THE DEVELOPMENTAL RATE OF NYMPHAL *LYCORMA DELICATULA,* AN ASSESSMENT OF THEIR DEVELOPMENTAL RESPONSE TO TEMPERATURE AND HOST PLANT

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ABSTRACT OF THE THESIS

The developmental rate of nymphal *Lycorma delicatula*, an assessment of their developmental response to temperature and host plant

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The spotted lanternfly *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) is an invasive planthopper species that was introduced into North America and is a threat to multiple plant industries. Being a recent introduction, not much was previously known regarding its phenology. For this thesis, the phenology of *L. delicatula* was examined through delving into its developmental response to temperature as well as the effect that host plant has on its development. Specimens, sourced from eggs hatched in the laboratory and field captured nymphs, were used in these experiments. To analyze its developmental response to temperature, nymphs were reared on *Ailanthus altissima* (Miller) (Sapindales: Simaroubaceae) at each of the following constant temperatures: 5, 10, 15, 20, 25, 30, 35, and 40°C. Developmental rate increased with temperature from 15 to 30°C for all instars, then declined again at higher temperatures. This study provided the first lower developmental threshold values for *L. delicatula* nymphs. Following this, the effect of host plant on the development of *L. delicatula* nymphs was assessed. Nymphs were

reared at a constant 25°C on the following hosts: *A. altissima*, *Vitis labrusca* (L.) (Vitales: Vitaceae), *Salix babylonica* (L.) (Malpighiales: Salicaceae), *Acer rubrum* (L.) (Sapindales: Sapindaceae), *Celastrus orbiculata* (Thunberg) (Celastrales: Celastraceae), *Ocimum basilicum* (L.) (Lamiales: Lamiaceae), and *Rosa multiflora* (Thunberg) (Rosales: Rosaceae). Host plant was found to have a significant effect on development time for nymphs in the first through third instar as well as on nymphal survival. This variability in development time by host plant can potentially impact phenology models, which should be updated to reflect these new insights. Both studies provide vital information for the development of phenology models for *L. delicatula* and can be used to refine existing models and make way for future studies, such as using field data to validate the development rate, looking into the impact on developmental rate of rearing on additional and multiple hosts, and calculating the developmental rate by rearing individual nymphs all the way to adults at various constant temperatures.

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Chapter 1

Introduction

The spotted lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), is an invasive planthopper that was first detected in North America in 2014 in Berks County, Pennsylvania (Barringer et al 2015). Its native range includes China, India, and Vietnam; however, it is also invasive in Japan and Korea. Since its detection its range in North America has expanded to fourteen states mostly located in the Northeast. Recent spread models have found that it has a high probability of reaching California by 2033 if no preventive measures are taken, which could devastate the Californian wine industry (Jones et al 2022). Despite being capable of dispersal through flight, human-mediated spread such as egg masses and hitchhiking individuals on cargo and vehicles has played a major role in its expansion (Ladin et al 2023).

Lycorma delicatula is univoltine throughout its entire range, both invaded and native, and has four instars. The first instar nymphs start to emerge from eggs in late April in North America (Barringer and Ciafre 2020). The first three instars are black and white in coloration in contrast to the fourth instar nymphs that have red coloration in addition to black and white. Adults can appear as early as late June and lay eggs starting in late September as the light cycle influences the timing of oviposition (Nixon et al 2022). Egg laying continues until temperatures reach levels that result in adult mortality. While adults have been observed to not immediately be capable of flight upon reaching adulthood, they do gain the capacity to fly before mating. Eggs are deposited in grayish to tan egg masses on various substrates such as bark, stone, wood fences, and bricks,

where they overwinter until spring. Female adults are capable of laying between one to three egg masses before dying (Nixon et al 2022).

Lycorma delicatula has a broad host range consisting of 103 plant species (Barringer and Ciafre 2020). It is important to note that *L. delicatula* has a preferred host, the tree of heaven, *Ailanthus altissima* (Miller) (Sapindales: Simarobaceae) (Dara et al 2015). *Ailanthus altissima* is widespread and commonly found in disturbed sites especially along train tracks, roads, and service stations. Despite this, *L. delicatula* doesn't require *A. altissima* to complete its lifecycle; however, the removal of *A. altissima* from its diet is associated with reduced fitness (Uyi et al 2020). An additional benefit of this broad host range is that it allows *L. delicatula* to feed in areas where *A. altissima* isn't available. This broad host range includes several major commodities, including grapes (*Vitis* spp. L. (Vitales:Vitaceae)). All life stages of *L. delicatula* are known to feed on *Vitis* spp. and adults have been observed depositing egg masses on this species. *Lycorma delicatula* has been identified as a major economic threat to the wine grape and table grape industry (Lee et al 2019). Despite *L. delicatula* being a rather recent introduction, the economic impacts to these industries are already visible with one vineyard in Pennsylvania reporting a 90% yield loss due to their feeding (Urban 2019). It was also determined that feeding by *L. delicatula* on grapevines can result in reduced root starch concentrations, which is associated with lower yield in the following years, thus raising concerns about potential long term effects of their feeding (Harner et al 2022). *Lycorma delicatula* also poses a threat to the tree fruit industry as *Malus* spp. (Miller) (Rosales: Rosaceae) and *Prunus* spp (L.) (Rosales: Rosaceae) are included in the host list. Many forest dwelling trees such as *Juglans nigra* (L.) (Fagales: Juglandaceae) are at risk

as well (Lee et al 2019). *Lycorma delicatula* feeds on the phloem of plants and by doing so damages the plant resulting in dieback and wilting. While feeding, they also exude excess sugar from their food source as honeydew that facilitates the gowth of sooty mold, which interferes with photosynthesis when present on foliage (Kim et al 2013). The honeydew is attractive to *Vespula* spp. (Panzer) (Hymenoptera: Vespidae), which can pose risks to humans when they are abundant (Dara et al 2015). The attraction of *Vespula* spp. also poses issues to agriculture and forestry in the form of a workplace hazard and possible impacts on agritourism, such as wine tasting and apple picking. Additionally, *L. delicatula* tends to aggregate in large numbers as adults on landscapes plants, which makes them a nuisance pest (Lee et al 2019).

Monitoring for *L. delicatula* is dependent on a limited number of methods at this time (Urban and Leach 2023). Mechanical traps such as glue traps wrapped around the trunk of trees were initially used to exploit their behavior to climb the tall silhouettes that they are attracted to (Baker et al 2021). These glue traps had issues with catching nontarget species such as lizards, birds, and mammals and were phased out in favor of circle trunk traps that exploited the same behavior while having similar catches and less nontargets captures (Francese et al 2020, Nixon et al 2020). Visual surveys are also used for detection; however, both visual surveys and trapping become less effective for montioring low density populations such as along the invasion front. Environmental DNA has successfully been used for detecting *L. delicatula* even at low densities and outperforms visual surveys (Valentin et al 2020, Allen et al 2021). There has also been success using canines to detect egg masses (Essler et al 2021). Chemical ecology research has found evidence of pheromone use by *L. delicatula* and that male adults are attracted

to volatiles from the honeydew of conspecific male adults showing that there is the potential to develop pheromone traps for *L. delicatula* (Faal et al 2022a, Faal et al 2022b). Pest control for *L. delicatula* is primarily done via the application of chemicals. Research has found that pyrethroids such as bifenthrin and beta-cyfluthrin as well as the neonicotinoid dinotefuran are effective against *L. delicatula* and suitable for use in vineyards (Leach 2021). New York State has successfully used backpack vacuum units for nymph and adult removal as an alternative to chemical applications (Bucello 2023). Biocontrol options are also being looked into for control options. *Batkoa major* (Thaxter) (Entomophthorales: Entomophthoraceae) and *Beauveria bassiana* (Balsamo-Crivelli) (Hypocreales: Cordysipitaceae) were both found to cause population reductions in *L. delicatula* in the wild in North America (Clifton et al 2019). *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae), which was introduced to North America in 1908 from Japan in an effort to control *Lymantria dispar* (L.) (Lepidoptera: Erebidae), was found to be an egg parasitoid of *L. delicatula* (Liu and Mottern 2017). Unfortunately, it has a low attack rate thus making it inadequate for suppressing *L. delicatula*. *Anastatus orientalis* (Yang and Choi) (Hymenoptera: Eupelmidae), a parasitoid of *L. delicatula* in its native range that has an attack rate of up to 15%, was found to be a promising option for biocontrol (Broadley et al 2021). Another potential biocontrol option is the parasitoid *Dryinus sinicus* (Olmi) (Hemiptera: Dryinidae), which was recorded to have parasitized 31.08% of a sample of *L. delicatula* nymphs collected from Tai'an, Shandong Province in China (Xin et al 2020).

The temperature of the surrounding environment has a major influence on ectotherms, such as insects, and has been shown to impact their physiology, development,

and growth. Insects tend to be adapted to the climate in their native range and exposure to temperatures outside of this range can lead to increased mortality and poor development. Temperature shifts outside this range has been indicated as one of the causes of recent insect decline in the tropics (García-Robledo and Baer 2021). This is especially important for invasive species as climate influences the areas that they can successfully establish in. Once established, temperature shifts could lead to a change in generations per year in the evaded area as seen with other invasive hemipterans (Céspedes et al 2019). While we know there is little risk of this happening with *L. delicatula*, this was a potential concern after its first detection in North America. Prior to detection in the United States, previous research on the phenology of *L. delicatula* was limited to a study from Korea where they were reared on bittersweet (*Pathenocissus quinquefolia* (L.) (Vitales: Vitaceae)) at room temperature (assumed to be approximately 20°C) (Park et al 2009). This knowledge gap drove the need for research to understand its response to temperature by establishing the upper and lower thresholds for development and determining what temperatures near those thresholds cause stress. Once this information is determined, the effect that temperature has on *L. delicatula* can be further understood by using degree-days, which are the accumulated number of degrees per day between the upper and lower developmental threshold for an insect (Baskervia and Emin 1969). The higher the temperature is above the lower developmental threshold, the less time is required for the insect to accumulate enough degree-days to reach the next stage in its development. Degree-days can be estimated once the upper and lower developmental thresholds of an insect are known as well as the amount of time it spends in each instar at a range of temperatures. While the study from Korea in 2009 found the duration of the first, second,

third, and fourth instars to be 18.8, 20.9, 20.8, and 22.2 days, respectively, it was only evaluated at a single temperature and therefore didn't provide enough data for determinating their degree-day requirements. A different study looked at its degree day requirements using phenology models on field data collected in 2017 from Pennsylvania; however, the lower developmental threshold wasn't determined and 10°C was used instead for the lower developmental threshold (Lui 2019). These degree-day requirements are particularly useful for monitoring without knowing the lower developmental threshold. The research presented in this thesis was done to help fill this knowledge gap on the developmental response to temperature of *L. delicatula*.

Another factor that can influence insect development is host plant, which is important to account for given the broad host range of *L. delicatula*. One way that host plant can influence developmental rate of insects is through their innate defensive compounds. The amounts of tannins and flavinoids observed in the host plants that *Erythroneura sudra* (distant) (Hemiptera: Cicadellidae) was reared on was found to have a positive correlation with their development time as well as a negative correlation with their survival rate (Huang at el 2020). Nitrogen content in host plants is known to effect the developmental rate and survival of insects. *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae) is known to develop faster and had higher survivorship when reared on corn plants fertilized with nitrogen compared to corn plants without additional nitrogen (Wang et al 2006). Since research has shown that host plant can significantly affect the developmental rate of insects, it should be considered when creating phenology models (Abarca et al 2018, Awmack and Leather 2002). This has been already observed in other invasive hemipterans with broad host ranges, such as the brown marmorated stink bug

(*Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)), where nymphs were found to develop faster when reared on *Prunus persica* (L.) (Rosales: Rosaceae) compared to when reared on *Malus domestica* (Borkhausen) (Rosales: Rosaceae) (Acebes-Doria et al 2016). The research on the effect of host plant presented in this thesis helps verify previously found degree-day information and determines if the accumulated degree-days required to complete each instar needs be adjusted to account for host plant.

The first objective of this research is to determine the upper and lower developmental thresholds of *L. delicatula* as well as its degree-day requirements through examining their response to range of temperatures. The rationale behind having this as an objective was that this information can be used to make degree-day models for determining when each life stage begins to appear in the field based on local climate. This is important as it can predict when the optimal timing for monitoring and/or application of control methods should occur. In addition, good phenology models based on their responses to a broad range of temperatures are important for determining its potential range across North America. These data can also be used to help optimize mass rearing protocols for *L. delicatula* for use in bioassays or biological control rearing.

The second objective of this research was to examine the effect of host plant on the development of *L. delicatula*. As the chemical profiles of plants tend to vary by species, it is important to account for this since it would be reflected in the developmental rate and survivorship of *L. delicatula* on various hosts. Furthermore, when comparing developmental rates between host plants, slower growth is an indictator of host resistance and thus there are merits in growers selecting varieties that *L. delicatula* develops slower

on. These data can also be used to determine if the previously calculated degree-day models are applicable to different crops or if they need to be adjusted per certain crops.

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Chapter 2

Effects of temperature on development and survival of nymphal *Lycorma delicatula* (Hemiptera: Fulgoridae)

Abstract

Lycorma delicatula (White), an invasive planthopper originally from Asia, is an emerging pest in North America. It is important to understand its phenology in order to determine its potential range in the United States. *Lycorma delicatula* nymphs were reared on *Ailanthus altissima* (Miller) (Sapindales: Simaroubaceae) at each of the following constant temperatures: 5, 10, 15, 20, 25, 30, 35, and 40°C. The time spent in each instar and survival were recorded. Developmental rate increased with temperature from 15 to 30°C for all instars, then declined again at higher temperatures. Nymphal survival was lower at 35°C than between 15 and 30°C for all instars, and first instars placed at 5, 10, and 40°C all died without molting. This suggests that *L. delicatula* survival and development may be affected in the Southern United States by high temperatures and developmental delays will occur under cool spring conditions. The lower developmental threshold was found to be 13.00 ± 0.42°C for first instars, 12.43 ± 2.09°C for second instars, 8.48 ± 2.99°C for third instars, and 6.29 ± 2.12°C for fourth instars. The degree-day (DD) requirement for nymphs in the previous instar to complete development to reach the second instar, third instar, fourth instar, and adult was 166.61, 208.75, 410.49, and 620.07 DD (base varied), respectively. These results provide key data to support the development of phenology models and help identify the potential range of *L. delicatula* in North America.

Keywords: spotted lanternfly, phenology, temperature, development time, degree-days

Introduction

The temperature of the surrounding environment has a major influence on ectotherms, such as insects, and can impact physiology, development, and growth. As insects tend to be adapted for the climate in their native range, exposure to temperatures outside of this range in invaded habitats can lead to increased mortality and poorer development during the establishment phase. Estimating the upper and lower thresholds for development and determining the temperature ranges near those thresholds at which the insect starts showing signs of stress, can teach us much about the potential geographic range the insect can invade. With the economic and ecological risk posed by many exotic insects, it can be crucial to look at their response to temperature in order to get a sense of the abiotic constraints that may define their range and provide the timing of stages for deployment of management and monitoring options.

The spotted lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), an invasive species native to China, India, and Vietnam, was first reported in North America in 2014 in Berks County, Pennsylvania (Barringer et al. 2015). Since then, its range in North America has expanded to include Delaware, New Jersey, New York, Pennsylvania, Virginia, and West Virginia and it has also been intercepted in additional states (Lee et al. 2019). *Lycorma delicatula* is currently univoltine and begins to emerge in late April in North America and has four nymphal instars. The first three instars are black and white in coloration while the fourth instar changes to red, black, and white (Dara et al. 2015). Adults tend to appear around mid-July and lay eggs from early September until the first frost. These eggs then overwinter. Both nymphs and adults feed from the phloem of plants, with 172 identified host plant species (Barringer and Ciafré 2020). Despite its

diverse host range, it is heavily associated with the invasive tree of heaven, *Ailanthus altissima* (Miller) (Sapindales: Simaroubaceae), which is one of its preferred hosts and is abundant in North America (Dara et al. 2015).

The broad host range of *L. delicatula* includes several major agricultural commodities including grapes (*Vitis spp*. L. (Vitales: Vitaceae)), which can host all stages of the insect, posing a major economic risk to the wine grape and table grape industry (Lee et al. 2019). Their full impact to the tree fruit industry is currently unknown. Many trees that are found in forests, such as *Juglans nigra* (L.) (Fagales: Juglandaceae), are also at risk (Lee et al. 2019). *Lycorma delicatula* damages plants through feeding, which can cause dieback and wilting. Furthermore, while feeding, they also exudes excess sugar from their food source as honeydew that facilitates sooty mold growth, which interferes with photosynthesis if it occurs on foliage (Kim et al. 2013). This honeydew is attractive to *Vespula spp.* Panzer (Hymenoptera: Vespidae), which can pose risks to humans at locations where they are abundant (Dara et al. 2015). In addition, *L. delicatula* tend to aggregate in large numbers as adults on street trees and other landscape plants making them a nuisance pest in addition to being an agricultural and forestry pest (Lee et al. 2019).

As *L. delicatula* spreads through the northeastern United States, it is important to discern what other regions of the country are likely to support populations. One method of understanding the effect of temperature on an organism is by using degree-days (DD), which are the accumulated number of degrees per day between the upper (T_{max}) and lower (T_{min}) developmental threshold of that organism (Baskervi and Emin 1969). The higher the temperature is above the T_{min}; the less time is required for the insect to

accumulate enough DD to reach the next stage in its development. DD can be estimated once the T_{max} and T_{min} of an insect is known along with the amount of time it spends in each instar at various temperatures.

Previous research has followed nymphal development of *L. delicatula* on Virginia creeper, *Parthenocissus quinquefolia* (L.) (Vitales: Vitaceae), at room temperature (assumed to be slightly above 20°C) and found the duration for first, second, third, and fourth instars was 18.8, 20.9, 20.8, and 22.2 d, respectively (Park et al. 2009). A more recent study looked at its DD requirements using phenology models based on field data from Pennsylvania in 2017; however, for this study, the T_{min} for *L. delicatula* was arbitrarily set at 10°C (Liu 2019). The lack of a T_{min} for each instar makes it difficult to use previously found DD requirements for monitoring. Furthermore, past studies did not evaluate its developmental rate at a variety of different temperatures and thus, that information is still needed in order to discern their DD requirements.

Good phenology models based on the responses of *L. delicatula* to a broad range of temperatures are vital to determining its potential range across North America, as well as for predicting when monitoring and/or application of control methods needs to occur. The information obtained from these models will also help develop protocols for rearing large numbers of *L. delicatula* for use in bioassays or biological control rearing.

The objectives of this study were to 1) examine how temperature affects the instar specific survival of nymphs, 2) calculate the developmental rate and temperature thresholds for each instar, and 3) to estimate instar-specific DD requirements.

Methods and Materials

Source Populations: In total, 164 *L. delicatula* egg masses were collected from eight different sites in Pennsylvania (Table 1). Egg masses were imported into the Forest Service quarantine laboratory in Ansonia, CT under a valid permit on 5 April 2019 and 16 October 2019. The 96 egg masses collected in April were held individually in 60 × 15 mm Petri dishes (Falcon 351007, Becton Dickinson Labware, Franklin Lakes, NJ) at 10°C until incubation on 13 May 2019 at 25°C and 60 % RH with a photoperiod of 16:8 (L:D) h to initiate hatch. The 68 egg masses collected in October 2019 were used to supplement the supply of fourth instars used in a later part of the study (as described subsequently). Hatch used in the fourth instar cage rearing came from egg masses that were held at 5°C (26 egg masses) or 10°C (37 egg masses) for 84 d followed by incubation at 25°C as well as from egg masses that were held at a constant 15°C (4 egg masses) for the entire duration, all with a photoperiod of 16:8 (L:D) h. Voucher specimens of adult *L. delicatula* were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT.

Nymphal Rearing at Different Temperatures: Rearing tubes were constructed from a 61.0 cm long section of 10.3 cm diameter thin-walled shipping tubes with clear caps (PRT.028-4.050 and PCC4.000, Cleartec Packaging, Park Hills, MO). Three mesh openings were added to each tube: two 10.2 × 2.5 cm openings in the side of the tube at 15.2 and 25.4 cm from the top along opposite sides, and a 5.1 × 5.1 cm opening in the middle of the cap (Fig. 1). *Ailanthus altissima* plants were grown from seeds collected from trees in Morgantown, WV in October 2018. Seeds were initially planted in Jiffy Plugs and then transferred to tree pots measuring 7.6 × 7.6 × 20.3 cm (CN-SS-TP-308, Greenhouse Megastore, Danville, IL) filled with soil (Premier BK25, Promix M, Premier

Horticultural Inc., Quakertown, PA) after sprouting. The *A. altissima* seedlings were provided Osmocote fertilizer (ICL Specialty Fertilizers, Summerville, SC) when first potted and monthly thereafter. A quart-mixing cup (KUS 932, TCP Global, San Diego, CA) was used at the bottom of the tube to hold the potted 30–45 cm tall *A. altissima* plant.

Upon hatching, 20 nymphs that hatched the same day were placed in a rearing tube containing a single potted *A. altissima* plant. Six rearing tubes containing first instar *L. delicatula* (120 total nymphs per treatment) were placed in growth chambers at each of the following temperatures: 10, 15, 20, 25, 30, and 35°C. Three shorter rearing tubes (40.0 cm with mesh openings at 7.6 and 19.1 cm from the top) were placed at 5 and 40°C (total of 60 nymphs per treatment). These smaller tubes were needed since the space available in the chambers running those temperatures was much more limited. All chambers had a chart recorder that was regularly calibrated to check for accuracy of temperature and humidity set points in the chambers. The photoperiod in all the chambers was 16:8 (L: D) h and the humidity was between 60 and 80% RH for temperatures 5–30°C and 40–50% RH for temperatures 35–40°C. The differing humidities were because only the 20 and 25°C chambers had humidity controls while the humidity in the other chambers was maintained by placing open bins of water in the bottom.

Nymphs were monitored daily for survival and molting (confirmed by a cast skin). Once molted, the new instars were relocated to a freshly prepared rearing tube with others that molted the same day for that temperature treatment. A maximum of 16 second instars and six third instars were put in a tube, so in some cases more than one tube was setup each day. Dead nymphs were regularly removed from the tubes in order to maintain

hygiene. This was repeated until nymphs reached the fourth instar, where they were held singly and a fresh concord grape (*Vitis labrusca* L. (Vitales: Vitaceae)) vine section without leaves in a water tube was also added to the rearing tube each Monday, Wednesday, and Friday, in order to provide an alternative food source. Plants were changed regularly (more frequently at higher temperatures and/or larger instars) to reduce potential stress caused by overfeeding on a stem. Plants were changed every 28 d for first and second instars. For third instars, plants were changed every 21 d at 20 and 25°C, every 14 d at 30°C, and every 10 d at 35°C. For fourth instars, plants were changed every 7 d. Plants were watered from the bottom without opening the tubes as needed to maintain soil moisture.

Nymphal Rearing to Get Temperature Responses for Fourth Instars in Cages: Due to low survival of the fourth instar in the first part of this study, the number of fourth instars that became adults was limited as only two (one male and one female) and five (all males) individuals at 25 and 30°C, respectively. In order to augment the study, 100 first instars that hatched from the egg masses collected in October 2019 were placed in 32.5 × 32.5 × 77.0 cm mesh cages (BugDorm 4S3074, MegaView Science Co., Ltd, Taichung, Taiwan) containing 1–2 60 cm tall potted *A. altissima* plants. In total, 10 cages were setup over a 22-d period. As nymphs molted to the second instar, they were moved to a larger mesh cage (60 × 60 × 120 cm; BugDorm 6S620, MegaView Science Co., Ltd, Taichung, Taiwan) with 2–3 100 cm tall potted *A. altissima* plants. A total of six cages, each with a maximum of 100 second instars, were setup over a 21-d period. New third instars were moved to new large cages (same setup as used for the second instars) with four 100 cm tall potted *A. altissima* plants and new plants were rotated in

weekly. A total of eight cages with a maximum of 60 third instars each were setup over a 15-d period of time. These cages were checked daily for new fourth instars and 4–6 nymphs that molted the same day were placed in small cages (same as used for the first instars) with 50–60 cm tall *A. altissima* plants. Cages containing the fourth instars were assigned to a temperature (15, 20, 25, 30, or 35°C) in sequential order from lowest to highest temperature to ensure that all temperature treatments had nymphs from across the 23-d period that molts occurred. In total, 30 fourth instars were placed at each temperature and were monitored daily for mortality and molting (confirmed by a cast skin). Each cage started with two trees and an additional tree was added weekly (20–35°C) or every 10 d (15°C). Once there were four trees, the oldest tree was removed when a new tree was added. Trees were watered daily. When a newly molted adult was found in the cage it was sexed, weighed, and then frozen. If the adult had not finished sclerotization, it was left at room temperature overnight, then frozen. Three measurements were made on each frozen adult: fore wing length, fore wing width, and hind tibia length.

Statistical Analysis: Statistical analyses were performed using SAS 9.4 (SAS Institute 2015). The normality of the data was evaluated using the Shapiro–Wilk and the Anderson–Darling tests. When the data did not fit a normal distribution PROC UNIVARIATE was used to assess the distributional fit of the data to a gamma distribution. The gamma distribution accommodates long right-skewed tails, which are common in this type of data.

PROC GLIMMIX was used to evaluate the fixed effect of temperature on the duration of each of the first three instars. Since the sex of the adults was known the fixed

effects of sex and the interaction between sex and temperature were added to the model to evaluate effects on the duration of the fourth instar. Adult body weight (g), fore wing length (mm) and width (mm), and hind tibia length (mm) were evaluated for both sexes separately using the temperature as a fixed effect. Adults obtained from both the first (tube-based) and supplemental (cage rearing) data sets were included in the analysis. The container the individuals were reared in was treated as a random effect in all models to account for between container effects. Each model was fitted to a gamma distribution with a log link function because the response variables had long right tails. When interaction effects were not significant the model was reduced to the significant effects and re-run. Differences among means were determined using the Tukey–Kramer post hoc analysis and $\alpha = 0.05$. For each model, residuals were evaluated for normality and the homogeneity of variance was assessed using Levene's test. This ensures that the assumptions for the statistical tests have been met or appropriate steps have been taken to account for unequal variance.

The relationship between temperature and developmental rate for each instar was estimated by fitting both a linear model (developmental rate = a + bT) using Statistix (2013) and the Briere non-linear model using PROC NLIN and Marquardt convergence method (Briere et al. 1999, SAS Institute 2015) (Table 3). Temperatures near the T_{min} or above the optimal temperature (temperature at which development was fastest) were included in the Briere models but were not included in the linear models. The T_{min} for the linear model was estimated as –a/b where a and b were estimated by least squares regression (Statistix 2013). The standard error for the T_{min} estimated using the linear model was calculated using the formula given in Campbell et al. (1974). The Briere

model is: developmental rate = $a \times T \times (T - T_{min}) \times ((T_{max} - T)^{(1\div b)})$.In the model T_{min} is the lower developmental threshold, a is an empirical constant, and b determines the inflection point and steepness of the decline when temperatures (T) approach the upper developmental threshold (T_{max}). The non-linear relationship (Briere et al. 1999) was evaluated for all instars, but not ultimately used for the last two instars due to its poor fit to the data (based on adjusted r^2 and a visual comparison to the data), especially near the optimal temperature. The optimum temperature for the Briere model was calculated by equating the first derivative to zero and solving for T. The T_{min} used in subsequent analyses was from the Briere model for the first two instars and from the linear model for the last two instars.

