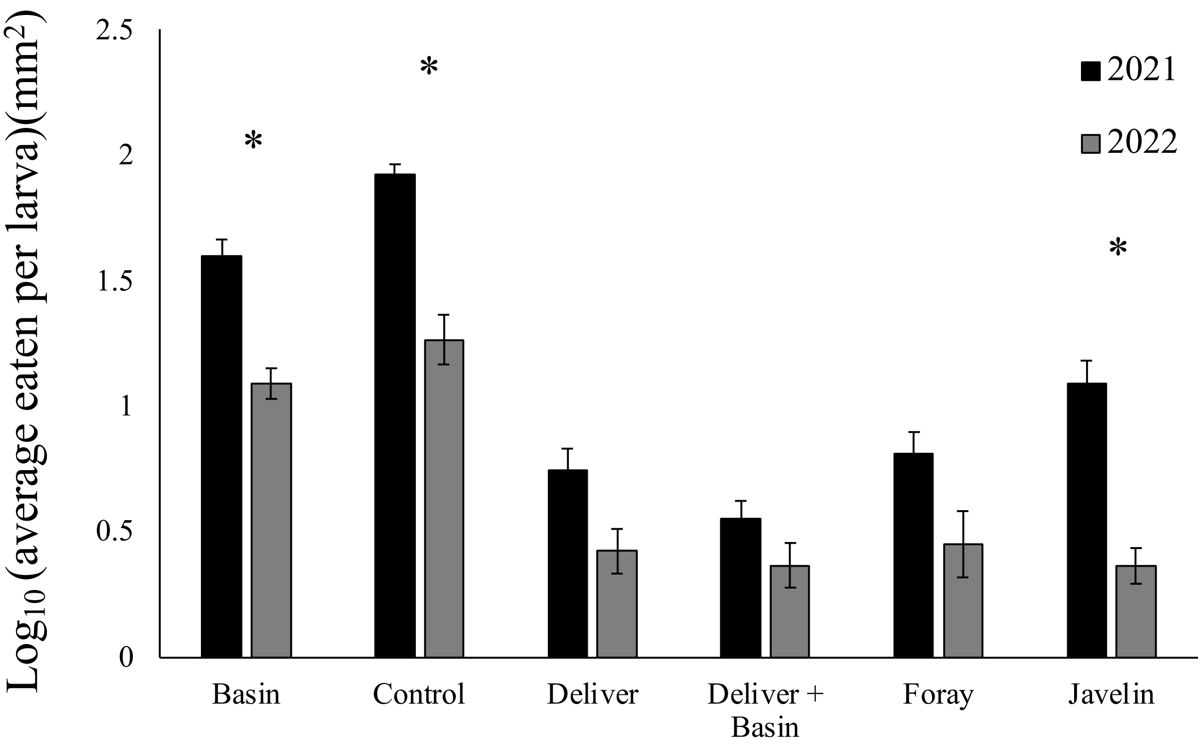

Figure 14. Average (± SE) amount of foliage eaten (Log_{10} mm^2) by browntail moth larvae per treatment in 2021 and 2022 after seven-day bioassay experiments (n = 6 replicates). Asterisk symbols indicate significant differences between years at the α = 0.05 level.

Discussion

Biopesticides can be an essential component of IPM programs for invasive insect species. When Lepidopteran larvae ingest Bt, feeding cessation is a precursor to mortality (van Frankenhuyzen & Nystrom, 1987). This work demonstrates that several commercially available Bt products can significantly reduce the amount of feeding by pre-diapause BTM larvae in lab bioassays. Bioassays are a key first step in management programs so that the effects of treatments on a species in a controlled laboratory setting can be observed. Unfortunately, there is no standard number of larvae or replicates to be used for comparing treatments in bioassays with BTM, with researchers of BTM, and closely related spongy moth, having used anywhere from 1-

100 larvae in bioassay experiments (Ruiu et al., 2012; Pavlushin et al., 2019; Groden et al., 2022).

It is essential for larvae to feed on treated leaves to observe how ingesting the different Bt products affects healthy larvae. Therefore, determining the optimal number of larvae in an experimental unit to ensure abundant feeding is necessary for experimental design. Limited feeding could cause misleading results by not representing field feeding habits. This research found that the number of larvae in a bioassay cup influenced both the amount of leaf material eaten and the mass per larva (Figs. 7, 8), indicating that results may vary based on the number of larvae chosen to include in trials. In addition, precision between replicates within a treatment was different based on the number of larvae per cup, with 50 larvae per cup having the highest precision (i.e., lowest variability) for both variables.

A positive linear relationship was found between the number of larvae per cup and the amount eaten per larva (Fig. 8). When the whole nest was included in the cup, each larva was found to have eaten more than when 50 or fewer larvae were in the cup. This effect could be evidence of a competition-driven behavioral effect; as competition for resources increases, the increased feeding response is initiated. Stressful interactions from crowding have been found to push larvae to consume more food and grow larger. In these cases, crowding was beneficial because it encouraged group cooperation of feeding facilitation and collective degradation of the leaf material (Fitzgerald & Costa, 1999; Rosa et al., 2017). Eventually, the number of larvae in the cup could have reached a point where the increased feeding response was limited by the amount of food available, but leaves were replenished in this study when they were severely defoliated, so BTM larvae were never limited by lack of food.

Although the peak mass per larva occurred when 50 larvae were in the cup, resulting in a quadratic rather than an expected linear relationship (Fig. 7a), there was a significant correlation between the amount eaten and mass per larva (Fig. 7b). This indicates that the number of larvae per cup may be indirectly affecting the mass per larva by influencing the amount eaten per larva. If the number of larvae per cup directly affects larval mass, regardless of the amount eaten, it may be due to the larva processing food inefficiently due to stress or increased effort demands, which would transform this food into energy instead of mass (Blanckenhorn, 1999; Rosa & Saastamoinen, 2017). Perhaps larvae with lower mass are expending more energy on behaviors such as locating food or activities that may be influenced by stressors such as separating them from their communal groups. Regardless, this research shows that there is a density-dependent behavioral effect caused by the number of early instar BTM larvae used in experiments.

BTM from the same egg mass feed together gregariously once they hatch until they enter diapause and for several weeks after they emerge from winter nests in the spring. As they mature, they disperse and feed independently until they reach the prepupal stage (Schaefer, 1974). Like many gregarious species, they can use pheromone trails and other chemical signals to help mediate group foraging and staying aggregated (Fitzgerald, 1976; Costa & Pierce, 1997; Costa et al., 2004). There are several potential benefits to gregarious feeding for larvae, such as thermoregulation which helps keep a constant and favorable temperature in the group, aiding digestion and improving growth rates, and reducing water loss in larvae (Allen, 2010; Pimentel et al., 2012). Aggregation of Lepidoptera larvae with urticating hairs such as BTM can also be a strategy for anti-predator defense that is most effective when there is a large group. Early instar larvae are particularly vulnerable to parasitism and predation, so even modest increases in mass or growth can reduce overall development time and reduce exposure to threats (Fiorentino et al., 2014). Separating gregarious larvae into smaller groups can reduce growth rates due to the loss of this behavior's benefits (Allen, 2010; Fiorentino et al., 2014). Previous research has shown that BTM larvae can be exceptionally sensitive to having their densities manually reduced to extremely low amounts. It was noted that BTM larvae were so stubborn when densities were manually separated from winter nests that they would often stop feeding and starve to death (Lau, 1996). The reduced feeding observed in the experimental treatments with fewer larvae in cups is not expected to translate to field trials where the larvae would not be manually separated and would be able to behave gregariously.

