The Pennsylvania State University

The Graduate School

MONITORING AND COLD TOLERANCE OF THE INVASIVE SPOTTED LANTERNFLY, *LYCORMA DELICATULA* (HEMIPTERA: FULGORIDAE)

A Thesis in
Entomology

by
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ABSTRACT

The spotted lanternfly (*Lycorma delicatula*) (SLF) has been an invasive species in the Eastern United States since 2014. It first established in Berks, Co. Pennsylvania and has been rapidly spreading to other states since. This insect is a phloem feeding planthopper that specifically impacts grapes as well as other native hardwoods. Not only has SLF become a nuisance to homeowners, but also an economically important pest. The goal of this thesis is to improve monitoring tools for regions where SLF persists and could distribute to.

This thesis has four chapters; the first chapter is a literature review to focus on the current research and knowledge of SLF since its arrival in the United States. The last three chapters describe research experiments on SLF management, monitoring, and biology.

The second chapter of this thesis is a three-year study at Green Hills Preserve in Mohnton, Pennsylvania using circle traps on different host species to determine the best deployment strategies for capturing SLF throughout the season. Using this monitoring method, we were able to better understand population presence, relative abundance, seasonal activity, and multi-year trends of SLF in an area near where the infestation first began. Our results showed that what is considered in practice to be the ideal host species to deploy traps on, *A. altissima*, did not significantly out-perform other tree species, as other species (e.g., *J. nigra*) can also yield strong capture. Traps are most effective when they are deployed before May and taken down at the end of November. In 2021, a strong decline in the population followed by a spike the following year was observed. Overall, circle traps are most effective in areas with high SLF populations, though similar tools have been successful in residential areas where there are high numbers on individual trees.

The third chapter of this thesis reports results of experiments on SLF first instar cold tolerance. Laboratory studies were designed to investigate four different cold tolerance factors of first instar nymphs to aid in determining where SLF can distribute and establish. The four factors included critical thermal minimum (CTmin), chill coma recovery time (CCRT), lower lethal temperature (LLT), and lethal time at low temperature (LLt). For first instar nymphs, the CTmin ranged from 7.3 to 7.8 °C, with a mean CTmin of 7.666 ± 0.017 °C (mean ± SEM). The CCRT ranged from 1 to 621 seconds, with a mean CCRT of 114 ± 18 seconds (mean ± SE). The LLT was near -7.5 °C for 100% death, while LLt is predicted to occur around 950 minutes at -2.5 °C

for 100% death based on results using logistic regression modeling. Overall, this study provides first insights of cold tolerance for SLF and helps to develop and inform models for understanding limits for first instar survival.

The fourth chapter of this thesis investigates evidence concerning whether and potentially how SLF generates heat. There is a crucial need for improved monitoring tool for SLF in areas of infestation as well as areas of potential establishment. It has been demonstrated that SLF are able to be detected using infrared cameras, suggesting that the insect seems to be generating heat. Yet, evidence concerning whether SLF is generating heat is limited, and if it is, potential explanations of how and under what conditions remains to be explored. In this study, adult SLF body temperatures are taken throughout the day, along with weather data, live body mass, and activity in order to gather more baseline evidence concerning whether SLF may be generating heat. This information sheds light on this potentially unusual aspect of SLF's basic biology, in order to inform further studies of possible endothermy, which in turn, could lead to the potential development of thermal monitoring for SLF.

Findings from this thesis help to improve monitoring tool for the invasive SLF in regions where it persists and could distribute to. Trends from the three-year trapping study demonstrates that host species can vary and still yield capture. The firsthand information on first instar cold tolerance of this species as well as investigating evidence into whether and potentially how SLF generates heat gives a better understanding of SLF's basic biology and the ability to determine better prediction of distribution, establishment, and potential control and monitoring methods. Altogether, this information can aid in the use of monitoring tools in infested areas as well as areas that are at risk of establishment.

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Chapter 1

Spotted Lanternfly Literature Review

Introduction

*Lycorma delicatula***,** commonly known as the spotted lanternfly (SLF), is an invasive planthopper in the Eastern United States (Dara et al. 2015) that is native to Asia, and was first detected in Berks County, Pennsylvania in 2014 (Dara et al. 2015, Bourgoin 2019). Since then, SLF populations have rapidly spread with current established populations of the insect known to occur in Pennsylvania as well as in fifteen nearby states (NYSIPM- Cornell 2023). Since its arrival, SLF has caused significant damages to a variety of agricultural industries as well as being a nuisance to homeowners. Chapter 1 reviews SLF's life cycle, management, agricultural and economic impacts, as well as relevant background concerning the need for improved monitoring that is the basis for the research presented in this thesis.

Host Range and Life Cycle

The spotted lanternfly (*Lycorma delicatula* (White) (Hemiptera: Fulgoridae)) (SLF) was first detected in Berks County Pennsylvania in 2014 (Dara et al. 2015). Survey information from the Pennsylvania Department of Agriculture and USDA APHIS indicates it is likely that SLF came to Pennsylvania on a shipment of stone as egg masses (Parra et al. 2017). SLF is native to China, Vietnam, and Japan (Dara et al. 2015, Bourgoin 2019). Since its arrival, SLF has spread from Pennsylvania and is established in a total of sixteen states in Northeastern US. To reduce likelihood of further spread, internal state quarantines have been introduced in Connecticut, Delaware, Maryland, New Jersey, Ohio, Pennsylvania, and Virginia. Other states that do not have infestations but have had detections of SLF (but with no known established populations) include New Hampshire and Vermont (**Figure 1-1**) (NYSIPM- Cornell 2023).

SLF is univoltine and its life cycle includes four nymphal stages, an adult stage, and an overwintering egg stage (**Figure 1-2**). Depending on seasonal temperatures, first instar nymphs begin emerging from their egg masses in early spring (Dara et al. 2015) and after 2-3 weeks, the insect will molt into the next instar. During the summer, adults begin to emerge and will lay eggs from August until early November (Dara et al. 2015). SLF overwinters in the egg stage, as egg masses are laid from September to November.

The identification of this insect can be done by color and size. First instar (4 mm), second instar (6 mm), and third instar (10 mm) nymphs are black with white spots. The fourth instar (12 mm) is red with black markings and white spots. Adult SLF are deep brown on their head and legs with a yellow abdomen with black bands. Forewings are grey with black spots with black veins on the wing tips. Hindwings are black and white anteriorly and bold red with black spots posteriorly (Dara et al. 2015). Males (21-22 mm) and females (24-27 mm) vary in size, with females being typically larger than males. Females can be identified by the red postero-caudal end of the abdomen, with males lacking the red color. When egg masses are laid, each contain about 30-50 eggs. The female covers the eggs in a tannish brown waxy layer, which then hardens to form an oothecum to protect the eggs for the winter (Dara et al. 2015). Egg masses can be seen on tree bark, stone, cinder blocks, automobiles, rail cars, and shipping pallets (Urban 2020, Dara et al. 2015). Egg masses on nonplant material adds to the risk of spread (Dara et al. 2015).

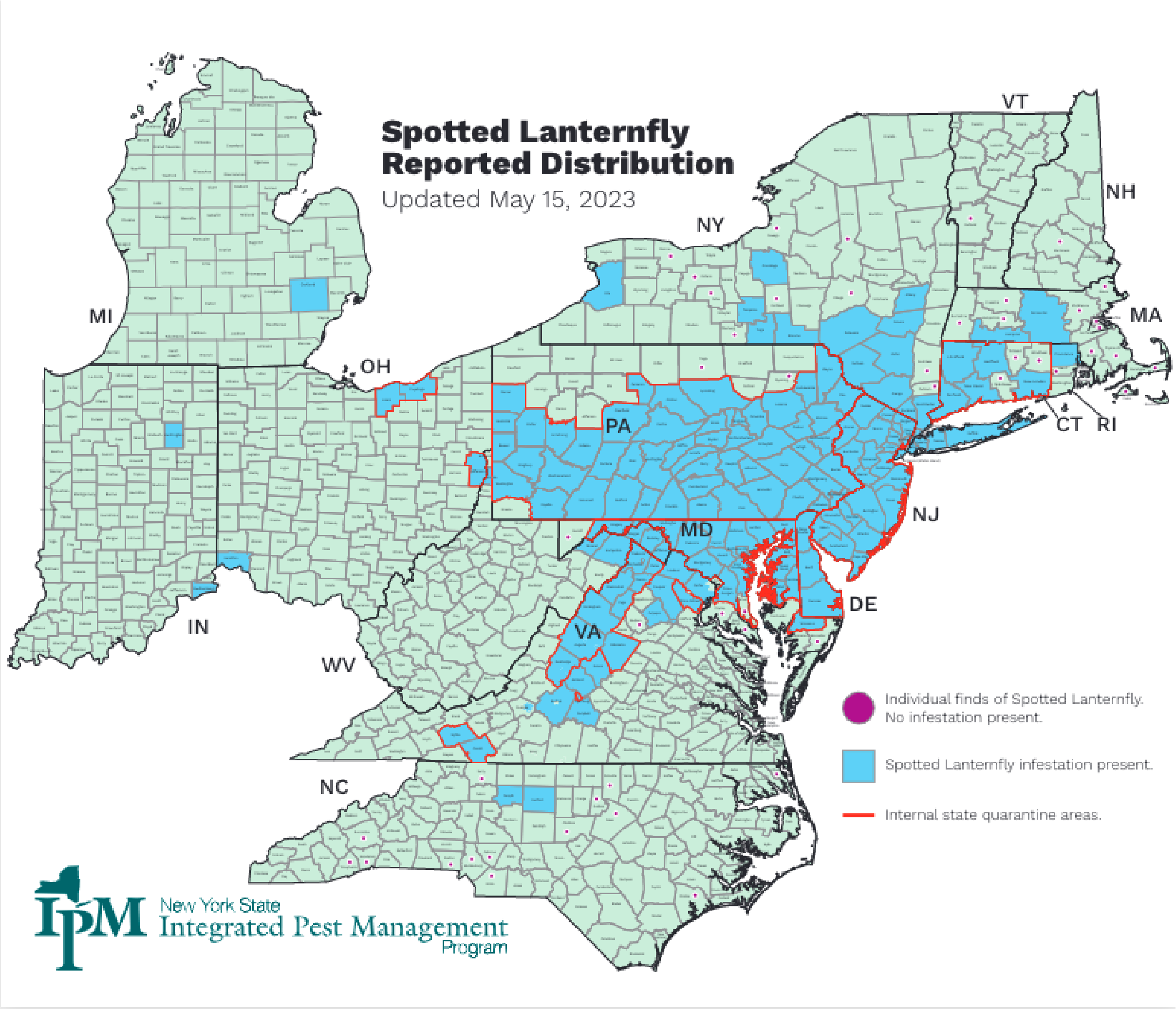

Figure 1-1: Reported distribution of SLF in the United States as of May 2023. (NYSIPM, 2023)

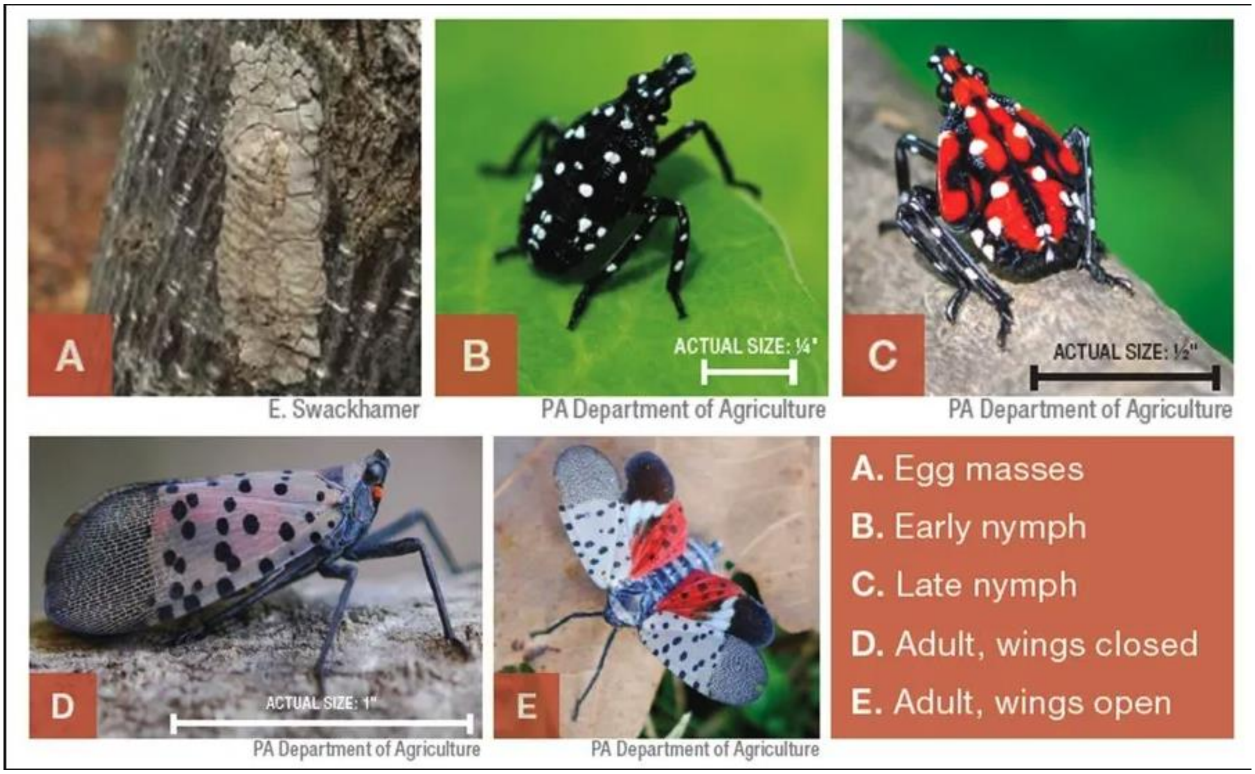

Figure 1-2: All life stages of the SLF, from egg to adult. (Penn State Extension, 2023)

Diet

This generalist phloem-feeding planthopper is typically known to feed on at least 56 taxa in North America and 103 taxa worldwide. Yet, the full host range of this species still remains unknown (Barringer and Ciafré 2020). However, as it goes through its lifecycle, its host range narrows, although it remains highly polyphagous across all life stages (Kim et al. 2011, Urban and Leach 2022). Despite the wide variety of feeding hosts, true reproductive hosts that SLF can successfully complete its life cycle on are thought to be narrower (Lee et al. 2019).

Unfortunately, in the case of SLF, some of these hosts include economically important crops such as grapevines (*Vitis* spp.) and several tree fruit species (*Malus* spp., *Prunus* spp.). Softwood and hardwood tree species are also at risk, including red maple (*Acer rubrum*), silver maple (*Acer saccharinum*), black walnut (*Julglans nigra*), American beech (*Fagus grandifolia*), chestnut oak (*Quercus montana*), sycamore (*Platanus occidentalis*), paper birch (*Betula papyrifera*), and multiple species of cottonwoods and poplars (*Populus* spp.) (Urban 2020). Tree of heaven (*Ailanthus altissima*) (TOH) has been reported to be a preferred host, not only for feeding but also egg laying. This tree host is favored likely due to its high sap and sugar content, as well as ailanthone (Lee et al. 2009, Song et al. 2018). Interestingly, ailanthone is a bitter quassinoid produced by TOH, that has been demonstrated to make SLF bitter tasting when ingested and distasteful to predators (Song et al. 2018). SLF may also seek out other hosts that produce defensive chemicals to protect themselves. In addition, SLF has been reported to sequester limonoids or toxic secondary metabolites for defense. Like ailanthone, limonoids are bitter compounds related to quassinoids (Roy and Saraf 2006). Other Fulgorid planthopper species have been shown to sequester limonoids to potentially make their bright coloration (Johnson and Foster 1986). In addition to TOH, SLF has been shown to have an affinity towards three families that produce limonoids: *Pricrasma quassioides* (D. Don) Benn. (Sapindales: Meliaceae), *Melia azedarach* L. (Sapindales: Meliaceae), *Celedra fissilis* Vell. (Sapindales: Meliaceae), *Toona sinensis* (A. Juss.) M. Roem. (Spindales: Meliaceae), *Phellodendron amurense* Rupr. (Sapindales: Rutaceae), *Tetradium daniellii* (Benn.) (Sapindales: Rutaceae), and *Zanthoxylum simulans* Hance (Sapindales: Rutaceae) (Barringer and Ciafré 2020).

Along with toxic secondary metabolites, SLF has been known to feed on hosts with high sucrose and fructose content like grapevines (*Vitis* sp.) (Lee et al. 2019). It has been reported that SLF likely prefers hosts with higher feeding qualities such as hosts with greater sap flow (Barringer and Ciafré 2020). There are a few physical changes that occur throughout SLF's lifecycle that might attribute to their feeding preferences. For example, the tarsi and arolia go through developmental changes. This may allow adults to physically attach to a wider range of vegetative substrates (Avanesyan et al. 2019). Another developmental change that occurs that might impact feeding preferences is the mouthparts. The stylets and labium become much bigger in adults that may allow them to penetrate thick bark that nymphs are unable to penetrate with their smaller mouthparts (Avanesyan et al. 2019).

SLF feeding is known to be a plant stressor, causing yellowing and branch die back as well as oozing. Generally, the effect of adult feeding is greater than nymphs on trees (Lavely et al. 2022). Heavy adult feeding on one specific part of the tree may suppresses gas exchange and reduce nitrogen concentrations in leaves, as well as reduce soluble sugars in branch wood during and after feeding (Lavely et al. 2022). While heavy feeding on trees suppresses gas exchange, it does not alter non-structural carbohydrates, nitrogen concentrations, or overall tree growth (Lavely et al. 2022). This suggests that moderate to heavy feeding by adult SLF on young sapling may impair tree growth (Lavely et al. 2022). SLF infestations have been reported to kill TOH as well as grapevines (*Vitis* sp.). Harner et al. 2022 noted greater densities of SLF can affect carbohydrate and nitrogen dynamics in two *Vitis* species. Feeding directly on the phloem affects starch and total nitrogen concentrations of woody roots. Prolonged exposure strongly reduced leaf gas exchange. Therefore, it was concluded intense late-season phloem feeding by large adult SLF population densities can induce carbon limitation, with the potential for negative year-after effects in cases of severe belowground carbon depletion (Harner et al. 2022).

