Management of Spotted Lanternfly (*Lycorma delicatula*) Overwintering Egg Masses and Multiple State Records of *Aculops ailanthi,* the Potential Biological Control Agent of Tree-of-Heaven (*Ailanthus altissima*)

Jason Tyler Bielski

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Entomology

Douglas G. Pfeiffer (Chair)

Stefan T. Jaronski

Thomas P. Kuhar

Scott M. Salom

Tracy C. Leskey

Jim C. Steffel

20 May 2024

Blacksburg, Virginia

Keywords: Spotted lanternfly, *Lycorma delicatula*, *Aculops ailanthi*, Tree-of-Heaven, *Ailanthus altissima*, Ovicide, Pesticide, Biopesticide, Biological Control, Invasive Species, Integrated Pest Management

Management of Spotted Lanternfly (*Lycorma delicatula*) Overwintering Egg Masses and Multiple State Records of *Aculops ailanthi*, the Potential Biological Control Agent of Tree-of-Heaven (*Ailanthus altissima*)

Jason Tyler Bielski

Abstract (Academic)

The spotted lanternfly (*Lycorma delicatula*) overwinters in egg masses for approximately eight months a year, representing the longest individual life stage. The immobile egg mass life stage constitutes a good candidate for management practices. Many insecticides and biopesticides have been demonstrated to provide control of nymphal and adult *L. delicatula*, but more research is needed on managing SLF egg masses. I conducted bioassays across three years (2021–2023) utilizing various insecticides and biopesticides against untreated and water checks at different application timings on SLF egg masses. Furthermore, in 2023, field trials of malathion and *Beauveria bassiana* biopesticides were investigated. I found substantial hatch reduction from malathion in all bioassays and field trials. Other pesticides tested in laboratory bioassays demonstrated varying hatch reductions across application timings and years. Laboratory bioassays suggested a single commercially available application of *B. bassiana* made directly on overwintering *L. delicatula* egg masses could subsequently infect hatching neonates. In laboratory studies, the optimal timing of spray applications on *L. delicatula* egg masses was approximately two weeks before hatch. Both field trials demonstrated that infection in hatching *L. delicatula* nymphs was greater than in laboratory bioassays. The intention of this research is to provide stakeholders with additional environmentally friendly tools to manage spotted lanternfly. In separate studies, I report the first detections of *Aculops ailanthi*, an exotic mite on tree-of-heaven, *Ailanthus altissima*, from Montgomery County, Virginia, and Wayne County, Michigan, USA.

Samples from both states were sent to USDA-ARS for identification, and scanning electron microscopy confirmed the species as *A. ailanthi* based on the morphological features. Moreover, I describe the impacts that high populations of *A. ailanthi* can have on *Ai. altissima,* in greenhouse settings, and its potential use as a biological control agent. I investigated the efficacy of various foliar insecticide treatments against *A. ailanthi* on potted *Ai. altissima* saplings to produce additional management recommendations for researchers struggling to cultivate *Ai. altissima* in greenhouse conditions due to the overwhelming injury produced by *A. ailanthi*. All pesticide treatments significantly reduced *A. ailanthi* populations and provided residual control for two weeks.

Management of Spotted Lanternfly (*Lycorma delicatula*) Overwintering Egg Masses and Multiple State Records of *Aculops ailanthi,* the Potential Biological Control Agent of Tree-of-Heaven (*Ailanthus altissima*)

Jason Tyler Bielski

Abstract (General Audience)

The invasive spotted lanternfly has spread to many States since it was introduced into the USA in 2014. Spotted lanternfly negatively influences many economic sectors, disrupting the distribution of commerce and requiring stakeholders to implement management options to reduce impacts on valuable commodities. Grapevines, a preferred host of spotted lanternfly, are at the greatest risk from spotted lanternfly. Currently, most spotted lanternfly management in vineyards targets the adult life stage as the adults aggregate in dense populations, feeding and excreting honeydew on vines. While many insecticides and biopesticides are effective at managing spotted lanternfly to some degree, commercial vineyards have reported an increase in the frequency of pesticide applications against spotted lanternfly. Spotted lanternfly survive the winter in egg masses, and despite remaining in egg masses for a large portion of the year, little research has been conducted on the management of spotted lanternfly egg masses. Here, I examined insecticide and biopesticide applications for spotted lanternfly egg masses. I applied a single application of pesticides to spotted lanternfly egg masses at various times during the overwinter life stage. I found many insecticidal treatments resulted in a reduction in the hatch of the spotted lanternfly. Furthermore, I observed signs of infection in recently emerged spotted lanternfly when egg masses were exposed to biopesticide treatments. In laboratory studies, I found that commercial insect pathogenic fungus applications made two weeks before hatch resulted in the most significant hatch

reduction and infection. Field trials of pesticides against overwintering spotted lanternfly egg masses demonstrated similar effects as those observed in laboratory studies. Finally, while growing tree-of-heaven for SLF research, I documented the presence of a mite, *Aculops ailanthi*, reporting multiple new state records and observations of potential biological control utility against tree-of-heaven.

Acknowledgments

I would like to express my sincere appreciation to my supervisor, Dr. Douglas Pfeiffer, for all his help, advice, and thoughtful criticism. His knowledge, commitment, and tolerance have greatly influenced this dissertation and my life. I am incredibly fortunate to have had the opportunity to work in his lab. I extend great appreciation to my dissertation committee, Dr. Kuhar, Dr. Salom, Dr. Jaronski, Dr. Leskey, and Jim Steffel, for their in-depth analysis and helpful recommendations, which have raised the caliber of this research. I am greatly indebted to Dr. Jaronski for teaching me and extending his insect pathology and microbial control expertise.

I also owe a debt of gratitude to the instructors and staff at Virginia Tech's Department of Entomology for providing me with a supportive learning environment and invaluable resources that have greatly aided in my research. I especially want to thank Kathy Shelor for her outstanding friendliness and helpfulness. I would like to thank the Invasive Species Collaborative at Virginia Tech for giving me a feeling of importance in a growing world.

I would like to extend my sincerest appreciation to Dr. Setliff and Dr. Underwood for inspiring me toward the wonders of entomology and setting me down a path of knowledge during my undergraduate degree at Kutztown University of Pennsylvania, providing me with my first scientific research and writing opportunities. With the guidance and mentorship of Jim Steffel and LABServices Inc., I established the skills necessary to conduct this dissertation. I would like to extend my thanks for the space provided for me to perform my research at the Alson H. Smith Jr.

Agricultural Research and Extension Center, Nathan Brandt for providing laboratory assistance, Mark Sutphin for help locating spotted lanternfly egg masses, which was critical to my research, Courtney Walls and her family for providing a field plot and allowing access to their properties for SLF egg mass collection, and Ryan Mays for sourcing supplies, providing assistance whenever needed and helping manage TOH in the greenhouses.

Loved ones can play the most instrumental roles in our lives. My parents, family, friends, and other loved ones have encouraged my abilities and provided their never-ending support and compassion; for that, I extend my greatest appreciation. Guardian Health and Performance for fostering a safe space for me when I needed it most, helping reinvigorate my passions in life, and giving me a role in a community.

I would like to express my gratitude to the Virginia Wine Board (Proposal Number PUVQHEEZ) and the USDA-NIFA CAP Award 2019-51181-30014 for their financial support, which allowed me to carry out the required research and collect the essential data for this study. Lastly, I would like to express my gratitude to the vineyard managers and owners for letting me conduct the research at their commercial locations and collect SLF egg masses around the property; without them, this study would not have been feasible.

In conclusion, with a deep sense of gratitude, I acknowledge the collective effort of everyone listed above and any others who have significantly impacted my academic and personal

life. Your contributions have been the cornerstone of my dissertation, and I am truly grateful for your participation. I would not have been here without every one of you. Thank you, everyone.

Table of Content

Chapter 1: Introduction

Jason Tyler Bielski

1.1 Distribution and Invasion Status

The spotted lanternfly (SLF), *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), is an introduced species of planthopper that is endemic to eastern regions of China, India, Taiwan, and Vietnam (Hua 2000, Jung et al. 2017). The earliest detection of SLF in North America was in Berks County, Pennsylvania, by the Pennsylvania Department of Agriculture in 2014 (Barringer et al. 2015). However, egg masses at the detection site suggest that SLF arrived earlier (Dara et al. 2015). SLF has become established in many counties in neighboring states, including Virginia, and was documented in 2018. Before becoming established in the USA, SLF quickly spread through South Korea and Japan in 2004 and 2006, respectively (Han et al. 2008, Kim et al. 2013, Tomisawa et al. 2013). Domestic populations of SLF in northern China were determined to be the source of introductions to Japan and South Korea (Kim et al. 2013, Zhang et al. 2019). Phylogenetic analysis indicates that SLF arrived in the USA via South Korean populations in a single event (Du et al. 2020). In contrast, South Korean SLF populations likely became established from multiple introductions from China and Japan (Du et al. 2020). The introduction of SLF was likely unintentional due to SLF's proclivity for human-assisted transportation and globalization, increasing long-range dispersal probability (Keller et al. 2020).

Model-based establishment risk is often utilized to predict areas of potentially high economic impact from pest species. Both CLIMEX and MAXENT establishment models have been used to indicate the suitable habitat globally for SLF (Jung et al. 2017, Byeon et al. 2020,

Wakie et al. 2020). CLIMEX modeling has suggested highly suitable habitats for SLF establishment in North America, South and Central America, Central Africa, western Asia, India, and Oceania, with little likelihood of establishment risk in Europe and Australia (Jung et al. 2017). Conversely, MAXENT modeling has predicted the high suitability risk of SLF establishment in Asia, Australia, Europe, North America, and South America (Wakie et al. 2020). Tropical habitats are not likely supportive of SLF establishment, indicating that they are more adapted to temperate climates (Wakie et al. 2020). Using MAXENT modeling, Wakie et al. (2020) suggest that many Northeastern, Central, Mid-Atlantic, and Pacific region states are highly susceptible to SLF establishment. SLF does not require the presence of TOH to survive, but utilization of TOH as a host plant does increase SLF survival fitness (Uyi et al. 2020). Furthermore, SLF can establish populations without TOH in the introduced landscape (Barringer and Ciafré 2020). Both aforementioned models considered TOH presence to be a requirement of the establishment. Despite the prevalence of one of the preferred host plants, TOH, in the mid-Atlantic USA, Ladin et al. (2023) modeled the dispersal of SLF. They determined that it is facilitated by human-mediated dispersal of egg masses and adults. Due to the risk of SLF spreading to high-stake areas of the world, such as West Coast wine grapes in the USA or European grape industries, a diverse set of integrated pest management tools must be available to help manage this pest.

1.2 Biology, Host Range, and Lifecycle

The SLF is a hemimetabolous insect with four nymphal instars that resemble the adult stage (Han et al. 2008, Dara et al. 2015). The first three instars are small (3–10 mm), black-bodied with white spots, whereas the fourth and final instar nymphs are larger (13–17 mm) and visually distinct with the addition of red patterns covering the body (Han et al. 2008, Park et al. 2009). Nymphal

SLF can be challenging to detect and is often confused with ticks; the adult stage is frequently mistaken for a moth (Dara et al. 2015). The adult life stage of SLF is much larger than that of nymphs, with a body size approximately 35 mm long and half that wide (Dara et al. 2015). Adult SLF forewings are gray with irregular black spots; the basal third of the forewing is patterned asymmetrically with black blocks and gray veins between each block (Park et al. 2009). Adult SLF hindwings are red with black spots posteriorly and striped black and white anteriorly (Park et al. 2009). Adult female SLF tend to be slightly larger than males, especially later in the season when the female's abdomen becomes swollen with fertilized eggs (Han et al. 2008). In addition to size, female adult SLF have red valvulae on the abdomen's posterior end, allowing easy distinguishment between males (Han et al. 2008).

The SLF is reported in all native and non-native ranges to be univoltine (Hua 2000, Dara et al. 2015, Jung et al. 2017). As SLF spreads further south, it is unlikely to have more generations per year (Urban 2019). The egg mass is the overwintering life stage of SLF. The egg masses of SLF closely resemble the spongy moth, *Lymantria dispar dispar* (Linnaeus) (Lepidoptera: Erebidae), egg masses but are not covered in scales (Urban 2019). Instead, a gray, mud-like proteinaceous coating produced by the female SLF protects the eggs (Park et al. 2009). Each SLF egg mass contains approximately 30–80 eggs (Dara et al. 2015, Liu 2019). Egg masses of SLF are laid on various organic and inorganic substrates (Kim et al. 2011, Liu 2019), increasing the risk of human-mediated spread and confounding sampling efforts (Keller et al. 2020). The SLF overwinters for up to eight months a year in the USA (Smyers et al. 2021, Dechaine et al. 2021); therefore, there is a large window of opportunity to manage this non-mobile egg stage. Multiple phenology studies have been done in Virginia and Pennsylvania to determine the cumulative

degree days for the first instar SLF to emerge (Liu 2019, Liu 2020, Dechaine et al. 2021). These studies found that SLF first started to emerge from egg masses at 111.5–270.0 cumulative degree days, with a lower degree threshold of 10°C, in the field.

The SLF displays a wide range of host-related feeding behaviors associated with herbaceous and woody plant taxa in native and non-native ranges (Barringer and Ciafré 2020, Dechaine et al. 2021). Barringer and Ciafré (2020) compiled field observations and suggested that SLF feed upon at least 56 of the known 103 worldwide host species in North America. The host range of SLF is broad in early development stages, but host preference tends to narrow as they develop (Kim et al. 2011). Host preference tests for SLF demonstrated higher nymphal survivability on tree-of-heaven (TOH), *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae), and grapevines (*Vitis* spp.) than other host species in South Korea and corroborated in North America (Lee et al. 2009, Dara et al. 2015). According to Elsensohn et al. (2023), On a diet of winegrape alone - winegrape or black walnut, *Juglans nigra* (Linnaeus) (Fagales: Juglandaceae), combined with TOH, SLF showed good development and excellent survival; additional host plants could be needed to provide SLFs with appropriate nutritional quality for survival. The only single plant diets shown to be enough for complete SLF development were winegrape and TOH (Elsensohn et al. 2023).

Overall, in both their native and non-native ranges, TOH seems to be the favored host for SLF feeding (Lee et al. 2019, Elsensohn et al. 2023). TOH is an invasive ornamental tree species on all continents, excluding Antarctica (Kowarik and Säumel 2007). Kowarik and Säumel (2007) stated that TOH is native to Asia and was introduced to the USA in 1784, where it has become

widely established. While TOH is a preferred host, it is not required for SLF to complete development or reproduce, but it does increase their survival fitness (Uyi et al. 2020, Elsensohn et al. 2023).

TOH and many other preferred host plants of SLF contain high levels of specialized organic metabolites contributing to their toxic nature (Ohmoto et al. 1983, Dara et al. 2015). This indicates that SLF has a propensity for plants with toxic secondary metabolites as a host (Lee et al. 2019). Juvenile SLF develops aposematic coloration at the final nymphal stage and is exaggerated in adulthood, following diet narrowing toward plants containing secondary metabolites (Song et al. 2018). Reports in China suggest that SLF is medicinal and consumed at low, non-toxic doses to help alleviate inflammation (Han et al. 2008).

All nymphal stages are active climbers and jumpers. SLF possesses specialized arolia, allowing for attachment to smooth surfaces if the tarsal claws slip or fail (Frantsevich et al. 2008). Immature SLF engages in repetitive falling and climbing behavior, dissipating with age (Kim et al. 2011). Though not strong fliers, adults can fly, preferring to climb tall materials and coast off air currents (Myrick and Baker 2019). Nymphs' dispersal distance is low relative to adult dispersal (Cooperband et al. 2019). Recently emerged adult SLF disperse short distances to seek sufficient host material but seldom travel more than 10 m (Kim et al. 2011, Cooperband et al. 2019).

1.3 Impacts

All life stages of SLF are phloem feeders, utilizing a piercing mouthpart to suck the plant's sap (Han et al. 2008). SLF feeding results in reduced photosynthetic function, decreasing the food supplies of the plant (Urban 2019). Heavy SLF feeding can induce the plant to ooze, stain, and crack bark, causing wilting and death (Han et al. 2009, Dara et al. 2015). SLF produces copious amounts of honeydew, a high-sugar liquid excrement (Dara et al. 2015). Honeydew can foster sooty mold growth, attracting undesirable insects (ants, wasps, flies, and other pests) and producing unpleasant odors. The development of sooty mold can further inhibit the plant's capability for photosynthesis by blocking sunlight.

Grapevine does not contain toxic secondary metabolites like TOH. This host is among the most affected agricultural commodities in regions of SLF invasion (Park et al. 2009, Barringer et al. 2015). Grapevines contain high sugar content, contributing to SLF preference (Lee et al. 2009). Adult SLF is the most problematic in vineyards in the USA during late summer (Urban 2019), while in South Korea, SLF has been an issue for both tree fruit and vineyards with the highest densities during early summer as nymphs (Han et al. 2008).

In the infestation area in the USA, SLF is usually present in vineyards throughout the season, generally in low populations (Urban 2019). In Pennsylvanian vineyards, outbreak populations of SLF can occur early in the vine-growing season during SLF egg hatch and again in mid-September, when adult SLF enter the vineyard from the surrounding landscape (Leach and Leach 2020). Leach and Leach (2020) found that SLF exhibits strong edge preference in vineyards, with >40% egg masses and adult SLF observed within the first 15 m of the vineyard. In 2018,

vineyards in Pennsylvania at the center of the infestation area reported nearly triple the insecticide applications and cost to manage influxes of adult SLF from the neighboring landscape (Urban 2019).

Nymphs commonly feed on vine shoots, whereas adult SLF typically feed on shoots, cordons, and vine trunks (Leach and Leach 2020). Grape berries are not fed upon by any SLF life stage. However, vegetative growth, yield, and bud set have been observed to significantly decrease from intense SLF feeding in vineyards (Urban 2019). If vines are not killed outright by high invasive pressure, winter vine mortality can increase due to reduced vine hardiness (Urban 2019, Leach and Leach 2020). Although Harner et al. (2022) found that large populations of SLF feed on grapevines might have a detrimental impact on the plant's nitrogen and carbon dynamics, it is yet unknown how much of an impact SLF has on grapevines. Plant pathogens or human diseases are not known to be transmitted by SLF (Lee et al. 2019, Brooks et al. 2020). Growers may require extra care to protect the fruit from honeydew and keep SLF out of the harvest.

Economic analysis indicates that SLF has already caused an annual financial loss of $50 million (USD) in Pennsylvania as of 2019 (Harper et al. 2019). Furthermore, numerous state Departments of Agriculture have implemented quarantine and eradication operations in regions infected with SLF since its entry into the USA. In the USA, the main objectives of quarantine programs are education, outreach, and controlling the flow of SLF and any goods that could contain different life stages (Urban 2019). When exporting products or carrying vehicles that promise not to convey SLF from contaminated regions, businesses operating in and out of quarantine zones are obliged to carry licenses and undergo training on SLF identification (Urban

2019). Companies that are discovered to be in breach of quarantine directives could face penalties. In order to prevent SLF from being inadvertently carried somewhere else, inhabitants in quarantined areas are also urged to examine cars and freight before leaving the area.

1.4 Management

Virginia Department of Agriculture and Consumer Services and other state agencies initially used sticky tree bands to monitor SLF, but they can catch non-target organisms, including vertebrates (Pfeiffer et al. 2018). In addition to the off-target effects of sticky bands, they are not very effective at trapping because SLF is predisposed to avoid obstacles (Cooperband 2019) and has a cyclic behavior of falling and climbing any tall potential host material (Kim et al. 2011). Sticky tree bands are non-reusable and tend to become ineffective at catching later nymphal stages and adult SLF (Kim et al. 2011, Francese et al. 2020). Alternatively, circle trunk traps reduce waste, increase SLF catch (specifically 3rd and 4th instars, and adults), and limit bycatch (Francese et al. 2020). Growers or homeowners can use tree bands or circle trunk traps to help monitor and reduce local SLF pressure. It is unknown if SLF utilizes pheromones or attractants. Scouting for SLF, specifically along the vineyard field edges closest to wooded areas, is crucial for effective management strategies (Leach and Leach 2020). Aviles-Rosa et al. (2023) demonstrated that dogs can easily distinguish between SLF egg masses and other environmental distractions where the trained dogs' average detections of SLF egg masses on bark were above 99% after eight months of training. Using detection dogs to help locate SLF egg masses in vineyards, port yards, or other high-risk areas could further isolate pesticide applications and help reduce early nymphal populations.

There are a variety of cultural control methods that can be employed against SLF. One cultural control method involves the mechanical removal of SLF egg masses. Although scraping egg masses can provide some control, Keller et al. (2020) found that most SLF egg masses oviposited on large trees are located 3m or higher. Whole tree chipping has been shown to reduce nymphal emergence by 100% (Cooperband et al. 2018). While tree chipping may be an efficacious method to destroy infected wood, it does not apply to large-area management. Instead, it could be a non-chemical method of reducing SLF populations in urban areas.

An Encyrtid egg parasitoid, *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae), was introduced in 1908 for the control of the invasive spongey moth and has been shown to parasitize SLF egg masses at low rates in the USA (Liu and Mottern 2017). Low parasitization rates and fluctuating population dynamics decrease the likelihood of *O. kuvanae* as a successful biological control agent (Liu and Mottern 2017, Liu 2019). Additionally, two parasitoids native to China, *Anastatus orientalis* (Yang & Choi) (Hymenoptera: Eupelmidae) and *Dryinus browni* (Olmi) (Hymenoptera: Dryinidae), are in the United States Department of Agriculture quarantine labs where their potential use as biological control agents against SLF is under evaluation (Choi et al. 2012, Broadley et al. 2020, Xin et al. 2020). *Anastatus orientalis* is another egg parasitoid of SLF, and *D. browni* parasitizes immature SLF. Generalist predators such as stink bugs, wheel bugs, mantids, and spiders have been observed in the field feeding on SLF but are unreliable as a control method (Barringer and Smyers 2016).

The trap tree approach is one of the most effective methods of lowering SLF populations locally and is incorporated by many state agencies (Pfeiffer et al. 2018). The trap tree approach involves removing small TOH, less than 15 cm in diameter at breast height (DBH), with an herbicide. Larger TOH (>15 cm DBH) are used as "lure" trees. SLF then tends to congregate and feed on the lure TOH, which is treated with a systemic insecticide to help reduce local populations of SLF.

Four naturally occurring entomopathogenic fungi, *Batkoa major* (Thaxt.) Humber (Entomophthorales: Entomophthoraceae), *Beauveria bassiana* (Bals.-Criv.) Vuill (Hypocreales: Cordycipitaceae), *Metarhizium pemphigi* (Driver and Milner) Kepler, Rehner and Humber (Hypocreales: Clavicipitaceae), and *Ophiocordyceps delicatula* (Clifton, Castrillo, and Hajek) (Hypocreales: Ophiocordycipitaceae), have been observed to cause localized populations of SLF to collapse in field sites in Pennsylvania (Clifton et al. 2019, Clifton et al. 2021). Of the four native fungi known to infect SLF, only *B. bassiana* is commercialized in multiple formulations and strains. These products are approved for organic production. Applications of *B. bassiana* mycoinsecticides, contact and residual, have demonstrated efficacy against nymph and adult SLF in laboratory and field trials (Clifton et al. 2020, Clifton and Hajek 2022). Control from these products alone will likely not be sufficient to suppress SLF populations enough to prevent damage but can complement other pest management strategies.

Many ecologically friendly agricultural practices (e.g., mycoinsecticides) and broad-spectrum pesticides can reduce populations of SLF at all life stages (Park et al. 2009, Kim et al. 2010, Shin et al. 2010, Lee et al. 2011, Choi et al. 2012, Leach et al. 2019). Growers have noted

that despite SLF's susceptibility to several pesticides, a greater frequency of pesticide treatments is required to manage populations in vineyards effectively (Urban 2019). Insecticide treatments targeting the adult life stage will temporarily lower SLF numbers in the vineyard, but they repopulate from nearby woods or other ecosystems, necessitating more pesticides (Park et al. 2009, Leach and Leach 2020). A greater frequency of broad-spectrum insecticide applications can damage beneficial organisms, which makes secondary pest infestations, like mites, more likely to occur (Urban 2019). Although a grapevine nutrient deficiency threshold of around 5–15 SLF per shoot exists, there is currently no action threshold for SLF in vineyards (Harner et al. 2022). Two key phenological events in vineyard management are the aggregation of adults and early hatching nymphs in the field (Leach et al. 2019, Lee et al. 2019, Urban 2019). Given that sizable populations of SLF frequently gather in vineyards during grape harvest season and that, after pesticide treatments, they will re-invade the surrounding terrain from neighboring populations, the majority of SLF management so far has been concentrated on the adult life stage (Leach and Leach 2020).

Numerous insecticides used against SLF, including neonicotinoids, are already being used in vineyards and orchards. Pollinators, specifically European honey bees *Apis mellifera* (Linnaeus) (Hymenoptera: Apidae), are susceptible to neonicotinoids, but Underwood et al. (2019) found no detectable level of neonicotinoids were present in honey, wax, or bees that feed upon the honeydew of SLF exposed to systemic neonicotinoid treatments. SLF developing insecticide resistance is unlikely due to having only one generation per year and large numbers of adult SLF constantly immigrating from outside the field. Rotation of pesticide mode of action remains essential to reduce resistance in other insects in the agroecosystem.

1.5 Virginia Wine Grape Industry

Virginia's grapevine industry stands as a flourishing cornerstone within the state's agricultural tapestry, exerting significant economic influence. With over 300 wineries and more than 3,700 acres of vineyards gracefully adorning its landscapes, Virginia boasts a rich viticultural heritage (Quinlan and Grube 2019). These vineyards, spanning regions like the Shenandoah Valley, Piedmont, and Northern Neck, cultivate an eclectic array of grape varieties, leveraging the state's diverse soil compositions and microclimates. Among the cultivars nurtured in Virginia's vineyards are classic European vinifera varieties such as Cabernet Franc, Viognier, and Petit Verdot, esteemed for their exceptional quality and expressive character (Schoenfeld 2018). Additionally, Virginia embraces the cultivation of native American hybrids like Norton and Vidal Blanc, contributing to its viticultural diversity and enriching its winemaking landscape (Schoenfeld 2018).

While it may not rival the scale of renowned wine regions like California's Napa Valley, Virginia's industry has experienced remarkable growth and garnered recognition for its quality wines and diverse offerings. Virginia's production volume is relatively smaller than other wine-producing states, such as California. In 2018 alone, Virginia wineries and vineyards collectively propelled an estimated economic impact exceeding $1.4 billion (USD), illuminating their pivotal role in the Commonwealth's economy (Quinlan and Grube 2019).