If the positive trend between the number of BTM larvae and the amount of leaf material eaten per larva observed in the lab bioassay translates to field populations, it could indicate a positive feedback loop. The presence of a high density of larvae could lead to increased feeding, which could contribute to larger and healthier larvae. Healthy larvae can reach maturity faster, evade predation and have greater chances of mating, with higher fecundity potential in females (Hainsworth et al., 1991; Barragan-Fonseca et al., 2018). This can ultimately lead to more larvae per nest, which contributes to a larger density of larvae feeding, etc.

It is recommended that lab bioassays using BTM should use either 50 larvae (for better precision) or whole nests (for better field representation) as their experimental unit. Unfortunately, using more larvae in bioassays increases the workload for researchers during initial larval collections and manual separation during bioassay setup. Working with whole nests (i.e., a high number of larvae) can also pose a high risk of discomfort to researchers due to the higher number of urticating hairs. Future research should compare treatment bioassay results, such as percent mortality, to determine whether there is a significant difference in results between using 50 larvae or entire nests. Ultimately, whole nests were selected for biopesticide trials in this study to ensure no confounding behavioral effects.

Previous research has found that BTM larvae are susceptible to Bt treatments (Dubois et al., 2001; Groden et al., 2022), which these results also support. In addition, peptide products have been shown to be effective on Lepidoptera species however, these products appear less effective on BTM. The peptide product, Spear-Lep, alone was not found to have significantly reduced the amount eaten per larva compared to water-sprayed leaves, and the peptide product, Basin, was inconclusive as it significantly reduced the amount consumed in trial 1 but not in trial 2 (Fig. 9, 12). Within product comparisons also found no difference if the peptides were added. The rate at which larvae ceased feeding was less consistent, with the inclusion of peptides improving some products (Agree and Leprotec) however, it made other products slower at reducing feeding (Foray). Field trials have had positive results in some studies (Fanning et al., 2018), indicating that future research using peptides in field studies may find different results than lab bioassays.

Entrust (spinosad) is currently a common biopesticide choice for effective BTM control in Maine. Results from this study found that Entrust was not significantly better than some Bt products, which is better from an ecological standpoint because products containing spinosad pose a threat to non-target insects, including beneficial pollinators and parasitoids (Biondi et al., 2012). The specificity of some Bt products to Lepidoptera larvae adds a layer of protection to beneficial insects that a broad-spectrum contact kill product cannot. Biological control agents and parasitoids of BTM have been identified and are important to consider in controlling this pest (Burgess & Crossman, 1929; Schaefer, 1974; Elkinton et al., 2006; Frago et al., 2012; Boyd, 2020). Although parasitoids are not regulating BTM populations since outbreaks continue as new populations establish, and older populations persist, they can offer some control in localized areas with low BTM densities (Elkinton et al., 2006; Boyd, 2020). The use of spinosad and other broad-spectrum insecticides threatens these natural enemies and the control they provide on natural BTM populations.

From an economic standpoint, Bt can be more cost effective than an Entrust treatment - a single application of Entrust is more than 5x more expensive than a single application of Deliver, one of the Bt products that were not statistically different (ARBICO organics, 2023). Field application studies of foliar treatments are limited for BTM. There must be an adequate amount of foliage surface area for the product to stick to and be applied prior to mass defoliation by herbivores, creating a limited treatment window. Groden et al. (2022) did not find adequate control in field trials with a single application of Btk (Foray 48B, Valent BioSciences, Libertyville, IL) due to the slow mortality rate following exposure to one round of treatment. They recommended that two applications be used for control, especially if the risk of exposure to the urticating hairs is the primary concern. A direct comparison of two treatments to a single treatment is more challenging to determine for field applications. The limited treatment window for BTM makes it difficult to provide two consecutive treatments within the recommended follow-up treatment window of 5-10 days, especially when hiring a licensed pesticide applicator where a second property visit will be necessary (Maine BPC, 2022).

Products used in this trial were selected based on their availability for immediate commercial use by applicators. The amount and types of Cry proteins used in the formulation of these Bt products was not a variable that we addressed. We observed that Deliver (Btk) alone and with peptides (Basin and Spear-Lep) was one of the products in our first trial that the reduction in the amount eaten by larvae was not significantly different from spinosad (Entrust).

Timeline comparisons of the proportion of nests with active feeding in both trials show a comparatively low rate of active feeding with Deliver, indicating quick cessation of feeding by larvae. Deliver had the highest percentage of active Bt strains (85%; Table 3) of all our tested products. Foray (Btk) alone was also not significantly different however, it contains a much lower amount of active ingredient (12.65%). On the other end of the spectrum, the product with the lowest amount of Cry proteins was Javelin (7.5%). It moderately reduced the amount of feeding in our treatment trials, however, it performed poorly in slowing active larval feeding. This suggests that products containing elevated amounts of Cry proteins may be the most effective for BTM control. Other studies have suggested that the specific Cry protein toxins can be more effective for BTM specifically, such as CryA1C (Dubois et al., 2001).

Products in this research were derived from two subspecies of Bt. Kustaki (Btk) was the primary subspecies, however, two products containing Cry toxins from subspecies Aizawa (Bta) were also studied. Bta products (Agree & Xentari) were less effective at reducing the amount eaten by larvae. Notably, Xentari had the second highest amount of Cry proteins (54%) yet maintained active feeding in all bioassay cups for five days (Fig. 10). This suggests that the subspecies of Bt interacting with the associated Cry toxins may play a larger role in their effectiveness on BTM larvae and should be taken in consideration with treatment decisions.

BTM larvae are polyphagous folivores of many deciduous trees and shrubs, especially those in the species *Rosaceae* (Schaefer, 1974). Early instar larvae that had recently formed winter nests in the fall were used in these trials, however, it is important to consider the feeding season that occurs when they first emerge from winter nests around the end of April through mid-June. Older larvae are larger and higher concentrations of Cry toxins from Bt are needed to be lethal as they age (Shahid et al., 2019). Further research into the lethal concentration of Bt to the different BTM larval instar stages could provide essential insight into the susceptibility at these different stages. BTM behavior is important to consider, especially when foliar spray applications may be hindered when larvae are protected under their silk webbing or winter nests. This may be more of a factor in the fall feeding season when the larvae are feeding and building in communal areas often protected by silk webbing before they eventually aggregate into winter nests. The webbing may protect the leaf surface below from adequate treatment coverage, reducing toxic effects on feeding larvae. In the spring, they are actively leaving the confines of their winter nest to feed beyond the protection of their silk, where leaves are more likely to have adequate coverage of Bt. Timing is an important consideration for these applications, and products may affect the early and late instar feeding seasons differently. Feeding habits and additional behaviors may also differ between larval stages, and understanding how these variables are affected by treatments could be a potential route for targeted seasonal applications in the field. Further research is needed to compare the treatment effects for both feeding seasons.

Results from bioassays were compared for repeat treatments from 2021 and 2022 (Fig. 14). All of the treatments had increased amounts eaten per larva in 2021, with the control, Basin, and Javelin eating significantly more in this trial. This indicates that other factors may affect the amount eaten, such as plant species. For example, in 2021, larvae in bioassay experiments were fed leaves from cherry trees; however, in 2022 leaves came from apple trees. The differences we are observing may be due to some host traits that improved larvae health or growth and reduced susceptibility to toxins that could be occurring when the larvae consumed cherry leaves in 2021 and were able to consume more leaf area before feeding cessation. An array of host traits can affect herbivory preferences, such as total tannin content (plant defensive compound), leaf toughness, leaf surface texture, protein content, and water content (Coley et al., 1985; Pearse, 2011). Many of these are important for overall larvae preference, however, factors such as surface texture can be more important for biopesticide application coverage or delivery of the treatment and how well it physically persists on the plant surface (Santos et al., 2019). For example, the leaf surface of the cherry may have limited the dispersal of treatment droplets and allowed for gaps of untreated surface area that the larvae could take advantage of and feed on. These are important considerations, but for the purposes of treatment comparisons, they should be limited. Feeding larvae the same host species will help remove some of these confounding host trait factors and improve result comparisons in future efforts.

In polyphagous Lepidopteran folivore species, the host species' diet can have a substantial effect on the microbial composition of the larval midgut (Broderick et al., 2004). Chemical composition and weight can also differ between host species (Schaefer, 1974). Some of these differences have previously been identified for BTM specifically (Pantyukov, 1967), however, less is known about host fidelity. In our laboratory bioassays, we collected larvae feeding on a host and then fed them leaves from a different host, except for trial 2, which had larvae from both apple and cherry. When changing their diet to a plant they were not used to feeding on, we may have captured some behavior that was an effect of host fidelity. There are mixed results on host fidelity in the insect community. From an evolutionary standpoint, some species prefer exotic plants because they are "naïve" and lack natural defenses (Morrison & Hay, 2011). On the other hand, some Lepidoptera have rejected plant hosts because they were exotic or unfamiliar to them, even if they were nutrient-rich (Karowe, 1990). Future efforts should test the effects on larvae that are fed the same host that they originally were collected from (e.g. apple-apple or cherry-cherry). Using larvae from the same host species and then feeding them a variety of different hosts (e.g. oak: apple, cherry, oak) could lead to some additional insight to the host preferences of BTM larvae. Alternatively, using larvae from all different species of deciduous hosts and feeding them a single species (e.g. apple, cherry, oak: cherry) could help describe potential benefits that preferred host species may provide the larvae.

An essential step forward in determining if biopesticides can effectively control BTM populations is field testing treatments. Products may break down faster or have altered effects on the BTM larvae when placed in a natural setting. Additionally, peptide products may have stronger effects when applied in field settings than those observed in our laboratory bioassays. Our results suggest that our top products could be effective in field applications, and they should be tested for overall efficacy against BTM larvae in a natural environment. Observing the effects of a single application compared to an additional repeat application in field trials may be essential for determining the overall effectiveness of Bt treatments on larvae.

In conclusion, results from this work increase our understanding of BTM behavior in a laboratory bioassay setting and demonstrate that the number of larvae in different treatment groups can affect variables such as total mass and amount eaten per larva. Our comparison of commercially available Bt products also found some treatments were as effective as spinosad, the common broad-spectrum biopesticide currently used as the industry standard in BTM control applications. The effectiveness of Bt products is likely due to the type and amount of Cry proteins present in the formulation and products with higher amounts and new strains should be tested for increased control of BTM larvae. Our methods can be used for improving future laboratory bioassay research and field management efforts using Bt against BTM larvae. Further work is needed to test Bt products in field trials and on mature host trees to determine effectiveness in a natural setting.

BIBLIOGRAPHY

Abbasi, Q. D., Jan, N. D., Mahar, A. N., Khuhro, R. D., Nizamani, S. M., & Panhwar, A. (2007). Monitoring of ambrosia bark beetle through installation of sticky color traps at different heights in mango trees. *International Journal of Fruit Science*, 7(3), 65–79. https://doi.org/10.1300/J492v07n03_07

Adams, C. G., Schenker, J. H., McGhee, P. S., Gut, L. J., Brunner, J. F., & Miller, J. R. (2017). Maximizing information yield from pheromone-baited monitoring traps: estimating plume reach, trapping radius, and absolute density of *Cydia pomonella* (Lepidoptera: Tortricidae) in Michigan apple. *Journal of Economic Entomology*, 110(2), 305–318. https://doi.org/10.1093/jee/tow258

Allen, P. E. (2010). Group size effects on survivorship and adult development in the gregarious larvae of *Euselasia chrysippe* (Lepidoptera, Riodinidae). *Insectes Sociaux*, *57*(2), 199–204. https://doi.org/10.1007/s00040-010-0068-3

ARBICO Organics (n.d.). Biopesticides. Retrieved January 17, 2023, from https://www.arbico-organics.com/category/biopesticides-biological-pest-control

Asaro, C., & Chamberlin, L. A. (2015). Outbreak history (1953-2014) of spring defoliators impacting oak-dominated forests in Virginia, with emphasis on gypsy moth (*Lymantria dispar* L.) and fall cankerworm (*Alsophila pometaria* Harris). *American Entomologist*, 61(3), 174-185. https://academic.oup.com/ae/article/61/3/174/2194501

Athanassiou, C. G., Kavallieratos, N. G., & Mazomenos, B. E. (2004). Effect of trap type, trap color, trapping location, and pheromone dispenser on captures of male *Palpita unionalis* (Lepidoptera: Pyralidae). *Journal of Economic Entomology*, 97(2), 321–329. https://doi.org/10.1603/0022-0493-97.2.321

Aukema, J. E., McCullough, D. G., Von Holle, B., Liebhold, A. M., Britton, K., & Frankel, S. J. (2010). Historical accumulation of nonindigenous forest pests in the continental United States. *BioScience*, 60(11), 886-897. https://doi.org/10.1525/bio.2010.60.11.5

Band, S. S., Vaishampayan, S., Patidar, S., Matcha, N. (2019). Comparative efficiency of ultraviolet black light lamp and mercury vapour lamp as a light source in light trap against major insect pest of Kharif crops. *Journal of Entomology and Zoology Studies*, 7(1), 532–537. https://www.entomoljournal.com/archives/2019/vol7issue1/PartH/6-5-345-196.pdf

Barnard, D. R., & Geden, A. J. (1993). Influence of larval density and temperature in poultry manure on development of the house fly (Diptera: Muscidae). *Environmental Entomology,* 22(5), 971-977. https://academic.oup.com/ee/article/22/5/971/2480730

Barragan-Fonseca, K. B., Dicke, M., & van Loon, J. J. A. (2018). Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). *Entomologia Experimentalis et Applicata*, 166(9), 761–770. https://doi.org/10.1111/eea.12716

Bierl, B. A., Beroza, M., & Collier, C. W. (1970). Potent sex attractant of the gypsy moth: its isolation, identification, and synthesis. *Science* 170(3953), 87-89. http://www.jstor.org/stable/1730179

Biondi, A., Mommaerts, V., Smagghe, G., Viñuela, E., Zappalà, L., & Desneux, N. (2012). The non-target impact of spinosyns on beneficial arthropods. *Pest Management Science*, 68(12), 1523–1536. https://doi.org/10.1002/ps.3396

Bjostad, L. B., Hibbard, B. E., & Cranshaw, W. S. (1993). Application of semiochemicals in integrated pest management programs, pp. 199-218. *In* S. O. Duke, J. J. Menn, & J. R. Plimmer (Ed.). *Pest Control With Enhanced Environmental Safety*. Retrieved from https://doi.org/10.1021/bk-1993-0524.ch014

