HOW SPECIALIST AND GENERALIST HERBIVORES ARE RESPONDING TO
INVASIVE PLANT THREATS

A thesis submitted in partial fulfillment of the
requirements for the degree of
Master of Science

By

LAUREN SHEWHART
B.S. Biology, University of Dayton, 2014

2016
Wright State University

WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

April 13, 2016

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER
MY SUPERVISION BY Lauren E. Shewhart ENTITLED How specialist and
generalist herbivores are responding to invasive plant threats BE ACCEPTED
IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE DEGREE
OF Master of Science.

_____
Don Cipollini, Ph.D.
Thesis Director

_____
David Goldstein, Ph.D.
Chair, Department of Biological
Sciences

Committee on
Final Examination

_____
Don Cipollini, Ph.D.

_____
John O. Stireman, Ph.D.

_____
Thomas P. Rooney, Ph.D.

_____
Robert E. W. Fyffe, Ph.D.
Dean, School of Graduate Studies

ABSTRACT

Shewhart, Lauren E. M.S., Department of Biological Sciences, Wright State University, 2016. How Specialist and Generalist Herbivores are Responding to Invasive Plant Threats.

The purpose of this study was to investigate novel interactions of native herbivores (*Abia inflata, Abia americana*, *Zaschizonyx montana*, and *Hyphantria* cunea) with non-native plants in Ohio. No-choice and choice bioassays were conducted with adults and larvae to examine life history traits, performance, and preference of these herbivores feeding exclusively on native and non-native species and damaged and undamaged foliage. It was found that all organisms in this study can perform well and complete their whole life cycle on *L. maackii.* Adult *A. americana* will oviposit in non-native hosts however newly emerged larvae have 100% larval mortality on *L. japonica*. *A. inflata* had a reduction in larval mass on herbivore damaged foliage but not artificially damaged foliage. Some non-native species (*L. maackii*, *L. tatarica* and *P. calleryana*) appear to be suitable host for *H. cunea,* whereas other non-native species (*L. japonica, E. alatus,* and *E. umbellata*) are unsuitable hosts for early larval development. When given a choice *H. cunea* caterpillars preferred native foliage. These studies could benefit efforts at using these native insects as biocontrol agents for *L. maackii* or other non-native, invaders.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

1. INTRODUCTION

1.1 INVASIVE SPECIES

Invasive species are exotic species introduced into a novel region that spread beyond their initial introduction area with the potential to cause environmental and economic harm. Exotic species are brought to the United States accidentally or purposefully for various reasons from ornamental landscaping to erosion control (Luken and Thieret 1996; Williamson and Fitter 1996; Hayes and Holzmueller 2012). However, only about one out of every one thousand exotic plant species becomes invasive due to the physiological, ecological, and evolutionary filters present when a species tries to establish in a new habitat (Williamson and Fitter 1996; Mack et al. 2000). Although only 1% of exotic plant species become invasive, the species that do become invasive cause major disruptions in the environments in which they invade. In order to be successful, invasive plants often have novel characteristics that allow them to outcompete native plants for resources such as light, nutrients, and pollinators; and even reduce native plant growth by disrupting biogeochemical cycles (Luken and McKnight 1993; Gordon 1998). Some invasive plants can create virtual monocultures in the forest floor displacing or eliminating many native plant species (Luken and McKnight 1993; McNeish et al. 2015).

Invasive species cause major environmental and economic harm, and are the second major threat to biodiversity after habitat destruction (Vitousek et al. 1997). Approximately 42 percent of threatened and endangered species in the United States are

at risk from invasive species (Sadler 2006). Not only are invasive species threatening our endangered organisms, they also cost the United States an estimated $120 billion in damages to agriculture and the environment (Pimentel et al. 2005). Invasive species are not only a problem in the United States, but they are a global problem with increasing costs to biodiversity and the economy worldwide (Pimentel et al. 2005). Better understanding of how native species interact with non-native, invasive species is very important to fully understand the impact of biological invasions and potential find native biological control agents.

1.2 NOVEL PLANT INSECT INTERACTIONS

Insects are very important for the health of native ecosystems, because they perform many services such as decomposing material, cycling nutrients, pollinating flowers, and providing a food source to other organisms (Saul 1999). Insects are responsible for keeping many plant populations in check by negatively affecting their growth and reproduction by reducing bud production, flower production, fruit production, and seed production; increasing seed and seedling mortality; and defoliating plants (Crawley 1989).

Many invasive plant species in their native ranges are often poor competitors with limited distribution (Keane and Crawley 2002; Lieurance 2012). However, in non-native ranges, these invasive plants become dominant due to an increased competitive ability (Keane and Crawley 2002; Lieurance 2012). One of many hypotheses to explain this phenomenon is known as the Enemy Release Hypothesis (Keane and Crawley 2002; Lieurance 2012). Invasive plant species in novel environments experience a release from

natural enemies, especially from co-evolved specialist herbivores not found in the novel environment, which results in the rapid increase in abundance, distribution, and vigor of invasive plant species (Keane and Crawley 2002; Colautti et al. 2004; Lieurance 2012).

As invasive plant species enter a new habitat, they interact with native fauna, especially insect communities. There are three types of interactions herbivores can have with invasive plants. First, a positive interaction could occur in which an insect benefits by successfully recognizing and using the invasive plant (Davis and Cipollini 2014). Second, nothing happens because the insect does not recognize the invasive as a food source and does not use it (Davis and Cipollini 2014). Lastly, insects can be negatively affected, because one or all life stages cannot use or have a lower fitness on the invasive species (Davis and Cipollini 2014). The type of novel plant insect interaction may depend on the type of herbivore interacting with the novel host.

Specialist herbivores vary widely in their host specificity, some consumes one host (monophagous) or a few closely related hosts (oligophagous). Over 90% of all herbivores are considered specialists (Price et al. 2011; Murphy and Loewy 2015). Many specialist herbivores have evolved mechanisms to tolerate the defensive chemicals of the native plant species they feed upon (Barbosa and Saunders 1985; Ali and Agrawal 2012). Some specialists can even incorporate plant toxins into their bodies to deter predators from eating them (Rhoades and Cates 1976; Ali and Agrawal 2012). Since specialist herbivores feed on relatively few, closely related species, they are particularly vulnerable to non-native plant invasions (Ali and Agrawal 2012). Invasive plants can outcompete and greatly reduce native plant populations used by specialists. Monophagous herbivores and specialist herbivores on rarer plants are especially threatened because if the herbivore

3

consumes all of its host plant it may not be able to find another. Along with this, adults may have to spend extra time searching for a rare hosts for oviposition, making her more vulnerable to predators (Murphy and Loewy 2015). Specialist herbivores may not be able to utilize invasive hosts due to novel defenses, elevated chemical defenses, or the lack of specific oviposition cues (Callaway and Ridenour 2004; Cappuccino and Arnason 2006; Jahner et al. 2011). Through time, specialists will need to evolve mechanisms to utilize or avoid new invasive plant species if they are to persist in extremely invaded habitats.

Generalist or polyphagous herbivores can consume a wide range of plant species that are not closely related to one another. Generalist herbivores can tolerate a wide variety of toxins, but are not specialized to deal with any particular plant defense (Bernays and Minkenberg 1997; Ali and Agrawal 2012). Being able to consume a wide range of host reduces the herbivores' exposure to high levels of allelochemicals, but producing a wide range of detoxification enzymes can be metabolically costly (Bernays and Minkenberg 1997; Ali and Agrawal 2012). Generalist herbivores, just like specialists, can be threatened by invasive plant invasions. Invasive plants often outcompete native plants and could potentially reduce the abundance of ideal foliage for the generalist herbivore. Generalists may be left eating lower quality food, which reduces the overall fitness and health of the organism. Generalists may be able to consume the invasive host but may not choose to consume it because they do not recognize the new host as a food source (Lankau et al. 2004). However, being able to eat a large range of species ensures that generalists will not become stranded if they run out of food on their original host, as they can just move onto the next native or even invasive species (Bernays and Minkenberg 1997). Also, females that can oviposit on non-native host will benefit from a

reduction in search time for and oviposition site, which decreases her vulnerability to predators (Murphy and Loewy 2015).

1.3 PLANT DEFENSES

Herbivory can greatly impact a plant's growth, survival, competitive success, and reproductive success (Crawley 1989; Lieurance 2012). The amount of herbivore damage needed to reduce the fitness of a plant varies by taxon, but research has shown that as little as 6-12% herbivory can reduce the growth and reproductive success of woody trees (Warrington and Whittaker 1985; Crawley 1989). Both native and non-native plants have many resistance mechanisms to deter herbivory such as mechanical defenses (thorns, high leaf toughness, etc.) and chemical defenses (Barbosa and Saunders 1985; Strauss and Agrawal 1999; Cappuccino and Arnason 2006). There are two main types of plant chemical defenses constitutive defenses which are always present in a plant and induced defenses that are only present after herbivory damage to the plant (Gurevitch et al. 2006). Plants often produce constitutive defenses to defend their most valuable tissues that are most likely to be attacked (Cates 1980; Barbosa and Saunders 1985; McCall and Fordyce 2010). Producing defensive chemicals is costly and energy taxing for a plant, so many plants will produce low levels of constitutive defenses if herbivory rates are low and put more energy into growth and reproduction (Gurevitch et al. 2006; McCall and Fordyce 2010).

Constitutive defenses are often not enough to prevent herbivores from eating a plant, so many plants must produce secondary inducible defenses. Plants have many inducible defenses to deter, slow down, or even kill herbivores (Barbosa and Saunders 1985). Plants can produce a wide range of secondary metabolites to protect against

5

herbivores and microbes by reducing the quality of the damaged leaves (Haukioja 1980; Gurevitch et al. 2006; Ali and Agrawal 2012). Damaged leaves are often a deterrent for herbivores because they have a lower leaf area, lower water and nutrient content, higher toughness, an increase in defensive chemicals, (Feeny 1970; Myers and Post 1981; Rhoades 1983) and are a visual and odor cue for predators (Anthony 1998; VanLaerhoven et al. 2000).

1.4 PLANT SPECIES

The overall goal of this experiment was to rear specialist and generalist herbivores on native and non-native plants found in Ohio. Below are listed all of the native and non-native species used in this study. Included in the description are the family and general characteristics of each species, known herbivores, and any other relevant information.

<u>Native Plants:</u>

<u>*Lonicera reticulata* (Grape Honeysuckle)</u> Family: Caprifoliaceae

Lonicera reticulata is a woody vine native to North America which can grow up to 15 feet tall (Hilty 2015). It often uses other vegetation around it for support. This plant is a host to many insects such as the *Hemaris diffinis* (Snowberry Clearwing), *Hemaris thysbe* (Hummingbird Clearwing), *Ypsolopha dentella* (Honeysuckle Moth); aphids such as the *Hyadaphis foeniculi* (Honeysuckle Aphid); and the sawfly *Abia americana* (Honeysuckle Sawfly) (Hilty 2015). Deer and birds also feed on *L. reticulata* (Hilty 2015).

Prunus serotina (Wild Black Cherry) Family: Rosaceae

Prunus serotina is a fast-growing, aggressive native tree that can grow up to 80 feet (Hilty 2015). *Prunus serotina* is a pioneer species that does very well in disturbed habitats. Many insects use *P. serotina* as a food source. Over 30 species of Lepidoptera feed on the leaves of *P. serotina*, along with many beetle and sawfly species (Hilty 2015). The fruit of *P. serotina* is eaten by many birds and small mammals (Hilty 2015).

Symphoricarpos albus (Common Snowberry) Family: Caprifoliaceae

Symphoricarpos albus is a small native shrub that can reach 20 feet in height and has characteristic white fruit later in the year (Favorite and Moore 2008). The caterpillars of the snowberry clearwing, hummingbird clearwing, and the larvae of the sawfly *A. americana* are known to feed on the leaves of *S. albus* (Favorite and Moore 2008). Some bird species and small mammals use *S. albus* for food and shelter (Favorite and Moore 2008).

Non-native plants:

All of the following plants have been introduced into the United States for various reasons. Most of them are considered invasive plant species and some are extremely invasive in Ohio. They all are threatening native vegetation, especially endangered native flora.

Elaeagnus umbellata (Autumn Olive) Family: Elaeagnaceae

This shrub is native to East Asia and can grow up to 20 feet tall (Hilty 2015). It was introduced into the North America for many reasons from beautifying highways to providing food for wildlife (Hilty 2015). Since its introduction *E. umbellata* has spread

into many states, often invading meadows and prairies. The flowers and fruit of *E. umbellata* provide food for many pollinators and gamebirds (Hilty 2015). Many mammals also eat the fruits from this invasive shrub (Hilty 2015).

Lonicera japonica (Japanese Honeysuckle) Family: Caprifoliaceae

Lonicera japonica is a non-native woody vine that was introduced into the United States from Eastern Asia for horticultural purposes (Munger 2002). This vine can grow up to 18 feet long. *Lonicera japonica* outcompetes native plants for light and nutrients (Munger 2002). This invasive vine can also kill native trees by climbing them, restricting light, and breaking limbs due to its weight. In some areas, *L. japonica* is evergreen or nearly evergreen. Many birds and small mammals feed on the berries of *L. japonica* and use the vine as shelter (Munger 2002).

Lonicera maackii (Amur Honeysuckle) Family: Caprifoliaceae

Lonicera maackii, is an invasive shrub from China that can grow up to 20 feet tall. *Lonicera maackii* has many invasive characteristics which allow it to outcompete native plants and negatively impact herbivores (Gorchov and Trisel 2003; McEwan et al. 2009b; Lieurance and Cipollini 2013a; Lieurance and Cipollini 2013b). *Lonicera maackii* has an extremely long growing season, it emerges from dormancy in spring before most native plants and does not lose its leaves until late fall to early winter (McEwan et al. 2009a). Many pollinators take advantage of the nectar produced by this shrub. Some aphids such as *Alphitoaphis lonicericola* suck plant juices from this honeysuckle species (Luken and Thieret 1996; Hilty 2015). Even with all these potential herbivores, Lieurance and Cipollini (2012) found that *L. maackii* receives little to no herbivore damage from

8

generalists and specialists in the field. In the laboratory, caterpillars of the generalist gypsy moth, which can clear entire tree canopies, cannot survive when only feeding on honeysuckle leaves (McEwan et al. 2009b). Another generalist, the army worm caterpillar, performs poorly when fed Amur honeysuckle (Lieurance and Cipollini 2013a).

Pyrus calleryana (Callery Pear) Family: Rosaceae

This tree was introduced to the United States from East Asia. *Pyrus calleryana* can grow up to 50 feet tall (Hilty 2015). This tree spreads into degraded woodlands and other disturbed habitats. There are about 20 cultivars of *P. calleryana* which are self-sterile, but when crossed with another cultivar can produce fertile fruit (Hilty 2015). The fruit of *P. calleryana* can be eaten by many birds (Hilty 2015).

1.5 HERBIVORE SPECIES

<u>Specialist Herbivores:</u>

Abia americana (Honeysuckle Sawfly) Family: Cimbicidae

Abia americana is a specialist sawfly native to North America that feeds on native honeysuckle and its relatives. Adult *Abia americana* emerge in late April to early May (Figure 2.1). The female uses her ovipositor to insert eggs into the edges of leaves. The larvae will hatch from the egg in 5-8 days and begin feeding on the edges of leaves. After about 3-4 weeks of development, the larvae pre-pupate in a fibrous cocoon in the soil and leaf litter. *Abia americana* overwinter in this pre-pupal form until emerging the next spring.

Abia inflata (Honeysuckle Sawfly) Family: Cimbicidae

Abia inflata is a specialist sawfly native to North America that feeds on native honeysuckle and its relatives (Lieurance and Cipollini 2013a). Adult *A. inflata* emerge in late April to early May (Figure 2.2). The female uses her ovipositor to insert eggs into the edges of leaves. The larvae will hatch from the egg in 5-8 days and begin feeding on the edges of leaves. After about 3-4 weeks of development, the larvae pre-pupate in a fibrous cocoon in the soil and leaf litter. *Abia inflata* overwinter in this pre-pupal form until emerging the next spring. In the laboratory, *A. inflata* larvae can also feed and survive on non-native *L. maackii* relatively well, but are very rarely found feeding on them in the field (Lieurance and Cipollini 2012; Lieurance and Cipollini 2013a; Lieurance and Cipollini 2013b; Stireman personal observation).

Zaschizonyx montana (Snowberry Sawfly) Family: Tenthredinidae

Zaschizonyx montana is a specialist sawfly native to North America and is only known to feed on native *Symphoricarpos* species (Smith and Gibson 1984). Adult *Z. montana* emerge in late April to early May (Fig 3.1). Adult *Z. montana* feed on the *Symphoricarpos* leaves before females oviposit eggs into the edges of leaves. The larvae will hatch from eggs in 5-8 days and begin feeding on the edges of leaves. After about 2-3 weeks of development, the larvae start to pre-pupate and turn lime green. Once the larvae hit this stage they do not feed anymore and remained curled underneath foliage. It is still not known if the larvae overwinter in their larval stage, pre-pupal stage, or pupal stage.

Generalist Herbivore:

Hyphantria cunea (Fall Webworm) Family: Arctiidae

Hyphantria cunea is a generalist herbivore native to North America that can feed on over 630 different plant species (Warren and Tadic 1970). *Hyphantria cunea* has become an invasive pest in Europe, China, and North Korea (Sourakov and Paris 2014).There are two races of fall webworm, the black and the red race (Loewy et al. 2013). The red race is more common in southern areas, whereas the black race is more common in more northern areas including Ohio (Loewy et al. 2013). In Ohio, adults emerge in late May to early July (Fig 4.1). Females lay egg masses of 400-1000 eggs on the underside of leaves (Sourakov and Paris 2014). Fall webworm caterpillars have 5-8 instars. The caterpillars of *H. cunea* stay in large groups and build webs on the outer branches of their host (Mason et al. 2011). *Hyphantria cunea* is known to feed on honeysuckle, and we have found it feeding on invasive *Lonicera maackii* and native *Lonicera reticulata* locally in the field (personal observation). Caterpillars of *H. cunea* have also been seen on other invasive hosts such as *L. japonica* and *P. calleryana* (personal observations). The red race caterpillars feed together their entire lives, the black race feed together for most of their lives until they reach later instars (Loewy et al. 2013). Then the caterpillars will disperse, mature, and pupate in the soil. Fall webworms in more northern areas, like Ohio, only have one to two generation per year, but more southern areas can have as many as four generations in one year (Gordon 1976).

1.6 GOALS AND IMPORTANCE

The goal of this project was to examine the performance and preference of all life stages of *A. americana*, *A. inflata, Z. montana*, and *H. cunea* on non-native, invasive species in relation to native congeners and other ecologically relevant species. Along with this, the cost of consuming damaged, native and non-native foliage was evaluated with the specialists *A. inflata* and *Z. montana.* The negative and positive aspects of group feeding were investigated with the generalist *H. cunea*.

This research is important in two ways. First, invasive species, especially *L. maackii, L. japonica, P. calleryana, E. umbellata* and *L. tatarica,* are quickly becoming a large part of the native ecosystems and outcompeting native plants. It is important to understand what happens to native herbivores, like *A. americana, A. inflata, Z. montana,* and *H. cunea*, when they attempt to consume these invasive plants. Understanding the consequences of an invasive plant species on the flora and fauna of ecosystems in which it invades is very important, before the ecosystems are altered beyond repair. Second, if all stages of *A. americana, A. inflata, Z. montana,* and *H. cunea* can utilize these invasive species and can be influenced to consume them, then these organisms could potentially be used as biological control agents to help reduce and control invasive species. Controlling non-native plant populations with native organisms instead of chemical or mechanical methods would be a great benefit to the economy and the environment.

1.7 REFERENCES

Ali JG, Agrawal AA (2012) Review: Specialist versus generalist insect herbivores and
plant defense. Trends Plant Sci 17:293-302

Anthony R (1998) The third trophic level of plant defence: Neotropical social wasps' use
of odours of freshly damaged leaves when hunting. Rev Bras Zool :1075

Barbosa P, Saunders JA (1985) Plant allelochemicals: Linkages between herbivores and
their natural enemies. Recent advances in phytochemistry 19:107-137

Bernays EA, Minkenberg OPJM (1997) Insect herbivores: Different reasons for being a
generalist. Ecology :1157

Callaway RM, Ridenour WM (2004) Novel weapons: Invasive success and the evolution
of increased competitive ability. Frontiers in Ecology and the Environment :436-
443

Cappuccino N, Arnason JT (2006) Novel chemistry of invasive exotic plants. Biol Lett
2:189-193

Cates RG (1980) Feeding patterns of monophagous, oligophagous, and polyphagous
Insect herbivores: The effect of resource abundance and plant chemistry.
Oecologia :22

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ, Rejmanek M (2004) Is invasion
success explained by the enemy release hypothesis? Ecol Lett 7:721-733

Crawley MJ (1989) Insect herbivores and plant-population dynamics. Annu Rev Entomol
34:531-564

Davis S, Cipollini D (2014) Do mothers always know best? Oviposition mistakes and

resulting larval failure of *Pieris virginiensis* on *Alliaria petiolata*, a novel, toxic

host. Biol Invasions 16:1941-1950

Favorite J, Moore L (2008) Plant fact sheet for snowberry [*Symphoricarpos albus* (L.)

Blake.].

Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring

feeding by winter moth caterpillars. Ecology :565

Gorchov DL, Trisel DE (2003) Competitive effects of the invasive shrub, *Lonicera*

maackii (Rupr.) Herder (Caprifoliaceae), on the growth and survival of native tree

seedlings. Plant Ecol :13

Gordon DR (1998) Effects of invasive, non-indigenous plant species on ecosystem

processes: lessons from Florida. Ecol Appl :975

Gordon SW (1976) Population studies of the fall webworm in Ohio with the development

of life tables in relation to temperature. Dissertation Master, Ohio State University

Gurevitch J, Scheiner SM, Fox GA (2006) The ecology of plants. Sinauer associates

incorporated, Sunderland, MA

Haukioja E (1980) On the role of plant defences in the fluctuation of herbivore

populations. Oikos :202

Hayes SJ, Holzmueller EJ (2012) Relationship between invasive plant species and forest

fauna in Eastern North America. Forests 3:840-852.

Hilty J (2015) Trees, shrubs, and woody vines of Illinois. In: Anonymous .

http://www.illinoiswildflowers.info/trees/tree_index.htm. Accessed January/7

2016

Jahner JP, Bonilla MM, Badik KJ, Shapiro AM, