Phenology, impact, and rearing of *Lycorma delicatula* (White) (Spotted Lanternfly) in Virginia

Andrew Chase Dechaine

Thesis submitted

to the faculty of the Department of Entomology at

the Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science in the Life Sciences

in

Entomology

Douglas G. Pfeiffer, Co-Chair

Thomas P. Kuhar, Co-Chair

Scott M. Salom

Tracy C. Leskey

1 February 2021

Blacksburg, Virginia

Keywords: phenology, dendrochronology, *Ailanthus altissima,* tree-of-heaven, rearing, quarantine

Phenology, impact, and rearing of *Lycorma delicatula* (White) (Spotted Lanternfly) in Virginia

Andrew C. Dechaine

Abstract (Academic)

The spotted lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), is a new invasive insect from Asia that is currently spreading in the Mid-Atlantic region of the US, where it has become a pest of economic concern for many industries in their invaded range. The purpose of this research was to document *L. delicatula* phenology in Virginia, their feeding impact to trees, and to test a rearing protocol in quarantine laboratory conditions.

First, using field plots and weekly observational surveys, *L. delicatula* phenology was documented in 2019 and 2020 in Winchester, Virginia. I showed that *L. delicatula* were active from late April into November for each sampling year, and that the timing of life stage events varied only slightly between years. I also calculated cumulative average growing degree days for each life stage event using a lower developmental threshold of 10°C. Additionally, I confirmed that the host range of *L. delicatula* narrows as they progress through development. These results will help growers and land managers develop integrated pest management plans targeting *L. delicatula.*

Second, dendrochronological methods were used to quantify *L. delicatula* feeding injury to *Ailanthus altissima* (Miller) Swingle (Sapindales: Simaroubaceae), *Juglans nigra* L. (Fagales: Juglandaceae), and *Liriodendron tulipifera* L. (Magnoliales: Magnoliaceae). Two sites in Pennsylvania that have experienced high populations and heavy feeding pressure from *L. delicatula* since 2016 were used to collect tree cores for analysis. I found evidence suggesting *L. delicatula* is capable of reducing the growth of *A. altissima*, but did not find the same evidence in the wood of the other tree species tested. Additionally, I found evidence that systemic insecticide treatments may reduce or prevent damage to *A. altissima* fed on by *L. delicatula.* Additional dendrochronological studies on the damage inflicted by *L. delicatula* feeding could shed light on the long-term impact of this new invasive tree pest.

Lastly, a protocol for rearing *L. delicatula* was tested inside Virginia Tech's Insect Quarantine Laboratory. Three different cuttings of *A. altissima* (apical meristems, epicormic shoots, and field collected foliage) were tested to determine the best food source for *L. delicatula* in quarantine laboratory conditions. Overall, I did not find a significant difference between food treatments, however a greater proportion of third instars developed into fourth instars in the apical meristem treatment. I suggest future *L. delicatula* rearing research include the use of potted plants and/or multiple species. Though I had low success in rearing adult *L. delicatula* to produce egg masses, this method may prove useful for rearing early instars from eggs or sustaining field collected specimens for short durations.

Research expanding our knowledge of *L. delicatula* will help us reach our goal of more effectively managing this pest species in the future.

Phenology, impact, and rearing of *Lycorma delicatula* (White) (Spotted Lanternfly) in Virginia

Andrew C. Dechaine

Abstract (Public)

The spotted lanternfly, *Lycorma delicatula* (White), is a new invasive pest species impacting the eastern and northeastern regions of the United States. This insect uses its straw-like mouth parts to feed on the sap of many different plants including fruit trees, grapes, and several important ornamental and timber trees. Though they do not bite or sting, feeding can result in wilting, the growth of sooty mold, and sometimes plant mortality, making them an economic and nuisance pest in their invaded range. This research primarily focused on studying the timing of the spotted lanternfly's life cycle, feeding impact to trees, and a method for raising them in the laboratory for research purposes. The life-cycle of the spotted lanternfly was documented over two growing seasons in Winchester, VA and the timing of each life stage was shown to be similar between years. Additionally, it was confirmed that the spotted lanternfly feeds on fewer host species as it develops into an adult. Tree-ring analysis was used to identify spotted lanternfly feeding injury to tree-of-heaven, black walnut, and tulip poplar. I found evidence suggesting spotted lanternfly feeding can cause reduced growth in tree-of-heaven, but did not find similar evidence in the other species tested. A laboratory colony of spotted lanternflies would prove beneficial for additional research on this insect. I tested three different types of branches cut from the tree-of-heaven to identify the best food source for spotted lanternfly in laboratory conditions. The findings of this research will help develop pest management strategies to reduce the impact of this new pest in the US.

Dedication

For my wife, Rachel, for whom I owe everlasting gratitude.

Acknowledgements

Table of Contents

Chapter 1: Introduction and literature review

1.1 Introduction

Insects can play a large positive or negative role in the health of forest ecosystems (Aukema et al. 2010). Non-native, invasive insects continue to be unintentionally distributed around the world as a result of globalization and increased world trade (Finley and Chhin 2016). Many of these insects pose a significant threat to various natural resources in the United States (Finley and Chhin 2016). For example, after the first identification of the introduced emerald ash borer [*Agrilus planipennis* (Fairmaire)] in Michigan in 2002, it has been estimated that the economic cost from damage and subsequent management of ash trees was about $1 billion USD annually in its first decade of spread, and will no doubt alter forest composition on a large scale (Aukema et al. 2011, McCullough 2020).

Similarly, the gypsy moth [*Lymantria dispar* (L.)], introduced around 1869 to the northeastern United States, has had a wide range of ecological and economic effects. In 1981, it was estimated that gypsy moth defoliation cost the Pennsylvania timber industry about $72 million USD in lost timber value (Alalouni et al. 2013). According the US Department of Labor, that would equate to approximately $215 million USD in 2020 (US Bureau of Labor Statistics 2020). Gypsy moth defoliation can also cause a shift in forest composition, having a positive or negative effect to wildlife and nutrient cycling (Alalouni et al. 2013).

Another recently introduced non-native insect in North America, the spotted lanternfly (Figure 1.1), *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), also known as the spot clothing wax cicada and the Chinese blister cicada (Dara et al. 2015), was originally described by Adam White in 1845 and is native to China, Taiwan, Vietnam, and India (Barringer et al. 2015, Dara et al. 2015, Lee et al. 2019). Although this insect has been reported to have medicinal use since the 12th Century (Barringer et al. 2015, Dara et al. 2015), rigorous study of this insect has only recently been facilitated by its introduction to South Korea, Japan, and the United States. The current literature regarding this insect's invasion and distribution, biology and life history, host preference, damage and impact, and management is summarized below.

1.2 Invasion and distribution

Lycorma delicatula was introduced to South Korea in 2004 and Japan in 2008 (Barringer et al. 2015), and it is thought that *L. delicatula* was transported to these countries unintentionally from Chinese shipments of ornamental vegetation (Hong et al. 2012, Lee et al. 2019). Using molecular techniques, Kim et al. (2013) confirmed that specimens collected in South Korea were genetically identical to those from locations in China confirming its origin (Kim et al. 2013). Following its introduction, *L. delicatula* was reported to be a sporadic pest to grapes, fruit trees, and ornamental trees in those countries resulting in annual economic impact (Han et al. 2008, Barringer et al. 2015).

The first record of *L. delicatula* in the North America was documented by the Pennsylvania State Department of Agriculture in 2014, which identified an established population in Berks County, PA (Barringer et al. 2015) (Figure 1.2). As of December 2020, *L. delicatula* has spread to 26 PA counties and populations can be found in New York, Connecticut, Ohio, New Jersey, Maryland, Delaware, West Virginia, and Virginia (Figure 1.2) (NYS IPM 2020). Similar to South Korea and Japan, it is hypothesized that *L. delicatula* was unintentionally introduced to the U.S. from a shipment of ornamental stone from China (Kingan et al. 2019). The stone shipment was likely infested with egg masses, as they are inconspicuous and resemble a smear of mud (Kingan et al. 2019).

Though efforts were initiated to eradicate this pest in the US, *L. delicatula* has proven to be very good at "hitchhiking" and human-aided transport has likely contributing to their long-range dispersal (Keller et al. 2020). Additionally, CLIMEX and MAXENT software have been used to predict the potential spatial distribution of *L. delicatula* based on suitable climate and habitat parameters (Jung et al. 2017, Wakie et al. 2020).

The results of the two models however, were somewhat different. The CLIMEX model used climate and habitat parameters from South Korea while the MAXENT model used parameters from China, South Korea, Japan, and the United States (Jung et al. 2017, Wakie et al. 2020). The resulting potential *L.*

delicatula distribution map produced by the CLIMEX model shows a greater likelihood for establishment in tropical regions of the globe, while the MAXENT model predicted a shift to more temperate regions (Jung et al. 2017, Wakie et al. 2020). Together, these models show that *L. delicatula* likely has the ability to establish in the majority of the Mid-Atlantic and Northeast regions of the United States, as well as large areas of the Pacific Northwest and California (Jung et al. 2017, Wakie et al. 2020). Furthermore, the models predicted suitable habitat in South America, Australia, Africa, Korea, Japan, and almost all of Europe (Jung et al. 2017, Wakie et al. 2020). Therefore, it seems likely that *L. delicatula* will continue to spread and establish outside of its native range.

1.3 Biology and life history

Lycorma delicatula is univoltine in its native and introduced range, overwintering as an egg. Egg masses are about 2.5-4 cm long and typically contain 30-50 eggs that are laid in rows and covered by a brownish waxy layer (Figure 1.3) (Dara et al. 2015). Eggs are laid in the fall and can be deposited on a variety of surfaces (e.g. stone, metal, fence posts, vehicles, and trailers) but are generally found on tree trunks and the underside of tree branches (Kim et al. 2011, Liu 2019). According to Liu and Mottern (2017), there are differences in egg laying behavior between Asian and North American populations. In China and South Korea, female *L. delicatula* are reported to oviposit shortly after adult eclosion, whereas Pennsylvania populations have been reported to oviposit approximately two months after eclosion (Liu and Mottern 2017). In North America, eggs are typically first observed in September or October (Dara et al. 2015, Liu 2020).

In South Korea, Lee et al. (2011) examined the minimum lethal winter temperature on egg masses by geographic location and found that egg mortality increased as mean winter temperature decreased. They concluded that increasing average winter temperatures as a result of climate change could be a factor in the range expansion of *L. delicatula* (Lee et al. 2011).

Lycorma delicatula develops through four nymphal instars with each instar lasting approximately 15-25 days (Park et al. 2009). The first, second, and third instar are black with white spots while the fourth instar can be identified by its distinct red color with black and white spots (Dara et al. 2015) (Figures 1.4 & 1.5). The average length of the first, second, third, and fourth instar is 4, 5.5, 8, and 12.5 mm, respectively (Dara et al. 2015). After eclosion, adult *L. delicatula* are approximately 3.8 cm long and can sometimes be mistaken for a moth (Dara et al. 2015) (Figure 1.1). *Lycorma delicatula* wings lie folded over the body resembling a tent. Forewings are tannish grey with black spots and reticulated tips, while hind wings are black, white and red. In addition, the abdomen changes from a black, white, and red coloration as 4th instars to alternating black and yellow stripes as an adult (Kang et al. 2016). In Pennsylvania, first, second, third, fourth, and adult *L. delicatula* were initially observed in May, June, June, July, and July, respectively (Dara et al. 2015, Liu 2020).

Once nymphs have hatched, they begin climbing up host plants and continue to do so until they find a suitable feeding site, encounter a barrier and jump off, or fall off from a disturbance (e.g. wind) (Kim et al. 2011). Once on the ground, they start moving and again begin climbing when they encounter a new host or vertical substrate. This falling-ascending cycle is thought to be a strategy for finding a suitable host as well as a way of dispersing over the landscape (Kim et al. 2011). *Lycorma delicatula* possess structures on their tarsi called arolia. Arolia are adhesive pads located between their claws and play an important role in climbing, especially on smooth surfaces (Kim et al. 2011). Early instars have small arolia and are more readily dislodged from their host (Kim et al. 2011). As *L. delicatula* grow, the arolium becomes larger and more effective at adhering to smooth surfaces and thus the falling and ascending cycle duration becomes longer and fall from their host less frequently (Kim et al. 2011).

Keller et al. (2020) investigated the ability of *L. delicatula* nymphs to disperse through a contiguous deciduous forest in Pennsylvania. Using a mark, release, and recapture study design, they found that all four instars were capable of traveling similar distances over time (Keller et al. 2020). However, third instars

moved the farthest on average at a median distance of 17 m over 7 days (Keller et al. 2020). As a fulgorid, *L. delicatula* are strong jumpers in all life stages, but have been reported as poor fliers as adults (Pfeiffer et al. 2018). The flight capabilities of *L. delicatula* have been described by Baker et al. (2019) and Wolfin et al. (2019). Both studies showed that *L. delicatula* were capable of flying between 10-50 m at any given time (Baker et al. 2019, Wolfin et al. 2019). Adults typically exhibited the same falling-ascending behavior observed in nymphs to disperse across the landscape to find adequate feeding sites (Wolfin et al. 2019) However, it was discovered that mated female *L. delicatula* were less capable of flight and only flew about 4 m on average, likely due to their added weight (Wolfin et al. 2019).

Baker et al. (2019) described *L. delicatula* courtship and mating that was observed in four PA field sites in 2017. Courtship was first observed on 31 August 2017 and consisted of a male *L. delicatula* performing a wing-vibration behavior adjacent to a female (Baker et al. 2019). After the initial observation, male courtship behavior was regularly documented in subsequent field visits (Baker et al. 2019). On 5 October, a courtship that led to copulation was observed, but it is still unknown if the male wing-vibration behavior is required for successful mating (Baker et al. 2019). During the study they documented 21 male-female pairs mating. In all observations, the male's wings were on top of the female's and their abdomens were swollen and yellow, with the female's abdomen more swollen than the male's (Baker et al. 2019) (Figure 1.6). From the 21 mating pairs, they estimated that copulation can last between 2 and 4 hours (Baker et al. 2019).

1.4 Host preference

Lycorma delicatula is a terrestrial phloem-feeding insect that lives in forested habitat with over 100 documented host plant species from 33 families (Dara et al. 2015, Barringer and Ciafré 2020). Many of these hosts are of agricultural, ornamental, and landscape importance (Dara et al. 2015). Examples of known host species in the US are: *Acer rubrum* L. (red maple), *Acer saccharinum* L. (silver maple), *Acer negundo* L. (boxelder), *Toxicodendron radicans* L. (poison ivy), *Rhus typhina* L. (staghorn sumac), *Hedera*

helix L. (English ivy), *Lonicera japonica* Thunberg (Japanese honeysuckle), *Robinia pseudoacacia* L. (black locust), *Gleditsia triacanthos* L. (honey locust), *Juglans nigra* L. (black walnut), *Prunus serotine* Ehrhart (wild cherry), *Prunus persica* L. (peach), *Rosa multiflora* Thunberg (multiflora rose), *Malus* spp. L. (apple), *Ailanthus altissima* (Miller) Swingle (tree-of-heaven), *Ulmus* spp. L. (elm), *Celtis occidentalis* L. (hackberry), *Vitis* spp. L. (wild, wine, and table grapes), *Parthenocissus quinquefolia* L. (Virginia creeper), and *Tetradium daniellii* (Bennett) Hartley (bee-bee tree or Korean evodia) (Dara et al. 2015, Barringer and Ciafré 2020, Liu 2020).

Numerous studies have reported that the host range of *L. delicatula* is broad in early life stages but narrows as the insect develops into an adult (Kim et al. 2011, Song et al. 2018, Lee et al. 2019, Uyi et al. 2020). Kim et al. (2011) conducted a study from April to December 2009 in Seoul, South Korea, and found *L. delicatula* nymphs on 12 of 13 species of marked trees on 16 July (Kim et al. 2011). However, from 6 September to 1 November 2009, adults were only collected from *T. daniellii* and *A. altissima* (Kim et al. 2011).

Ailanthus altissima is a fast-growing invasive tree species found across the continental U.S., but is native to China and Taiwan (Kowarik and Säumel 2007). This tree can reach 27-30 m in height and is found in forest openings, urban landscapes, disturbed areas, and areas bordering agricultural fields (Kowarik and Säumel 2007). Evidence suggests *A. altissima* is a preferred host of *L. delicatula* and may accelerate its spread within the U.S. (Dara et al. 2015). Recent research done in Pennsylvania showed that although *A. altissima* is a preferred host of *L. delicatula*, it does not require *A. altissima* to complete development and reproduce (Uyi et al. 2020). However, insects that were not allowed to feed on *A. altissima* exhibited reduced fitness compared to insects that were allowed to feed on *A. altissima* (Uyi et al. 2020).

1.5 Injury and impact

Lycorma delicatula use their piercing and sucking mouthparts to tap into the phloem of plants (Hao et al. 2016). Injury manifests itself in several forms. Feeding on leaves, small branches and vines can cause

wilting and branch death (Lee et al. 2009). Similar to other hemipteran species, *L. delicatula* secretes the waste product honeydew, which is high in sugar content, attracts other insects, and is colonized by sooty mold fungi (Dara et al. 2015). In heavily infested areas, later instars and adult *L. delicatula* aggregate and feed in large numbers (personal observation), and the honeydew excreted covers the ground and surrounding vegetation (Dara et al. 2015). Sooty mold fungi are darkly pigmented, so colonized plants have reduced photosynthetic capabilities and can wither and die (Lee et al. 2009). Adults and late instar nymphs observed feeding in groups have also been reported to cause weeping or oozing wounds on the tree trunk, resulting in wilting and branch death (Dara et al. 2015). Direct injury from feeding on fruits or seeds has not been observed.

Lycorma delicatula has been described as a pest of many tree and shrub species in China (Dara et al. 2015). However, there are no data suggesting that *L. delicatula* is a pest of economic status in China, presumably due to natural enemies present in its native range that keep populations in check or its coevolved history with its host plants (Richardson et al. 2011). In 2004, *L. delicatula* was introduced to South Korea and rapidly spread throughout that country (Dara et al. 2015). Following this introduction, South Korea reported annual growth in economic impact related to grapes, fruit trees, and as an emerging urban pest (Park et al. 2009, Choi et al. 2011, Dara et al. 2015).

Lycorma delicatula populations have exploded in their introduced range in North America. Vineyards in areas of Pennsylvania, where populations of *L. delicatula* are high, have reported up to 90% loss in grape yield as a result of repeated infestations and the number of insecticide applications have tripled (Leach et al. 2019, Urban 2020). Harper et al. (2019) calculated the potential economic impact of *L. delicatula* on agriculture and forestry in Pennsylvania. They determined that if *L. delicatula* continues to spread throughout PA, an annual economic impact of $99.1 and $236.3 million USD for the agriculture and forestry industries, respectively could occur (Harper et al. 2019).

Specifically, in Virginia in 2017, there were 272 wineries that generated about $5 billion in economic activity in the state (John Dunham & Associates 2017). Additionally, Virginia is the sixth largest apple producer in the U.S. with approximately 6,475 ha in production (Pfeiffer 2020a, The Virginia Apple Board 2020). From the first detection of *L. delicatula* into VA in January 2018 until October 2020, there were no reports from vineyards or orchards experiencing infestations. However, in October 2020, *L. delicatula* was discovered at a commercial wine grape vineyard north of Winchester (Pfeiffer 2020b). It was only a matter of time as eradication efforts have largely been ineffective and the infestation area has increased from 15 km^2 in 2018 to 280 km^2 in 2020 (Pfeiffer 2020b).

Future research needs to be done to investigate long term impact of *L. delicatula* feeding on trees. One such method could be through the use of dendrochronology. Dendrochronology is the study and dating of annual tree rings (Fritts 1976). Using tree cores, dendrochronology allows for the identification and quantification of past ecological events such as climate change, historic wildfires, defoliation events, and insect outbreaks (Morrow and Lamarche 1978, Hogg et al. 2002, Huang et al. 2008). Morrow and Lamarche (1978) were able to experimentally show that continuous feeding by several insect species reduced the growth of subalpine Eucalyptus (Morrow and Lamarche 1978). Similarly, dendrochronology has been used to quantify reduced radial growth of many tree species by defoliating insects in the US and Canada (Swetnam et al. 1985).

1.6 Management

Since the introduction of *L. delicatula* into the United States, quarantine and eradication programs have been established by multiple state Departments of Agriculture in infested areas such as Pennsylvania, Delaware, New Jersey, Maryland, and Virginia (NYS IPM 2020). Quarantine programs in the US are largely aimed at education, outreach, and regulating the movement of live *L. delicatula* and any commodities that may harbor various life stages (Urban 2020). Businesses operating in and out of quarantined areas are required to obtain training on *L. delicatula* identification and carry permits when

shipping goods and transporting vehicles stating they will not move *L. delicatula* from infested areas (Urban 2020). Businesses found in violation of quarantine orders may be subject to a fine. Additionally, residents in quarantined zones are encouraged to inspect vehicles and cargo before moving out of quarantine zones so *L. delicatula* is not mistakenly transported elsewhere (Urban 2020).

Eradication efforts focus primarily at the perimeter of quarantine zones as well as areas with high densities of *L. delicatula* (Urban 2020). There is currently no trap specific to *L. delicatula*, however recent research suggests that modified Circle traps, originally developed for weevils, are effective at capturing all life stages in the field (Nixon et al. 2020). Additionally, an attract-and-kill system, combining *A. altissima* with the treatment of a systemic insecticide, is being used to manage populations (Lee et al. 2019, Urban 2020). As a preferred host of *L. delicatula*, *A. altissima* is identified in quarantined areas and either removed or treated with systemic insecticide (Lee et al. 2019, Urban 2020). Treatment generally occurs during later *L. delicatula* life stages because studies have shown a shift in feeding toward *A. altissima* occurs with the onset of red fourth instars (Song et al. 2018). Additional management tactics such as surveying, tree banding, egg scraping, host removal, sanitation, and insecticide treatments are also being used for management (Urban 2020).

Multiple studies have shown that insecticides are a powerful tool for controlling *L. delicatula*. In South Korea, Park et al. (2009) and Shin et al. (2010) evaluated a number of insecticides against *L. delicatula* nymphs and eggs. Park et al. (2009) tested deltamethrin, imidacloprid, clothianidin, fenitrothion, and thiacloprid diluted at one part per thousand on second and third instar nymphs. Their results showed that deltamethrin and clothianidin resulted in 100% mortality after two hours, fenitrothion and imidacloprid resulted in 100% mortality after 24 hours, while thiacloprid was less effective, although percentages were not reported (Park et al. 2009). Shin et al. (2010) tested 26 different insecticides on *L. delicatula* in laboratory bioassays. Results showed that only chlorpyrifos caused 100% mortality in eggs if applied

before late April and all 26 insecticides tested on first and second instars caused 100% mortality (Shin et al. 2010).

In North America, Leach et al. (2019) evaluated the efficacy of multiple insecticides in laboratory and field bioassays at controlling *L. delicatula*. Chlorpyrifos was the only insecticide that caused 100% mortality on egg masses, while paraffinic oil caused about 71% mortality (Leach et al. 2019). Compounds including pyrethroids, neonicotinoids, carbamates, and organophosphates were all effective at killing *L. delicatula* nymphs and adults, but only thiamethoxam and bifenthrin exhibited residual activity up to 14 days after treatment (Leach et al. 2019). None of the tested compounds approved for organic production were effective at controlling any life stage of *L. delicatula* (Leach et al. 2019).