Blair, C. P. (1979). The browntail moth, its caterpillar and their rash. *Clinical and Experimental Dermatology*, 4(2), 215–222. https://doi.org/10.1111/j.1365-2230.1979.tb01621.x

Blanckenhorn, W. U. (1999). Different growth responses to temperature and resource limitation in three fly species with similar life histories. *Evolutionary Ecology*, 13, 395-409. https://doi.org/10.1023/A:1006741222586

Bouwer, M. C., Slippers, B., Wingfield, M. J., Allison, J. D., & Rohwer, E. R. (2017). Optimization of pheromone traps for *Coryphodema tristis* (Lepidoptera: Cossidae). *Journal of Economic Entomology*, *110*(4), 1603–1610. https://doi.org/10.1093/jee/tox171

Boukouvala, M. C., Kavallieratos, N. G., Skourti, A., Pons, X., Alonso, C. L., Eizaguirre, M., Fernandez, E. B., Solera, E. D., Fita, S., Bohinc, T., Trdan, S., Agrafioti, P., & Athanassiou, C. G. (2022). *Lymantria dispar* (L.) (Lepidoptera: Erebidae): Current status of biology, ecology, and management in Europe with notes from North America. *Insects,* 13(854), 1-35. https://doi.org/10.3390/insects13090854

Boyd, K. S. (2020). The relative abundance and diversity of parasitoids of the Browntail Moth (*Euproctis chrysorrhoea* L.) and factors that influence their population dynamics. *Electronic Theses and Dissertations*. 3172. https://digitalcommons.library.umaine.edu/etd/3172

Boyd, K. S., Drummond, F., Donahue, C., & Groden, E. (2021). Factors Influencing the Population Fluctuations of *Euproctis chrysorrhoea* (Lepidoptera: Erebidae) in Maine. *Environmental Entomology,* 50(5), 1203–1216. https://doi.org/10.1093/ee/nvab060

Bradbury, R. L. (1995). Efficacy trials of Foray 48B against early larval instars of the browntail moth, *Euproctis chrysorrhoea* (L.). Maine Department of Conservation, Maine Forest Service, Insect & Disease Management Division, Technical Report 35. Augusta, Maine, U.S.

Bradbury, R. L. (1999). The browntail moth, Euproctis chrysorrhoea, summary of Maine Forest Service activities for 1996. Maine Department of Conservation, Maine Forest Service, Insect & Disease Management Division, Technical Report 40. Augusta, Maine, U.S.

Bradshaw, C. J. A., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., Barbet-Massin, M., Salles, J. M., Simard, F., & Courchamp, F. (2016). Massive yet grossly underestimated global costs of invasive insects. *Nature Communications*, 7. https://doi.org/10.1038/ncomms12986

Bravo, A., Sarabia, S., Lopez, L., Ontiveros, H., Abarca, C., Ortiz, A., Lina, L., Villalobos, F. J., Peña, G., Nuñez-Valdez, M., Soberón, M., & Quintero, R. (1998). Characterization of cry genes in a Mexican Bacillus thuringiensis strain collection. *Applied and Environmental Microbiology*, 64(12), 4965-4972. https://doi.org/10.1128/AEM.64.12.4965-4972.1998

Brezolin, A. N., Martinazzo, J., Muenchen, D. K., de Cezaro, A. M., Rigo, A. A., Steffens, C., Steffens, J., Blassioli-Moraes, M. C., & 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

Broderick, N. A., Raffa, K. F., Goodman, R. M., & Handelsman, J. (2004). Census of the bacterial community of the Gypsy Moth larval midgut by using culturing and culture-independent methods. *Applied and Environmental Microbiology*, 70(1), 293–300. https://doi.org/10.1128/AEM.70.1.293-300.2004

Burgess, A. F. (1936). Recent work in control of gypsy moth and brown tail moth. *Journal of Economic Entomology*, 29(4), 773-778. https://doi.org/10.1093/jee/29.4.773

Burgess, A. F., & Baker, W. L. (1938). The gypsy and brown-tail moths and their control. Bulletin No. 464. U.S. Department of Agriculture, Washington D.C.

Burgess, A. F., & Crossman, S. S. (1929). Imported insect enemies of the gipsy moth and the brown-tail moth. Bulletin No. 86. U.S. Department of Agriculture, Washington D.C.

Butenandt, A., Beckmann, R., Stamm, D., & Hecker, E. (1959). Über den sexual-lockstoff des seidensspinners *Bombyx mori*. Reindarstellung und Konstitution. *Zeitschrift für Naturforschung*, 14b:283-284.

Cardé, R. T., & Haynes, K. F. (2004). Structure of the pheromone communication channel in moths, pp. 283-332. *In* Cardé, R. T., & Millar, J. G. (Eds.). *Advances in insect chemical ecology*. Cambridge University Press. https://ebookcentral.proquest.com/lib/umaine/detail.action?docID=266518

Clausen, C. P. (1956). Biological control of insect pests in the continental United States. USDA Technical Bulletin 1139. Washington, D.C., U.S.

Coley, P. D., Bryant, J. P., & Chapin, F. S. (1985). Resource availability and plant antiherbivore defense. *Science,* 22(4728), 895-899. https://www.jstor.org/stable/1695984

Costa, J. T., Fitzgerald, T. D., & Janzen, D. H. (2004). Trail-following behavior and natural history of the social caterpillar of *Arsenura armida* in Costa Rica (Lepidoptera: Saturniidae: Arsenurinae). Tropical Lepidoptera 12(1-2), 17-23. http://www.troplep.org/TLR/12-1-2/pdf003.pdf

Costa, J. T., & Pierce, N. E. (1997). Social evolution in the Lepidoptera: ecological context and communication in larval societies, pp. 407-442. *In* Choe, J. C. & Crespi, B. J. (Eds.). *The Evolution of Social Behavior in Insects and Arachnids* Cambridge University Press, Cambridge. https://piercelab.oeb.harvard.edu/publications/social-evolution-lepidoptera-ecological-context-and-communication-larval

David, C. T., Kennedy, J. S., & Ludlow, A. R. (1983). Finding of a sex pheromone source by gypsy moths released in the field. *Nature*, 303, 804-806. https://doi.org/10.1038/303804a0

de Jong, M. C. J. M., Bleumink, E. & Nater, J. P. (1975). Investigative studies of the dermatitis caused by the larva of the brown-tail moth *(Euproctis chrysorrhoea Linn.)*. *Archives Dermatological Research,* 253, 287–300. https://doi.org/10.1007/BF00561154

Dodds, K. J., Aoki, C. F., Arango-Velez, A., Cancelliere, J., D'Amato, A. W., DiGirolomo, M. F., & Rabaglia, R. J. (2018). Expansion of southern pine beetle into northeastern forests: Management and impact of a primary bark beetle in a new region. *Journal of Forestry*, 116(2), 178–191. https://doi.org/10.1093/jofore/fvx009

Dormont, L., Baltensweiler, W., Choquet, R., & Roques, A. (2006). Larch- and pine-feeding host races of the larch bud moth (*Zeiraphera diniana*) have cyclic and synchronous population fluctuations. *Oikos,* 115(2), 299-307. https://doi.org/10.1111/j.2006.0030-1299.15010.x