Agricultural and Economical Impacts

SLF is a known pest in China and Korea, attacking a diverse range of important agriculture and forestry plants including shrubs and trees, orchards, and vineyards (Dara et al. 2015). This piercing-sucking feeder attacks young stems and bark tissue. Older nymphs and adults can be seen feeding in large groups, this can cause wounds resulting in oozing, wilting, and death of branches (Dara et al. 2015). SLF excretes a sticky sugary substance called honeydew, which consists of sugar and amino acids (Nixon et al 2020). Honeydew can cover anything below the insects (vegetation, decks, cars, etc.). Honeydew grows a visible black mold called sooty mold, it has been known to impair leaf photosynthesis and weaken plants over time (Urban 2020, Nixon et al. 2020). This sweet-smelling substance attracts ants, bees, and wasps (Dara et al. 2015).

This pest has become quite a nuisance to tourism, businesses, and residential sectors and has received a large media attention (Urban and Leach 2022). Homeowners and businesses have invested in pesticides, traps, and tree removal to help combat SLF (Urban and Leach 2022).

SLF is predicted to cause significant economic damages in Pennsylvania. Worst-case scenario estimates that the expected overall annual impact of SLF damage on Pennsylvania agriculture is $99.1 million statewide (Harper et al. 2019). Although to date, SLF direct feeding only kills TOH and grape species, it still impacts several agriculture operations; nursery operators, fruit growers, and Christmas tree growers (Harper et al. 2019). In the forestry area, hardwood tree species are taking the biggest hit from SLF. Insecticide cost alone has nearly tripled in the grape industry (Urban 2020). The U.S. may have a greater pressure on plants and greater spillover to other plants hosts, potentially increasing the economic impact (Urban and

Leach 2022). As a pest of growing economic concern in the USA, it is important that SLF activity is evaluated for improved management and monitoring (Leach and Leach 2020).

Management

SLF has drastically spread since its arrival in 2014, mostly via artificial human-meditated transport of egg masses or adults themselves (Dara et al. 2015). Numerous studies have been conducted to identify effective insecticides as well as biological controls to help combat this invasive species (Urban and Leach 2022). Several states have also established quarantine zones to try to prevent further spread of SLF populations (NYSIPM- Cornell 2023). To control established populations of SLF, chemical control has been relied upon until biological or other cultural control measures are discovered and implemented (Leach et al. 2019). The main method of management of SLF is the use of insecticides. SLF are particularly susceptible to broad-spectrum pyrethroids, organophosphates, and neonicotinoids (Dara et al. 2015, Leach et al. 2019). Though it is no longer on the market, the compound chlorpyrifos has shown to provide 100% mortality of egg masses in the laboratory and field bioassays, while paraffinic oil showed only a 71% mortality (Leach et al. 2019, Leach et al. 2021). As for adults and nymphs, pyrethroids, neonicotinoids, carbamates, and organophosphates demonstrated strong knockdown in semi-field and field bioassays. Currently, only thiamethoxam and bifenthrin are able to control 50% of adults up to 14 days after application (Leach et al. 2019). After SLF hatch, the insect ascends upwards on vegetation so methods to eliminate nymphs such as foliar sprays and systemic trunk drenches have also been applied to host trees (Parra et al. 2018). TOH removal paired with a trap tree approach has also been used. This approach removes trees within a 0.4 km radius of a SLF infestation and then the TOH is treated with insecticides such as dinotefuran or imidacloprid via basal trunk spray or trunk injection. With all other TOH removed in the area, SLF will seek out the trap tree to feed and die from the insecticides (Urban 2020).

Using chemical control in vineyards has been more challenging as SLF can re-populate vines quickly after insecticide application due to large resident populations in adjacent non-crop habitat (Park et al. 2009, Urban 2020). It has been shown in semi-field and field bioassays that chlorpyrifos, etofenprox/diazinon, and dinotefuran have excellent knockdown activity against nymphs and lasted up to 14 days in vineyards (Leach et al. 2019). Due to adult reinfestation, Penn State Extension vineyard management guide recommends using a longer-residual product, like pyrethroids, during the heaviest period of reinfestation which tends to be in September (Leach et al. 2021). For treatments applied closer to harvest, Penn State Extension vineyard management guide recommends repeated applications of products with shorter preharvest intervals. Repeated applications for adequate control might be required. Also, it has been reported that SLF have a strong edge effect, and sometimes treating just the edges can kill adults while also saving time and money (Urban and Leach 2022). After harvest, it is recommended to apply a postharvest insecticide application to control adult populations and to actively scout out and remove egg masses to prepare for next year (Leach et al. 2021). There is a need to develop long-term holistic control for SLF in vineyards as adults come in during harvest time and spraying fruit with insecticides is not ideal.

Outreach efforts undertaken by USDA and states department of agriculture have implemented messaging campaigns advocating "if you see it, squash it" for reducing spread of SLF adults and destruction via physical scraping of egg masses in order to increase public awareness and limit human-mediated transport and spread of SLF (Urban et al. 2021). However, these methods are impractical for large scale management (Leach et al. 2021) but can be used as control measures by residents and the general public. Penn State Extension management guide suggests to homeowners to first determine if their infestation is worth treating. If the infestation is manageable, use cultural, physical, or biological controls such as removing hosts and scrape or smash egg masses and the insect itself. If the infestation is unmanageable, treat with the least toxic but effective option like soaps and oils and always read the label (Leach et al. 2021).

Monitoring

Monitoring for insect populations is considered a foundation of developing integrated pest management tactics as they predict pest damage, monitor populations, and determine when to enact a management strategy (Flint 2012). There is a critical need for improved monitoring tools for SLF management (Urban 2020). Estimating population size may be an essential first and critical step in improving current understanding of SLF population dynamics. It is certainly needed to quantitatively determine the efficacy and effectiveness of any control efforts (Urban 2020). It is vital to develop tools for monitoring population presence, relative abundance, and seasonal activity of SLF (Nixon et al. 2020). What drives dispersal and how far the insect can move within a generation through natural dispersal mechanisms is not yet fully understood (Urban et al. 2021).

In pest management, a typical management approach that has been used for several decades is “lure and kill” (El-Sayed et al. 2009). With this approach, the pest is attracted by a semiochemical lure and subjected to a killing agent (El-Sayed et al. 2009). For SLF, volatiles emitted from known preferred hosts, like TOH and grapevine, were used to create a lure (Cooperband et al. 2019). These compounds include methyl salicylate, (Z)-3-hexenol, and (E, E)-α-farnesene, and all three compounds were found to be highly attractive to SLF in laboratory behavioral bioassays (Cooperband et al. 2019) Methyl salicylate was attractive to all stages of SLF, younger nymphs were not as attractive to (Z)-3-hexenol and (E, E)-α-farnesene. Overall, methyl salicylate attracted the most SLF and in the field produced a two- to four- fold increase in capture compared to unbaited controls (Cooperband et al. 2019). To evaluate the reactiveness of methyl salicylate as a lure for SLF, baited and unbaited traps were compared in field settings (Nixon et al. 2020). However, the presence of the methyl salicylate lure did not increase capture of traps deployed on host trees or on sticky bands deployed on vertical tree-mimicking posts (Nixon et al. 2020). This might be due to competition from surrounding habitat and host plants that may reduce SLF response to methyl salicylate lures (Nixon et al. 2020). Without an attractive lure, current development of traps are considered to be passive traps and collect SLF that happen to crawl upwards on the host with a trap (Nixon et al. 2020).

The current traps that are in use for SLF are based upon the innate tendency of the insect to walk upward on surfaces (Kim et al. 2011). These traps include sticky bands and diverse types of tree traps. Brown sticky bands are wrapped around tree trunks and have been shown to be the most attractive color to SLF (Choi et al. 2012). These bands are easy to install and can be used in various landscapes. Drawbacks of the sticky bands include that they need to be frequently changed but are also messy and difficult to manage. The bands also capture nontarget vertebrates, such as birds and mammals, as well as nontarget invertebrates (Francese et al. 2020, Leach et al. 2021, Urban and Leach 2022). It has also been noted that fourth instars nymphs and adults have the ability to escape or avoid sticky bands (Francese et al. 2020). A commercially available tree band (BugBarrier) has been used to target larger developmental stages like fourth instar nymphs as well as adults (Francese et al. 2020). Because of these difficulties associated with sticky bands, other traps have been assessed to determine if they could yield comparable capture rates. In tests performed using cone traps, it was found these yielded captures that were equivalent to or exceeded captures of SLF on sticky bands (Nixon et al. 2020). These traps do not require the use of insect-trapping adhesives such as those found in sticky bands (Francese et al. 2020). The cone trap also takes advantage of the insect's tendency to climb upwards and funnels the insects into a plastic container where they are dispatched with a kill strip. In comparison to BugBarrier, cone traps caught more third and fourth instar nymphs as well as adults (Francese et al. 2020). Cone traps also significantly lowered nontarget capture and require less frequent changes compared to sticky bands (Nixon et al. 2020, Urban and Leach 2022, Nixon et al. 2023). Nixon et al. 2020 evaluated various modifications of cone traps and found that the modified circle trap was the most successful due to its larger collection jar and its ability to capture adult SLF as well as nymphs (Nixon et al. 2020). Based on this work the modified circle trap has been recommended for its effectiveness at capturing SLF as well as their relative ease-of-use and reusability. Because there is no lure, these traps are passive, and carry with them a significant downside: they yield the best results in areas where SLF populations are high. They are less effective in areas where SLF populations are known to be low, and therefore by extension, are less effective in areas not yet infested by SLF, and where early detection may be critical to reduce the likelihood of SLF population establishment. In addition, best results of these traps were obtained when deployed on ornamental tree species including TOH, maples, and black walnut, and on plant species of economic important, such as grapevines, for which these traps have not yet been modified (Urban and Leach 2022).

In addition to traps, other monitoring approaches have been developed and evaluated for SLF. One such approach seeks to sample environmental deoxyribonucleic acid (eDNA) to detect SLF (Allen et al. 2021). Since SLF is known to profusely excrete honeydew (sugary excrement) upon substrates on and near what it feeds upon, this provides an opportunity to capture DNA excreted with honeydew sampled from these substrates. This approach uses a "roller" method to capture DNA off substrates, like tree surfaces, using a commercial paint roller. For SLF, the roller is rolled over tree surfaces, dipped in a solution to remove the eDNA from the roller, and then samples are run in a polymerase chain reaction (PCR) system to amplify any sampled SLF DNA present (Peterson et al. 2022). This use of eDNA detection improves the ability to delimit invasion fronts, identify satellite populations, and confirm local eradications (Allen et al. 2021).

However, it has broad-use limitations in general monitoring, specifically because it is only able to detect SLF DNA within 2 weeks after it is deposited, and it can be somewhat costly depending upon the number of samples tested.

Another monitoring approach for detecting SLF spread focuses on human-mediated transport of the pest and uses canines to detect the smell of egg masses. Canines are trained to detect dead egg masses by scent and are eventually trained to be able to detect live egg masses. These trained dogs have been shown to have an accuracy of at least 90% (Essler et al 2021). This demonstrates that SLF have a smell that is detectable by dogs. Detection canines have exhibited the ability to detect and discriminate SLF egg masses from other environmental distractors, further confirming that detection canines can be used as an effective partner to help in eradication efforts (Aviles-Rose et al. 2023). However, this approach is costly with respect to time and resources needed and is only applicable to SLF egg masses that are transported on goods and conveyances.

Given SLF's propensity to spread to new areas via human mediated transport, performing the cost effective, broad range monitoring necessary for early detection of new SLF introductions across a wide geographic scale (e.g., throughout western grape growing regions in California, Oregon, and Washington, as well as in other states throughout the US) is currently highly limited and in urgent need of new solutions. One promising approach may come from using knowledge of the insect's biology to delimit areas where it can potentially survive and establish versus those with environmental parameters where it could not. This would allow for available resources to be targeted for monitoring only those locations, and only at particular time periods, at which particular life stages of SLF could survive and establish if transported there.

Most introduced species are not adapted to their unfamiliar environment and can result in reduced fitness and potential extinction (Lombardo and Elkinton 2017). A strong invasive has high stress resistance to abiotic factors such as temperature, relative humidity, precipitation, atmospheric pressure, and wind (Nyamukondiwa et al. 2022). This high stress resistance to abiotic factors allows invasive insects to adapt easily to different environments. Temperature is often regarded as the most important abiotic factor in determining species distribution (Kelley 2014). Extreme temperatures can increase mortality and limit development of a species geographic niche and can, in part, determine the potential distribution of invasive species in novel habitats (Keena et al. 2023). Temperature can also determine the timing of life stages as well as viability of eggs (Gilbert et al. 2004).

To date, limited work has been conducted concerning identification of areas that are suitable to SLF survival of particular life stages based on temperature constraints. Parra et al. 2017 estimated unsuitable areas for SLF establishment in the United States based on mortality of eggs from three different regions in South Korea (Urban and Leach 2023). Egg masses collected had different minimum winter temperatures and minimum January temperatures of -13.9 °C was considered unsuitable. The northern United States was considered to be unsuitable for SLF establishment. States included Maine, Wisconsin, North Dakota, South Dakota, and higher-elevation sites in the Adirondack and Rocky Mountains (Parra et al. 2017)

Although other studies on aspects of SLF thermal requirements have been conducted, these have primarily focused on effects of temperature on rates of egg and nymphal development (Kreitman et al 2021, Keena et al. 2023), or on overall matching of climatic conditions to those with known established SLF populations (Jung et al. 2017, Wakie et al. 2017) to predict and identify new locations potentially suitable to SLF establishment. However, some insights can be gleaned from some of the temperatures tested in these developmental studies. For example, Krietman et al., 2021 reared nymphs (first-fourth) in the lab at constant temperatures of 5, 10, 15, 20, 25, 30, 35, and 40 °C. First instars died at 5, 10, and 40 °C while survival was poor at 35 °C. This suggests that the southern United States expansion of SLF populations might be limited by higher temperatures or even be delayed under cool spring conditions. However, these authors indicated the need for further studies that specifically examine SLF's ability to withstand sudden cold snaps in order to get a better understanding of its potential range (Kreitman et al. 2021).

Given that SLF can lay eggs on a diverse array of substrates and because it persists in the egg stage for up to 7 months per year, there is a particularly high risk of SLF spread through human-mediated transport of egg masses. New reports of SLF detections in multiple states (e.g., New Hampshire, Michigan) (NYSIPM- Cornell 2023) indicate that transport of egg masses on nursery stock has occurred with regularity and is an area of concern expressed by members of the National Plant Board (SLF Summit 2023). To date, either insecticidal treatments or physical inspection and removal of SLF is the primary practice (Urban 2020). Insecticides are typically used to treat nursery stock to prevent SLF from moving onto plants, whereas physically removal of SLF is done right before shipment, physical removal is not always accurate and increases associated labor costs for the nursery (Urban 2020). However, egg masses can go undetected particularly when laid on branches and trunks covered in full foliage. It is only upon arrival in a new location, after being exposed to permissible temperature (e.g., potentially warmer temperature conditions in greenhouses settings), would egg masses hatch to yield first instars. As such, it is possible that a better understanding of thermal limits of SLF, particularly of first instars, to compare to areas where nursey stock is being shipped will afford better means to predict where SLF can potentially establish to improve SLF control.

To better understand how insects respond to cold temperature, specifically cold snaps, an insect's cold tolerance can be investigated (Ring and Riegert 1991, Sømme 2000, Sinclair et al. 2015). Investigating thermal tolerance is the first step in determine whether a population may establish in novel environments and can also explain the role of temperature in driving insect population abundance (Nyamukondiwa et al. 2010, Terblanche et al. 2015, Motswagole et al. 2019). Insects can adapt to cold environments in a variety of ways that include extension of locomotory activity to low temperature, enhancement of metabolic rate, and maintenance of a positive energy balance whenever possible (Block 1990). Temperatures which are lethal to insects are a function of both duration and severity of exposure to the particular temperature (Chown and Nicolson 2004, Delinger and Lee 2010, Gunderson et al. 2017, Motswagole et al. 2019). Extreme low temperatures degrade and destabilize many of the insect's molecular components, such as carbohydrates and membranes (Angilletta 2009, Motswagole et al. 2019), as well as increase the risk tissue freezing (Block 1990). A greater understanding of the ways in which temperature acts through physiological processes to determine ecological outcomes has formed the basis for a range of applications of insect thermal biology (Bale 2002). For example, forecasting systems for pest and disease outbreaks (Werker et al. 1998), analysis of the establishment potential of alien species in new environments (Bale and Walter 2001), and use of ice-nucleating behavior as a novel method of pest management (Lee et al. 1998, Bale 2002). Low temperature is one of the key limiting factors for distribution of an invasive insect (Sinclair et al. 2015). For instance, geographic distribution, insect fitness, and seasonal activity of insects can be based upon temperature constraints (Chown and Nicolson 2004, Anderson et al. 2015). The main reasons to determine an uninvestigated insect's cold tolerance is to provide a detailed description of how the insect responses to cold temperature in the context of invasion. Understanding cold tolerance of invasive species could thus facilitate the development of mechanistic distribution models that can predict current and future geographic ranges, guide invasive management and mitigation strategies (Bykova and Sage 2012, Sobek-Swant et al. 2012), and provide a measure of low temperature performance that can be compared among populations (Sinclair et al. 2015). Therefore, determine cold tolerance for SLF would be informative for predictive distribution maps and enhanced monitoring tools, particularly for early life stages that are easily transported to new regions where they could establish.

Cold treatments have also been shown to be an option for controlling insects, such as atmospheric cold plasma (ACP) (Donohue et al. 2006, Abd El-Aziz et al. 2014, Radhakrishnan et al. 2016, Los et al. 2018, Ramanan et al. 2018, Ziuzina et al. 2021). ACP is increasingly being investigated to address bio-contaminations and sustainability issues in the agri-food chains (Ziuzina et al. 2021). This approach is an alternative approach to traditional fumigation methods for the preservation of agricultural commodities (Ziuzina et al. 2021). It was shown that cold plasma can be effectively applied for the elimination of pests (*Tribolium castaneum)* in stored foods by exposing the insects to the ACP treatment (15 °C) for 5 minutes (Ziuzina et al. 2021). By exposing the insect to cold temperatures, the ACP treatment reduces the respiration rate and the weight of the insect affecting levels of oxidative stress markers in adults. While this approach is relatively underexplored and less understood as an insecticidal approach, it demonstrates that cold treatments can be used for controlling insect populations.