Biological control can be an effective tool in an integrated pest management program. There are ongoing efforts to identify possible biological control agents for *L. delicatula* from its native range. *Anastatus orientalis* Yang & Choi (Hymenoptera: Eupelmidae) is an egg parasitoid from China and has been reported to cause up to 80% parasitism in *L. delicatula* egg masses (Choi et al. 2014). As of November 2020, *A. orientalis* is being reared and tested in quarantine as a possible control agent in the US (Broadley et al. 2020). Similarly, *Dryinus browni* (Hymenoptera: Dryinidae) is another Chinese parasitoid of *L. delicatula* that has been imported to quarantine labs in the US for host range testing (Urban 2020). Unlike *A. orientalis* however, *D. browni* is a nymphal parasitoid that targets first and second instars and has been recorded to parasitize 12.5-43.5% of *L. delicatula* (Lee et al. 2019).

In China, generalist predators that have been documented feeding on *L. delicatula* include: *Monomorium pharaonis* L. (pharaoh ant), *Nephila clavata* Koch (orb weaver), *Araneus ventricosus* Koch (orb weaver), and *Paratenodera sinensis* Saussure (praying mantis) (Hou 2013). Additionally, a few native generalist predators of *L. delicatula* have been documented in the United States. Two hemipteran generalist predators, a wheel bug (*Arilus cristatus* L.) and stink bug (*Apoecilus cynicus* Say), were documented in Pennsylvania in September and November 2015, respectively (Barringer and Smyers

11

2016). There have also been studies investigating predation by birds. These studies have reported that aposematic coloration of fourth instars and adults along with distasteful chemicals sequestered by *L. delicatula* feeding on *A. altissima* deter bird predation (Kang et al. 2011, Song et al. 2018).

1.7 Conclusion

Lycorma delicatula will likely spread to much of the United States and continue to impact vineyards, orchards, nurseries, and timber lands in the years to come. Research on *L. delicatula* phenology, its impact to trees, and development of rearing methods are explored in the following chapters.

1.8 References

Alalouni, U., M. Schädler, and R. Brandl. **2013**. Natural enemies and environmental factors affecting the population dynamics of the gypsy moth. J. Appl. Entomol. 137: 721–738.

Aukema, J. E., B. Leung, K. Kovacs, C. Chivers, K. O. Britton, J. Englin, S. J. Frankel, R. G. Haight, T. P. Holmes, A. M. Liebhold, D. G. McCullough, and B. von Holle. **2011**. Economic impacts of non-native forest insects in the continental United States. PLoS One. 6: 1–7.

Aukema, J. E., D. G. McCullough, B. Von Holle, A. M. Liebhold, K. Britton, and S. J. Frankel. **2010**. Historical accumulation of nonindigenous forest pests in the continental United States. Bioscience. 60: 886–897.

Baker, T. C., E. C. Smyers, J. M. Urban, Z. Meng, K. J. Pagadala Damadaram, A. J. Myrick, M. F. Cooperband, and M. J. Domingue. **2019**. Progression of seasonal activities of adults of the spotted lanternfly, *Lycorma delicatula*, during the 2017 season of mass flight dispersal behavior in eastern Pennsylvania. J. Asia-Pac. Entomol. 22: 705–713.

Barringer, L., and C. M. Ciafré. **2020**. Worldwide feeding host plants of spotted lanternfly, with significant additions from North America. Environ. Entomol. 49: 999–1011.

Barringer, L. E., L. R. Donovall, S.-E. Spichiger, D. Lynch, and D. Henry. **2015**. The first New World record of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae). Entomol. News. 125: 20–23.

Barringer, L. E., and E. Smyers. **2016**. Predation of the spotted lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) by two native Hemiptera. Entomol. News. 126: 71–73.

Broadley, H. J., J. R. Gould, L. T. Sullivan, X. Wang, K. A. Hoelmer, M. L. Hickin, and J. S. Elkinton. **2020**. Life history and rearing of *Anastatus orientalis* (Hymenoptera: Eupelmidae), an egg parasitoid of the spotted lanternfly (Hemiptera: Fulgoridae). Environ. Entomol. nvaa124: 1–8.

Choi, D., K. H. Kim, and Y. Jang. **2011**. Agonistic interactions between nymphs of *Lycorma delicatula* (Hemiptera: Fulgoridae). J. Asia-Pac. Entomol. 14: 21–25.

Choi, M.-Y., Z.-Q. Yang, X.-Y. Wang, Y.-L. Tang, Z.-R. Hou, J. H. Kim, and Y. W. Byeon. **2014**. Parasitism rate of egg parasitoid *Anastatus orientalis* (Hymenoptera: Eupelmidae) on *Lycorma delicatula* (Hemiptera: Fulgoridae) in China. Korean J. Appl. Entomol. 53: 135–139.

Dara, S. K., L. Barringer, and S. P. Arthurs. **2015**. *Lycorma delicatula* (Hemiptera: Fulgoridae): A new invasive pest in the United States. J. Integr. Pest Manag. 6: 1–6.

Finley, K., and S. Chhin. **2016**. Forest health management and detection of invasive forest insects. Resources. 5: 18.

Fritts, H. C. 1976. Tree rings and climate. Academic Press, London.

Han, J. M., H. Kim, E. J. Lim, S. Lee, Y. J. Kwon, and S. Cho. **2008**. *Lycorma delicatula* (Hemiptera: Auchenorrhyncha: Fulgoridae: Aphaeninae) finally, but suddenly arrived in Korea. Entomol. Res. 38: 281–286.

Hao, Y., C. H. Dietrich, and W. Dai. **2016**. Structure and sensilla of the mouthparts of the spotted lanternfly *Lycorma delicatula* (Hemiptera: Fulgoromorpha: Fulgoridae), a polyphagous invasive planthopper. PLoS One. 11: 1–22.

Harper, J. K., W. Stone, T. W. Kelsey, and L. F. Kime. **2019**. Potential economic impact of the spotted lanternfly on agriculture and forestry in Pennsylvania. Cent. Rural Pennsylvania. 1–84.

Hogg, E. H., J. P. Brandt, and B. Kochtubajda. **2002**. Growth and dieback of aspen forests in northwestern

Alberta, Canada, in relation to climate and insects. Can. J. For. Res. 32: 823–832.

Hong, K. J., J. H. Lee, G. S. Lee, and S. Lee. **2012**. The status quo of invasive alien insect species and plant quarantine in Korea. J. Asia-Pac. Entomol. 15: 521–532.

Hou, Z. 2013. Study on the *Lycorma delicatula* and egg parasitoids. M.S., Chinese Academy of Forestry.

Huang, J. G., J. Tardif, B. Denneler, Y. Bergeron, and F. Berninger. **2008**. Tree-ring evidence extends the historic northern range limit of severe defoliation by insects in the aspen stands of western Quebec, Canada. Can. J. For. Res. 38: 2535–2544.

John Dunham & Associates. **2017**. The wine industry boosts the Virginia economy by $5.0 billion in 2017. Brooklyn, New York.

Jung, J. M., S. Jung, D. hyeon Byeon, and W. H. Lee. **2017**. Model-based prediction of potential distribution of the invasive insect pest, spotted lanternfly *Lycorma delicatula* (Hemiptera: Fulgoridae), by using CLIMEX. J. Asia-Pac. Biodivers. 10: 532–538.

Kang, C. K., S. I. Lee, and P. G. Jablonski. **2011**. Effect of sex and bright coloration on survival and predator-induced wing damage in an aposematic lantern fly with startle display. Ecol. Entomol. 36: 709–716.

Kang, C., H. Moon, T. N. Sherratt, S.-I. Lee, and P. G. Jablonski. **2016**. Multiple lines of anti-predator defence in the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). Biol. J. Linn. Soc. 115–124.

Keller, J. A., A. E. Johnson, O. Uyi, S. Wurzbacher, D. Long, and K. Hoover. **2020**. Dispersal of *Lycorma delicatula* (Hemiptera: Fulgoridae) nymphs through contiguous, deciduous forest. Environ. Entomol. 49: 1012–1018.

Kim, H., M. Kim, D. H. Kwon, S. Park, Y. Lee, J. Huang, S. Kai, H. S. Lee, K. J. Hong, Y. Jang, and S. Lee. **2013**. Molecular comparison of *Lycorma delicatula* (Hemiptera: Fulgoridae) isolates in Korea, China, and Japan. J. Asia-Pac. Entomol. 16: 503–506.

Kim, J. G., E. H. Lee, Y. M. Seo, and N. Y. Kim. **2011**. Cyclic behavior of *Lycorma delicatula* (Insecta:

Hemiptera: Fulgoridae) on host plants. J. Insect Behav. 24: 423–435.

Kingan, S. B., J. Urban, C. C. Lambert, P. Baybayan, A. K. Childers, B. Coates, B. Scheffler, K. Hackett, J. Korlach, and S. M. Geib. 2019. A high-quality genome assembly from a single, field-collected spotted lanternfly (*Lycorma delicatula*) using the PacBio Sequel II system. Gigascience. 8: 1–10.

Kowarik, I., and I. Säumel. 2007. Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. Perspect. Plant Ecol. Evol. Syst. 8: 207–237.

Leach, H., D. J. Biddinger, G. Krawczyk, E. Smyers, and J. M. Urban. 2019. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula*, (Hemiptera: Fulgoridae), a new pest of fruit in the northeastern U.S. Crop Prot. 124: 104833.

Lee, D. H., Y. L. Park, and T. C. Leskey. 2019. A review of biology and management of *Lycorma delicatula* (Hemiptera: Fulgoridae), an emerging global invasive species. J. Asia-Pac. Entomol. 22: 589–596.

Lee, J., I.-K. Kim, S. Koh, S. J. Cho, S.-J. Jang, S.-H. Pyo, and W. IL Choi. 2011. Impact of minimum winter temperature on *Lycorma delicatula* (Hemiptera: Fulgoridae) egg mortality. J. Asia-Pac. Entomol. 14: 123–125.

Lee, J., S. Moon, H. Ahn, S. Cho, and J. Yang. 2009. Feeding behavior of *Lycorma delicatula* (Hemiptera: Fulgoridae) and response on feeding stimulants of some plants. Korean J. Appl. Entomol. 48: 467–477.

Liu, H. 2019. Oviposition substrate selection, egg mass characteristics, host preference, and life history of the spotted lanternfly (Hemiptera: Fulgoridae) in North America. Environ. Entomol. 48: 1452–1468.

Liu, H. 2020. Seasonal development, cumulative growing degree-days, and population density of spotted lanternfly (Hemiptera: Fulgoridae) on selected hosts and substrates. Environ. Entomol. 49: 1171–1184.

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

America. J. Insect Sci. 17.

McCullough, D. G. 2020. Challenges, tactics and integrated management of emerald ash borer in North America. Forestry. 93: 197–211.

Morrow, A. P. A., and V. C. Lamarche. **1978**. Tree ring evidence for chronic insect suppression of productivity in subalpine Eucalyptus. Science (80-.). 201: 1244–1246.

Nixon, L. J., H. Leach, C. Barnes, J. Urban, D. M. Kirkpatrick, D. C. Ludwick, B. Short, D. G. Pfeiffer, and T. C. Leskey. 2020. Development of behaviorally based monitoring and biosurveillance tools for the invasive spotted lanternfly (Hemiptera: Fulgoridae). Environ. Entomol. 49: 1117-1126.

NYS IPM. **2020**. New York State Integrated Pest Management. Spotted lanternfly. (https://nysipm.cornell.edu/environment/invasive-species-exotic-pests/spotted-lanternfly/).

Park, J.-D., M.-Y. Kim, S.-G. Lee, S.-C. Shin, J.-H. Kim, and I.-K. Park. **2009**. Biological characteristics of *Lycorma delicatula* and the control effects of some insecticides. Korean J. Appl. Entomol. 48: 53–57.

Pfeiffer, D. G. 2020a. Virginia Apples. Virginia Tech Virginia Fruit Web Site. (https://www.virginiafruit.ento.vt.edu/VirginiaAppleSite.html).

Pfeiffer, D. G. 2020b. Spotted lanternfly advancing: Control measures take various forms. Grape Press (Va. Vineyards Assoc.) 36(4): 1, 11-12.

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

Richardson, D. M., P. Pyšek, and J. T. Carlton. **2011**. A compendium of essential concepts and terminology in invasion ecology, pp. 409–420. *In* Fifty Years Invasion Ecol. Leg. Charles Elt. Blackwell Publishing Ltd.

Shin, Y.-H., S.-R. Moon, C. Yoon, S. Ahn, Ki, and G.-H. Kim. **2010**. Insecticidal activity of 26 insecticides against eggs and nymphs of *Lycorma delicatula* (Hemiptera: Fulgoridae). Korean J. Pestic. Sci. 14: 157–163.

Song, S., S. Kim, S. W. Kwon, S. Lee, and P. G. Jablonski. **2018**. Defense sequestration associated with narrowing of diet and ontogenetic change to aposematic colours in the spotted lanternfly. Sci. Rep. 8: 1–11.

Swetnam, T. W., M. A. Thompson, and E. K. Sutherland. **1985**. Spruce Budworms Handbook: Using dendrochronology to measure radial growth of defoliated trees, United States Dep. Agric. U.S. Department of Agriculture, Forest Service, Cooperative State Research Service.

The Virginia Apple Board. **2020**. About Virginia Apples. Virginia Apple Ind. (https://www.virginiaapples.net/virginia-apples).

Urban, J. M. 2020. Perspective: Shedding light on spotted lanternfly impacts in the USA. Pest Manag. Sci. 76: 10–17.

US Bureau of Labor Statistics. 2020. CPI Inflation Calculator. United States Dep. Labor. (https://www.bls.gov/data/inflation_calculator.htm).

Uyi, O., J. A. Keller, A. Johnson, D. Long, B. Walsh, and K. Hoover. **2020**. Spotted lanternfly (Hemiptera: Fulgoridae) can complete development and reproduce without access to the preferred host, *Ailanthus altissima*. Environ. Entomol. 49: 1185–1190.

Wakie, T. T., L. G. Neven, W. L. Yee, and Z. Lu. **2020**. The establishment risk of *Lycorma delicatula* (Hemiptera: Fulgoridae) in the United States and globally. J. Econ. Entomol. 113: 306–314.

Wolfin, M. S., M. Binyameen, Y. Wang, J. M. Urban, D. C. Roberts, and T. C. Baker. **2019**. Flight dispersal capabilities of female spotted lanternflies (*Lycorma delicatula*) related to size and mating status. J. Insect Behav. 32: 188–200.

1.9 Figures

Figure 1.1: Adult *Lycorma delicatula*. (Photo by Lawrence Barringer, Bugwoog.org).
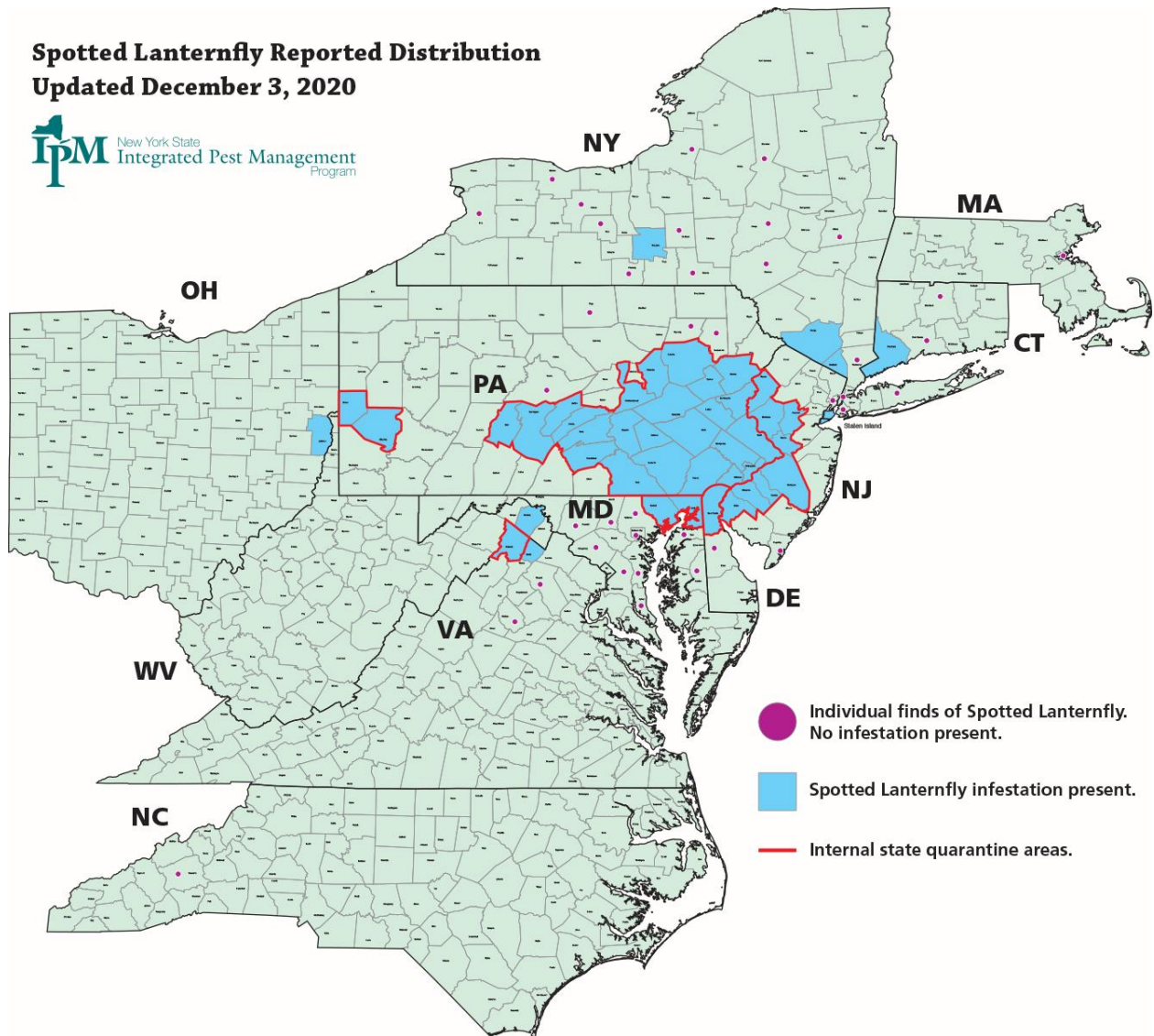
Figure 1.2: The distribution of *Lycorma delicatula* in the United States as of December 3, 2020. (NYS IPM 2020).

Figure 1.3: Egg masses and adult *L. delicatula.*

Figure 1.4: *Lycorma delicatula* nymphs. First, second, and third instars are black with white spots. (Photo by Lawrence Barringer, Bugwood.org).

Figure 1.5: *Lycorma delicatula* fourth instars are red and black with white spots. (Photo by Eric Day, Bugwood.org).

Figure 1.6: *Lycorma delicatula* in copula showing the male's wings over the female's wings (A), the same mating pair showing the female's swollen yellow and black striped abdomen (B). 5 September 2019. Winchester, VA.

Chapter 2: Phenology of *Lycorma delicatula* (White) in Virginia

Abstract:

Lycorma delicatula (White) (Hemiptera: Fulgoridae) is an invasive planthopper that was first discovered in North America in Berks County, Pennsylvania in 2014. Since its introduction, *L. delicatula* has spread to eight additional states and threatens agricultural, ornamental, and timber commodities throughout the US. The timing of insect life events is very important in the development of pest management tools and strategies. In 2019 and 2020, *L. delicatula* phenology was successfully documented in Winchester, Virginia using five-minute observational surveys at established monitoring plots. Each year, *L. delicatula* were active from May to November with initial detections of first, second, third, fourth, and adults occurring in May, May, June, June, and July, respectively. Cumulative average growing degree days were also calculated for each *L. delicatula* life stage using local weather data and a lower developmental threshold of 10°C. Combined, these data can be used by growers and land managers to facilitate development of effective pest management strategies.

2.1 Introduction

Lycorma delicatula (White) (Hemiptera: Fulgoridae), also known as the spotted lanternfly, is an invasive species native to China that was first discovered in North America in Berks County, PA in September 2014 (Barringer et al. 2015, Dara et al. 2015). As of November 2020, this insect has spread to 26 PA counties and populations can be found in New York, Connecticut, New Jersey, Maryland, Delaware, Ohio, West Virginia, and Virginia (NYS IPM 2020). This rapid spread is likely the result of *L. delicatula's* ability to occupy a broad range of climactic conditions and feed on more than 100 identified host species worldwide from 33 families (Barringer and Ciafré 2020). This insect has also shown the ability to hitchhike on vehicles (e.g. trains, trucks, trailers), allowing it to travel long distances and projections have shown that *L. delicatula* has the potential to establish populations throughout large regions of the United States as well as Europe and South America (Jung et al. 2017, Wakie et al. 2020).

Lycorma delicatula is a univoltine insect that overwinters as an egg. Eggs are laid in clusters of 30-50, covered with a brownish waxy substance that hardens over time, and can be deposited on a wide range of smooth living and non-living substrates such as trees, stones, fence posts, and rusty metal (Dara et al. 2015, Liu 2019). In PA, *L. delicatula* has been reported to hatch in late spring and develop through four instars before eclosion into adults in July (Figure 2.1), with mating observed to begin in September (Lee et al. 2019). This insect utilizes a broad host range as nymphs, with their host range narrowing in their latter instars (Kim et al. 2011, Lee et al. 2019).

Lycorma delicatula is a phloem-feeding planthopper that lives in forested habitats. As a phloem feeder, it uses its piercing sucking mouthparts to extract large amounts of sap from trees and other plants, which can result in wilting, branch death, and oozing wounds (Dara et al. 2015). Honeydew, their sugar-rich waste product from feeding, can coat everything below them including other plants. This honeydew can result in the growth of sooty mold and impede a plants ability to photosynthesize (Lee et al. 2019). With such a broad host range and its potential to establish throughout the country, this insect poses a

significant risk to the agriculture, fruit, nursery, and timber industries of the United States (Harper et al. 2019, Liu 2020). Thus far, vineyards in Pennsylvania have experienced the highest impact from *L. delicatula*, reporting high yield loss, vine death, and an increase of insecticide applications due to heavy infestations (Leach and Leach 2020, Urban 2020).

In January 2018, *L. delicatula* egg masses were discovered at a stone yard in northern Winchester, Frederick County, VA (Pfeiffer et al. 2018). Since its discovery, *L. delicatula* has spread throughout Winchester and the rest of Frederick County, and now into the neighboring Clarke County (Virginia Department of Agriculture and Consumer Services (VDACS) 2019). As of 2017, Frederick County was the leading producer of fruits, tree nuts, and berries in Virginia, with a market value of about $15 million USD (United States Department of Agriculture (USDA) 2017). *L. delicatula* poses a large threat to this industry and continued monitoring and management will be important in minimizing the impact of this invasive insect.

Determining the seasonal phenology of a new invasive pest is very important in the development of integrated pest management strategies to mitigate impact (Herms 2004). The goal of this study was to document the seasonal phenology of *L. delicatula* in Winchester, VA to ultimately provide information for the improvement of monitoring and integrated pest management.

2.2 Materials and methods

2.2.1 Study site

Lycorma delicatula phenology was documented in the northern portion of Winchester, VA during the growing seasons of 2019 and 2020. The study site was approximately a 2.6 km^2 area (39.206768, -78.155772) that consisted of mainly light industrial, manufacturing, and commercially developed land (Figure 2.2). It was a highly trafficked area with railroad lines, eighteen wheel tractor-trailers, US Rt. 11, and Interstate 81 about 1.6 km to the east. Many documented *L. delicatula* host plants were present throughout the study area.