Dubois, N., McManus, M., Huntley, P., & Newman, D. (2001). Implementation of a program to optimize the use of bacillus thuringiensis against the browntail moth (*Euproctis chrysorrhoea*), pp. 37-44. *In* Liebhold, A. M., McManus, M. L., Otvos, I. S., and Fosbroke, S. L. C. (Eds.) *Proceedings: Integrated management and dynamics of forest defoliating insects*, 1999, Gen. Tech. Rep. NE-277. U.S. Department of Agriculture, Forest Service, Northeastern Research Station, Newtown Square, PA. https://books.google.com/books?id=sV4ioV8DziEC&dq=Bacillus%20thuringiensis%2C%20euproctis%20chrysorrhoea&lr&pg=PA37#v=onepage&q=Bacillus%20thuringiensis,%20 euproctis%20 chrysorrhoea &f=false

Dukes, J. S., Pontius, J., Orwig, D., Garnas, J. R., Rodgers, V. L., Brazee, N., Cooke, B., Theoharides, K. A., Stange, E. E., Harrington, R., Ehrenfeld, J., Gurevitch, J., Lerdau, M., Stinson, K., Wick, R., & Ayres, M. (2009). Responses of insect pests, pathogens, and invasive plant species to climate change in the forests of northeastern North America:

What can we predict? *Canadian Journal of Forest Research,* 39(2), 231-248. https://doi.org/10.1139/X08-171

Elkinton, J. S., & Childs, R. D. (1983). Efficiency of Two Gypsy Moth *(*Lepidoptera: Lymantriidae*)* Pheromone-Baited Traps. *Environmental Entomology*, 12(5), 1519-1525. https://academic.oup.com/ee/article/12/5/1519/2393584

Elkinton, J. S., Parry, D., Boettner, G. H., & Boettner1, G. H. (2006). Implicating an introduced generalist parasitoid in the invasive Browntail Moth's enigmatic demise. *Ecology*, 87(10), 2662-2672. http://www.jstor.org/stable/20069276

Elkinton, J. S., Preisser, E., Boettner, G., & Parry, D. (2008). Factors influencing larval survival of the invasive browntail moth (Lepidoptera: Lymantriidae) in relict North American populations. *Environmental Entomology*, 37(6), 1429-1437. https://doi.org/10.1603/0046-225X-37.6.1429

El-Sayed, A. M., Suckling, D. M., Byers, J. A., Jang, E. B., & Wearing, C. H. (2009). Potential of "lure and kill" in long-term pest management and eradication of invasive species. *Journal of Economic Entomology*, 102(3), 815-835. https://doi.org/10.1603/029.102.0301

El-Sayed, A. M., Suckling, D. M., Wearing, C. H., & Byers, J. A. (2006). Potential of mass trapping for long-term pest management and eradication of invasive species. *Journal of Economic Entomology*, 99(5), 1550–1564. https://doi.org/10.1603/0022-0493-99.5.1550

El-Sayed, A. M. (2023). *Semiochemicals - Sex pheromones*. The pherobase: database of pheromones and semiochemicals. https://www.pherobase.com/database/compound/compounds-index.php

El-Shafie, H. A. & Faleiro, J. R. (2017). Semiochemicals and their potential use in pest management, pp. 3-22. *In* Shields, V. D. C. (Ed.). *Biological Control of Pest and Vector Insects.* IntechOpenScience, Online. https://doi.org/10.5772/66463

Epsky, N. D., Morrill, W. L., Mankin, R. (2004). Traps for capturing insects, pp. 2319-2329. *In* Capinera, J. L. (Ed.). *Encyclopedia of Entomology*. Springer, Dordrecht. https://doi.org/10.1007/0-306-48380-7_4376

Fajer, E. D., Bowers, M. D., & Bazzaz, F. A. (1991). The effects of enriched CO_2 atmospheres on the Buckeye Butterfly, *Junonia coenia. Ecology*, 72(2), 751-754. https://doi.org/10.2307/2937217

Fanning, P. D., VanWoerkom, A., Wise, J. C., & Isaacs, R. (2018). Assessment of a commercial spider venom peptide against spotted-wing Drosophila and interaction with adjuvants. *Journal of Pest Science*, *91*(4), 1279–1290. https://doi.org/10.1007/s10340-018-1016-7

Fernald, C., & Kirkland, A. (1903). *The brown-tail moth, Euproctis chrysorrhoea: a report on the life history and habits of the imported brown-tail moth*. Boston, Massachusetts, U.S.:

Wright and Potter Printing http://archives.lib.state.ma.us/bitstream/handle/2452/784075/ocm02688384.pdf?sequence=1&isAllowed=y

Fiorentino, V. L., Murphy, S. M., Stoepler, T. M., & Lill, J. T. (2014). Facilitative effects of group feeding on performance of the saddleback caterpillar (Lepidoptera: Limacodidae). *Environmental Entomology*, 43(1), 131–138. https://doi.org/10.1603/EN13144

Fitzgerald, T. D. (1976). Trail marking by larvae of the Eastern Tent Caterpillar. *Science*, 194(4268), 961-963. https://www.jstor.org/stable/1743393

Fitzgerald, T. D., Costa, J. T. (1999). Collective behavior in social caterpillars, pp. 379-400. *In* Detrain, C., Deneubourg, J. L., Pasteels, J. M. (Eds.). *Information Processing in Social Insects*. Birkhäuser, Basel. https://doi.org/10.1007/978-3-0348-8739-7_20

Frago, E., Pujade-Villar, J., Guara, M., & Selfa, J. (2012). Hyperparasitism and seasonal patterns of parasitism as potential causes of low top-down control in *Euproctis chrysorrhoea* L. (Lymantriidae). *Biological Control*, 60(2), 123–131. https://doi.org/10.1016/j.biocontrol.2011.11.013

Frago, E., Wang, H. L., Svensson, G. P., Marques, J. F., Hódar, J. A., Boettner, G. H., Ciornei, C., Dormont, L., Elkinton, J. S., Franzén, M., Khrimian, A., Marianelli, L., Marziali, L., Mas, H., Perez Laorga, E., Pérez-López, J., Roques, A., Simonca, V., & Anderbrant, O. (2019). Common pheromone use among host-associated populations of the browntail moth, *Euproctis chrysorrhoea*, displaying different adult phenologies. *Entomologia Generalis*, 39(3–4), 295–306. https://doi.org/10.1127/entomologia/2019/0774

Frewin, A. J., Adams, C., Judd, G., & Hazell, J. (2022). Wind tunnel and field evaluation of trapping efficiency of semiochemical baited camera-traps for capturing codling moth (Lepidoptera: Tortricidae). *Journal of Economic Entomology*, 115(6), 2004–2012. https://doi.org/10.1093/jee/toac132

Gould, F. (1998). Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology*, 43(1), 701-726. https://doi.org/10.1146/annurev.ento.43.1.701

Groden, E., Boyd, K. S., & Donahue, C. (2022). Evaluation of biorational insecticides for management of *Euproctis chrysorrhoea* (Lepidoptera: Erebidae). *Journal of Economic Entomology*, 115(6), 1877-1885. https://doi.org/10.1093/jee/toac153

Hainsworth, F. R., Precup, E., & Hamill, T. (1991). Feeding, energy processing rates and egg production in painted lady butterflies. *Journal of Experimental Biology,* 156, 249-265. https://doi.org/10.1242/jeb.156.1.249

Hara, A. H. (2000). Finding alternative ways to control alien pests-Part 2: New insecticides introduced to fight old pests. *Hawaii Landscape*, *4*(1), 5-7.