Virginia's wines have garnered widespread acclaim, earning numerous awards and accolades in esteemed wine competitions globally (Schoenfeld 2018). Moreover, beyond their economic significance, Virginia's vineyards play a pivotal role in preserving rural heritage and fostering community bonds. Many of these vineyards are family-owned and operated, embodying a deep-rooted commitment to sustainable agriculture and environmental stewardship (Quinlan and Grube 2019). Furthermore, the proliferation of vineyards has revitalized historic rural areas, stimulating local tourism and bolstering regional economies (Schoenfeld 2018). In terms of wine tourism and consumer interest, Virginia has emerged as a compelling destination for wine enthusiasts. The state's picturesque vineyards, coupled with its rich history and vibrant culinary scene, attract visitors from near and far. In essence, Virginia's grapevine industry epitomizes a harmonious fusion of tradition, innovation, and stewardship, poised to further elevate its stature as a leading wine-producing region in the USA.

1.6 Call to Action

Strömbom and Pandey (2021) modeled SLF lifecycles under the influence of various management strategies. They discovered that at least 35% of all SLF life phases needed to be controlled to induce population reduction. This included treating post-diapause SLF egg masses with chlorpyrifos. Therefore, SLF management should focus not only on the adult life stage in vineyards (Leach and Leach 2020) but also on all life stages.

The intention of this research is to provide stakeholders with additional environmentally friendly tools to manage SLF. This dissertation had three primary objectives:

1. Determine if a single insecticide treatment applied to overwintering SLF egg masses at various application timings will influence the SLF hatch.
2. Determine if a single application of commercially available biopesticides to overwintering SLF egg masses will infect hatching nymphs.
3. Investigate Aculops ailanthi distribution and management.

1.7 References

Aviles-Rosa, E. O., M. Nita, E. Feuerbacher, and N. J. Hall. 2023. An evaluation of spotted lanternfly (*Lycorma delicatula*) detection dog training and performance. Applied Animal Behaviour Science 258(105816) 1–11. doi.org/10.1016/j.applanim.2022.105816

Barringer, L. E., L. R. Donovall, S. E. Spichiger, D. Lynch, and D. Henry. 2015. The first new world record of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae). Entomological News 125(1) 20–23. https://doi.org/10.3157/021.125.0105

Barringer, L. E., and E. Smyers. 2016. Predation of the spotted lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) by two native Hemiptera. Entomological News 126(1) 71–73. https://doi.org/10.3157/021.126.0109

Barringer, L., and C. M. Ciafré. 2020. Worldwide feeding host plants of spotted lanternfly, with significant additions from north America. Environmental Entomology 49(5) 999–1011. https://doi.org/10.1093/ee/nvaa093

Broadley, H. J., J. R. Gould, L. T. Sullivan, X. Y. Wang, K. A. Hoelmer, M. L. Hickin, and J. S. Elkinton. 2020. Life history and rearing of *Anastatus orientalis* (Hymenoptera:

Eupelmidae), an egg parasitoid of the spotted lanternfly (Hemiptera: Fulgoridae). Environmental Entomology 50(1) 28–35. https://doi.org/10.1093/ee/nvaa124

Brooks, R. K., A. Toland, A. C. Dechaine, T. McAvoy, and S. Salom. 2020. The inability of spotted lanternfly (*Lycorma delicatula*) to vector a plant pathogen between its preferred host, *Ailanthus altissima*, in a laboratory setting. Insects 11(8) 515. https://doi.org/10.3390/insects11080515

Byeon, D., J.-M. Jung, S. Jung, and W.-H. Lee. (2020). Effect of types of meteorological data on species distribution predicted by the CLIMEX model using an example of *Lycorma delicatula* (Hemiptera: Fulgoridae). Journal of Asia-Pacific Biodiversity 13(1) 1–6. https://doi.org/10.1016/j.japb.2019.11.010

Choi, D.-S., D.-I. Kim, S.-J. Ko, B.-R. Kang, J.-D. Park, S.-G. Kim, and K.-J. Choi. 2012. Environmentally-friendly control methods and forecasting the hatching time *Lycorma delicatula* (Hemiptera: Fulgoridae) in Jeonnam Province. Korean Journal of Applied Entomology 51(4) 371–376. https://doi.org/10.5656/ksae.2012.09.0.022

Clifton, E. H., L. A. Castrillo, A. Gryganskyi, and A. E. Hajek. 2019. A pair of native fungal pathogens drives decline of a new invasive herbivore. Proceedings of the National Academy of Sciences of the United States of America. 116(19) 9178–9180. https://doi.org/10.1073/pnas.1903579116

Clifton, E. H., A. E. Hajek, N. E. Jenkins, R. T. Roush, J. P. Rost, D. J. Biddinger, and P. Nachappa. 2020. Applications of *Beauveria bassiana* (Hypocreales: Cordycipitaceae) to control populations of spotted lanternfly (Hemiptera: Fulgoridae), in semi-natural landscapes and on grapevines. Environmental Entomology 49(4) 854–864. https://doi.org/10.1093/ee/nvaa064

Clifton, E. H., L. A. Castrillo, and A. E. Hajek. 2021. Discovery of two hypocrealean fungi infecting spotted lanternflies, *Lycorma delicatula*: *Metarhizium pemphigus* and a novel species, *Ophiocordyceps delicatula*. Journal of Invertebrate Pathology 186. https://doi.org/https://doi.org/10.1016/j.jip.2021.107689

Clifton, E. H., and A. E. Hajek. 2022. Efficacy of *Beauveria bassiana* and *Cordyceps javanica* mycoinsecticides against spotted lanternflies, *Lycorma delicatula*, in laboratory bioassays. Biocontrol Science and Technology 32(7) 824–836. https://doi.org/10.1080/09583157.2022.2052804

Cooperband, M. F., R. Mack, and S. E. Spichiger. 2018. Chipping to destroy egg masses of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). Journal of Insect Science 18(3). https://doi.org/10.1093/jisesa/iey049

Cooperband, M. F., J. Wickham, K. Cleary, S.-E. Spichiger, L. Zhang, J. Baker, I. Canlas, N. Derstine, and D. Carrillo. 2019. Discovery of Three Kairomones in Relation to Trap and Lure Development for Spotted Lanternfly (Hemiptera: Fulgoridae). Journal of Economic Entomology 112(2). https://doi.org/10.1093/jee/toy412

Dara, S. K., L. Barringer, and S. P. Arthurs. 2015. *Lycorma delicatula* (Hemiptera: Fulgoridae): A new invasive pest in the United States. Journal of Integrated Pest Management 6(1). https://doi.org/10.1093/jipm/pmv021

Dechaine, A. C., M. Sutphin, T. C. Leskey, S. M. Salom, T. P. Kuhar, and D. G. Pfeiffer. 2021. Phenology of *Lycorma delicatula* (Hemiptera: Fulgoridae) in Virginia, USA. Environmental Entomology 50(6) 1267–1275. doi: 10.1093/ee/nvab107.

Du, Z., Y. Wu, Z. Chen, L. Cao, T. Ishikawa, S. Kamitani, T. Sota, F. Song, L. Tian, W. Cai, and H. Li. 2020. Global phylogeography and invasion history of the spotted lanternfly revealed

by mitochondrial phylogenomics. Evolutionary Applications https://doi.org/10.1111/eva.13170

Elsensohn, J. E., L. J. Nixon, J. Urban, S. K. Jones, and T. C. Leskey. 2023. Survival and development of *Lycorma delicatula* (Hemiptera: Fulgoridae) on common secondary host plants differ by life stage under controlled conditions. Frontiers in Insect Science 3:1134070. https://doi.org/10.3389/finsc.2023.1134070

Francese, J. A., M. F. Cooperband, K. M. Murman, S. L. Cannon, E. G. Booth, S. M. Devine, and M. S. Wallace. 2020. Developing traps for the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). Environmental Entomology 49(2) 269–276. https://doi.org/10.1093/ee/nvz166

Frantsevich, L., A. Ji, Z. Dai, J. Wang, L. Frantsevich, L, and S. N. Gorb. 2008. Adhesive properties of the arolium of a lantern-fly, *Lycorma delicatula* (Auchenorrhyncha, Fulgoridae). Journal of Insect Physiology 54(5) 818–827. https://doi.org/10.1016/j.jinsphys.2008.03.005

Han, J. M., H. Kim, E. J. Lim, S. Lee, Y. J. Kwon, and S. Cho. 2008. *Lycorma delicatula* (Hemiptera: Auchenorrhyncha: Fulgoridae: Aphaeninae) finally, but suddenly arrived in Korea. Entomological Research 38(4) 281–286. https://doi.org/10.1111/j.1748-5967.2008.00188.x

Harner, A. D., H. L. Leach, L. Briggs, and M. Centinari. 2022. Prolonged phloem feeding by the spotted lanternfly, an invasive planthopper, alters resource allocation and inhibits gas exchange in grapevines. Plant Direct 6(10) 1–18. doi.org/10.1002/pld3.452.

Harper, J. K., W. Stone, T. W. Kelsey, and L. F. Kime. 2019. Potential economic impact of the spotted lanternfly on agriculture and forestry in Pennsylvania. www.rural.palegislature.us.

Hua, L. Z. 2000. List of Chinese insects. Zhongshan (Sun Yat-sen) University Press, Guangzhou 3(1).

Jung, J.-M., S. Jung, D.-H. Byeon, and W.-H. Lee. 2017. Model-based prediction of potential distribution of the invasive insect pest, spotted lanternfly *Lycorma delicatula* (Hemiptera: Fulgoridae), by using CLIMEX. Journal of Asia-Pacific Biodiversity 10(4) 532–538. https://doi.org/10.1016/j.japb.2017.070.001

Keller, J., J. Rost, K. Hoover, J. Urban, H. Leach H, M. Porras, B. Walsh, M. Bosold, and D. Calvin. 2020. Dispersion patterns and sample size estimates for egg masses of spotted lanternfly (Hemiptera: Fulgoridae). Environmental Entomology 49(6) 1462–1472. https://doi.org/10.1093/ee/nvaa107

Kim, S.-K., G.-Y. Lee, Y.-H. Shin, and G.-B. Kim. 2010. Chemical control effect against spot clothing wax cicada, *Lycorma delicatula* (Hemiptera: Fulgoridae) nymphs and adults. The Korean Journal of Pesticide Science 14(4) 440–445.

Kim, J.-G., E.-H. Lee, Y.-M. Seo, and N.-Y. Kim. 2011. Cyclic behavior of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae) on host plants. Journal of Insect Behavior 24(6) 423–435. https://doi.org/10.1007/s10905-011-9266-8

Kim, H., M. Kim, D. H. Kwon, S. Park, Y. Lee, J. Huang, S. Kai, H. S. Lee, K. J. Hong, Y. Jang, and S. Lee. 2013. Molecular comparison of *Lycorma delicatula* (Hemiptera: Fulgoridae) isolates in Korea, China, and Japan. Journal of Asia-Pacific Entomology 16(4) 503–506. https://doi.org/10.1016/J.ASPEN.2013.07.003

Kowarik I., and I. Säumel. 2007. Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. Perspectives in Plant Ecology, Evolution and Systematics 8(4) 207–237. doi:10.1016/j.ppees.2007.03.002

Ladin, Z. S., D. A. Eggen, T. L. E. Trammell, and V. D'Amico. 2023. Human-mediated dispersal drives the spread of the spotted lanternfly (*Lycorma delicatula*). Scientific Reports 13(1) 1098(1) 1–15. doi.org/10.1038/s41598-022-25989-3

Leach, H., D. J. Biddinger, G. Krawczyk, E. Smyers, and J. M. Urban. 2019. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula*, (Hemiptera: Fulgoridae), a new pest of fruit in the Northeastern U.S. Crop Protection 124(1) 1–6. https://doi.org/10.1016/j.cropro.2019.05.027

Leach, H., and A. Leach. 2020. Seasonal phenology and activity of spotted lanternfly *(Lycorma delicatula*) in eastern US vineyards. Journal of Pest Science 93(4) 1215–1224. https://doi.org/10.1007/s10340-020-01233-7

Lee, D. H., Y. L. Park, and T. C. Leskey. 2019. A review of biology and management of *Lycorma delicatula* (Hemiptera: Fulgoridae), an emerging global invasive species. Journal of Asia-Pacific Entomology 22(2) 589–596. https://doi.org/10.1016/j.aspen.2019.03.004

Lee, J.-E., S.-R. Moon, H.-G. Ahn, S.-R. Cho, J.-O. Yang, C. Yoon, and G.-H. Kim. 2009. Feeding behavior of *Lycorma delicatula* (Hemiptera: Fulgoridae) and response on feeding stimulants of some plants. Korean Journal of Applied Entomology 4(1) 467–477. doi:10.5656/KSAE

Lee, K.-Y., S.-K. Kim, I.-H. Kim, and K.-S. Kim. 2011. Seasonal occurrence of spot clothing wax cicada, *Lycorma delicatula* (Hemiptera: Fulgoridae) and it's control efficacy using EFAM at the vineyards. The Korean Journal of Pesticide Science 15(3) 303–309.

Liu, H., and J. Mottern. 2017. An old remedy for a new problem? Identification of *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae), an egg parasitoid of *Lycorma delicatula* (Hemiptera:

Fulgoridae) in North America. Journal of Insect Science 17(1). doi.org/10.1093/jisesa/iew114

Liu, H. 2019. Oviposition substrate selection, egg mass characteristics, host preference, and life history of the spotted lanternfly (Hemiptera: Fulgoridae) in North America. Environmental Entomology 48(6) 1452–1468. https://doi.org/10.1093/ee/nvz123

Liu, H. 2020. Seasonal development, cumulative growing degree-days, and population density of spotted lanternfly (Hemiptera: Fulgoridae) on selected hosts and substrates. Environmental Entomology 49(5) 1171–1184. doi.org/10.1093/ee/nvaa074

Myrick, A. J., and T. C. Baker. 2019. Analysis of anemotactic flight tendencies of the spotted lanternfly (*Lycorma delicatula*) during the 2017 mass dispersal flights in Pennsylvania. Journal of Insect Behavior 32(1) 11–23. https://doi.org/10.1007/s10905-019-09708-x

Ohmoto, K., K. Koike, and Y. Sakamoto. 1981. Studies on the constituents of *Ailanthus altissima* Swingle. II. alkaloidal constituents. Chemical and Pharmaceutical Bulletin 29(2) 390–395. https://doi.org/10.1248/cpb.29.390

Park, J. D., M. Y. Kim, S. G. Lee, S. C. Shin, J. H. Kim, and I. K. Park. 2009. Biological characteristics of *Lycorma delicatula* and the control effects of some insecticides. Korean Journal of Applied Entomology 48(1) 53–57. Doi:10.5656/KSAE.2009.48.1.053

Pfeiffer, D. G., E. R. Day, and T. A. Dellinger. 2018. Spotted lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae). Virginia Cooperative Extension Fact Sheet Publication ENTO-180NP. 1–2.

Quinlan, M. B., and A. M. Grube. 2019. Economic contributions of Virginia wineries and vineyards in 2018. Virginia Vineyard Association.

Schoenfeld, A. 2018. Virginia wine industry economic impact study. The Weldon Cooper Center for Public Service, University of Virginia.

Shin, Y.-H., S.-R. Moon, C. Yoon, K.-S. Ahn, and G.-H. Kim. 2010. Insecticidal activity of 26 insectcides against eggs and nymphs of *Lycorma delicatula* (Hemiptera: Fulgoridae). The Korean Journal of Pesticide Science 14(2) 157–163.

Smyers, E. C., J. M. Urban, A. C. Dechaine, D. G. Pfeiffer, S. R. Crawford, and D. D. Calvin. 2021. Spatio-temporal model for predicting spring hatch of the spotted lanternfly (Hemiptera: Fulgoridae). Environmental Entomology 50(1) 126–137. https://doi.org/10.1093/ee/nvaa129

Song, S., S. Kim, S. W. Kwon, S. I. Lee, and P. G. Jablonski. (2018. Defense sequestration associated with narrowing of diet and ontogenetic change to aposematic colours in the spotted lanternfly. Scientific Reports 8(1). https://doi.org/10.1038/s41598-018-34946-y

Strömbom, D., and S. Pandey. 2021. Modeling the life cycle of the spotted lanternfly (*Lycorma delicatula*) with management implications. Mathematical Biosciences 340(1) 108670. doi.org/10.1016/j.mbs.2021.108670

Tomisawa, A., S. Ohmiya, H. Fukutomi, K. Hayashi, and T. Ishikawa. 2013. Biological notes on *Lycorma delicatula* (White) (Hemiptera, Fulgoridae) in Ishikawa Prefecture, Japan. Japanese Journal of Entomology 16(1) 3–14.

Underwood, R., B. Breeman, J. Benton, J. Bielski, J. Palkendo, and T. Betts. 2019. Are non-target honey bees (Hymenoptera: Apidae) exposed to dinotefuran from spotted lanternfly (Hemiptera: Fulgoridae) trap trees? Journal of Economic Entomology 112(6) 2993–2996. https://doi.org/10.1093/jee/toz176

Urban, J. M. 2019. Perspective: shedding light on spotted lanternfly impacts in the USA. Pest Management Science 76(1) 10–17. https://doi.org/10.1002/ps.5619

Uyi, O., J. A. Keller, A. Johnson, D. Long, B. Walsh, and K. Hoover. 2020. Spotted lanternfly (Hemiptera: Fulgoridae) can complete development and reproduce without access to the preferred host, *Ailanthus altissima*. Environmental Entomology 49(5) 1185–1190. https://doi.org/10.1093/ee/nvaa083

Wakie, T. T., L. G. Neven, W. L. Yee, and Z. Lu. 2020. The establishment risk of *Lycorma delicatul*a (Hemiptera: Fulgoridae) in the United States and globally. Journal of Economic Entomology 113(1) 306–314. https://doi.org/10.1093/jee/toz259

Xin, B., Y. L. Zhang, X. Y. Wang, L. M. Cao, K. A. Hoelmer, H. J. Broadley, and J. R. Gould. 2020. Exploratory survey of spotted lanternfly (Hemiptera: Fulgoridae) and its natural enemies in China. Environmental Entomology 50(1) 36–45. https://doi.org/10.1093/ee/nvaa137

Zhang, L., W. Zhao, F. Wang, and D. Qin. 2019. Genetic diversity and population structure of natural *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) populations in China as revealed by microsatellite and mitochondrial markers. Insects 10(10). https://doi.org/10.3390/insects10100312

Chapter 2: Bioassay and field trial of pesticides against spotted lanternfly (*Lycorma delicatula*) egg masses at different overwintering application timings

Jason Tyler Bielski

2.1 Abstract

Spotted lanternfly (*Lycorma delicatula*) overwinter in egg masses for approximately eight months a year, representing the longest individual life stage. Therefore, the inactive egg mass life stage constitutes a good candidate for management practices. Egg masses were collected from various locations in Winchester, VA, and stored in climate-controlled incubators simulating winter conditions (25°C, 65% relative humidity, and a 16:8 light-to-dark ratio). The egg masses were treated at three periods during the overwintering phase: winter, early spring, or late spring. I conducted bioassays across three years (2021–2023) utilizing 13 insecticides, across multiple modes of action, compared to an untreated and water check. Furthermore, in 2023, a field trial of malathion against an untreated check was investigated. Egg mass hatch reduction was analyzed to determine the efficacy of the various treatments and application timings. In all bioassays, I found strong reductions in survivorship from malathion and chlorpyrifos. Other pesticides tested in laboratory bioassays demonstrated varying, lesser hatch reductions across application timings and years. In the field trial, I found strong evidence that malathion provides ovicidal efficacy against hatching SLF nymphs.

Key Words: integrated pest management, invasive species, ovicides, spotted lanternfly

2.2 Introduction

Invasive insects are organisms that have been introduced to a new geographic range, where they negatively impact the native ecology and/or disrupt human economics or health (Exec. Order No. 13112, 1999). The visually apparent spotted lanternfly (SLF), *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), is an Asian native that has become an issue for many economic sectors in South Korea, Japan, and the northeastern United States (Lee et al. 2019). This phloem-feeding planthopper has a wide host range (Barringer and Ciafré 2020, Dechaine et al. 2021) but is the most problematic pest for vineyards among agricultural crops in the eastern USA (Urban 2019, Urban and Leach 2023). In vineyards, SLF has been observed to negatively influence overall vine hardiness, reduce yield, and promote sooty mold growth through honeydew production (Dara et al. 2015, Urban 2019, Leach and Leach 2020). Harner et al. (2022) found that high populations of SLF can negatively affect the carbon and nitrogen dynamics of the plant. However, the full ramifications of the effects SLF plays on grapevines are still unclear. It is estimated that the annual economic impact of SLF is $50 million (USD) in Pennsylvania's regions under economic quarantine due to the presence of this invasive species (Harper et al. 2019).

SLF egg masses, nymphs, and adults are vulnerable to many environmentally friendly agricultural methods (such as mycoinsecticides) and broad-spectrum insecticides such as pyrethroid, neonicotinoid, carbamate, and organophosphate classes (Park et al. 2009, Kim et al. 2010, Shin et al. 2010, Lee et al. 2011, Choi et al. 2012, Leach et al. 2019). Many pesticides already applied in traditional vineyard programs are toxic to SLF and will provide some level of short-term crop protection (Leach et al. 2019, Leach and Leach 2020). Several chemical control studies have demonstrated that chlorpyrifos has high efficacy against all SLF life stages (Park et al. 2009,

Kim et al. 2010, Shin et al. 2010, Leach et al. 2019), although environmental, human health, and phytotoxicity hazards can occur when using this insecticide (Rauh et al. 2006, Rauh et al. 2011, Rauh et al. 2015).

In South Korea, Shin et al. (2009) found that SLF egg masses were highly susceptible to applications of chlorpyrifos (100% mortality); further investigation into the effects of chlorpyrifos demonstrated that egg mass hatch rate and nymphal survival were significantly reduced (22.0–100.0%) when SLF egg masses were treated from February to May 2010, with greater control observed when applications were applied earlier in the overwintering period. Surprisingly, other organophosphates that Shin et al. (2010) tested, such as diazinon, fenitrothion, methidathion, and phenthoate, did not have ovicidal effects (0.0–34.1%). Leach et al. (2021) also found strong ovicidal efficacy with chlorpyrifos. Chlorpyrifos tolerances have been uncertain, with total complete restrictions on all crops in 2021 and restored in 2023; therefore, I seek a suitable replacement to demonstrate similar ovicidal effects on SLF. I used chlorpyrifos (Lorsban®75WG, Gowan® USA) and malathion (Malathion 8 Flowable, Gowan® USA) in this study. I included chlorpyrifos as a positive control and used malathion because it has demonstrated ovicidal efficacy on the brown rice planthopper *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) (Salim and Heinrichs 1987).

Leach et al. (2019) conducted field and laboratory bioassays against SLF egg masses and found that all five pesticides used resulted in a reduction in hatch compared to the control (8.20–100.0%); specifically, they found that chlorpyrifos resulted in 100.0% control, paraffinic oil with the subsequent greatest reduction in hatch at 45.0%, and both the neonicotinoids and the soybean

oil resulting in 8.2–26.0% control. In both laboratory and field ovicidal trials conducted by Leach et al. (2019), the egg masses were treated in April 2018. Furthermore, Chase and Wright (2023) demonstrated that spiromesifen, abamectin, mineral oil, neem oil, and insecticidal soaps have little ovicidal efficacy on SLF egg masses. Previously, it has been demonstrated that some oil-based pesticide applications, such as paraffinic oil, soybean oil, and mineral oil, all have some level of efficacy against overwintering SLF (Shin et al. 2010, Leach et al. 2019).

In this research, I aimed to reinvestigate some previously researched insecticides against overwintering SLF egg masses and also probe for other potential ovicidal candidates. The oil-based treatments incorporated in this bioassay included a clarified hydrophobic extract of neem oil (Trilogy®, Certis Bio LLC.), paraffinic oil (JMS Stylet Oil, JMS Flower Farms, Inc.), methylated soybean oil (MSO, Southern Ag®), and mineral oil (Damoil™, Drexel Chemical, and Mite-E-Oil®, HELENA®). Neem oil, paraffinic oil, methylated soybean oil, and mineral oil have all demonstrated ovicidal efficacy against the brown rice planthopper (Koul and Isman 1991, Zehnder and Norris 1995, Cohen and Alam 1998, Kannan and Subramanian 2004). In addition to chlorpyrifos and malathion, my laboratory bioassay experiments used bifenthrin (Talstar®P, FMC Corporation), zeta-cypermethrin (Mustang® Maxx, FMC Corporation), and dinotefuran (Scorpion® 35SL, Gowan® USA). Furthermore, these insecticides have also demonstrated ovicidal efficacy against brown rice planthopper (Wang et al. 2007, Srinivasan and Suresh 2009, Ghosal et al. 2013)

The insect growth regulator (IGR) buprofezin (IRAC group 16) is a chitin synthesis inhibitor targeting many homopterans and mites. When used as an ovicide, buprofezin has

demonstrated reduced hatchability on the brown rice planthopper (Asai et al. 1983). In susceptible adult insects, such as the brown rice planthopper, buprofezin indirectly acts on the adult female by diminishing the female lifespan, impeding oviposition rates, and decreasing the successful hatch of the next generation (Asai et al. 1985, Kanaoka et al. 1996). Interestingly, even at sub-lethal doses, buprofezin has demonstrated the ability to hinder the oviposition and lifespan of brown rice planthopper (Kanaoka et al. 1996).

Buprofezin is recommended against SLF in South Carolina and Georgia (Coyle et al. 2019). Shin et al. (2010) looked at the effects of some IGRs against hatching SLF, including bistrifluron and teflubenzuron (IRAC 15–inhibitors of chitin biosynthesis). Although not within IRAC group 16, bistrifluron and teflubenzuron share a similar mode of action with buprofezin–chitin biosynthesis inhibitors. The efficacy of bistrifluron and teflubenzuron against hatching SLF was 26.0, and 14.9% corrected mortality, respectively (Shin et al. 2010). Leach et al. (2019) investigated the efficacy of tebufenozide (IRAC 18–ecdysone receptor agonist) against adult SLF, resulting in 12.5% adjusted mortality 48 hours after direct exposure to applications. More research is necessary to determine the efficacy of IGRs against SLF fully. Unfortunately, the selectiveness of buprofezin can be a double-edged sword. While it can help prevent the indiscriminate control of insects within a managed system, it could also prove ineffective against SLF. I utilized (Applaud 70DF, Nichino America, Inc.) in this study to investigate its ovicidal efficacy against SLF in laboratory bioassays.