26

2.2.2 Insect sampling 2019

In early April 2019, the infestation zone was scouted for observational plot locations. Observation plots were 4 m^2 and required the presence of at least three known host species and five egg masses per plot. It was determined that these requirements would be adequate to track the phenology of *L. delicatula* throughout the growing season. On 20 April 2019, twenty observational plots (n = 20) were established in northern Winchester, VA (Table 2.1). Plot dimensions were measured using a meter tape (Keson Open Reel Fiberglass Tape, Model OTR-50M) and each corner was marked with a uniquely numbered flag corresponding to that plot. Plot locations were generally located in edge habitat along railroad lines, farm fields, and various forest edges. An inventory of woody and herbaceous plant species, the number of visible *L. delicatula* egg masses per host, and the coordinates of each plot were determined. Annual forb and grass species were not documented as available literature does not suggest *L. delicatula* utilize those as host plants. In 2019 a total of 30 host species from 21 families were used to track *L. delicatula* phenology (Table 2.2). If plots were destroyed or found to not possess *L. delicatula* populations during the season, they were replaced with new nearby plots of woody vegetation when possible.

Beginning on 24 April 2019, plots were visited weekly. Five-minute observational insect surveys were conducted at each plot to record *L. delicatula* life stage (eggs, nymphs (instars 1-4), and adults) and the number of *L. delicatula* present per host species. A standard clicker (Ktrio Metal Tally Counter) was used to keep track of the number of individuals present. When more than one life stage were observed, a labeled multiple unit tally counter (Denominator Company 1X4MT) was used to keep track of each life stage present. Multiple vantage points, and 10x42 binoculars (Nikon Prostaff 3S) were used to obtain representative data from each plot. Egg masses counted at the beginning of the season were deposited the previous year (2018), while those counted after observed adult eclosion were deposited the same data collection year (2019). Host phenology observations were also recorded (e.g. bud swell, leaves emerging, flowering) to determine if there was a match between host and insect phenology that may

ultimately aid growers and managers to identify the timing of *L. delicatula* life events. Weekly data collection continued until 5 November 2019 when very few live adults were observed (n = 26 observation dates).

2.2.3 Insect sampling 2020

This observational study was repeated in 2020. On 17 March 2020, additional plot locations were identified using the requirements outlined in 2019 for a total of 22 plots (Table 2.1). In 2020 a total of 29 host species from 19 families were used to document *L. delicatula* phenology (Table 2.3). Due to Virginia Tech-wide travel restrictions implemented in the early stages of the COVID-19 pandemic in 2020, I coordinated with a Frederick County Virginia Cooperative Extension agent to collect the remainder of the 2020 *L. delicatula* phenology data using the same techniques developed in 2019. Weekly data collection for 2020 began on 24 April and continued until 4 November when very few live *L. delicatula* adults were observed (n = 27 observation dates).

2.2.4 Cumulative average growing degree days

At the time of this study, the population of *L. delicatula* in Winchester, Virginia was the southernmost extent known in the North America. Therefore, it was thought that cumulative average growing degree days calculated for the onset of each life stage for *L. delicatula* would be meaningful information. Cumulative average growing degree days were calculated using the 2019 and 2020 *L. delicatula* phenology data and weather data obtained from the Winchester regional weather station KOKV METAR (lat/long: 39.150, -78.150, elev: 221.9 m).

Laboratory experiments conducted in South Korea concluded that the lower developmental threshold for *L. delicatula* from egg to eclosion of first instars was between 8.14°C and 11.13°C (Choi et al. 2012, Park 2015). Similarly, Smyers et al. (Advanced access 2020) recently conducted comparable experiments in the United States. Based on laboratory and field observations in Pennsylvania and Virginia, a good estimate for the lower developmental threshold of *L. delicatula* was calculated to be 10.2°C for the mid-

Atlantic region (Smyers et al. Advanced access 2020). At the time of this work, an upper developmental threshold has not been experimentally confirmed for *L. delicatula*. This study assumes there is no upper threshold and was set to 54°C for degree day calculation. There is currently no degree day model specific to *L. delicatula*, thus cumulative average growing degree days were calculated using a lower developmental threshold of 10°C signifying the consensus of lab results and field observations from South Korea and the United States (Choi et al. 2012, Park 2015, Liu 2020, Smyers et al. Advanced access 2020) (Phenology and Degree Day Models at http://uspest.org/wea/).

2.2.5 Data analysis

R statistical software version 3.5.1, along with the ggplot package, were used to summarize and visualize *L. delicatula* phenology for 2019 and 2020 (Wickham 2016, R Core Team 2018).

2.3 Results

2.3.1 *Lycorma delicatula* phenology 2019

In 2019, *L. delicatula's* first observed hatch was 1 May. Eggs continued to hatch into early June with peak observations of first instars occurring during the week of 15 May. First instar nymphs were recorded to be present on a total of 28 host plants (Table 2.2). Second instar nymphs were detected on 24 May with peak numbers occurring the week of 29 May on a total of 25 host plants. Third instar nymphs were initially detected on 4 June with their peak occurring the week of 21 June on a total of 19 host plants. The distinct transition into fourth instars was observed on 26 June with peak numbers occurring during the week of 9 July on a total of 19 host plants. Adult *L. delicatula* eclosion was first detected on 9 July, the same observation week as peak fourth instar occurrence. Adults continued to emerge into September with peak observations recorded on 5 September on a total of 18 host plants. Egg masses deposited in 2019 were first detected on 11 September. New egg masses were continually observed into November before cold temperatures killed the remaining adults. A total of 23,358 *L. delicatula* were observed over

29

26 weeks in Winchester, VA in 2019 (Table 2.2). *Lycorma delicatula's* seasonal development in Winchester, VA for 2019 is summarized in Figures 2.3 and 2.5.

2.3.2 *Lycorma delicatula* phenology 2020

In 2020, *L. delicatula's* first observed hatch was 7 May. First instar nymphs were continually observed into late June with peak observations occurring the week of 25 May and were present on a total of 27 hosts (Table 2.3). Second instars were detected on 15 May. Peak observations for second instars occurred during the week of 8 June and were present on a total of 25 host plants. Third instars were initially observed on 5 June with peak observations occurring the week of 29 June. Third instars were found present on a total of 24 hosts. Red and black fourth instar nymphs were first observed on 29 June with their peak observations occurring the week of 13 July and were present on a total of 21 hosts. Eclosion into adult *L. delicatula* was initially detected on 20 July with peak observations recorded on 15 September. Egg masses deposited in 2020 were first seen on 15 September. New egg masses were continually deposited into November before cold temperatures killed the remaining adults. A total of 24,901 *L. delicatula* were observed over 27 weeks in Winchester, VA in 2020 (Table 2.3). *Lycorma delicatula's* seasonal development in Winchester, VA for 2020 is summarized in Figures 2.4 and 2.6.

2.3.3 Degree days

In 2019, cumulative average growing degree days calculated for the onset of each *L. delicatula* life stage were 135, 300, 413, and 649 for first, second, third, and fourth instars, respectively. Adults were first observed in the field when cumulative average growing degree days reached 835 and egg masses were detected at 1,673.5 degree days in Winchester, VA. A total of 1,998.5 cumulative average growing degree days were recorded on the last day of sampling in 2019.

In 2020, the cumulative average growing degree days were calculated to be 111.5, 129, 304, 566, for the onset of first, second, third, and fourth instars, respectively. Adult *L. delicatula* were first observed when cumulative average growing degree days reached 887 and egg masses were detected at 1611.5. In

total, 1,781.7 cumulative average growing degree days were recorded for the 2020 *L. delicatula* data collection season.

2.4 Discussion

2.4.1 *Lycorma delicatula* phenology in Winchester, VA

This study documented the seasonal phenology of *L. delicatula* in Winchester, VA during the years 2019 and 2020. It was found that active *L. delicatula* were present beginning in early May and persisted into November for each sampling year with abundant observations each week. In both 2019 and 2020, *L. delicatula* peak life stage observations were approximately two weeks apart between first, second, third, and fourth instars respectively. In 2019 and 2020, the highest recorded number of adults for a single week of sampling was recorded approximately eight or nine weeks after the peak of fourth instars across all plots, respectively. Egg mass deposition was first detected approximately eight weeks after initial adult eclosion in both years (Figures 2.3 – 2.6).

Two other studies documented the 2019 seasonal development of *L. delicatula* at multiple sites in Pennsylvania (Leach and Leach 2020, Liu 2020) located to the north in areas that experience cooler weather conditions than our site in Virginia despite being in the same plant hardiness zone of 6b (http://planthardiness.ars.usda.gov). *Lycorma delicatula* eggs in Winchester, VA appear to have hatched approximately 3 weeks before *L. delicatula* in the areas of Pennsylvania where their studies took place (Leach and Leach 2020, Liu 2020). However, once hatched, *L. delicatula* phenology at all sites in 2019 appeared to follow a similar timing, but delayed by 2 – 3 weeks. None of these studies, including this one, indicated more than a single generation of *L. delicatula* per year.

Additionally, Smyers et al. (Advanced access 2020) studied the effect of temperature on the development of *L. delicatula* eggs in Pennsylvania. First, they completed laboratory experiments on *L. delicatula* eggs collected from Pennsylvania to determine the minimum temperature threshold for

development using linear regression (Smyers et al. Advanced access 2020). They then compared this model to documented field observations from Pennsylvania and Virginia, including the 2019 first instar data from our study (Smyers et al. Advanced access 2020). They determined that a good estimate of the minimum temperature threshold for egg development was 10.2°C and that an estimated 259 degree-days would be required to achieve 50% egg hatch in Virginia (Smyers et al. Advanced access 2020). These findings align with what I observed in Winchester, VA from first instar observations in 2019 and 2020.

Initial detection and peak observations of each life stage in Winchester differed from year to year but were likely affected by seasonal weather variability (Hodgson et al. 2011). For example, in 2020, *L. delicatula* eggs were observed to hatch about one week later compared to 2019. The cumulative average growing degree days (CAGDD) calculated for the hatch date in 2020 were less than the degree days calculated for the hatch date in 2019, indicating a delayed hatch in 2020 was possibly due to cooler weather. However, CAGDD clearly doesn't explain all of the variation, as the first observation of third instars for both years occurred during the same sampling week, despite there being a difference of about 100 CAGDD between years. Liu (2020) discussed the complications of only using CAGDD to predict *L. delicatula* development on a broad scale, matching the discrepancy in CAGDD observed in Virginia. Additionally, relying on weekly observations from specific $4m^2$ plots limited our ability to detect the first occurrence of each life stage. Broader and more frequent sampling surveys coupled with a degree day model specific to *L. delicatula* may better predict phenology events, such as the degree day models used for *Cydia pomonella* L. (codling moth) (Phenology and Degree Day Models at http://uspest.org/wea/).

Some of the variation observed between instar counts was likely caused by physical changes at field sites. For example, between sampling events on 29 May and 4 June in 2019, three observational plots were destroyed. The destruction of those plots may explain the reduced number of observations of second instars during that time period (Figure 2.3). Similarly, late in the sampling season of 2019 the only plot containing Korean evodia, *Tetradium daniellii* (Benn.) T.G. Hartley, which is a known host of *L.*

32

delicatula in China, Korea, and the United States (Kim et al. 2011, Barringer and Ciafré 2020), was destroyed. Therefore, all sampling events afterwards did not include observations of *T. daniellii* for that year, until a replacement plot was added in 2020. Likewise, insecticide treatments may also have influenced some numbers. For example, the newly added *T. daniellii* that was being observed in 2020 was treated during the 17 August sampling week, reducing the number of observed insects the following week from more than 400 to three (Figure 2.6). This treatment could also explain why there were very few egg masses documented on this species. Even with these unforeseen circumstances, the general trend of *L. delicatula* phenology was clearly observed.

2.4.2 Host range of *Lycorma delicatula* narrows from first instar to adult

This experiment was not specifically designed to test host preference; however, a few trends were observed. First, the host range of *L. delicatula* decreased from first instar to adult. For example, first instars were observed on 33 different plant species, while host observations of the later life stages gradually narrowed to 25 plant species. More than 90% of all fourth instars and about 85% of all adult *L. delicatula* in both sampling years were found on just three plant species (*Ailanthus altissima, Tetradium daniellii*, and *Vitis* spp.) (Tables 2.2 and 2.3). This narrowing pattern of *L. delicatula's* host range through development has been reported by others (Dara et al. 2015, Song et al. 2018, Lee et al. 2019). It should also be noted that over half of all the egg masses observed in both years were found on *A. altissima*. During this study, feeding was not recorded, but frequent high numbers of individual *L. delicatula* observed on the same plants over numerous weeks and life stages, along with the presence of honeydew and sooty mold, was a strong indication that extended periods of feeding was occurring. In fact, all of these plant species observed have been recorded elsewhere as known hosts (Dara et al. 2015, Barringer and Ciafré 2020).

Second, *L. delicatula* clearly preferred *A. altissima, Vitis* spp*.,* and *T. daniellii*, with the highest numbers occurring later in the season when fourth instars and adults were most prevalent (Table 2.2 and 2.3). This

observation was obvious despite the fact that our experimental units were plots and not plants, therefore not all host species were represented equally. This preference is consistent with Kim et al. (2011), where later life stages were only found on *A. altissima* and *T. daniellii* in a host preference experiment involving 13 species conducted in South Korea.

2.4.3 Management

Knowledge of *L. delicatula's* seasonal phenology is important for management in order to determine when growers or land managers should initiate specific pest management strategies. For example, sticky band traps can be an effective tool at capturing early instar nymphs, but non-adhesive modified Circle traps have shown to be more effective at capturing fourth instars and adults (Francese et al. 2020, Nixon et al. 2020). Phenology data presented here can be used to help land managers and growers know when and where to deploy specific traps targeted at *L. delicatula* while minimizing non-target impact. Additionally, broad spectrum insecticides are an effective way to kill large numbers of *L. delicatula* (Leach et al. 2019), but the timing of application should be precise to limit impact to beneficial insects. According to our data, basal bark applications of systemic insecticides would likely be most effective when *L. delicatula* have developed into later life stages and have moved to preferred host species such as *A. altissima*.

The phenology of *L. delicatula* may vary where climate differs or in years with unique weather patterns. Therefore, I suggest that using cumulative average growing degree days to predict the timing of *L. delicatula* life stages may be useful in those regions. I also suggest that host phenology could be a good monitoring and prediction tool for *L. delicatula* life events, as host phenology also varies based on local growing degree days and weather conditions. For example, based on our host phenology observations that were recorded weekly, *L. delicatula* eggs were recorded to begin hatching between bud break and leaf emergence of *A. altissima*. A similar trend was observed with *Vitis*, where *L. delicatula* were initially recorded hatching when leaves began to break bud. Furthermore, in both sampling years the transition

of fourth instars into adults occurred after *A. altissima* were done flowering and seeds were beginning to form. A similar trend was again observed in relation to *Vitis*. When adult *L. delicatula* were emerging, fruit was beginning to form on vines in the field. Lastly, the seeds of *A. altissima* were observed to be drying and turning brown when egg masses were first recorded in both sampling years. Since *A. altissima* and *Vitis* are both widely distributed and easily observed, this could help land managers easily predict *L. delicatula* activity elsewhere.

2.5 Conclusion

This study documented the 2019 and 2020 seasonal phenology of *L. delicatula* in its southern limit in North America. It also confirmed that *L. delicatula* has a broad host range as early instars but gradually narrows as the insect develops into an adult. Similarly, this study confirmed that *L. delicatula's* preferred host species is *A. altissima*. This research will provide baseline data to land managers in Virginia to help in monitoring and developing effective integrated pest management strategies for *L. delicatula*.

2.6 Acknowledgements

2.7 References

Barringer, L., and C. M. Ciafré. **2020**. Worldwide feeding host plants of spotted lanternfly, with significant additions from North America. Environ. Entomol. 49: 999–1011.

Barringer, L. E., L. R. Donovall, S.-E. Spichiger, D. Lynch, and D. Henry. **2015**. The first New World record of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae). Entomol. News. 125: 20–23.

Choi, D.-S., D.-I. Kim, S.-J. Ko, B.-R. Kang, J.-D. Park, S.-G. Kim, and K.-J. Choi. **2012**. Environmentally-friendly control methods and forecasting the hatching time *Lycorma delicatula* (Hemiptera:

Fulgoridae) in Jeonnam Province. Korean J. Appl. Entomol. 51: 371–376.

Dara, S. K., L. Barringer, and S. P. Arthurs. 2015. *Lycorma delicatula* (Hemiptera: Fulgoridae): A new invasive pest in the United States. J. Integr. Pest Manag. 6: 1–6.

Francese, J. A., M. F. Cooperband, K. M. Murman, S. L. Cannon, E. G. Booth, S. M. Devine, and M. S. Wallace. 2020. Developing traps for the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). Environ. Entomol. 49: 269–276.

Harper, J. K., W. Stone, T. W. Kelsey, and L. F. Kime. 2019. Potential economic impact of the spotted lanternfly on agriculture and forestry in Pennsylvania. Cent. Rural Pennsylvania. 1–84.

Herms, D. 2004. Using degree-days and plant phenology to predict pest activity, pp. 49–59. *In* Integr. Pest Manag. Midwest Landscapes. Minnesota Agricultural Experiment Station Publication SB-07645.

Hodgson, J. A., C. D. Thomas, T. H. Oliver, B. J. Anderson, T. M. Brereton, and E. E. Crone. 2011. Predicting insect phenology across space and time. Glob. Chang. Biol. 17: 1289–1300.

Jung, J. M., S. Jung, D. hyeon Byeon, and W. H. Lee. 2017. Model-based prediction of potential distribution of the invasive insect pest, spotted lanternfly *Lycorma delicatula* (Hemiptera: Fulgoridae), by using CLIMEX. J. Asia-Pac. Biodivers. 10: 532–538.

Kim, J. G., E. H. Lee, Y. M. Seo, and N. Y. Kim. 2011. Cyclic behavior of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae) on host plants. J. Insect Behav. 24: 423–435.

Leach, H., D. J. Biddinger, G. Krawczyk, E. Smyers, and J. M. Urban. 2019. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula*, (Hemiptera: Fulgoridae), a new pest of fruit in the northeastern U.S. Crop Prot. 124: 104833.

Leach, H., and A. Leach. 2020. Seasonal phenology and activity of spotted lanternfly (*Lycorma delicatula*) in eastern US vineyards. J. Pest Sci. 93: 1215–1224.

Lee, D. H., Y. L. Park, and T. C. Leskey. 2019. A review of biology and management of *Lycorma delicatula* (Hemiptera: Fulgoridae), an emerging global invasive species. J. Asia-Pac. Entomol. 22: 589–596.

Liu, H. 2019. Oviposition substrate selection, egg mass characteristics, host preference, and life history of the spotted lanternfly (Hemiptera: Fulgoridae) in North America. Environ. Entomol. 2015: 1452–1468.

Liu, H. 2020. Seasonal development, cumulative growing degree-days, and population density of spotted lanternfly (Hemiptera: Fulgoridae) on selected hosts and substrates. Environ. Entomol. 49: 1171–1184.

Nixon, L. J., H. Leach, C. Barnes, J. Urban, D. M. Kirkpatrick, D. C. Ludwick, B. Short, D. G. Pfeiffer, and T. C. Leskey. 2020. Development of behaviorally based monitoring and biosurveillance tools for the invasive spotted lanternfly (Hemiptera: Fulgoridae). Environ. Entomol. 49: 1117-1126.

NYS IPM. **2020**. New York State Integrated Pest Management. Spotted lanternfly. (https://nysipm.cornell.edu/environment/invasive-species-exotic-pests/spotted-lanternfly/).

Park, M. 2015. Overwintering ecology and population genetics of *Lycorma delicatula* (Hemiptera: Fulgoridae) in Korea. Seoul Natl. Univ. 228.

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

R Core Team. **2018**. R: A language and environment for statistical computing. R foundation for stistical computing, Vienna, Austria.

Smyers, E. C., J. M. Urban, A. C. Dechaine, D. G. Pfeiffer, S. R. Crawford, and D. C. Calvin. **2020**. Spatial-temporal model for predicting spring hatch of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). Environ. Entomol. Advanced access.

Song, S., S. Kim, S. W. Kwon, S. Lee, and P. G. Jablonski. **2018**. Defense sequestration associated with narrowing of diet and ontogenetic change to aposematic colours in the spotted lanternfly. Sci. Rep. 8: 1–11.

United States Department of Agriculture (USDA). **2017**. 2017 Census of Agriculture Frederick County –

Virginia.

Urban, J. M. 2020. Perspective: Shedding light on spotted lanternfly impacts in the USA. Pest Manag. Sci. 76: 10–17.

Virginia Department of Agriculture and Consumer Services (VDACS). **2019**. Spotted Lanternfly Detected in Clarke County, VA. Press Release. (https://www.vdacs.virginia.gov/press-releases-191105-spotted-lanternfly-detected-in-clarke-county.shtml)

Wakie, T. T., L. G. Neven, W. L. Yee, and Z. Lu. **2020**. The establishment risk of *Lycorma delicatula* (Hemiptera: Fulgoridae) in the United States and globally. J. Econ. Entomol. 113: 306–314.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Anlysis. Springer-Verlag New York.

2.8 Tables and figures

Table 2.1: *Lycorma delicatula* phenology observational plot locations in Winchester, VA.

Plot	Latitude (decimal degrees)	Longitude (decimal degrees)
1 [ab]	39.20914	-78.1472
2 [ac]	39.20611	-78.1504
3 [ab]	39.20641	-78.1500
4 [ab]	39.20552	-78.1510
5 [ac]	39.20528	-78.1514
6 [ac]	39.20465	-78.1521
7 [ab]	39.20191	-78.1528
8 [ab]	39.20217	-78.1537
9 [ab]	39.20307	-78.1528
10 [ad]	39.20186	-78.1524
11 [ab]	39.21091	-78.1567
12 [ab]	39.21089	-78.1572
13 [ab]	39.21257	-78.1549
14 [ab]	39.20673	-78.1548
15 [a]	39.20790	-78.1545
16 [ab]	39.20904	-78.1527
17 [a]	39.20323	-78.1597
18 [ab]	39.20915	-78.1525
19 [ab]	39.20327	-78.1539
20 [ab]	39.20313	-78.1542
21 [ab]	39.20222	-78.1533
22 [b]	39.21049	-78.1571
23 [b]	39.21196	-78.1583
24 [b]	39.20628	-78.1501
25 [b]	39.21222	-78.1563
26 [b]	39.20074	-78.1533
27 [b]	39.20060	-78.1535
28 [b]	39.20081	-78.1539

[a] plot used in 2019
[b] plot used in 2020
[c] plot destroyed partway through 2019, replaced by plot 21
[d] plot destroyed late 2019, was not replaced

Table 1.2 Total *Lycorma delicatula* observed per host species in Winchester, VA in 2019.