Objectives

The objective of this thesis is to overall improve monitoring tools for the newly invasive SLF in regions where it persists and could distribute to. Chapter 2 will focus on the modified circle trap and how it is best deployed to yield the strongest capture rates. Chapter 2 will also focus on population dynamics, seasonal abundances, as well as multi-year trends in an area near where the infestation first began. Chapter 3 will focus on determining cold tolerance of first instar nymphs. Factors of cold tolerance include critical thermal minimum, chill coma recovery time, lower lethal limit, and lethal time at low temperature. Determining these factors will help to better predict SLF distribution, establishment, and potential control methods. Lastly, Chapter 4 will focus on investigating evidence concerning whether and potentially how SLF generates heat.

Not only will this help to better understand SLF’s basic biology, but it could also be used for developing potential monitoring tools. Altogether, this information can aid in the use of monitoring tools in infested areas as well as areas that are at risk of SLF establishment.

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Chapter 2

Monitoring Spotted Lanternfly using Circle Traps

Summary

The invasive *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) (SLF) was first detected in Berks, County Pennsylvania in 2014. Since then, the SLF has been spreading through the Eastern United States. A three-year study at Green Hills Preserve (201 acres) in Mohnton, Pennsylvania using circle traps on different host species was done to determine the best deployment strategies for capturing SLF throughout the season. Using this monitoring method, we were also able to better understand population presence, relative abundance, seasonal activity, and multi-year trends of SLF near where the infestation first began. Our results showed that what is considered in practice the ideal host species to deploy traps on is *Ailanthus altissima* (tree of heaven (TOH)), it did not significantly out-perform other tree species, as other species (e.g., *J. nigra*) can also yield strong capture. Traps are most effective when they are deployed before May and taken down at the end of November. In 2021, a strong decline in the population followed by a spike the following year was observed. Overall, circle traps are most effective in areas with high SLF populations, though similar tools have been successful in residential areas where there are high numbers on individual trees.

Some of the data collected for the present thesis, and presented here (as described below), have been include with data collected from other locations and published in the following peer reviewed publication:

Nixon, Laura J. Caitlin Barnes, Elizabeth Deecher, Katarzyna Madalinska, Anne Nielsen, Julie Urban, Tracy C Leskey. 2023. Evaluating deployment strategies for spotted lanternfly (*Lycorma delicatula* Hemiptera: Fulgoridae) traps, *Journal of Economic Entomology*, 116(2), 426-434.

Introduction

In 2014 in Berks County Pennsylvania, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) was first detected (Barringer et al. 2015, Dara et al. 2015). The spotted lanternfly (SLF) is native to China, India, and Vietnam (Lee et al. 2019). Since its arrival in Pennsylvania, it has been detected in fifteen nearby states and has established itself in seven states (NYSIPM-Cornell 2023). It is predicted that SLF is climatically suitable for establishment in most of the United States (Jung et al. 2017, Wakie et al. 2020, Keena et al. 2023).

The SLF is a polyphagous, phloem-feeding, planthopper that has demonstrated feeding behaviors associated with 103 plant taxa across 33 families and 17 orders. In North America, SLF feeds on 56 plant taxa that include native, cultivated, and nonnative species (Barringer and Ciafré 2020). Preferred hosts have shown to be tree of heaven (TOH) (*Ailanthus altissima* [Sapindales: Simaroubaceae]), grapevines (*Vitis* spp. [Vitales: Vitaceae]), and several common hardwood tree species (Lavely et al. 2022). It has been shown that SLF is a plant stressor and moderate to heavy feeding by SLF may impair tree growth in young saplings (Lavely et al. 2022). SLF has been shown to damage grapevine species with intensive late-season phloem feeding by large adult SLF population densities. With these large population densities, feeding can induce carbon limitations with the potential for negative year-after effects in cases of severe belowground carbon depletion (Harner et al. 2022).

There is a critical need for improved monitoring tools for SLF management (Urban 2020). It is vital to develop tools for monitoring population presence, relative abundance, and seasonal activity of SLF (Nixon et al. 2020). Monitoring for insect populations is considered a foundation of developing integrated pest management tactics as they predict pest damage, interpret populations, and determine when to enact a management strategy (Flint 2012). Estimating population size may be an essential first and critical step in improving current understanding of SLF population dynamics. In doing so, it can quantitatively determine the efficacy and effectiveness of any control efforts (Urban 2020).

A commonly used and inexpensive trap is the brown sticky band trap. Sticky bands are used to monitor and capture SLF by taking advantage of their negative gravitaxis (Kim et al. 2011) of walking upwards on trees (Nixon et al. 2020). Sticky bands have shown to be useful in capturing various life stages of SLF and are easy to obtain and install. There are drawbacks to these bands though, nontarget vertebrates and invertebrates have been frequently caught, causing distress to not only the nontarget but also homeowners (Nixon et al. 2020). Also, sticky bands need to be frequently changed for the band to remain sticky. A variety of cone traps have also been used as a monitoring tool for SLF. These traps have vinyl that is wrapped around the tree and takes advantage of SLF's negative gravitaxis by funneling SLF into a container that contains a kill strip. The use of cone traps, specifically the modified circle trap, has yielded captures that are equivalent to or exceeded capture by sticky bands (Francese et al. 2020). Additionally, nontarget captures are significantly lower for circle traps compared to sticky bands and less frequently required replacement (Nixon et al. 2020). To yield the best results for monitoring as well as capturing, it is recommended to have the circle traps deployed before May and conclude trapping by the end of November. This ensures early capture of first instars as well as late season adults. Mass trapping has been shown to successfully reduce densities of pest insects in isolated areas (El-Sayved et al. 2006, Nixon et al. 2023). The use of circle traps has been recommended for residential areas to reduce SLF from specific hosts (Urban et al. 2020).

As SLF rapidly spreads throughout the Eastern United States, it is essential to develop monitoring tools for SLF population presence, relative abundance, and seasonal activity (Nixon et al. 2020). Monitoring SLF populations over several seasons in an area near where the infestation first began could help improve understanding and predict SLF population dynamics, including seasonal population spikes (egg hatch and adult emergence), along with relative abundance and host preference of tree species. Circle traps could also help early detection in nearby states that are at risk of SLF. Understanding which tree species to affix the traps to would be beneficial.

This chapter will focus on the modified circle trap and how it is best deployed to yield the strongest capture rates. A two-year study was conducted in Pennsylvania and Virginia to evaluate the efficacy of modified circle trap designs. An additional third year in Pennsylvania was conducted to better understand seasonal activity, host preferences, and population dynamics. While results of the two-year study have been published (Nixon et al. 2021, Nixon et al. 2023), here we present an analysis of the three years of data collected in Pennsylvania in order to better examine any multi-year trends in trap capture.

Methods

Research Site

This study was conducted by deploying traps on trees, as described below, at Green Hills Preserve- Natural Lands in Mohnton, PA from July 2020 to November 2022. The field site was located at coordinates 40.25 N, -75. 91 W in an area of approximately 201 acres of land surrounded by rolling farm fields, woodlands, and wetlands within the Pennsylvania SLF quarantine zone. This site was chosen because it had a known high population of SLF the previous year and when the traps were first deployed.

Modified Circle Traps

Modified circle traps were deployed in areas with known high or low densities of SLF prior to egg hatch in the spring in Pennsylvania. Modified circle traps consisted of a folded coated polyester screen (‘Pet Resistant’, Saint-Gobain, Malvern, PA) with a large collection jar top (AgBio Inc.). Traps were secured onto trees by screwing a plank of wood into the tree. The vinyl was then wrapped around the tree and held in place by staples. The plastic container was held upright by a bungee cord wrapped around the tree (**Figure 2-1**). DDVP kill strips were placed in the plastic containers and changed every two weeks. Traps were deployed on five host tree species common to Berks County, PA. Three replicates of traps were affixed to selected host trees. Tree hosts included were Tree of Heaven (*Ailanthus altissima*), Black Walnut (*Juglans nigra*), Red Maple (*Acer rubrum*), Northern Red Oak (*Quercus rubra*), and Sweet Pignut Hickory (*Carya glabra*) (**Figure 2-2**). Each tree species was included in all replications with a total of 15 trees. For all three years of this study, traps were affixed to the same 15 trees. The average diameter at breast height (DBH) of each species were Tree of Heaven 9.98 inches, Black Walnut 10.93 inches, Red Maple 7.86 inches, Northern Red Oak 19 inches, Pignut Hickory 13.7 inches. All traps were emptied weekly, and nymphal and adult captures were recorded from May to November. This study was repeated for three consecutive years in 2020, 2021, and 2022.

Statistics

Data was analyzed independently for each year, and within those datasets nymphal and adult SLF captures were separated. The weekly captures of SLF were analyzed using a univariate repeated measures ANOVA. The model included tree species and time as explanatory factors; the replicate was included as a nested variable with tree species to account for random variation from location. To analyze the likelihood of SLF presence on each tree species, all datasets were transformed to binomial data for presence and absence; separate Pearson's chi-squared tests were performed for each dataset.

Figure 2-1: Modified circle trap with DDVP kill strip affixed on Tree of Heaven

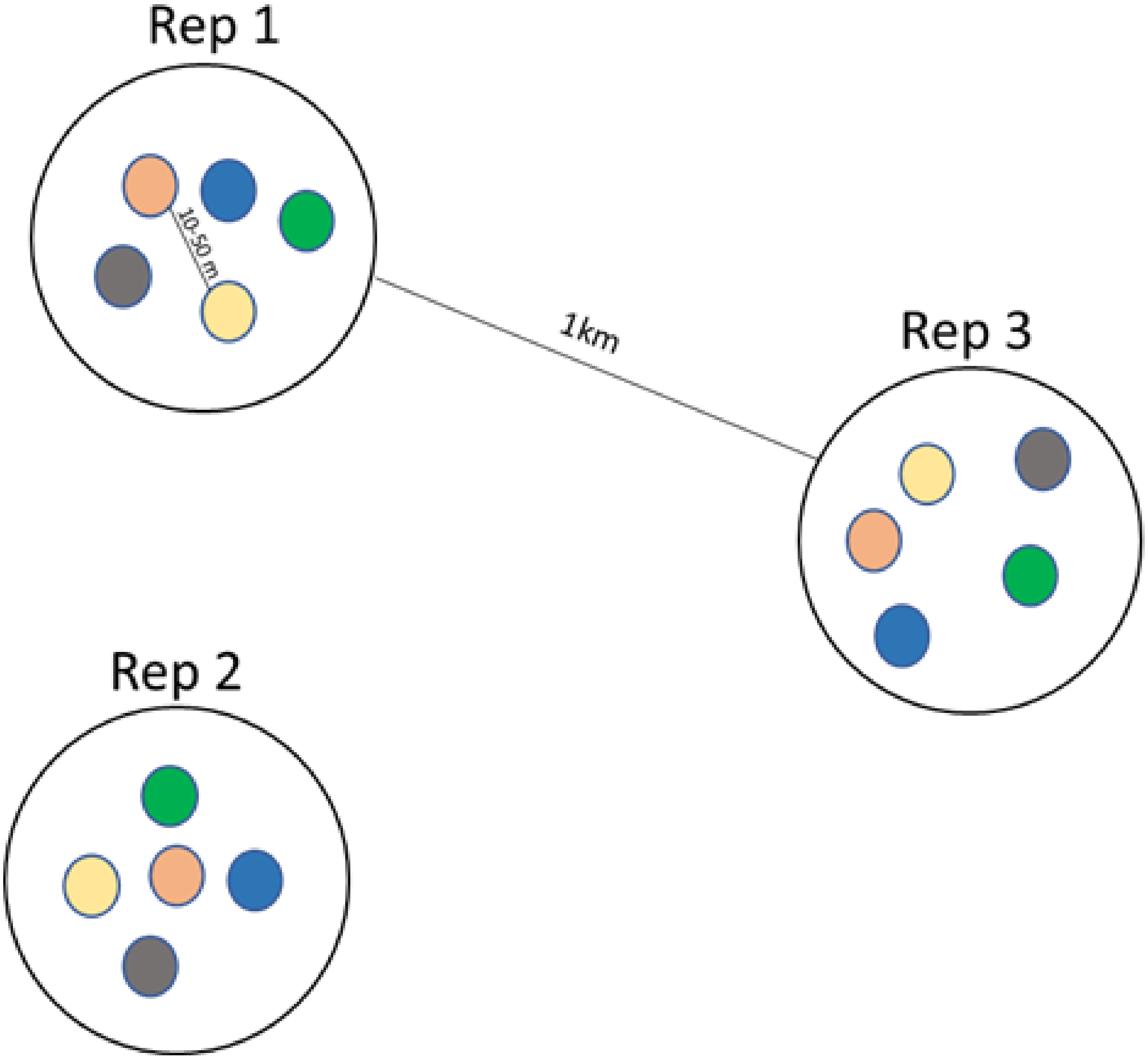

Figure 2-2: Modified circle trap set up; three replications consisting of five different tree species.

Results

In 2020, the first nymphal (first and second instars) capture was recorded on July 7th and capturing continued until October 2nd (**Figure 2-3**). In 2021, the first nymphal (first and second instars) capture was recorded on June 9th and continued until September 6th (**Figure 2-4**). In

2022, the first nymphal (first instars) capture was recorded on May 18th and continued until September 27th (**Figure 2-5**). In 2020, the first adult capture was recorded on July 30th and capturing continued until November 13th. In 2021, adult capture began on August 4th and continued until November 8th. In 2022, the first adult capture was recorded on July 26th and continued until November 15th (**Table 2-1**).

In 2020, tree species had no significant effect on either nymphal or adult SLF captures in traps ($F = 1.4$; d.f. = 4, 10; $P = 0.31$ and $F = 0.9$; d.f. = 4, 10; $P = 0.52$, respectively). Time had a significant effect on both nymphal and adult SLF capture in traps ($F = 9.3$; d.f. = 10, 100; $P < 0.0001$ and $F = 3.7$; d.f. = 14, 140; $P < 0.0001$, respectively). There were no differences in the likelihood of presence of nymphs and adults ($\chi^2 = 5.3$, d.f. = 4, $n = 165$, $P = 0.26$ and $\chi^2 = 8.4$, d.f. = 4, $n = 225$, $P = 0.079$, respectively) (Nixon et al. 2023).

In 2021, tree species had no significant effect on either nymphal or adult SLF captures in traps ($F = 0.8$; d.f. = 4, 10; $P = 0.56$ and $F = 1.8$; d.f. = 4, 10; $P = 0.20$, respectively). Time had a significant effect on both nymphal and adult SLF captures in traps ($F = 3.6$; d.f. = 9, 90; $P < 0.001$ and $F = 2.3$; d.f. = 10, 100; $P = 0.02$, respectively). Nymphs were significantly less likely to be present in *A. rubrum* and *C. glabra* than the other species ($\chi^2 = 15.6$, d.f. = 4, $n = 150$, $P = 0.004$), while adults were significantly more likely to be present on *A. altissima* and *A. rubrum* than other species ($\chi^2 = 13.4$, d.f. = 4, $n = 165$, $P = 0.01$) (Nixon et al. 2023).

In 2022, tree species had no significant effect on either nymphal or adult SLF capture in traps ($F = 0.29$; d.f. = 4, 10; $P = 0.876$ and $F = 0.83$; d.f. = 4, 10; $P = 0.535$, respectively). Time had a significant effect on both nymphal and adult SLF captures in traps ($F = 13.8$; d.f. = 26, 260; $P < 0.001$ and $F = 4.05$; d.f. = 26, 260; $P < 0.001$, respectively). There was no difference in likelihood of presence of nymphs ($\chi^2 = 4.5$, d.f. = 4, $n = 405$, $P = 0.35$), while adults were significantly more likely to be present on *Q. rubra* than other species ($\chi^2 = 15.9$, d.f. = 4, $n = 405$, $P < 0.001$).

In the three-year span, numerically there was a large decrease in the SLF population in 2021 at the site (<1000 SLF collected). In 2022, the population came back and largely increased compared to the prior year (>8000) (**Figure 2-6**). Generally, first, second, third, and fourth SLF instars had the greatest capture on *J. nigra*, with adult's greatest capture on *A. altissima*. Overall, although *A. altissima* traps numerically captured the greatest number of SLF (**Figure 2-7**), this was not a statistically significant effect (i.e., tree species) in any year of our study.

	2020	2021	2022
First Instars	7/10-7/10	6/9-7/7	5/18-7/26
Second Instars	7/10-7/30	6/9-7/7	6/2-7/26
Third Instars	7/10-8/28	6/23-7/29	6/15-8/16
Fourth Instars	7/10-10/2	7/14-9/6	7/10-9/27
Adult Males	7/30-11/6	8/4-11/8	7/26-11/8
Adult Females	7/30-11/13	8/11-11/8	8/2-11/15

Table 2-1: First captures of each life stage of SLF for each year of the study

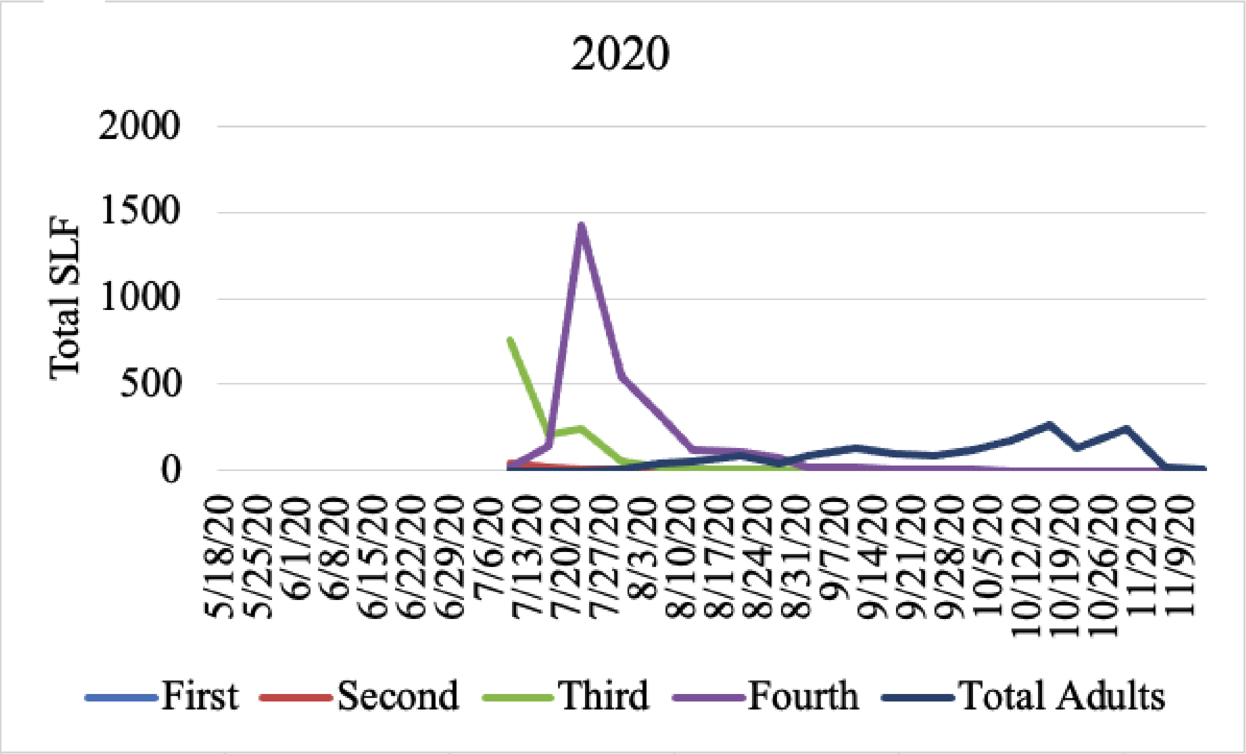

Figure 2-3: Timeline of SLF life stages in 2020. Note: traps in the first year of the study were not deployed until early July.