Although SLF are susceptible to many insecticides, growers have reported an increase in the frequency of pesticide applications needed to manage populations successfully (Urban 2019).

More pesticide applications are required because although the insecticide applications reduce SLF populations in the vineyard, they are repopulated from adjacent forests or other environments (Park et al. 2009, Leach and Leach 2020). In addition to the increased number of applications, broad-spectrum pesticides kill beneficial organisms, increasing the susceptibility of secondary infestations of pests such as mites (Urban 2019). Currently, no action threshold is established for SLF in vineyards at any life stage, but there is a grapevine nutrient deficiency threshold of about 5–15 SLF per shoot (Harner et al. 2022). Product, labor expenses, and pesticide label restrictions are critical factors for growers to consider when it comes to pest management, making it imperative that effective integrated pest management techniques become defined for stakeholders at risk to SLF.

In vineyards, there are two crucial phenological events for managing SLF: early hatching nymphs and adults as they aggregate in the field (Leach et al. 2019, Lee et al. 2019, Urban 2019). To date, most pesticide management of SLF is focused on the adult life stage because large populations of SLF tend to aggregate in vineyards coinciding with the grape harvest and, after pesticide applications, will reinvade from local populations in the surrounding landscape (Leach and Leach 2020). Little egg mass life stage management is performed in vineyards despite being immobile and the longest individual life stage. In this study, I aimed to investigate further the efficacy of various pesticides against overwintering SLF egg masses both in laboratory bioassays and a field efficacy trial. Furthermore, I examined the effects of application timing on hatch when SLF egg masses were treated at different times during the overwintering period.

2.3 Material and Methods

2.3.1 SLF Egg Masses Collection and Storage for Laboratory Bioassays

In 2021, 2022, and 2023, SLF egg masses were collected at various field sites within Frederick County, Virginia, where SLF has been present for several years. Egg masses were sampled from the landscape, mainly, but not exclusively from tree-of-heaven (TOH), *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae). The SLF egg masses were collected individually and removed from the plant substrate. Egg masses were chiseled around, and then, using a knife, gently peeled off the plant material with the SLF egg mass attached to a small piece of bark. In all years, the SLF egg masses were collected in January. Only SLF egg masses that appeared undamaged from removal, and with intact protective proteinaceous covering, were selected for the experiment (Figure 2.1). Immediately following collection, the SLF egg masses were stored individually in Petri dishes (various sizes) in an environmental incubator. The incubators were held at 10°C, 65% relative humidity, and a 16:8 light-to-dark ratio. Egg masses were stored thus for at least 100 days, and then induced to hatch by exposing them to 25°C, 65% relative humidity, and a 16:8 light-to-dark ratio (Keena and Nielsen 2021). A predicted SLF hatch was estimated using 200 cumulative growing degree days, with an upper degree threshold of 35°C and a lower degree threshold of 10°C (Liu 2020). All the SLF egg masses were visually inspected for signs of parasitism by comparing the exit holes of SLF to those of *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae), which are more circular in appearance compared to the oval exit hole produced by SLF emergence (Liu and Mottern 2017).

2.3.2 Laboratory Bioassays of 13 Pesticides against Overwintering SLF Egg Masses

In each year, 2021–2023, ten SLF egg masses were randomly selected for each treatment and application timing. The SLF egg masses were treated at one of three different periods during the overwintering life stage: winter (January), early spring (March), and late spring (May). The winter application timing, or 20 weeks before hatch (WBH), represents when growers may have more flexible time to treat egg masses. The early and late spring application timing are 12 and 2 WBH, respectively. The early spring and late spring application timings correlate with growers doing vineyard maintenance, such as vine pruning, dormant oil applications, and fungicide applications (Pfeiffer et al. 2024). I tested an untreated and water check against 13 pesticides, across multiple modes of action, at the highest label rate on grapevines (Table 2.1). All application rates were calculated using 935.4 L ha^{-1} (100 gal ac^{-1}), a typical vineyard application rate in the USA. Applications were made using a 100 mL plastic hand spray bottle. Sprays were made approximately 15–20 cm from the egg mass surface and were applied until runoff. This constituted about 4–5 pumps of the plastic sprayer (approximately 1 mL per pump), aiming to mimic typical bark applications. Egg masses treated during the winter or early spring application timing were briefly removed from cold storage. Egg masses treated during the late spring application timing were not put back into cold storage but instead induced to hatch immediately following the methods mentioned above. All egg masses were held in cold storage for at least 100 days before the hatch was induced. The SLF egg masses were given four weeks to hatch before hatch survivorship data were collected.

Egg mass hatch reduction was analyzed to determine the treatment efficacy. A small fine bristle paint brush was used to remove the proteinaceous covering of each egg mass so that the

number of eggs that hatched or not could be visually quantified. To determine hatch proportions for each replicate, the number of nymphs to hatch was divided by the number of eggs per egg mass. Therefore, a mean hatch percentage closer to 100% indicates that a greater proportion of the SLF emerged from egg masses; conversely, a lower mean percentage indicates fewer SLF nymphs hatched. For each year of the bioassay, the mean percent SLF hatch per application timing and treatment were rank transformed and analyzed with an independent-sample Kruskal-Wallis test performed to determine statistical differences. If statistical differences were observed, a Dunn's step-down pairwise comparison post-hoc test was performed based on the rank sum of each treatment. A Bonferroni correction was performed because of the many pairwise comparisons performed and used to determine significant differences between treatments ($\alpha = 0.05$). All statistics were performed using SPSS 23.

2.3.3 Field Trial of Malathion Against Overwintering SLF Egg Masses

In 2023, two field plots separated by 50 m were in Winchester, VA. 100 SLF egg masses were located and flagged in each field plot for use in the field trial. 50 SLF egg masses within each field plot were allocated to winter or spring application timing groups. Colored push pins were placed adjacent to the egg mass to differentiate between SLF egg masses in the trial and those not in the trial within the same field plot. Each field plot was allocated to one treatment: untreated check or malathion (Malathion 8 Flowable, Gowan® USA). Malathion treatments were applied with a 1 L plastic hand sprayer short of runoff using the maximum label rate of 935.4 L ha^{-1} (100 gal ac^{-1}). Applications were made on 05 January 2023 for the winter treatment and 20 April 2023 for the spring application timing. Egg masses were treated during only one application timing.

The SLF hatch was first observed on 01 May 2023. One month following the observed hatch, SLF egg masses were analyzed one month after the observed hatch to determine treatment effect. The mean percentage hatch was calculated by quantifying the hatch of each egg mass following the methodology mentioned above. The percent hatch was quantified for each egg mass by dividing the total number of eggs by the number of hatched eggs. An independent-sample Mann-Whitney U test was performed to compare differences in mean treatment hatch rates at each application timing. I did not observe signs of parasitism in the 2023 field efficacy study. All statistics were performed using SPSS 23.

2.4 Results

2.4.1 Laboratory Bioassays of 13 Pesticides against Overwintering SLF Egg Masses

450 SLF egg masses were used each year, resulting in a total of 1350 SLF egg masses used in lab bioassays. All egg masses were held in cold storage for 126 days (18 weeks × 7 days) before being induced to hatch. Kruskal Wallis nonparametric tests indicate that the distribution of mean percent hatch was not the same across all years. I observed significant differences in the distribution of mean percent hatch during each year and each application timing (Table 2.2). Across all years and application timings, chlorpyrifos resulted in a 0.00% mean hatch (Table 2.3). Similarly, malathion resulted in a 0.00–3.38% mean hatch during all years and application timings (Table 2.3). The untreated and water checks each resulted in a mean hatch of 32.75–83.80% and 30.23–79.81% respectively (Table 2.3).

Across all years and application timings of the bioassays against overwintering SLF egg masses, I observed a significant difference between the mean hatch of untreated and water checks (Table 2.2). Specifically, in the untreated and water check treatments, the lowest mean percent hatch during the 2022 year of the bioassay, 32.75–59.71% and 30.23–47.83% (Table 2.3), respectively. This trend of lower hatch rates can be observed in all treatments and application timings during 2022.

2.4.2 Field Trial of Malathion Against Overwintering SLF Egg Masses

200 SLF egg masses were used in the field test. I performed four independent-sample Mann-Whitney U tests on the application timing and the treatments. The first two Mann-Whitney U tests indicated that the distribution of mean percent hatch was not the same across all treatments during each application timing (*Winter* $U=2441.50$, $p<0.001$; *Spring* $U=2500.00$, $p<0.001$). Specifically, in the untreated check, I observed mean hatch rates of 95.4–96.8% in the winter and spring application timing (Table 2.4). Conversely, significant mean hatch reductions in the malathion treatments compared to the untreated check during the spring and winter application timings, 6.7% and 1.4%, respectively (Table 2.4). Independent-sample Mann-Whitney U test indicated that the distribution of mean percent hatch in the malathion treatment was not the same across application timings $U=929.00$, $p=0.009$. I did not observe significant differences between the mean percent hatch of the untreated check across the winter and spring application timing $U=1413.00$, $p<0.241$.

2.5 Discussion and Conclusion

Of all the chemical treatments tested in bioassays, chlorpyrifos and malathion were the most effective at 20, 12, and 2 WBH across all three years of the study. At each treatment timing interval, chlorpyrifos (Lorsban®75WG, Gowan® USA) and malathion (Malathion 8 Flowable, Gowan® USA) had significantly reduced mean hatch survivability than the untreated and water check (Table 2.2 and 2.3). Ovicidal effects were observed from chlorpyrifos and malathion treatments, being the only two treatments to cause significant mean hatch reduction when applications were made 20 WBH across all three years (Table 2.2 and 2.3). In 2021 and 2022, chlorpyrifos and malathion treatments were the only treatments that resulted in significant differences between the untreated and water checks (Table 2.2 and 2.3). Both chlorpyrifos and malathion are organophosphates (IRAC group 1B); therefore, similar efficacy could be expected. I included chlorpyrifos as a positive control in the bioassay because it has demonstrated significant ovicidal effects against SLF egg masses (Shin et al. 2010, Leach et al. 2019). Due to court rulings, chlorpyrifos tolerances are reinstated for 2024; the future is uncertain. Stakeholders must identify a viable substitute replicating similar ovicidal effects. This bioassay suggests that a single application of malathion to SLF egg masses before hatch is comparable to chlorpyrifos at all application timings across all years.

In 2023, during each application timing of the bioassay, there were significant treatment effects with all treatments except hexythiazox (in both Savey® 35 SL, Gowan® USA, and Onager® Optek, Gowan® USA) (Table 2.2 and 2.3). I only observed significant differences from the mean percent hatch rates of the untreated and water check, and both hexythiazox treatments during the 12 WBH application timing in 2021 (Table 2.2 and 2.3). Therefore, I do not recommend

applications to overwintering SLF egg masses due to the limited treatment efficacy of both hexythiazox treatments across all years and application timings. Both hexythiazox treatments are typically utilized to control mite eggs and larvae, functioning as a mite growth regulator.

Likewise, the insect growth regulator buprofezin did not show significant treatment effects in 2021 and 2022, excluding 12 WBH in 2021 (Table 2.2 and 2.3). Significant treatment effects were observed for buprofezin during all application timings in 2023, with 41.50-49.34% mean percent hatch rates (Table 2.2 and 2.3). While the hatch reduction of buprofezin in 2023 is significant compared to both checks, they are biologically insignificant compared to the chlorpyrifos and malathion treatments. I propose further investigation be made into the efficacy of buprofezin and other IGRs against SLF. In addition to continuing the ovicidal efficacy of buprofezin against overwintering SLF, the effects of buprofezin against all SLF nymphal stages should also be examined. Furthermore, the lifespan, oviposition rates, and future generation hatch rates of SLF exposed to buprofezin should be explored. Ultimately, the goal was to determine whether this selective insecticide could play a role in the pest management of SLF. Specifically, even though buprofezin may not exhibit the best ovicidal efficacy against SLF, it may hinder the oviposition and lifespan of those SLF surviving ovicidal applications. If determined to be an efficacious insecticide against SLF, buprofezin could provide growers with another tool in the pest management toolbox. Although buprofezin has been shown to have efficacy against hemipterans, in terms of planthopper management, it has demonstrated significant control of the brown rice planthopper (Uchida et al. 1985). The SLF is within an entirely different family of planthoppers, Fulgoridae. Broadening the understanding of buprofezin against additional insect families is essential for understanding the full scope of its control potential. While reports of resistance to

buprofezin and other insecticides have been reported in *N. lugens* (Wang et al. 2008), the SLF is unlikely to develop such resistance due to its univoltine life cycle (Urban 2019). The indirect physiological effects of buprofezin on future brown rice planthopper progeny provided more long-term control than direct control (Nagatai 1986)

All the oil-based pesticides investigated in this bioassay demonstrated 0.0–69.8% mean hatch reduction to overwintering SLF compared to the untreated and water check across all years and application timings. We did observe statistical differences between the two mineral oil treatments across application timing and years, indicating some variability in formulation or SLF egg mass quality. The variability observed from all the oil-based pesticides suggests that some biotic or abiotic variability influences the efficacy against SLF egg masses.

The remaining pesticide treatments, bifenthrin, zeta-cypermethrin, and dinotefuran, followed similar trends observed with the oil-based treatments in laboratory bioassays against overwintering SLF egg masses. Chase and Wright (2023) found that pre-emergent applications of bifenthrin to SLF egg masses did not prevent hatch but killed nymphs shortly after contact with residues applied to the surface of egg masses. The experimental design did not account for any residual efficacy the pesticide treatment may provide. Instead, I only investigated the ovicidal efficacy. Future research should be done to establish the residue efficacy against SLF egg masses.

The field trial in 2023 indicated a significant reduction in the mean percent hatch of SLF egg masses treated with malathion compared with an untreated check at both winter and spring application timings. I found a significant difference between the hatch of SLF egg masses treated

with malathion in winter and spring. However, those significant differences are not biologically substantial, considering both mean percent hatch were 6.69 and 1.35% (Table 2.4). The untreated check did not demonstrate significant differences between application timing, indicating that the natural hatch survivorship of SLF did not vary between plots.

Collection methods of the SLF egg masses likely resulted in undetectable levels of damage to the overwintering embryos, resulting in a reduction in overall hatch across all treatments. Furthermore, each year, I observed that applications at 20 WBH resulted in the lowest mean percent hatch rate for all treatments, including the untreated and water check. Applications made 20 WBH may be efficacious, but overall, I observed the most significant treatment effects when applications were made 2 WBH during all years.

The SLF egg masses hatched within two weeks of being induced to hatch via increased temperature in the environmental chamber. Since I controlled the timing of the SLF hatch, I could apply pesticides accurately within the narrow timeframes. Parasitism was not observed in the field trial. Although parasitism by *O. kuvanae* was detected in the USA on SLF egg masses (Liu 2017), Liu and Mottern (2017) found that parasitism rates were low in the field sites observed from 2016 and 2017.

It is evident from this research that treatments of malathion to overwintering SLF egg masses can significantly reduce egg hatch, providing strong ovicidal effects in both laboratory and field bioassays. While other pesticides did provide strong effects in some years and application timings in laboratory bioassay, the results were less consistent than those offered by malathion.

The intended application method from the data generated would be for licensed stakeholders to make small backpack sprayer applications of malathion to vineyard edges or other areas of high SLF egg mass populations. Strömbom and Pandey (2021) modeled SLF lifecycles under the effects of various management techniques. They found that a minimum of 35% of all SLF life stages must be managed to cause population decline, including treatments of post-diapause SLF egg masses with chlorpyrifos. My study can further aid such models as Strömbom and Pandey (2021) described.

2.6 Acknowledgments

This project was funded by the USDA-NIFA CAP Award 2019-51181-30014 and the Virginia Wine Board (Proposal Number PUVQHEEZ).

2.7 References

Asai, T., M. Fukada, S. Maekawa, K. Ikeda, and H. Kanno. 1983. Studies on the mode of action of buprofezin I. nymphicidal and ovicidal activities on the brown rice planthopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae). Applied Entomology and Zoology 18(4) 550–552. https://doi.org/10.1303/aez.18.550

Asai, T., O. Kajirhara, M. Fukada, and S. Maekawa. 1985. Studies on the mode of action of buprofezin II. effects on reproduction of the brown planthopper, *Nilaparvata lugens* Stål

(Homoptera: Delphacidae). Applied Entomology and Zoology 20(2) 111–117. https://doi.org/10.1303/aez.20.111

Barringer, L., and C. M. Ciafré. 2020. Worldwide feeding host plants of spotted lanternfly, with significant additions from north America. Environmental Entomology 49(5) 999–1011. https://doi.org/10.1093/ee/nvaa093

Chase, K. D., and S. E. Wright. 2023. Preemergent control of spotted lanternfly, 2020. Arthropod Management Tests 48(1)1–2. doi.org/10.1093/amt/tsad001

Choi, D.-S., D.-I. Kim, S.-J. Ko, B.-R. Kang, J.-D. Park, S.-G. Kim, and K.-J. Choi. 2012. Environmentally-friendly control methods and forecasting the hatching time *Lycorma delicatula* (Hemiptera: Fulgoridae) in Jeonnam Province. Korean Journal of Applied Entomology 51(4) 371–376. https://doi.org/10.5656/ksae.2012.09.0.022

Cohen, M. B., and S. N. Alam. 1998. Ovicidal effects of petroleum spray oil on the brown planthopper, *Nilaparvata lugens*. International Rice Research Notes 23(1) 5-6.

Cooperband, M. F., R. Mack, and S. E. Spichiger. 2018. Chipping to destroy egg masses of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). Journal of Insect Science 18(3). https://doi.org/10.1093/jisesa/iey049

Coyle, D. R., J.-H. Chong, and B. A. Blaauw. 2019. Spotted lanternfly management in nurseries, orchards, vineyards, and natural areas in South Carolina and Georgia. www.lgpress.clemson.edu

Dara, S. K., L. Barringer, and S. P. Arthurs. 2015. *Lycorma delicatula* (Hemiptera: Fulgoridae): A new invasive pest in the United States. Journal of Integrated Pest Management 6(1). https://doi.org/10.1093/jipm/pmv021

Dechaine, A. C., M. Sutphin, T. C. Leskey, S. M. Salom, T. P. Kuhar, and D. G. Pfeiffer. 2021. Phenology of *Lycorma delicatula* (Hemiptera: Fulgoridae) in Virginia, USA. Environmental Entomology 50(6) 1267–1275. doi: 10.1093/ee/nvab107.

Executive Order number 13112. 1999. 3 C.F.R. 64(25) 6183–6186. https://www.federalregister.gov/documents/1999/02/08/99-3184/invasive-species.

Ghosal, A., M. L. Chatterjee, and S. K. Mukherjee. 2013. Evaluation of newer insecticides against brown planthopper, *Nilaparvata lugens* (Stal.) on rice. Journal of Crop and Weed 9(1) 116-119.

Harner, A. D., H. L. Leach, L. Briggs, and M. Centinari. 2022. Prolonged phloem feeding by the spotted lanternfly, an invasive planthopper, alters resource allocation and inhibits gas exchange in grapevines. Plant Direct 6(10) 1–18. doi.org/10.1002/pld3.452.

Harper, J. K., W. Stone, T. W. Kelsey, and L. F. Kime. 2019. Potential economic impact of the spotted lanternfly on agriculture and forestry in Pennsylvania. www.rural.palegislature.us.

Hua, L. Z. 2000. List of Chinese insects. Zhongshan (Sun Yat-sen) University Press, Guangzhou 3(1).

Ishaaya, I., and D. Degheele. 1988. Insecticides with novel modes of action. Applied Agriculture 16 385–407.

Izawa, Y., M., Uchida, and M. Yasui. 1986. Mode of action of buprofezin on the twenty-eight-spotted ladybird, *Henosepilachna vigintioctopunctata Fabricius*. Agricultural and Biological Chemistry 50(5) 1369–1371. https://doi.org/10.1080/00021369.1986.10867580

Kanaoka, A., R. Yamaguchi, and T. Konno. 1996. Effect of buprofezin on oviposition of brown planthopper, *Nilaparvata lugens*, at sub-lethal dose. Journal of Pesticide Science 21(1) 153–157.

Kannan, R., and S. Subramanian. 2004. Efficacy of paraffinic oil against the brown planthopper, *Nilaparvata lugens* (Stål). Journal of Applied Entomology 128(3) 177-180. doi:10.1111/j.1439-0418.2004.00830.x

Keena, M. A., and A. L. Nielsen. 2021. Comparison of the hatch of newly laid *Lycorma delicatula* (Hemiptera: Fulgoridae) eggs from the United States after exposure to different temperatures and durations of low temperature. Environmental Entomology 50(2) 410–417. https://doi.org/10.1093/ee/nvaa177

Keller, J., J. Rost, K. Hoover, J. Urban, H. Leach H, M. Porras, B. Walsh, M. Bosold, and D. Calvin. 2020. Dispersion patterns and sample size estimates for egg masses of spotted lanternfly (Hemiptera: Fulgoridae). Environmental Entomology 49(6) 1462–1472. https://doi.org/10.1093/ee/nvaa107

Kim, S.-K., G.-Y. Lee, Y.-H. Shin, and G.-B. Kim. 2010. Chemical control effect against spot clothing wax cicada, *Lycorma delicatula* (Hemiptera: Fulgoridae) nymphs and adults. The Korean Journal of Pesticide Science 14(4) 440–445.

Koul, O., and M. B. Isman. 1991. Efficacy of neem seed extracts against the brown planthopper, *Nilaparvata lugens*. Journal of Economic Entomology 84(2) 680-686. doi:10.1093/jee/84.2.680

Leach, H., D. J. Biddinger, G. Krawczyk, E. Smyers, and J. M. Urban. 2019. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula*, (Hemiptera: Fulgoridae), a new pest of fruit in the Northeastern U.S. Crop Protection 124(1) 1–6. https://doi.org/10.1016/j.cropro.2019.05.027

Leach, H., and A. Leach. 2020. Seasonal phenology and activity of spotted lanternfly *(Lycorma delicatula*) in eastern US vineyards. Journal of Pest Science 93(4) 1215–1224. https://doi.org/10.1007/s10340-020-01233-7

Lee, D. H., Y. L. Park, and T. C. Leskey. 2019. A review of biology and management of *Lycorma delicatula* (Hemiptera: Fulgoridae), an emerging global invasive species. Journal of Asia-Pacific Entomology 22(2) 589–596. https://doi.org/10.1016/j.aspen.2019.03.004

Lee, J.-E., S.-R. Moon, H.-G. Ahn, S.-R. Cho, J.-O. Yang, C. Yoon, and G.-H. Kim. 2009. Feeding behavior of *Lycorma delicatula* (Hemiptera: Fulgoridae) and response on feeding stimulants of some plants. Korean Journal of Applied Entomology 4(1) 467–477. doi:10.5656/KSAE

Lee, K.-Y., S.-K. Kim, I.-H. Kim, and K.-S. Kim. 2011. Seasonal occurrence of spot clothing wax cicada, *Lycorma delicatula* (Hemiptera: Fulgoridae) and it's control efficacy using EFAM at the vineyards. The Korean Journal of Pesticide Science 15(3) 303–309.

Liu, H., and J. Mottern. 2017. An old remedy for a new problem? Identification of *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae), an egg parasitoid of *Lycorma delicatula* (Hemiptera: Fulgoridae) in North America. Journal of Insect Science 17(1). doi.org/10.1093/jisesa/iew114

Liu, H. 2019. Oviposition substrate selection, egg mass characteristics, host preference, and life history of the spotted lanternfly (Hemiptera: Fulgoridae) in North America. Environmental Entomology 48(6) 1452–1468. https://doi.org/10.1093/ee/nvz123

Liu, H. 2020. Seasonal development, cumulative growing degree-days, and population density of spotted lanternfly (Hemiptera: Fulgoridae) on selected hosts and substrates. Environmental Entomology 49(5) 1171–1184. doi.org/10.1093/ee/nvaa074

Nagatai, T. (1986). Timing of buprofezin application for control of the brown planthopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae). Applied Entomology and Zoology 21(3) 357–362.

Park, J. D., M. Y. Kim, S. G. Lee, S. C. Shin, J. H. Kim, and I. K. Park. 2009. Biological characteristics of *Lycorma delicatula* and the control effects of some insecticides. Korean Journal of Applied Entomology 48(1) 53–57. Doi:10.5656/KSAE.2009.48.1.053

Pfeiffer, D. G., A. B. Baudoin, K. B. Rice and M. Nita. 2024. Grapes: diseases and insects in vineyards. 2024 Pest Management Guide for Horticultural and Forest Crops. Virginia Cooperative Extension Publication 456-017 3-1 – 3-16.

Rauh, V. A., R. Garfinkel, F. P. Perera, H. F. Andrews, L. Hoepner, D. B. Barr, R. Whitehead, D. Tang, R. W. Whyatt. 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118 e1845-e1859. doi: 10.1542/peds.2006-0338

Rauh, V., S. Arunajadai, M. Horton, F. Perera, L. Hoepner, D. B. Barr, and R. Whyatt. 2011. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environmental Health Perspectives 119 1196-1201. doi: 10.1289/ehp.1003160.

Rauh, V. A., W. E. Garcia, R. M. Whyatt, M. K. Horton, D. B. Barr, and E. D. Louis. 2015. Prenatal exposure to the organophosphate pesticide chlorpyrifos and childhood tremor. NeuroToxicology 51 80-86. doi: 10.1016/j.neuro.2015.09.004.

Salim, M., and E. A. Heinrichs. 1987. Toxicity of insecticides to the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), and its predators: effects of rates and

timing of application. Journal of Economic Entomology 80(1) 6-12. doi:10.1093/jee/80.1.6.

Shin, Y.-H., S.-R. Moon, C. Yoon, K.-S. Ahn, and G.-H. Kim. 2010. Insecticidal activity of 26 insectcides against eggs and nymphs of *Lycorma delicatula* (Hemiptera: Fulgoridae). The Korean Journal of Pesticide Science 14(2) 157–163.

Smyers, E. C., J. M. Urban, A. C. Dechaine, D. G. Pfeiffer, S. R. Crawford, and D. D. Calvin. 2021. Spatio-temporal model for predicting spring hatch of the spotted lanternfly (Hemiptera: Fulgoridae). Environmental Entomology 50(1) 126–137. https://doi.org/10.1093/ee/nvaa129

Srinivasan, R., and S. Suresh. 2009. Ovicidal activity of zeta-cypermethrin against brown planthopper, *Nilaparvata lugens*. Indian Journal of Plant Protection 37(2) 127-130.