Host species	1st Instar	2nd Instar	3rd Instar	4th Instar	Adult	Egg Masses	Total # Observed*
Acer negundo	215	24	7	0	6	1	252
Acer platanoides	137	22	54	71	697	133	981
Acer saccharinum	1	2	15	7	339	184	364
Ailanthus altissima	255	151	1432	1516	8375	1238	11729
Alliaria petiolata	12	4	1	1	0	0	18
Allium spp.	0	0	0	0	0	0	0
Arctium spp.	3	0	0	0	0	0	3
Celtis occidentalis	169	66	68	30	84	285	417
Elaeagnus umbellata	2	4	4	0	1	1	11
Euonymus alatus	8	21	1	0	0	0	30
Fraxinus spp.	0	0	0	0	0	0	0
Gleditsia triacanthos	34	4	16	79	39	67	172
Hedera helix	3	1	0	0	2	0	6
Juglans nigra	44	5	0	1	10	7	60
Lonicera japonica	111	30	7	2	1	0	151
Lonicera spp.	743	183	99	68	87	0	1180
Morus alba	65	10	44	24	25	42	168
Parthenocissus quinquefolia	236	39	16	50	338	0	679
Prunus serotina	9	2	0	3	0	2	14
Prunus spp.	133	29	11	5	16	39	194
Quercus spp.	3	0	0	1	0	0	4
Robinia pseudoacacia	4	0	0	0	0	6	4
Rosa multiflora	89	45	80	0	0	0	214
Rubus occidentalis	21	1	0	0	0	0	22
Sassafras spp.	106	20	24	15	28	52	193
Tetradium daniellii	31	117	933	1921	935	0	3937
Toxicodendron radicans	5	2	0	2	0	0	9
Ulmus pumila	66	15	4	4	18	24	107
Ulmus spp.	116	16	0	0	0	0	132
Vitis spp.	72	121	944	572	598	0	2307
Grand Total	2693	934	3760	4372	11599	2081	23358

* Number of new egg masses were not included in total number of *Lycorma delicatula* observed

Table 2.2: Total *Lycorma delicatula* observed per host species in Winchester, VA in 2020.

Host species	1st Instar	2nd Instar	3rd Instar	4th Instar	Adult	Egg Masses	Total # Observed*
Acer negundo	10	1	0	0	0	0	11
Acer platanoides	69	17	9	12	904	57	1011
Acer saccharinum	93	40	9	4	133	92	279
Ailanthus altissima	1104	562	845	559	8264	961	11334
Alliaria petiolata	7	0	1	0	0	0	8
Castanea mollissima	113	3	1	3	7	5	127
Celtis occidentalis	409	54	46	26	55	424	590
Elaeagnus umbellata	3	9	2	0	1	1	15
Euonymus japonicus	20	0	0	0	1	0	21
Fraxinus spp.	74	36	19	16	25	47	170
Gleditsia triacanthos	15	15	12	11	31	91	84
Hedera helix	0	0	0	0	0	0	0
Juglans nigra	72	127	164	511	516	151	1390
Lonicera japonica	74	10	8	2	10	0	104
Lonicera spp.	474	177	108	47	50	0	856
Morus alba	46	4	7	0	3	32	60
Parthenocissus quinquefolia	300	208	83	121	325	0	1037
Prunus spp.	125	10	9	17	36	514	197
Pyrus calleryana	11	9	6	24	12	2	62
Quercus spp.	0	0	0	0	0	0	0
Robinia pseudoacacia	1	1	1	0	10	3	13
Rosa multiflora	68	4	9	30	0	0	111
Rubus occidentalis	11	26	14	4	2	0	57
Rubus phoenicolasius	1	2	3	1	0	0	7
Sassafras spp.	18	30	24	10	6	0	88
Tetradium daniellii	41	216	331	885	1184	15	2657
Toxicodendron radicans	68	3	2	1	2	0	76
Ulmus pumila	7	7	0	3	17	24	34
Vitis spp.	580	572	517	862	1971	1	4502
Grand Total	3814	2143	2230	3149	13565	2420	24901

* Number of new egg masses were not included in total number of *Lycorma delicatula* observed

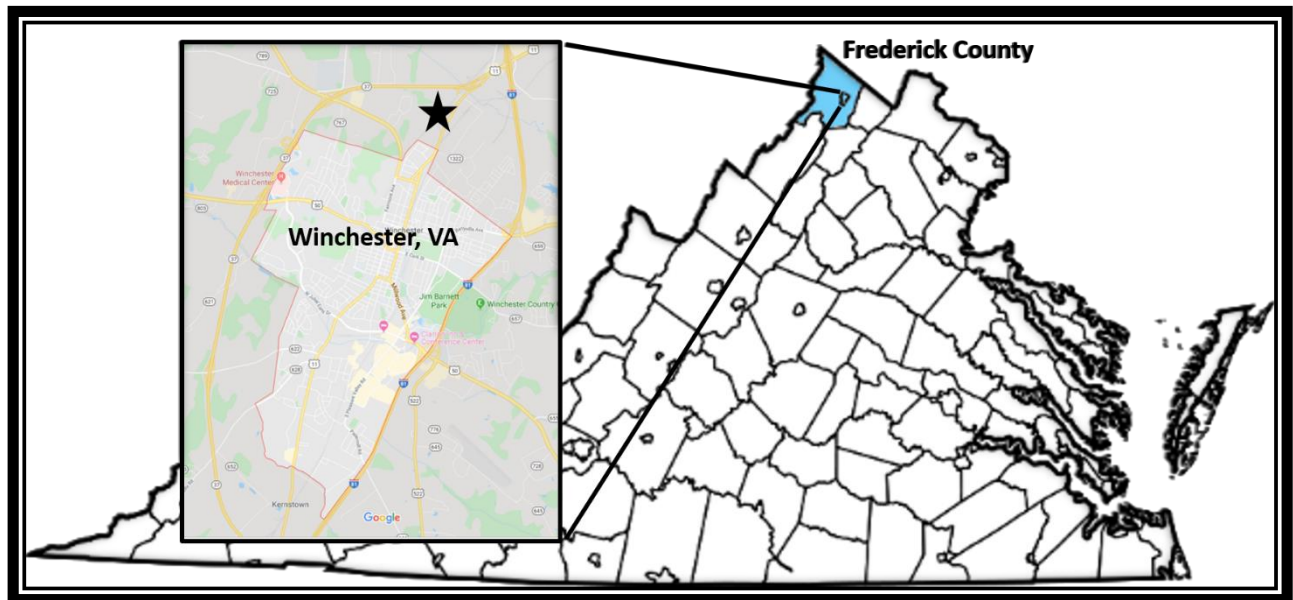**Figure 2.1:** *Lycorma delicatula* life stages.

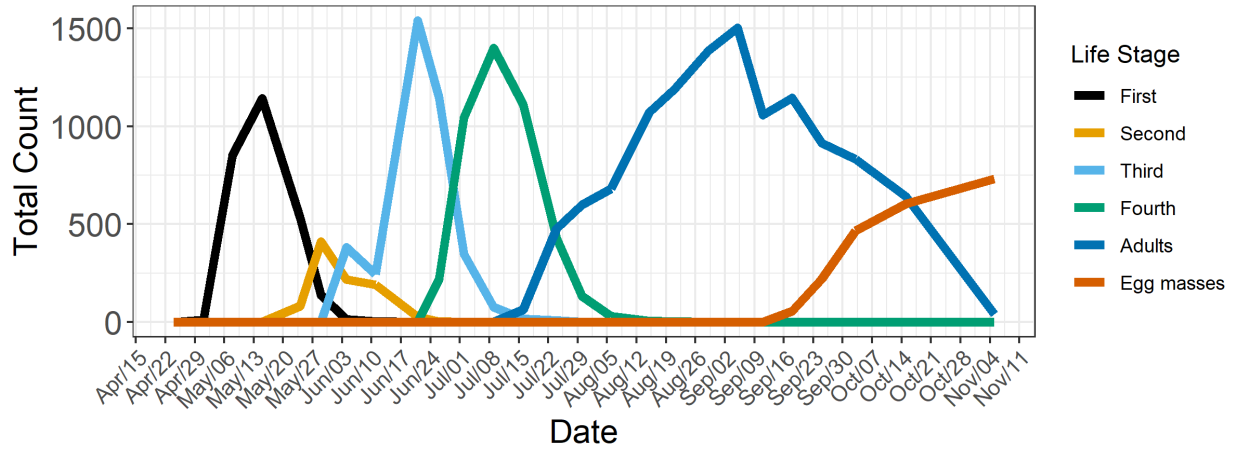**Figure 2.7**: A map showing *Lycorma delicatula* study site in Winchester, VA.

Figure 2.8: 2019 seasonal development of *Lycorma delicatula* in Winchester, VA.
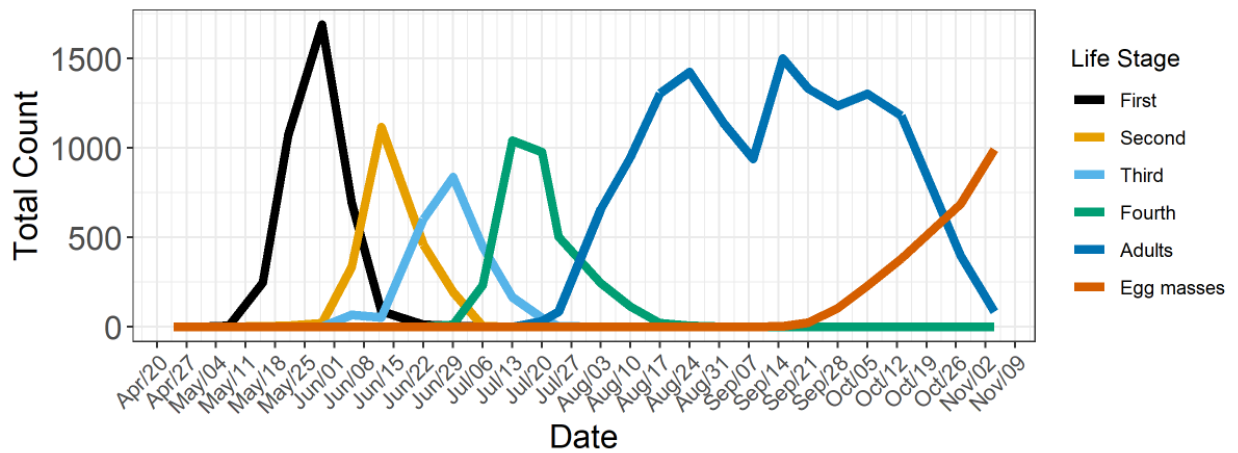
Figure 2.9: 2020 seasonal development of *Lycorma delicatula* in Winchester, VA.

43
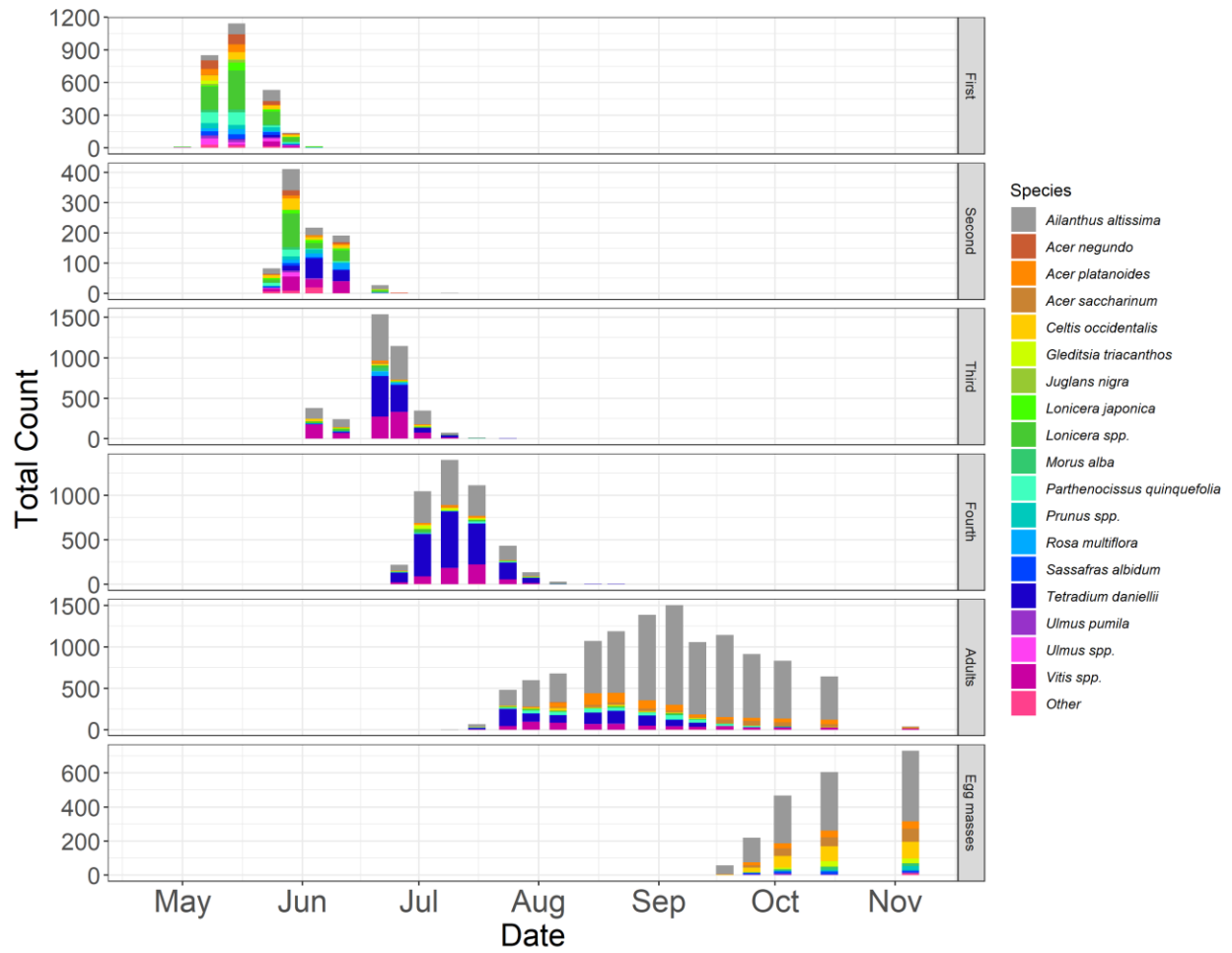
Figure 2.10: 2019 total counts of *Lycorma delicatula* life stage per host species. "Other" were host species that had fewer than 50 observed individuals in 2019.
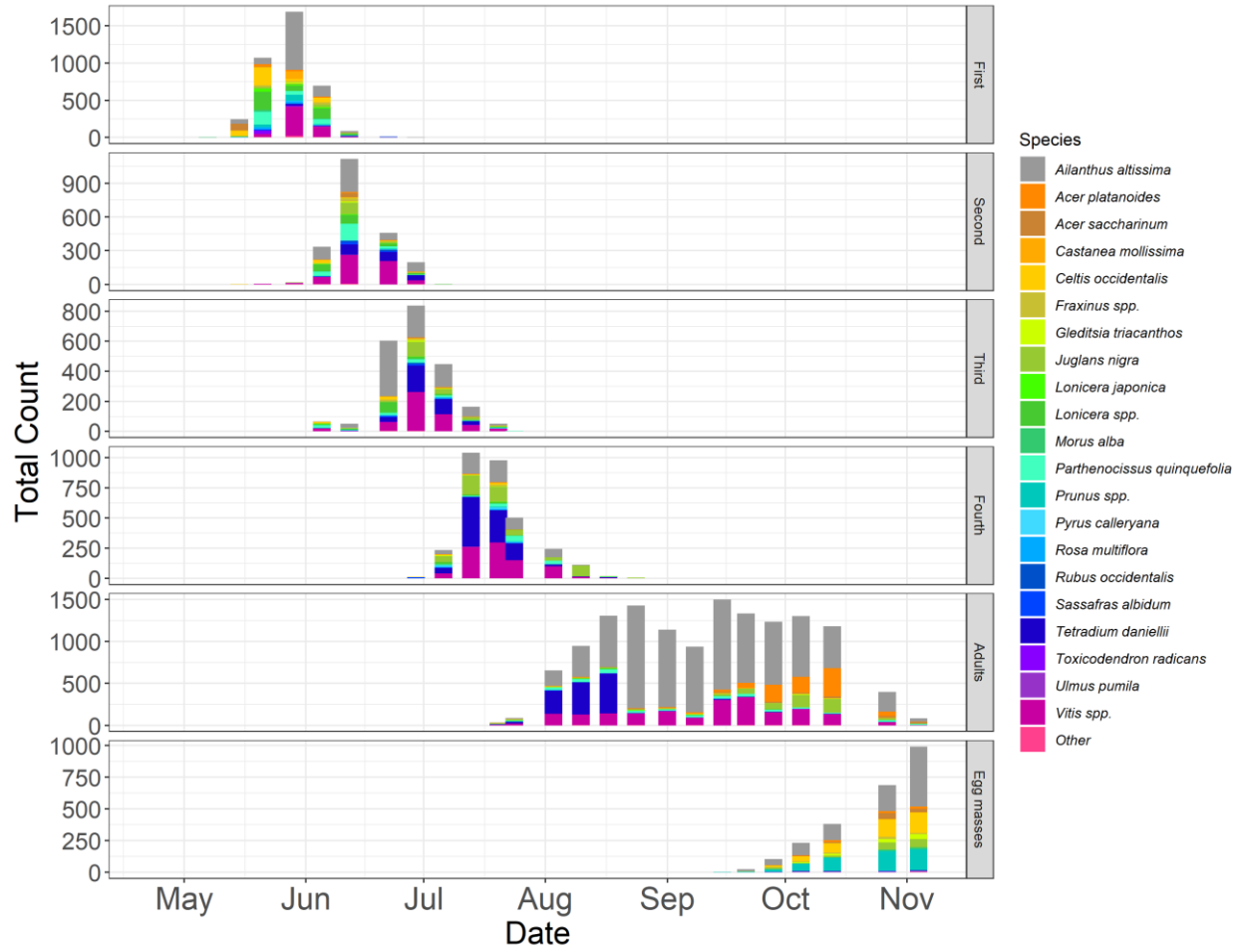
Figure 2.11: 2020 total counts of *Lycorma delicatula* life stage per host species. "Other" were host species that had fewer than 50 observed individuals in 2020.

Chapter 3: Using dendrochronology to quantify *Lycorma delicatula* (White) feeding impact to trees

Abstract

Lycorma delicatula (White) (Hemiptera: Fulgoridae) was first detected in the United States in Berks County, Pennsylvania in 2014. Native to China, this phloem-feeding planthopper has the potential to impact the agricultural, ornamental, nursery, and timber industries in its invaded range. Using tree cores and standard dendrochronological methods, I attempted to quantify *L. delicatula* feeding impact to four tree species in Pennsylvania: *Ailanthus altissima, Juglans nigra, Liriodendron tulipifera, and Acer rubrum*. I found evidence that suggests *L. delicatula* feeding reduces the annual growth of *A. altissima,* but not the annual growth in the other tree species. I also found evidence that suggests systemic insecticide treatment to *A. altissima* reduces the impact of *L. delicatula* on tree growth.

3.1 Introduction

Lycorma delicatula (White) (Hemiptera: Fulgoridae), also known as the spotted lanternfly, is an insect species native to China that was first detected in the United States in 2014 in Berks County, Pennsylvania (Dara et al. 2015). *L. delicatula* is a phytophagous, phloem-feeder with over 100 identified host species worldwide and 56 host species confirmed in North America, although this list will likely increase dramatically as this invasive species spreads (Barringer and Ciafré 2020). As a phloem-feeder, *L. delicatula* has the potential to cause high economic and ecological impact (Finley and Chhin 2016).

In Pennsylvania, *L. delicatula* has proven to be a major pest to grapevines. Some vineyards in the heart of the infestation zone that have experienced repeated seasons of high pest pressure from *L. delicatula* have reported up to 90% yield loss and the number of insecticide applications have tripled (Urban 2020). Studies have shown that insecticides are effective at killing *L. delicatula*, but immigration of adult insects continue to invade from forests and vegetation surrounding the vineyard in late summer (Leach et al. 2019, Leach and Leach 2020).

It is believed that *L. delicatula* is largely feeding and developing through their life cycle on forest tree species. Forest stands in the mid-Atlantic US are often composed of mixed hardwood species, many of which are suitable hosts for *L. delicatula* (Stolte et al. 2012, Barringer and Ciafré 2020). Among them are important ecological and timber species such as black walnut (*Juglans nigra* L.), maples (*Acer* L.), oaks (*Quercus* L.), and tulip poplar (*Liriodendron tulipifera* L.). For example, in the previous chapter, *L. delicatula* phenology was recorded in Winchester, Virginia from observations of insects feeding on a total of 33 host species, including many trees (See Table 2.2 & 2.3) It has been estimated that the economic impact of *L. delicatula* feeding on forest trees could reach $152.6 million USD annually in Pennsylvania alone (Harper et al. 2019).

Tree-of-heaven, *Ailanthus altissima* (Miller) Swingle (Sapindales: Simaroubaceae), is a tree species native to China that was introduced into the United States in the late 1700's (Kowarik and Säumel 2007).

48

Since *L. delicatula's* introduction, it has become very apparent that this insect displays a preference for *A. altissima*. A recent study showed that *L. delicatula* does not require this tree to complete its development, but it does make up a major component of their diet and they develop best on a combination of *A. altissima* and other plant species (Uyi et al. 2020).

Given their broad host range and the absence of effective natural enemies in North America (Dara et al. 2015), *L. delicatula* populations have exploded, widening their infested range. The impact *L. delicatula* is having on forest tree species in infested areas is not yet known. Some studies have reported the insect's ability to severely damage or kill a tree (Cooperband et al. 2019), but to our knowledge there has not been a formal study quantifying the impact of *L. delicatula* feeding on trees.

Most studies of *L. delicatula* have focused on morphology, reproduction, impact to vineyards, management, host range, distribution suitability, and economic impact. Very few have looked into the effect of *L. delicatula* on the radial growth of host trees. Dendrochronology, the study of using annual tree rings to date past events (Fritts 1976), has been routinely used to identify past defoliation events and beetle outbreaks in forests throughout the United States (Morrow and Lamarche 1978, Hogg et al. 2002, Huang et al. 2008). However, methods for determining damage to trees induced by phytophagous phloem-feeding hemipteran insects have not been well documented in the literature (Yang and Karban 2009). This study was conducted to determine if established dendrochronological methods could be used to quantify *L. delicatula* feeding injury to host trees.

Phloem is primarily comprised of carbohydrates and is low in available nitrogen (mainly amino acids) that are needed for the production of proteins; thus, *L. delicatula* needs to ingest large amounts of phloem sap in order to acquire these important compounds (Gamper 2012). Large aggregations of *L. delicatula* feeding on a tree effectively remove large quantities of important nutrients from the tree that were made during photosynthesis.

3.2 Materials and methods

3.2.1 Study area

To investigate the impact of heavy *L. delicatula* feeding on the radial growth of trees, I collected samples from two sites in Pennsylvania where *L. delicatula* has been established since 2016 (Figure 3.01).

3.2.2 Montgomery County, PA Site

On 7 January 2020, tree cores were collected from locations in Pennsburg, Upper Hanover Township of Montgomery County, Pennsylvania (40.36672, -75.54746). This area of PA has experienced large populations of *L. delicatula* since 2016. With the help of a Penn State Extension agent, tree cores were collected from *A. altissima* (n=10)*, Acer rubrum* L. (n=8)*, Juglans nigra* L. (n=8)*,* and *Liriodendron tulipifera* L. (n=5) that were annually observed to have high populations of *L. delicatula* feeding on them since 2016.