https://www.ctahr.hawaii.edu/haraa/documents/2000HILandscapeFindingAlternateWaysPart2.pdf

Hendrichs, J., Robinson, A. S., Kenmore, P., Vreysen, M. J. B. (2007). Area-wide integrated pest management (AW-IPM): Principles, practice and prospects, pp. 3-33. *In* Vreysen, M. J. B., Robinson, A. S., Hendrichs, J. (Eds.). *Area-Wide Control of Insect Pests*. Springer, Dordrecht, Netherlands. https://doi.org/10.1007/978-1-4020-6059-5_1

Hitchings, E. F. (1908). Report of the State Entomologist (Maine) in 'Agriculture of Maine', Sixth Annual Report of the Commissioner of Agriculture of the State of Maine, pp. 205-306. Sentinel Publishing Company, Waterville, ME. http://lldc.mainelegislature.org/Open/Rpts/PubDocs/PubDocs1908v1/PD1908v1_02.pdf

Holbrook, R. F., Beroza, M., & Burgess, E. D. (1960). Gypsy moth (*Porthetria dispar*) detection with the natural female sex lure. *Journal of Economic Entomology*, 53(5), 751–756. https://doi.org/10.1093/jee/53.5.751

Howard, L. O. (1899). Three insect enemies of shade trees. Retrieved from https://www.google.com/books/edition/Three_Insect_Enemies_of_Shade_Trees/LIMoAAAAYAAJ?hl=en&gbpv=1&printsec=frontcover

Jackman, S. (2020). pscl: Classes and methods for R developed in the political science computational laboratory. United States Studies Centre, University of Sydney. Sydney, New South Wales, Australia. R package version 1.5.5. https://github.com/atahk/pscl/

Karlson, P., & Lüscher, M. (1959). 'Pheromones': a new term for a class of biologically active substances. *Nature*, 183(4653), 55-56. https://doi-org.wv-o-ursus-proxy02.ursus.maine.edu/10.1038/183055a0

Karowe, D. N. (1990). Predicting host range evolution: colonization of *Coronilla varia* by *Colias philodice* (Lepidoptera: Pieridae). *Evolution*, 44(6), 1637–1647. https://doi.org/10.1111/j.1558-5646.1990.tb03852.x

Kelly, L., & Debinski, D. M. (1999). Effects of larval food-limitation on *Vanessa cardui* Linnaeus (Lepidoptera: Nymphalidae). *The American Midland Naturalist,* 141(2), 315-322. https://www.montana.edu/ecology/diane_debinski/documents/AmMidNat-KelD99.pdf

Khrimian, A., Lance, D. R., Schwarz, M., Leonhardt, B. A., & Mastro, V. C. (2008). Sex pheromone of browntail moth, *Euproctis chrysorrhea* (L.): synthesis and field deployment. *Journal of Agricultural and Food Chemistry*, 56(7), 2452–2456. https://doi.org/10.1021/jf073161w

King, G. F. (2019). Tying pest insects into knots: the deployment of spider-venom-derived knottins as biopesticides. *Pest Management Science*, 75(9), 2437-2445. https://doi.org/10.1002/ps.5452

King, G. F., & Hardy, M. C. (2013). Spider-venom peptides: Structure, pharmacology, and potential for control of insect pests. *Annual Review of Entomology*, 58, 475–496. https://doi.org/10.1146/annurev-ento-120811-153650

Klapwijk, M. J., Csóka, G., Hirka, A., & Bjöorkman, C. (2013). Forest insects and climate change: Long-term trends in herbivore damage. *Ecology and Evolution*, 3(12), 4183–4196. https://doi.org/10.1002/ece3.717

Krist, F. J., Ellenwood, J. R., Woods, M. E., McMahan, A. J., Cowardin, J. P., Ryerson, D. E., Sapio, F. J., Zweifler, M. O., & Romero, S. A. (2015). 2013-2027 National Insect and Disease Forest Risk Assessment: Summary and data access. *In* K. M. Potter & B. L. Conkling (Eds.), *Forest Health Monitoring: National Status, Trends and Analysis, 2014* (pp. 87-92). U.S. Department of Agriculture, Forest Service, Southern Research Station. https://www.srs.fs.usda.gov/pubs/gtr/gtr_srs209.pdf

Lau, E. (1996). Moth-killing bacteria may be a safer pesticide alternative, a Connecticut lab works on a natural foe to caterpillars as chemicals rain on casco bay islands. Portland Press Herald, Portland, ME.

Leatherman, D. A., Farmer, D. S. & Hill, D. S. (1995). *Insect series: Gypsy moth*. Colorado State University. https://static.colostate.edu/client-files/csfs/documents/05539.pdf

Lenth, R. V. (2022). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.2. https://CRAN.R-project.org/package=emmeans

Leonhardt, B. A., Mastro, V. C., Schwarz, M., Tang, J. D., Charlton, R. E., Pellegrini-Toole, A., Warthen, J. D., Jr, Schwalbe, C. P., & Cardé, R. T. (1991). Identification of sex pheromone of browntail moth, *Euproctis chrysorrhoea* (L.) (Lepidoptera: Lymantriidae). *Journal of Chemical Ecology*, 17(5), 897–910. https://doi.org/10.1007/BF01395598

Lidwell-Durnin, J. (2022). Plague, Crisis, and Scientific Authority during the London Caterpillar Outbreak of 1782. *Historical Journal*, 1-23. https://doi.org/10.1017/S0018246X22000048

Liebhold, A., & Bentz, B. (2011). *Insect Disturbance and Climate Change*. Retrieved from https://www.fs.usda.gov/ccrc/topics/insect-disturbance-and-climate-change

Liebhold, A., Luzader, E., Reardon, R., Bullard, A., Roberts, A., Ravlin, W., Delost, S., & Spears, B. (1996). Use of a geographic information system to evaluate regional treatment effects in a gypsy moth (Lepidoptera: Lymantriidae) management program. *Journal of Economic Entomology*, 89(5), 1192-1203. https://doi-org.wv-o-ursus-proxy02.ursus.maine.edu/10.1093/jee/89.5.1192

Liebhold, A. M., & Tobin, P. C. (2008). Population ecology of insect invasions and their management. *Annual Review of Entomology,* 53, 387–408. https://doi.org/10.1146/annurev.ento.52.110405.091401

Lewis, T., & Macaulay, E. D. M. (1976). Design and elevation of sex-attractant traps for pea moth, *Cydia nigricana* (Steph.) and the effect of plume shape on catches. *Ecological. Entomology*. 1(3), 175–187. https://doi.org/10.1111/j.1365-2311.1976.tb01221.x

Logan, J. A., Régnière, J., & Powell, J. A. (2003). Assessing the impacts of global warming on forest pest dynamics. *Frontiers in Ecology and the Environment*, 1(3), 130-137. https://doi.org/10.1890/1540-9295(2003)001[0130:ATIOGW]2.0.CO;2

Lovett, G. M., Weiss, M., Liebhold, A. M., Holmes, T. P., Leung, B., Lambert, K. F., Orwig, D. A., Campbell, F. T., Rosenthal, J., McCullough, D. G., Wildova, R., Ayers, M. P., Canham, C. D., Foster, D. R., LaDeau, S. L., & Weldy, T. (2016). Nonnative forest insects and pathogens in the United States: Impacts and policy options. *Ecological Applications*, 26(5), 1437-1455. https://doi.org/10.1890/15-1176