DD requirements for each instar were estimated using the function: DD = [constant temperature – T_L] × Dt, where Dt is the total number of days required by individual nymph to reach the next instar. The relationships between accumulated DD and cumulative proportion of individuals to reach each instar were estimated using the Gompertz function, $P = \exp [- \exp (- b * DD + a]$ (Brown and Mayer 1988) with the Marquardt convergence method. In the function a and b are constants that determine the shape of the curve. Accumulated DD required by 10, 50, and 90% of nymphs to reach each instar were calculated.

Results

Survival: At 10°C, most first instar nymphs died between 21 and 35 d (mean 22.7 ± 0.6 d) (Fig. 2). At 40°C, the first instar nymphs all died in the first 5 d (1.8 ± 0.1 d). Mean survival of first instar nymphs at 5°C was 5.1 ± 0.6 d; however, some survived longer than 2 wk. At 35°C, first instars that were unable to molt, died within 35 d (mean

8.6 ± 0.7 d). First instar survival at 15 to 30°C was similar for the first 35 d. However, at 15°C mortality increased again at 63 d. In the second instar, mortality for 15 and 35°C was similar except that a very small percentage at 15°C survived for much longer (Fig. 2). Greater than 70% of the first and second instars held at 20–30°C were able to molt (Fig. 2). Survival of second instars at 30°C was lower than at 20 and 25°C. In rearing tubes, fewer than 50% of the third or fourth instars were able to molt. The survival of fourth instars was higher in cages than in the rearing tubes. Survival of fourth instars in cages was similar at 15 and 20°C (Fig. 3). More males than females survived to complete the fourth instar at both 15 and 30°C. In cages, greater than 70% of fourth instars at 15, 20, and 25°C were able to molt to adult.

Developmental Rate and Temperature Thresholds for Each Instar: The mean time (days) spent in each of the four instars decreased as the temperature increased from 15 or 20 to 30°C and then began to increase again at higher temperatures for the first and second instars (Table 2). There were significant differences between some of the temperatures for days between molts, but it varied between instars. When the number of days spent in the fourth instar for both sexes was evaluated, temperature had significant effects ($F = 33.30$; $df = 3, 22.15$; $P < 0.0001$) and sex ($F = 20.08$; $df = 1, 73.40$; $P < 0.0001$), while the interaction between the two did not ($F = 0.25$; $df = 3, 71.36$; $P = 0.8630$), although, females tended to spend longer in the fourth instar than males. The parameter values for the linear and Briere models used to describe the relationship between temperature and developmental rate are given in Table 3 and the lines/curves are plotted with the raw data in Fig. 4. The Briere model was a good fit for the data for the first and second instars, but the linear model (excluding temperatures above the optimal)

was better for third and fourth instars. Another reason for choosing the linear model for these instars was that data for temperatures above the optimum was lacking for the third instar and only one temperature that resulted in 100% mortality was evaluated above the optimum for the fourth instar. The fit was evaluated both based on adjusted r^2 and by a visual examination of the fit. The T_{min} for development decreased with instar (using the model that was better for each instar) and the upper threshold for development was highest for the first instar at 43.81 ± 2.59°C and then remained close to 35°C for the other instars (Fig. 4). The optimal temperature for the first and second instars were 31.4 and 30.0°C, respectively.

Estimated DD Requirements: Using the estimated developmental thresholds, the DD requirements for each of the instars were estimated using the Gompertz function (Table 4 and Fig. 5). DD requirements increased with each instar, partly due to the decreasing lower developmental threshold. The difference in the mean number of DD required for 10–90% of the population to reach the next life stage increased between the first and third instars, but was similar for third and fourth instars (92, 148, 335, and 364 DD for the first, second, third, and fourth instars, respectively).

Adult Mass and Morphometrics: Adult weight differed significantly by sex ($F = 114.14$; $df = 1, 74.59$; $P < 0.0001$) but not temperature ($F = 0.38$; $df = 3, 13.41$; $P = 0.7710$) or the interaction between sex and temperature ($F = 1.34$; $df = 3, 72.94$; $P = 0.2668$) with females weighing more than males (Table 5). The length of the hind tibia also differed significantly by sex ($F = 47,61$; $df = 1, 80.63$; $P < 0.0001$) and not temperature ($F = 1.57$; $df = 3, 14.55$; $P = 0.2396$) or the interaction between sex and temperature ($F = 0.47$; $df = 3, 78.88$; $P = 0.7021$) (Table 5). Forewing length was

significantly affected by sex ($F = 70.56$; $df = 1, 79.79$; $P < 0.0001$) and temperature ($F = 5.22$; $df = 1, 20.67$; $P = 0.0077$) but not the interaction between the two ($F = 2.33$; $df = 1, 77.54$; $P = 0.0810$). Among males, forewing length of males at 15°C was shorter than for males at all other temperatures. Among females, forewing length of females at 15°C was shorter than for females at all other temperatures (Table 5). Forewing width was significantly affected only by sex ($F = 47.39$; $df = 1, 61.17$; $P < 0.0001$) and temperature ($F = 4.18$; $df = 1, 19.79$; $P = 0.0191$) but not the interaction between the two ($F = 0.31$; $df = 1, 60.58$; $P = 0.8213$. Forewing width of females was wider than that of males (Table 5). Among males, males at both 15 and 30°C had narrower wings than males at 20 and 25°C. At 15°C, some males did not fully expand their wings, resulting in shorter, narrower wings.

Discussion

Temperature had a significant effect on *L. delicatula* developmental rate, which increased with temperature from 15 to 30°C for all instars, then declined again at higher temperatures. Survival was poor at 35°C for all instars, which suggests that this temperature is near or exceeds the T_{max}. The mean time spent in each instar increased with each successive molt. The estimated T_{min} based on the linear model also decreased as they progressed through the instars; however, this trend was not observed when the T_{min} were estimated based on the Briere model. Likewise, the T_{max} showed a decreasing trend as they progressed through the nymphal instars. At temperatures near both the T_{max} and T_{min} nymphs showed signs of stress; decreased survival, more incomplete molts, and more adults with partially expanded wings. The range of DD that were required for 10–90% of nymphs in an instar to complete development increased with instar and the

extremes tended to come from individuals held at the temperatures closest to the thresholds.

The T_{max} estimated for first instars is probably higher than the true T_{max} since first instars placed at 40°C only survived for a few days. The complete mortality of first instars at 40°C makes sense as that temperature would not normally be reached during the spring when they first emerge. The estimated T_{min} is likely accurate, but it is difficult to discern where the lower lethal threshold is, since the survival trend suggests that the first instars did persist at lower temperatures. Survival of hatchlings at low temperatures could be critical during cold snaps that may occur during hatch. Other insects like the spongy moth (*Lymantria dispar* (L.) (Lepidoptera: Erebidae)) often spend longer near the egg masses until temperatures rise above 10°C when they can disperse and begin feeding (McManus 1973, Keena and Shi 2019).

Previous MAXENT modeling for *L. delicatula* has used 11°C as the lower developmental threshold (Wakie et al. 2020). Another study set the lower developmental threshold to be 10°C and the upper developmental threshold to be 35°C calculating DD when development was followed in the field (Liu 2019). The T_{min} reported here are similar to those previously used; however, it is important to note that these data showed that the T_{min} varied for each instar. The T_{max} from this study for second through fourth instars was similar to that previously used; however, under the conditions (temperature and humidity) assessed in this study the T_{max} for fourth instars falls between 30 and 35°C as there was complete mortality at 35°C for fourth instars. Under different humidities or varying temperatures, the nymphs may be able to survive higher temperatures.

Mortality of nymphs that occurred during the beginning of each instar was likely due to temperature stress or variation in temperature sensitivity within the population, while later mortality may have been due to molting issues or resource depletion. Other factors may also have impacted *L. delicatula* survival rates including an infestation of eulophid mites on the *A. altissima* in the greenhouse during the last month of data collection. In addition, the plants in cages maintained their vigor better when watered from the top, which could not be done in the rearing tubes. Humidity differences between the temperatures may also have impacted observed mortality since at warmer temperatures moisture occasionally was evident on the inside of the containers. In most cases, however, the nymphs were able to move to more optimal humidity locations in the tubes but if they got trapped in the moisture they would die. Also, nymphs showed preferences for some plants over others so providing them with a choice within the cage may be a better option. This host plant choosiness of *L. delicatula* has been documented in the field and may be tied to nutrient differences between plants within a species (Mason et al. 2020).

It was interesting that temperature did not have a significant effect on adult weight or size (as measured by the hind tibia length). This may indicate that nymphs must reach a certain size before they can molt and so must compensate for temperature differences by extending the length of time in the instar at lower temperatures where the metabolism will be the slowest. Temperature, however, did influence wing size significantly in males and in females. The reduction in wing size at 15 and 30°C appeared to be due to issues in fully expanding the wings in both sexes at these temperatures which were close to the T_{min} or T_{max} for fourth instar nymphs. Previous research has shown that

wing area does not have a significant effect on the flight capabilities of female *L. delicatula* adults (Wolfin et al. 2019). However, since this reduction in wing size was due to issue with fully expanding their wings, it would still affect flight capabilities.

For the second and third instars, there was a bimodal distribution for the time in instar. It is possible that this is a difference between sexes; however, this could not be confirmed, as individual nymphs were not followed. Females spent significantly longer as fourth instars at 20, 25, and 30°C, which supports this hypothesis. Field observations also found that adult populations were skewed to males when the first adults start appearing (Baker et al 2019).

Previous research found that at 20°C, the duration for first, second, third, and fourth instars was 18.8, 20.9, 20.8, and 22.2 d, respectively on bittersweet (Park et al. 2009), which is considerably shorter than our study. This was especially true for third and fourth instars. This could be due to nutritional differences between hosts or potentially because the temperature in the previously study was assumed, not documented, to be 20°C. Also, in a preliminary trial using left over nymphs from this study, fourth instars at 25°C on *V. labrusca* only took an average of 23 d compared with the 38 d on *A. altissima* (unpublished data), so further work on the effects of host on developmental rates is merited.

It is important to note that *L. delicatula* takes longer to develop than other planthoppers. For example, *Nilaparvata lugens* (Stål), a multivoltine planthopper in the family Delphacidae (Hemiptera), was found to take an average of 5.6, 4.9, 5.1, 4.7 and 4.4 d to complete the first, second, third, fourth, and fifth instars at 20°C (Vattikuti et al. 2019). As *L. delicatula* only has one generation per year, it makes sense that it would

take longer to develop than species that have multiple generations per year. The large size of *L. delicatula* likely contributes to this difference as well. This also suggests that there is a low risk of *L. delicatula* having multiple generations per year in any part of its range due to its relatively long development time and the fact that it overwinters as eggs.

Previous research determined the range of cumulative DD requirements for first, second, third, and fourth instars in Pennsylvania to be 153–652, 340–881, 567–1020, and 738–1227 DD_{10}, respectively (Liu 2019). If the data from the current study were converted to T_{min} of 10°C then the range of cumulative DD for each instar would approximately be (not exact since we did not follow individuals) 165–490, 315–1165, 525–1865, and 740–2525 DD_{10}, for first, second, third, and fourth instars, respectively. The expanded DD ranges may be due to including temperatures near the upper and lower thresholds or to restricting nymphal feed to a single food source since there is some evidence that they may grow faster when they have access to multiple hosts.

This study suggests that *L. delicatula* experiences thermal stress when exposed to temperatures greater than 30°C but can develop slowly at temperatures as low as 15°C. This ability may help buffer the nymphs against the periods of low temperature that might be expected in the early spring when egg hatch begins. Based on these results, *L. delicatula* probably will not perform very well in extremely hot climates in the southern United States where temperature often go above 30°C. Research on the invasive *L. dispar* has suggested that its poorer performance at temperatures greater than 28°C contributes to limiting its potential geographic range in North America (Thompson et al 2017). Further studies that look at its ability to withstand sudden cold snaps or heat waves are needed to get a better idea of its potential range.

The current study also provides data that can be useful in conjunction with the field data from Liu (2019) for developing a phenology model for *L. delicatula*, to aid in stage-specific prediction for management as individuals leave forests and invading nearby vineyards or other crops. The model will be further aided by incorporation of data on egg diapause to more accurately predict potential range for this insect in North America. These data may be useful for developing optimal laboratory rearing methods. Without an efficient laboratory rearing methodology for *L. delicatula*, mass rearing of discovered natural enemies needed for field-testing or release will be limited, especially outside the infested zone. Efforts to screen pesticides are equally restricted by the resources needed to obtain the large numbers of even aged individuals from natural populations. Year-round mass rearing would allow rapid screening of many pesticides and other monitoring or control methods.

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Table 1. Approximate location (latitude and longitude), collection date, and hosts from which the egg masses of spotted lanternfly used in this study were obtained.

Collection location	Collection date	Host (number of masses)	Latitude	Longitude
Bally, PA	11/10/2018	*Acer Saccharinum* (L.) (7)	40.39ºN	75.59 ºW
Audubon, PA	3/30/2019	*Ailanthus altissima* (Miller) (11), *Prunus serotina* (Ehrhart) (6)	40.12ºN	75.42 ºW
Audubon, PA	3/30/2019	*Acer rubrum* (L.) (12), *Pinus strobus* (L.) (5)	40.12ºN	75.42 ºW
Wyomissing, PA	4/2/2019	*Acer rubrum* (L.) (25)	40.35ºN	75.99 ºW
Pennsburg, PA	4/4/2019	*Acer saccharinum* (L.) (30)	40.36ºN	75.52 ºW
Hellertown, PA	10/15/2019	*Acer negundo* (L.) (1), *Malus domestica*, (Borkhausen) (1), *Ailanthus altisima* (Miller) (6)	40.58ºN	75.34 ºW
Whitehall township	10/15/2019	*Ailanthus altisima* (Miller) (6)	40.67ºN	75.51 ºW
Pennsburg, PA	10/16/2019	*Ailanthus altisima* (Miller) (53)	40.41ºN	75.48 ºW

Table 2. Mean [± SE (*n*)] time spend (days) by *Lycorma delicatula* in each instar at different temperatures.