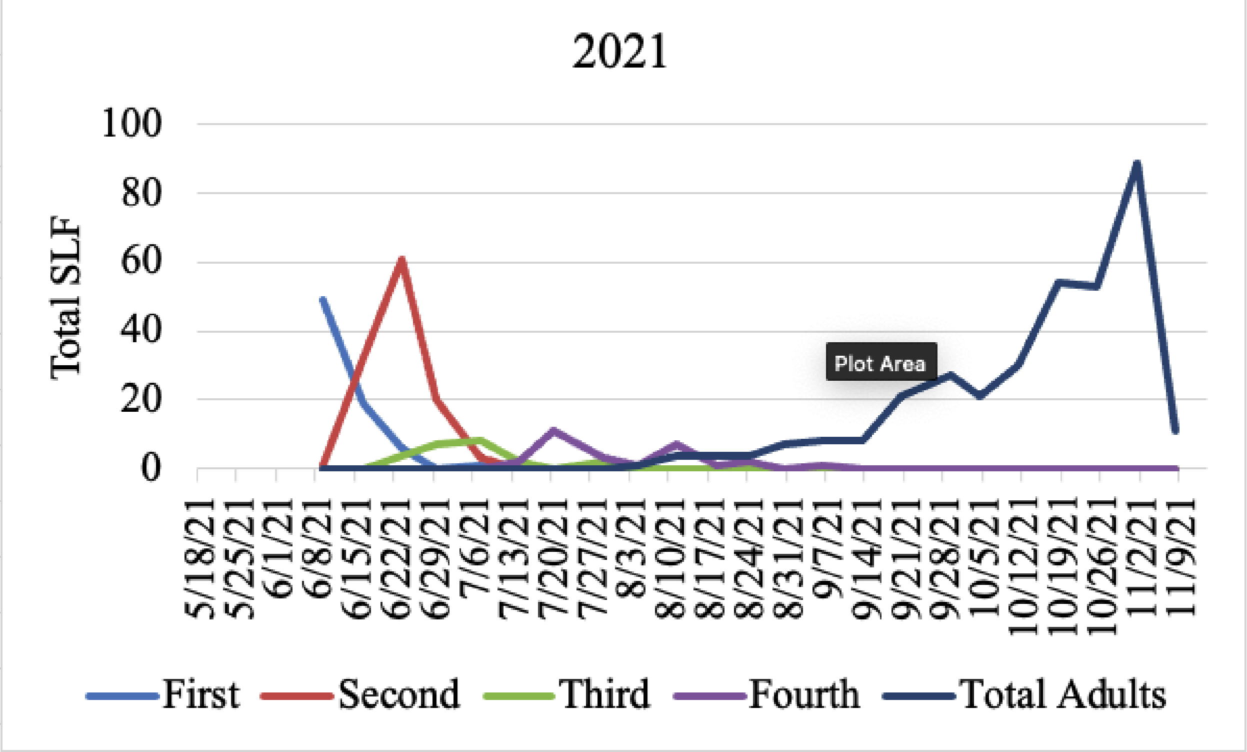

Figure 2-4: Timeline of SLF life stages in 2021. Note: traps in the second year of the study were not deployed until early June.

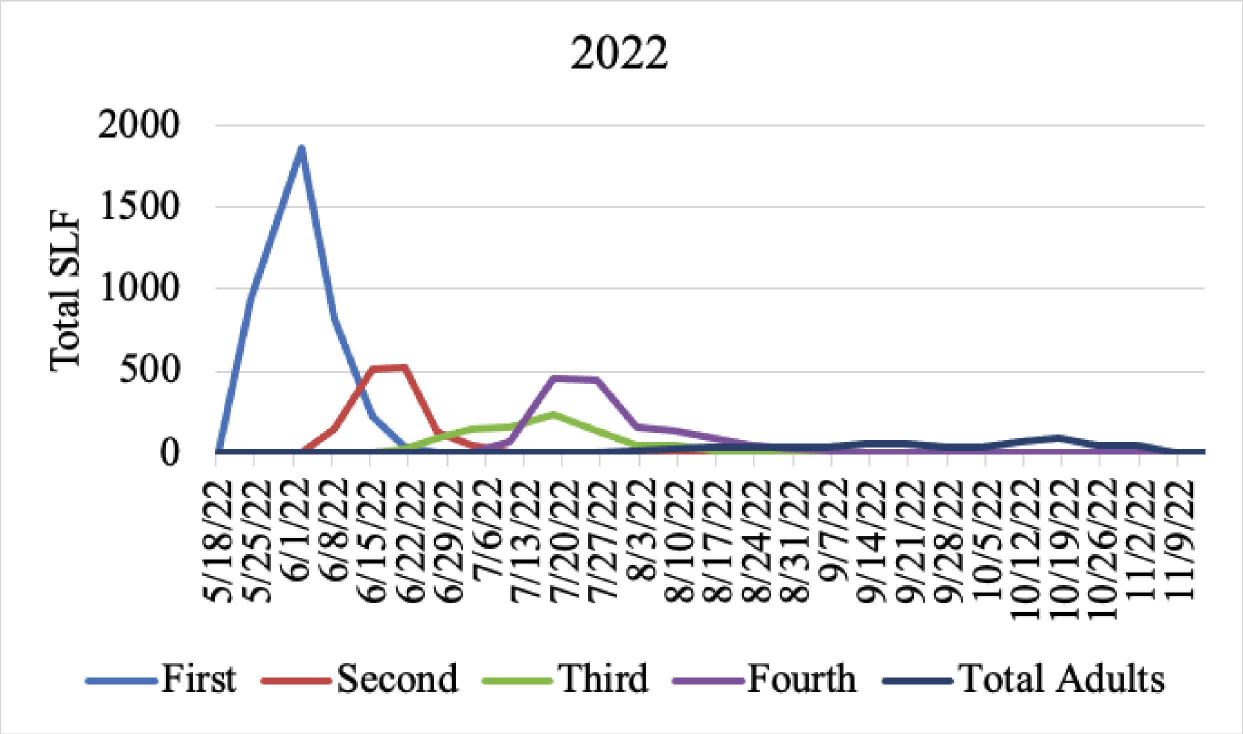

Figure 2-5: Timeline of SLF life stages in 2022.

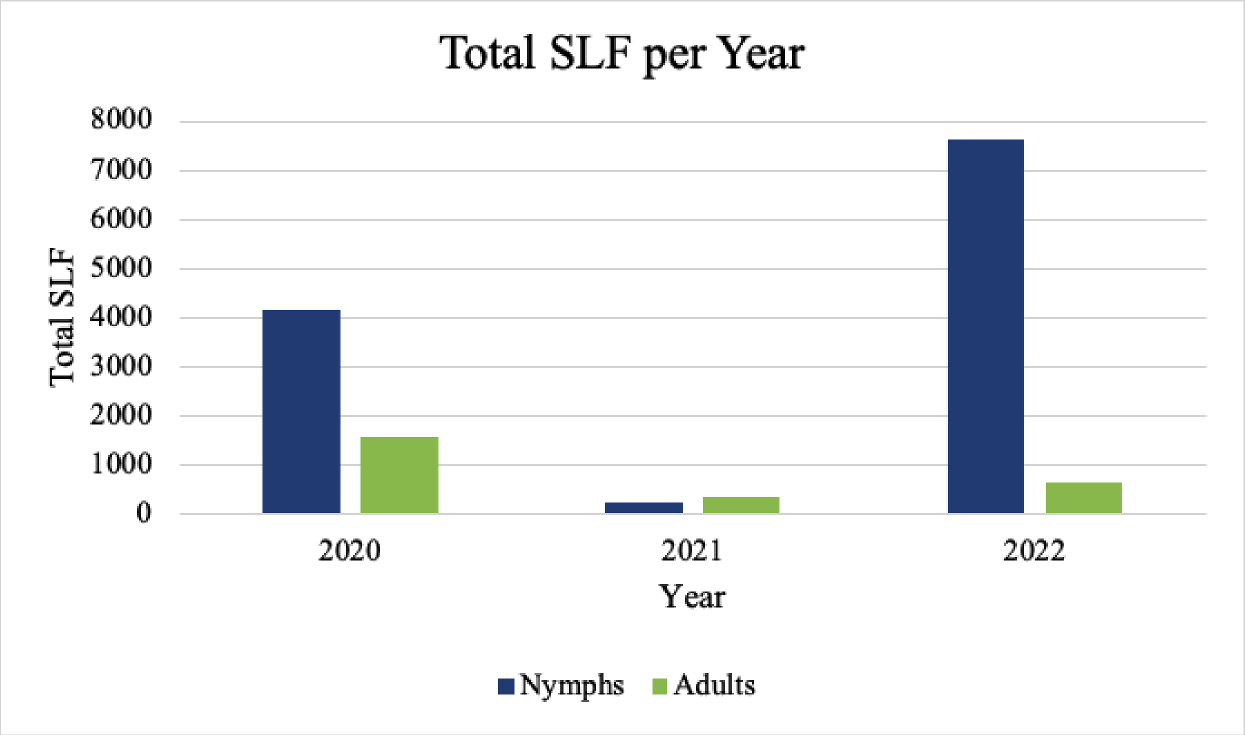

Figure 2-6: Total number of nymphs and adults SLF captured per year in modified circle traps in Berks County, PA during 2020, 2021, and 2022.

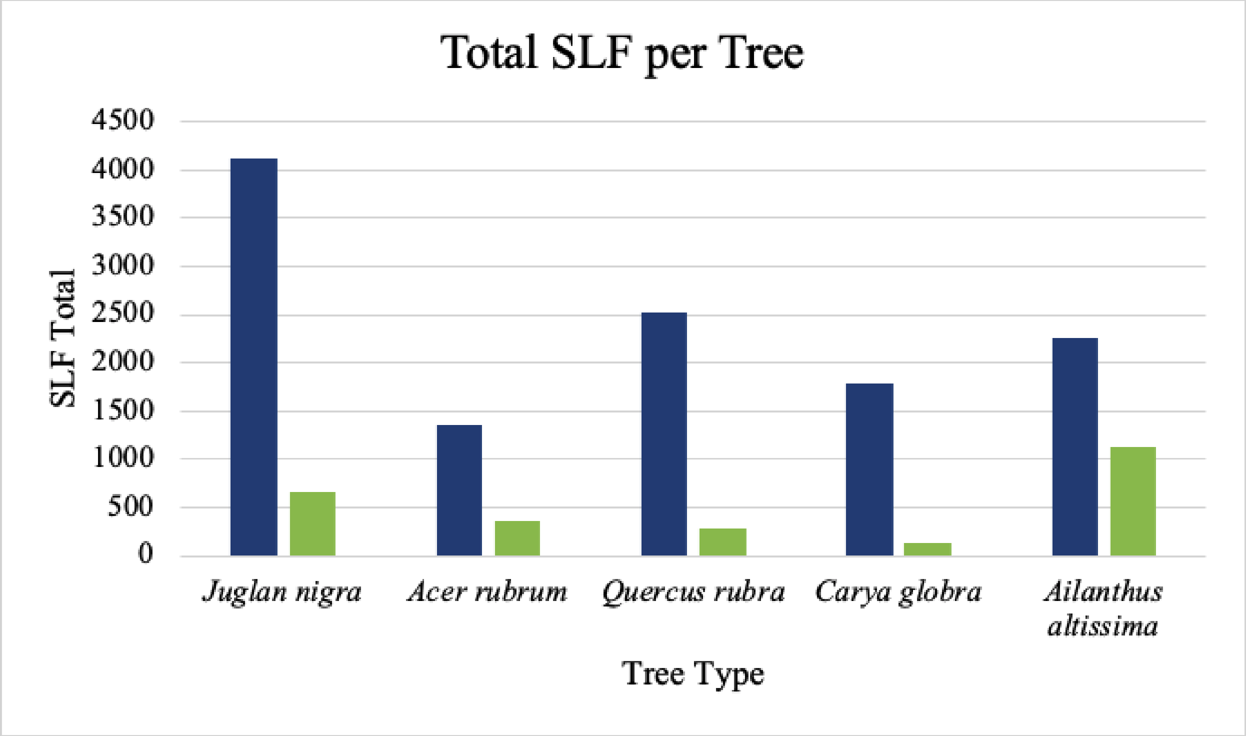

Figure 2-7: Total number of SLF captured in modified circle traps on host plants in Berks County, PA during 2020, 2021, and 2022.

Discussion

Previous studies have demonstrated that circle traps are an effective tool for capturing and monitoring SLF in areas with known high populations (Nixon et al. 2020). In this study, we observed more nymphs captured on *J. nigra* and *A. altissima* overall, and more adults were captured on *A. altissima*. However, we did not observe any statistically significant effect of tree species in any of the three-year study. It is known that SLF nymphs have a wide variety of host plants that narrows as they go through their lifecycle (Kim et al. 2011), with TOH often being reported as a preferred host throughout their lifecycle (Nixon et al 2023). However, in this study, the evidence does not provide support that only TOH should be used for monitoring SLF using circle traps. Because all of the tree species did capture SLF in all three years, this could suggest that the optimal method of trap deployment would be to monitor using a range of tree species if possible. If resources are limited, requiring trap deployment on only select tree species, the results suggest that perhaps using *J. nigra* in addition to TOH may be a useful alternative approach, particularly for capturing nymphal SLF, which may aid in early detection and provide greater opportunity to treat with insecticides earlier in the season.

It is unknown why there was such a decrease in the population in 2021 followed by a rebound in 2022. Potential explanations for the decline in 2021 include depleted nutrients, SLF population expansion, and 17-year Brood X cicadas. Late season adults tend to heavily feed which can leave host plants stressed and nutrient depleted (Urban 2020). Due to this, adults may lay eggs in new areas for the next generation to seek out stronger hosts with better nutrient contents (Mason et al. 2020). This could be one of the reasons there was a decrease in the population in 2021. Furthermore, in 2021, billions of the 17-year Brood X cicadas (*Magicicada septendecim*, *M. cassinii*, and *M. sependecula*) emerged from the soil throughout the midwestern and eastern United States (Graber-Stiehl 2021, Ficklin et al. 2023). SLF and cicada species are similar in body structure, how they feed, and how they move across landscapes (Avanesyan et al. 2019). Reading, PA saw a large emergence specifically at Green Hills Preserve. Brood X was recorded to emerge from mid-April all the way until the first week of July with a peak in emergence in the last week of May (Kritsky 2021). Brood X's emergences coincides with first instar SLF hatching from egg masses. The preserve was densely populated with cicadas and circle traps were filled with cicadas for continuous weeks. It is unclear whether the Brood X population directly impacted SLF capture rates. However, by comparing this data with that collected by other collaborators (e.g., in Virginia and New Jersey) who also observed a decline in SLF populations in 2021 (Nixon et al. 2023), it can be observed whether or not a high influx of cicadas was also present in those areas (and if they were also prevalent in their SLF circle traps).

There is an expectation among grape growers and the landscaping industry that populations are high for several years and then decline. It has been seen that when SLF populations move into a new area, the populations are high at first for approximately 2-3 years and then decline (personal observation). However, the data that is presented here shows a rebound in population after decline in the area of original infestation. SLF population fluctuations could be due to a number of factors; lack of host fitness and resource availability, control efforts, seasonal weather patterns, and/or predation. To understand if these factors are affecting SLF populations, trapping results in other regions/states need to be compared.

This three-year study provides practical insights into biosurveillance tools long term for SLF. These results do not provide strong support for placement of circle traps only on TOH as is currently the practice employed by the USDA APHIS (Francese et al. 2020). A more effective approach could be to deploy traps on multiple tree species if sufficient resources are available. It should be noted that the first two years of the study traps were deployed beyond the beginning of May, this demonstrated a decline in capturing first and second instars. Throughout the three-year study, when the traps are initially deployed, a large influx of nymphs are captured. Specifically in 2022, the beginning of May showed the largest influx of first instar nymphs, while in 2020 and 2021 it was missed due to late deployment. Therefore, circle traps are best deployed before the month of May and taken down at the end of November in order to capture any active life stage of SLF that may be present in (or move into) the area that is being monitored. Development of better monitoring and management tools for SLF for various landscapes remains a priority as current methods are limited for areas with very low SLF populations in order to improve early detection of SLF introduction into new areas.