Maine Department of Agriculture. Conservation & Forestry Board of Pesticides Control. (2022). *Pesticide laws, regulations & policies.* https://www.maine.gov/dacf/php/pesticides/laws.shtml

Maine Forest Service (2022). Browntail Moth (BTM) Dashboard. Retrieved from https://www.arcgis.com/apps/dashboards/8f2931a691374ac9853636e71cbb1f40

Marques, J. F., Wang, H. L., Svensson, G. P., Frago, E., & Anderbrant, O. (2014). Genetic divergence and evidence for sympatric host-races in the highly polyphagous brown tail moth, *Euproctis chrysorrhoea* (Lepidoptera: Erebidae). *Evolutionary Ecology* 28, 829–848. https://doi-org.wv-o-ursus-proxy02.ursus.maine.edu/10.1007/s10682-014-9701-3

Martel, V., Morin, O., Monckton, S. K., Eiseman, C. S., Béliveau, C., Cusson, M., & Blank, S. M. (2021). Elm zigzag sawfly, *Aproceros leucopoda* (Hymenoptera: Argidae), recorded for the first time in North America through community science. *The Canadian Entomologist*, 154, 1-18. https://doi.org/10.4039/tce.2021.44

Mayo, J. H., Straka, T. J., & Leonard, D. S. (2003). The cost of slowing the spread of the gypsy moth (Lepidoptera: Lymantriidae). *Journal of Economic Entomology,* 96(5), 1448-1454. https://academic.oup.com/jee/article/96/5/1448/2217856

Miller, J. R., & Gut, L. J. (2015). Mating disruption for the 21st century: Matching technology with mechanism. *Environmental Entomology*, 44(3), 427–453. https://doi.org/10.1093/ee/nvv052

Morrison, W. E., & Hay, M. E. (2011). Herbivore preference for native vs. exotic plants: Generalist herbivores from multiple continents prefer exotic plants that are evolutionarily Naïve. *PLoS ONE*, 6(3). https://doi.org/10.1371/journal.pone.0017227

Mujezinović, O., Mešan, M., Dautbašić, M., & Zahirović, K. (2017). Monitoring of populations of Browntail Moth (*Euproctis chrysorrhoea* L.) in the Central Bosnia. *Sumarski List*, *141*(7–8), 387–393. https://doi.org/10.31298/sl.141.7-8.5

Myers, J. H. (1988). Can a general hypothesis explain population cycles of forest lepidoptera? *Advances in Ecological Research*, 18, 179–242. https://doi.org/10.1016/S0065-2504(08)60181-6

Myers, J. H. (1998). Synchrony in outbreaks of forest lepidoptera: A possible example of the moran effect. *Ecology*, 79(3), 1111-1117. https://doi-org.wv-o-ursus-proxy02.ursus.maine.edu/10.2307/176606

Myers, J. H., & Cory, J. S. (2013). Population cycles in forest lepidoptera revisited. *Annual Review of Ecology, Evolution, and Systematics*, 44, 565-592. https://doi.org/10.1146/annurev-ecolsys-110512-135858

Onufrieva, K. S., Hickman, A. D., Leonard, D. S., & Tobin, P. C. (2015). Efficacies and second-year effects of SPLAT GM^{TM} and SPLAT GM^{TM} organic formulations. *Insects*, 6(1), 1-12. https://doi.org/10.3390/insects6010001

Onufrieva, K. S., Onufriev, A. v., Hickman, A. D., & Miller, J. R. (2020). Bounds on absolute gypsy moth (*Lymantria dispar dispar*) (Lepidoptera: Erebidae) population density as derived from counts in single milk carton traps. *Insects*, 11(10), 1–17. https://doi.org/10.3390/insects11100673

Pantyukhov, G. A. (1962). The effect of positive temperatures upon different populations of the brown-tail moth *Euproctis chrysorrhoea* L. and the gipsy moth *Lymantria dispar* L. (Lepidoptera, Orgyidae). *Entomologicheskoe Obozrenie*, 41, 274–284.

Pavlushin, S. v., Belousova, I. A., Chertkova, E. A., Kryukova, N. A., Glupov, V. v., & Martemyanov, V. v. (2019). The effect of population density of *Lymantria dispar* (Lepidoptera: Erebidae) on its fitness, physiology and activation of the covert nucleopolyhedrovirus. *European Journal of Entomology*, 116, 85–91. https://doi.org/10.14411/EJE.2019.009

Pearse, I. S. (2011). The role of leaf defensive traits in oaks on the preference and performance of a polyphagous herbivore, *Orgyia vetusta*. *Ecological Entomology*, 36(5), 635–642. https://doi.org/10.1111/j.1365-2311.2011.01308.x

Percival, G. C. (2016). The browntail-moth (*Euproctis chrysorrhoea L.).* Plant Health Care Program, Bartlett Tree Research Laboratories, U.K. and Ireland. https://www.bartlett.com/resources/Brown-Tail-Moth_UK.pdf

Pimentel, C., Santos, M., Ferreira, C., & Nilsson, J.-Å. (2012). Temperature, size, reproductive allocation, and life-history evolution in a gregarious caterpillar. *Biological Journal of the*

Linnean Society, 105, 340-349. https://academic.oup.com/biolinnean/article/105/2/340/2452604

Plata-Rueda, A., Quintero, H. A., Serrão, J. E., & Martínez, L. C. (2020). Insecticidal activity of bacillus thuringiensis strains on the nettle caterpillar, *Euprosterna elaeasa* (Lepidoptera: Limacodidae). *Insects*, 11(5), 310-320. https://doi.org/10.3390/insects11050310

Poland, T. M., Petrice, T. R., & Ciaramitaro, T. M. (2019). Trap designs, colors, and lures for emerald ash borer detection. *Frontiers in Forests and Global Change*, 2(80), 1-11. https://doi.org/10.3389/ffgc.2019.00080

Ravlin, F. W. (1991). Development of monitoring and decision-support systems for integrated pest management of forest defoliators in North America. *Forest Ecology and Management*, 39, 3-13. https://doi.org/10.1016/0378-1127(91)90156-P

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Régnière, J., Cooke, B. J., Béchard, A., Dupont, A., & Therrien, P. (2019). Dynamics and management of rising outbreak spruce budworm populations. *Forests*, 10(748), 1-19. https://doi.org/10.3390/f10090748

Reiff, W. (1909). The relation of sex heliotropism in the brown-tail moth. Psyche, 16(6), 115-118. https://downloads.hindawi.com/journals/psyche/1909/203674.pdf

Rennie, J. (1869). *Insect Architecture* (2nd ed.). London, England: Bell and Daldy.

Rosa, E., & Saastamoinen, M. (2017). Sex-dependent effects of larval food stress on adult performance under semi-natural conditions: only a matter of size? *Oecologia*, 184, 633-642. https://doi.org/10.1007/s00442-017-3903-7

Rosa, E., van Nouhuys, S., & Saastamoinen, M. (2017). The more the merrier: Conspecific density improves performance of gregarious larvae and reduces susceptibility to a pupal parasitoid. *Ecology and Evolution*, 7(24), 10710–10720. https://doi.org/10.1002/ece3.3571

Ruiu, L., Lentini, A., Coinu, M., Loi, A., Serra, G., & Luciano, P. (2012). Comparative applications of *Bacillus thuringiensis* formulations against *Lymantria dispar* in Sardinian forests. *International Organisation for Biological and Integrated Control*, 76, 185-190. https://www.researchgate.net/profile/Pietro-Luciano/publication/268504800_Comparative_applications_of_Bacillus_thuringiensis_formulations_against_Lymantria_dispar_in_Sardinian_forests/links/560bc78208aed467d7adac06/Comparative-applications-of-Bacillus-thuringiensis-formulations-against-Lymantria-dispar-in-Sardinian-forests.pdf

Russell V. Lenth (2022). emmeans: Estimated marginal means, aka least-squares means. R package version 1.8.2. https://CRAN.R-project.org/package=emmeans

Santos, C. A. M. dos, Santos, R. T. da S., Della'Vechia, J. F., Griesang, F., Polanczyk, R. A., & Ferreira, M. da C. (2019). Effect of addition of adjuvants on physical and chemical characteristics of Bt bioinsecticide mixture. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-48939-y

SAS Institute Inc. (2021). Using JMP 16. Cary, NC, USA.