3.2.3 Berks County, PA Site

On 5 March 2020, *A. altissima* tree cores were collected from Blue Marsh Lake Recreation Area located in northwest Berks County, Pennsylvania (40.380709, -76.028454). Blue Marsh is maintained by the Philadelphia District U.S. Army Corps of Engineers. *L. delicatula* was initially discovered at Blue Marsh in 2016. In 2018 and 2019 high populations of *L. delicatula* were observed and led managers to treat some, but not all, *A. altissima* on Blue Marsh property. Individual trees selected for insecticide treatment were solely based on observed high infestations of *L. delicatula* on those particular trees. Treated trees were sprayed until runoff with the systemic insecticide dinotefuran (Transtect 70 WSP Insecticide, Rainbow Treecare Scientific Advancements, Minnetonka, MN) as a basal bark application at 37.34 g AI/liter from the ground to 30-38 cm on the trunk and 360 degrees around the tree.

Some *A. altissima* were treated in 2018 and 2019 (two years of insecticide treatment), some were treated only in 2019 (one year of insecticide treatment), and many were left untreated. The initial treatment date in 2018 was 6 October. The same trees were then treated a second time on 26 July 2019.

Trees with only one year of insecticide treatment were sprayed on 16 August 2019. Ten cores were collected from each treatment for a total of thirty *A. altissima* cores.

3.2.4 Core collection and laboratory processing

All trees were cored at breast height using a Jim-Gem 35 cm increment borer (Forestry Suppliers, model 63084, Jackson, MS, USA) with a core diameter of 5.15 mm. Extracted cores, were immediately placed in labeled plastic straws. The straws had holes punched in them to allow the cores to remain straight while drying. Cores were air-dried on a baking sheet at room temperature for two weeks.

Once dried, cores were processed using standard dendrochronological methods (Stokes and Smiley 1968). Cores were removed from the straws and individually mounted at the correct orientation to 25 cm wood blocks with a grove cut down the center to accommodate the core. The grove was approximately 2 mm deep, allowing at least 50% of the core to remain exposed. The exposed surface of each core was then sanded with 220 grit sand paper using a random orbital sander (Dewalt model DWE6420, Baltimore, MD, USA) for approximately 10-15 seconds to create a flat working surface. Each core was then sanded with progressively finer grit paper (320, 400, and 1500, respectively) for two minutes per core. This was done to remove scratches from the previous grit and create a prepared surface with clearly defined rings and wood cells for dating and measurement under the scope (Phipps 1985).

3.2.5 Core measurement

Tree cores were visually cross-dated using the list method by matching narrow rings between cores to ensure accurate dating (Yamaguchi 1991). For cores that were collected in Montgomery County, PA, ring widths were measured to the nearest 0.01 mm using a dissecting microscope and Velmex measuring system. A sliding-stage was incrementally moved via a small crank and a crosshair in the microscope was used to visually delimit the ring boundaries when taking measurements. The sliding-stage micrometer was connected to a computer and measurements were recorded in MeasureJ2X software (VoorTech Consulting, Holderness, NH, USA).

Due to university building access restrictions as a result of COVID-19, *A. altissima* cores collected from Berks County, PA were measured digitally. Cores were placed under a dissecting scope equipped with a 9-megapixel digital camera (AmScope SKU: MU900, Irvine, CA, USA) connected to a computer. Scope calibration and measurements were collected on-screen using AmScope software (version x64, 3.7.7303, Irvine, CA, USA). Calibration was done at 1x zoom using a 0.01 mm stage micrometer (AmScope SKU: MR096, Irvine, CA, USA). All ring widths were measured to the nearest 0.01 mm

Once ring widths were measured for all cores, the computer program COFECHA was used to statistically evaluate the accuracy of dated cores (Holmes 1983, Grissino-Mayer 2001). COFECHA applies a 32-year cubic smoothing spline across all the data to create a master chronology for each site and species (Speer 2010). It then compares each individual tree core series to the master chronology by splitting it into 50-year segments and uses 25 years of overlap to calculate the series inter-correlation for that site and species (Speer 2010). Any flags or potential errors identified by COFECHA were investigated and corrected. If any dating errors could not be resolved, that specific core was excluded from further analysis.

3.2.6 Data analysis

I wanted to compare pre- and post- *L. delicatula* infestation years to determine if there were detectable differences in radial tree growth. There was observational conformation of high *L. delicatula* populations in this area beginning in 2016, suggesting there was at least a partial population in this area in 2015. For the purposes of this research, the distinction between pre- and post- *L. delicatula* infestation is defined here as the year 2015. Thus, ring widths associated with the years 2015 – 2019 were considered infested rings. All tree growth prior to 2015 was considered uninfested rings.

To perform the analysis, I used paired *t* tests to compare radial growth of infested years (2015 – 2019) to uninfested years (2010 – 2014) for each site and species. I also compared the radial growth of earlier uninfested years (2005 – 2009) to the uninfested years (2010 – 2014) to act as a control.

As trees grow, ring width tends to decrease (Swetnam et al. 1985). This is a result of the tree annually putting on wood to a growing circumference. The goal of standardization in dendrochronology is to remove the age and size dependence so trees of differing ages can be compared to one another (Veblen et al. 1991). Standardizing ring-width chronologies has shown to be useful in understanding the impacts of insects, climate, and other various environmental pressures on tree growth (Swetnam et al. 1985). There are various methods for standardizing ring-width chronologies depending on the data and hypothesis being tested. Standardizing is done by plotting raw-ring width as a function of time and then fitting a curve to the data (Swetnam et al. 1985). The raw-ring width value is then divided by the fitted curve value for each measurement, resulting in a dimensionless ring-width index (RWI) with average growth centered around one (Swetnam et al. 1985). If the master chronology's RWI is greater than one, greater than average growth occurred for that year and vice versa for RWI values that are less than one. The negative exponential curve is typically considered the most conservative method and widely used by dendrochronologists to help identify insect outbreaks and climate events dating hundreds of years into the past (Cook and Kairiukstis 1990).

Raw-ring width chronologies were standardized by fitting a negative exponential curve to the data using the computer program ARSTAN (Cook and Holmes 1986). If the negative exponential curve did not fit, a horizontal line through the mean was used for standardization (Veblen et al. 1991, 1992). Ring-width indices were summed for all cores to form a master chronology for each site and species. Data were imported into R (R Core Team 2018), where the distribution was checked for normality using the Shapiro Wilk test. If normality was met, a standard paired *t* test was performed. If normality was not met, the non-parametric paired Wilcoxon test was calculated from the RWI to determine if there was a significant difference in tree growth pre- and post- *L. delicatula* invasion (Pichler and Oberhuber 2007).

3.2.7 Climate variables and tree growth

Climate variables can also influence tree growth (Cook and Kairiukstis 1990, Hogg et al. 2002, Girardin et al. 2008, Speer 2010). Here, I attempted to remove the strongest correlated climate variable in order to strengthen and clarify the possible *L. delicatula* feeding signal in the wood. Each state in the lower 48 has been divided into various climate divisions. Each division has a network of sampling stations that collect data from multiple climactic variables. The data from all the sampling stations are averaged together to form a monthly divisional dataset for each year going back to 1895 (Guttman and Quayle 1996). Our sampling sites were both located in Pennsylvania climate division three, the Southeastern Piedmont (National Centers for Environmental Information 2020).

The climate divisional dataset for Pennsylvania division three was obtained from the National Oceanic and Atmospheric Administration (NOAA) databank (https://catalog.data.gov/dataset/noaas-climate-divisional-database-nclimdiv). This dataset consisted of monthly averages for minimum temperature, maximum temperature, average temperature, precipitation, and Palmer Drought Severity Index (PDSI) from 1895 – 2019.

To perform a climate response analysis, dominant climate variables that affected tree growth were identified using a correlation matrix in Excel. I compared each site and species standardized chronology to each climactic parameter for PA climate division three (Speer et al. 2010). Once the dominant climate variable was identified, all data for that variable was divided by its average to create a dimensionless climate index. To normalize by climate and to remove the dominant climate signal, the climate index was subtracted from the standardized chronology (RWI) for each site and species. Paired *t* tests were then calculated to determine if there was a significant difference in tree growth pre- and post- *L. delicatula* infestation.

3.3 Results

3.3.1 Montgomery County, PA

3.3.1.1 *Ailanthus altissima*

Ailanthus altissima chronologies obtained from Montgomery County were relatively young and ranged in length from 16 to 48 years. A single chronology did not correlate well with the other series' and was removed from the analysis. All other chronologies correlated well with the master chronology in COFECHA with an interseries correlation of 0.483 (Table 3.1, Figure 3.02).

I attempted to standardize *A. altissima* chronologies by fitting a negative exponential curve to the raw ring-width data, but later year growth was close to zero and unrealistically skewed the RWI's. Therefore, to equalize the variance across series, I standardized *A. altissima* chronologies by fitting a horizontal line through the mean and the distribution of RWIs were then shown to be approximately normal. Using a Students' paired *t* test, a difference in RWI was found between pre- and post-infested years, with infested years having significantly smaller RWI's compared to uninfested years (t = 4.0634, df = 4, p-value = 0.01531). I then compared two preceding periods of uninfested years prior to *L. delicatula* invasion. Again, using the Students' paired *t* test, there was no significant difference in RWI between the two periods of uninfested years (t = 2.4107, df = 4, p-value = 0.0735).

For a two-tailed correlation with a sample size of 47 years at a confidence level of 0.05, the critical value for Pearson's correlation coefficient was identified as 0.2816 (Rohlf and Sokal 1995). All climactic variables were correlated with the standardized *A. altissima* chronology and the September average temperature had the highest negative correlation of -0.576. A linear regression was calculated for September average temperature versus the standardized chronology (Figure 3.03). The regression showed about 33% (R^2 = 0.3314, p-value < 0.05, n = 47) of the tree's reduced growth could be attributed to September average temperature. After subtracting the normalized climate index from the standardized chronology, a Student's paired *t* test showed a significant reduction in growth after *L. delicatula*

infestation (t = 4.4239, df = 4, p-value = 0.01148). The two periods of uninfested years prior to *L. delicatula* invasion did not show a significant difference (t = 2.3663, df = 4, p-value = 0.07713; Figure 3.04).

3.3.1.2 *Juglans nigra*

Juglans nigra chronologies collected from Montgomery County contained series' ranging from 26 to 81 years. Dating discrepancies from three tree series that did not correlate well with the master chronology calculated in COFECHA could not be resolved and were excluded from further analysis. The remaining five series had a satisfactory interseries correlation of 0.307 (Table 3.1, Figure 3.02).

To maintain consistency for the Montgomery County site, all *J. nigra* series were standardized in ARSTAN by fitting a horizontal line through the mean to equalize variance across series and the RWI distribution was shown to be approximately normal. A paired student's *t* test did not show a significant difference in RWI between infested versus uninfested years (t = 0.67804, df = 4, p-value = 0.5349). However, there was a significant difference in RWI between uninfested time periods prior to *L. delicatula* invasion (t = 4.6143, df = 4, p-value = 0.009924).

For a two-tailed correlation with a sample size of 81 years at a confidence level of 0.05, the critical value for Pearson's correlation coefficient was identified as 0.2159 (Rohlf and Sokal 1995). All climate variables were correlated with the standardized chronology for *J. nigra* and September minimum temperature had the largest negative correlation of -0.262. A linear regression was calculated for September minimum temperature versus the standardized chronology (Figure 3.05). The regression showed that about 7% (R^2 = 0.0687, p-value < 0.05, n = 81) of the tree's reduced growth could be attributed to September minimum temperature. After subtracting the normalized climate index from the standardized chronology, a student's paired *t* test showed no significant reduction in growth after *L. delicatula* infestation (t = 2.0557, df = 4, p-value = 0.109). However, the two uninfested time periods prior to *L. delicatula* invasion did shown a significant reduction in RWI when the dominant climate variable was removed from the chronology (t = 3.5586, df = 4, p-value = 0.02361; Figure 3.06).

3.3.1.3 *Liriodendron tulipifera*

Liriodendron tulipifera chronologies collected from Montgomery county consisted of series ranging from 17 to 40 years. All series correlated well with the master chronology calculated in COFECHA and had an interseries correlation of 0.529 (Table 3.1, Figure 3.02).

Liriodendron tulipifera was standardized in ARSTAN by fitting a horizontal line through the mean and RWI distribution was shown to be approximately normal. A student's paired *t* test did not show a significant difference in RWI between infested versus uninfested years (t = -2.3516, df = 4, p-value = 0.07838). Similarly, there was no significant difference in RWI when comparing the two uninfested time periods (t = 2.0495, df = 4, p-value = 0.1098).

For a two-tailed correlation with a sample size of 40 years at a confidence level of 0.05, the critical value for Pearson's correlation coefficient was identified as 0.3008 (Rohlf and Sokal 1995). All climate variables were correlated with the standardized chronology for *L. tulipifera* and July maximum temperature had the largest negative correlation of -0.474. A linear regression was calculated for July maximum temperature verses the standardized chronology (Figure 3.07). The regression showed that about 22% (R^2 = 0.2251, p-value < 0.05, n = 40) of the trees reduced growth could be attributed to July maximum temperature. After subtracting the normalized climate index from the standardized chronology, a student's paired *t* test showed a significant reduction in growth of *L. tulipifera* after *L. delicatula* infestation (t = -2.9614, df = 4, p-value = 0.0415). However, there was no significant difference in the two uninfested time periods when the dominant climate variable was removed (t = 2.2884, df = 4, p-value = 0.084; Figure 3.08).

3.3.1.4 *Acer rubrum*

Acer rubrum chronologies obtained from Montgomery County had a high degree of variation in ring width and ranged from 20 to 152 years. None of the seven trees sampled correlated well with the master chronology created in COFECHA (Table 3.1, Figure 3.02). I revisited the cores to determine if mistakes

were made during dating, but could not find any apparent discrepancies. Since I could not confidently say these tree cores were dated correctly, they were ultimately excluded from further analysis.

3.3.2 Blue Marsh Recreation Area, Berks County, PA

3.3.2.1 *Ailanthus altissima*

Ailanthus altissima samples collected from Blue Marsh were relatively young and ranged from 5 to 37 years. A total of eight cores did not date well with the master chronology. Discrepancies in the wood could not be identified and the cores were removed from further analysis. All other series dated well in COFECHA with an interseries correlation of 0.485 (Table 3.1, Figure 3.02). To remain consistent, I standardized Blue Marsh *A. altissima* chronologies by fitting a horizontal line through the mean and the distribution of RWIs were found to be approximately normal.

To identify the dominant climate variable influencing tree growth at the Blue Marsh site, all data, including different treatments, were run in a correlation matrix together. For a two-tailed correlation with a sample size of 38 years at a confidence interval of 0.05, the critical value for Pearson's correlation coefficient was identified as 0.312 (Rohlf and Sokal 1995). All climate variables were correlated against the standardized chronology for *A. altissima* and it was found that June and July average temperature had the largest negative correlation at -0.520 and -0.447, respectively. A linear regression was calculated for June and July average temperature versus the standardized chronology (Figure 3.09). The regression showed that about 32% (R^2 = 0.3226, p-value < 0.05, n = 38) of reduced tree growth could be attributed to June and July average temperature. After subtracting the normalized climate index from the standardized master chronology, the data were broken up into treatment for analysis using Students' paired *t* test (zero, one, and two years of insecticide treatment respectively).

3.3.2.2 No insecticide treatment

A Students' paired *t* test showed there was a significant difference in RWI between pre- and post-infested years, with infested years consisting of smaller RWIs compared to uninfested years (t = 3.9575,

df = 4, p-value = 0.01671). However, when I compared two preceding time periods of uninfested years prior to *L. delicatula* invasion, no significant difference in RWI was identified (t = 1.2411, df = 4, p-value = 0.2824).

After accounting for the dominant climate variables, *A. altissima* without insecticide treatment showed a significant reduction in RWI after *L. delicatula* introduction (t = 3.5129, df = 4, p-value = 0.02461). However, no significant difference in RWI was found when I compared the two preceding periods of uninfested years prior to *L. delicatula* invasion (t = 1.3079, df = 4, p-value = 0.261; Figure 3.10).

3.3.2.3 One year of insecticide treatment

After one year of insecticide treatment, using a Students' paired *t* test, there was no significant difference in RWI between pre- and post-infested years (t = -0.14744, df = 4, p-value = 0.8899). Similarly, when I compared two preceding time periods of uninfested years prior to the introduction of *L. delicatula*, there was no significant difference between RWI (t = 1.6672, df = 4, p-value = 0.1708).

After accounting for the dominant climate variables, *A. altissima* that received one year of insecticide treatment did not show a significant reduction in RWI after the introduction of *L. delicatula* (t = -0.26362, df = 4, p-value = 0.8051). Similarly, no significant difference in RWI was found when I compared the two preceding periods of uninfested years prior to *L. delicatula* invasion (t = 1.8175, df = 4, p-value = 0.1433; Figure 3.11).

3.3.2.4 Two years of insecticide treatment

After two consecutive years of insecticide treatment, using a Students' paired *t* test, there was no significant difference in RWI between pre- and post-infested years (t = -2.3156, df = 4, p-value = 0.07838). When two preceding time periods of uninfested years prior to *L. delicatula* invasion were compared, no significant difference in RWI was detected (t = 2.0495, df = 4, p-value = 0.1098).

After accounting for the dominant climate variables, *A. altissima* that received two years of insecticide treatment did not show a significant reduction in RWI post *L. delicatula* invasion (t = -2.6123, df = 4, p-

value = 0.05928). Similarly, no significant difference in RWI was found when I compared the two preceding periods of uninfested years prior to *L. delicatula* invasion (t = 2.1527, df = 4, p-value = 0.09768; Figure 3.12).

3.4 Discussion

3.4.1 *Lycorma delicatula* impact on *Ailanthus altissima*

I found evidence suggesting that *L. delicatula* reduces the annual growth of *A. altissima*. This impact was seen in the reduction on radial growth in *A. altissima* tree rings at both field sites post *L. delicatula* invasion excluding insecticide treated trees. Beginning in 2015 and continuing to 2019, a severe suppression event was evident in the data and I propose it was the result of *L. delicatula* feeding. Furthermore, the difference in radial growth was not observed in the two preceding five-year time periods prior to *L. delicatula* invasion nor on the insecticide-treated *A. altissima*.

Similar impacts to trees have been reported in related systems. Research conducted in Mexico used dendrochronological methods and found that a phloem-feeding scale insect, *Stigmacoccus garmilleri* Foldi*,* negatively effects the growth of oak trees at increasing densities (Gamper 2012). *Adelges tsugae* Annand (hemlock woolly adelgid) is an invasive insect that feeds in high numbers on *Tsuga canadensis* L. (eastern hemlock) in the eastern United States. Although *A. tsugae* feeds on the tree's xylem, dendrochronological research has shown that *T. canadensis* exhibits a sharp reduction in growth immediately following *A. tsugae* infestation (Walker et al. 2014). Tree-ring analysis has also shown that increasing densities of periodical cicada, a xylem feeder, can negatively affect growth in many tree species (Yang and Karban 2009, Speer et al. 2010).

Trees cored at these sites were relatively young (age < 30 years) compared to those generally used in dendrochronology to identify past insect outbreaks and climate events. However, *L. delicatula's* introduction to this system is very recent and the need to look hundreds of years into the past is unnecessary. Also, given *L. delicatula's* apparent preference to *A. altissima* (Han et al. 2008, Park et al.

2009, Dara et al. 2015, Lee et al. 2019), which is a relatively short-lived tree (Kowarik and Säumel 2007), these are a useful indicator to use for this study. To account for these trees' age limitations, and the potential reduced growth from *L. delicatula* that might drive a negative exponential fit to zero, thus under representing the impact, I fit a horizontal line through the mean to preserve the year-to-year variation while still allowing comparison of different trees (Veblen et al. 1991, 1992). This allowed us to be able to account for a younger tree's more vigorous growth rate.

Reduced tree growth driven by climate instead of *L. delicatula* was accounted for by the normalization and subtraction of the dominant climactic variable from the standardized master chronology that was affecting the growth of *A. altissima* for each site. As climate is often a main driver of tree growth (Girardin et al. 2008), this allowed us to minimize the influence of climate on our comparisons. Additional tree cores from more *A. altissima* as well as a non-*L. delicatula* host species would help confirm this in future studies (Rolland et al. 2001).

Not all observed variation for *A. altissima* RWI seen in the master chronology from Upper Hanover Site (Figure 3.13) can be attributed to *L. delicatula* feeding. This is not surprising because there are many variables that affect tree growth (Girardin et al. 2008). For example, a suppression in growth can be seen in the data beginning in 2007 (Figure 3.13). This suppression event occurred before the introduction of *L. delicatula* and therefore cannot be attributed to their feeding. During the summer and fall of 2007 in the mid-Atlantic region, severe drought affected the area where our sampling sites were located (NOAA National Centers for Environmental Information 2007). This drought potentially caused the reduction in growth for the following years as the trees recovered.

3.4.2 Insecticide treatment minimized *Lycorma delicatula* impact to *Ailanthus altissima*

I found evidence that suggests treating *A. altissima* with the insecticide dinotefuran reduces the impact of *L. delicatula* on tree growth. This difference was tested at the Blue Marsh site where *A. altissima* treated with insecticide did not exhibit reduced growth post- *L. delicatula* infestation, while those left

untreated showed reduced ring width. Since systemic insecticides are effective at quickly killing large numbers of *L. delicatula* (Leach et al. 2019, Lee et al. 2019), this study essentially compares *A. altissima* trees that experienced sustained feeding by *L. delicatula*, to *A. altissima* trees where feeding was quickly halted. I was therefore able to compare radial growth between *A. altissima* over the same time period and location with the only difference being heavy *L. delicatula* feeding influenced by insecticide treatment.

In 2010, the efficacy of dinotefuran at controlling armored scales in Christmas tree plantations was explored in Berlin, Connecticut (Cowles 2010). Cowles concluded that a sprayed basal bark application of dinotefuran up to 25 cm on the tree provided good efficacy at controlling scale populations while not significantly impacting natural enemies (Cowles 2010). Similarly, a study looking at the effect of various insecticides for the control of emerald ash borer was conducted over a six-year period in Michigan beginning 2007 (McCullough et al. 2019). They found that annual basal bark treatments of dinotefuran were effective at keeping larval densities low enough to prevent damage to ash trees (McCullough et al. 2019).

In its invaded range in North America, there are very few arthropod enemies associated with *A. altissima* (Ding et al. 2006). *Atteva aurea* (Cramer), the Ailanthus webworm, has been reported as a non-native herbivore to *A. altissima*, but severe damage has only rarely been documented on seedlings and young saplings (Ding et al. 2006). All trees sampled in this study measured greater than 25 cm DBH. Therefore, it is unlikely that an additional herbivore of *A. altissima* was responsible for the decreased growth observed in untreated trees at Blue Marsh. This may indicate, where warranted, that treating high value trees, such as timber or ornamental species, may help reduce the impacts of *L. delicatula*

3.4.3 *Lycorma delicatula* impact to other host species

I did not find any evidence suggesting *L. delicatula* significantly reduced the growth of *J. nigra* using dendrochronological methods. *Juglans nigra* had sample chronologies that correlated well with their

master chronology in COFECHA indicating they were accurately dated (Table 3.1). I did detect a significant reduction in growth for *J. nigra* between the two preceding time periods prior to *L. delicatula* introduction. This growth suppression appears to have begun in 2010 (Figure 3.14), and may be the result of a late season drought experienced in the sampling area. A similar decrease in growth can be seen in the master chronology of *A. altissima* from Upper Hanover, but did not appear to effect significance of *A. altissima*.