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Chapter 3

Cold Tolerance of First Instar Spotted Lanternfly

Summary

The spotted lanternfly *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) (SLF) is an invasive planthopper species in the Eastern United States. First documented in Berks County, Pennsylvania in 2014, this species continues to spread and is a concern for the United States. Temperature plays a significant role on invasive species based on where they can distribute and establish. Laboratory studies were designed to investigate critical thermal minimum (CTmin), chill coma recovery time (CCRT), lower lethal temperature (LLT), and lethal time at low temperature (LLt) for SLF first instar nymphs. The CTmin ranged from 7.3 to 7.8 °C, with a mean CTmin of 7.666 ± 0.017 °C (mean ± SEM). The CCRT ranged from 1 to 621 seconds, with a mean CCRT of 114 ± 18 seconds (mean ± SE). The LLT was near -7.5 °C for 100% death, while LLt is predicted to occur around 950 minutes at -2.5 °C for 100% death based on results using logistic regression modeling. Overall, this study provides first insights of cold tolerance for SLF first instar nymphs and helps to develop and inform models for understanding limits to survival.

Introduction

According to a review by Nyamukondiwa et al. 2022, there are typically four common biological and physiological traits that make a strong invasive pest. First, the species is polyphagous, this gives them the ability to feed on a variety of hosts; second, possessing strong dispersal skills, either passively or as stowaways; third, having high fitness that allows them to quickly adapt to new environments; and fourth, having high stress resistance to varying temperatures and humidity (Nyamukondiwa et al. 2022). This latter category includes several abiotic factors such as temperature, relative humidity, precipitation, atmospheric pressure, and wind (Nyamukondiwa et al. 2022). Understanding how an invading organism might respond to variation in environmental temperature can bring about significant insight regarding the patterns and processes of species invasion (Kelley 2014).

Spotted Lanternfly (SLF) (*Lycorma delicatula*) (White) (Hemiptera: Fulgoridae) was first detected in Berk County, Pennsylvania in 2014 (Dara et al. 2015). This invasive phloem-feeding planthopper is originally from Asia. Since its arrival, it has spread rapidly throughout the Eastern United States (NYSIPM- Cornell 2023). SLF's drastic spread has mostly been via human-mediated transport, including firewood, trains, plant material, etc. (Urban 2020). SLF is expected to continue its spread throughout the United States to places that are climatically suitable (Jones et al. 2022). This invasive pest feeds on a wide variety of hosts and has been known to stress and damage economically important hosts (Barringer and Ciafré 2020).

With a diverse range of host species, SLF feeding can take place in a variety of habitats impacting many industries including grape, tree fruit, ornamentals, timber, and even homeowners. As a result of this diverse feeding habit, SLF moves across multiple habitats and impacts hosts in extremely diverse landscapes (agricultural, suburban, urban, managed, and natural forested areas) (Urban et al. 2020). This makes SLF the perfect hitchhiker and increases the probability of spreading. SLF therefore possesses two of the traits, high polyphagy and strong ability to hitchhike, identified by Nyamukondiwa et al. 2022.

Due to SLF's ability to spread via human-mediated transport, it has become a problem in nurseries (Dara et al. 2015, Urban 2020). Not only can SLF feed on a variety of nursery stock, but state quarantines put in place to reduce risk of SLF spread require that plants must be free of SLF before shipment. This has caused nurseries to spend extra time and money either inspecting their nursery stock or spraying insecticides before shipment (Urban 2020). It is a concern that these types of shipments could lead to further spreading of SLF. An egg mass could go unnoticed on the plants themselves or even the pots they are in. Once these egg masses get shipped out to different states, these unseen egg masses could hatch in states that do not already have SLF. As long as there is a food source and the temperature is livable, SLF could establish in this new area. Since SLF is known to also lay eggs on non-plant material, this is also a concern outside of nurseries. Thermal limits still need to be determined where SLF nymphs and adults can survive after transport. In addition, most nursery stock is shipped in a refrigerator truck. If the truck is cold enough to kill SLF, this could help nurseys limit spraying insecticides as well as labor for inspections.

Since SLF's establishment in Pennsylvania, potential distribution estimations based on temperature have only been done on egg masses in South Korea by both Parra et al. 2017 and Jung et al. 2017. That is, these are estimates of the cold temperature that are the lower limits that SLF can survive in the egg stage. Parra et al. 2017 estimated unsuitable areas for SLF establishment based on mortality of egg masses that were collected in various regions with different minimum winter temperatures in South Korea. A minimum January temperature below -13.9 °C was used to define environmental unsuitability and resulted in regions in the Northern U.S. to be unsuitable. In Jung et al. 2017, distribution was determined by using a CLIMEX model. Data on the occurrence of eggs were reported by the Korea Forest Service in 2013. For temperature index, the lower temperature threshold was 8 °C and the upper temperature threshold was 33 °C. These numbers were based on at what point eggs did not hatch (Choi et al. 2012) as well as adults' ability to survive up to 33 °C. Samples from South Korea may not have sufficient range of climate data to fully understand SLF's global distribution (Urban and Leach 2022). Kreitman et al. 2020 investigated SLF nymphal development at various constant temperatures. The lower developmental threshold was found to be 13 ± 0.42 °C. It is suggested that SLF can develop slowly at temperatures as low as 15 °C and developmental delays will occur under cool spring conditions. Therefore, further studies that look at SLF nymph's ability to withstand sudden cold snaps are needed to get a better idea of its potential range (Kreitman et al 2020). Cold tolerance helps to understand how insects respond to and survive exposure to a given set of conditions at low temperatures. It can also help indicate potential prediction and control methods (Sinclair et al. 2015, Cira et al. 2016). Understanding insect cold tolerance allows us to predict the establishment and spread of insect pests and biological control agents (Sinclair et al. 2015).

For the purpose of this study, a few measures that are most often used to characterize cold tolerance of chill susceptible species were investigated: critical thermal minimum (CTmin), chill coma recovery time (CCRT), lower lethal temperature (LLT), and lethal time at low temperature (LLt). CTmin or chill coma onset temperature is the temperature at which the insect enters a reversible state where neuromuscular transmission and movement ceases (e.g., insect is unable to feed, reproduce, or evade predators) when exposed to cold temperature (David et al. 1998, Gilbert et al. 2001, Hazell and Bale 2011, MacMillan and Sinclair 2011, Andersen et al. 2015). At the insect's CTmin, they begin to enter what is called chill coma (Hazell and Bale

2011, MacMillan and Sinclair 2011, Sinclair et al. 2015). Chill coma is likely driven by an inability to maintain ionic homeostasis through the effects of temperature on ion- motive ATPases, ion channel gating mechanisms, and/or the lipid membrane, leading to a loss of nerve and muscle excitability (MacMillan and Sinclair 2011). The amount of time it takes to come out of chill coma and recover coordinated neuromuscular function when rewarmed is called chill coma recovery time (CCRT) (David et al. 1998, Gilbert et al. 2001, MacMillan and Sinclair 2011, Sinclair et al. 2015). Both CTmin and CCRT are measurements of resistance to the effect of cold and are useful for comparing insects' thermal biology (Andersen et al. 2015, Sinclair et al. 2015). Comparing to other insects can be useful in terms of host range which can determine if SLF is capable of surviving in a given area. Comparing SLF to other invasive species could utilize control methods that might be useful in SLF control.

Testing lower lethal limits demonstrates temperatures under specific conditions (typically 1-2 hours short cold exposure) that kill a species or insect population (Clarke et al. 2013, Sinclair et al. 2015). Estimating an insect's reaction when exposed to low temperature can in turn provide information about the survival under field conditions and therefore has been shown to be highly ecologically relevant (Hatherly et al. 2005, Sinclair et al. 2015). The lower lethal temperature is the temperature at which all individuals are killed and provides information about the likelihood of survival under a given set of conditions (Sinclair et al. 2015). Mortality associated with acute exposure to low temperature has been associated with cellular apoptosis (Yi and Jr Lee 2004, Yi et al. 2007) and may involve a phase change of the cellular membranes (Kostal et al. 2004). Lethal time at low temperature is the amount of time at cold temperature at which all individuals are killed. LLt is relevant to assessing risk for insects that may be exposed to prolonged cold during refrigerated shipping (Beaudry et al. 1998, Sinclair et al. 2015). Understanding insect cold tolerance allows prediction of establishment and spread of invasive insects as well as potential biological control agents (Sinclair et al. 2015).

With SLF's strong hitchhiking abilities along with the economic damages they can cause, it is fundamental to better understand this pest in anticipation of slowing down the spread in North America. The goal of this study is to determine cold tolerance of first-instar nymphs, including, CTmin, CCRT, LLT, and LLt. Determining cold tolerance of SLF nymphs can help more accurately predict geographic distribution and establishment in North America. With this information it is possible to rule out places SLF could persist. Overall, this study will provide information about SLF’s basic biology to help better understand, control, and slow the spread of this invasive planthopper.

Methods

Animal Sources and Maintenance

SLF egg masses were collected in winter of 2022 and 2023. Masses were collected by cutting off chunks of bark or tree limbs, primarily from *Ailanthus altissima,* at sites in Sinking Springs and Reading, Pennsylvania (**Figure 3-1**). Egg masses were separated (using a chisel to remove egg mass and underlying bark) and stored in a refrigerator at 4 °C until incubated for use in the study. Several weeks prior to performances of our experiments, egg masses were removed from refrigeration and carefully hot glued vertically to pieces of wood (**Figure 17**). This was done to keep egg masses vertically as well as prevent mold growth. Wood pieces were wedged into pots that contained tree of heaven and placed in either the greenhouse (28-30 °C, 67% humidity) or a growth chamber (28 °C, 60-65% humidity) for 2-3 weeks until hatch. Once nymphs hatched, they were allowed at least one day of feeding on potted tree of heaven plants (approximately 25-30 cm tall). First instar nymphs were used to conduct CTmin, CCRT, LLT, and LLt, respectively.

Figure 3-1*:* Egg masses removed from *Ailanthus altissima* from winter 2022 and 2023 used for CTmin, CCRT, LLT, and LLt (Left). Egg masses hot glued on pieces of wood for hatching used for CTmin, CCRT, LLT, and LLt (Right).

Critical Thermal Minimum (CTmin)

Following guidelines used by Sinclair et al. 2015 and Anderson 2014 procedure, a circulating water bath (VWR- 7L low profile, refrigerated circulator with MX controller containing a mix of ethylene glycol and water in equal amounts) was set to 15 °C (**Figure 3-2**). One of each ten lab-reared first instar SLF nymphs were placed into a 13mm x 100mm Pyrex glass test tube. Using the eraser end of a pencil, a rectangular swatch of fine fabric mesh screen for windows (Saint-Gobain ADFORS. Home Depot) approximately 3 cm x 7 cm was pushed into the test tube approximately 5 cm above the SLF nymph to prevent escape. The tubes were wrapped with wire that held the tubes in place in the bath. A thermocouple (Type K) was placed in one of the test tubes with the nymph to track the SLF's body temperature (**Figure 3-2**). SLF were observed the whole time for body movements. After the insects were immersed in the water bath, they remained there with the temperature setting kept at 15 °C for two minutes to allow the insects to acclimate. At that point, the bath temperature was decreased by 2.5 °C and once the water bath came to a reading of 12.5 °C, the insects were held in the bath at that temperature for two minutes. This process continued, decreasing the water bath temperature setting in increments of 2.5 °C, with a two-minute acclimation period after every temperature change. According to Sinclair et al. 2015, it is possible to expedite measurements by rapidly reducing the temperature from the rearing temperature to an intermediate temperature (e.g., 10 °C), allowing equilibration before cooling at the predetermined rate.

Once the temperature reached 7.5 °C (preliminary data suggested this temperature induced the onset of chill-coma) and the two-minute acclimation period was over, bugs were quickly taken out and a stimulus test was done. The stimulus test was done by slightly tapping the test tube on the countertop. If the SLF showed any signs of body movement (walking, leg twitch, etc.) it was considered not to be in a chill coma yet. If SLF showed no movement, it was considered to be in chill coma and time, bath temperature, and thermocouple temperature were recorded. After all nymphs were observed, bath temperature was then decreased by 0.1 °C, the insects were given the two-minute acclimation period, and then stimulus test was performed again on only those insects that had not yet reached chill coma. This was repeated until all insects feel into chill coma. Note: when each individual nymph reached chill coma (i.e., was observed to not move when given the stimulus test), the temperature of the thermocouple at that time was recorded as the CTmin for that insect. The insect was then returned to the water bath until all nymphs reached chill coma in order to allow all insects to be exposed to the same temperatures for the same time duration. The CTmin process was replicated five times, ten nymphs per replicate (n= 5).

Figure 3-2: Circulating water bath (VWR- 7L low profile, refrigerated circulator with MX controller containing a mix of ethylene glycol and water in equal amounts) used for CTmin, LLT, and LLt (Left). Ten lab-reared first instar SLF nymphs submerged into bath in 13mm x 100mm Pyrex glass test tube, with one thermocouple (Type K) for CTmin (Right).

Chill Coma Recovery Time (CCRT)

Once all ten insects fell into a chill coma, the insects were simultaneously removed from the water bath, timing began on a stopwatch, and each insect was immediately observed (**Figure 3-3**). When the first movement was observed from an individual, time elapsed was recorded. Nymphs were observed and recovery time recorded until all nymphs came out of chill coma. Observation time was 20-30 minutes. The CCRT process was replicated five times (10 nymphs per replicate). This similarly followed the Sinclair et al. (2015) and Anderson (2014) procedure.

Lower Lethal Temperature (LLT)

Following Anderson 2014, lower lethal temperature (LLT) was determined by exposing a set of ten first instar SLF nymphs to each of one of seven temperatures: (7.5, 5, 2.5, 0, -2.5, -5, -7.5 °C) for one hour each in a water bath (VWR- 7L low profile, refrigerated circulator with MX controller; ethylene glycol mixed 1:1 with water). The upper temperature was determined by CTmin data and when insects fell into chill coma. The range was selected to be equidistant around 0 °C as a mid-point, as it was expected to be a common temperature at which many home and commercial freezers would be set. After one hour, SLF was removed and returned to room temperature and observed for movement as an indicator of survival. Nymphs were observed and survival was recorded for approximately two hours; any movement indicated that the insect was alive. If the SLF never moved, it was recorded as dead. Each of the seven temperature ranges were replicated three times, ten nymphs per replicate (n= 3).

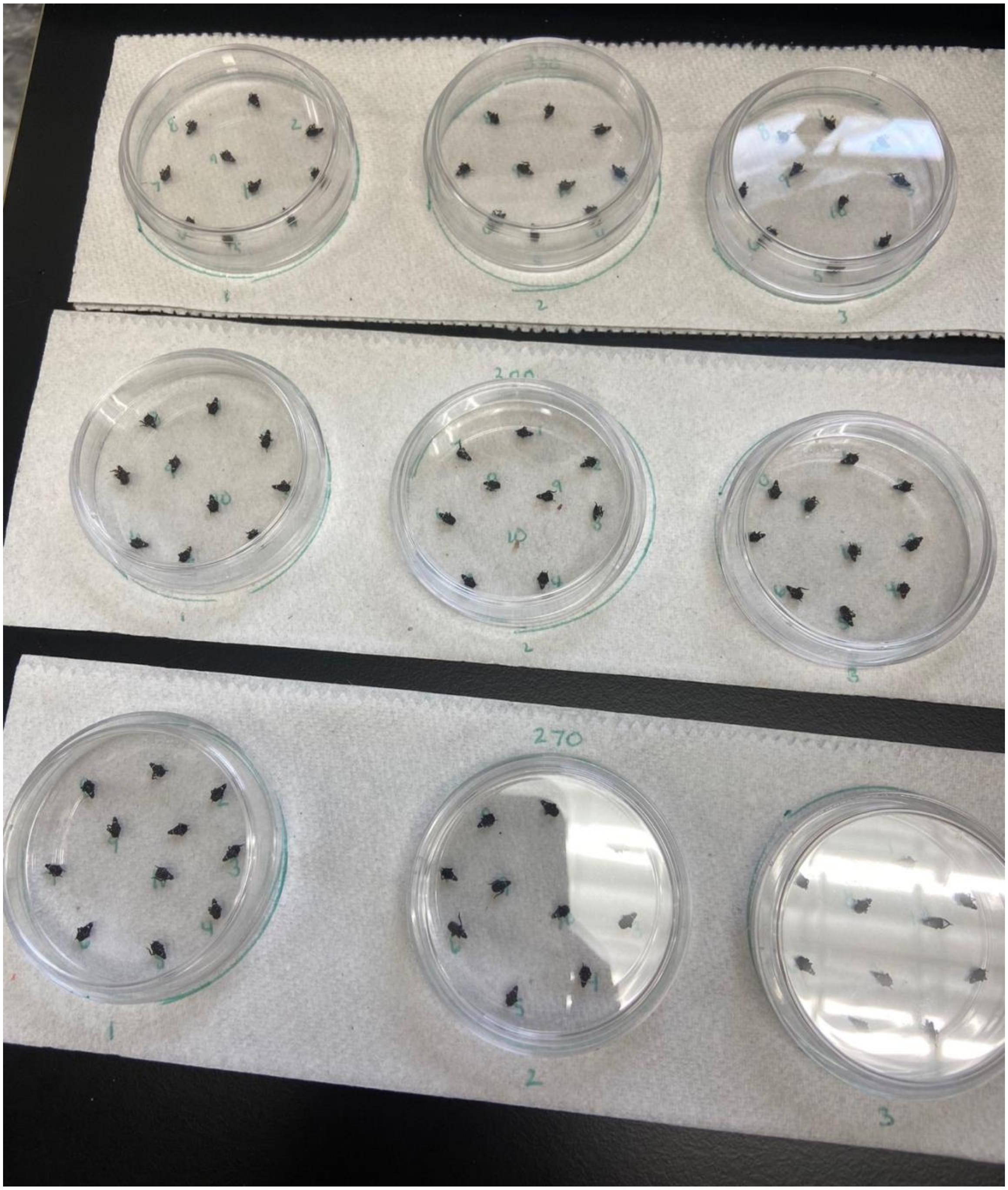

Figure 3-3: Observation setup of lab-reared first instar SLF for CCRT, LLT, and LLt.

Low Time at Lower Temperature (LLt)

Comparably following Anderson 2014 procedure to estimate LLt, a similar approach to LLT was taken, but with exposures to a set temperature (-2.5 °C) across a range of pre-determined time periods. The range of pre-determined time periods were as follows; 60, 90, 120, 150, 180, 210, 240, 270, 300, and 330 minutes. To allow for more insects to go in the bath, 0.5 mL plastic tubes were used with foam floats (**Figure 3-4**). Ten first instar nymphs were exposed to one of each of the time periods at -2.5 °C. All nymphs were submerged at once in the bath (VWR- 7L low profile, refrigerated circulator with MX controller. Ethylene glycol mixed 1:1 with water) and removed after their designated time period. Each of the ten time periods were replicated three times, ten nymphs per replicate (n= 3).