Strömbom, D., and S. Pandey. 2021. Modeling the life cycle of the spotted lanternfly (*Lycorma delicatula*) with management implications. Mathematical Biosciences 340(1) 108670. doi.org/10.1016/j.mbs.2021.108670

Uchida, M., T. Asai, and T. Sugimoto. 1985. Inhibition of cuticle deposition and chitin biosynthesis by a new insect growth regulator, buprofezin, in *Nilaparvata lugens* Stål. Agricultural and Biological Chemistry 49(4) 1233–1234. https://doi.org/10.1080/00021369.1985.10866885

Urban, J. M. 2019. Perspective: shedding light on spotted lanternfly impacts in the USA. Pest Management Science 76(1) 10–17. https://doi.org/10.1002/ps.5619

Urban, J. M., and H. Leach. 2023. Biology and management of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae), in the United States. Annual Review of Entomology 68: 151–167. doi.org/10.1146/annurev-ento-120220-111140.

Uyi, O., J. A. Keller, A. Johnson, D. Long, B. Walsh, and K. Hoover. 2020. Spotted lanternfly (Hemiptera: Fulgoridae) can complete development and reproduce without access to the preferred host, *Ailanthus altissima*. Environmental Entomology 49(5) 1185–1190. https://doi.org/10.1093/ee/nvaa083

Wang, Y., S. Wu, and C. Ma. 2007. The ovicidal activity of bifenthrin against brown planthopper, *Nilaparvata lugens*. Acta Entomologica Sinica 50(1) 15-20.

Wang, Y., C. Gao, Z. Xu, C. Z. Yu, J. Zhang, W. Li, D. Dai, Y. Lin, W. Zhou, and J. Shen. 2008. buprofezin susceptibility survey, resistance selection and preliminary determination of the resistance mechanism in *Nilaparvata lugens* (Homoptera: Delphacidae). Pest Management Science 64(10) 1050–1056. https://doi.org/10.1002/ps.1606

Zehnder, G. W., and D. M Norris. 1995. Methylated soybean oil as an ovicide for the brown planthopper, *Nilaparvata lugens*. Pest Management Science 45(1) 37-42. doi:10.1002/ps.2780450107

2.8 Figures and Tables

Figure 2.1 Individual SLF egg masses collected for laboratory bioassays.

Table 2.1 The treatment list utilized for the 2021–2013 laboratory bioassays of 13 pesticides against overwintering SLF egg masses. The treatment list (product name) indicates the active ingredient and IRAC groups associated with each. The rates used for each treatment were based on the highest label rate. An untreated and water check was used as a comparison.

Treatment	Manufacturer	Active Ingredient	IRAC Group	Percent AI	Rate per ha (g or ml)
Untreated Check	-	-	-	-	-
Water Check	-	-	-	-	-
Lorsban®75WG	Gowan® USA	chlorpyrifos	1B	75.00	1491
Malathion 8 Flowable	Gowan® USA	malathion	1B	79.50	2198
Talstar®P	FMC Corporation	bifenthrin	3A	7.90	2923
Mustang® Maxx	FMC Corporation	zeta-cypermetherin	3A	9.15	292
Scorpion® 35SL	Gowan® USA	dinotefuran	4A	35.00	384
Savey® 35 SL	Gowan® USA	hexythiazox	10A	50.00	420
Onager® Optek	Gowan® USA	hexythiazox	10A	11.93	1754
Applaud 70DF	Nichino America, Inc.	buprofezin	16	70.00	1681
Trilogy®	Certis Bio LLC.	clarified hydrophobic extract of neem oil	UNE	70.00	18707
JMS Stylet Oil	JMS Flower Farms, Inc.	paraffinic oil	UNE	97.10	18707
MSO	Southern Ag®	methylated soybean oil and surfactant blend	UNE	100.00	2338
Damoil™	Drexel Chemical	mineral oil	UNM	70.00	28061
Mite-E-Oil®	HELENA®	mineral oil	UNM	90.00	28061

Table 2.2 Kruskal-Wallis nonparametric statistical tests for laboratory bioassays against SLF egg masses from 2021–2023.

Year	Weeks Before Hatch (WBH)	Test Statistic (H)	DF	p-value
2021	2	77.554	14	<.001
2021	12	82.864	14	<.001
2021	20	59.693	14	<.001
2022	2	97.220	14	<.001
2022	12	64.656	14	<.001
2022	20	48.011	14	<.001
2023	2	108.860	14	<.001
2023	12	95.326	14	<.001
2023	20	100.535	14	<.001

Table 2.3 Percent mean hatch in laboratory bioassays of 13 pesticides at 20, 12, and 2 weeks before hatch (WBH) from 2021–2023.[1]

	2021			2022			2023		
	Weeks Before Hatch			Weeks Before Hatch			Weeks Before Hatch		
	20	12	2	20	12	2	20	12	2
Treatment	Mean Hatch (%)			Mean Hatch (%)			Mean Hatch (%)		
Untreated Check	52.12 ab	76.94 a	80.56 ab	32.75 a	49.11 a	59.71 a	78.21 a	83.80 a	83.54 a
Water Check	49.77 a	79.38 a	82.11 a	30.23 a	47.89 a	47.12 b	75.86 a	77.82 a	79.51 a
Lorsban®75WG	0.00 c	0.00 d	0.00 e	0.00 c	0.00 c	0.00 g	0.00 f	0.00 d	0.00 c
Malathion 8 Flowable	0.00 c	0.00 d	0.00 e	1.59 b	0.31 c	1.52 efg	3.38 f	0.00 d	0.00 c
Talstar®P	18.96 b	14.48 bc	63.09 abcd	23.76 a	49.21 a	12.80 cde	36.05 de	43.71 bc	30.68 b
Mustang® Maxx	18.63 b	31.42 bc	66.19 bcd	22.49 a	42.15 ab	6.79 defg	45.80 cde	42.48 c	34.68 b
Scorpion® 35SL	21.32 ab	12.10 bc	51.86 d	28.00 a	19.64 b	16.74 cdef	39.01 cde	32.82 c	31.69 b
Savey® 35 SL	33.52 ab	15.09 b	80.00 abcd	27.32 a	46.31 a	40.29 bc	67.76 ab	70.50 a	82.42 a
Onager® Optek	32.85 ab	39.54 bc	81.48 abc	28.98 a	47.20 ab	36.82 bc	72.63 abc	76.14 ab	79.10 a
Applaud 70DF	22.60 b	30.78 bc	80.17 a	26.04 a	24.90 ab	24.51 bcde	49.34 cde	45.89 bc	41.50 b
Trilogy®	24.36 ab	34.37 b	55.06 d	22.14 a	39.88 ab	26.64 bcde	52.99 bcd	51.82 bc	38.00 b
JMS Stylet Oil	16.69 b	39.11 b	49.41 d	21.49 a	49.13 a	0.00 g	51.10 cd	53.42 bc	19.93 b
MSO	23.88 ab	34.65 bc	63.13 cd	23.20 a	47.06 a	0.00 fg	48.83 cde	52.40 bc	23.60 b
Damoil™	17.43 b	6.44 c	66.20 cd	15.20 a	26.78 ab	0.00 efg	28.12 e	32.29 c	21.62 b
Mite-E-Oil®	19.68 b	24.81 bc	69.75 abcd	15.74 a	24.96 ab	4.84 gfed	30.34 de	29.44 c	23.51 b

[1] Data were ranked sum transformed for an independent sample Kruskal-Wallis test, and untransformed mean percent data is shown. Dunn's pairwise comparison post-hoc test was performed based on the rank sum of each treatment. A Bonferroni correction was performed; therefore, more conservative significant differences were observed. Data within columns followed by a letter in common are not significantly different; $p<0.05$.

Table 2.4 Field efficacy of malathion against applied to overwintering SLF egg masses.[1]

	Application Timing			
	Winter		Spring	
Treatment	Hatched Eggs	Mean Hatch (%)	Hatched Eggs	Mean Hatch (%)
Malathion 8 Flowable	104	6.69 b	20	1.35 b
Untreated Check	1741	95.37 a	1775	96.75 a

[1]An independent-sample Mann-Whitney U test was performed to compare differences in mean treatment hatch rates at each application timing. Data within columns followed by a letter in common are not significantly different; $p<0.05$.

Chapter 3: Bioassays and field trials of commercially available *Beauveria bassiana* mycoinsecticides against overwintering spotted lanternfly (*Lycorma delicatula*) egg masses

Jason Tyler Bielski

3.1 Abstract

The efficacy of *Beauveria bassiana* against nymphal and adult spotted lanternfly has previously been demonstrated in laboratory and field settings. In 2021–2023, I conducted laboratory bioassays that suggested treatments of commercially available biopesticidal fungi, made to spotted lanternfly (*Lycorma delicatula*) egg masses, resulting in newly emerged neonates infection. Specifically, a commercially available application of *B. bassiana* was made directly on overwintering egg masses, exposing the hatching neonates to enough viable spores to become infected. In laboratory studies, the optimal timing of spray applications on spotted lanternfly egg masses was approximately two weeks before hatch. However, infection was observed in newly emerged nymphs when treatments were applied even 20 weeks before the hatch was induced. Two field trials were conducted to demonstrate the real-world applicability of mycoinsecticide treatments to overwintering spotted lanternfly egg masses. Both field trials demonstrated infection of hatching nymphs at levels greater than those observed in laboratory bioassays. The intention of this research is to provide stakeholders with additional novel tools to manage spotted lanternfly. This management approach would provide growers with a viable, low-toxicity, and OMRI-rated solution for managing spotted lanternfly as they hatch with applications of mycoinsecticides months to weeks before the predicted hatch of SLF.

3.2 Introduction

The spotted lanternfly (SLF), *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), is an optically remarkable planthopper native to the eastern regions of Asia (Lee et al. 2019). In the past few decades, this fulgorid has developed an infamous reputation due to its propensity for human-mediated spread (Keller et al. 2020). Non-native populations of SLF were first detected in Berks County, Pennsylvania, USA, by the Pennsylvania Department of Agriculture in 2014 (Barringer et al. 2015). Before their introduction into the USA, SLF had rapidly spread throughout Korea and Japan, where these insects were problematic for tree fruit and vineyards (Han et al. 2008, Tomisawa et al. 2013). In the USA, South Korea, and Japan, SLF has caused the most significant economic impact on grape vineyards (Park et al. 2009, Barringer et al. 2015, Urban 2019). Economic analysis in Pennsylvania suggested that the SLF has already caused an annual loss of hundreds of jobs and over $50 million to the state, with financial losses projected to drastically increase as the SLF spreads (Harper et al. 2019).

All life stages of SLF are commonly seen aggregating in dense populations on various host plants (Urban 2019). Typical of phloem-feeding insects, SLF produces copious quantities of honeydew, resulting in the growth of sooty mold, further reducing photosynthesis and potentially making the fruit unmarketable (Dara et al. 2015). Large populations of SLF can be seen in infested vineyards between spring and late summer (Leach and Leach 2020). In their native ranges, SLF do not pose much of an economic disturbance but have been documented to cause some damage to trees and small brush (Zhang 1993). Conversely, in their non-native geographic range, SLF has been observed to prefer grapevines and other fruit trees (Lee et al. 2009, Barringer et al. 2015,

Urban 2023). Harner et al. (2022) found that high populations of SLF can disrupt grapevine gas exchange and change resource allocation when they feed for extended periods. Intense feeding has been documented in vineyards to influence vine hardiness negatively and significantly reduce vegetative growth, yield, and bud set (Urban 2019, Leach and Leach 2020).

SLF nymphs and adults are vulnerable to various insecticidal modes of action (Leach et al. 2019). In vineyards, SLF adults are the critical target life stage for pest management tactics because the adults tend to aggregate in vineyards around the time of grape harvest (Leach and Leach 2020). In 2018, vineyards in Pennsylvania at the center of the infestation area reported nearly triple the insecticide applications and cost to manage influxes of adult SLF from the neighboring landscape (Urban 2019).

Unfortunately, limited organic and biological control methods are currently available for SLF management in the USA. Naturally occurring entomopathogenic fungi, *Batkoa major* (Thaxt.) Humber (Entomophthorales: Entomophthoraceae), and *Beauveria bassiana* (Bals.-Criv.) Vuill (Hypocreales: Cordycipitaceae) has been observed to cause the collapse of SLF adults in the localized population in PA, USA (Clifton et al. 2019). Upon further investigation, Clifton et al. (2021) identified two additional Hypocrealean fungi, *Metarhizium pemphigi* (Driver and Milner) Kepler, Rehner and Humber (Hypocreales: Clavicipitaceae), and *Ophiocordyceps delicatula* (Clifton, Castrillo, and Hajek) (Hypocreales: Ophiocordycipitaceae), naturally infecting SLF in PA, USA. From 2018–2020, *Ba. major* displayed the most significant local population decline across the two field sites in PA, USA (<0.5 ha) (Clifton et al. 2019, Clifton et al. 2021). Although

Ba. major has been observed in the USA to infect five insect orders (Gryganskyi et al. 2022), Entomophthorales fungi species are not well understood and are thought to have a limited host range (Boomsma et al. 2014, Hajek et al. 2022). Hajek et al. (2022) conducted laboratory bioassays with cultured *Ba. major* and found that all life stages of SLF were susceptible to infection. Although commercial production of Entomophthorales fungi has yet to be achieved, Hajek et al. (2022) demonstrated that despite complex culturing and production challenges, further research into the utilization of *Ba. major* should be investigated.

Unlike *B. bassiana,* the other species (*Ba. major*, *M. pemphigi, and O. delicatula*) are not commercially available. Several different formulations and strains of *B. bassiana* are commercially available and organically certified. Furthermore, more is needed to know about the natural distribution of *B. bassiana, Ba. major*, *M. pemphigi, and O. delicatula* in the USA. In laboratory bioassays, Clifton and Hajek (2022) utilized conditions optimal for infection with mycoinsecticides in laboratory bioassays and found that direct applications against SLF nymphs and adults resulted in significant mortality and cadaver sporulation after 14 days. In semi-field trials on potted grapevines and field trials, multiple strains and formulations of the biopesticides demonstrated considerable efficacy against SLF adult populations with contact and residual applications (Clifton et al. 2020). In the same study, Clifton et al. (2020) demonstrated that area-wide applications of *B. bassiana* GHA (BoteGHA®, Certis Bio LLC.) significantly reduced fourth instar SLF when compared to untreated plots; furthermore, few impacts were observed in nontarget arthropods in treated field sites. Additionally, *Cordyceps javanica* (Frieder and Bally) Samson & Hywel-Jones (Hypocreales: Cordycipitaceae) (strain PFR-97®, Certis Bio LLC.) biopesticide was

also investigated in laboratory bioassay and field studies, and while infection rates were not as high, *B. bassiana* infection did occur (Clifton et al. 2020, Clifton and Hajek 2022).

Although much research has focused on nymph and adult SLF management, little has focused on overwintering SLF egg masses, representing the longest immobile individual life stage (Shin et al. 2010, Leach et al. 2020). To date, no research has investigated whether applications of *B. bassiana* to overwintering SLF egg masses can survive until the predicted hatch and subsequently infect the hatching nymphs. Here, I examined the efficacy of different formulations and strains of *B. bassiana* at multiple seasonal timings during the SLF overwintering period.

Laboratory bioassays and field efficacy studies were conducted from 2021–2023 to investigate these objectives. I started with laboratory bioassays, where SLF egg masses were collected and treated with several *B. bassiana* strains and formulations in winter and spring. The infected SLF egg masses were then held under laboratory conditions and subsequently stimulated to hatch, then reared on tree-of-heaven (TOH), *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae), and were observed for signs of infection. I hypothesized that SLF neonates would encounter enough viable *B. bassiana* spores contaminating the egg masses' surface for infection and subsequently becoming infected, resulting in death within a few days. In addition to laboratory bioassays, I also examined the efficacy of applications of *B. bassiana* under field conditions where I anticipated infection to occur but at reduced compared incidences to laboratory bioassays. Lastly, I examined the capability of commercially available *B. bassiana* to survive on the surface of SLF

egg masses when exposed to natural freeze-thaw cycles of winter, simulating the effects of winter application of the fungus to egg masses.

As outlined in Ortiz-Urquiza et al. (2013), infections from *B. bassiana* begins with the attachment of spores to the insect's cuticle, usually within 24–48 hours after contact. Germination of the spore depends on the host, host life stage, virulence, and environmental factors. Penetration through the insect cuticle can occur within 1–2 days. Generally, penetration is focused on areas of the cuticle that are thin and less sclerotized. Vegetative growth begins within the host, overcoming host defenses (3–4 days generally, but can take longer for larger insects, up to 2–4 weeks) and eventually developing a mycelium network throughout the infected insect. With *Beauveria*, the host subsequently dies due to the sapping of nutrients in the host's hemolymph and fat stores; additionally, the production of secondary metabolites produced by the fungus can result in the mortality of the infected host. External growth on the surface of the cadaver and conidial production will occur if environmental conditions (100% humidity for several days) are adequate. In this research, I utilized *B. bassiana* mycoinsecticides against overwintering SLF, where I anticipated the hatching nymphs would come into contact with viable spores and become infected. Spot treatment of egg masses with fungus sprays also fits within the vineyard management regime, especially during periods of minimal workload from other tasks.

3.3 Methods

3.3.1 SLF Egg Masses Collection and Storage for Laboratory Bioassays

From 2021–2023, the SLF egg masses were collected at various Frederick County, Virginia field sites. Egg masses were conveniently sampled from the landscape, mainly from TOH, but not exclusively. In 2021 and 2022, the SLF egg masses were collected individually and removed from the plant substrate with the bark attached. Egg masses were chiseled around, and then the bark was gently peeled off with the SLF egg mass attached. In 2023, I opted for a technique to collect SLF egg masses that would reduce the likelihood of unforeseen damage to the egg masses due to extraction from plant bark. Therefore, no individual SLF egg masses were removed from plant material; infested plant material, such as low-hanging branches, was removed instead. Plant material infested with SLF egg masses was further cut down to fit into insect-rearing cages to standardize the number of SLF egg masses per replicate.

For 2021 and 2022 laboratory bioassays, all SLF egg masses were collected in December of the year prior (2020 and 2021, respectively) and then moved to climate-controlled cold storage (Table 3.1). In 2023, the SLF egg masses were collected on 01–03 March 2023 and were stored in a climate-controlled cold storage until 20 April 2023 (Table 3.1). Only SLF egg masses with intact protective proteinaceous covering were selected for the experiment in all years. Individual egg masses were stored in Petri dishes of various sizes; alternatively, infested branches were stored in plastic totes.

The SLF egg masses were brought to the Alson H. Smith Jr. Agricultural Research and Extension Center, Winchester, VA, and stored in a climate-controlled incubator. The incubators were held at 10°C, 65% relative humidity, and a 16:8 light-to-dark ratio to simulate overwintering conditions. In 2021 and 2022, egg masses were stored for at least 100 days and were induced to hatch by removing from the climate-controlled incubator and exposed to 25°C, 65% relative humidity, and a 16:8 light-to-dark ratio (Keena and Nielsen 2021). The predicted SLF hatch was estimated using 200 cumulative growing degree days, with an upper degree threshold of 35°C and a lower degree threshold of 10°C (Liu 2020). In 2023, SLF egg masses were stored in cold storage until local populations from the collection site were observed to hatch naturally.

3.3.2 Tree-of-Heaven Propagation for Laboratory Bioassays

TOH was propagated in the Virginia Tech greenhouses. The TOH, a preferred host plant, was intended to supply recently emerged SLF nymphs a feeding source during the infection period of the laboratory bioassays in 2021–2023. An infection period was required to quantify the proportion of infection rather than relying on the mortality rate. The infection period, or post treatment observation period, allowed the SLF nymphs sufficient time to come into contact with the spores as they hatch and become infected with *B. bassiana*. The applied *B. bassiana* spores required adequate time to germinate, penetrate the insect cuticle, overcome host defenses, and develop a mycelium network (Zimmerman 2007, Ortiz-Urquiza et al. 2013). All TOH utilized in the study were grown from seeds locally collected in Blacksburg, VA. Seeds were stored in the freezer for at least a month before germination. Germination was achieved in seed trays (0.5 x 0.25 m), where 16 seeds were planted directly into damp Miracle-Gro® Potting Mix (Marysville, Ohio);

this soil medium was utilized for the entire study. After seedlings reached 10–15 cm in height, they were transferred to 3.75 L black plastic horticultural pots with drain holes. Plants were watered as needed, and no additional fertilizer was used. If necessary, pesticides were used to reduce greenhouse pests such as *Aculops ailanthi* (Lin, Jin, and Kuang) (Trombidiformes: Eriophyidae). Pesticide applications were of low toxicity and consisted of rotations of neem oil, mineral oil, and insecticidal soaps. Pesticide applications were halted one month before the TOH seedlings were utilized in the study. Seedlings, at least one year after germination and 20–60 cm in height, were brought to the Alson H. Smith Jr. Agricultural Research and Extension Center, Winchester, VA, for utilization in the laboratory bioassay trials.

In 2021, TOH was only six months past germination. Potted TOH were placed in insect-rearing cages (45.7 x 45.7 x 61.0 cm) (Figure 3.1) to allow hatching SLF nymphs to feed on a stable food source between exposure to infectious spores and either death or collection for further analysis. Potted and caged TOH were exposed to a plant grow light at a 16:8 light-dark cycle. Each potted TOH was covered with a mesh paint strainer to prevent the nymphs from walking on the soil medium or falling into the water runoff tray. In 2021 and 2022, one SLF egg mass and potted TOH were placed together in an insect-rearing cage. In 2023, two potted TOH were placed with SLF egg mass-infested plant materials. More TOH were utilized in 2023 because of the increased number of egg masses per infested plant material replicate.

3.3.3 Surface Sterilization of SLF Cadavers and Plating to Determine Infection with *B. bassiana*

Cadavers previously collected following a laboratory bioassay or field trial were thawed out for five minutes. Once thawed, the SLF cadavers were immersed and agitated in diluted 0.5–0.8% sodium hypochlorite solution for one minute. Next, cadavers were transferred to another solution of deionized water, where they were immersed and agitated for 15–20 sec. Then, cadavers were transferred to a third solution of deionized water for 5–10 sec. Finally, the excessive solution was removed from the surface-sterilized SLF nymphs on filter paper in a Buchner funnel, and excess fluid was drawn off under a vacuum pump. Surface sterilized cadavers were placed and transferred to a dodine and potato dextrose agar. A dodine addition, with 150 mg L^{-1} chloramphenicol, crystal violet, or similar antibiotics, was utilized to prevent cross-contamination of spore samples (Chase et al. 1986). Plates were stored at room temperature in the dark. Plated nymphs were observed for four days to allow any internal *Beauveria* infections to emerge and sporulate if they were present within the insects; if they were present, the insect would be assumed to be infected. Any more than four days and contaminants would begin to grow on the plate. Infection signs for *B. bassiana* were easily identifiable due to the white, powdery mycelium characteristics of *B. bassiana* emerging from intersegmental regions of the cadavers (Figure 3.2). The spore shape and other microscopic characteristics could visually verify investigation of questionable infections. Cadavers with infections that could not be verified as *B. bassiana* were considered uninfected.

3.3.4 Laboratory Bioassays of *B. bassiana* Biopesticides Against Overwintering SLF Egg Masses

In 2021, ten SLF egg masses were randomly selected for each application timing (winter and spring) and treatment. One strain of *B. bassiana* was utilized, GHA (the active ingredient in BotaniGard®, Certis Bio LLC.). Two formulations were investigated: a wettable powder (BotaniGard® 22WP, Certis Bio LLC.) and an emulsifiable suspension (BoteGHA® ES, Certis Bio LLC.) (Table 3.2). The highest label rate of *B. bassiana* GHA ES was utilized to standardize the application rates, 2.4 kg ha^{-1} (equal to $1.06x10^{14}$ conidia ha^{-1}). All other treatments were standardized by the spores mL^{-1} to the same power of ten (Table 3.2). A standard vineyard application rate of 935.40 L ha^{-1} (100 gal ac^{-1}) is a typical vineyard application rate in the USA. All the *B. bassiana* treatments were compared against an untreated check and a water check. An organosilicon surfactant (Silwet L77® surfactant, Loveland Chemicals Inc.) was added to the WP treatment to help better suspend the spores in the solution at a concentration of 0.03%. SLF egg masses were treated at two different timings, 20 (winter) and 2 (spring) weeks before the induced hatch. These two applications correspond with a winter application and a spring application. The spring application was intended to be as close to the predicted hatch as possible. In contrast, the winter application would represent a period that a vineyard may be in maintenance mode. Applications were made with 100 mL plastic hand spray misters. Each egg mass was sprayed approximately 10–15 cm away from the egg mass surface. Egg masses were sprayed until just before the product ran off from the surface of the egg mass. This constituted about 4–5 pumps of the plastic sprayer (approximately 1 mL per pump), aiming to mimic typical bark applications. The winter application was made on 08 February 2021, and the spring application was made on 14 June 2021 (Table 3.1). Egg masses treated in winter were temporarily removed from cold

storage for applications and immediately returned to cold storage. Winter-treated egg masses were then allowed to dry thoroughly before being put back into climate-controlled storage. Spring treatments were not put back into cold storage but were immediately induced to hatch. Once the hatch occurred, SLF were given seven days in cages before being collected for analysis. The nymphs were collected utilizing a hand-held manual aspirator. After collection, SLF were artificially killed with cold, and then frozen to preserve the specimen cadavers for further analysis.

In 2022, ten SLF egg masses were randomly selected for each application timing and treatment. Two strains of *B. bassiana* were utilized, GHA (BotaniGard®, Certis Bio LLC.) and ANT03 (BioCeres®, Anatis Bioprotection). Two formulations of each *B. bassiana* strain were investigated: a wettable powder (WP) (GHA and ANT03), an emulsifiable solution (ES) (GHA), and an emulsifiable concentration (EC) (ANT03) (Table 3.2). Winter treatment (20 WBH) was applied on 15 February 2022, while spring (2 WBH) treatments were applied on 21 June 2022 (Table 3.1). Application techniques and specimen sampling methodology were replicated in 2021.

In 2023, ten branches with SLF egg masses were randomly selected for application for each treatment. Only the spring application timing was investigated in 2023. Treatments were the same as in 2022, including two strains and two formulations of each strain of *B. bassiana*. Specimen sampling techniques were replicated from 2021 and 2022. Mycoinsecticide applications were made on 20 April 2023 (Table 3.1).