Instar	**Temperature (°C)**					**Statistics**		
	15	**20**	**25**	**30**	**35**	**F**	**df**	***P***
First	71.3 ± 2.57a (34)	23.4 ± 0.73b (71)	12.6 ± 0.39c (79)	10.9 ± 0.33cd (89)	11.7 ± 0.41d (49)	519.9	4, 25.31	<0.0001
Second	NA	24.0 ± 1.92a (56)	20.2 ± 1.70a (65)	14.2 ± 1.25b (59)	27 ab (1)	6.76	3, 24.01	0.0018
Third	NA	40.4 ± 4.38a (14)	24.6 ± 2.03b (21)	21.0 ± 1.95b (26)	NA	11.09	2, 27.64	0.0003
Fourth Male	64.6 ± 4.93a (14)	39.5 ± 2.93b (14)	34.0 ± 2.29b (13)	24.2 ± 1.67c (12)	NA	31.15	3, 17.28	<0.0001
Fourth Fem	85.5 ± 8.04a (7)	50.1 ± 4.21b (12)	38.6 ± 3.25bc (10)	28.4 ± 3.70c (7)	NA	20.16	3, 11.77	<0.0001

Means within a row followed by a different letter are significantly different from each other at $P < 0.05$ using Tukey-Kramer post hoc test. Sample size (n) is the number of survivors.

Table 3. Parameter values for linear and nonlinear models used to describe the relationship between temperature (°C) and developmental rate of *Lycorma delicatula* nymphs.

Development Period	Temperature range	Model	a	b	T_{min}	T_{max}	n	$_{Adj}R^2$
Days in First instar	10 to 30	Linear	0.00529± 0.000129	-0.06069 ± 0.00317	11.47 ± 0.42	NA	272	0.8613
	10 to 35	Briere	0.000011± 0.0000009	0.9335 ± 0.2035	13.05 ± 0.42	43.81 ± 2.59	322	0.8699
Days in Second instar	20 to 30	Linear	0.00379± 0.000307	-0.03526 ± 0.00781	9.30 ± 1.35	NA	180	0.4581
	20 to 30	Briere	0.000062± 0.000016	1.9766 ± 0.6338	12.43 ± 2.09	35.58 ± 0.82	180	0.5124
Days in Third Instar	20 to 30	Linear	0.00250± 0.000419	-0.02121 ± 0.01104	8.48 ± 2.99	NA	59	0.3692
	20 to 35	Briere	0.000073± 0.000014	6.4742 ± 12.3743	9.87 ± 8.76	35.00 ± 0.00	60	0.3704
Days in Fourth Instar	15 to 30	Linear	0.00164± 0.000013	-0.00103 ± 0.00299	6.29 ± 2.12	NA	87	0.6470

	15 to 35	Briere	0.00065± 0.000011	9.6873 ± 12.8048	10.00 ± 2.65	35.00 ± 0.00	88	0.4136

See methods for details of the analysis.

Table 4. Estimated accumulated DD (± SE) required to reach each instar by *Lycorma delicatula* nymphs for 10, 50, and 90 of the population.

Life stage reached	**Degree-day (± SE) requirement for nymphs (%) to reach different life stages after egg hatch**			**T_{min} Used**	**R^2_{Adj}**
	10%	**50%**	**90%**		
2nd instar	130.77 (130.01 - 131.49)	166.61 (166.46 - 166.76)	222.84 (221.34 - 224.42)	13.05	0.9911
3rd instar	151.31 (150.48 - 152.12)	208.75 (208.69 - 208.82)	298.89 (297.46 - 300.36)	12.43	0.9967
4th instar	280.14 (277.41 - 282.75)	410.49 (410.27 - 410.72)	615.03 (610.39 - 619.89)	8.48	0.993
Adult	478.33 (481.46 – 475.04)	620.07 (619.90 – 620.25)	842.49 (837.14 – 848.10)	6.29	0.9890

T_{min} = Lower temperature thresholds. R^2_{Adj} value is based on the relationship between degree-days and cumulative proportion attaining each instar using the Gompertz function, $P = \exp [- \exp(- b * DD + a]$.

Table 5. Mean [± SE (*n*)] adult *Lycorma delicatula* body weight (g), fore wing length (mm) and width (mm), and hind tibia length (mm) at different temperatures.

Sex	Measure	Temperature (°C)				Statistics		
		15	20	25	30	F	df	*P*
Male	Weight (g)	0.118 ± 0.006a (14)	0.127 ± 0.007a (14)	0.126 ± 0.007a (12)	0.112 ± 0.008a (8)	0.89	3, 13.96	0.4718
	Fore wing length (mm)	13.7 ± 0.70b (14)	17.0 ± 0.87a (14)	17.6 ± 0.78a (13)	17.2 ± 0.76a (12)	5.36	3, 20.2	0.0071
	Fore wing width (mm)	6.78 ± 0.16b (14)	7.58 ± 0.18a (14)	7.58 ± 0.17a (13)	7.35 ± 0.17ab (12)	4.77	3, 15.82	0.0148
	Hind tibia length (mm)	9.19 ± 0.19a (14)	9.54 ± 0.19a (14)	9.45 ± 0.18a (13)	9.42 ± 0.18a (12)	0.61	3, 14.27	0.6189
Female	Weight (g)	0.165 ± 0.012a (7)	0.165 ± 0.011a (12)	0.173 ± 0.012a (11)	0.175 ± 0.017a (7)	0.18	3, 9.641	0.9058
	Fore wing length (mm)	16.5 ± 0.74b (7)	19.7 ± 0.77ab (12)	21.2 ± 0.84a (11)	21.4 ± 1.17a (7)	6.99	3, 9.187	0.0096
	Fore wing	8.09 ± 0.54a (7)	9.60 ± 0.52a (12)	9.08 ± 0.51a (11)	8.81 ± 0.63a (7)	1.34	3, 11.65	0.3098

width (mm)							
Hind tibia length (mm)	9.8 ± 0.41a (7)	10.5 ± 0.38a (12)	10.6 ± 0.39a (11)	10.8 ± 0.58a (7)	1.08	3, 9.935	0.3999

Means within a row followed by a different letter are significantly different from each other at $P < 0.05$ using Tukey-Kramer *post hoc* test. Sample size (n) is the number of survivors.

Fig. 1. Photo of the tube rearing method used.

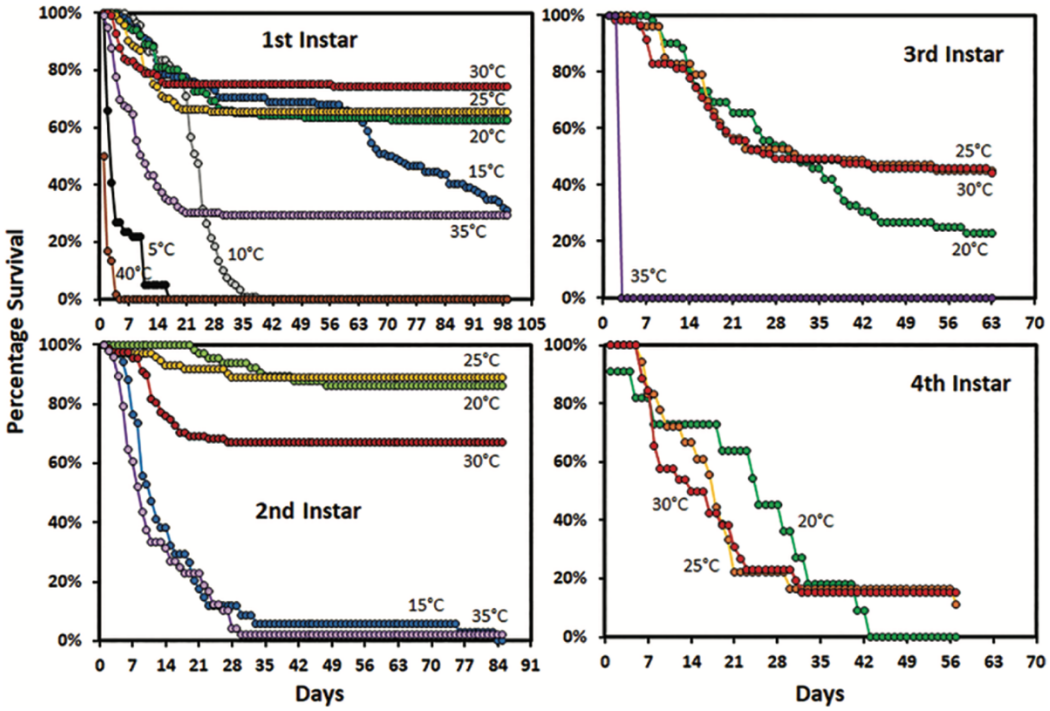

Fig. 2. Survivorship curves for *Lycorma delicatula* nymphs reared in tubes by instar and temperature.

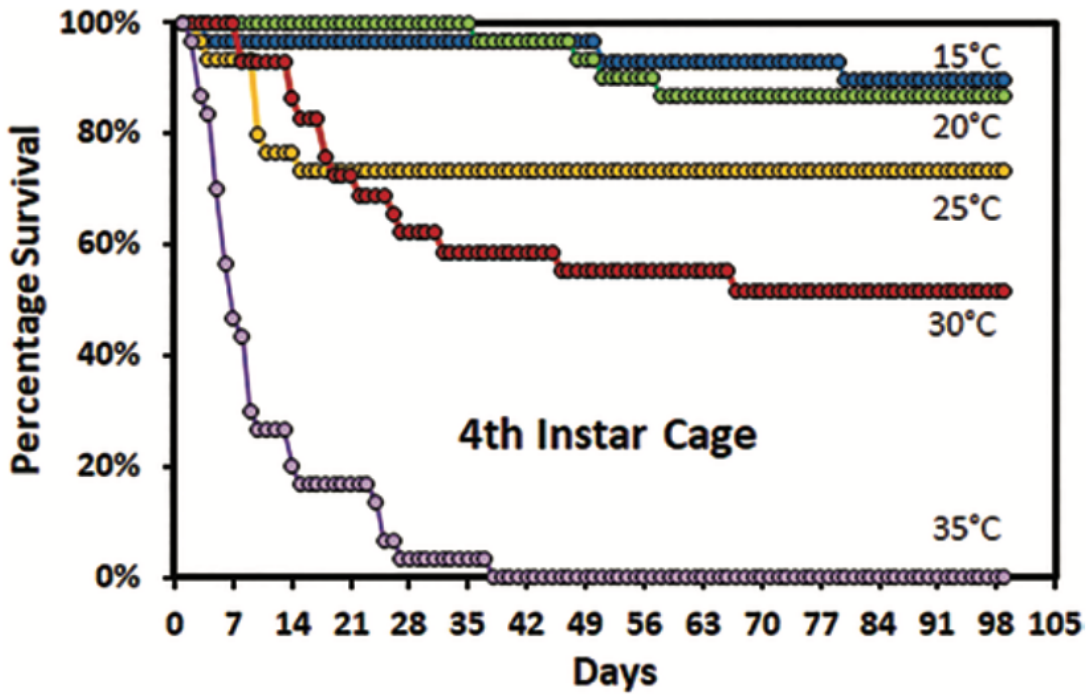

Fig. 3. Survivorship curves for fourth instar *Lycorma delicatula* nymphs reared in cages by temperature.

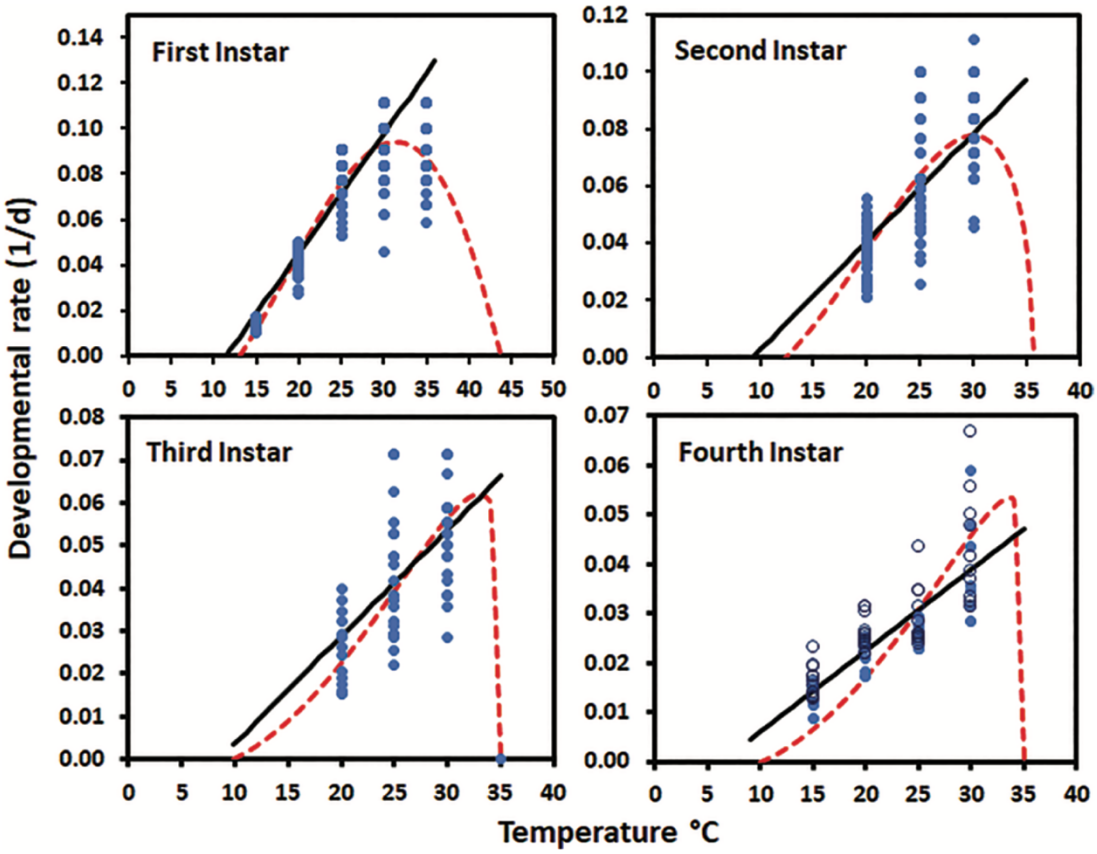

Fig. 4. Relationship between temperature and the developmental rates (1/d) for *Lycorma delicatula* nymphs by instar. Dots represent the observed individual values for each nymph (fourth instar open female and closed male). The solid line is the linear and dashed the Briere predicted relationships between developmental rate and temperature.