Liriodendron tulipifera also had cores that correlated well with their master chronology in COFECHA (Table 3.1). Once the dominant climate factor of July maximum temperature for *L. tulipifera* was removed, there was evidence suggesting a significant increase in growth occurred after *L. delicatula* invasion (Figure 3.15). This phenomenon could be evidence that some species are benefiting from the *L. delicatula* invasion. Yang (2004) tested a hypothesis where he looked at the effect of periodical cicada (*Magicicada* spp. Davis) density on the growth of American bellflower (*Campanulastrum americanum* L.) (Yang 2004). He enriched the soil of American bellflowers with different densities of periodical cicada carcasses that resulted in larger seed, leaves, and higher nitrogen concentration in leaves compared to controls (Yang 2004, Speer et al. 2010).

Impact of *L. delicatula* to *J. nigra* and *L. tulipifera* may still be occurring, despite no impact being detected using our methods. For example, both *L. tulipifera* and *J. nigra* are often less infested than *A. altissima* (authors observation), and therefore growth impacts may not become detectable in just five years. Additionally, since there were a large number of *A. altissima* at this Upper Hanover Site, *L. delicatula* impact may have been focused on *A. altissima*, while a site excluding *A. altissima* may have *L. tulipifera* and *J. nigra* show impact. This difference in impact level between *A. altissima* versus *L. tulipifera* and *J. nigra* could be explained as well because *L. delicatula* feeds throughout its life cycle on *A. altissima*, while reports of them feeding on *J. nigra* and *L. tulipifera* are limited to earlier life stages. A larger sample size that includes a diversity of different sites could help clarify if *L. delicatula* does impact growth in non-*A. altissima* tree hosts and ensure I was not just looking at trees that had escaped herbivory. Additionally,

since *L. delicatula* are often found feeding in the canopy of trees, stem analysis of canopy branches may provide useful information in future studies (Stephens and Westoby 2015).

The samples collected from *Acer rubrum* in Montgomery County, PA had a large amount of year-to-year variation and the interseries correlation from COFECHA was below an acceptable threshold. I therefore excluded these samples from our analysis. These trees sampled were all growing in edge habitat and subject to many factors that could contribute to abnormal growth patterns such as, watering, fertilizer, herbicide, and root compaction from vehicles. *Acer rubrum* is also a very difficult species to date accurately. The diffuse porous wood does not exhibit clear boundaries between early and late season growth as is seen in ring porous species (Speer 2010). Additional sampling and very careful crossdating of *A. rubrum* from numerous infested sites could account for this variation.

3.5 Conclusion

It appears that dendrochronology can be used to identify and quantify long-term *L. delicatula* feeding injury to certain trees such as *A. altissima*, supporting the use of these methods with other phloem feeders or invasive tree-feeding herbivores. However, standard dendrochronology methods may not be the most effective way to identify a feeding signal and study the long-term impacts of *L. delicatula* on *J. nigra* and *L. tulipifera*, as I was unable to quantify impact to those species. It is also possible that these tree species are simply not as affected by *L. delicatula* feeding. Basal insecticide applications of dinotefuran appear to reduce and prevent damage to trees that experience heavy feeding from *L. delicatula.*

3.6 Acknowledgments

3.7 References

Barringer, L., and C. M. Ciafré. **2020**. Worldwide feeding host plants of spotted lanternfly, with significant additions from North America. Environ. Entomol. 49: 999–1011.

Cook, E. R., and R. L. Holmes. **1986**. ARSTAN: Guide for computer program ARSTAN, pp. 50–65. *In* Users Man. Progr. ARSTAN, Tree-Ring Chronol. West. North Am. California, East. Oregon North. Gt. Basin. Laboratory of TreeRing Research, The University of Arizona, Tucson, Arizona.

Cook, E. R., and L. A. Kairiukstis. **1990**. Tree-ring standardization and growth-trend estimation, pp. 104–123. *In* Cook, E.R., Kairiukstis, L.A. (eds.), Methods Dendrochronology. Appl. Environ. Sci. Springer-Science+Business Media, B.V.

Cooperband, M. F., J. Wickham, K. Cleary, S. E. Spichiger, L. Zhang, J. Baker, I. Canlas, N. Derstine, and D. Carrillo. **2019**. Discovery of three kairomones in relation to trap and lure development for spotted lanternfly (Hemiptera: Fulgoridae). J. Econ. Entomol. 112: 671–682.

Cowles, R. S. 2010. Optimizing a basal bark spray of dinotefuran to manage armored scales (Hemiptera: Diaspididae) in Christmas tree plantations. J. Econ. Entomol. 103: 1735–1743.

Dara, S. K., L. Barringer, and S. P. Arthurs. **2015**. *Lycorma delicatula* (Hemiptera: Fulgoridae): A new invasive pest in the United States. J. Integr. Pest Manag. 6: 1–6.

Ding, J., Y. Wu, H. Zheng, W. Fu, R. Reardon, and M. Liu. **2006**. Assessing potential biological control of the invasive plant, tree-of-heaven, *Ailanthus altissima*. Biocontrol Sci. Technol. 16: 547–566.

Finley, K., and S. Chhin. **2016**. Forest health management and detection of invasive forest insects. Resources. 5: 18.

Fritts, H. C. 1976. Tree Rings and Climate. Academic Press, London.

Gamper, H. A. 2012. Effects of scale insects on forest dynamics in tropical montane oak forests of Veracruz, Mexico. Flordia State Univ. Libr.

Girardin, M. P., F. Raulier, P. Y. Bernier, and J. C. Tardif. **2008**. Response of tree growth to a changing

climate in boreal central Canada: A comparison of empirical, process-based, and hybrid modelling approaches. Ecol. Modell. 213: 209–228.

Grissino-Mayer, H. 2001. Evaluating crossdating accuracy: A manual and tutorial for the computer program COFECHA. Tree-Ring Res. 57: 205–221.

Guttman, N. B., and R. G. Quayle. **1996**. A historical perspective of U.S. climate divisions. Bull. Am. Meteorol. Soc. 77: 293–303.

Han, J. M., H. Kim, E. J. Lim, S. Lee, Y. J. Kwon, and S. Cho. **2008**. *Lycorma delicatula* (Hemiptera: Auchenorrhyncha: Fulgoridae: Aphaeninae) finally, but suddenly arrived in Korea. Entomol. Res. 38: 281–286.

Harper, J. K., W. Stone, T. W. Kelsey, and L. F. Kime. **2019**. Potential economic impact of the spotted lanternfly on agriculture and forestry in Pennsylvania. Cent. Rural Pennsylvania. 1–84.

Hogg, E. H., J. P. Brandt, and B. Kochtubajda. **2002**. Growth and dieback of aspen forests in northwestern Alberta, Canada, in relation to climate and insects. Can. J. For. Res. 32: 823–832.

Holmes, R. 1983. Computer-assisted quality control in tree-ring dating and measurement. Tree-Ring Bull. 43: 69–75.

Huang, J. G., J. Tardif, B. Denneler, Y. Bergeron, and F. Berninger. **2008**. Tree-ring evidence extends the historic northern range limit of severe defoliation by insects in the aspen stands of western Quebec, Canada. Can. J. For. Res. 38: 2535–2544.

Kowarik, I., and I. Säumel. **2007**. Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. Perspect. Plant Ecol. Evol. Syst. 8: 207–237.

Leach, H., D. J. Biddinger, G. Krawczyk, E. Smyers, and J. M. Urban. **2019**. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula*, (Hemiptera: Fulgoridae), a new pest of fruit in the northeastern U.S. Crop Prot. 124: 104833.

Leach, H., and A. Leach. **2020**. Seasonal phenology and activity of spotted lanternfly (*Lycorma delicatula*)

in eastern US vineyards. J. Pest Sci. 93: 1215–1224.

Lee, D. H., Y. L. Park, and T. C. Leskey. **2019**. A review of biology and management of *Lycorma delicatula* (Hemiptera: Fulgoridae), an emerging global invasive species. J. Asia-Pac. Entomol. 22: 589–596.

McCullough, D. G., T. M. Poland, A. R. Tluczek, A. Anulewicz, J. Wieferich, and N. W. Siegert. **2019**. Emerald ash borer (Coleoptera: Buprestidae) densities over a 6-yr period on untreated trees and trees treated with systemic insecticides at 1-, 2-, and 3-yr intervals in a central michigan forest. J. Econ. Entomol. 112: 201–212.

Morrow, A. P. A., and V. C. Lamarche. **1978**. Tree ring evidence for chronic insect suppression of productivity in subalpine Eucalyptus. Science. 201: 1244–1246.

National Centers for Environmental Information. **2020**. U.S. Climate Divisions. (https://www.ncdc.noaa.gov/monitoring-references/maps/us-climate-divisions.php).

NOAA National Centers for Environmental Information. **2007**. State of the Climate: Drought for September 2007. State Clim. (https://www.ncdc.noaa.gov/sotc/drought/200709).

NYS IPM. **2020**. New York State Integrated Pest Management. Spotted lanternfly. (https://nysipm.cornell.edu/environment/invasive-species-exotic-pests/spotted-lanternfly/).

Park, J.-D., M.-Y. Kim, S.-G. Lee, S.-C. Shin, J.-H. Kim, and I.-K. Park. **2009**. Biological characteristics of *Lycorma delicatula* and the control effects of some insecticides. Korean J. Appl. Entomol. 48: 53–57.

Phipps, R. L. 1985. Collecting, preparing, crossdating, and measuring tree increment cores. US Geol. Surv. Water Resour. Investig. Rep. 85-4148. 48.

Pichler, P., and W. Oberhuber. **2007**. Radial growth response of coniferous forest trees in an inner Alpine environment to heat-wave in 2003. For. Ecol. Manage. 242: 688–699.

R Core Team. **2018**. R: A language and environment for statistical computing. R foundation for stistical computing, Vienna, Austria.

Rohlf, F. J., and R. R. Sokal. **1995**. Critical values for correlation coefficients, pp. 123–125. *In* State

University of New York at Stony Brook (ed.), Stat. Tables. W. H. Freeman and Company, Stony Brook University, New York.

Rolland, C., W. Baltensweiler, and V. Petitcolas. **2001**. The potential for using *Larix decidua* ring widths in reconstructions of larch budmoth (*Zeiraphera diniana*) outbreak history: Dendrochronological estimates compared with insect surveys. Trees - Struct. Funct. 15: 414–424.

Speer, J. H. 2010. Fundamentals of Tree-Ring Research, Univ. Arizona Press. Tuscon.

Speer, J. H., K. Clay, G. Bishop, and M. Creech. **2010**. The effect of periodical cicadas on growth of five tree species in midwestern deciduous forests. Am. Midl. Nat. 164: 173–186.

Stephens, A. E. A., and M. Westoby. 2015. Effects of insect attack to stems on plant survival, growth, reproduction and photosynthesis. Oikos. 124: 266–273.

Stokes, M. A., and T. L. Smiley. **1968**. An Introduction to Tree-ring Dating. University of Chicago Press.

Stolte, K. W., B. L. Conkling, S. Fulton, and M. P. Bradley. **2012**. State of Mid-Atlantic region forests in 2000., Gen. Tech. Rep. - South. Res. Station. USDA For. Serv. Ashville, NC.

Swetnam, T. W., M. A. Thompson, and E. K. Sutherland. **1985**. Spruce Budworms Handbook: Using dendrochronology to measure radial growth of defoliated trees, United States Dep. Agric. U.S. Department of Agriculture, Forest Service, Cooperative State Research Service.

Urban, J. M. 2020. Perspective: Shedding light on spotted lanternfly impacts in the USA. Pest Manag. Sci. 76: 10–17.

Uyi, O., J. A. Keller, A. Johnson, D. Long, B. Walsh, and K. Hoover. **2020**. Spotted lanternfly (Hemiptera: Fulgoridae) can complete development and reproduce without access to the preferred host, *Ailanthus altissima*. Environ. Entomol. 49: 1185–1190.

Veblen, T. T., K. S. Hadley, M. S. Reid, and A. J. Rebertus. **1991**. Methods of detecting past spruce beetle outbreaks in Rocky Mountain subalpine forests. Can. J. For. Res. 21: 242–254.

Veblen, T. T., T. Kitzberger, and A. Lara. 1992. Disturbance and forest dynamics along a transsect from

Andean rain forest to Patagonian shrubland. J. Veg. Sci. 3: 507–520.

Walker, D. M., C. A. Copenheaver, and A. Zink-Sharp. **2014**. Radial growth changes following hemlock

woolly adelgid infestation of eastern hemlock. Ann. For. Sci. 71: 595–602.

Yamaguchi, D. K. 1991. A simple method for cross-dating increment cores from living trees. Can. J. For.

Res. 21: 414–416.

Yang, L. H. 2004. Periodical cicadas as resource pulses in North American forests. Science (80-.). 306:

1565–1567.

Yang, L. H., and R. Karban. **2009**. Long-term habitat selection and chronic root herbivory: Explaining the

relationship between periodical cicada density and tree growth. Am. Nat. 173: 105–112.

3.8 Tables and figures

Table 3.1: Summary of COFECHA results including site name, tree species (species), number of cores taken (Ncores), mean ring width (mm), series intercorrelation, and mean sensitivity.

Site	Species	N$_{cores}$	Mean ring width (mm)	Series intercorrelation	Mean sensitivity
Upper Hanover	*Ailanthus altissima*	8	3.80	0.483	0.290
Upper Hanover	*Acer rubrum*	7	1.95	-0.103	0.347
Upper Hanover	*Juglans nigra*	5	2.46	0.307	0.361
Upper Hanover	*Liriodendron tulipifera*	5	5.74	0.592	0.298
Blue Marsh	*Ailanthus altissima*	22	4.79	0.485	0.327
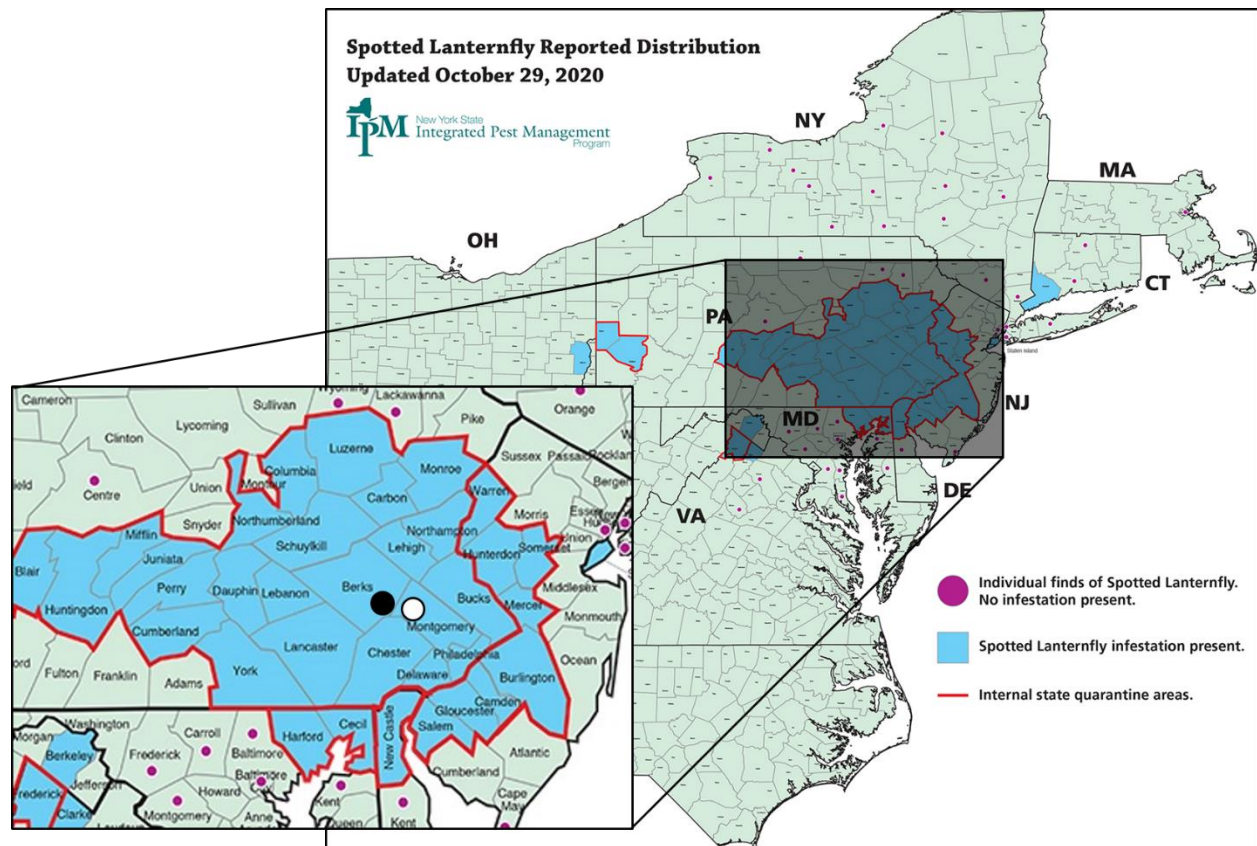
Figure 3.01: Map of study site location. Upper Hanover Township, Montgomery County, PA (white dot), Blue Marsh Recreation Area, Berks County, PA (black dot) (NYS IPM 2020).
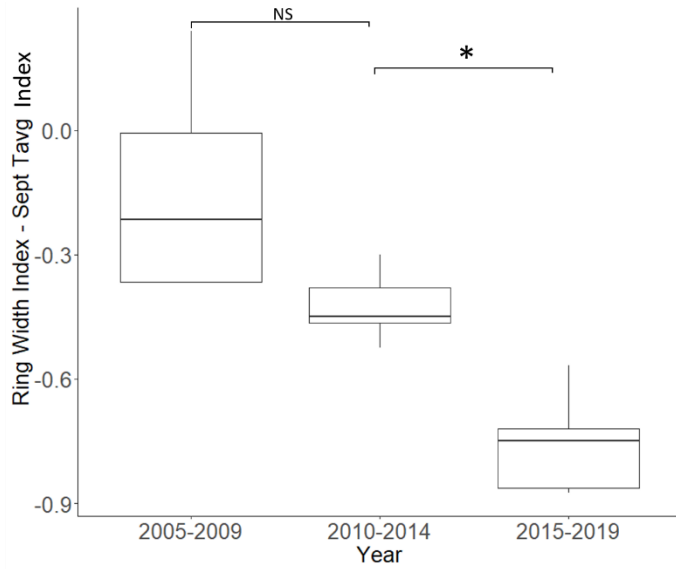
Figure 3.02: Representative core of each species. *Ailanthus altissima* (A), *Juglans nigra* (B), *Liriodendron tulipifera* (C), *Acer rubrum* (D).



y=-0.1332x + 9.913
R^2=0.3314
p<0.05
n=48
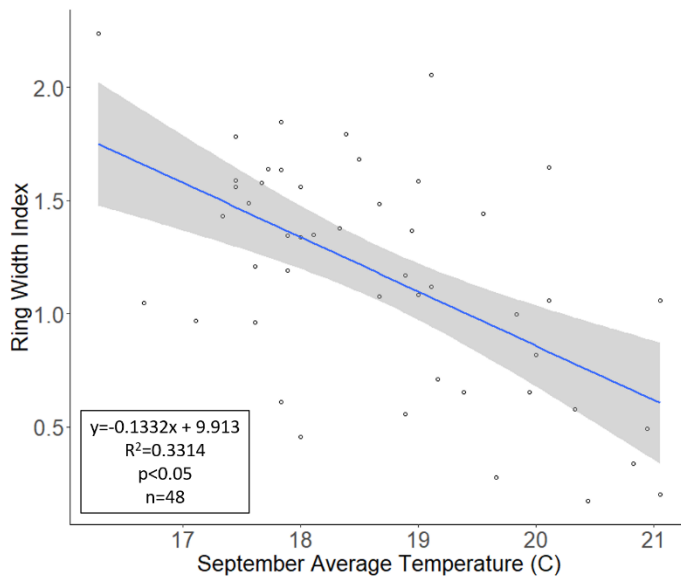
Figure 3.03: Regression analysis of *A. altissima* ring-width index and September average temperature. Upper Hanover Township, PA.
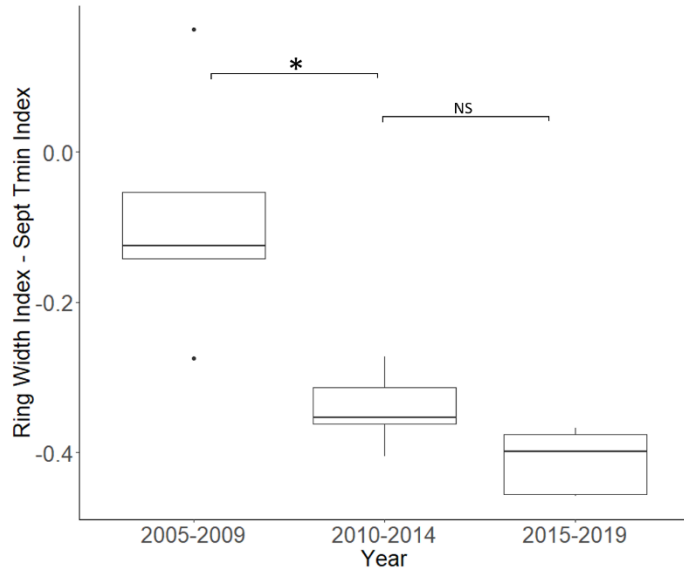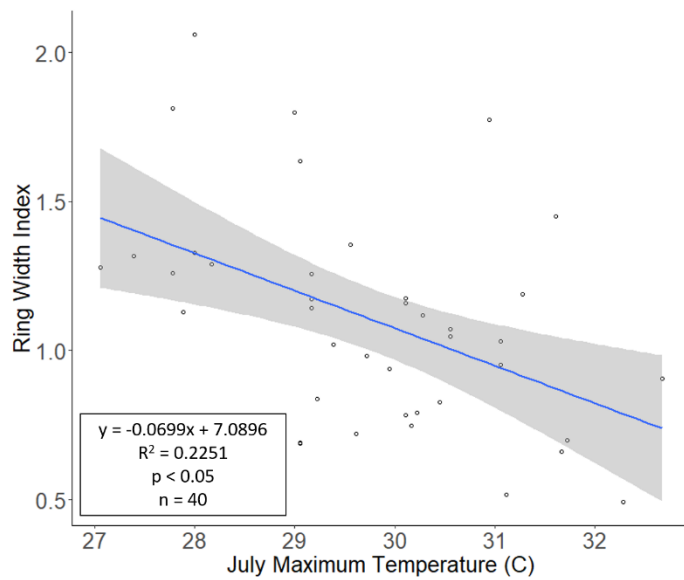
Figure 3.04: *Ailanthus altissima* ring-width index with the dominant climate variable removed pre- and post- *L. delicatula* infestation. Upper Hanover Township, PA. Significance level = 0.05.