All egg masses were visually inspected to quantify the number of SLF to hatch per egg mass. To quantify the proportion of SLF to hatch, any remaining proteinaceous covering from the egg mass was removed with a fine-bristled paintbrush, and exit holes were quantified. Parasitism holes were differentiated from regular occlusion holes by their more circular appearance compared to the elliptical appearance of the latter (Liu and Mottern 2017). To determine hatch proportions for each replicate, the number of nymphs to hatch was divided by the number of eggs per egg mass. Therefore, a mean hatch percentage closer to 100% indicates that a greater proportion of the SLF emerged from egg masses; conversely, a lower mean percentage indicates fewer SLF nymphs hatched. Mean hatch and infection were quantified for each treatment and application timing. An independent-sample Kruskal-Wallis test was performed to determine statistical differences between each treatment's mean percent hatch and mean percent infection during each application time. Separate Kruskal-Wallis tests were performed for each application timing (winter and spring) in 2021 and 2022. If statistical differences were observed, Dunn's step-down pairwise comparison post-hoc test was performed based on the rank sum of each treatment. A Bonferroni correction was performed because of the high number of pairwise comparisons performed and used to determine significant differences between treatments ($\alpha = 0.05$). All statistics were performed using SPSS 23.

3.3.5 Field Trial of a *B. bassiana* Biopesticide Against Overwintering SLF Egg Masses

In spring 2023, two field plots separated by 50 m were located in Winchester, VA. 50 SLF egg masses were found and flagged within each field plot for use (Table 3.5). Colored push pins were placed adjacent to the egg mass to differentiate between SLF egg masses in the trial and those

not in the trial within the same field site. Each field plot was allocated to one treatment: untreated check or *B. bassiana* GHA ES (BoteGHA® ES, Certis Bio LLC.). Treatments were applied with a 1 L hand sprayer until just short of runoff using the maximum label rate (4.68 L ha^{-1}, $1x10^{14}$ conidia ha^{-1}) at 935.40 L ha^{-1}. SLF egg masses were treated on 20 April 2023, and hatch was observed on 01 May 2023. One week following the observed hatch, SLF nymphs were sampled from a square meter surrounding the treated egg masses. The nymphs were collected utilizing a handheld vacuum aspirator. Collected SLF were artificially killed with cold exposure and held in a freezer for further analysis.

In the 2023 field trial against overwintering SLF egg masses, the mean percentage hatch was calculated by quantifying each egg mass hatch proportion, following the methodology mentioned above. The proportion of infection was quantified for each treatment by dividing the total number of infected nymphs by the total number of nymphs collected. An independent sample Mann-Whitney U test was performed to compare differences in mean treatment hatch rates. A Fisher's Exact Test was conducted to determine if there were significant differences in the percentage of infection between treatments. All statistics were performed using SPSS 23.

3.3.6 Field Trial of a *B. bassiana* Biopesticide Against Hatching SLF Nymphs

Six red maple, *Acer rubrum* (Linnaeus) (Sapindales: Sapindaceae), trees were located in a single field plot in Winchester, VA. Each tree was visually inspected before the trial to determine that a high population of overwintering SLF was present. Each tree was randomly assigned to one

of two treatment groups: untreated check or *B. bassiana* GHA ES (BoteGHA® ES (Table 3.6). Treatments were applied when the SLF hatch was detected at the field plot. Hatch was observed on May 1, 2023. Trees were treated using a Tomahawk® 14L backpack sprayer (Tomahawk Power LLC.), utilizing the maximum label rate of *B. bassiana* GHA ES (4.68 L ha^{-1}) at 935.40 L ha^{-1}. Each *B. bassiana* GHA ES treatment tree received two full spray tanks to achieve full coverage. The output of the airblast sprayer was 103.45 mL sec^{-1} (6.21 L min^{-1}). Nymphs were collected, and 3-minute visual counts of SLF populations on each tree were performed at 0, 5, and 10 days after treatment (DAT). No visual aid tools were utilized for the timed visual counts. Nymphs were collected by climbing trees and using a handheld vacuum aspirator to sample as many nymphs as possible conveniently. Collected nymphs were killed with cold exposure and held in a freezer for further analysis.

In the 2023 field trial against hatching nymphs, 3-minute visual counts of SLF populations on each tree were performed at 0, 5, and 10 DAT. The mean SLF population per treatment was calculated. The mean percent infection was quantified from each treatment at each DAT interval. To calculate the mean proportion of infected nymphs, the quantity of collected infected nymphs was divided by the number of collected nymphs per replicate. An independent sample Mann-Whitney U test was performed to determine significant differences between mean infection and visual counts against treatment across each sampled DAT. An independent-sample Kruskal-Wallis test was performed to determine significant differences in the total nymphs collected across all treatments between 0, 5, and 10 DAT. Dunn's pairwise comparison post-hoc test was performed based on the rank sum of each treatment. A Bonferroni correction was performed because of the

many pairwise comparisons performed and used to determine significant differences between treatments ($\alpha = 0.05$). All statistics were performed using SPSS 23.

3.3.7 *Beauveria bassiana* Biopesticide Winter Survival on SLF Egg Masses

In 2021, three SLF egg masses were randomly selected and utilized per treatment (Table 2.7). Treatments were applied following the same methodology outlined for the laboratory bioassay in 2021–2023 (Table 3.2). In 2021, the winter survivability of one strain of *B. bassiana* was investigated, GHA (the active ingredient in BotaniGard®, Certis Bio LLC.). Two formulations were investigated: a wettable powder (BotaniGard® 22WP, Certis Bio LLC.) and an emulsifiable suspension (BoteGHA® ES, Certis Bio LLC.) (Table 3.2). The SLF egg masses were treated at 20 WBH with or without natural winter exposure. To mimic natural overwintering conditions, SLF egg masses were held outside and exposed to winter conditions or in an environmental chamber to limit extraneous ecological factors. Egg masses exposed to actual winter conditions were held at a field plot in Hamburg, PA (LABServices Inc.). SLF egg masses kept in an environmental chamber were exposed to 10℃, 65% relative humidity, and an 18:6 hr light-dark cycle. The SLF egg masses were treated on 04 January 2021 and stored until 24 May 2021.

In 2022, 6-16 SLF egg masses were randomly utilized per treatment (Table 3.7). Two strains of *B. bassiana* were utilized, GHA (BotaniGard®) and ANT03 (BioCeres®, Anatis Bioprotection). Two formulations of each *B. bassiana* strain were investigated: a wettable powder (WP) (GHA and ANT03), an emulsifiable solution (ES) (GHA), and an emulsifiable concentration

(EC) (ANT03) (Table 3.2). Each treatment was compared to an untreated check. Additionally, another group of egg masses was treated not at 20 WBH, but at 2 WBH and not exposed to natural field conditions to act as a positive control for spore germination when applied to SLF egg masses. The SLF egg masses were treated on 03 January 2022 (20 WBH) and 09 May 2023 (2 WBH). Eggs were stored until 23 May 2022.

In both years, treatments followed those utilized in the laboratory bioassays (Table 2). After exposure to natural environmental conditions or simulated winter conditions, the SLF egg masses were rinsed of any remaining spores using a 0.05% organosilicon surfactant (Silwet L-77®). Spores were removed by gently rolling a sterile cotton swab, moistened with Silwet, against the egg mass, then transferring any collected spores into a solution of 0.05% Silwet. 1 mL of the spore-laden solution was spread plated onto potato dextrose agar with dodine (as Syllit 65W, 0.92g L^{-1} agar), where potential germination was allowed to occur. Plates were stored and incubated in dark conditions, at room temperature, for 18 hours. After 18 hours, 200 spores were randomly counted from each replicate and visually inspected for evidence of germination utilizing 400X phase contrast microscopy. Agar plugs were stained with lactophenol cotton blue to aid in distinguishing germinated spores. Those spores that successfully germinated were considered to have survived the treatment exposure. This method of sampling SLF egg masses was destructive to the SLF egg mass.

In both years, an independent-sample Kruskal-Wallis test was performed to determine statistical differences between treatments during each application timing and environmental

exposure. A Dunn post-hoc test was performed for multiple pairwise comparisons of treatments. A Bonferroni correction was performed to determine significant differences between treatments ($\alpha = 0.05$). All statistics were performed using SPSS 23.

3.4 Results

3.4.1 Laboratory Bioassays of *B. bassiana* Biopesticides Against Overwintering SLF Egg Masses

In 2021, signs of infection were observed in 65 surface sterilized cadavers throughout the trial bioassay. There was no significant difference between the mean percent hatch between treatments at either application timing, *20 WBH* $H(3)=2.263$, $p=0.520$; *2 WBH* $H(3)=6.158$, $p=0.104$ (Table 3.3). The mean percent hatch of the untreated and water check was low between both application timings at 10.1–23.6% (Table 3.3). I did not observe signs of infection in any surface sterilized cadavers in either the untreated or water check during both application timings. Furthermore, significant differences were not observed between all treatments' mean percent infection at 20 WBH, $H(3)=6.178$, $p=0.103$ (Table 3.4). Although infection occurred at the 20 WBH application timing, the mean percent infection was <1% (Table 3.4). Significant differences between all treatments mean percent infection were observed at 2 WBH application timing, $H(3)=18.466$, $p<0.001$ (Table 3.4). At the 2 WBH application timing I observed mean percent infection rates from 2.6–4.6% (Table 3.4). Although significantly different from both checks, I did not observe significant differences between either *B. bassiana* GHA formulation during the 2 WBH application timing.

In 2022, signs of infection were observed in 180 surface sterilized cadavers throughout the trial bioassay. A low mean percent hatch (<10%) was observed from SLF egg masses during the 20 WBH application time (Table 3.3). The mean percent hatch increased during the spring application timing, ranging from 18.0–68.7% (Table 3.3). There were no statistical differences between treatment mean percent hatch during the winter application timing, $H(5)=10.289$, $p=0.067$, while during the spring application timing, treatment differences were significant $H(5)=20.507$, $p=0.001$ (Table 3.3). Neither untreated nor water checks resulted in infection during both application timings. Conversely, infection did occur in each *B. bassiana* treatment at both application timings, despite strain or formulation. Excluding both check treatments, the mean percent infection during the winter and spring application ranged from 1.1–44.0% to 18.2–43.1% (Table 3.4). Significant differences were observed between the mean percent infection across treatments during winter [$H(5)=18.804$, $p=0.002$] and spring [$H(5)=21.570$, $p<0.001$] applications (Table 3.4).

In 2023, signs of infection were observed in 133 surface sterilized cadavers. The mean percent hatch ranged from 52.2–76.4% (Table 3.4). Kruskal-Wallis tests indicated significant differences between treatments and mean percent hatch $H(5)=29.311$, $p<0.001$. Specifically, all the *B. bassiana* treatments resulted in a hatch reduction at 2 WBH. After surface sterilization and plating of collected cadavers, no signs of infection were detected in either of the check treatments. The mean percent infection of all the *B. bassiana* treatments ranged from 5.8–7.3% (Table 3.4).

Significant differences between all *B. bassiana* treatments and the checks were observed $H(5)=39.054$, $p<0.001$, but not among the *B. bassiana* treatments.

3.4.2 Field Trial of a *B. bassiana* Biopesticide Against Overwintering SLF Egg Masses

100 SLF egg masses were utilized for this field trial across two sites. 1197 SLF nymphs were collected from the areas directly proximal to the treated egg mass, no more than 1 meter away from the treated egg mass. All SLF egg masses utilized in this field trial were located and capable of quantifying the mean percent hatch. The mean percent hatch rates for the untreated check and *B. bassiana* GHA ES treatments were 96.3% and 93.4% (Table 3.5). An independent sample Mann-Whitney U test indicated a significant difference between the mean percent hatch rates of the untreated check and *B. bassiana* GHA ES treatments $U=908$, $p=0.017$ (Table 3.5). No infection was detected in any of the nymphs collected from the UTC field site, while the percent infection observed in the *B. bassiana* GHA ES treatments was 31.5% (Table 3.5). A Fisher's Exact Test found a significant difference between infected nymphs in the UTC and BotaniGard ES treatments $p<0.001$.

3.4.3 Field Trial of a *B. bassiana* Biopesticide Against Hatching SLF Nymphs

A total of 2521 first instar SLF nymphs were visually observed across the entire 10-day observational period. At 0 DAT, 773 SLF nymphs were observed for the untreated check and the *B. bassiana* GHA ES treatments, 305 and 468 SLF nymphs, respectively (Table 3.6). At 5 DAT, 574 and 1124 SLF nymphs were observed between the untreated check and the *B. bassiana* GHA

ES treatments, respectively (Table 3.6). At 10 DAT, 50 SLF nymphs were collected across all the untreated check and *B. bassiana* GHA ES treatments, 28 and 22, respectively (Table 3.6). No significant differences were observed in nymphs in visual counts among treatments at 0, 5, and 10 DAT $U(6)=1.000$, $p=0.200$; $U(6)=0.000$, $p=0.100$; $U(6)=6.000$, $p=0.700$ (Table 3.6). Significant differences were observed between the total number of nymphs from both treatments at each DAT interval $H(18)=14.392$, $p<0.001$ (Table 3.6). Specifically, post-hoc pairwise comparisons indicated that the total population of SLF nymphs visually observed between 5 and 10 DAT were significantly different at $\alpha = 0.05$. Due to low populations of SLF nymphs on 10 DAT, I could not collect any specimens to determine infection. At 0 and 5 DAT, 270 and 807 individual first-instar SLF nymphs were collected from the untreated check and *B. bassiana* GHA ES replicates. At 0 DAT, no collected SLF nymphs across both treatments were found to be infected, while at 5 DAT, 281 total SLF nymphs, or 43.9% (Table 3.6), from the *B. bassiana* GHA ES treatment were observed to be infected. No significant differences between mean infection rate and treatments were observed at 0 DAT $U(6)=8460.50$, $p=1.00$, but at 5 DAT, treatment differences were observed $U(6)=29880.000$, $p<0.001$ (Table 3.6).

3.4.4 *Beauveria bassiana* Biopesticide Winter Survivability on SLF Egg Masses

In 2021, eighteen SLF egg masses were utilized to investigate the winter survivability of all formulations of *B. bassiana* GHA on SLF egg masses. I found significant differences between all treatments for mean percent germination (spore viability) regardless of winter exposure $H(2)=7.448$, $p=0.024$ (Table 3.7). Regardless of winter exposure, I observed no spores in the untreated check treatments. I observed the most significant mean percent germination in *B.*

bassiana GHA WP and ES when SLF egg masses were treated and not exposed to natural winter conditions, ranging from 81.5–90.8% (Table 3.7). The mean percent germination reduction was observed when SLF egg masses were treated and exposed to winter conditions, ranging from 15.8–57.2% (Table 3.7).

In 2022, 130 SLF egg masses were used across the various treatments and environmental exposure. I found significant differences between all treatments and mean percent germination independent of application timing; 20 WBH application timing + winter exposure $H(4)=65.207$, $p=<0.001$, 20 WBH application timing + no winter exposure $H(4)=27.609$, $p=<0.001$, 2 wk application timing + no winter exposure, $H(4)=20.716$, $p=0.024$ (Table 3.7). Across all application timings, all untreated checks resulted in no germination. The positive control (2 WBH application timing + no winter exposure) resulted in the most significant mean percent germination across all *B. bassiana* biopesticidal treatments at rates greater than 80% (Table 3.7). SLF egg masses treated 20 WBH and not exposed to winter conditions resulted in mean percent germination rates ranging from 27.6–79.4% (Table 3.7). Likewise, SLF egg masses treated 20 WBH and exposed to winter conditions mean percent germination rates ranged from 11.3–51.9% (Table 3.7). At all treatment timings, I found that the *B. bassiana* GHA WP resulted in the most significant mean percent germination ranging from 52.0–86.9% (Table 3.7).

3.5 Discussion and Conclusion

In 2021, the laboratory bioassay of *B. bassiana* GHA applied to overwintering SLF was a proof of concept, warranting further investigation. While low, the mean percent infection data generated in 2021 indicated that some treatment effect occurred. In 2021, I utilized significantly younger potted TOH per replicate and experienced high nymphal mortality after hatching in all treatments, including the checks. Although I did experience high nymphal mortality rates in 2022 and 2023, mortality was reduced by providing larger TOH to emerged nymphs. The excessive hatch reduction observed in 2021 is likely attributed to collection methodology, specifically, overhandling and damaging the SLF egg masses during removal from trees, in transit, or during trial processes. The bioassay collection techniques improved in later years, and I observed greater hatch rates.

In the 2022 and 2023 laboratory bioassays, a higher mean percent infection was observed during the spring application than any other timing for all mycoinsecticide treatments. However, both GHA and ANT03 strains resulted in some infection during the winter application timing. This situation could result from small replicate size, low hatch rates, and residual capability of *B. bassiana* spores applied. Likely, the number of viable spores cm^{-2} on the SLF egg masses following spring applications was greater, which resulted in higher infection rates. In the 2023 laboratory bioassays, I only investigated the spring (2 WBH) application timing. Because prior research from 2021 and 2022 indicated that *B. bassiana* spores applied to SLF egg masses in winter will survive until spring at reduced viability, I focused on the spring application timing because the laboratory bioassay from 2022 indicated much more potent, consistent treatment effects. The overall

percentage of infection in 2023 was lower than previously observed in the 2022 laboratory bioassay. In 2023, due to the improved SLF egg mass collection techniques, the hatched nymphs may have been healthier than in prior years of the bioassay. If SLF nymphs hatched in healthier conditions, infection from *B. bassiana* biopesticides could have been prevented by SLF defenses. Nevertheless, it demonstrated that applications of *B. bassiana* mycoinsecticide to overwintering SLF egg masses can infect hatching nymphs.

Overall, fewer total nymphs and egg masses were utilized in 2021 and 2022 than in 2023 laboratory bioassays. This is because I utilized infested plant material rather than collected individual egg masses in 2023. However, the hatch survivorship of treated egg masses was difficult to quantify accurately because of how densely the SLF egg masses were oviposition the infested plant material, a greater mean percent hatch was observed. Standardization of the SLF egg mass infested plant material utilized for the 2023 laboratory bioassay trial was not possible, and all treatments and replicates varied in the number of egg masses utilized per infested plant material. The SLF egg masses were collected much later into the overwintering period in 2023 than in the previous year of bioassays; this allowed for egg masses to be exposed to climate storage for less time mimicking natural conditions for more time.

In the 2023 laboratory bioassay, I observed significant reductions in the mean percent hatch rate, indicating some level of ovicidal activity from all treatments compared to the check treatments. This trend was not observed in the 2021 or 2022 bioassays. Furthermore, regardless of strain, both oil treatments resulted in a numerically greater hatch reduction, although the

improvement was not statistically significant. I observed significant reductions in the hatch in the 2023 field trial against SLF egg masses, although not at a biologically substantial level because both treatment hatch rates were greater than 90%. Overall, this indicates that some level of ovicidal effect was induced by *B. bassiana* formulations, which should be investigated further.

In the 2023 field trial against hatching SLF nymphs, the rate of *B. bassiana* GHA ES was doubled from that utilized in the laboratory bioassays but was within the maximum label rate. Greater hatch in field trials relative to laboratory bioassay was observed; this is likely associated with handling the delicate SLF egg masses in the latter case. Alternatively, egg masses collected in prior years were potentially exposed to environmental variables that reduced hatch. I found a significant difference between the mean percent hatch rates of those egg masses treated with *B. bassiana* GHA ES and those not, indicating an ovicidal treatment effect was observed. However, the mean percentage of hatch rates in both treatments still exceeded 90%. Laboratory bioassays in 2023 showed a similar ovicidal effect from *B. bassiana* GHA ES treatments. A significant total percent infection (31.5%) suggests that treatments of *B. bassiana* GHA ES before the hatch resulted in subsequent infection of hatching nymphs. Future field trials should attempt only to collect SLF nymphs exposed to the treatments; the method of collecting nymphs from a 1-meter area around the treated egg mass successfully demonstrated that infection occurred but was likely inaccurate due to sampling methods. Despite the large collection radius, considerably greater infection in cadavers was observed in the field trials targeting overwintering SLF egg masses as opposed to the 2023 laboratory bioassay.

In 2023, we found infection of collected SLF nymphs at 5 DAT in the field trial where *B. bassiana* GHA ES was applied against hatching SLF nymphs on red maple trees. No infection was observed in the untreated check, while 43.9% was observed within the *B. bassiana* GHA ES treatment at 5 DAT. No infection was observed at 0 DAT in either treatment, indicating no baseline level of native *B. bassiana* in the environment. Timed visual counts indicated a population crash of SLF at 10 DAT. This population crash is likely attributed to dispersal away from red maple and resulting departure of the insects from the treated and control trees. Smaller trees should be used in future research to ensure complete treatment coverage. Furthermore, sampling was difficult in higher portions of the tree. Additionally, the spore cm^{-2} should have been collected pre- and post-application to determine if there was no baseline level of *B. bassiana* was naturally present, no drift occurred, and to determine the infectious dose of *B. bassiana* delivered to each replicate. The application of *B. bassiana* mycoinsecticides against hatching SLF should be further investigated.

It is typical for high populations of SLF to aggregate on red maple (Liu 2019). Liu (2019) demonstrated that red maple was not a preferred oviposition host of SLF when more preferred hosts were present. Despite low oviposition rates on red maple (Liu 2019), the presence of SLF egg masses suggests that when other preferred hosts are not present, such as in urbanized areas, red maple could be a suitable host for high populations of overwintering SLF. I observed high populations of SLF egg masses on red maple in one field plot lacking the presence of other preferred hosts. High oviposition rates and host migration suggest that red maple could be used as a trap tree method for overwintering populations of SLF. Applications of commercially available *B. bassiana* against a large population of hatching SLFs before they disperse toward a broader,

more suitable host range could provide stakeholders with an organic biopesticide management option.

The winter survivability of *B. bassiana* spores applied to SLF egg masses and exposed to natural overwintering conditions demonstrated that *B. bassiana* (GHA and ANT03) spores could survive and subsequently germinate during the warmer spring, although at reduced rates. Additionally, when SLF egg masses were treated at 20 WBH and not exposed to winter conditions, I saw a greater mean percent of germination than when exposed to natural winter conditions. I anticipated the ES and EC treatments in the 2022 germination survivability would have the greatest mean percent germination due to additional oils in the formulation, but the opposite was observed, with the *B. bassiana* GHA WP treatment resulting in the greatest germination rate. Furthermore, various abiotic and biotic factors, most significantly UV radiation, temperature, and moisture levels, can influence spores' efficacy, virulence, and survivability when utilizing biopesticides (Jaronski 2010).

Both laboratory bioassays and field trials resulted in positive infection, suggesting that applications of *B. bassiana* mycoinsecticides are efficacious as a management tactic when applied to overwintering or hatching SLF nymphs. All the *B. bassiana* biopesticides utilized resulted in infection, although across all years of laboratory bioassays, I did not find a single formulation or strain that was more efficacious. It has already been demonstrated that SLF nymphs and adults are susceptible to *B. bassiana* mycoinsecticides (Clifton et al. 2020, Clifton and Hajek 2022). Specifically, these data suggest that SLF nymphs can pick up the *B. bassiana* spores upon hatch

and subsequently become infected. Wraight and Ramos (2002) demonstrated that formulations of biopesticides can influence the efficacy against select pests. Leach et al. (2019) investigated the effectiveness of organic materials like azadirachtin and spinosad against nymphal SLF and observed 8.0% and 58.0% efficacy, respectively.

This research suggests that *B. bassiana* spores applied to SLF egg masses could survive until the SLF eggs hatch and infect nymphs as they come into contact with spores on the surface of the egg masses. While applications 20 WBH did demonstrate some infection levels, I suggest applications timings 2 WBH or as close to the predicted hatch as possible to result in the greatest efficacy and survivability of the *B. bassiana* spores. This application method would provide stakeholders with a convenient window of opportunity to manage early populations of SLF. Furthermore, applying *B. bassiana* during the winter or spring months may be more convenient to practitioners and allow consistent coverage of spores to egg masses due to the lack of foliage and easier detection. More field testing with greater replicates and field sites should be done in future research before integrated pest management recommendations can be made.

In both laboratory bioassays and field trials, the lack of infection in the untreated or water checks indicated that the surface sterilization methodology was adequate at removing surface contaminants while preserving any potential internal infection from *B. bassiana* treatments. This suggests that surface sterilization and plating methods were sufficient to demonstrate infection after nymphal exposure to infectious spores. This technique was instrumental because the rate of natural morality for the laboratory-reared SLF was high and would have created a bias in the

mortality data; therefore, using infection as the quantification metric was more accurate and demonstrated actual treatment effects.

Strömbom and Pandey (2021) modeled SLF life cycles under various management techniques. Within the limitations of their model, they concluded that while the utilization of biological and cultural control tactics alone would not be sufficient to lower SLF populations below replacement thresholds, they could provide sustained suppression of existing populations. The data generated from my dissertation can further aid such models as described by Strömbom and Pandey (2021). Jaronski (2010) suggested that pests should be exposed to high concentrations of infectious materials before outbreak populations are established, helping thwart outbreaks. Should this novel technique be implemented in integrated pesticide management, the intended application methodology would involve applications of *B. bassiana* biopesticides made with a backpack sprayer to the perimeter of vineyards or at-risk areas. It has been documented that SLF egg masses are more typically observed around the vineyard perimeter adjacent to hedgerows or forested environments (Leach and Leach 2020). This tendency may allow targeted, more efficient application of *Beauveria* instead of broadcast sprays of mycoinsecticides such as those investigated in this research.

3.6 Acknowledgments

The USDA-NIFA CAP Award 2019-51181-30014 and the Virginia Wine Board (Proposal Number PUVQHEEZ) provided funding for this project.