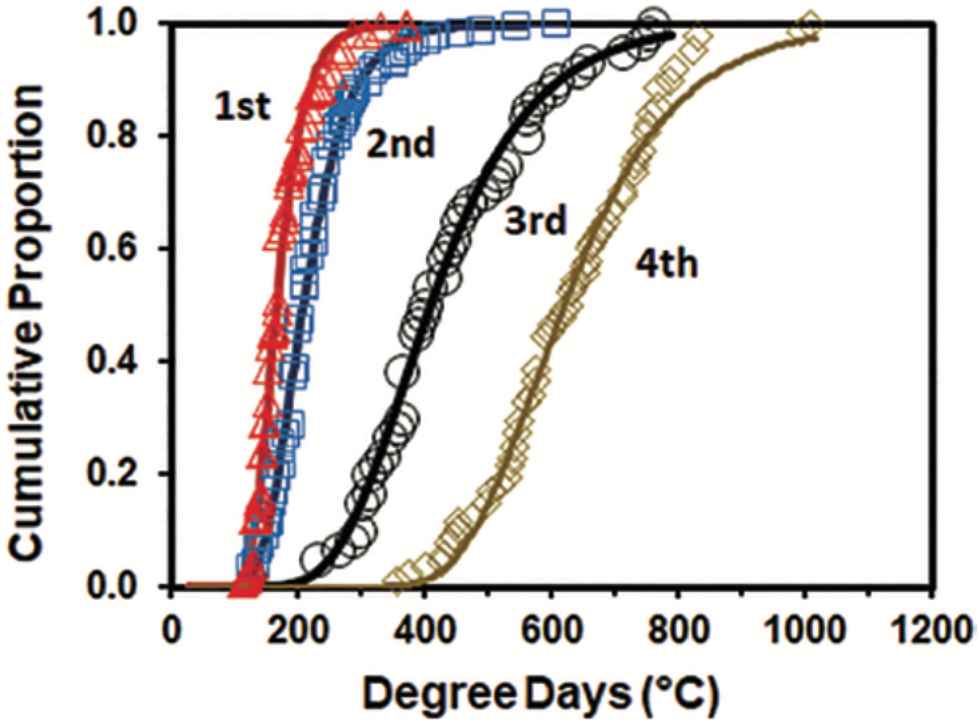

Fig. 5. Cumulative proportion of *Lycorma delicatula* nymphs to complete each instar reared at 15, 20, 25, 30, and 35°C over accumulated DD. Open triangles, squares, circles, and diamonds represent first, second, third, and fourth instar individuals, respectively. Solid lines were fitted to cumulative the proportion of individuals using Gompertz function, $P = \exp[-\exp(-bDD + a]$.

Chapter 3

The Impact of Host Plant Species on Instar Duration and Body Weight of Nymphal *Lycorma delicatula*

Abstract

The spotted lanternfly *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) is an invasive species of planthopper that was introduced to North America and a threat to multiple industries. Nymphs and egg masses were collected to assess each instar's rate of development at a constant 25°C on the following hosts: *Ailanthus altissima* (Miller) (Sapindales: Simaroubaceae), *Vitis labrusca* (L.) (Vitales: Vitaceae), *Salix babylonica* (L.) (Malpighiales: Salicaceae), *Acer rubrum* (L.) (Sapindales: Sapindaceae), *Celastrus orbiculata* (Thunberg) (Celastrales: Celastraceae), *Ocimum basilicum* (L.) (Lamiales: Lamiaceae), and *Rosa multiflora* (Thunberg) (Rosales: Rosaceae). Host plant was found to have significant effect on development time for nymphs in the first through third instar as well as on nymphal survival. Nymphs failed to develop through the second instar on *O. basilicum* and the third and fourth instar on *A. rubrum*. Host plant also had a significant effect on the mean weight of nymphs in the first, second, and fourth instar, but not the third instar and on adult hind tibia length and forewing width. This variability in *L. delicatula* development time by host plant can potentially impact phenology models and should be updated to reflect these new insights. Rearing practices should also be refined to account for host plant influences on their physiology as well.

Keywords: phenology, survival, development, hostplant, lanternfly

Introduction

The spotted lanternfly *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) is an invasive species first detected in the United States in the summer of 2014. Native to China, Vietnam, and India, the United States is one of three countries invaded by *L. deliculata* together with Japan and South Korea (Barringer et al 2015). Since its initial detection in Pennsylvania, *L. delicatula* has spread across the northeastern United States establishing in multiple states (Barringer et al 2015).

Lycorma delicatula has four instars. The first instar nymphs start to emerge from eggs in late April in North America (Barringer and Ciafré 2020). The first three instars are black and white in coloration in contrast to the fourth instar nymphs, which are black, white, and red in coloration. Adults appear around mid-July and lay eggs from early September until temperatures reach levels that kill the adults. The eggs are deposited in a grayish to tan colored egg masses on various substrates such as bark, stone, wood fences, and brick, where the egg masses then overwinter until the next spring.

Lycorma delicatula has a broad host range consisting of 103 plant species (Barringer and Ciafré 2020). Despite this, *L. delicatula* has a preferred host, which is tree of heaven, *Ailanthus altissima* (Miller) (Sapindales: Simarobaceae) (Dara et al 2015). Recently, it was found that *L. delicatula* does not require *A. altissima* to complete its lifecycle, however, the removal of *A. altissima* from its diet is associated with reduced fitness (Uyi et al 2020). Despite being widespread and commonly found in disturbed sites, *A. altissima* will not always be available as a host for *L. delicatula*.

External temperature has a major influence on the development and growth of insects; however, other factors can still influence their growth. Previous research has shown that the host plant can affect an insect's phenology and should be considered when

producing phenology models (Abarca et al 2018, Awmack and Leather 2002). For example, Oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) larvae develop faster when feeding on *Prunus persica* (L.) (Rosales: Rosaceae) than when feeding on *Malus domestica* (Borkhausen) (Rosales: Rosaceae) (Myers et al 2007). Likewise, the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) nymphs from the second instar onward were found to develop faster when reared on *P. persica* than when reared on *M. domestica* (Acebes-Doria et al 2016).

The phenology of *L. delicatula* has been previously determined on *A. altissima* and *Parthenocissus quinquefolia* (L.) (Vitales: Vitaceae) (Kreitman et al 2020, Park et al 2009). In the latter study, when *L. delicatula* was reared on *P. quinquefolia* at room temperature (assumed to be slightly above 20°C), it was found that the duration for first, second, third, and fourth instar nymphs was 18.8, 20.9, 20.8, and 22.2 days, respectively (Park et al 2009). When reared on *A. altissima* at that temperature, it was found that the duration for first, second, and third instar nymphs was 23.4, 24.0, and 40.4 days, respectively (Kreitman et al 2020). The data for the fourth instar nymphs were separated for males and females with it taking 39.5 and 50.1 days to complete that instar, respectively. These differences in results with *L. delicatula* taking less time to develop on *P. quinquefolia* than *A. altissima* at 20°C suggests that host plant also influences their development. In addition, fourth instar nymphs at 25°C were found to take fewer days to develop when reared on fox grape, *Vitis labrusca* (L.) (Vitales: Vitaceae) compared to *A. altissima* in an unpublished trial which further stresses the importance of examining the development rate of *L. delicatula* on different host plants.

To further understand the effect of host plant on the development of *L. delicatula*, it is important to rear nymphs on a variety of different hosts. In this study the development of *L. delicatula* was examined using one of the following plants as a host: tree of heaven (*A. altissima*), fox grape (*V. labrusca*), weeping willow (*Salix babylonica* (L.) Malpighiales: Salicaceae), red maple, (*Acer rubrum* (L.) Sapindales: Sapindaceae), Oriental bittersweet, (*Celastrus orbiculata* (Thunberg) Celastrales: Celastraceae), basil (*Ocimum basilicum* (L.) Lamiales: Lamiaceae), and multiflora rose (*Rosa multiflora* (Thunberg) Rosales: Rosaceae). These plants were each selected for various reasons. *Ailanthus altissima* was selected because it is a preferred host making it a good reference for comparison with other host plants. Since *L. delicatula* poses a significant threat to wine grapes, it is important to see if being reared on *V. labrusca* influences their developmental rate. *Salix babylonica* was selected because it is a common landscape tree and was one of the trees used in the study that showed this insect could complete development without *A. altissima* (Uyi et al 2020). *Celastrus orbiculata* was selected based on previous literature and the early instars are commonly found using it as a host. *Ocimum basilicum* is a common garden plant and *R. multiflora* is a common forest plant, both of which *L. delicatula* has been found feeding on.

Methods

Source Populations: On June 17th, 2020, *L. delicatula* first (n=140) and second (n=63) instar nymphs were collected at a site in Hunterdon County, New Jersey USA (Riegelsville, NJ). The site had *Vitis* spp., *Rosa* spp., *C. orbiculata*, *A. altissima*, *Celtis occidentalis* (L.) (Rosales: Cannabaceae) and *Juglans nigra* (Fagales: Juglandaceae) as well as other assorted unidentified shrubbery. The nymphs were found mostly in the

shade and egg masses were observed on site. The nymphs were transferred to a quarantine facility located in Ansonia, Connecticut USA as per USDA APHIS permits in containers containing a single 50-70 cm long sprig of wild grape, *Vitis volpina* L. (Vitales: Vitaceae) to sustain them for the trip. At the quarantine facility, the nymphs were sorted by instar and placed into a large mesh cage (60 × 60 × 120 cm; BugDorm 6S620, MegaView Science Co., Ltd, Taichung, Taiwan) with 2–3 100 cm tall potted *A. altissima* plants and a single *V. labrusca* plant and held at 25°C with a photoperiod of 16:8 (L:D) and a relative humidity between 60 and 80%. Once the nymphs began molting to the next instar, ten that molted the same day were taken and set up in a smaller 32.5 × 32.5 × 77.0 cm mesh cage (BugDorm 4S3074, MegaView Science Co., Ltd, Taichung, Taiwan) containing two host plants of the same type for use in experiments. These smaller cages were held at the same photoperiod, temperature, and humidity as the other larger cages. Any additional nymphs that molted were transferred to the large BugDorm cages and allowed to develop into later instars.

Plant Rearing: *Ailanthus altissima* were grown from seeds collected in Wallingford, Connecticut USA in October 2019. Seeds were initially planted in Jiffy Plugs and then transferred to tree pots measuring 7.6 × 7.6 × 20.3 cm (CN-SS-TP-308, Greenhouse Megastore, Danville, Illinois) filled with soil (Premier BK25, Promix M, Premier Horticultural Inc., Quakertown, Pennsylvania) after sprouting. The *A. altissima* seedlings were provided 5-10 g (amount depends on the size of the pot) of Osmocote fertilizer (ICL Specialty Fertilizers, Summerville, South Carolina) when first put into tree pots and monthly thereafter. Otherwise, the *A. altissima* seedlings were reared as described in Kreitman et al (2020).

Celastrus orbiculate were grown from cuttings from multiple plants obtained from Wallingford and Ansonia, Connecticut USA. *Rosa multiflora* was grown from cuttings obtained from multiple plants from Wallingford, Connecticut and from at least ten individual plants from Ansonia, Connecticut. Both plants were obtained during the summer of 2020. *Ocimum basilicum* plants were grown from "hybrid herb, basil Prospera organic" seeds purchased from Seedway LLC (Hall, New York) using the same method as the *A. altissma* plants. *Celastrus orbiculate*, *R. multiflora*, and *O. basilicum* were all grown in the same soil and tree pots as the *A. altissima* plants.

The *A. rubrum* and *S. babylonica* plants were purchased from Cold Stream Farm LLC (Freesoil, Michigan) in late March 2020. The *V. labrusca* bare root plants were purchased from Double A Vineyard (Fredonia, New York) and were shipped in the Spring of 2020.

2020 Nymphal Rearing: For this first year, the host plants were *A. altissima*, *V. labrusca*, *S. babylonica*, and *A. rubrum*. Three cages of each host plant treatment were set up for ten second instar nymphs as well as for ten third instar nymphs, both sourced from the rearing cages containing the field collected nymphs. For the fourth instar nymphs, five individuals that molted the same day were placed in a small cage for each host with ten replicates of each over a period of twelve days. New plants of the same host were added to the cages every seven days for second through third instar nymphs and every four days for fourth instar nymphs. Nymphs were monitored daily for survival and molting, which was confirmed by a cast skin. Any newly molted individuals were removed from the cages, weighed, and then preserved by freezing for later sexing. In the case of the second instar nymphs, they were preserved in ethanol, and we were unable to

determine their sex. Measurements of forewing length, forewing width, and hind tibia length were taken of all frozen adults under a dissecting microscope.