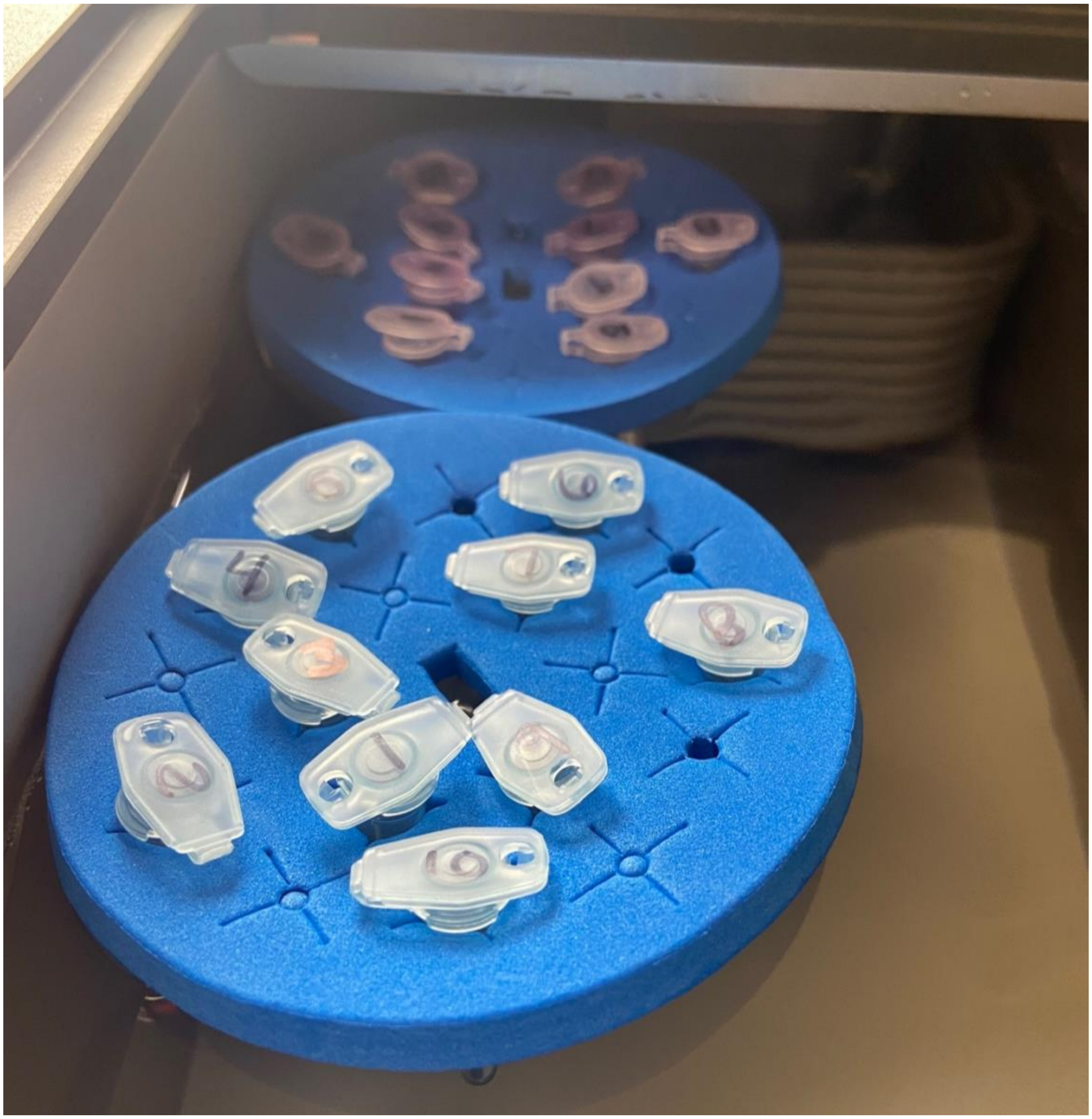

Figure 3-4: Foam floats with 0.5 mL tubes in the circulating water bath used for LLT and LLt.

Statistics

For both CTmin and CCRT, values are reported as the mean ± SEM. For both LLT and LLt, a logistic regression was done to compare and predict survival at various temperature and timepoints.

Results

Critical Thermal Minimum (CTmin)

The CTmin temperature for first instar nymphs of SLF ranged from 7.3 to 7.8 °C, respectively. The mean critical thermal minimum temperature for first instar nymphs of SLF was 7.666 ± 0.017 °C (mean ± SEM).

Chill Coma Recovery Time (CCRT)

The CCRT for first instar nymphs of SLF ranged from 1 to 621 seconds, respectively. The mean chill coma recovery time for first instar nymphs of SLF was 114 ± 18 seconds (mean ± SE). All nymphs made a recovery, none resulted in death.

Lower Lethal Temperature (LLT)

Logistic regression was used to analyze the relationship between low temperature and the probability of it resulting in either the insect being alive or dead. Temperature was shown to have a significant effect ($P < 0.001$) on whether the nymph was alive or dead. For every one unit change in temperature, the log odds of being dead (vs. alive) increases by 1.183, with 95% confident that this range covers the true odds ratio. The overall effect of temperature was statistically significant ($\chi^2 = 32.2$; d.f. = 2; $P < 0.001$, respectively). For one unit decrease in temperature, the odds of being dead (vs. alive) increases by a factor of 3.264. Our results demonstrated 50% death of first instar around -5 °C and 100% death at -7.5 °C (**Table 3-1**). The model predicts 50% death around -4.5 °C and 100% death around -9.5 °C (**Figure 3-5**).

Temperature (°C)	Survival (%)
7.5 °C	100%
5 °C	100%
2.5 °C	96%
0 °C	83%
-2.5 °C	96%
-5 °C	40%
-7.5 °C	0%

Table 3-1: Survival rate of lab reared first instar SLF at low temperatures for LLT.

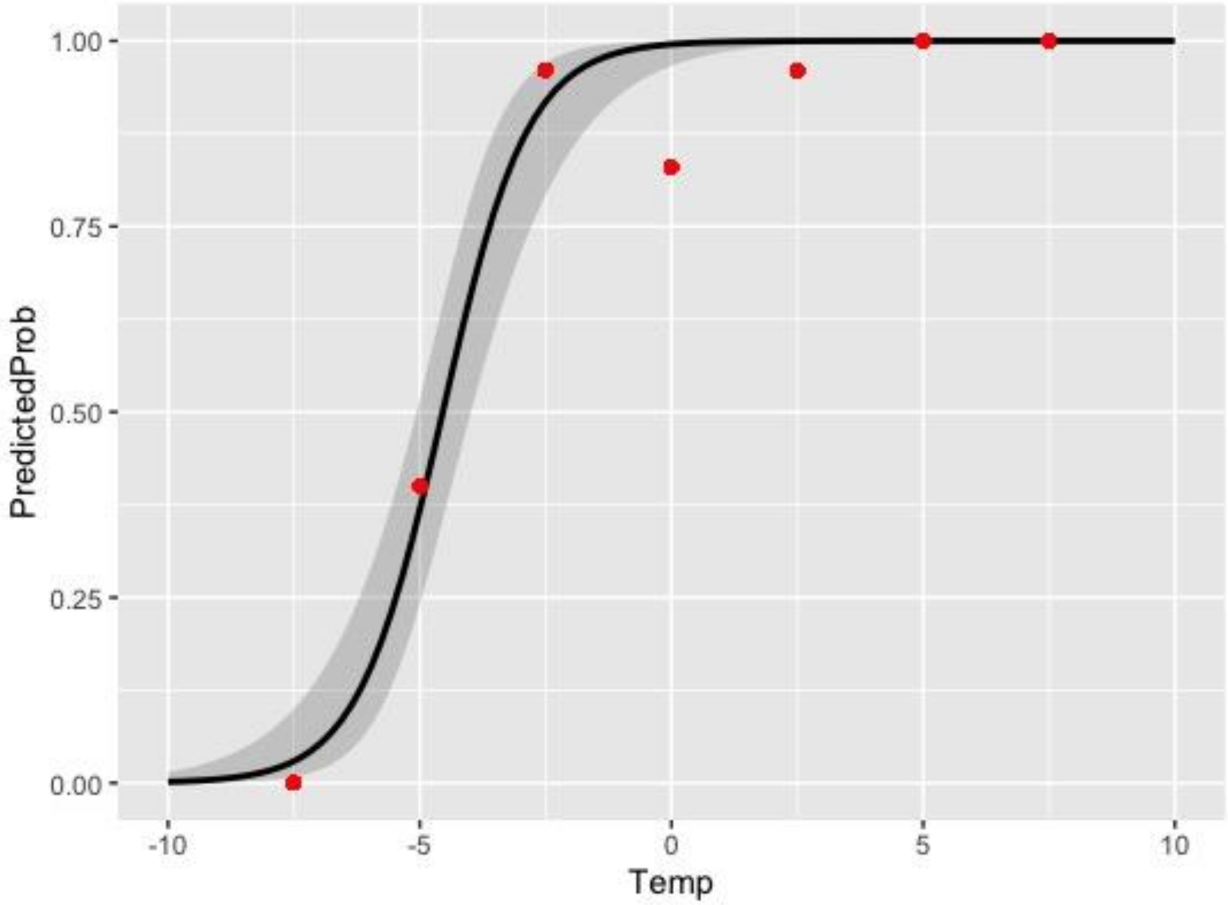

Figure 3-5: Logistic regression prediction for LLT.

Low Time at Lower Temperature (LLt)

The results from this section of the study can be found in the table below (**Table 3-2**), 50% death was not achieved during this experiment. Logistic regression was used to analyze the relationship between time at low temperature and the probability of it resulting in either the insect being alive or dead. Time was shown to have a significant effect ($P < 0.001$) on whether the nymph was alive or dead. For every one unit change in time, the log odds of being dead (vs. alive) increase by -0.009, with a 95% confident that this range covers the true odds ratio. The overall effect of time was shown to be statistically significant ($\chi^2 = 15.78$; d.f. = 2; $P < 0.001$, respectively). For one unit increase in time, the odds of being dead (vs. alive) increase by a factor of 0.99. The model predicts 50% death around 430 minutes and 100% death around 950 minutes (**Figure 3-6**).

Time (minutes)	Survival at -2.5 °C (%)
60 min	100%
90 min	96%
120 min	96%
150 min	90%
180 min	80%
210 min	96%
240 min	93%
270 min	76%
300 min	63%
330 min	76%

Table 2-2: Survival rate of lab reared first instar SLF at -2.5 °C for various lengths of time for LLt.

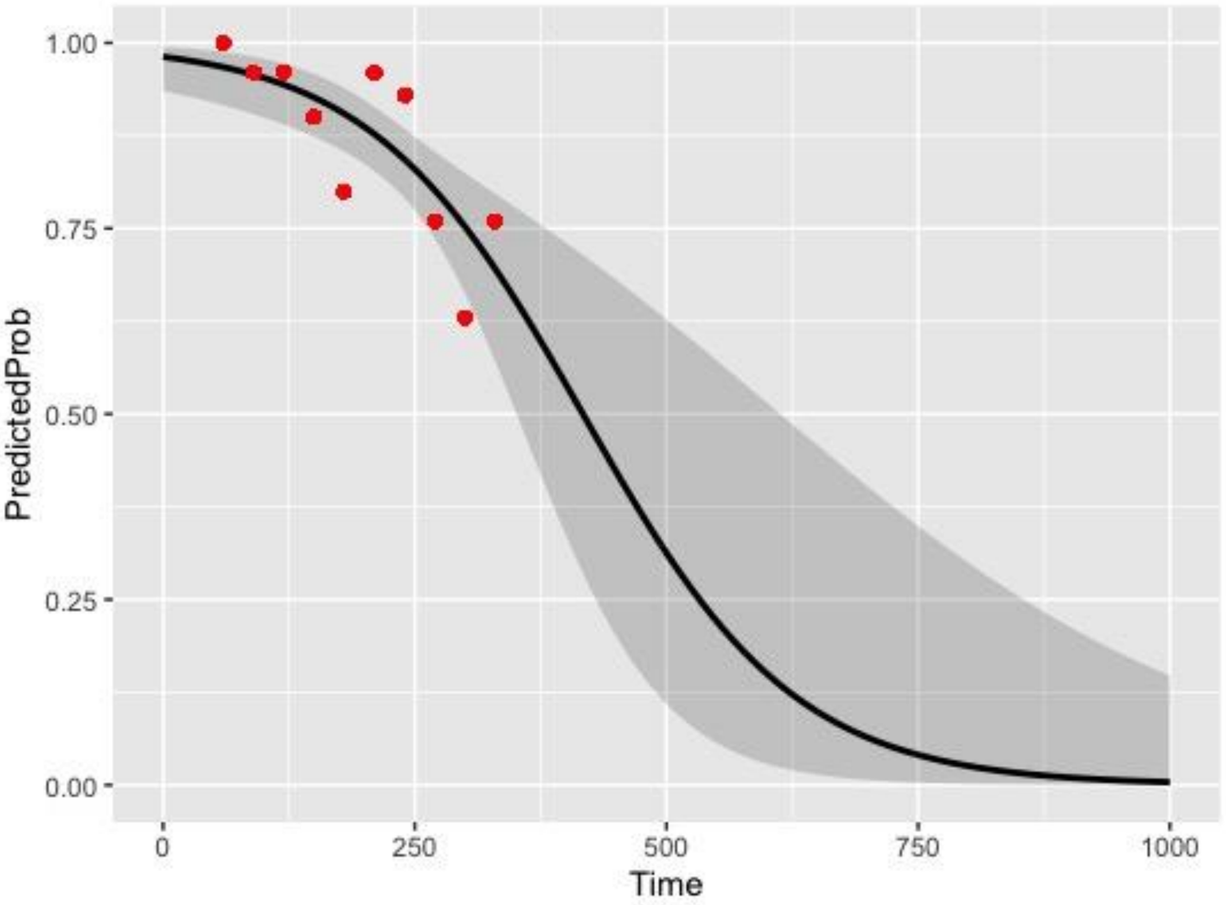

Figure 3-6: Logistic regression prediction for LLt

Discussion

Temperature is often regarded as the most important abiotic factor in determining species distribution (Kelley 2014). Understanding how an invading organism might respond to variation in environmental temperature can bring about significant insight regarding the patterns and processes of species invasion (Kelley 2014). SLF is an invasive planthopper species that is expanding its range further into the Eastern United States (Dara et al. 2015). This study provides the firsthand information on first instar cold tolerance of this species. With this information, a better understanding of SLF's basic biology can help to control and slow the spread of this invasive planthopper. Overall, this study can provide a more accurate prediction of SLF's geographic distribution and establishment in North America.

CTmin provides a useful estimate of thermal biology and can be used for comparative purposes (Anderson et al. 2015, Sinclair et al. 2015). The results from the present study showed that first instar SLF nymphs on average fall into chill coma at 7.6 °C (45.7 °F) respectively. In comparison, the brown planthopper *Nilaparvata lugens* (St˚al) (Hemiptera: Delphacidae), which is a pest to rice, has an average CTmin for second instar nymphs of 15.3 °C (59.5 °F) (Piyaphongkul et al. 2014). The redbanded stink bug *Piezodorus guilinii* (Westwood) (Hemiptera: Pentatomidae), which is an invasive in the US, has an average CTmin for lab-reared third, fourth, and fifth nymphs combined of 8.9 °C (48.02 °F) (Bastola and Davis 2018). *Rhodnius prolixus* and *Triatoma infestans* are both known to be insect vectors in South America. *R. prolixus* fifth instar nymphs have an average CTmin of 6.16 °C (43.09 °F) and *T. infestans* fifth instar nymphs have an average CTmin of 1.03 °C (33.9 °F) (Belliard et al. 2019). It can be difficult to make comparisons when there is limited data on closely related species to SLF, but comparing with other Hemipterans is still valuable. Compared to another planthopper, SLF first instars are less sensitive to this aspect of temperature. In our experiment, CTmin was evaluated only from a laboratory colony. It has been reported that CTmin is influenced by bioclimatic variables (Käfer et al. 2020). For instance, acclimatization to colder temperatures can increase critical thermal limits in insects (Calhoun 1960, Allen et al. 2012, Bastola and Davis 2018). It has also been suggested that host plant may strongly mediate lower critical thermal limits (Kleynhans et al. 2013). In addition, difference in thermal tolerance has been reported in insects based on their life stages (Bowler and Terblanche 2008, Marais et al. 2009, Bastola and Davis 2018). Therefore, further experiments with field acclimated insect populations as well as other life stages on various host plants will clarify the importance of CTmin in terms of SLF's biology. In addition, determining critical thermal maximum (CTmax) would also aid in understanding SLF upper thermal limits.

For CCRT, all first instar nymphs were able to recover from their chill coma. This information may help to gain a better understanding of their physiology, fundamental niche, and dispersion potential, as well as to predict more accurately their distribution. Other life stages as well as field acclimated insects should be evaluated to compare if CCRT is similar.

The LLT to kill 100% of SLF first instar nymphs in one hour in our experiment is -7.5 °C (18.5 °F). With this information, there is a better understanding of SLF's potential range and likelihood of survival under a given set of conditions (Hatherly et al. 2005, Sinclair et al. 2015, Kreitman et al 2021). In comparison, second instars of the brown planthopper (St̊al) (Hemiptera: Delphacidae) experienced 50% death (LTT50) at 2.3 ± 0.4 °C (Piyaphongkul et al. 2014), while in this study SLF showed around 50% death at -5 °C. As such, again SLF shows greater cold tolerance compared to the most closely related species examined to date. With milder winters, it could be predicted that adult SLF would be able to extend their adult stage, and in turn lay more eggs that when hatch, could survive more extreme cold snaps that those pervasive in the current known distribution. Though other stressors like starvation and desiccation may also affect winter survival (Terblanche et al. 2011, Bastola and Davis 2018). These temperature constraints can be used in temperature models to predict and develop reliable pest establishment risk maps for SLF. If the laboratory conditions do not reflect the salient features of the conditions in nature, laboratory derived LLT estimates may over- or under-estimate the probability of mortality in the field (Sinclair et al. 2015). For future studies, it would be beneficial to know if LLT is the same for all life stages as well as field acclimated insects. In addition, due to the low temperature SLF is able to withstand, it could be beneficial to identify the supercooling point of this insect to determine if it can tolerate internal freezing.