3.7 References

Boomsma, J. J., A. B. Jensen, N. V. Meyling, and J. Eilenberg. 2014. Evolutionary interaction networks of insect pathogenic fungi. Annual Review of Entomology 50 467–485. doi:10.1146/annurev-ento-011613-162054

Chase, A. R., L. S. Osborne, and V. M. Ferguson. 1986. Selective isolation of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* from an artificial potting medium. The Florida Entomologist 69(2) 285–292. https://doi.org/10.2307/3494930

Clifton, E. H., L. A. Castrillo, A. Gryganskyi, and A. E. Hajek. 2019. A pair of native fungal pathogens drives decline of a new invasive herbivore. Proceedings of the National Academy of Sciences of the United States of America. 116(19) 9178–9180. https://doi.org/10.1073/pnas.1903579116

Clifton, E. H., A. E. Hajek, N. E. Jenkins, R. T. Roush, J. P. Rost, D. J. Biddinger, and P. Nachappa. 2020. Applications of *Beauveria bassiana* (Hypocreales: Cordycipitaceae) to control populations of spotted lanternfly (Hemiptera: Fulgoridae), in semi-natural landscapes and on grapevines. Environmental Entomology 49(4) 854–864. https://doi.org/10.1093/ee/nvaa064

Clifton, E. H., L. A. Castrillo, and A. E. Hajek. 2021. Discovery of two hypocrealean fungi infecting spotted lanternflies, *Lycorma delicatula*: *Metarhizium pemphigus* and a novel

species, *Ophiocordyceps delicatula*. Journal of Invertebrate Pathology 186. https://doi.org/https://doi.org/10.1016/j.jip.2021.107689

Clifton, E. H., and A. E. Hajek. 2022. Efficacy of *Beauveria bassiana* and *Cordyceps javanica* mycoinsecticides against spotted lanternflies, *Lycorma delicatula*, in laboratory bioassays. Biocontrol Science and Technology 32(7) 824–836. https://doi.org/10.1080/09583157.2022.2052804

Dara, S. K., L. Barringer, and S. P. Arthurs. 2015. *Lycorma delicatula* (Hemiptera: Fulgoridae): A new invasive pest in the United States. Journal of Integrated Pest Management. 6(1) 1–7. https://doi.org/10.1093/jipm/pmv021

Gryganskyi, A.P., J. Golan, and A. E. J. Hajek. 2022. Season-long infection of diverse hosts by the entomopathogenic fungus *Batkoa major*. PloS One 17(5). doi: 10.1371/journal.pone.0261912

Hajek, A. E., E. H. Clifton, S. E. Stefanik, and D. C. Harris. 2022. *Batkoa major* infecting the invasive planthopper *Lycorma delicatula*. Journal of Invertebrate Pathology 194. DOI: 10.1016/j.jip.2022.107821

Han, J. M., H. Kim, E. J. Lim, S. Lee, Y. J. Kwon, and S. Cho. 2008. *Lycorma delicatula* (Hemiptera: Auchenorrhyncha: Fulgoridae: Aphaeninae) finally, but suddenly arrived in Korea. Entomological Research. 38: 281–286. https://doi.org/10.1111/j.1748-5967.2008.00188.x

Harner, A. D., H. L. Leach, L. Briggs, and M. Centinari. 2022. Prolonged phloem feeding by the spotted lanternfly, an invasive planthopper, alters resource allocation and inhibits gas exchange in grapevines. Plant Direct 6(10) 1–18. doi.org/10.1002/pld3.452.

Harper, J. K., W. Stone, T. W. Kelsey, and L. F. Kime. 2019. Potential economic impact of the spotted lanternfly on agriculture and forestry in Pennsylvania. www.rural.palegislature.us.

Jaronski, S. T. (2010). Ecological factors in the inundative use of fungal entomopathogens. BioControl 55(1) 159–185. https://doi.org/10.1007/s10526-009-9248-3

Keena, M. A., and A. L. Nielsen. 2021. Comparison of the hatch of newly laid *Lycorma delicatula* (Hemiptera: Fulgoridae) eggs from the United States after exposure to different temperatures and durations of low temperature. Environmental Entomology 50(2) 410–417. https://doi.org/10.1093/ee/nvaa177

Keller, J., J. Rost, K. Hoover, J. Urban, H. Leach H, M. Porras, B. Walsh, M. Bosold, and D. Calvin. 2020. Dispersion patterns and sample size estimates for egg masses of spotted lanternfly (Hemiptera: Fulgoridae). Environmental Entomology 49(6) 1462–1472. https://doi.org/10.1093/ee/nvaa107

Leach, H., D. J. Biddinger, G. Krawczyk, E. Smyers, and J. M. Urban. 2019. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula*, (Hemiptera: Fulgoridae), a new pest of fruit in the northeastern U.S. Crop Protection 124 1–6.

Leach, H., and A. Leach. 2020. Seasonal phenology and activity of spotted lanternfly (*Lycorma delicatula*) in eastern US vineyards. Journal of Pest Science 93(12) 1215–1224. DOI:10.1007/s10340-020-01233-7

Lee, D. H., Y. L. Park, and T. C. Leskey. 2019. A review of biology and management of *Lycorma delicatula* (Hemiptera: Fulgoridae), an emerging global invasive species. Journal of Asia-Pacific Entomology 22(2) 589–596. https://doi.org/10.1016/j.aspen.2019.03.004

Lee, J.-E., S.-R. Moon, H.-G. Ahn, S.-R. Cho, J.-O. Yang, C. Yoon, and G.-H. Kim. 2009. Feeding behavior of *Lycorma delicatula* (Hemiptera: Fulgoridae) and response on feeding stimulants of some plants. Korean Journal of Applied Entomology 48(4) 467–477. DOI:10.5656/KSAE.2009.48.4.467

Liu, H. 2020. Seasonal development, cumulative growing degree-days, and population density of spotted lanternfly (Hemiptera: Fulgoridae) on selected hosts and substrates. Environmental Entomology 49 (5) 1171–1184. https://doi.org/10.1093/ee/nvaa074

Liu, H., and J. Mottern. 2017. An old remedy for a new problem? Identification of *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae), an egg parasitoid of *Lycorma delicatula* (Hemiptera: Fulgoridae) in North America. Journal of Insect Science 17(1). doi.org/10.1093/jisesa/iew114

Liu, H., and R. J. Hartlieb. 2019. Spatial distribution of *Lycorma delicatula* (Hemiptera: Fulgoridae) egg masses on tree-of-heaven, black walnut, and Siberian elm in North America. Journal of Economic Entomology 113 1028–1032.

Milner, R. J., R. J. Huppatz, and C. S. Sandra. 1991. A new method for assessment of germination of metarhizium conidia. Journal of Invertebrate Pathology 57 121–123.

Ortiz-Urquiza, A.,and N. O. Keyhani. 2013. Action on the surface: entomopathogenic fungi versus the insect cuticle. Insects 4(3) 357-374. https://doi.org/10.3390/insects4030357

Park, M. 2015. Overwintering ecology and population genetics of *Lycorma delicatula* (Hemiptera: Fulgoridae). Korea Ph. D Thesis, Seoul National University.

Strömbom, D., and S. Pandey. 2021. Modeling the life cycle of the spotted lanternfly (*Lycorma delicatula*) with management implications. Mathematical Biosciences 340(1) 108670. doi.org/10.1016/j.mbs.2021.108670

Tomisawa, A., S. Ohmiya, H. Fukutomi, K. Hayashi, and T. Ishikawa. 2013. Biological notes on *Lycorma delicatula* (White) (Hemiptera, Fulgoridae) in Ishikawa prefecture, Japan. Japanese Journal of Entomology 16(1) 3–14. https://doi.org/10.20848/kontyu.16.1_3

Urban, J. M. 2019. Perspective: Shedding light on spotted lanternfly impacts in the USA. Pest Management Science 76 10–17. https://doi.org/10.1002/ps.5619

Urban, J. M., and H. Leach. 2023. Biology and management of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae), in the United States. Annual Review of Entomology 68 151–167. doi.org/10.1146/annurev-ento-120220-111140.

Uyi, O., J. A. Keller, A. Johnson, D. Long, B. Walsh, and K. Hoover. 2020. Spotted lanternfly (Hemiptera: Fulgoridae) can complete development and reproduce without access to the preferred host, *Ailanthus altissima*. Environmental Entomology 49(5) 1185–1190. https://doi.org/10.1093/ee/nvaa083

Wraight, S. P., and Ramos, M. E. 2002. Application parameters affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle *Leptinotarsa decemlineata*. Biological Control 23(2) 164–178. https://doi.org/10.1006/bcon.2001.1004

Zhang, Z.Z. 1993. Forest Insects. China Forestry Publishing House, Beijing, China. 164.

Zimmermann, G. 2007. Biocontrol Science and Technology Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology 17(6) 553–596. https://doi.org/10.1080/09583150701309006

3.8 Figures and Tables

Figure 3.1 Insect rearing cage with a potted TOH and SLF egg mass per cage. This cage design was utilized for laboratory bioassays in 2021–2023.

Figure 3.2 SLF nymphs with visible signs of infection from *B. bassiana*.

Table 3.1 Collection and application dates of the laboratory bioassay of *B. bassiana* against overwintering SLF egg masses in 2021–2023. Application dates were at 20 and 2 weeks before hatch (WBH).

		Application Date	
Laboratory Bioassay (yr)	**Collection Date**	**20 WBH**	**2 WBH**
2021	December, 2020	February 8, 2021	June 14, 2021
2022	December, 2021	February 15, 2022	June 21, 2022
2023	March 1–3, 2023	-	April 20, 2023

Table 3.2 The treatment for the 2021–2023 laboratory bioassays of *B. bassiana* biopesticides against overwintering SLF egg masses. The rates used during applications were standardized within the same power of ten spores per mL.

Treatment	Manufacturer	Active Ingredient	Spores per ml	Rate (kg or L/ha)
BotaniGard® 22WP	Certis Bio LLC.	*Beauveria bassiana* GHA	4.80E+07	2.24
BoteGHA® ES	Certis Bio LLC.	*Beauveria bassiana* GHA	5.00E+07	2.34
BioCeres® WP	Anatis Bioprotection	*Beauveria bassiana* ANT03	7.20E+07	3.36
BioCeres® ES	Anatis Bioprotection	*Beauveria bassiana* ANT03	5.00E+07	2.34
Untreated Check	-	-	-	-
Water Check	-	-	-	-

Table 3.3 The mean percent (±) SEM hatch of SLF egg masses from the laboratory bioassays of *B. bassiana* against overwintering SLF egg masses in 2021–2023.[1]

		2021			
		Winter		Spring	
Treament	n	Hatched Nymphs	Hatch Mean (%) ± SEM	Hatched Nymphs	Hatch Mean (%) ± SEM
BotaniGard® 22WP	10	100	10.00 ± 2.19 b	172	21.50 ± 4.13 ab
BoteGHA® ES	10	79	7.90 ± 2.98 a	108	13.50 ± 4.25 a
Untreated Check	10	101	10.10 ± 2.81 ab	132	16.50 ± 4.99 ab
Water Check	10	105	10.50 ± 2.72 ab	189	23.63 ± 1.40 b
		2022			
		Winter		Spring	
Treament	n	Hatched Nymphs	Hatch Mean (%) ± SEM	Hatched Nymphs	Hatch Mean (%) ± SEM
BotaniGard® 22WP	10	24	7.62 ± 4.81 ab	152	23.94 ± 8.20 b
BoteGHA® ES	10	3	3.13 ± 2.57 ab	154	36.57 ± 7.73 b
BioCeres® WP	10	55	8.63 ± 4.54 ab	59	18.04 ± 6.27 b
BioCeres® ES	10	25	6.01 ± 3.55 ab	110	25.67 ± 5.94 b
Untreated Check	10	26	8.08 ± 3.10 b	235	68.74 ± 2.02 a
Water Check	10	0	0.00 ± 0.00 a	210	50.80 ± 10.38 ab
		2023			
		Winter		Spring	
Treament	n	Hatched Nymphs	Hatch Mean (%) ± SEM	Hatched Nymphs	Hatch Mean (%) ± SEM
BotaniGard® 22WP	10	-	-	563	60.96 ± 2.00 ab
BoteGHA® ES	10	-	-	668	54.55 ± 2.22 ab
BioCeres® WP	10	-	-	330	63.11 ± 2.77 b
BioCeres® ES	10	-	-	524	52.20 ± 3.03 a
Untreated Check	10	-	-	453	76.37 ± 2.99 c
Water Check	10	-	-	413	74.18 ± 3.78 c

[1]Data were ranked sum transformed for an independent sample Kruskal-Wallis test, and untransformed mean percent data is shown. Dunn's pairwise comparison post-hoc test was performed based on the rank sum of each treatment. A Bonferroni correction was performed. Data within columns followed by a letter in common are not significantly different; $p<0.05$.

Table 3.4 The mean percent (±) SEM infection of *B. bassiana* visually observed in SLF from the laboratory bioassays against overwintering SLF egg masses in 2021–2023. No winter application timing was investigated in 2023.[1]

		2021			
		Winter		Spring	
Treament	n	Total Infected Nymphs	Infection Mean (%) ± SEM	Total Infected Nymphs	Infection Mean (%) ± SEM
BotaniGard® 22WI	10	5	0.50 ± 0.27 b	37	4,.63 ± 1.40 b
BoteGHA® ES	10	2	0.20 ± 0.13 ab	21	2.63 ± 1.22 b
Untreated Check	10	0	0.00 ± 0.00 a	0	0.00 ± 0.00 a
Water Check	10	0	0.00 ± 0.00 ab	0	0.00 ± 0.00 a
		2022			
		Winter		Spring	
Treament	n	Total Infected Nymphs	Infection Mean (%) ± SEM	Total Infected Nymphs	Infection Mean (%) ± SEM
BotaniGard® 22WI	10	3	3.00 ± 3.00 a	51	43.09 ± 12.61 b
BoteGHA® ES	10	1	1.11 ± 1.11 a	35	18.12 ± 4.29 b
BioCeres® WP	10	16	43.99 ± 15.03 b	26	28.86 ± 11.99 b
BioCeres® ES	10	18	16.19 ± 10.24 ab	28	26.07 ± 10.24 b
Untreated Check	10	0	0.00 ± 0.00 a	0	0.00 ± 0.00 a
Water Check	10	0	0.00 ± 0.00 a	0	0.00 ± 0.00 a
		2023			
		Winter		Spring	
Treament	n	Total Infected Nymphs	Infection Mean (%) ± SEM	Total Infected Nymphs	Infection Mean (%) ± SEM
BotaniGard® 22WI	10	-	-	38	6.33 ± 0.50 b
BoteGHA® ES	10	-	-	43	7.30 ± 0.63 b
BioCeres® WP	10	-	-	22	6.83 ± 1.20 b
BioCeres® ES	10	-	-	30	5.79 ± 0.79 b
Untreated Check	10	-	-	0	0.00 ± 0.00 a
Water Check	10	-	-	0	0.00 ± 0.00 a

[1]Data were ranked sum transformed for an independent sample Kruskal-Wallis test, and untransformed mean percent data is shown. Dunn's pairwise comparison post-hoc test was performed based on the rank sum of each treatment. A Bonferroni correction was performed. Data within columns followed by a letter in common are not significantly different; $p<0.05$.

Table 3.5 The mean percent hatch and standard error of SLF during the 2023 field trial of a *B. bassiana* biopesticide against overwintering SLF egg masses.[1]

		Hatch		Infection		
Treatment	n	Mean (%)	Standard Error	Total Nymphs	Total (%)	Standard Error
BoteGHA® ES	50	93.38 b	0.82	816	31.50 b	0.19
UTC	50	96.25 a	0.48	381	0.00 a	0.00

[1]An independent sample Mann-Whitney U test was performed to determine if statistical differences between treatment mean percent hatch rates were present. A Fisher's Exact Test was performed to determine if there were significant differences in the total percentage of infection between treatments. Data within columns followed by a letter in common are not significantly different; $p<0.05$.

Table 3.6 The field trial of a *B. bassiana* biopesticide against hatching SLF nymphs in 2023 on red maple trees. The mean count of SLF was visually observed over 3 minutes, and the mean infection of *B. bassiana* observed on collected, surface sterilized SLF cadavers are displayed.[1]

		Timed Visual Counts (Mean)			Mean Infection (%)	
Treatment	n	0 DAT	5 DAT	10 DAT	0 DAT	5 DAT
BoteGHA® ES	3	156.00 a	374.67 a	7.33 a	0.00 a	43.84 b
Untreated Check	3	101.67 a	191.33 a	9.33 a	0.00 a	0.00 a

[1]An independent sample Mann-Whitney U test was performed to determine significant differences between mean percent infection and mean visual counts against treatment across each sampled DAT. Data within columns followed by a letter in common are not significantly different; $p<0.05$.

Table 3.7 Mean percent (±) SEM germination of *B. bassiana* from 2021–2022 winter survivability field and laboratory study of mycoinsecticides applied to SLF egg masses.[1]

	Mean Germination (%)									
	2021				2022					
	20 WBH				20 WBH				2 WBH	
Treatment	n	No winter exposure	n	Winter exposure	n	No winter exposure	n	Winter exposure	n	No winter exposure
Untreated check	3	0.00 ± 0.00 a	3	0.00 ± 0.00 a	6	0.00 ± 0.00 a	15	0.00 ± 0.00 a	5	0.00 ± 0.00 a
BotaniGard® 22WP	3	90.83 ± 1.36 c	3	57.17 ± 5.90 c	6	79.42 ± 3.71 e	16	51.94 ± 0.77 e	6	86.92 ± 2.37 c
BoteGHA® ES	3	81.50 ± 1.80 b	3	15.83 ± 2.46 b	6	33.42 ± 1.40 c	15	14.17 ± 0.92 c	6	80.67 ± 1.33 b
BioCeres® WP	-	-	-	-	6	60.33 ± 1.19 d	14	42.43 ± 0.51 d	6	86.00 ± 1.29 c
BioCeres® ES	-	-	-	-	6	27.58 ± 1.53 b	10	11.30 ± 0.50 b	6	78.50 ± 1.06 b

[1] Data were ranked sum transformed for an independent Kruskal-Wallis test, and untransformed mean percent data is shown. Dunn's pairwise comparison post-hoc test was performed based on the rank sum of each treatment. A Bonferroni correction was performed; therefore, more conservative significant differences were observed. Data within columns followed by a letter in common are not significantly different; $p<0.05$.

Chapter 4: New State Records of *Aculops ailanthi* (Lin, Jin, and Kuang) (Acariformes; Trombidiformes: Prostigmata: Eriophyidae), in USA; A Pest or Biological Control Agent of *Ailanthus altissima* (Mill.) Swingle?

In Review Proceedings of the Entomological Society of Washington

JASON BIELSKI, DOUGLAS PFEIFFER, RON OCHOA, ALEXANDER BROWN AND ANDREW ULSAMER

(JB) Department of Entomology, The Virginia Polytechnic Institute and State University, 216 Price Hall, Blacksburg, VA 24061, USA (jbiel654@vt.edu); (DP) Department of Entomology, The Virginia Polytechnic Institute and State University, 216 Price Hall, Blacksburg, VA, 24061, USA (dgpfeiff@vt.edu); (RO) USDA-ARS Systematic Entomology Laboratory, 10300 Baltimore Ave, Building 003, BARC-West, Beltsville, MD, 20705, USA (ron.ochoa@usda.gov); (AB) MRP-APHIS 11200 Metro Airport Center Drive, Romulus, MI, 48174, USA; (alexander.a.brown@usda.gov) (AU) USDA-ARS Systematic Entomology Laboratory, 10300 Baltimore Ave, Building 003, BARC-West, Beltsville, MD, 20705, USA (andrew.ulsamer@usda.gov)

Abstract.—We report the first detections of *Aculops ailanthi* Lin, Jin, and Kuang (1997) (Acariformes: Trombidiformes: Prostigmata: Eriophyidae) from Montgomery County, Virginia,

and Wayne County, Michigan, USA; the fourth and fifth states to report this non-gall forming rust mite on *Ailanthus altissima* (Mill.) Swingle. We initially became aware of the pest in Virginia due to severe symptomology on greenhouse-cultivated *Ai. altissima*. In Michigan, similar observations from a field survey led to the mites' discovery. We tentatively identified the pest as an eriophyid mite of unknown species. Samples from both states were sent to USDA-ARS for identification and, using scanning electron microscopy, confirmed the species as *A. ailanthi* based on the morphological features. Moreover, we describe the impacts that high populations of *A. ailanthi* can have on *Ai. altissima* in greenhouse settings and potential use as a biological control agent. Field observations from Michigan are encouraging that *A. ailanthi* can affect *Ai. altissima* outside of greenhouse settings.

Key Words: Tree-of-heaven; New state records; Eriophyid mites

Tree-of-heaven (hereafter ToH), *Ailanthus altissima* (Mill.) Swingle, is a fast-growing deciduous tree native to China infamous for establishing itself in disturbed environments. Excluding Antarctica, ToH can be found on every continent (Kowarik and Säumel 2007). In North America, ToH was first introduced for ornamental purposes in 1784; since its introduction, it has become widespread across the USA and is listed as a noxious weed in many states because it can easily outcompete native species (USDA NRCS 2014). Additionally, existing ToH stands provided ample host material for devastating invasive insects such as *Halyomorpha halys* (Stål), brown marmorated stink bug, and *Lycorma delicatula* (White), spotted lanternfly, in the USA

(Hoebeke and Carter 2003; Barringer et al. 2015). Despite being a prolific plant species in the landscape, especially in disturbed forest areas (Nature Conservancy 2020), cultivating ToH in a greenhouse for research on biocontrol agents has been challenging due to unforeseen mite pest problems.

In China, Ding et al. (2006) documented 46 species of herbivorous invertebrates, 16 fungi, and one potyvirus that utilize ToH; seven of those phytophagous arthropods have been introduced and detected in the USA to some degree (Ding et al. 2006; Skvarla et al. 2021). Additionally, in the USA, *Atteva aurea* (Fitch, 1857) (*Ailanthus* webworm moth) is an indigenous species that feeds upon many plants within the Simaroubaceae family and has adapted to ToH (Becker 2009). Two weevil species, *Eucryptorrhynchus brandti* (Harold) and *E. chinensis* (Olivier), show considerable promise as biological control agents, but releases have still not been approved in the USA (Ding et al. 2006; Herrick et al. 2012; Marini et al. 2022). Two species of *Verticillium, V. nonalfalfae,* and *V. dahliae,* have been observed to infect ToH in the USA and Europe. They caused withering, yellowing, premature leaf drops, and dieback; the use of either *Verticillium* species as a biological control agent is still in development (Shall and Davis 2006; Brooks et al. 2020; Pisuttu et al. 2020). Furthermore, Snyder et al. (2012) determined that *E. brandti* can transfer *V. nonalfalfae* to ToH.

Eriophyid mites (Acari: Eriophyoidea) typically have a high degree of host specificity and, despite their near microscopic stature, can severely impact their associated host (Skoracka et al.

2010; de Lillo et al. 2018). Four eriophyid mite species in two genera, *Aculops* and *Aculus*, are documented to have associations with ToH: *Aculops ailanthi* Lin, Jin, and Kuang from China; *Aculops taihangensis* Hong and Xue from China; *Aculus altissimae* Xue and Hong from China; *Aculus mosoniensis* Ripka and Ersek from Hungary. Two of these four mite species, *A. ailanthi* and *A. mosoniensis* , reported from the USA and Europe respectively, show promise as biological control agents (Gardener 2008; Ripka and Ersek 2014; Skvarla et al. 2021; de Lillo et al. 2022). ~~The taxonomy separating these two genera, *Aculops* and *Aculus*, is currently under investigation (de Lillo et al. 2017; Marini et al. 2021; Skvarla et al. 2021; de Lillo et al. 2022). Two of these four mite species show promise as biological control agents, *A. ailanthi* and *A. mosoniensis* (Skvarla et al. 2021; de Lillo et al. 2022), reported from the USA and Europe respectively (Gardener 2008; Ripka and Ersek 2014; Skvarla et al. 2021; de Lillo et al. 2022). However, these mites are found outside the native range of ToH~~. Both mites cause significant damage to ToH saplings and new green growth in greenhouse environments (Gardener 2008; Skvarla et al. 2021; Marini et al. 2022). ~~Ripka and Ersek (2014) studied ToH and described a new species, *A. mosoniensis*, which appeared novel from Europe. Unfortunately, a careful comparison of eriophyid mites associated with ToH was not conducted. Using molecular analysis and morphological observations, de Lillo et al. (2022) indicated *Aculus mosoniensis* belongs to the genus *Aculops* and that *A. mosoniensis* and *A. taihangensis* are taxonomically synonymous. Skvarla et al. (2021) proposed that *A. altissimae*, based on the morphological differences cited in its marginal and unreliable description, is a junior synonym of *A. ailanthi*. These findings suggest a further molecular comparison of *A. ailanthi* with *A. mosoniensis*, as it is likely that the latter is a synonym. In addition to taxonomic confusion, little is documented about *A. ailanthi* phenology; for example, the overwintering life stage is unknown.~~

To date, *A. ailanthi* has been documented in Maryland, Pennsylvania, and West Virginia (Gardener 2008; Skvarla et al. 2021). As previously mentioned, the cultivation of ToH for research on associated invasive arthropod species and biocontrol agents has become an area of growing interest.

MATERIALS AND METHODS

Samples of infested leaves from Virginia were stored in 30 ml glass vials and then submerged with 70% ethanol; additionally, mite-infested leaves were sent on ice to preserve live specimens for identification. From Michigan, samples of live material were sealed in plastic bags and shipped with frozen gel packs. All specimens were sent to USDA-ARS Systematic Entomology Laboratory for species confirmation.

Tabletop SEM observations were made using a TM 3030Plus scanning electron microscope (Hitachi High Technologies America, Pleasanton, CA, USA) equipped with a Deben cooler system (Angstrom Scientific Inc., Ramsey, NJ, USA). Eriophyid specimens *in situ* on plant material were attached to 32 mm aluminum specimen holders with carbon sticky pads. Individual mites were removed from the plant material and mounted ventrally. The samples were cooled to -25 C. An accelerating voltage of 5 kV was used to view the specimens.

RESULTS

In the spring of 2022, potted ToH was grown from seed at Virginia Tech greenhouses, Blacksburg, Virginia, USA, for the rearing of *L. delicatula* within quarantine facilities. Shortly after emergence, we observed young ToH seedlings exhibit leaf deformation (like herbicide damage, see Figs. 1, 2), leaf yellowing, leaf rolling upward, premature leaf drop, drying and necrosis of apical meristem, and seedling death. Infested leaves were observed for surface pests using a stereomicroscope and tentatively identified as an unknown species of non-gall-forming eriophyid mites. We suspected the eriophyid mite in question was *A. ailanthi* due to the host specificity of eriophyid mites (Skoracka et al. 2010), lack of galls, and the recent detection of *A. ailanthi* in surrounding states (Gardner 2008; Skvarla et al. 2021).

Scan images were captured at a resolution of 1,280 x 960 pixels (Fig. 3). The mites were compared with the original descriptions as well as previously collected material and identified as *A. ailanthi*. We document observations and identification of *A. ailanthi* in southwest Virginia in 2022 and Michigan in 2023.