$y = -0.0555x + 4.0264$
$R^2 = 0.0687$
$p < 0.05$
$n = 81$
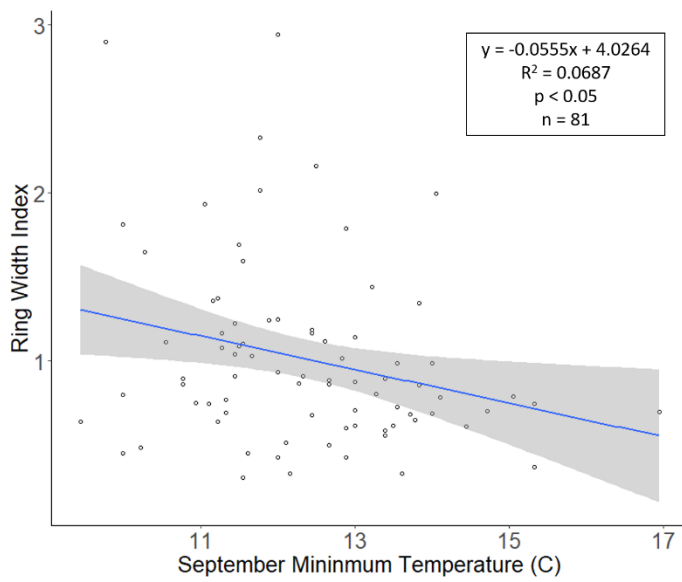
Figure 3.05: Regression analysis of *Juglans nigra* ring-width index and September minimum temperature. Upper Hanover Township, PA.
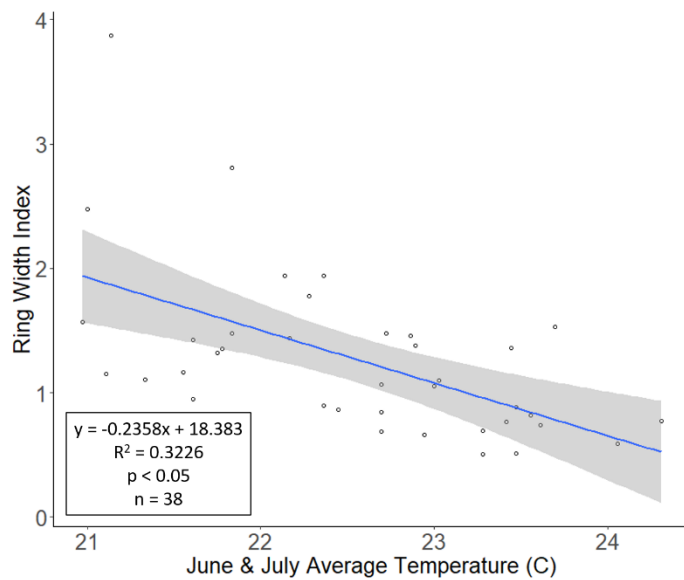
Figure 3.06: *Juglans nigra* ring-width index with the dominant climate variable removed pre- and post- *L. delicatula* infestation. Upper Hanover Township, PA. Significance level = 0.05.

$$y = -0.0699x + 7.0896$$
$$R^2 = 0.2251$$
$$p < 0.05$$
$$n = 40$$

Figure 3.07: Regression analysis of *Liriodendron tulipifera* ring-width index and July maximum temperature. Upper Hanover Township, PA.
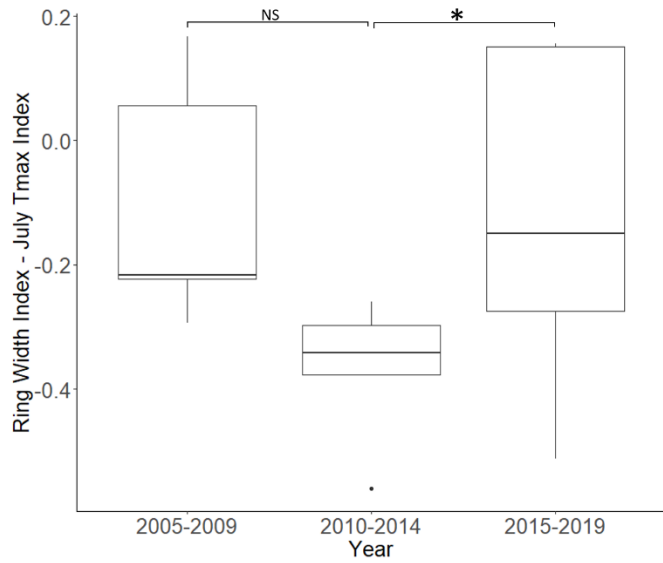
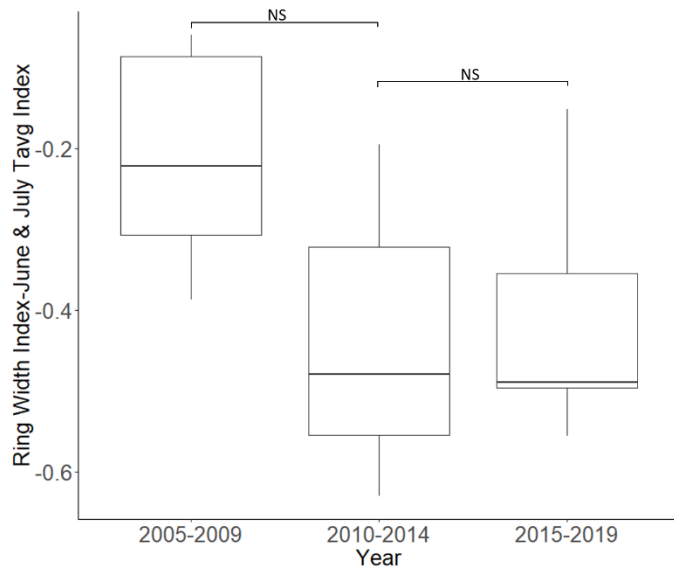**Figure 3.08:** *Liriodendron tulipifera* ring-width index with the dominant climate variable removed pre- and post- *L. delicatula* infestation. Upper Hanover Township, PA. Significance level = 0.05.

y = -0.2358x + 18.383
R^2 = 0.3226
p < 0.05
n = 38

Figure 3.09: Regression analysis of *A. altissima* ring-width index and the average temperature for June and July. Blue Marsh Recreation Area, Berks County, PA.
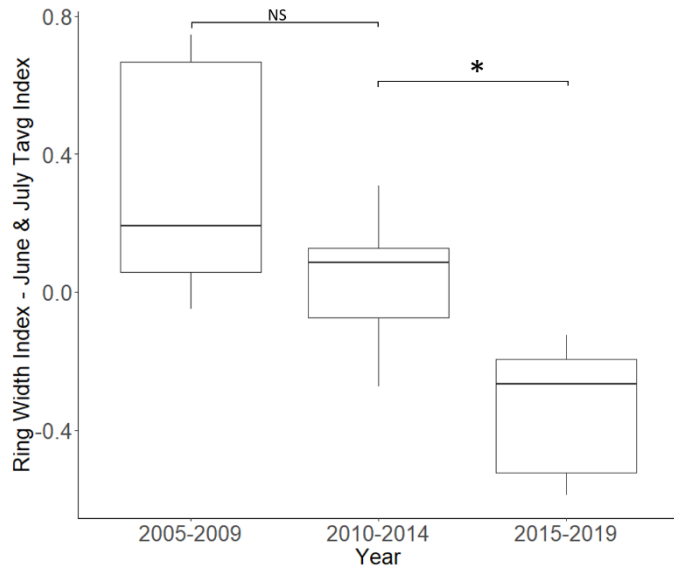
Figure 3.10: *Ailanthus altissima,* no insecticide treatment, ring-width index with the dominant climate variable removed. Comparisons between pre and post *L. delicatula* infestation. Blue Marsh Recreation Area, Berks County, PA. Significance level = 0.05.

Figure 3.11: *Ailanthus altissima*, one year of basal bark treatment with dinotefuran, ring-width index with the dominant climate variable removed. Comparisons between pre- and post- *L. delicatula* infestation. Blue Marsh Recreation Area, Berks County, PA. Significance level = 0.05.
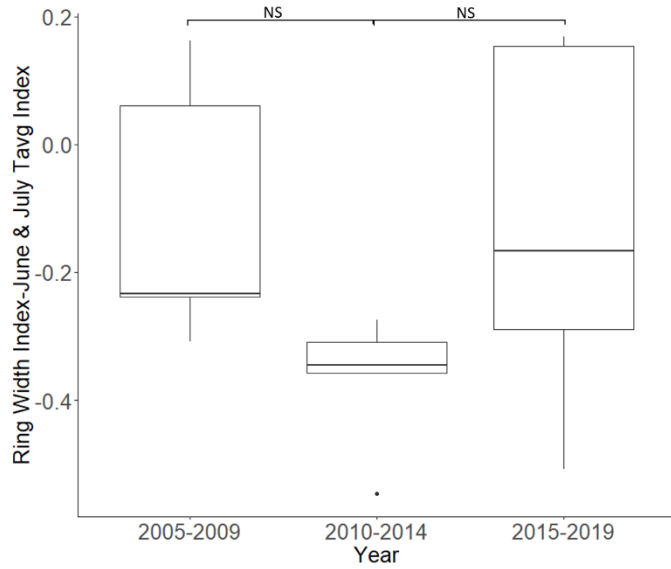
Figure 3.12: *Ailanthus altissima*, two years of basal bark treatment with dinotefuran, ring-width index with the *dominant* climate variable removed. Comparisons between pre- and post- *L. delicatula* infestation. Blue Marsh Recreation Area, Berks County, PA. Significance level = 0.05.
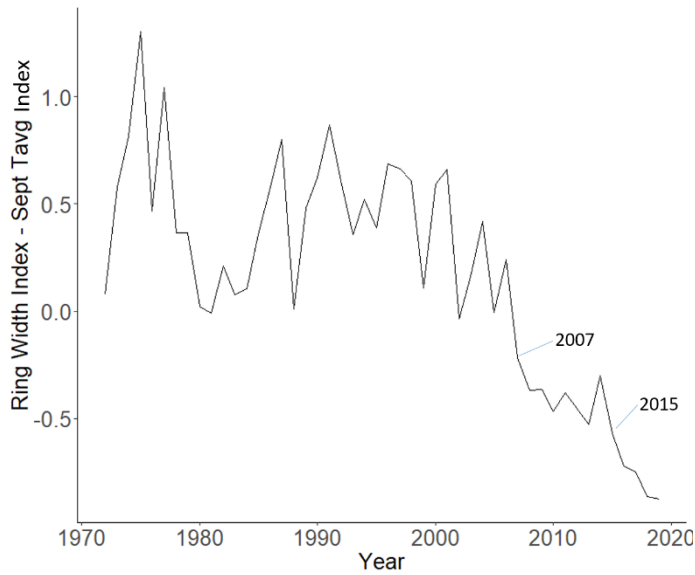
Figure 3.13: *Ailanthus altissima* master chronology showing a growth suppression in 2007 and 2015. Growth suppression in 2007 was likely from severe drought experienced in the sampling area that year. Growth suppression in 2015 was likely a result of heavy *L. delicatula* feeding. Upper Hanover Township, PA.
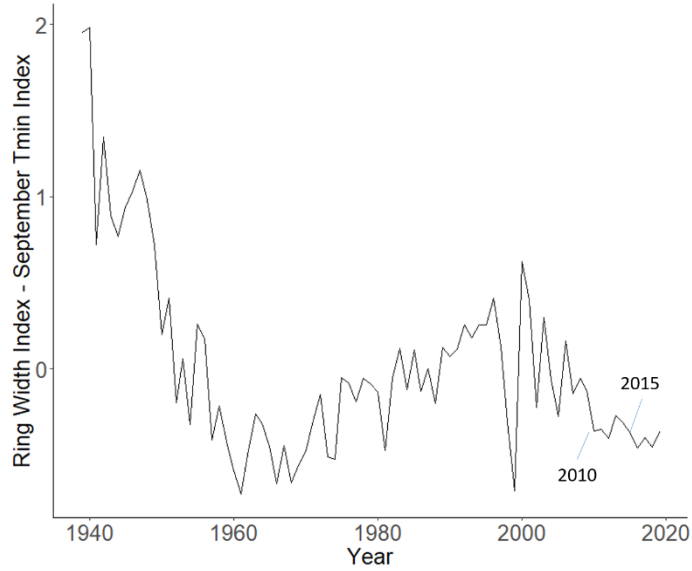
Figure 3.14: *Juglans nigra* master chronology showing a growth suppression in 2010. This may have been due to a late season drought experienced in that area. Upper Hanover Township, PA.
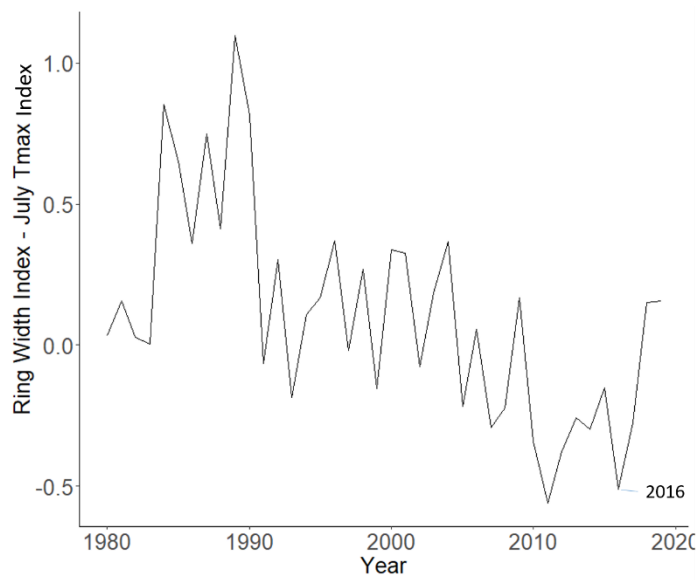
Figure 3.15: *Liriodendron tulipifera* master chronology showing a significant increase in growth after *L. delicatula* invasion. Upper Hanover Township, PA.

Chapter 4: Testing a *Lycorma delicatula* (White) rearing protocol in quarantine laboratory conditions

Abstract

Lycorma delicatula (White) (Hemiptera: Fulgoridae) was first detected in the United States in Berks County, Pennsylvania in 2014. This phloem-feeding insect has since spread to eight surrounding states and poses a significant threat to various industries in the Northeast US including grapes and timber. A continuous laboratory colony of *L. delicatula* would enable year-round research on this new invasive insect. In 2018 and 2019, I tested a rearing protocol for *L. delicatula* in two separate quarantine facilities. Three different cuttings of the preferred host tree-of-heaven, *Ailanthus altissima* (Miller) Swingle, were used as a food source, apical meristems, epicormic shoots, and field collected foliage. *Lycorma delicatula* were successfully reared from egg-to-egg in each treatment with a greater proportion of third instars developing into fourth instars on apical meristems. A total of 30 adults and 3 egg masses were produced from approximately 600 first instars. I determined that this rearing method may be useful for early instar development and short-term maintenance of field collected specimens, but is inefficient to maintain a continuous *L. delicatula* laboratory colony. I suggest changes to this rearing method that should be explored in future studies.

Keywords: *Lycorma delicatula,* spotted lanternfly, *Ailanthus altissima,* tree-of-heaven, rearing, quarantine laboratory

4.1 Introduction

Lycorma delicatula, also known as the spotted lanternfly, is an invasive insect that was first discovered in the United States in 2014 by the Pennsylvania Department of Agriculture in Berks County, PA (Barringer et al. 2015, Dara et al. 2015). Since its discovery, *L. delicatula* has spread to 26 Pennsylvania counties as well as the surrounding states of New York, Connecticut, Ohio, New Jersey, Maryland, Delaware, West Virginia, and Virginia (NYS IPM 2020). *Lycorma delicatula* is a phloem-feeding insect with more than 100 documented host species worldwide (Barringer and Ciafré 2020). As a phloem-feeder, *L. delicatula* has the potential to cause serious economic impact to fruit, agriculture, ornamental, nursery, and timber industries across the U.S. (Finley and Chhin 2016, Harper et al. 2019, Wakie et al. 2020). To date, Pennsylvania vineyards have experienced the largest impact from *L. delicatula* with reports of up to 90% loss of grape yield in some areas (Urban 2020).

Though they do not bite or sting, *L. delicatula* has also proven to be a residential nuisance pest in its invaded range. Sometimes numbering in the thousands, large aggregations of these conspicuous insects have been reported to reduce resident's quality of life as well as potentially decrease property value (Urban 2020). As *L. delicatula* feed, they excrete copious amounts of honeydew that will coat any surface beneath them. This layer of honeydew promotes the growth of sooty mold that can reduce a plants photosynthetic capability and stain some materials (Dara et al. 2015, Lee et al. 2019, Murman et al. 2020).

Great strides have been taken to determine effective management of *L. delicatula* in the United States. Most studies have taken place in a field setting to identify host species, effective insecticides, potential predators, and trap and lure development, just to name a few. However, laboratory colonies of insects play a significant role in scientific research, and can be attributed to large advances across many disciplines such as genetics, behavioral ecology, plant-insect interaction, insecticide development, and biological control (Roe et al. 2018). Since its introduction into the United States, researchers have been

trying to rear *L. delicatula* in the lab with limited success and only brief mention of rearing methods in the literature (Cooperband et al. 2019, Leach et al. 2019, Derstine et al. 2020).

It has been documented that *L. delicatula* tends to prefer tree-of-heaven [*Ailanthus altissima* (Miller) Swingle] as a host species (Dara et al. 2015, Song et al. 2018, Lee et al. 2019). In 2018 and 2019, a *L. delicatula* rearing protocol, developed by the USDA-APHIS Otis Lab in MA, was tested. This protocol used three different cuttings of *A. altissima* as a food source for *L. delicatula*; apical meristems, epicormic shoots, and field-collected foliage. The goal of this study was to determine the survivorship of *L. delicatula* on three *A. altissima* food treatments in a quarantine lab setting.

4.2 Materials and methods

4.2.1 Insect collection

Lycorma delicatula egg masses that were deposited in the fall of 2018 were collected on 11 April 2019 from a heavily infested, small forested area in Winchester, Virginia (39.208147, -78.154378). Strips of tree bark with egg masses were placed in zip-lock bags and transported in a sealed cooler to the Virginia Tech Insect Quarantine Laboratory in Blacksburg, VA (37.211793, -80.489576). Once in quarantine, individual egg masses were placed in Petri dishes and sealed with tape. All petri dishes containing egg masses were stored in an environmental chamber (Percival Model I36LL, Perry, IA, USA) set to 5°C until needed. All appropriate permits were obtained from Virginia Department of Agriculture and Consumer Services (Permit #: VASLF-19031) and the United States Department of Agriculture (Permit #: P526P-18-02138) prior to egg mass collection.

4.2.2 Food collection

Apical meristem and epicormic shoot material were collected from Virginia Tech's Kentland Farm in Blacksburg, VA (37.202071, -80.591499) before bud break in April 2019. Using a chainsaw, *Ailanthus altissima* trees were felled, sectioned, and divided into two categories. Apical meristems consisted of terminal branches measuring approximately 0.5-1 m in length from the terminal bud up the branch.

Epicormic shoot material consisted of main stem bolts measuring approximately 0.5-0.75 m in length and ranging in diameter from 5-13 cm. Apical meristem and epicormic shoot material were immediately placed in 18.9 L (5 gallon) buckets at the correct orientation and filled with approximately 15 cm of water. All material was stored in a 5°C walk-in cold storage unit at Kentland Farm until needed. Water in the buckets was replaced every two weeks.

Material presented to *L. delicatula* in quarantine was taken from cold storage, transported to Virginia Tech's Washington Street greenhouse (37.220515, -80.423777), provided fresh water, and allowed to sprout. It took approximately three weeks for apical meristems to produce enough foliage to present to insects in quarantine and about four weeks for epicormic shoots to emerge from bolts. Once apical meristem and epicormic shoots sprouted, they were transported to the Quarantine Lab and presented to *L. delicatula*. Fresh field-collected *A. altissima* foliage was collected and cut to size as needed from the surrounding property outside of the Quarantine Lab.

4.2.3 Rearing *Lycorma delicatula*

Egg masses were taken from cold storage and placed in an environmental chamber at 25°C, 16:8 L:D and about 65% relative humidity to induce *L. delicatula* egg hatch. Upon egg hatch, first instars were placed in mesh rearing cages (BioQuip, 34x34x60 cm, 1466BV, Rancho Dominguez, CA, USA) that contained one of three food treatments. All food was cut to less than 60 cm in length so it would fit inside the rearing cage. Stems were placed in 946 ml deli containers (Comfy Package, 00643129338540, Brooklyn, NY, USA), containing a solution of about 473 ml of water and MaxiGro hydroponic fertilizer (General Hydroponics, GH1211, Santa Rosa, CA, USA). Food measurements consisted of number of stems, shoots, stem diameter, and stem length. Deli containers were sealed using Parafilm (PM996) (Figure 4.1). 20-50 first instar nymphs were introduced to each rearing cage per treatment.

New apical meristems and field-collected foliage were provided every 2-3 days. *Ailanthus altissima* bolts with epicormic shoots were replaced at least once per week. New food was placed in the cage with

the old food allowing nymphs to move to the new food within 24 hours. This step was done to limit the amount of disturbance experienced by nymphs. Old food was then removed, checked for any remaining live *L. delicatula* or exuviae and discarded.

Every 2-3 days *L. delicatula* life stage and survivorship data were collected from each treatment cage. Temperature and light conditions in the rearing room were also altered based on climate data from 2018 to mimic field conditions in Reading, PA where *L. delicatula* populations have thrived. Life stage was determined by the presence of exuviae after a molting event. Exuviae and dead individuals were removed using an aspirator, documented, and retained for the duration of the study.

4.2.4 Data analysis

Data collected by the USDA-APHIS Otis Lab and Virginia Tech were combined and the number of individuals that reached each life stage were totaled for each treatment.

In 2018 the USDA-APHIS Otis Lab, located in Buzzards Bay, Massachusetts, conducted the same experiment and provided their data for comparison. Data from both experiments were combined in Excel and the number of individuals that reached each life stage were totaled for each treatment. After checking that our data satisfied assumptions, I tested the significance of food treatment on the survivorship of *L. delicatula* using a one-way analysis of variance (ANOVA) with location added as a predictor. If the overall model was significant, differences between treatments were determined using Tukey's honestly significant difference (HSD) post-hoc test. All data were analyzed using R statistical software (R Core Team 2018).

4.3 Results

4.3.1 General trends

Overall, I saw a high amount of variation in survival between treatments and a low number of *L. delicatula* surviving to adults across all treatments. Out of a total of 579 newly hatched first instar nymphs, 403, 291, 151, and 30 *L. delicatula* survived to the second, third, fourth, and adult life stages, respectively

(Table 4.1). There were no egg masses laid in the 2018 trial, however a total of three egg masses were laid in 2019 (none of the eggs deposited in 2019 hatched). The number of days each replication lasted also varied with the shortest being 38 days and the longest being 169 days before all *L. delicatula* died (Figure 4.3).

4.3.2 Proportion survival analysis

Our analysis showed that there was no significant difference between treatments for *L. delicatula* surviving from first to second instars (F = 1.536; df = 3,11; p = 0.2602) (Figure 4.2). I did observe a significant difference in our model of the proportion of second instars surviving to third instars (F = 3.892; df = 3,11; p = 0.04049), however our post-hoc comparison did not reveal a significant difference between treatments or location. There was a significant difference detected in our model for the proportion of third instars surviving to fourth instars (F = 7.685; df = 3,11; p = 0.004805), and our post-hoc comparison revealed that insects in the apical meristem treatment did better compared to insects in the epicormic shoot or field collected material treatments. Location was also significant (p = 0.00843) with more third instars surviving to fourths at Virginia Tech. Our model calculated a significant difference for the proportion of fourth instars surviving to adults (F = 6.239; df = 3,11; p = 0.009882), however post-hoc comparison did not detect a significant difference between treatments. Location was again calculated as significant (p = 0.00237) with more fourth instars surviving to adults at Virginia Tech. In 2019, two egg masses were laid in the apical meristem treatment and one was laid in the epicormic shoot treatment in Virginia Tech's Quarantine Lab (Table 4.1).