LLT is particularly relevant for risk assessment for insects that may be exposed to prolonged cold during refrigerated shipping (Beaudry et al. 1998, Sinclair et al. 2015). This is particularly relevant for nurseries that ship out plants and run the risk of shipping SLF with the cargo. The predicted LLt for first instar nymphs at -2.5 °C (27.5 °F) based on the logistic regression is 950 minutes. A diesel-powered refrigeration unit (reefer trailer) for shipping plants is best at 0.56 °C (33 °F). Therefore, for first instar nymphs to be killed on plants in a refrigerator truck, it would take almost 16 hours. In comparison, the invasive redbanded stink bug *Piezodorus guildinni* (Westwood) (Hemiptera: Pentatomidae) LLt at -2 °C died in an average of 56.81 hours (Bastola and Davis 2018). Further studies on first instar nymphs are needed to determine if the logistic regression prediction is accurate, as well as other life stages and field acclimated insects.

In conclusion, this study provides the first insights into the cold tolerance of the invasive SLF in the United States. Information on cold tolerance of SLF is critical for understanding their possible geographic range, relative abundance, season activity, and distribution patterns. Studying cold stress on this species has the potential to illuminate new modes of management. This information will form the basis for developing a model to forecast first instar nymphal hatch survival. Further investigation on other life stages as well as field acclimated populations will help to fully understand SLF cold tolerance.

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Chapter 4

Investigating Adult Spotted Lanternfly Temperature Variation

Summary

Spotted lanternfly (*Lycorma delicatula* (SLF)) was first detected in Berks County, Pennsylvania in 2014. Since its detection, it has spread into a total of 16 states and have negatively impacted the grape industry. There is a crucial need for improved monitoring tool for SLF in areas of infestation as well as areas of potential establishment, specifically in vineyards. It has been demonstrated that adult SLF are able to be detected using infrared cameras, suggesting that the insect seems to be generating heat. Yet, evidence concerning whether SLF is generating heat is limited, and if it is, potential explanations of how and under what conditions SLF does so remain to be explored. In this study, adult SLF body temperatures are taken throughout the day, along with weather data, body mass, and activity in order to gather more baseline evidence concerning whether SLF may be generating heat. This information sheds light on this potentially unusual aspect of SLF's basic biology, in order to inform further studies of possible endothermy, which in turn, could lead to the potential development of thermal monitoring for SLF.

Introduction

In Berks County, Pennsylvania in 2014, spotted lanternfly (*Lycorma delicatula* (SLF)) was first detected. Since then, the invasive phloem feeding planthopper has drastically spread throughout the Eastern United States. This insect feeds on a wide variety of hosts but has negatively impacted the grape industry the most. There is a crucial need for better monitoring tools for SLF (Urban 2020). Studies have demonstrated that SLF can be detected using thermal cameras (Dinets 2021, Liu et al. 2021), which suggest that SLF may be generating heat. As such, these authors have suggested that thermal imaging may provide a novel avenue for monitoring and potential early detection of SLF. Although a review of the application of thermal imaging for insect detection is beyond the scope of the current thesis, the unusual findings reported by Dinets

2021 and Liu et al. 2021 demonstrated the need for additional baseline data to be collected to better examine evidence of heat generation by SLF adults.

Liu et al. 2021 demonstrated that adult SLF could be detected in field habitats (e.g., mixed hardwoods) using infrared thermography at long wavelength infrared (8-14 μm) in Pennsylvania. Two different infrared cameras were evaluated on various bark surfaces, boxelder tree (*Acer negundo* L. [Sapindales: Sapindaceae]), Chinese privet shrub (*Ligustrum sinense* Lour. [Lamiales: Oleaceae]), tree of heaven (*Ailanths altissima*), and red maple (*Acer rubrum*). Adult SLF were able to be detected by infrared thermography (September to October 2019) sampled in the study (Liu et al. 2021). Observations were made between 4:30-7:30 AM to reduce potential interference of solar radiation for better quality thermograms. Detection rate differed significantly between times of observation, detection stayed almost unchanged between 4:30-6:00 AM (approximately 40% detection rate), dipped slightly from 6:00-7:00 AM (approximately 30% detection rate), and dropped significantly by 7:30 AM (approximately 10% detection rate). A significant temperature gradient was observed for adults at close range (4 m) with the highest temperature in the head, followed by the thorax, portions of the wings, and legs. Because SLF could be detected during this time period, when the sun was not available to provide direct heat source, these images suggest (and was presumed by the authors to show) that SLF was generating heat at this time. However, no direct measurements of insect temperature were taken in this study.

Liu et al. 2021 suggests that active feeding and rapid hemolymph circulation in adults are the primary causes of the thermal signal coming from SLF that allows it to be detected by thermal cameras. The cibarial pump is used to extract copious quantities of fluid from the host plant (Naskrecki and Nishida 2007) and is powered by substantial dilator muscles in the head (Dugravot et al. 2008). These muscles are constantly contracting during feeding which the authors assert as the most likely mechanism being used to generates heat (Liu et al. 2021). Hemolymph circulation in SLF adults during active feeding would also presumably pass the heat from the head to the thorax, legs, wings, and the abdomen making SLF detectable to thermal cameras (Liu et al. 2021). Liu et al. 2021 goes on to suggest that vineyards may provide the ideal sites for large-scale testing in the field as highly active adult populations are usually observed between September and October (Leach and Leach 2020). It is also suggested that the focus should be placed on times when most adults are feeding aggressively to increase detection rate, both during the day and at night (Liu et al. 2021). In addition to observation time, the role of adult age and sex needs to be investigated as well (Liu et al. 2021).

However, no data was presented to support Liu's hypotheses concerning the source of this thermal signal. Moreover, these recommendations are based on highly limited data (e.g., measurements being taken only during the same three-hour window in early morning). In New Jersey, Dinets 2021 subsequently observed a total of nine insects, of which four were observed at least four times a day for five days, and one was observed every 2-24 hours during August and September. A Pulsar Quantum Lite XQ30V thermal imager (Yukon Advanced Optics Worldwide, Ltd.) and TP30 laser thermometer (ThermoPro) were used for these observations. Results showed that trees covered with adult SLF could be seen through the thermal imager from over 100 m away. Later in the season, SLF were no longer measurably different from ambient temperature so they could not be distinguished via thermal imaging (Dinets 2021). Dinets 2021 suggests that the heat signature is due to the insect becoming homeothermic within 24 hours after metamorphosis into adults and maintaining homeothermy for 2-3 weeks. The author suggests that SLF's constant access to an unlimited supply of energy in the form of sugar-rich sap may contribute to endothermy, which would require a high metabolic rate for an endothermic insect (McNab 2009, Dinets 2021). This study does not explain why SLF stopped being endothermic later in the adult life stage, but the author suggests that lower body temperature is required for successful egg development (Dinets 2021). Dinets 2021 suggests that this finding could be useful in thermal detection of SLF. But as with the Liu et al. 2021 study, much remains unexplained about this phenomenon.

Therefore, the goal of the present study is to gather additional data to substantiate possible heat generation more thoroughly by SLF. Body temperatures of adult SLF were recorded at multiple intervals throughout the day once per week in Pennsylvania vineyards in 2021 from October to November. In addition to body temperatures, weather data, vine location, activity, sex, and live mass were also recorded in order to examine some factors that might possibly explain, or covary with temperature. That is, to further explore evidence that feeding or mating (e.g., wing fluttering) or other activities may be associated with temperature variation, SLF activity (behavior prior to temperature sampling) was recorded. We hypothesize that because SLF adults, females in particular, substantially increase in size and fat deposition as they become reproductively mature, heat generation may be related to an aspect of reproductive development that is occurring in this life stage (e.g., increased fat metabolism or overall metabolism associated with reproductive development).

Methods

Research Sites

This study was conducted under field conditions at Vynecrest Vineyard & Winery in Breinigsville, PA and an independent grower in Coopersburg, PA within the Pennsylvania SLF quarantine zone in October 2021. The two field sites were located at coordinates 40.56 N, -75.64 W and 40.49 N, -75.45 W in an area with a variety of grape cultivars surrounded by farming fields. Adult SLF populations did not persist at Vynecrest, therefore a switch to an independent grower took place in late October 2021.

Weather Data

Air temperature (°C), heat index (°C), relative humidity (%), station pressure (mb) (pressure observed at specific elevation and is the true barometric pressure of a location), and dew point (°C) were collected once a week in October 2021 using a Kestrel (5500AG Agriculture Weather Meter).

Adult Body Temperature

Once a week during SLF adult lifecycle (October 2021), body temperatures of ten males and ten females were taken in Pennsylvania vineyards at each of five timepoints (8 AM, 10 AM, 12 PM, 2 PM and 4 PM). As the season went on, there was less available males therefore sometimes less than ten were surveyed in a timepoint. A Kestrel (5500AG Agriculture Weather Meter) was used to record weather data at the beginning of each timepoint. SLF were randomly selected throughout a row of grapevines on the edge of a vineyard. Prior to taking the temperature reading of each insect, SLF’s activity was recorded (inactive- sitting/resting (I), walking (W), feeding (F), courting (C), mating (M), or ovipositing (O)). Feeding activity was defined as having the proboscis contacting the surface of the plant. When the stylet was not visible, a probe was used to determine whether the SLF was feeding. This was done by slightly lifting the insect with the probe to determine if the mouthparts were in the vine. If the mouthparts remained in the plant, the behavior was considered feeding. If the SLF mouthparts readily broke contact with the plant, the behavior was recorded as inactivity; this follows Leach and Leach 2020 protocol. SLF were caught in a 5 mL flat bottom test tube by angling the tube in front of the insect causing it to jump into it, the insect was then weighed in the test tube. After weighing, body temperature was taken using an infrared thermometer (Industrial IR with Circle Laser (IRK-2)) and a thermocouple (Type K) attached, fitted with a 0.38 inch by 1.0-inch flat elongated probe (**Figure 4-1**). The SLF was contained in the flat bottom test tube and manipulated using the probe onto their back along the side of the tube. This was done so there was no heat transfer from hands to the insect and all temperatures were taken in the shade. Temperatures were taken by firmly pressing the probe against the ventral surface of the abdomen for 30 seconds (Hanson and Venette 2013, Cira et al. 2016). To take the insects temperature, the thermocouple was pressed against the abdomen and the temperature was read on the infrared thermometer. Prior lab testing using an infrared camera showed that there was a strong heat signature coming from the abdomen of adult SLF imaged on a host plant in the quarantine lab (**Figure 4-2**). This area of the body also allowed for full direct contact of the entire thermocouple probe surface to the insect's body. This is why it was chosen to use the probe on the abdomen in anticipation of recording the best temperature. SLF were euthanized after their temperature was taken and the probe was cleaned by wiping it with ethanol prior to sampling the next insect.

Figure 4-1*:* Scale and infrared thermometer (Industrial IR with Circle Laser (IRK-2)) and a thermocouple (Type K) attached, fitted with a 0.38 inch by 1.0-inch flat elongated probe at Private Growers in Coopersburg, PA.

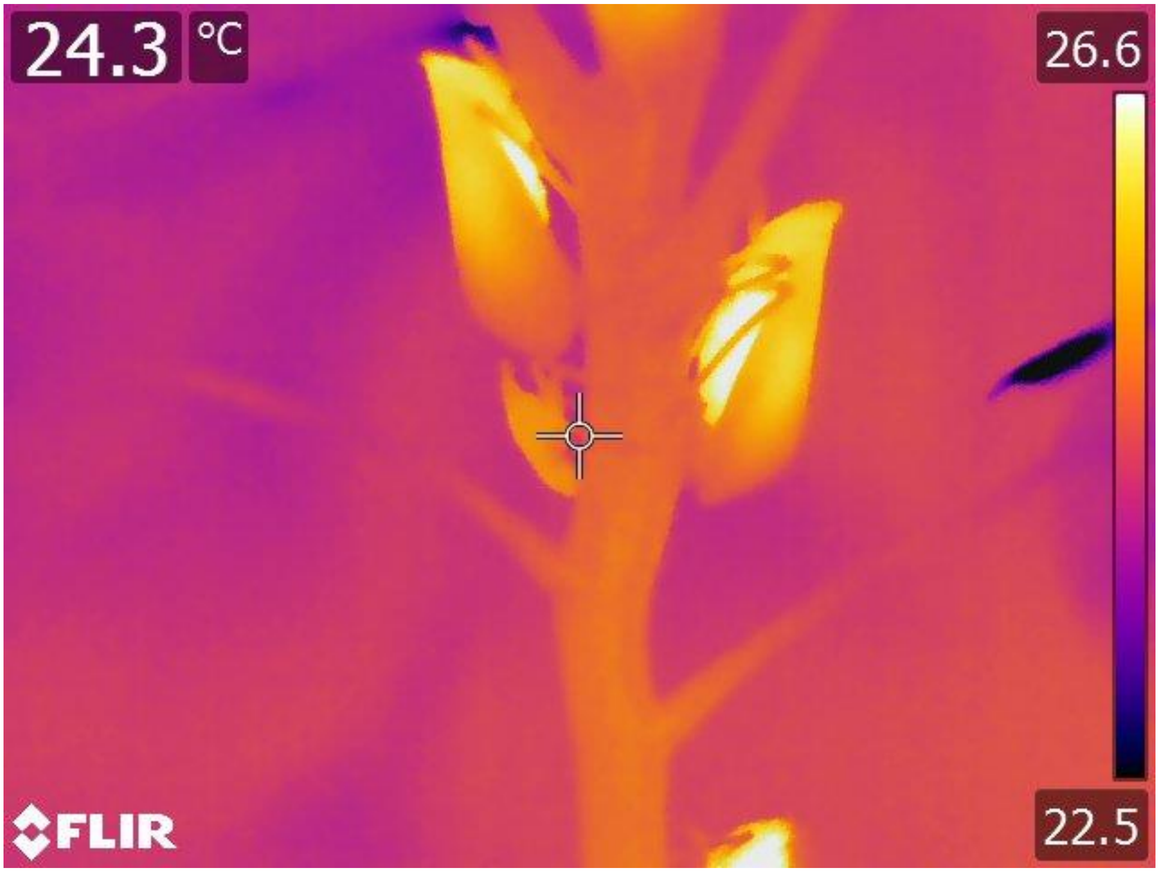

Figure 4-2: Prior lab testing using an infrared camera showing strong heat signature coming from the abdomen of adult SLF imaged on a host plant in a quarantine lab (Rudolf Schilder, Julie Urban, Heather Leach).

Stab Thermocouple Validation

To validate the body temperatures measurements taken in 2021 with the thermocuple flat probe applied to the external ventral surface of the abdomen (as described above), a stab thermocouple was used to measure the internal abdominal temperature of SLF adults more directly. In August 2022, from 11 AM to 4 PM, at Vynecrest Winery in Breinigsville PA, both the flat probe and the stab probe were paired with a thermocouple thermometer: RTD/Thermocouple (SDL200-Nist) was used to sample body temperatures. Air temperature (°C), heat index (°C), relative humidity (%), station pressure (mb), and dew point (°C) were collected every hour throughout the day using a Kestrel (5500AG Agriculture Weather Meter)

(**Figure 4-3**). Individually, 100 adult SLF were contained in a 5 mL test tube and manipulated using the probe onto their backs along the side of the test tube. Body temperature was first recorded using the flat probe thermocouple, followed by the stab thermocouple. Each thermocouple was held in place for 30 seconds and then temperature was recorded. Protocols for the stab thermocouple followed Stone and Willmer 1989 and for the flat thermocouple followed Cira et al 2016. SLF were euthanized after their temperature was taken and probes were cleaned before the next insect. This data was compared to the flat probe method.

Figure 4-3*:* Kestrel (5500AG Agriculture Weather Meter) and thermocouple thermometer (RTD/Thermocouple (SDL200-Nist)) paired with the flat probe and the stab probe thermocouples at Vynecrest Winery in Breinigsville, PA.

Statistics

To determine whether there was a significant association between measured variables, a Pearson's Correlation Test and Kruskal-Wallis Test were computed between insect body temperature and other measured variables. For the flat probe and stab probe thermocouple, mean and standard deviation as well as a t-Test was computed to determine if the methods yielded comparable results.

Results

In this study, during the month of October, the insect's body temperature ranged from 13-33 °C while the ambient temperature ranged from 11-31 °C (**Figure 4-4**). Insect temperatures ranged from 2 to 9 °C above ambient temperature throughout the course of the day (**Figure 4-5**). Pearson's correlation test showed a significant positive correlation between SLF body temperature and air temperature, dew point, and heat index ($r = 0.926$; $P < 0.001$, $r = 0.91$; $P < 0.001$, and $r = 0.9$; $P < 0.001$, respectively). SLF body temperature showed a significant negative correlation with body mass and relative humidity ($r = -0.237$; $P < 0.001$ and $r = -0.503$; $P < 0.001$, respectively). Kruskal-Wallis test showed significant correlation of SLF body temperature and date collected, vineyard, timepoint, and activity ($\chi^2 = 296.1$; d.f. = 3; $P < 0.001$, $\chi^2 = 257.92$; d.f. = 1; $P < 0.001$, $\chi^2 = 23.88$; d.f. = 4; $P < 0.001$, and $\chi^2 = 11.162$; d.f. = 4; $P = 0.0248$, respectively). There was no significant correlation between SLF body temperature and station pressure and sex ($r = 0.008$; $P < 0.891$ and $\chi^2 = 0.17666$; d.f. = 1; $P = 0.6743$, respectively). For the different activities recorded, mean SLF body temperature was highest when insects were observed to be courting and showed the least amount of variation. Similarly, mating also had an elevated temperature with more variation than courting. While feeding, insects had the lowest average temperature and showed the most variation (**Figure 4-6**). There was no evidence of a specific activity taking place at a specific timepoint throughout the day (**Figure 4-7**).