Schaefer, P. W. (1974). The population ecology of the browntail moth (*Euproctis chrysorrhoea*) (Lepidoptera: Lymantriidae) in North America. Ph.D dissertation. University of Maine, Orono, ME.

Shahid, M. I., Arshad, M., Hasan, M. u., & Khan, M. A. (2019). Efficacy of Cry1Ac toxin from *Bacillus thuringiensis* against the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 29(55), 1-5. https://doi.org/10.1186/s41938-019-0157-1

Sharov, A. A., Liebhold, A. M. (1998). Model of slowing the spread of gypsy moth (Lepidoptera: Lymantriidae) with a barrier zone. *Ecological Applications,* 8(4), 1170-1179.
https://doi.org/10.2307/2640970

Sharov, A. A., Liebhold, A. M., & Roberts, E. A. (1997). Methods for monitoring the spread of gypsy moth (Lepidoptera: Lymantriidae) populations in the Appalachian mountains. *Journal of Economic Entomology,* 90(5), 1259-1266. https://doi.org/10.1093/jee/90.5.1259

Sparks, T. C., Storer, N., Porter, A., Slater, R., & Nauen, R. (2021). Insecticide resistance management and industry: the origins and evolution of the Insecticide Resistance Action Committee (IRAC) and the mode of action classification scheme. *Pest Management Science*, 77(6), 2609–2619. https://doi.org/10.1002/ps.6254

Stern, V. M., Smith, R., van den Bosch, R., & Hagen, K. (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

Stevens, L. J., & Beroza, M. (1972). Mating-inhibition field tests using disparlure, the synthetic gypsy moth sex pheromone. *Journal of Economic Entomology*, 65(4), 1090–1095. https://doi.org/10.1093/jee/65.4.1090

Suckling, D. M., Stringer, L. D., Stephens, A. E., Woods, B., Williams, D. G., Baker, G., & El-Sayed, A. M. (2014). From integrated pest management to integrated pest eradication: Technologies and future needs. *Pest Management Science*, 70(2), 179–189. https://doi.org/10.1002/ps.3670

Sun, Y., Fu, Z., He, X., Yuan, C., Ding, X., & Xia, L. (2016). Enhancement of *Bacillus thuringiensis* insecticidal activity by combining Cry1Ac and bi-functional toxin HWTX-XI from spider. *Journal of Invertebrate Pathology*, 135, 60–62. https://doi.org/10.1016/j.jip.2015.02.005

Tabakovic-Tosic, M., Milosavljevic, M., & Georgiev, G. (2018). Entomophaga aulicae-new entomopathogenic fungus in the Republic of Serbia. *Acta Zoologica Bulgaria*, 70 (1), 133-137. https://www.researchgate.net/publication/324968889

Tcheslavskaia, K. S., Thorpe, K. W., Brewster, C. C., Sharov, A. A., Leonard, D. S., Reardon, R. C., Mastro, V. C., Sellers, P., & Roberts, E. A. (2005). Optimization of pheromone dosage for gypsy moth mating disruption. *Entomologia Experimentalis et Applicata*, 115(3), 355–361. https://doi.org/10.1111/j.1570-7458.2005.00266.x

United States of America, Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Special Review and Reregistration Division. Amended Reregistration Eligibility Decision (RED) for Carbaryl. Environmental Protection Agency, August 2008. https://archive.epa.gov/pesticides/reregistration/web/pdf/carbaryl-red-amended.pdf

United States of America, Department of Agriculture, Animal and Plant Health Inspection Service. (1985). Laboratory Report. Otis Methods Development Center, Otis Air National Guard Base, Massachusetts.

Urban, J. M. & Leach, H.. (2022). Biology and management of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae), in the United States. *Annual Review of Entomology*, 68, 151-167. https://doi.org/10.1146/annurev-ento-120220-111140

van Frankenhuyzen, K., & Nystrom, C. W. (1987). Effect of temperature on mortality and recovery of spruce budworm (lepidoptera: Tortricidae) exposed to *Bacillus thuringiensis* Berliner. *The Canadian Entomologist*, *119*(10), 941–954. https://doi.org/10.4039/Ent119941-10

White, P. J. T., Glover, K., Stewart, J., & Rice, A. (2016). The technical and performance characteristics of a low-cost, simply constructed, black light moth trap. *Journal of Insect Science*, 16(1), 1-9. https://doi.org/10.1093/jisesa/iew011

Wilson, D. T. R. (2015). The Venom of Australian Spiders. P., Gopalakrishnakone (Ed.). *Spider Venoms* (1st ed., pp. 21-46). Retrieved from https://www.researchgate.net/publication/299640819_Spider_Venoms

Wilson, H., Maccaro, J. J., & Daane, K. M. (2020). Optimizing trap characteristics to monitor the leaffooted bug *Leptoglossus zonatus* (Heteroptera: Coreidae) in orchards. *Insects*, *11*(6), 1–9. https://doi.org/10.3390/insects11060358

Windley, M. J., Herzig, V., Dziemborowicz, S. A., Hardy, M. C., King, G. F., & Nicholson, G. M. (2012). Spider-venom peptides as bioinsecticides. *Toxins*, 4(3), 191–227. https://doi.org/10.3390/toxins4030191

Witzgall, P., Kirsch, P., & 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

Wright, R. H. (1964). After pesticides–what? *Nature,* 204, 121-125. https://doi.org/10.1038/204121a0

Zeileis, A., Kleiber, C., & Jackman, S. (2008). Regression models for count data in R. *Journal of Statistical Software,* 27(8). http://www.jstatsoft.org/v27/i08/.

BIOGRAPHY OF THE AUTHOR

Sadia Jane Crosby was born in Bath, Maine on June 19th, 1995. She was raised in Georgetown, Maine and graduated from Morse High School in 2013. She attended Roger Williams University and graduated in 2017 with a Bachelor of Science in Environmental Science. She returned to Maine and began working at Hawkes Tree Service, where she received training and became a licensed arborist. Her experience working with trees in Midcoast, Maine during this time coincided with the beginning of the current outbreak of browntail moth (*Euproctis chrysorrhoea*; BTM). Her interests shifted to providing care for trees threatened and damaged by BTM, so she obtained her commercial master pesticide applicator license and began providing control plans and applications for the pest at residential and commercial properties. Sadia decided to further her knowledge about integrated pest management strategies for BTM and entered the School of Biology and Ecology, Master of Science program at the University of Maine in January of 2021. Her research focus has been on control and monitoring strategies for BTM with biopesticides and pheromone trapping. Sadia is a candidate for the Master of Science degree in Entomology from the University of Maine in May 2023.