2021 Nymphal Rearing: In 2021, we added first instar nymph reared from field collected egg masses. The egg masses were collected by removing both the egg mass and bark substrate that it was on using a chisel and hammer from two sites in Pennsylvania and one site in New Jersey on 20 October 2020 (Table 1). These egg masses were held individually in 60 × 15 mm Petri dishes (Falcon 351007, Becton Dickinson Labware, Franklin Lakes, New Jersey) at 15°C until they hatched. Upon hatching, twenty nymphs that hatched on the same day from egg masses from the same collection site were placed in a small BugDorm cage (32.5 × 32.5 × 77.0 cm) containing two plants of the same species. The species of plants used were *A. altissima*, *V. labrusca*, *S. babylonica*, *A. rubrum*, *C. orbiculata*, *O. basilicum*, and *R. multiflora*. Additional hatch from those egg masses was placed in the larger BugDorm cages (60 × 60 × 120 cm) with 2–3 100 cm tall potted *A. altissima* plants and a single *V. labrusca* and held at 25°C for rearing to be used as second instar nymphs. For both first and second instar nymphs, two cages were set up using nymphs from the New Jersey site and one cage using nymphs from each Pennsylvania site for a total of four cages for each host. Voucher specimens were preserved in a freezer for reference in addition to the voucher specimens of adult *L. delicatula* that were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, Connecticut USA.

Statistical Analysis: Statistical analyses were performed using SAS 9.4 (SAS institute 2015). Data did not fit assumptions of normality per the Shapiro-Wilk and Anderson-Darling tests. PROC Univariate was then used to assess the fit of the data to a

gamma distribution. PROC GLIMMIX was used to evaluate the fixed effect of host on the duration and body mass of each instar. For instars where the sex was known, the fixed effects of sex and the interaction between sex and host plant were added to the model. The state the egg masses were collected and the cage were treated as random effects. Each model was fitted to a gamma distribution with a log link function because the response variables had long right tails. Differences among means were determined using the Tukey-Kramer post hoc analysis and $\alpha = 0.05$. Statistical comparisons of nymphal survival between host plants for each instar were done using a Peto-Wilcoxon test in Statistix 10.0 (Statistix 2013). For this analysis any nymphs that molted or were inadvertently killed were censored.

Results

Survival: Overall, host plant had an impact on nymphal survival. In 2020, second instar nymphs had the lowest percent survival on *A. rubrum* when compared to other hosts (Fig. 1). There was no significant difference in survival between host plants for second instar nymphs in 2020 ($\chi = 4.98$, DF = 3, P=0.173). Third and fourth instar nymphs reared on *A. rubrum* and *S. babylonica* had the lowest percent survival. The survival between host plants for both third and fourth instar nymphs in 2020 was significantly different ($\chi = 114.44$, DF = 3, P<0.000 for third instars and $\chi = 100.48$, DF = 3, P<0.000 for fourth instars). In 2021, survival was highest for all instars on *A. altissima* except for first instar nymphs (Fig. 2). There was a significant difference in survival between host plants for both first and second instar nymphs in 2021 ($\chi = 87.12$, DF = 6, P<0.000 for first instars and $\chi = 55.00$, DF = 6, P<0.000 for second instars). First instar nymphs had the highest percent survival when reared on *C. orbiculata* followed by *A.*

rubrum. In addition, first and second instar nymphs reared on *O. basilicum* had the lowest percent survival and second instar nymphs had a high percent survival on more hosts than first instar nymphs.

Nymphal Development: Host did not have a significant effect on the mean time spent in second instar in 2020 ($F_{3, 78} = 2.49$; $P = 0.066$) (Fig. 3). Second instar nymphs reared on *A. rubrum* spent significantly more time in that instar than nymphs reared on *V. labrusca*. In addition, host did not have a significant effect on the weight of the nymphs upon completing the second instar ($F_{3, 78} = 0.91$; $P = 0.440$).

For third instar nymphs, the mean development time was significantly affected by sex ($F_{1, 186} = 30.87$; $P > 0.0001$) with female nymphs having longer development time (Fig. 3). Host had a significant effect on the mean development time in third instar ($F_{2, 186} = 8.98$; $P = 0.0003$); however, there was no significant difference for nymphs reared on *A. altissima* and *V. labrusca*. There was no significant interaction between host and sex for the third instar ($F_{2, 186} = 0.35$; $P = 0.7029$). Female nymphs reared on *S. babylonica* spent significantly longer in third instar than all the other nymphs. None of the nymphs reared on *A. rubrum* were able to complete the third instar.

Sex had a significant effect on the mean weight of nymphs that completed the third instar ($F_{1, 186} = 126.8$; $P < 0.0001$) (Fig. 3). For each host, the female nymphs weighed significantly more than the male nymphs reared on the same host. Both host ($F_{2, 186} = 1.68$; $P = 0.193$) and the interaction of host and sex ($F_{2, 186} = 1.99$; $P = 0.143$) did not have a significant effect on the mean weight of nymphs that completed the third instar. No significant difference was found for the mean weight of male or female nymphs reared on any of these hosts. In addition, female nymphs that were reared on *A. altissima*

weighed significantly more that male nymphs that were reared on either *S. babylonica* or *V. labrusca*. Female nymphs reared on *V. labrusca* also weighed significantly more than male nymphs that were reared on *S. babylonica*.

Sex had a significant effect on the mean development time spent in fourth instar ($F_{1, 59} = 7.26$; $P = 0.009$) (Fig. 3); however, host did not have significant effect on the mean time spent in the fourth instar ($F_{2, 59} = 0.88$; $P = 0.4195$). Likewise, the interaction of host and sex also did not have a significant effect on the mean time spent in the fourth instar ($F_{2, 59} = 0.6$; $P = 0.5526$). Female nymphs reared on *A. altissima* developed significantly longer than male nymphs that were reared on the same host plant. None of the fourth instar nymphs reared on *A. rubrum* were able to complete the fourth instar.

For first instar nymphs, host plant had a significant effect on the mean time in the first instar ($F_{6, 262} = 24.21$; $P < 0.0001$) (Fig. 4) with significantly longer development on *A. rubrum* than all other hosts except for *O. basilicum*. There was no significant difference in mean development time in first instar between *A. altissima*, *V. labrusca*, and *C. orbiculata*. First instar nymphs also spent significantly less time in the first instar when using *A. altissima* as a host compared to *R. multiflora*, *S. babylonica*, *A. rubrum*, and *O. basilicum*. The weight of first instar nymphs was also significant affected by host plant ($F_{6, 271} = 22.41$; $P > 0.0001$). No significant differences were observed between first instar nymphs reared on *A. altissima*, *V. labrusca*, *R. multiflora*, and *S. babylonica*. Nymphs reared on *C. orbiculata* weighed significantly less than those reared on *V. labrusca* or *A. altissima*; however, no significant difference in weight was seen when compared to those reared on *R. multiflora* or *S. babylonica*. Nymphs reared on *O.*

basilicum and *A. rubrum* weighed significantly less than nymphs reared on all other hosts.

For second instar nymphs, both host ($F_{5, 186} = 9.25$; $P < 0.0001$) (Fig. 4) and the interaction of host and sex ($F_{5, 186} = 3.25$; $P = 0.008$) had significant effects on the mean development time in the second instar. Sex alone did not have a significant effect ($F_{1, 186} = 0.62$; $P = 0.432$) on the mean time in the second instar. When reared on *A. rubrum*, males spent significantly longer in that instar than females. In addition, male nymphs reared on *A. rubrum* took significantly longer than second instar nymphs reared on any of the other host, except for *R. multiflora*. None of the nymphs reared on *O. basilicum* were able to complete the second instar stage.

For the second instar nymphs, sex had a significant effect on the mean weight ($F_{1, 186} = 113.27$; $P < 0.0001$) (Fig. 4) with female nymphs weighing more than male nymphs. Host also significantly impacted mean weight ($F_{1, 186} = 22.25$; $P < 0.0001$); however, the interaction between host and sex was not significant ($F_{1, 186} = 0.22$; $P = 0.956$). Female nymphs reared on *S. babylonica* weighed significantly more than female nymphs reared on *C. orbiculata* but not between female nymphs reared on *V. labrusca*, *A. altissima*, and *S. babylonica*. Female nymphs reared on these hosts weighed significantly more than female nymphs reared on *C. orbiculata*, *A. rubrum*, or *R. multiflora*. No significant difference was found between male nymphs reared on *V. labrusca*, *C. orbiculata*, *A. altissima*, and *S. babylonica*. Male nymphs reared on either *V. labrusca*, *A. altissima*, or *S. babylonica* weighed more than male nymphs reared on either *R. multiflora* or *A. rubrum*.

Adult Mass and Morphometrics: Host ($F_{2, 59} = 3.97$; $P = 0.024$), sex ($F_{1, 59} = 32.42$; $P < 0.0001$), and the interaction of host and sex ($F_{2, 59} = 8.02$; $P = 0.001$) all had a significant effect on the mean weight of adults that completed the fourth instar (Table 2). Adult females that completed the fourth instar on either *A. altissima* or *V. labrusca* weighed significantly more than male adults that completed the fourth instar on either *A. altissima*, *V. labrusca*, or *S. babylonica* as well as female adults completing the fourth instar on *S. babylonica*. Likewise, the mean weight of adults that completed the fourth instar were not significantly different between female adults reared on *A. altissima* or *V. labrusca*. No significant difference in mean weight of adult males that completed the fourth instar on *A. altissima*, *V. labrusca*, *S. babylonica*, or female adults that completed the fourth instar on *S. babylonica* was observed.

Sex had a significant effect on adult forewing length ($F_{1, 64.92} = 28.16$; $P < 0.0001$) while host ($F_{2, 20.14} = 2.81$; $P = 0.084$) and the interaction of host and sex ($F_{2, 68.73} = 2.42$, $P = 0.097$) did not (Table 2). Both host ($F_{2, 75} = 3.71$; $P = 0.029$) and sex ($F_{1, 75} = 13.32$; $P = 0.001$) had a significant effect on the forewing width of adults, however, the interaction of host and sex ($F_{2,75} = 2.01$; $P = 0.142$) did not. Female adults reared on either *A. altissima* or *V. labrusca* had significantly wider and longer forewings than males reared on the same host. Likewise, host ($F_{2, 19.75} = 3.93$; $P = 0.037$) and sex ($F_{1, 64.45} = 10.49$; $P = 0.002$) had a significant effect on adult hind tibia length while the interaction of host and sex ($F_{2, 69.31} = 2.84$; $P = 0.066$) had no significant effect. The hind tibia length of female adults that completed development on either *A. altissima* or *V. labrusca* was significantly longer than the hind tibia length of adult male reared on these two hosts.

Discussion

Host plant had an effect on nymphal survival. Nymphs survived equally well on *A. altissima* and *V. labrusca*, but survival was decreased on *R. multiflora*, *A. rubrum*, and *O. basilicum*. Nymphs failed to develop through the second instar on *O. basilicum* and the third and fourth instar on *A. rubrum*. Host plant was found to have a significant effect on the development time of *L. delicatula* nymphs in the first through third instars. Host plant was also found to have a significant effect on the mean weight of nymphs in the first, second, and fourth instar, but not the third instar. Host had a significant effect on adult hind tibia length and forewing width. These findings should be incorporated into phenology models for *L. delicatula* to account for host effects.

First instar nymphs took longest to develop on *A. rubrum* and *O. basilicum* and had the lowest weights as second instar nymphs. The inability of second instar nymphs to complete the second instar on *O. basilicum* while third and fourth instar nymphs were not able to complete development on *A. rubrum* suggests that the performance of earlier instars is indicative of host viability for later instars. Declining host viability as nymphal development progresses was also seen in the percent survival for second though fourth instar nymphs in 2020, where second instar nymphs reared on *S. babylonica* had similar survival to nymphs reared on either *A. altissima* or *V. labrusca*. This trend of reduced viability as development progresses was seen in previous research where a shift away from *R. multiflora* as the dominant host was observed in the *L. delicatula* third instar stage (Lui 2019). In addition, this trend of reduced survival on hosts where nymphs take longer to develop in earlier instars is also seen in *H. halys* nymphs (Acebes-Doria et al 2016). Another study found that *L. delicatula* nymphs could complete their first instar on more plants than the second instar (Murman et al 2020). Additionally, in that study,

second instar nymphs failed to complete that instar on *A. rubrum* and third instar nymphs failed to complete that instar on *S. babylonica* supporting the results for later instar nymphs in this study on those hosts. Interestingly, *L. delicatula* has consistently good survival during all instars on its preferred host, *A. altissima*. Furthermore, life stages where host plant had a significant effect on the mean time in instar, *A. altissima* was one of the hosts that nymphs spent the least amount of time feeding on during each instar as inferred from the slow-growth, high mortality hypothesis (Benry and Denno 1997). In many cases, nymphs reared on *V. labrusca* and *A. altissima* had no significant difference in mean development time in instar, thus suggesting that *V. labrusca* is a comparable host to *A. altissima* for *L. delicatula* nymphs. The interaction of host and sex only had a significant effect on the mean time in instar for second instar nymphs; however, that is most likely a result of male second instar nymphs reared on *A. rubrum* spending significantly longer in that instar than the nymphs reared on other hosts, excluding *R. multiflora*.