In comparing the flat probe versus the stab probe, the mean value of the stab probe (33.224 ± 1.329 (mean ± SD)) was not significantly different than the flat probe mean (33.272 ± 1.125 (mean ± SD)) (d.f. = 198; $P < 0.001$, respectively). Therefore, we determined that using the flat probe does not introduce substantial error to the temperature measurements and allows us to perform the non-lethal sampling needed to measure a large sample size of insects over time.

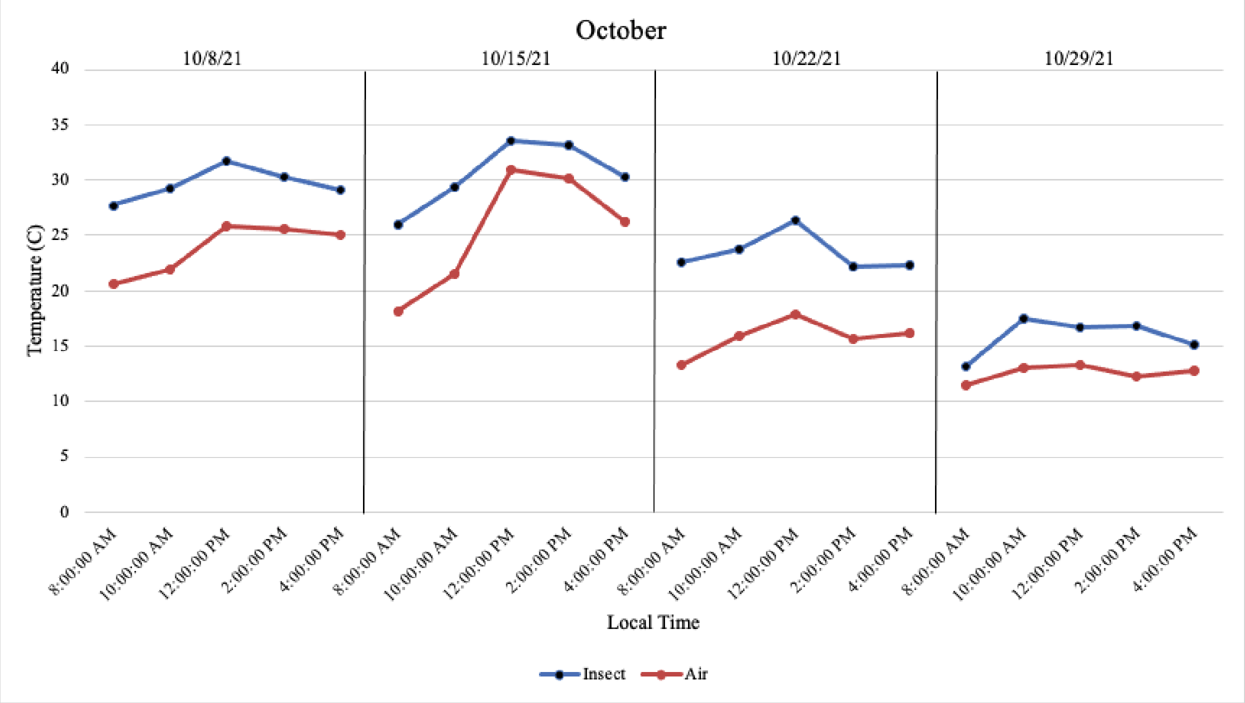

Figure 4-4*:* Average body temperature of adult SLF (measured every 2 h with infrared thermometer (Industrial IR with Circle Laser (IRK-2) and a thermocouple (Type K) attached, fitted with a 0.38 inch by 1.0 inch flat elongated probe)) and air temperature (measured every 2 h with Kestrel (5500AG Agriculture Weather Meter)) once a week in October 2021, Pennsylvania, USA.

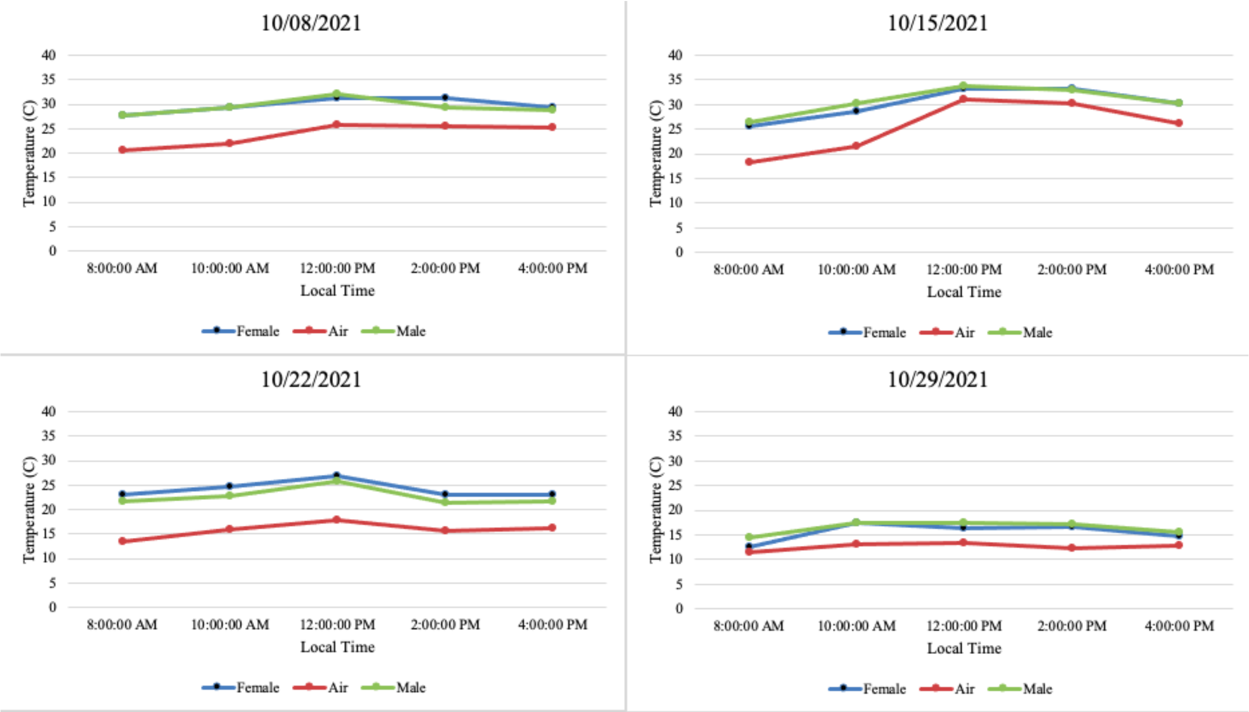

Figure 4-5: Average body temperature of adult male and female SLF (measured every 2 h with thermocouple attached to infrared thermometer) and air temperature (measured every 2 h with Kestrel) once a week in October 2021, Pennsylvania, USA. Maintaining a body temperature within 13-33 °C range, while the ambient temperature ranged within 11–31 °C range.

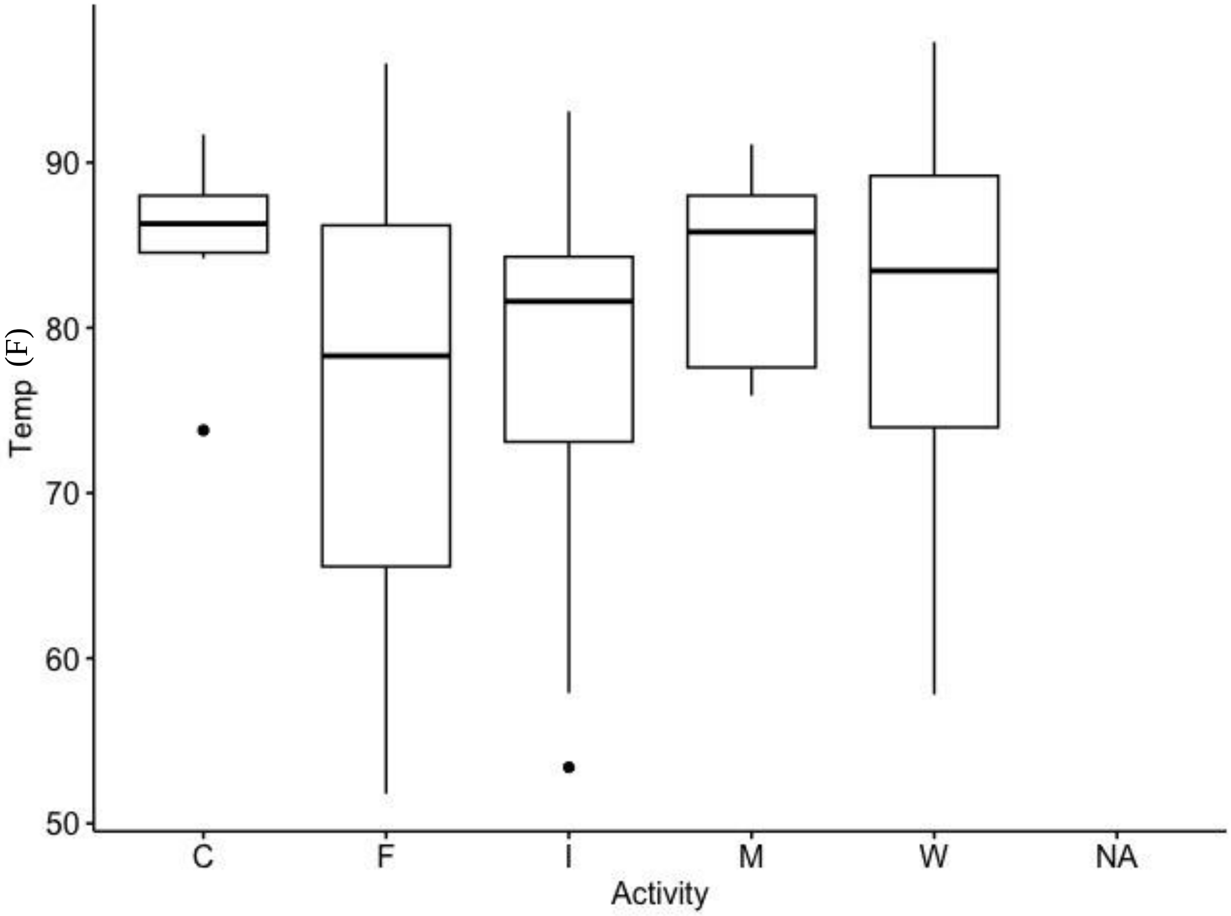

Figure 3-6*:* Body temperatures of adult SLF during different activities: Courting, Feeding, Inactive, Mating, and Walking

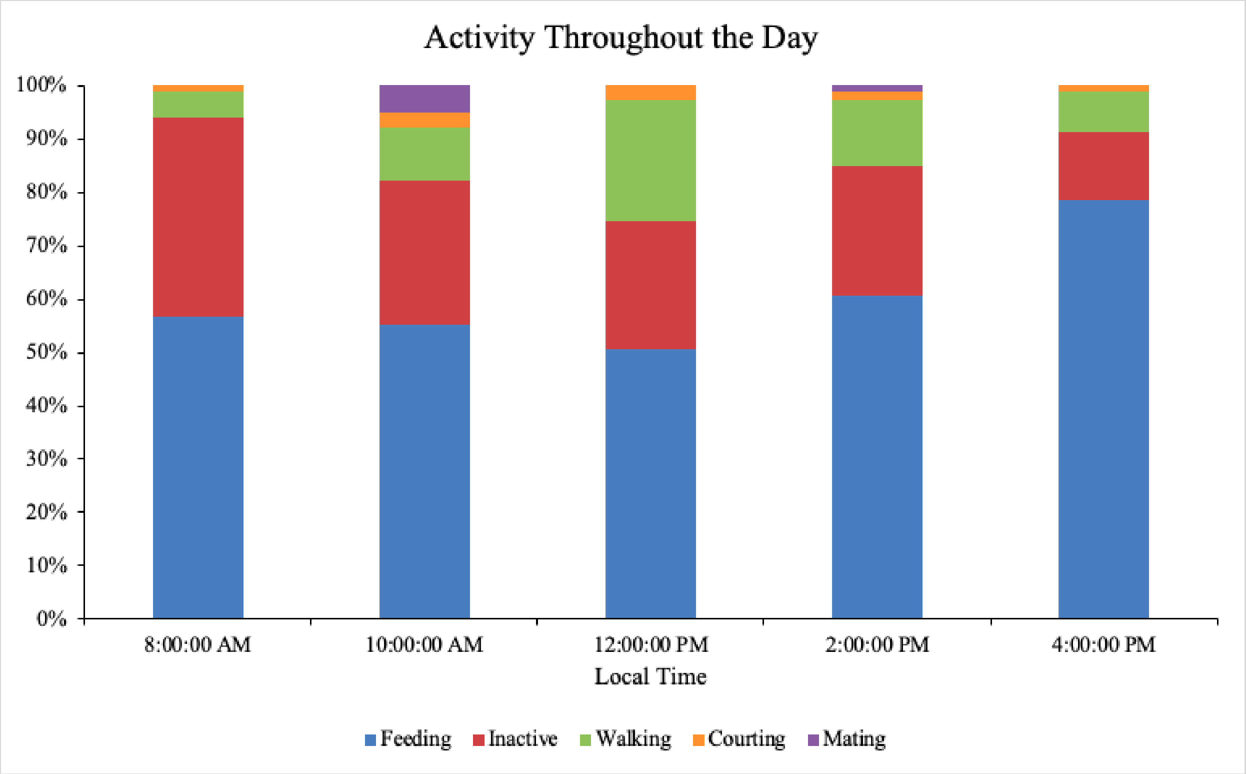

Figure 4-7: Activity of male and female SLF at different timepoints during the month of October 2021

Discussion

This study provided additional data concerning adult SLF patterns in temperature over multiple sampling events during the day. Numerous factors including weather data, SLF activity, sex, live mass, and timepoints throughout the day were investigated to better understand how adult SLF might be generating heat. Like most insects, SLF's body temperature was generally similar to ambient temperature throughout the month of October. Adult SLF averaged 5.46 °C warmer than ambient temperature. The largest temperature gap between ambient and the insect, 6.48 °C, was seen during the 10 AM timepoint, while the smallest temperature gap, 4.17 °C, was seen during the 4 PM timepoint. Mean adult SLF body temperatures stayed consistent above ambient temperature, throughout the day as well as the month of October. In Dinets 2021, adult SLF also remained at a relatively constant temperature that was warmer than ambient temperature; ambient temperature also showed more fluctuation than adult SLF. The gap between mean adult SLF body temperature and ambient temperature in September reported by Dinets 2021 ranged from 0-6 °C warmer than ambient temperature, whereas in our study, mean adult SLF body temperature was 3-9 °C warmer than ambient temperature.

Because we observed significantly positive correlations between body temperature and air temperature, heat index, and dew point, this indicates that SLF might depend on external sources, like sunbathing, as an energy source for its body heat rather than internal sources (Davenport 2012, Sinclair et al. 2015). However, in both this current study and Dinets 2021, it has been shown that adult SLF maintains an elevated body temperature overnight (Dinets 2021) and in the early morning hours (present study and Dinets 2021). This insect seems to be staying warm longer than if sunbathing is the only source of heat, suggesting that another source of heat might be in use. Heat might be generated through muscle contraction associated with wing movements or calling behavior, as well as feeding. For example, cicadas use the flight muscles and tymbal muscles to produce heat to raise body temperature and flight muscles are well suited for heat production. Some cicada species are also known to increase body temperature with their fight muscles that move the wings (Sanborn 2000). Endogenous heat production in the dessert cicada, *Tibicen winnemanna,* functions to raise and maintain thoracic temperature at a level necessary for reproductive activity when exogenous heat sources are unavailable (Sanborn 2000). Adult male SLF demonstrate a shiver-like movement, but this is typically displayed in what is generally presumed to be courting behavior between males and females. It could be that the male uses the shiver-like movements to heat up for mating. It was observed that the highest body temperature with the least amount of variation was seen during the activity of courting. However, there were few males observed exhibiting courting behavior (<10) and female and male body temperatures were seen to be similar throughout October. Relatively higher temperatures were also recorded for males directly after mating. However, as seen in courting, mating behavior was also observed at an overall low frequency (<10 individuals) (**Figure 15**).

As previously described, Liu et al. 2021 suggest that active feeding and rapid hemolymph circulation in adult SLF is the primary causes of the thermal signal coming from adult SLF that allow it to be detected by thermal cameras. Similarly, Dinets 2021 suggests SLF is able to maintain elevated body temperature by having constant access to an unlimited supply of energy from sugar-rich sap which in turn could require a high metabolic rate (McNab 2009). Our findings show that the most frequently observed behavior SLF was engaged in just prior to recording of each individual's temperature was feeding. The next most frequently observed behavior was inactivity. Therefore, while our results do not refute the hypotheses of Liu et al. 2021 and Dinets 2021 that SLF heat generation may be associated in some way with feeding, they do not provide compelling evidence supporting it. The sap being ingested by the insects is another potential source of heat. Seemingly to address this consideration, Dinets 2021 measured the temperature of the substrate, and found that the air temperature and the substrate of the tree follow similar temperature patterns. Because our study insects fed upon a different food source, grapevine, it is possible that grapevine might be more sugar-rich than the substrate (trees in a broadleaf forest) assessed in Dinets 2021. Therefore, grapevine might be warmer, or could contribute to indirect heat generating effects (e.g., this food source might require a higher metabolic rate for digestion). Substrate temperature was not taken in this study but could be considered for inclusion in further study.

In our study, it was also shown that the different vineyard locations had a significant effect on the insect's body temperature. While this is something to consider as they are about 20 miles apart and likely vary in elevation and weather characteristics, the change in vineyard location (which was what this variable addressed), took place halfway through the study. Therefore, the significance in body temperature change was likely due to decreasing air temperatures as the month of October progressed, which co-occurred with this change in vineyard location.

To determine how adult SLF are thermoregulating, further studies need to be done to investigate gaps that remain based on ours' and these previous studies' (Dinets 2021, Liu et al. 2021) findings. In particular, increased observations (with respect to number of individuals and across weeks) that include data collection overnight are needed to further substantiate that adult SLF is generating heat, and if this is associated with some particular behavior (e.g., increased feeding rates). Additional insights could also be gained by measuring additional variables, such as substrate temperature and metabolic rate. Once it is sufficiently demonstrated that adult SLF are generating heat, and further, mechanisms by which this is achieved are discovered, heat generation might then be able to be capitalized upon for the development of thermal monitoring tools.

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