4.4 Discussion

4.4.1 Rearing *Lycorma delicatula* on *Ailanthus altissima* cuttings in quarantine is possible

I demonstrated that it is possible to successfully rear *L. delicatula* in quarantine laboratory conditions. This included rearing *L. delicatula* to adults in all three food source treatments with the apical meristem treatment being the most successful of the three (Figure 4.3). In addition, oviposition occurred in two

treatments, the apical meristem and epicormic shoot treatments, producing a total of three egg masses. All of these treatments were especially successful in rearing large numbers of first and second instars, with approximately 70% of first instars developing into second instars across all *A. altissima* food treatments (Table 4.1).

The benefit of this rearing strategy is that I was able to utilize the preferred host of *L. delicatula*, *A. altissima*, throughout this entire experiment. This continuous supply of fresh green foliage, not available naturally year-round, allowed us to provide representative resources for the development of *L. delicatula* regardless of season. This method was also efficient in regards to egg mass collection, storage, and hatch initiation. Egg masses were easily collected from heavily infested field sites during *L. delicatula's* overwintering period and stored in an environmental chamber for multiple months. Although the duration that these egg masses remain viable in storage is currently unknown, I found that egg masses could be stored for at least three months after field collection. First instar eclosion could then be initiated by warming the egg masses for approximately two weeks at any point.

4.4.2 No significant differences observed between food treatments

Overall, I found that the ability of *L. delicatula* to successfully develop into adults did not significantly differ between any of the three food source treatments. The one exception seen was that a greater proportion of *L. delicatula* survived to fourth instars on apical meristems compared to epicormic shoots and field collected foliage (Figure 4.2). However, this trend was not consistently shown for other life stages, including adults, so the implication of this finding is minimal. Additionally, other small differences between treatments were observed regarding oviposition, with egg masses only being produced in apical meristem and epicormic shoot treatments. However, only three egg masses were produced in this entire experiment, which limited our ability to analyze these differences or to make inferences about treatment quality.

This lack of difference in treatments was unsurprising, as there was a high amount of variability in the data. For example, two replications of the epicormic shoot treatment showed very different results. One replication from the Otis Lab had about 2% of all nymphs in that cage survive to fourth instars, while one replication from Virginia Tech had about 90% survival (Table 4.1).

Some of this variation can be explained by the fact that, in 2018 the Otis Lab used an initial number of 50 first instars during their rearing experiments compared to the smaller numbers used in 2019. After our 2018 experience, it was recommended that an initial number of about 20 first instars be used in future experiments to make it easier to track development, reduce competition between individuals, and potentially reduce within treatment variation. This could explain why location was a significant factor in our analysis for fourth instars (Figure 4.2).

Another inconsistent variable in this study was the quality of the food sources presented. For example, fresh *A. altissima* material was readily available outside the Virginia Tech quarantine facility throughout the growing season. This meant that though material could be collected as needed and immediately presented to *L. delicatula*, the age of the foliage could not be controlled throughout the season and the field collected treatments could only be run during the growing season and not later in the year. This could have been especially true in early fall as senescence of trees was beginning to be observed. In contrast, the apical meristem and epicormic shoot treatments were available for use throughout the year, though the food quality likely changed as storage time in cold rooms lengthened.

4.4.3 Rearing methods proved inefficient with high inputs and low outputs

This method of rearing *L. delicatula* should not be used with the goal of raising adults or to produce multiple generations. Significant effort at two quarantine facilities reared only 30 adult *L. delicatula* and 3 egg masses from nearly 600 first instars over a two-year period (Table 4.1).

In order to provide enough food for this effort, significant planning and resources were needed. Maintaining a continuous supply of apical meristems and epicormic shoots required planning no later than

86

March, as *A. altissima* cuttings needed to be collected and stored in a walk-in cooler before they broke bud. Then at least 3-4 weeks before the material was needed, cuttings had to be moved to a greenhouse and allowed to sprout. Once sprouted, these apical meristems and epicormic shoots only survived for a few weeks in the greenhouse and in the quarantine facility resulting in a continual need to transport material to various locations. This process required access to a large *A. altissima* stand, adequate space in a walk-in cooler, access to a heated greenhouse facility year-round, and an authorized quarantine facility with plenty of space for rearing cages. The combination of constant insect feeding and perishable food sources, made this a very complicated, labor intensive, and inefficient method for rearing *L. delicatula* to later life stages*.

Some possible changes could improve the efficiency and output of rearing *L. delicatula* in quarantine. For example, Acebes-Doria et al. (2016) conducted research on host plant effects on brown marmorated stink bug [*Halyomorpha halys* (Stål)] development. Results showed that *H. halys* nymphs developed faster and survived longer on a mixed-host diet opposed to a single host (Acebes-Doria et al. 2016). Similarly, recent research of *L. delicatula's* gut content from field-collected third and fourth instars revealed DNA from multiple host species (Avanesyan and Lamp 2020). Approximately 93% of nymphs sampled in that study had ingested plant DNA from a host species other than the host from which they were collected (Avanesyan and Lamp 2020). Additionally, Uyi et al. (2020) investigated whether or not *L. delicatula* requires *A. altissima* to complete development. They determined that *L. delicatula* can complete development and reproduce in the absence of *A. altissima*, however fitness and survivorship increased when *A. altissima* was part of their diet (Uyi et al. 2020). This would suggest that a *L. delicatula* mass-rearing program would benefit from having multiple host plants as a food source.

Furthermore, potted plants would likely provide a longer lasting more robust food source for the insects than cuttings alone. During this experiment, fewer insects survived to subsequent life stages, suggesting that food treatments were inadequate at supporting later life stages. For example, *L. delicatula*

do not have a cibarial pump like those found in cicadas (Snodgrass 1947), and therefore must rely on phloem pressure to feed. Cut *A. altissima* likely has a severely reduced phloem pressure reducing the ability of *L. delicatula* to obtain adequate nutrients.

4.5 Conclusion

I determined that exclusively using *A. altissima* cuttings to rear *L. delicatula* from eggs to later life stages in a quarantine setting is possible, though time consuming and inefficient. Using this method to rear early life stages or supplement food for field collected specimens of any life stage in the short term, may prove useful. I suggest future *L. delicatula* mass rearing studies include multiple host species and the use of fully potted plants.

4.6 Acknowledgments

4.7 Data

The USDA-APHIS Otis Laboratory in Buzzards Bay, MA provided the data collected in 2018 that was used in this analysis with permission from Juli Gould.

4.8 References

Acebes-Doria, A. L., T. C. Leskey, and J. C. Bergh. **2016**. Host plant effects on *Halyomorpha halys* (Hemiptera: Pentatomidae) nymphal development and survivorship. Environ. Entomol. 45: 663–670.

Avanesyan, A., and W. O. Lamp. **2020**. Use of molecular gut content analysis to decipher the range of food plants of the invasive spotted lanternfly, *Lycorma delicatula*. Insects. 11: 215.

Barringer, L., and C. M. Ciafré. **2020**. Worldwide feeding host plants of spotted lanternfly, with significant

additions from North America. Environ. Entomol. 49: 999–1011.

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

Cooperband, M. F., J. Wickham, K. Cleary, S. E. Spichiger, L. Zhang, J. Baker, I. Canlas, N. Derstine, and D. Carrillo. **2019**. Discovery of three kairomones in relation to trap and lure development for spotted lanternfly (Hemiptera: Fulgoridae). J. Econ. Entomol. 112: 671–682.

Dara, S. K., L. Barringer, and S. P. Arthurs. **2015**. *Lycorma delicatula* (Hemiptera: Fulgoridae): A new invasive pest in the United States. J. Integr. Pest Manag. 6: 1–6.

Derstine, N. T., L. Meier, I. Canlas, K. Murman, S. Cannon, D. Carrillo, M. Wallace, and M. F. Cooperband. **2020**. Plant volatiles help mediate host plant selection and attraction of the spotted lanternfly (Hemiptera: Fulgoridae): a generalist with a preferred host. Environ. Entomol. 49: 1049–1062.

Finley, K., and S. Chhin. **2016**. Forest health management and detection of invasive forest insects. Resources. 5: 18.

Harper, J. K., W. Stone, T. W. Kelsey, and L. F. Kime. **2019**. Potential economic impact of the spotted lanternfly on agriculture and forestry in Pennsylvania. Cent. Rural Pennsylvania. 1–84.

Leach, H., D. J. Biddinger, G. Krawczyk, E. Smyers, and J. M. Urban. **2019**. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula*, (Hemiptera: Fulgoridae), a new pest of fruit in the northeastern U.S. Crop Prot. 124: 104833.

Lee, D. H., Y. L. Park, and T. C. Leskey. **2019**. A review of biology and management of *Lycorma delicatula* (Hemiptera: Fulgoridae), an emerging global invasive species. J. Asia-Pac. Entomol. 22: 589–596.

Murman, K., G. P. Setliff, C. V Pugh, M. J. Toolan, I. Canlas, S. Cannon, L. Abreu, M. Fetchen, L. Zhang, M. L. Warden, M. Wallace, J. Wickham, S.-E. Spichiger, E. Swackhamer, D. Carrillo, A. Cornell, N. T. Derstine, L. Barringer, and M. F. Cooperband. **2020**. Distribution, survival, and development of spotted lanternfly on host plants found in North America. Environ. Entomol. 1–12.

NYS IPM. **2020**. New York State Integrated Pest Management. Spotted lanternfly. (https://nysipm.cornell.edu/environment/invasive-species-exotic-pests/spotted-lanternfly/).

R Core Team. **2018**. R: A language and environment for statistical computing. R foundation for stistical computing, Vienna, Austria.

Roe, A. D., M. Demidovich, and J. Dedes. **2018**. Origins and history of laboratory insect stocks in a multispecies insect production facility, with the proposal of standardized nomenclature and designation of formal standard names. J. Insect Sci. 18: 1–9.

Snodgrass, R. E. 1947. The insect cranium and the "epicranial suture," Smithson. Misc. Collect. The Lord Baltimore Press, Baltimore, MD.

Song, S., S. Kim, S. W. Kwon, S. Lee, and P. G. Jablonski. **2018**. Defense sequestration associated with narrowing of diet and ontogenetic change to aposematic colours in the spotted lanternfly. Sci. Rep. 8: 1–11.

Urban, J. M. 2020. Perspective: Shedding light on spotted lanternfly impacts in the USA. Pest Manag. Sci. 76: 10–17.

Uyi, O., J. A. Keller, A. Johnson, D. Long, B. Walsh, and K. Hoover. 2020. Spotted lanternfly (Hemiptera: Fulgoridae) can complete development and reproduce without access to the preferred host, *Ailanthus altissima*. Environ. Entomol. 49: 1185–1190.

Wakie, T. T., L. G. Neven, W. L. Yee, and Z. Lu. **2020**. The establishment risk of *Lycorma delicatula* (Hemiptera: Fulgoridae) in the United States and globally. J. Econ. Entomol. 113: 306–314.

4.9 Tables and figures

Table 4.3: Results of a *Lycorma delicatula* rearing experiment in 2018 and 2019 showing the number of surviving individuals per life stage for each food treatment.

Cage	Year	Treatment	Location	First	Second	Third	Fourth	Adult	Egg Mass
Cage A	2019	Apical meristem	VT	25	20	19	16	7	2
RAM 1	2018	Apical meristem	Otis	50	31	25	24	3	0
RAM 2	2018	Apical meristem	Otis	50	31	26	22	6	0
RAM 3	2018	Apical meristem	Otis	50	31	30	21	0	0
Cage B	2019	Epicormic shoot	VT	16	16	16	1	0	0
Cage D	2019	Epicormic shoot	VT	21	5	5	4	4	1
Cage F	2019	Epicormic shoot	VT	27	6	4	2	2	0
ES1	2018	Epicormic shoot	Otis	50	34	1	0	0	0
ES2	2018	Epicormic shoot	Otis	50	32	6	1	0	0
Cage C	2019	Field collected	VT	20	19	18	18	6	0
Cage E	2019	Field collected	VT	20	19	17	15	2	0
FC 1	2018	Field collected	Otis	50	40	36	23	0	0
FC 2	2018	Field collected	Otis	50	45	43	1	0	0
FC 3	2018	Field collected	Otis	50	27	11	3	0	0
FC 4	2018	Field collected	Otis	50	47	34	0	0	0
Total				579	403	291	151	30	3

Figure 4.12: Photo depicting food treatments used in the *Lycorma delicatula* rearing experiment, apical meristems (A), epicormic shoots (B), and field collected foliage (C).
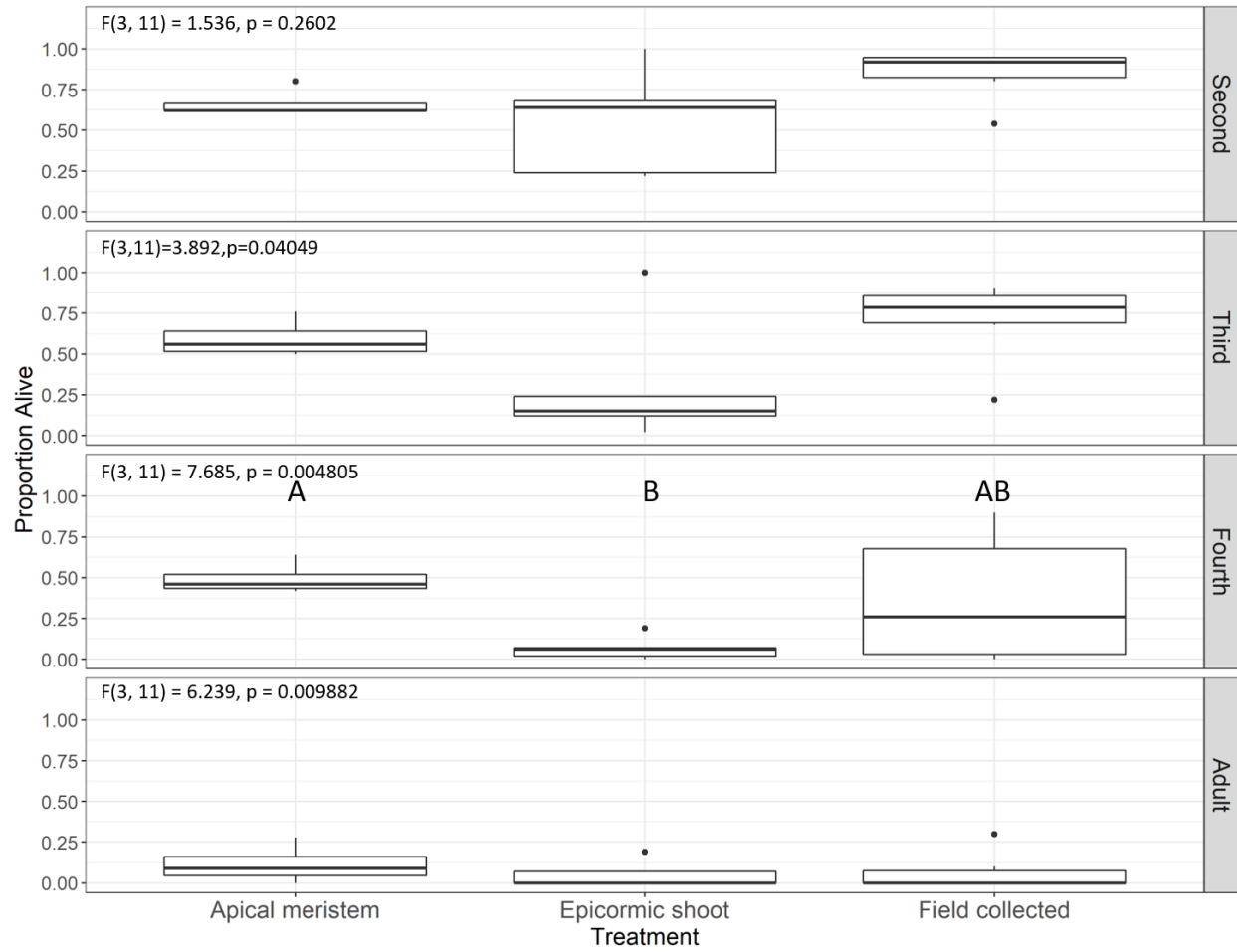
Figure 4.13: Proportion of *Lycorma delicatula* life stage surviving in cages containing *Ailanthus altissima* food treatment, apical meristem (n = 4 cages), epicormic shoot (n = 5 cages), field collected (n = 6). ANOVA with Tukey HSD post-hoc analysis.
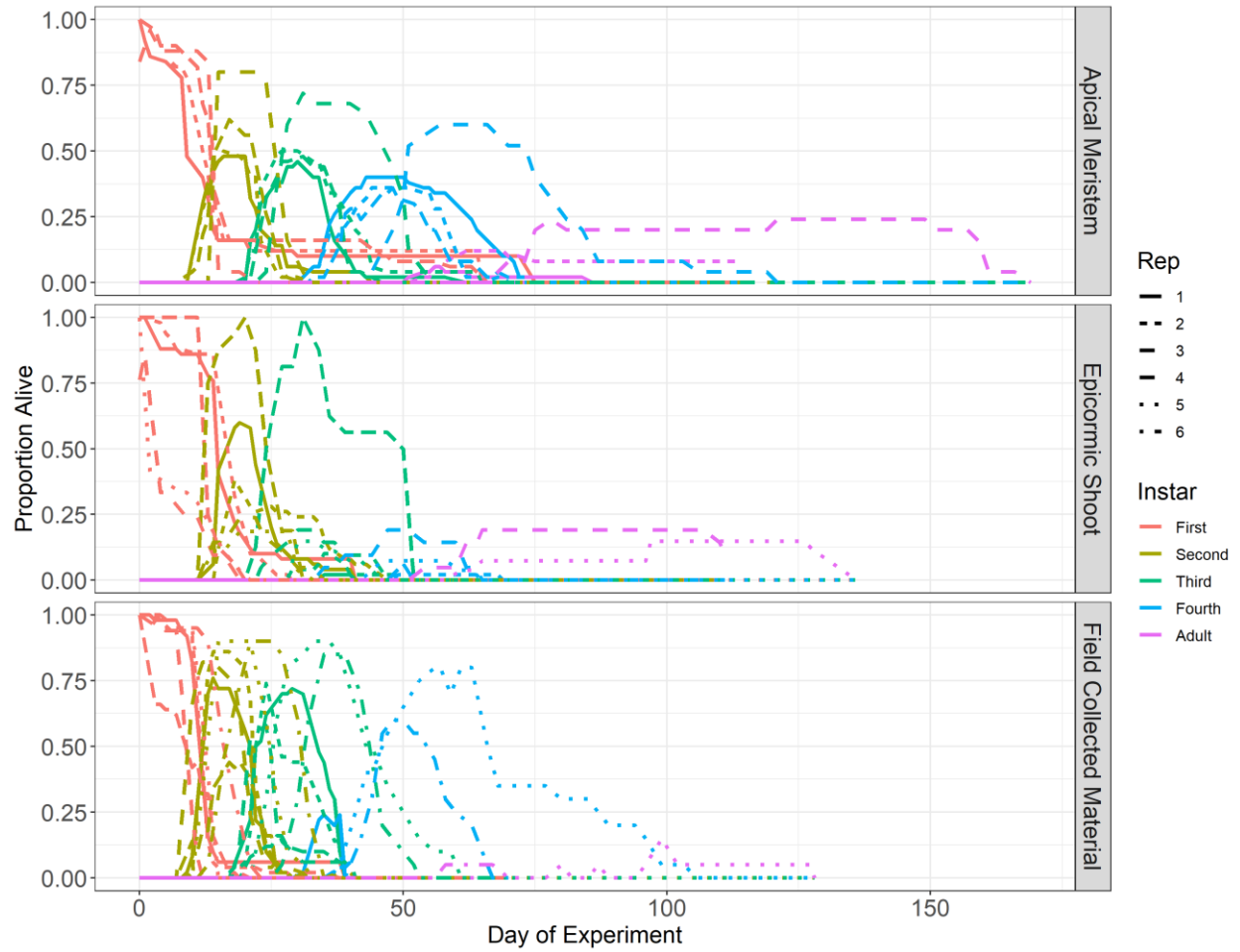
Figure 4.14: Proportion of *Lycorma delicatula* life stage alive each day of the experiment for each food treatment.

Chapter 5: Final conclusions and future research

The phenology study, conducted in Winchester, VA, demonstrated that the timing of events in the life-cycle of *L. delicatula* are fairly predictable and should be used to our advantage when planning management activities. The results of this study also suggest that the further south *L. delicatula* spreads, the earlier they will hatch and be active in the environment. In an attempt to help land managers in other areas, I included some notes about host phenology that may be able to help them predict these differences and I suggest future research continue to collect host phenology data. Though this was not a study investigating the host range of *L. delicatula*, the data I collected confirms that their host range narrows as they develop through the season. I think future research on the biology and requirements of *L. delicatula* diapause would be very beneficial. For example, it is still not known whether *L. delicatula* is capable of having more than one generation per year which would have major implications for warmer regions of the US.

Results from my feeding impact study suggests *L. delicatula* are capable of reducing the annual growth of *A. altissima*. While I did not find evidence of reduced growth in the other species tested, I think more research is needed on this subject. I demonstrated that dendrochronology can be used to identify a *L. delicatula* feeding signal, but a more robust study with a higher sample size is required for more ecologically or economically important tree species. This would provide an accurate picture of the impact we can expect in the long term. Additionally, a more robust study would include: two tree cores per tree, at least 30 trees per site, at least 2 sites, and the addition of non-host trees for climate analysis. The coordination with a dendrochronologist, such as Dr. Jim Speer from Indiana State University, prior to sampling is highly recommended. Until this is done, our results should be considered preliminary. A more robust study would be a good addition to what we know about *L. delicatula*, and would give us insight into the long-term impacts of this invasive insect that are not readily apparent.

A laboratory-reared colony would no doubt help us further our understanding *L. delicatula*, as this would allow work to continue throughout the year in a controlled environment. Though I demonstrated

it is possible to rear *L. delicatula* in the lab using *A. altissima* cuttings, the methods tested in our experiment were not effective enough to pursue further. I would like to see future rearing experiments include multiple species of potted trees presented to *L. delicatula* in the same cage. Since it has been documented that *L. delicatula* feed on multiple host species in the field, I think this would be very effective at sustaining a laboratory population. Furthermore, the use of potted plants would likely increase survivorship because one would not be starting with a food source that already has a limited "shelf life" such as *A. altissima* cuttings. In the meantime, I highly suggest researchers to work within quarantine zones in order to take advantage of non-quarantine research facilities, such as rearing in greenhouses. Additionally, we should all take advantage of utilizing field collected specimens whenever possible, as they are relatively easy to collect, safely transport, and keep alive for short periods of time. Despite not being effective, portions of these methods, such as how to collect and grow *A. altissima* foliage out of season can still be useful to future research.

Finally, after two years of working with this insect, it seems apparent that *L. delicatula* will likely persist and spread to many suitable regions of the United States. However, slowing the spread is very important because every year we learn more and gain insight on additional management and monitoring strategies making the research time and effort worthwhile. Lastly, I think it is important to take advantage of the public's interest. Since *L. delicatula* is so visible and apparent, their presence can be used an opportunity for entomology education and outreach.