Host plant also had a significant effect on the mean weight of *L. delicatula* nymphs in the first and second instars. This difference in weight is more likely explained by nutritional differences in the host rather than differences in plant defensive compounds as *L. delicatula* is known to sequester defensive compounds (Song et al 2018). Mean weight was also significantly affected by sex in the second, third, and fourth instar, suggesting that weight could be potentially useful for sexing third and fourth instar specimens. The significant effect on mean weight by the interaction of host and sex on fourth instar nymphs may limit the usefulness of this concept to only third instar nymphs, where that interaction did not have a significant effect. The difference between sex in

development time and weight was reflective of each other as the lighter males took less time to develop than the heavier females. Lower weights in fourth instar nymphs were also associated with less optimal temperatures in previous research, which further hints at *S. babylonica* being a less optimal host than either *A. altissima* or *V. labrusca* for female fourth instar nymphs (Kreitman et al 2020). The general similarities between the mean time spent in an instar for first and second instar nymphs between different hosts suggests that weight might be a better indicator of host suitability for those instars. For first instar nymphs, the host plants that took the longest to develop on also produced nymphs with the lowest weights. Growth rate affects the size of an individual, but the final size is determined by factors that terminate growth and lead to a molt. Many insects have a critical weight they must achieve before they molt and when not reached, they do not survive. The critical weight has been determined for *Manduca sexta* L. (Lepidoptera: Sphingidae) and molting frequency is associated with growth rate such that individuals that grow slower spend longer in each instar than individuals that grow faster (Callier and Nijhout 2013). In addition, slower growth rate has been seen in *M. sexta* in response to suboptimal temperatures or nutrition, which matches the trend shown in this study for *L. delicatula*. Thus, the nymphs may be only reaching the critical weights for each instar before molting on the suboptimal hosts.

Adult morphometrics differed by sex further suggesting that there is size-based sexual dimorphism in *L. delicatula* adults. Host had a significant effect on hind tibia length and forewing width of *L. delicatula* adults, which are more indicative of size than forewing length, which has been shown to affect their flight capabilities (Wolfin et al 2020). In *L. delicatula* adults, weight appears to be a proxy for sex and nourishment.

Nourishment could have a potential impact on flight capabilities with regard to dispersal as extra nourishment could be used to sustain longer flights (Johnson 1963). Heavier weights can also allow adult individuals to persist longer without food sources as seen with other Hemipterans and thus increases the odds of individuals surviving dispersal events that are human mediated such as on a plane or cargo ship (Gergs and Jager 2014). Landscape level decisions in terms of host quality for *L. delicatula* could also play a role in dispersal through shipping hubs, ports, and airfields.

The results of this study have implications for phenology models for *L. delicatula* because the phenology is affected by the host plant individuals feed on. Dynamic models accounting for host preference by instar are needed moving forward to have accurate predictions. Since the mean development time did not differ significantly between nymphs reared on either *A. altissima* or *V. labrusca*, the degree-day requirements from Kreitman et al (2020) should be viable for degree-day modeling for monitoring the growth of *L. delicatula* in vineyards, which take 12.6 – 12.77 d to complete development on grape. Regardless of which host that the second instar nymphs were reared on, the time spent in that instar was shorter than the time spent in the second instar at 25°C for the previously mentioned study. As the previous study didn't account for sex in that instar, it could potentially not be a true comparison. The use of plastic tubes in that study compared to the BugDorm cages used in this one also makes it harder to compare because the cages held more and larger hostplants. This same trend was also observed for nearly every host plant in the third instar nymphs with the exception of female third instar nymphs reared on willow. The same trend was apparent with fourth instar nymphs, which in both studies accounted for their sex. This difference only solidifies the flaws of using

plastic tubes over other containers for rearing nymphs. This is different from previous research that found that both first and second instar nymphs took longer to develop on *Vitis rotundifolia var.* Carlos (Michaux) than on *A. altissima* (Nixon et al 2022). This suggests that *L. delicatula* nymphs perform differently depending on the variant and species of *Vitis*. Further studies that look at different host plants and use a combination of host plants similar to forest and landscape compositions are needed to get a better idea of how host plant influences *L. delicatula* development.

Overall, this study shows that the development of *L. delicatula* can be influenced by host plant. Moving forward it is important to consider potential host options when developing management strategies for *L. delicatula* control as well as host when developing phenology models. Furthermore, this research can be extrapolated to identify what nutrients *L. delicatula* requires to complete development based on host utilization. The differences in development time varying by plant indicates a potential issue regarding using phenology models to predict the current life stage. Sampling for field data to use for validating phenology models is important and might be affected at a site level by the host plants that are present. As certain host plants result in nymphs with lower weight, this study can also inform the host choice when mass rearing *L. delicatula* for potential parasitoid use. The risk of *L. delicatula* damage to *O. basilicum* in homeowner gardens seems to be minimal as it does not appear to be a viable host for later instar nymphs. Further research that evaluates *L. delicatula* nymphal utilization of other species of *Vitis* is important since it is a major threat to vineyards.

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Table 1. Approximate locations (latitude and longitude), collection date, and hosts from which the egg masses of *Lycorma delicatula* used in this study were obtained.

Collection location	Collection date	Host (number of egg masses)	Latitude	Longitude
Spruce Run Reservoir Clinton, NJ, USA	10 October 2020	*Betula pendula* Roth (93) and dead trees (23)	40°39′ 47.03″N	74°55′36.02″ W
The Woodlands, Philadelphia, PA, USA	10 October 2020	*Prunus* spp. (110), *Broussonentia papyrifera* (L. Vent.) (Rosales: Moraceae) (8), *Acer platanoides* (L.) (Sapindales: Sapindaceae) (7), and *Crataegus* spp. (Rosales: Rosaceae) (10)	39°5′ 45.86″N	75°12′19.37″ W
Neshaminy State Park, Bensalem, PA, USA	10 October 2020	*Betula nigra* (L.) (Fagales: Betulaceae) (33), *Betula lenta* (L.) (Fagales: Betulaceae) (18), *Acer rubrum* (25), *Prunus* spp. (18), and *Pinus strobus* (L.) (Pinales: Pinaceae) (24)	40°4′ 31.87″N	75°55′0.59″ W

Table 2. Mean [± SE (*n*)] adult *Lycorma delicatula* body weight (g), forewing length (mm) and width (mm), and hind tibia length (mm) at different combinations of host plant species and sex in *L. delicatula* reared on three host plants in 2020.

Measure	Host plant species and sex[a]						Statistics		
	Ailanthus altissima		*Vitis labrusca*		*Salix babylonica*				
	Male	Female	Male	Female	Male	Female	*F*	d.f.	*p*-value
Weight (g)	0.120 ± 0.003b (32)	0.175 ± 0.006a (14)	0.124 ± 0.004b (12)	0.187 ± 0.006a (20)	0.124 ± 0.01b (4)	0.115b (1)	8.02	2,59	0.001
Forewing length (mm)	17.43 ± 0.22b (31)	21.43 ± 0.39a (13)	18.01 ± 0.35b (12)	21.55 ± 0.33a (20)	17.65 ± 0.59b (4)	18.39ab (1)	2.42	2,68.73	0.097
Forewing width (mm)	7.683 ± 0.11b (31)	9.18 ± 0.19a (13)	7.852 ± 0.18b (12)	9.361 ± 0.11a (20)	7.585 ± 0.30b (4)	7.56ab (1)	2.01	2,75	0.142
Hind tibia length (mm)	9.875 ± 0.10b (31)	11.05 ± 0.16a (13)	10.08 ± 0.15b (12)	11.15 ± 0.14a (20)	9.79 ± 0.28b (4)	9.58ab (1)	2.84	2,69.31	0.066

[a]Means followed by the same letter are not significantly different at a p-value ≤ 0.05 using Tukey–Kramer grouping. Sample size (N) is the number of survivors. d.f., degrees of freedom.

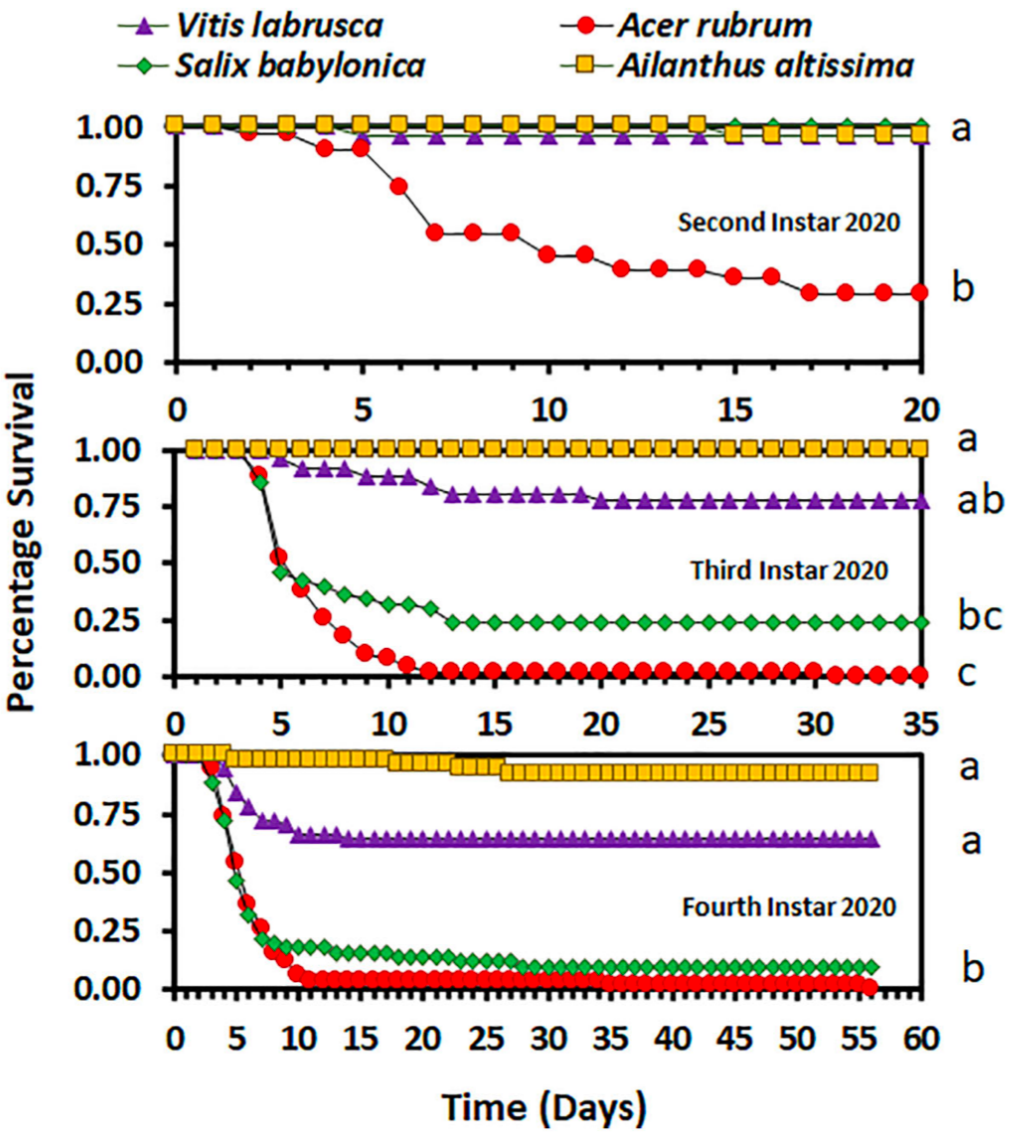

Fig. 1. Survivorship curves for *Lycorma delicatula* nymphs reared in 2020 by instar and host plant species. Different letters to the right of the graphs indicate differences between the overall survival for the host plant species.

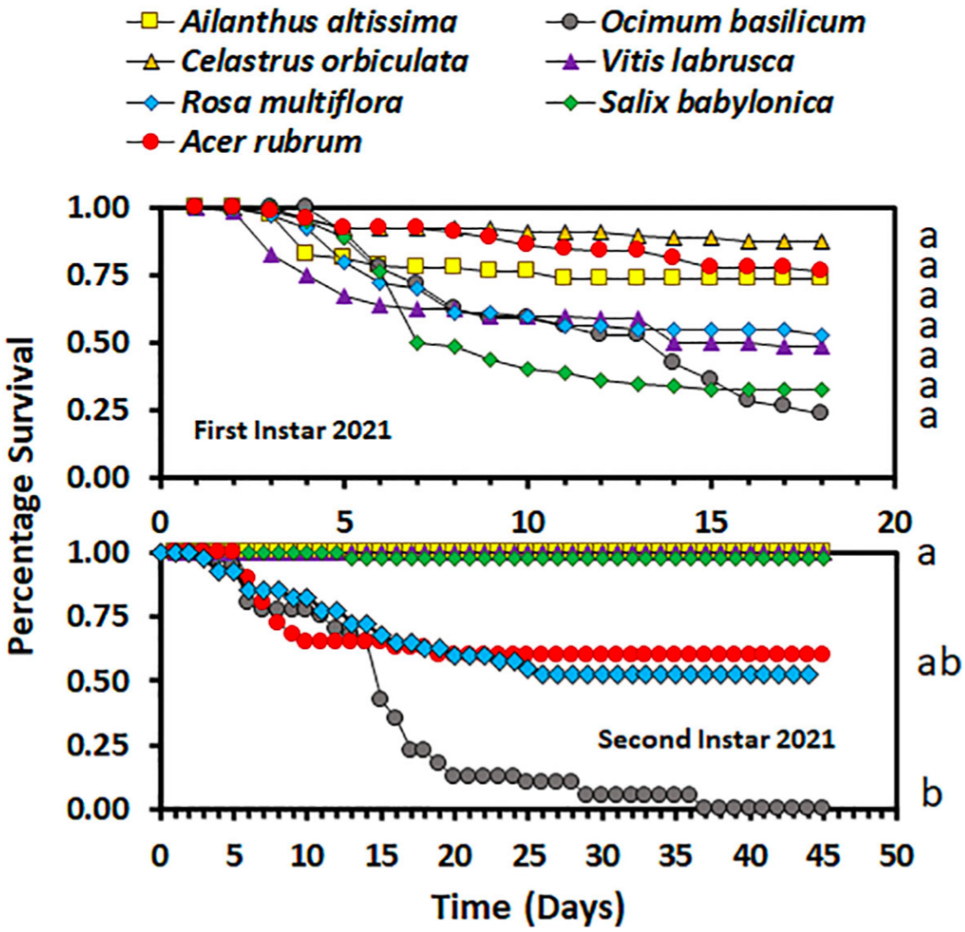

Fig. 2. Survivorship curves for *Lycorma delicatula* nymphs reared in 2021 by instar and host plant species. Different letters to the right of the graph indicate differences between the overall survival for the host plant species.