DISCUSSION

It is possible that *A. ailanthi* is present in North America within the distribution of ToH but has not been detected or surveyed for. Ozman-Sullivan et al. (2023) indicated the presence of *A. taihangensis* in Turkey and perhaps in the Middle East. This recent report and our findings support the need to carefully review the original species from China, where ToH is originally from.

Furthermore, the use of *A. ailanthi* as a potential biological control agent against ToH is still being studied at Virginia Tech and Pennsylvania State University (Skvarla et al. 2021). In

greenhouse settings, with a lack of natural predators, high populations of *A. ailanthi* can severely impact the growth of ToH (Skvarla et al. 2021). It is unknown if natural populations of *A. ailanthi* outside of a greenhouse setting can equally devastate established stands of ToH. Anecdotally, greenhouse-reared ToH infested with *A. ailanthi* were taken outside and exposed to natural conditions, mite populations subsequently declined. On the other hand, the observations from Michigan of heavily infested new and young growth of naturally established ToH (Fig. 4) offer evidence that there is potential for biological control outside of a greenhouse environment. The material from Michigan had the leaves dorsally and ventrally infested with all the mite stages; most of the eggs were located on the leaf's ventral surface. This may indicate that naturally occurring populations of *A. ailanthi* could build up the population density required to suppress ToH new growth and saplings. Conversely, *A. ailanthi* was only detected on the ventral surface of plant materials sampled from Virginia. The collapse of the Virginia populations of *A. ailanthi* on plant material taken outside the greenhouse points to the need for a greater understanding of the effects of wind and other natural conditions on *A. ailanthi* behavior. Another reason for further studies of the presence of the eriophyid mites is a possibility of an association with a virus that could help to restrict the spreading of this invasive plant (Ding et al. 2006).

The taxonomy separating these two genera, *Aculops* and *Aculus*, is currently under investigation (de Lillo et al. 2017; Marini et al. 2021; Skvarla et al. 2021; de Lillo et al. 2022). Unfortunately, a careful comparison of eriophyid mites associated with ToH was not conducted. Using molecular analysis and morphological observations, de Lillo et al. (2022) indicated *Aculus mosoniensis* belongs to the genus *Aculop*s and that *A. mosoniensis and A. taihangensis* are taxonomically synonymous. Skvarla et al. (2021) proposed that *A. altissimae*, based on the

morphological differences cited in its marginal and unreliable description, is a junior synonym of *A. ailanthi*. These findings suggest a further molecular comparison of *A. ailanthi* with *A. mosoniensis*, as it is likely that the latter is a synonym. In addition to taxonomic confusion, little is documented about *A. ailanthi* phenology; for example, the overwintering life stage is unknown.

We suggest conducting further surveys to determine the full range of *A. ailanthi* in the USA. The geographic and environmental variance in North America may influence the genotypical expression of *A. ailanthi,* resulting in better-suited phenotypes for the biological control of ToH. The known populations of *A. ailanthi* within the USA should be compared to determine if a superior phenotype for biological control on ToH exists.

ACKNOWLEDGEMENTS

Thank you to Armando Rosario-Lebron, APHIS-USDA, for guidance on Table Top SEM preparations. To Tobias Glik, APHIS-PPQ, for help and contact information from Michigan. To Dr. Shifu Zhao for comments and revision of the article. To the Smithsonian Natural History Museum and National Agricultural Library (NAL-USDA), SEL-USDA for support and assistance with specimens and references. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA; USDA is an equal opportunity provider and employer. The USDA-NIFA CAP Award 2019-51181-30014 and the Virginia Wine Board provided funding for this project.

LITERATURE CITED

Barringer L. E., L. R. Donovall, S. E. Spichiger, D. Lynch, and D. Henry. 2015. The first new world record of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae). Entomological News 125(1): 20–23. doi:10.3157/021.125.0105.

Becker V. O. 2009. A review of the New World *Atteva* Walker moths (Yponomeutidae, Attevinae). Revista Brasileira de Entomologia 53(3): 349–355. doi:10.1590/S0085-56262009000300007.

Brooks R. K., A. L. Snyder, E. A. Bush, S. M. Salom, A. Baudoin. 2020. First report of *Verticillium* Wilt caused by *Verticillium dahliae* impacting *Ailanthus altissima* in Virginia, U.S.A. Plant Disease 104(5): 1558–1560. doi:10.1094/PDIS-10-19-2064-PDN.

de Lillo E., O. Panzarino, P. Loverre, D. Valenzano, C. Mattia, F. Marini, M. Augé, and M. Cristofaro. 2017. New eriophyoid mites from Italy. IV. mites associated with weed plants. Systematic and Applied Acarology 22(12): 2256–2271. doi:10.11158/saa.22.12.15.

de Lillo E., A. Pozzebon, D. Valenzano, and C. Duso. 2018. An intimate relationship between eriophyoid mites and their host plants. Frontiers in Plant Science 9: 1–14. doi:10.3389/fpls.2018.01786.

de Lillo E., F. Marini, M. Cristofaro, D. Valenzano, R. Petanović, B. Vidović, T. Cvrković, and M. Bon. 2022. Integrative taxonomy and synonymization of *Aculus mosoniensis* (Acari: Eriophyidae), a potential biological control agent for tree of heaven (*Ailanthus altissima*). Insects 13(5): 1–16. doi:10.3390/insects13050489.

Ding J, Y. Wu, H. Zheng, W. Fu, R. Reardon, and M. Liu. 2006. Assessing potential biological control of the invasive plant, tree-of-heaven, *Ailanthus altissima*. Biocontrol Science and Technology 16(6): 547–566. doi:10.1080/09583150500531909.

Fitch A. 1857. Third report on the noxious and other insects of the state of New York. Transactions of the New York State Agricultural Society 16: 315–490.

Gardner, R.T. 2008. Native and Indigenous Biocontrols for *Ailanthus altissima*. Master's Thesis, University of Maryland, College Park, MD, USA. Pp 140. Thesis.

Herrick N. J., T. J. McAvoy, A. L. Snyder, S. M. Salom, and L. T. Kok. 2012. Host-range testing of *Eucryptorrhynchus brandti* (Coleoptera: Curculionidae), a candidate for biological control of tree-of-heaven, *Ailanthus altissima*. Environmental Entomology 41(1): 118–124. doi:10.1603/EN11153.

Hoebeke E., and M. E. Carter. 2003. *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): A polyphagous plant pest from Asia newly detected in North America. Proceedings of the Entomological Society of Washington 105: 225–237.

Kowarik I., and I. Säumel. 2007. Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. Perspectives in Plant Ecology, Evolution and Systematics 8(4): 207–237. doi:10.1016/j.ppees.2007.03.002.

Marini F., E. Profeta, B. Vidović, R. Petanović, E. de Lillo, P. Weyl, H. L. Hinz, C. E. Moffat, M. Bon, T. Cvrković, J. Kashefi, R. Sforza, and M. Cristofaro. 2021. Field assessment of the

host range of *Aculus mosoniensis* (Acari: Eriophyidae), a biological control agent of the tree of heaven (*Ailanthus altissima*). Insects 12(7): 1–16. doi:10.3390/insects12070637.

Marini F., M. Cristofaro, G. Mecca, B. Vidović, H. Williams, C. Moffat, H. L. Hinz, and P. Weyl. 2022. Biological control of tree of heaven, *Ailanthus altissima*. Annual Report 2021 CABI.

Nature Conservancy. 2020. Stories in Indiana: Tree of Heaven. https://www.nature.org/en-us/about-us/where-we-work/united-states/indiana/stories-in-indiana/journey-with-nature--tree-of-heaven/ Accessed on March 27, 2024.

Ozman-Sullivan S. K., G. T. Sullivan, P. E. Chetverikov, and E. Kaplan. 2023. *Aculus taihangensis* (Acari: Prostigmata: Eriophyidae), a potential biological control agent identified from the highly invasive pest plant, tree of heaven, in Türkiye. Acarological Studies, 5(2): 99–104. doi.org/10.47121/acarolstud.1290818.

Pisuttu C., A. Marchica, R. Bernardi, A. Calzone, L. Cotrozzi, C. Nali, E. Pellegrini, and G. Lorenzini. 2020. Verticillium wilt of *Ailanthus altissima* in Italy caused by *V. dahliae*: new outbreaks from Tuscany. IForest - Biogeosciences and Forestry 13(1): 238–245. doi:10.3832/ifor3238-013.

Ripka G., and L. Ersek. 2014. A new *Aculops* species (Acari: Prostigmata: Eriophyoidea) on *Ailanthus altissima* from Hungary. Acta Phytopathologica et Entomologica Hungarica 49(1): 49–56. doi:10.1556/APhyt.49.2014.1.5.

Schall M. J., and D. D. Davis. 2009. *Ailanthus altissima* wilt and mortality: etiology. Plant Disease 93(7): 747–751. doi:10.1094/PDIS-93-7-0747.

Skoracka A., L. Smith, G. Oldfield, M. Cristofaro, and J. W. Amrine. 2010. Host-plant specificity and specialization in eriophyoid mites and their importance for the use of eriophyoid mites as biocontrol agents of weeds. Experimental and Applied Acarology 51(1): 93–113. doi:10.1007/s10493-009-9323-6.

Skvarla M. J., R. Ochoa, A. Ulsamer, and J. Amrine. (2021), The eriophyid mite *Aculops ailanthi* Lin, Jin, and Kuang, 1997 (Acariformes: Prostigmata: Eriophyidae) from tree-of-heaven in the United States – new state records and morphological observations. Acarologia 61(1): 121–127. doi:10.24349/acarologia/20214421.

Snyder A. L., S. M. Salom, L. T. Kok, G. J. Griffin, and D.D. Davis. 2012. Assessing *Eucryptorrhynchus brandti* (Coleoptera: Curculionidae) as a potential carrier for *Verticillium nonalfalfae* (Phyllachorales) from infected *Ailanthus altissima*. Biocontrol Science and Technology 22(9): 1005–1019. doi:10.1080/09583157.2012.707639.

Swingle, W. T. (1916). The early European history and the botanical name of the Tree of Heaven, *Ailanthus altissima*. Journal of the Washington Academy of Sciences 6(14): 490–498.

USDA, NRCS. 2014. Field guide for managing tree-of-heaven in the Southwest *Ailanthus altissim*a (Mill.) Swingle.

FIGURE CAPTIONS

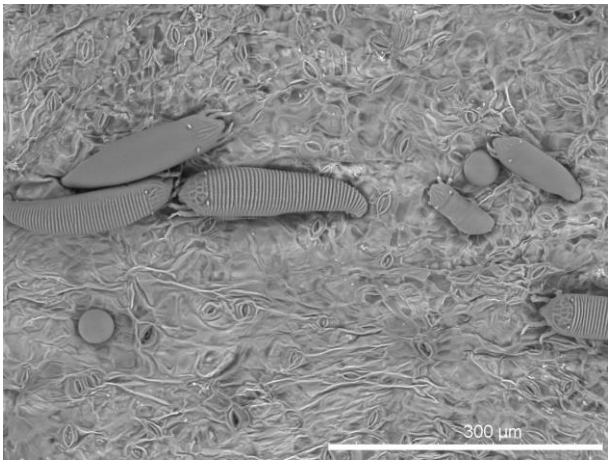
Fig. 1. Mite-infested, greenhouse-reared, ToH on the left. Healthy ToH on the right. Each ToH seedling was germinated and planted on the same date. Both seedlings were exposed to the same

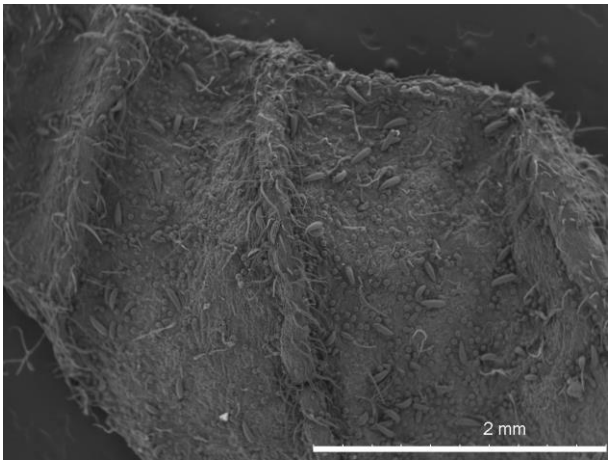
growing conditions, were six months from germination, and were approximately 0.5 meters in height.

Fig. 2. Mite-infested ToH sapling, approximately two meters in height, and observed in field conditions from Wayne County, Michigan, USA.

Fig. 3. Scanning electron microscope image of various life stages of *A. ailanthi in situ* on the ventral surface of a leaf of ToH (*Ai. Altissima*), including eggs, larvae, nymphs, and adults.

Fig. 4. Scanning electron microscope image of high population density of *A. ailanthi in situ* on the ventral surface of a leaf of ToH (*Ai. Altissima*).


300 µm


2 mm

Chapter 5: Foliar Insecticidal Management of *Aculops ailanthi* (Lin, Jin, and Kuang) (Acariformes; Trombidiformes: Prostigmata: Eriophyidae), on Potted *Ailanthus altissima* (Mill.) Swingle

Jason Tyler Bielski

5.1 Abstract

Native to Asia and first introduced to the USA as an ornamental tree species in 1784, tree-of-heaven, *Ailanthus altissima*, has become a noxious weed typical of disturbed sites, railroads, and roadways. The prevalence of *A. altissima* in the USA has facilitated the establishment of other destructive invasive species, such as the brown marmorated stink bug (*Halyomorpha halys*) and spotted lanternfly (*Lycorma delicatula*), both of which utilize *A. altissima* as a primary host species. While cultivation of *A. altissima* is uncommon in the USA, although it was previously grown as an ornamental species, it is frequently grown for research purposes on invasive species such as *H. halys* and *L. delicatula*. Here, I investigated the efficacy of various foliar insecticide treatments against *Aculops ailanthi* (Acariformes: Trombidiformes: Prostigmata: Eriophyidae) on potted *A. altissima* saplings to produce additional management recommendations for researchers struggling to cultivate *A. altissima* in greenhouse conditions due to the overwhelming injury produced by this under documented mite. Foliar application of insecticidal soap, neem oil extract, avermectin, or chlorfenapyr significantly reduced *Ac. ailanthi* populations on *A. altissima* and provided residual control for two weeks.

Key Words: Pesticide application, Eriophyid mite, Management, Tree-of-Heaven, Eriophyidae

5.2 Introduction

In many states, including California, Connecticut, North Carolina, and Michigan, tree-of-heaven (TOH), *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae), is legally considered a noxious or invasive weed species (Fryer 2010). Although TOH was once grown as an ornamental tree in the USA, it has become a noxious weed, typical among disturbed sites, railroads, and roadways (Kowarik and Säumel 2007). Noxious weeds are plants designated by law as harmful to crops, ecosystems, livestock, or humans. Control measures may be implemented to manage or eradicate noxious species. Control measures for noxious species may include trade quarantines banning distribution, importation, transportation, purchase, sale, transplanting, and cultivation. Invasive species are non-native plants, animals, or microorganisms that when introduced to a new habitat, can cause significant harm to the environment, economy, or human health. Invasive plants like TOH often outcompete native vegetation, disrupt ecosystems, and can lead to declining biodiversity (Kowarik and Säumel 2007).

Although a weed species may be labeled invasive in a state, legal restrictions are not yet in place to help negate the ramifications. The extensive distribution of TOH in the USA has helped facilitate the spread of multiple invasive species, such as the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), and spotted lanternfly (SLF), *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) (Hoebeke and Carter 2003, Barringer et al.

2015). In its native range in East Asia, despite its toxic characteristics, TOH is highly regarded in China for its medicinal purposes and the quality of wood (Hu 1979).

The facilitation of TOH plant material for research purposes can be complex due to its noxious or invasive labeling. Those researching TOH resort to growing their own TOH seedlings because nursery-grade saplings are unavailable. Consequently, many potted TOH were cultivated at the Virginia Tech greenhouses, Blacksburg, Virginia, USA, for research on managing invasive SLF. Eriophyid mites were observed, causing extensive injury to young potted TOH in those greenhouses in September 2021 (Chapter 4). Injury symptoms included leaf yellowing, leaf deformation, premature leaf drop, reduced growth, and even death in some plants. It was initially hypothesized that the infestation was due to *Aculops ailanthi* (Lin, Jin, and Kuang) (Trombidiformes: Eriophyidae) because of the lack of galls typical of eriophyid mites (Skvarla et al. 2021). The suspect mites were identified to be *A. ailanthi* (Bielski et al. *in press*). To date, *A. ailanthi* has only been recorded in Ohio, Maryland, Pennsylvania, West Virginia, Virginia, and Michigan (Gardner 2008, Skvarla et al. 2021, Bielski et al. *in press*). It is likely that *A. ailanthi* is present in more states with TOH, but this has yet to be determined. Close examination of herbivores on TOH has yet to be well documented (Gardner 2008), and likewise, even less literature is available for management strategies of pests on TOH. See Ding et al. (2006) and Gardner (2008).

Here, I investigated the efficacy of a foliar application of insecticidal soap (M-Pede®, Gowan® USA), neem oil extract (Trilogy®, Certis Bio LLC.), avermectin (Avid® 0.15 EC, Syngenta®), or chlorfenapyr (Phantom, BASF) against *A. ailanthi* on potted TOH to gather a better

understanding of the pesticide applications that can be utilized against this pest. Due to the intended research use of the TOH being grown, management of *A. ailanthi* needed to be achieved swiftly to prevent further damage and management techniques that would be minimally disruptive to SLF research. Skvarla et al. (2021) noted that the management of *A. ailanthi* was achieved by applying insecticidal soaps. Furthermore, other researchers working with potted TOH for SLF research have indicated that the use of foliar applications of chlorfenapyr can be efficacious against *A. ailanthi* with little effect on SLF at all life stages; Shin et al. (2010) found that SLF egg masses treated with chlorfenapyr exhibited 34% reduced hatch rate.

Avermectin, chlorfenapyr, and neem oil have all demonstrated control of another eriophyid, citrus mites, *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae) ((McCoy et al. 1982, Bullock and Pelosi 1998, Sonalkar et al. 2014). Avermectin has demonstrated significant control of citrus mites and has been utilized in the Florida citrus industry for decades (McCoy et al. 1982). Similarly, Bullock and Pelosi (1998) evaluated the novel pesticide chlorfenapyr against citrus mites and demonstrated comparable efficacy to industry standards in Florida citrus. Finally, neem oil, a biorational pesticide, has been shown in citrus orchards to reduce citrus mite populations, but was not as effective as other biorational pesticides investigated (Sonalkar et al. 2014).

5.3 Material and Methods

TOH was initially grown for pest management research on the invasive SLF. The Virginia Tech greenhouses were used to propagate trees of heaven. Every TOH used in the study was

cultivated using locally gathered seeds sourced directly from female trees in September 2020 in Blacksburg, Virginia. Before germination, seeds were kept in a freezer for at least one month. Sixteen seeds were placed directly into a moist soil medium in 0.50–0.25 m seed trays to induce germination. The seedlings were transplanted into 20 L black plastic horticultural pots with drain holes once they had grown to a height of 10–15 cm. Seedlings were allowed a year of growth before utilization in the trial. All plants were grown with Miracle-Gro® Potting Mix (Marysville, Ohio), and no additional fertilizers were incorporated into the soil medium.

All TOH used in the study were naturally infested with *A. ailanthi,* though the source of the infestation is unknown. Sapling replicates were at various stages of infestation, ranging from mild to severe. All TOH included in the trial were greater than 0.5 m in height and started the trial with a full canopy. Insecticidal treatments included insecticidal soap (M-Pede®, Gowan® USA), neem oil extract (Trilogy®, Certis Bio LLC.), avermectin (Avid® 0.15 EC, Syngenta®), or chlorfenapyr (Phantom, BASF) (Table 5.1). Additionally, an untreated check and a water check were incorporated into the trial. Each treatment consisted of eight replicates of randomly selected mite-infested potted TOH. A single insecticidal application was applied with an 8 L hand pump sprayer (HDC model #:1502HDXA) at the highest label rate (Table 5.1). The study followed a completely randomized block design with ten potted TOH per block and ten blocks for a total of 100 plants. Each treatment replicate was separated by a non-treated buffer potted TOH to avoid pesticide drift during application and as a source of natural reinfestation of *A. ailanthi.* and exposure to the residual activity. Therefore, forty-eight potted TOH were allocated to treatments; the remaining were buffer plants. A 1 m tall mesh fence surrounded the field plot to prevent deer and other curious vertebrates from tampering (Figure 5.1). The plot blocks were grouped in two

and separated by 0.5 m to allow for the collection of foliage and watering. Potted TOH replicates were watered when the potting medium became dry, or roughly every other day for 500–1000 mL per pot, or until 5–10% runoff was achieved. The potted TOH was brought outside the greenhouse, and the treatment applications were applied on October 1, 2021.

The infestation levels of eriophyid and predatory mites were quantified to determine treatment efficacy. Three leaves from each replicate were removed and stored in plastic zip bags for temporary storage. Leaf samples were taken during each predesignated observational window: pre-treatment (0 days after treatment), 3, 7, and 14 days after treatment (DAT). A mite brush machine (Henderson and McBurnie 1943, 1943 #1417) (Leedom Enterprises) was used to quantify mite populations on the surface of a leaf. A leaf sample was defined as three entire leaves, including all leaflets on the leaf. Leaf samples were processed on the day of collection. Each leaflet of a leaf sample was held into the top aperture of the mite brush machine and passed between a pair of revolving brushes. The leaflet sample was removed and discarded after 3–4 passes in the machine. Mites present on the surface of the leaf sample were brushed off and deposited on a glass disc atop a rotating table below the bottom opening. The glass disc was coated with corn syrup to adhere the collected specimens to the glass and inhibit movement. A complete leaf sample, all leaflets, was brushed onto a single collection plate and visually sampled for mite populations. The glass discs were visually inspected and quantified with a dissection microscope.

The mean predatory and eriophyid mites per leaf per treatment were compiled per the DAT observational window. An independent-sample Kruskal-Wallis test was employed to determine treatment effects during each observational window. If statistical differences were found, a Dunn

post-hoc test was performed for multiple pairwise comparisons. A Bonferroni correction was used to determine significant treatment differences (α=0.05). In addition, phytotoxicity was monitored and ranked on a scale of 0–10 based on severity, with a score of zero indicating no phytotoxicity damage was observed. If phytotoxicity injury was observed, the treatment mean ranking was quantified. The trial ended on 14 DAT (15 October 2021). All statistics were performed using SPSS 23.

5.4 Results

Each insecticidal treatment caused a significant reduction in *A. ailanthi* populations compared to mean populations per leaf from the untreated and water check treatments during all observation periods [$H(48) = 27.137$, $p<0.001$; $H(48)=27.849$, $p<0.001$; $H(48)=34.449$, $p<0.001$] (Table 5.1). There were no significant differences detected between any insecticidal treatments during any observation timing [$H(32)=0.567$, $p=0.904$; $H(32)=3.407$, $p=0.333$; $H(32)=0.893$, $p=0.844$; $H(32) =1.419$, $p=0.701$] (Table 5.1). I observed no statistically significant reduction in the mean population of *A. ailanthi* per leaf in the untreated and water check during all observational windows [$H(32)=2.152$, $p=0.542$; $H(32)=1.108$, $p=0.775$] (Table 5.1). Alternatively, each insecticidal treatment caused a statistically significant reduction in the mean population of *A. ailanthi* per leaf [$H(32)=15.260$, $p=0.002$; $H(32)=11.646$, $p=0.009$; $H(32)=12.592$, $p=0.006$; $H(32)=18.359$, $p<0.001$] (Table 5.1). Pre-treatment counts of the mean population of *A. ailanthi* per leaf found more than 100 mites per leaf. By 14 DAT, the insecticidal treatments had, on average, less than ten eriophyid mites detected per leaf compared to both checks, which still had populations of *A. ailanthi* greater than 50 individuals per leaf.

No predatory mites were detected across all observation periods, and no phytotoxicity was detected from any insecticidal treatments across the 14 DAT. During the pre-treatment observational window, no significant differences were observed for the mean population of *A. ailanthi* per leaf between all treatments [H(48)=0.792, p=0.978].

5.5 Discussion and Conclusion

By the end of the 14-day observation period, most TOH replicates were stripped of all foliage (Figure 5.1, 5.2, 5.3); future research should use larger TOH replicates or those with more robust canopies to allow more extended observation of *A. ailanthi* populations. The lack of significant differences in the mean population of *A. ailanthi* per leaf between all treatments during the pre-treatment observational window indicates that all TOH replicates were infested with similar populations of eriophyid mites. I observed an immediate drop in *A. ailanthi* populations in all treatments 3 DAT, including both checks, immediately upon the potted TOH replicates being removed from the greenhouse environment. This population decline and the lack of predatory mites indicate that some environmental factors influenced the eriophyid mite populations. Environmental factors such as landscape setting, relative humidity, ambient temperature, leaf temperature, and precipitation can all influence eriophyid mite populations (Skoracka et al. 2010, de Lillo et al. 2018). Temperatures were above 32 °C in the greenhouse, which could contribute to the fast-growing mite population I observed (de Lillo et al. 2018). Furthermore, it is probable that mite populations were blown away from heavy winds, further reducing populations as seen after 3 DAT in all treatments, a typically observed dispersal method (de Lillo et al. 2018).

However, more needs to be understood about the life history and biology of *A. ailanthi*, which further creates difficulties with pest management or biological control.

The mite brush machine utilized was initially designed for predatory mite quantification. Despite the intended use, the machine was sufficient to quantify *A. ailanthi* for this trial. The mite brush methodology outlined was more efficient than counting mites on individual leaves with a dissection microscope, as I attempted in preliminary screenings. Future research on *A. ailanthi* should consider quantification methods to reduce sampling time and better standardize each sample. Monfreda et al. (2010) did a meta-analysis on eriophyid mite sampling techniques. They found that the most frequently employed methods involved some variation of washing the infested plant material in a solution (tap water, organosilicon surfactant, ethanol, bleach), shaking the solution for some time, then sieving the solution to separate the plant material, mites, and eggs. The wash, shake, and sieve method for quantifying eriophyid mites outlined by Monfreda et al. (2007) is non-destructive and can be used with a variety of solutions and sieves; thus, it can be helpful for a variety of purposes, including slide mounting specimens, biological studies, molecular analysis, taxonomical studies, bioassays, rearing, host specificity, and mass release. To further standardize replicate sampling, the total leaf area per replicate should be measured to determine the mean number of mites per leaf area. Finally, the number of mite eggs per leaf sample should be quantified to understand population dynamics better.

I anticipated that the populations of predatory mites would increase and *A. ailanthi* would decrease after removal from the greenhouse and exposure to natural conditions, regardless of insecticidal treatments. Although I did observe a natural reduction in *A. ailanthi* populations in

both check treatments, no predatory mites were detected on any leaf samples during the entire trial period. The scarcity of predatory mites indicates the absence of populations within the greenhouse environment where the TOH was initially grown. Furthermore, the insecticidal residue may control both potential predatory mites and *A. ailanthi* populations; however, that does not account for the lack of predatory mites within both check treatments. Typically, mite brush machines are used to accurately quantify mite populations, such as spider mites and predatory mites (MacMillan and Costello 2015); therefore, it is unlikely that the sampling methodology resulted in a lack of predatory mite detections.

This research demonstrated that *A. ailanthi* populations are highly susceptible to all the insecticide treatments in this trial. Utilizing a spray program that alters the active ingredient regularly will be paramount to prevent breeding resistance in *A. ailanthi* in greenhouse populations. Further investigation into the use of commercially available predatory mites to manage the impacts of *A. ailanthi* on TOH grown for research or ornamental purposes could help mitigate the risks associated with insecticidal applications. Using predatory mite species could provide control of the damaging rust mite while resulting in no harm to SLF (Elhalawany et al. 2021). Cultural control methods, such as ventilation screens and environmental control inside and outside the greenhouse environment, should be incorporated to help reduce the probability of *A. ailanthi* infestations.

Furthermore, this research helps demonstrate the potential use of *A. ailanthi* as a biological control agent for TOH due to the extensive injury observed in the check replicates. The extent of injury caused by *A. ailanthi* on mature TOH was not investigated here, and future work is needed

to document the full potential of this biological control agent. The spread of several invasive species like BMSB and SLF has brought the research community's attention to the lack of scientific research associated with TOH. Consequently, more observations of *A. ailanthi* are being documented in the USA, which could further support the utilization of it as a potential biological control agent. As Bielski et al. (i*n press*) outlined, *A. ailanthi*, *Aculops taihangensis* (Hong and Xue), *Oculus altissima* (Xue and Hong), *Aculus mosoniensis* (Ripka and Ersek) are suspected to be the same species. This claim is further corroborated by Skvarla et al. (2021) and de Lillo et al. (2022) based on morphological and molecular analysis. These mites have been reported to cause injury to TOH saplings, which have demonstrated potential as a biological control agent against TOH in European field trials (Gardener 2008, Skvarla et al. 2021, Marini et al. 2022).

5.6 Acknowledgments

This project was funded by the USDA-NIFA CAP Award 2019-51181-30014 and the Virginia Wine Board (Proposal Number PUVQHEEZ).

5.7 References

Barringer, L. E., L. R. Donovall, S. E. Spichiger, D. Lynch, and D. Henry. 2015. The first new world record of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae). Entomological News 125(1) 20–23. Doi:10.3157/021.125.0105.

Bielski, J., D. Pfeiffer, R. Ochoa, A. Brown, and A. Ulsamer. *In Press*. New state records of *Aculops ailanthi* (Lin, Jin, and Kuang) (Acariformes; Trombidiformes; Prostigmata: Eriophyidae) in USA; a pest or biological control agent of *Ailanthus altissima* (Mill.) Swingle? Proceedings of the Entomological Society of Washington.

Bullock, R. C., and R. R. Pelosi. 1998. Alert^{R} (Chlorfenapyr): A new miticide for use in Florida Citrus. Proceedings of the Florida State Horticultural Society 111 159–163.

de Lillo, E., A. Pozzebon, D. Valenzano, and C. Duso. 2018. An intimate relationship between eriophyoid mites and their host plants. Frontiers in Plant Science 9 1–14. Doi:10.3389/fpls.2018.01786.

Ding, J., Y. Wu, H. Zheng, W. Fu, R. Reardon, and M. Liu. 2006. Assessing potential biological control of the invasive plant, tree-of-heaven, *Ailanthus altissima*. Biocontrol Science and Technology 16(6) 547–566. https://doi.org/10.1080/09583150500531909

Elhalawany, A. S., R. I. A. Abo-Shnaf, and A. S. Sanad. 2021. Release of predatory mite, *Neoseiulus barkeri* (Acari: Phytoseiidae) for its suppression two species of eriophyid mites (Acari: Eriophyidae) on olive seedlings in Egypt. International Journal of Acarology 47 35–40. https://doi.org/10.1080/01647954.2020.1870547

Fryer, J. L. 2010. *Ailanthus altissima*. Fire Effects Information System. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. https://www.fs.usda.gov/database/feis/plants/tree/ailalt/all.html

Gardner, R.T. 2008. Native and Indigenous Biocontrols for *Ailanthus altissima*. Master's Thesis, University of Maryland, College Park, MD, USA. Pp 140. [Thesis]

Hoebeke, E., and M. E. Carter. 2003. *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): A polyphagous plant pest from Asia newly detected in North America. Proceedings of the Entomological Society of Washington 105 225–237.

Hu, Shiu-ying. 1979. *Ailanthus altissima*. Arnoldia. 39(2): 29–50.

Kowarik, I., and I. Säumel. 2007. Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. Perspectives in Plant Ecology, Evolution and Systematics 8(4) 207–237. doi:10.1016/j.ppees.2007.03.002.

Lin, F. P., C. Jin, and H. Y. Kuang. 1997. A new genus and six new species of Eriophyidae from China (Acari: Eriophyoidea). Acta Entomologica Sinica 40(1) 86–93.

MacMillan, C. D., and M. J. Costello. 2015. Evaluation of a brushing machine for estimating density of spider mites on grape leaves. Experimental and Applied Acarology 67(4) 583–594. https://doi.org/10.1007/s10493-015-9972-6

Marini, F., M. Cristofaro, G. Mecca, B. Vidović, H. Williams, C. Moffat, H. L. Hinz, and P. Weyl. 2022. Biological control of tree of heaven, *Ailanthus altissima.* Annual Report 2021 CABI.

McCoy, C. W., R. C. Bullock, and R. A. Dybas. 1982. Avermectin B_1: A novel miticide active against citrus mites in Florida. Proceedings of the Florida State Horticultural Society 95 51–56.

Monfreda, R., Nuzzaci G., de Lillo E. 2007. Detection, extraction, and collection of eriophyoid mites. Zootaxa 1662 35–43.

Monfreda, R., M. Lekveishvili, R. Petanovic, and J. W. Amrine. 2010. Collection and detection of eriophyoid mites. Experimental and Applied Acarology 51(1) 273–282. https://doi.org/10.1007/978-90-481-9562-6_14

Skoracka, A., L. Smith, G. Oldfield, M. Cristofaro, and J. W. Amrine. 2010. Host-plant specificity and specialization in eriophyoid mites and their importance for the use of eriophyoid mites as biocontrol agents of weeds. Experimental and Applied Acarology 51(1) 93–113. Doi:10.1007/s10493-009-9323-6.

Skvarla, M. J., R. Ochoa, A. Ulsamer, and J. Amrine. 2021, The eriophyid mite *Aculops ailanthi* Lin, Jin, and Kuang, 1997 (Acariformes: Prostigmata: Eriophyidae) from tree-of-heaven in the United States – new state records and morphological observations. Acarologia 61(1) 121–127. doi:10.24349/acarologia/20214421.

Sonalkar, V. U., A. K. Sadawarte, M. V. Totawar, P. K. Nagre, and P. B. Wankhade. 2014. Management of citrus mites with bio-rational pesticides. The Punjabrao Krishi Vidyapeeth Research Journal 38(1) 26–31.

5.8 Figures and Tables

Figure 5.1–5.3 5.1 Image of field plot with a completely random block design. The field plot contained five replicates per block and ten blocks total. Each block was composed of ten plants, for a total of 100 plants. Each treatment replicate was separated by a non-treated buffer TOH. The image was captured at the end of the 14 DAT observational window. Most of the TOH foliage was removed for the trial or due to damage from *A. ailanthi*. 5.2 Potted TOH heavily infested with *A. ailanthi* at 14 DAT. Apical meristem decay and withering can be observed, in addition to leaf defoliation and deformation. 5.3 New apical growth has occurred in this healthy potted TOH replicate 14 DAT.

Table 5.1 Mean (±) SEM *A. ailanthi* mites per leaf on potted TOH seedlings in the field following application of insecticides.[1]

					Mean (±) SEM *A. ailanthi* per Leaf			
Treatment	**Manufacturer**	**Active Ingredient**	**Application Rate (mL/L)**	**n**	**0 DAT**	**3 DAT**	**7 DAT**	**14 DAT**
Untreated Check	-	-	-	3	130 ± 43.8 a	194 ± 79.8 a	76 ± 16.5 a	66 ± 16.3 a
Water Check	-	-	-	3	259 ± 115.8 a	99 ± 25.9 a	59 ± 15.7 a	78 ± 18.9 a
M-Pede®	Gowan® USA	insecticidal soap	20.0	3	111 ± 41.5 a	14 ± 5.2 b	11 ± 6.6 b	4 ± 2.1 b
Trilogy®	Certis Bio LLC.	neem oil	20.0	3	146 ± 70.1 a	20 ± 5.6 b	10 ± 4.7 b	7 ± 3.4 b
Avid® 0.15 EC	Syngenta®	avermectin	3.1	3	251 ± 100.8 a	21 ± 15.4 b	4 ± 2.9 b	3 ± 2.5 b
Phantom	BASF	chlorfenapyr	0.2	3	126 ± 31.7 a	7 ± 2.9 b	8 ± 4.0 b	4 ± 2.1 b

[1]Data were rank transformed for Kruskal-Wallis analysis; untransformed data are shown and separated by each observation window (0, 3, 7, 14 DAT). Data within columns followed by a letter in common are not significantly different; $p < 0.05$.

Chapter 6: Summary, Implications, and Suggestions for Future Research

Jason Tyler Bielski

6.1 Summary and Implications

There is a long window of opportunity to control this inactive egg life stage because spotted lanternfly (SLF), *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), overwinter for up to eight months a year in the USA (Smyers et al. 2021). Despite this long, inactive life stage, most SLF management in vineyards is focused on the adult life stage (Leach and Leach 2020). While nymphs and adult SLF are susceptible to a vast array of insecticides (Leach et al. 2019, Lee et al. 2019), it has been observed that vineyards have increased the frequency of pesticide applications up to three times to manage SLF (Urban 2019). The high frequency of pesticide applications to manage SLF populations in vineyards is a result of the SLF from the surrounding landscape immigrating into vineyards, in conjunction with the typical grape harvest in the Mid-Atlantic regions (Leach and Leach 2020) and the preharvest interval of many preferred pesticides against SLF. While no disease transmission has been demonstrated from SLF (Brooks et al. 2020), passive transmission of bacteria, such as fire blight by phloem-feeding leafhoppers, is possible (Pfeiffer et al. 1999). While no action thresholds currently exist for SLF, Harner et al. (2022) suggested a grape nutrient threshold of 5-15 SLF per vine because it was observed that dense aggregations of SLF per vine can negatively impact grape vine nutrition and dynamics. More robust management options must be available if no-tolerance or low-tolerance thresholds for SLF are ever required.

Alternative methods for managing SLF should be incorporated into existing integrated pest management programs. It has been observed that SLF displays a strong edge effect in vineyards, specifically during the overwintering life stage, early nymphs, and adults (Leach and Leach 2020). In forested settings, a significant portion of SLF egg masses are laid above three meters, outside the reach of egg scraping (Keller et al. 2020). According to Strömbom and Pandey's (2021) modeling of SLF life cycles under the influence of different management strategies, population reduction can only occur if at least 35% of all SLF life stages are managed. This means that the management of SLF populations only at the adult life stage is inadequate. Few ovicidal studies have been done against SLF (Shin et al. 2010, Leach et al. 2019) despite the long window of opportunity against overwintering SLF egg masses. Here, I further investigated insecticide ovicidal and biopesticide effects against overwintering SLF.

The research conducted for this dissertation demonstrated additional pest management tactics that can aid stakeholders in reducing the population of the invasive SLF. Specifically, I investigated the efficacy of 13 pesticides across various modes of action against SLF egg masses when applied at one of three different overwintering timings under laboratory settings. I found that while most insecticidal treatments provided some degree of ovicidal effects, only chlorpyrifos and malathion provided near complete control of hatching SLF at all application timings. Field tests also corroborated laboratory bioassay findings that showed that malathion demonstrated strong ovicidal effects. In laboratory and field research, I found that applications 2 weeks before hatch (WBH) had the greatest ovicidal effects, although I observed hatch reductions when applications were made 20 WBH. It has been previously documented that chlorpyrifos displayed strong

ovicidal effects against SLF (Shine et al. 2010, Leach et al. 2019). For regulatory reasons, chlorpyrifos is no longer available for use on food crops in the USA. Therefore, stakeholders need to find a workable replacement with comparable ovicidal effects.

In addition to conventional ovicidal research, this dissertation examined the effects of applications of *Beauveria bassiana* (Hypocreales: Cordycipitaceae) (Bals.-Criv.) Vuill biopesticides against overwintering SLF. Specifically, I examined if a single application of *B. bassiana* mycoinsecticides applied to SLF egg masses during the overwintering period could result in infection from recently emerged neonates. It has previously been demonstrated that SLF nymphs and adults are susceptible to multiple strains and formulations of *B. bassiana* in lab and field studies (Clifton et al. 2020, Clifton and Hajek 2022). In laboratory bioassays, I examined the effects of two application timing. Regardless of application timing, all strains and formulations of *B. bassiana* applied to SLF egg masses resulted in infection of exposed nymphs. More consistent infection rates in cadavers when SLF egg masses were treated at 2 WBH. Still, sporulation of cadavers was also observed when SLF egg masses were exposed to *B. bassiana* mycoinsecticides 20 WBH. In two field trials, I observed infection in exposed nymphs at rates higher than laboratory bioassays. However, in the field trials, egg masses were only exposed to mycoinsecticide applications 2 WBH or during hatch. The novel technique of applying biopesticides directly to overwintering egg masses demonstrated here could provide alternative organic management options for SLF. In contrast, the infection rates observed were not as significant as the hatch reduction from ovicidal applications of malathion; applications of mycoinsecticides result in fewer environmental and human health ramifications than organophosphate treatments. Furthermore,

applications of biopesticides to overwintering SLF egg masses can provide stakeholders with a management technique to help reduce SLF populations before they become outbreak levels and save pesticide applicators an application of limited-use pesticides that could be saved for later season populations of adult SLF.

Should this innovative approach be incorporated into integrated pesticide management, the planned strategy would be to use a backpack sprayer to apply pesticides to the edges of vineyards or other high-risk locations. This methodology would give stakeholders a low-cost, high-output strategy to manage SLF during their immobile life stage. Furthermore, during the time of SLF overwintering, vineyard maintenance may be lower, allowing for more time to scout the high-risk areas for high populations of SLF egg masses. Additionally, the lack of foliage in winter can enable applications with better spray coverage. This technique could apply to applications of malathion.

I announced the first findings of *Aculops ailanthi* (Lin, Jin, and Kuang) (Trombidiformes: Eriophyidae), the non-gall-producing rust mite on tree-of-heaven (TOH), *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae), State records from Wayne County, Michigan, and Montgomery County, Virginia, USA. These are the fourth and fifth states to report the presence of this mite. The pest was first detected in Virginia because of severe symptoms on TOH seedlings grown in greenhouses. The mites were discovered in Michigan due to similar findings during a field study. USDA-ARS received samples from both states and used scanning electron microscopy to confirm the species' identity as *A. ailanthi* based on morphological characteristics.

There currently is some taxonomic confusion regarding the two genera of TOH feeding mites, *Aculops* and *Aculus*. *Aculops ailanthi, Aculops taihangensis* (Hong and Xue), *Aculus altissimae* (Xue and Hong), *Aculus mosoniensis* (Ripka and Ersek) have all been observed to feed on TOH (Gardener 2008, Ripka and Ersek 2014, Skvarla et al. 2021, de Lillo et al. 2022, Marini et al. 2021). Nevertheless, all four species are suspected to be the same mite, *Aculops ailanthi*, the senior synonym in this set of names. The difficulty of preparing specimens to show the true character of the mites has resulted in several authors thinking they had found a new species. This confusion is relatively common in the Eriophyoidea, and all acarologists must scrutinize specimens from as many aspects as possible. Scanning electron microscopy images can resolve the generic assignment when high magnification and high resolution are used, and the anterior prodorsal section of the mite is observed from several views on several specimens. DNA typing is beginning to show the errors in synonymy (personal communication with de Lillo, 2023); respective samples collected from Virginia and Michigan *A. ailanthi* populations have been sent to de Lillo's lab for further genetic analysis. This taxonomic problem can only be resolved by DNA-typing of collections of the mites, especially from several areas in China, including the type locality and other regions worldwide. In combination with DNA typing, thorough and careful microscopic examination and photography of many specimens from each location must be made. These mites all belong to the genus *Aculops*, which was accurately determined by Lin et al. (1997). Some slide preparations indicate the genus *Aculus*, but these have not provided details of the apical-most portion of the frontal lobe; semi-dorsal preparations at high magnification usually reveal the sharply pointed declivus frontal lobe (personal communication with Ron Ochoa USDA).

I initially became aware of *A. ailanthi* due to the pest pressure exhibited on the TOH cultivated for SLF bioassays. I conducted a foliar insecticide field trial to determine the effective management of *A. ailanthi* on TOH. A single application of any tested pesticide significantly controlled *A. ailanthi* populations. In this case, *A. ailanthi* is considered a pest, but in another context, it can be considered a potential biological control agent for TOH. Damage from *A. ailanthi* primarily impacts younger TOH. It is possible that significant area-wide control of TOH can be achieved with *A. ailanthi*, in conjunction with other biological control agents such as *Eucryptorrhynchus brandti* (Harold) (Coleoptera: Curculionidae), and *Verticillium nonalfalfae* [Inderb. (formerly *Verticillium albo-atrum* Reinke and Berthold) (Hypocreales: Plectosphaerellaceae). Furthermore, eliminating one of the SLF preferred host plants could provide unseen ramifications for SLF management.

6.2 Suggestions for Future Research

There are many limitations when working with SLF. First, the invasive status of SLF can limit the geographic range of research. State "Stop the Spread" campaigns can limit invasive species range expansion but create difficulties for researchers relying on wild-caught populations of SLF to conduct research. Second, SLF is univoltine; therefore, only one generation of the population is available for research during any given year. Since this dissertation research focused on the overwintering life stage, I had a narrow time window to collect SLF egg masses and then

make appropriate treatments. Finally, SLF are exceptionally difficult to lab rear, further complicating research that can be done.

In this dissertation research methodology, I did not rear out the SLF nymphs after hatch. Instead, I only quantified the hatch rates associated with ovicidal effects from insecticidal treatments. Future research should further investigate the residual impact of insecticide when treated to SLF egg masses. According to Chase and Wright (2023), applying bifenthrin to SLF egg masses before hatching did not stop hatching, but it quickly killed nymphs as they came into contact with residues. I found strong ovicidal effects from chlorpyrifos and malathion when applied to overwintering SLF at all application times. Although some of the insecticidal treatments did not demonstrate as strong, consistent ovicidal effects, they could demonstrate additional nymphal mortality due to residual contact. Furthermore, it is possible that insect growth regulators, such as buprofezin, tested in this dissertation could provide subsequent life table impacts to SLF when overwintering egg masses are treated (Nagatai 1986).

In laboratory and field studies, I found evidence that SLF egg masses treated with *B. bassiana* biopesticides are susceptible to infection. I suggest further field research to establish the most efficacious application timing, application methods (such as application rate and spraying technique), biopesticide strain, and formulation against overwintering SLF egg masses. Furthermore, I suggest a greater number of replicates be incorporated. Collecting individual egg masses can require excessive amounts of time and damage the egg masses, further skewing hatch

effects. Although I found strong effects in field studies, I recommend further investigation into the impact of both insecticidal and biopesticide treatments on SLF egg masses, specifically within a vineyard setting.

I recommend undertaking additional surveys to ascertain the complete range of *A. ailanthi* in the USA. Regional and environmental variation may influence the genetic expression of *A. ailanthi*, making phenotypes more appropriate for the biological control of TOH. If a better phenotype for biological control on TOH exists, it should be compared to the known populations of *A. ailanthi* in the USA. Finally, determining the full taxonomic relations of *Aculops* and *Aculus* is imperative to establishing further biological control potential against TOH.

6.3 References

Brooks, R. K., A. L. Snyder, E. A. Bush, S. M. Salom, and A. Baudoin. 2020. First report of *Verticillium* Wilt caused by *Verticillium dahliae* impacting *Ailanthus altissima* in Virginia, U.S.A. Plant Disease 104(5) 1558–1560. doi:10.1094/PDIS-10-19-2064-PDN.

Chase, K. D., and S. E. Wright. 2023. Preemergent control of spotted lanternfly, 2020. Arthropod Management Tests 48(1) 1–2. doi.org/10.1093/amt/tsad001

Clifton, E. H., A. E. Hajek, N. E. Jenkins, R. T. Roush, J. P. Rost, D. J. Biddinger, and P. Nachappa. 2020. Applications of *Beauveria bassiana* (Hypocreales: Cordycipitaceae) to control populations of spotted lanternfly (Hemiptera: Fulgoridae), in semi-natural landscapes and

on grapevines. Environmental Entomology 49(4) 854–864. https://doi.org/10.1093/ee/nvaa064

Clifton, E. H., and A. E. Hajek. 2022. Efficacy of *Beauveria bassiana* and *Cordyceps javanica* mycoinsecticides against spotted lanternflies, *Lycorma delicatula*, in laboratory bioassays. Biocontrol Science and Technology 32(7) 824–836. https://doi.org/10.1080/09583157.2022.2052804

de Lillo, E., F. Marini, M. Cristofaro, D. Valenzano, R. Petanović, B. Vidović, T. Cvrković, and M. Bon. 2022. Integrative taxonomy and synonymization of *Aculus mosoniensis* (Acari: Eriophyidae), a potential biological control agent for tree of heaven (*Ailanthus altissima*). Insects 13(5) 1–16. doi:10.3390/insects13050489.

Gardner, R.T. 2008. Native and Indigenous Biocontrols for *Ailanthus altissima*. Master's Thesis, University of Maryland, College Park, MD, USA. Pp 140. [Thesis]

Harner, A. D., H. L. Leach, L. Briggs, and M. Centinari. 2022. Prolonged phloem feeding by the spotted lanternfly, an invasive planthopper, alters resource allocation and inhibits gas exchange in grapevines. Plant Direct 6(10) 1–18. doi.org/10.1002/pld3.452.

Keller, J., J. Rost, K. Hoover, J. Urban, H. Leach H, M. Porras, B. Walsh, M. Bosold, and D. Calvin. 2020. Dispersion patterns and sample size estimates for egg masses of spotted lanternfly (Hemiptera: Fulgoridae). Environmental Entomology 49(6) 1462–1472. https://doi.org/10.1093/ee/nvaa107

Leach, H., D. J. Biddinger, G. Krawczyk, E. Smyers, and J. M. Urban. 2019. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula*, (Hemiptera:

Fulgoridae), a new pest of fruit in the Northeastern U.S. Crop Protection 124(1) 1–6. https://doi.org/10.1016/j.cropro.2019.05.027

Leach, H., and A. Leach. 2020. Seasonal phenology and activity of spotted lanternfly *(Lycorma delicatula*) in eastern US vineyards. Journal of Pest Science 93(4) 1215–1224. https://doi.org/10.1007/s10340-020-01233-7

Lee, D. H., Y. L. Park, and T. C. Leskey. 2019. A review of biology and management of *Lycorma delicatula* (Hemiptera: Fulgoridae), an emerging global invasive species. Journal of Asia-Pacific Entomology 22(2) 589–596. https://doi.org/10.1016/j.aspen.2019.03.004

Lin, F. P., C. Jin, and H. Y. Kuang. 1997. A new genus and six new species of Eriophyidae from China (Acari: Eriophyoidea). Acta Entomologica Sinica 40(1) 86–93.

Marini, F., M. Cristofaro, G. Mecca, B. Vidović, H. Williams, C. Moffat, H. L. Hinz, and P. Weyl. 2022. Biological control of tree of heaven, *Ailanthus altissima*. Annual Report 2021 CABI.

Nagatai, T. (1986). Timing of buprofezin application for control of the brown planthopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae). Applied Entomology and Zoology 21(3) 357–362.

Pfeiffer, D.G., Killian, J.C., Yoder, K.S., 1999. Clarifying the roles of white apple leafhopper and potato leafhopper (Homoptera: Cicadellidae) in fire blight transmission in apple. Journal of Entomological Science 34(3) 314–321. https://doi.org/10.18474/0749-8004-34.3.314

Ripka, G., and L. Ersek. 2014. A new *Aculops* species (Acari: Prostigmata: Eriophyoidea) on *Ailanthus altissima* from Hungary. Acta Phytopathologica et Entomologica Hungarica 49(1) 49–56. doi:10.1556/APhyt.49.2014.1.5.

Shin, Y.-H., S.-R. Moon, C. Yoon, K.-S. Ahn, and G.-H. Kim. 2010. Insecticidal activity of 26 insectcides against eggs and nymphs of *Lycorma delicatula* (Hemiptera: Fulgoridae). The Korean Journal of Pesticide Science 14(2) 157–163.

Skvarla, M. J., R. Ochoa, A. Ulsamer, and J. Amrine. (2021), The eriophyid mite *Aculops ailanthi* Lin, Jin, and Kuang, 1997 (Acariformes: Prostigmata: Eriophyidae) from tree-of-heaven in the United States – new state records and morphological observations. Acarologia 61(1) 121–127. doi:10.24349/acarologia/20214421.

Smyers, E. C., J. M. Urban, A. C. Dechaine, D. G. Pfeiffer, S. R. Crawford, and D. D. Calvin. 2021. Spatio-temporal model for predicting spring hatch of the spotted lanternfly (Hemiptera: Fulgoridae). Environmental Entomology 50(1) 126–137. https://doi.org/10.1093/ee/nvaa129

Strömbom, D., and S. Pandey. 2021. Modeling the life cycle of the spotted lanternfly (*Lycorma delicatula*) with management implications. Mathematical Biosciences 340(1) 108670. doi.org/10.1016/j.mbs.2021.108670

Urban, J. M. 2019. Perspective: Shedding light on spotted lanternfly impacts in the USA. Pest Management Science 76 10–17. https://doi.org/10.1002/ps.5619