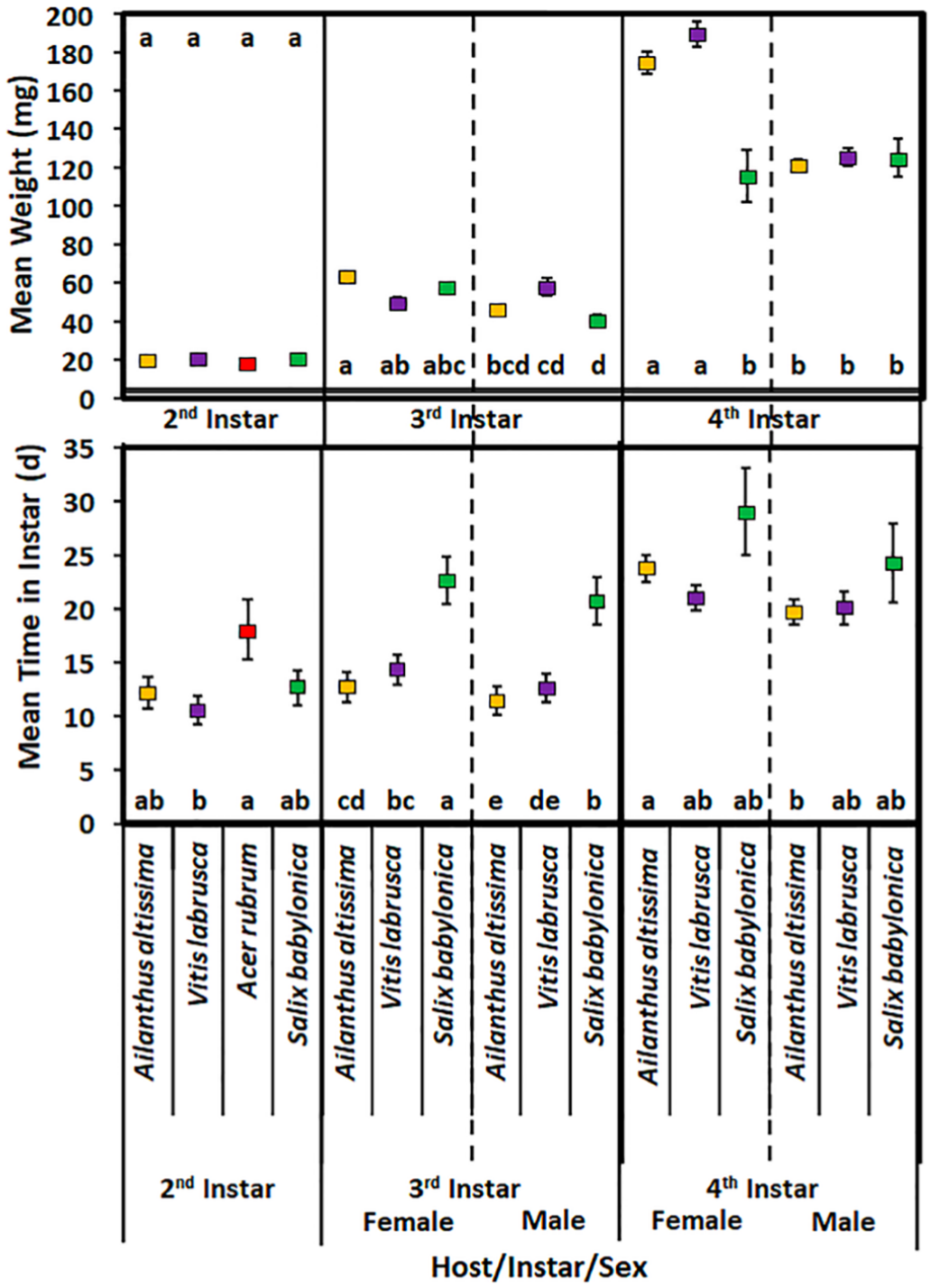

Fig. 3. Mean time (days) spent in instar and weight (mg) of *Lycorma delicatula* nymphs reared in 2020 by host plant species, instar, and sex. Means with a different letter are significantly different from each other at a *p*-value < .0.05 using Tukey-Kramer grouping.

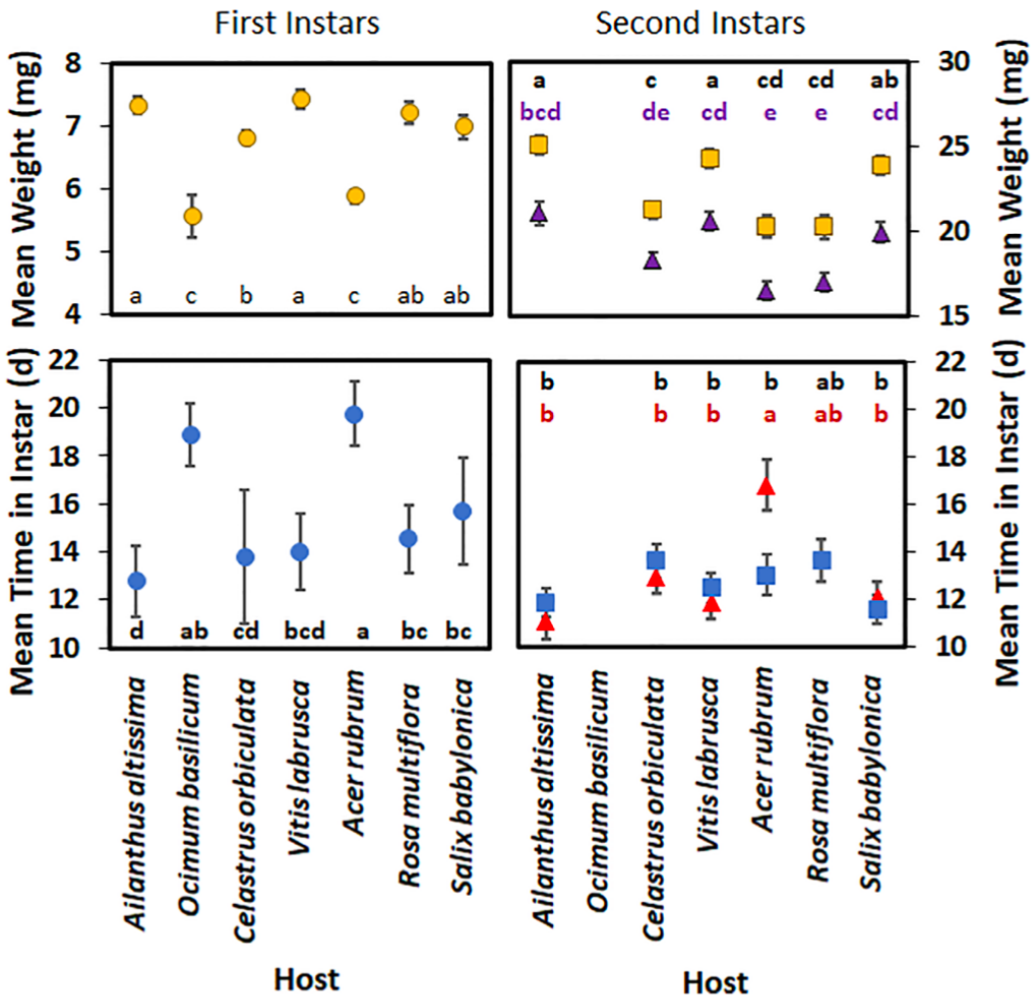

Fig. 4. Mean time (days) spent in instar and weight (mg) of *Lycorma delicatula* nymphs reared in 2021 by hot plant species and instar. Triangles represent males whereas squares represent females for second instar nymphs. Means with a different letter are significantly different from each other at a p-value < 0.05 using Tukey-Kramer grouping.

Chapter 4

Conclusions

Through these two studies, it was determined that both temperature and host plant influence the development rate of *Lycorma delicatula*. It was shown that sex also had an effect on their developmental rate resulting in a bimodal distribution when the response for both sexes were clumped together. The development rate when reared on either *Ailanthus altissima* or *Vitis labrusca* was similar, indicating that the upper and lower developmental thresholds derived from the temperature response study don't have to be adjusted to account for host plant differences when developing degree-day models that are useful for growers of *V. labrusca*. The lower threshold values can be used to keep track of accumulated degree-days for field populations and can be further used to predict when each life will appear by comparing that field estimate to the number of accumulated degree-days needed for 10% of the population to complete different life stages. The data from the temperature response study shows that *L. delicatula* has a drastic decrease in both survival and developmental rate at the upper end of its thermal range, while having a gradual decrease in both survival and developmental rate at the lower end. In a recent paper looking at their thermal response to heat waves and cold snaps, it was concluded that *L. delicatula* has more variation in thermal response allowing them to tolerate more climates than were first anticipated (Keena et al 2023). In addition, the temperature response study was used to model the potential range of *L. delicatula* in both Australia and Europe by Maino et al (2022). The data from both studies can be used to refine degree-day and phenology models for *L. delicatula* allowing for more accurate prediction of when certain life stages will appear and inform current advancements with

management options including biological control. Field data is still important for validating the developmental rate estimates as in Maino et al (2022), where the authors readjusted the developmental rate found in the temperature response study to better reflect the available field data. Recently, it was shown that all life stages of *L. delicatula* are susceptible to the entomopathogenic fungus *Batkoa major* with adult males being the most susceptible (Hajek et al 2022). Knowing this, degree-day models can be used to predict when adults will start appearing and time the applications of *B. major* to optimize efficiency. The ability to manipulate *L. delicatula*'s developmental rate by controlling the temperature that the nymphs are reared at allows for year-round availability of any life stage making it more viable to run serial passage of entomopathogenic fungi through individuals of a selected life stage in order to encourage better specificity and virulence when developing commercial strains of that fungus. Accurate models also help determine the best time and which host plants to monitor for different *L. delicatula* life stages. The host plant developmental response study has implications for future research into the landscape-level implications for control and has the potential to augment preexisting control methods. Selecting plants for the landscape that *L. delicatula* develops slower on and that are at lower agricultural risk from *L. delicatula* creates more opportunities for *L. delicatula* mortality. First instars nymphs are known to feed on less optimal hosts based on gut content analysis (McPherson et al 2022). Later instar nymphs have a narrower host range and are observed on fewer species plants in the field suggesting that there would potentially be some dispersal of later instar nymphs from sites lacking optimal host plants (Lui 2019). With this in mind, action thresholds could be readjusted for later instars to potentially account for their lower population density making detection more difficult. In

addition, recent research has found that *L. delicatula* developmental rate and survivorship varies between two different grape species: *V. labrusca* and *Vitis vinifera* (L.) (Vitales: Vitaceae) (Laveaga 2022). This same study also observed improved survivorship on a combined diet of *A. altissima* and either of the two grapevine cultivars compared to any of the plants alone suggesting that the removal of *A. altissima* from the local landscape around vineyard can be used as a management tool.

While recent advancements in research help improve successful management of *L. delicatula*, strategies to address it should go beyond mitigating as much of the negative impacts as possible and focus on a comprehensive strategy like the slow the spread program developed for *Lymantria dispar* (Tobin and Blackburn 2002). The slow the spread program consists of multiple tactics depending on location in reference to the distribution of *L. dispar.* Elimination is used for isolated populations that arise beyond the invasion front. This strategy could be used in places such as California where *L. delicatula* has yet to establish and are far away from the invasion front. For elimination, the tactics used need to be based around reducing their rate of increase (R_o) below one so the population cannot maintain their numbers and eventually dies out, which is an example of the Allee effect. Along the invasion front, heavy control such as egg mass removal and trapping needs to be used in order to hinder the rate of expansion. While egg mass removal is a great tool as it works against overwintering *L. delicatula*, it has limitations. A vast majority of egg masses on trees are located at heights greater than six meters with only 1.78% of egg masses being found below three meters (Keller et al 2020). This constraint can be potentially addressed by developing unmanned aerial vehicles that are capable of removing egg masses that are located out of the reach of

humans. Unmanned aerial vehicles have already been successfully used to detect other insects and there is ongoing research to use them to detect *L. delicatula* (Park et al 2021). Monitoring and a rapid response to detection at transportation hubs such as train stations, truck stops, rest stations, and airports, and along transportation routes such as train tracks and highways is also vital for this to work. While mating disruption is an important component of the stop the spread program for *L. dispar*, that tool is currently not available for *L. delicatula* due to limited understanding of its chemical ecology. There is evidence of pheromone use by *L. delicatula*, which increases the probability of future development of mating disruption for *L. delicatula* (Faal et al 2022). Where *L. delicatula* is currently established, there needs to be suppression of numbers to reduce populations below damaging levels. Exclusion netting can be used to prevent *L. delicatula* from becoming a pest in agricultural sites; however, their use is not viable in forests and urban and suburban environments (Leach et al 2021). In light of the report of a 90% yield loss in a vineyard associated with *L. delicatula* infestation in Pennsylvania, precautionary principle should be adhered to even if it has been only reported from a single site (Urban 2019). Even though it is unknown if *L. delicatula* can induce similar yield loss levels in other agricultural commodities and forests, there are concerns about potential long effects of their feeding in those systems. Furthermore, the economic burdens that are associated with procedures such as inspection requirements that are required in quarantine areas is detrimental to businesses (Urban and Leach 2023). Further advancements in research and technology are needed to adequately control *L. delicatula* to address these issues.

In conclusion, the research on the phenology of *L. delicatula* presented in this thesis helps provide the groundwork needed to create improved phenology and degree-

day models. These models will help inform growers when management options should be implemented and thus lead to better use of the current management options available for control of *L. delicatula*.

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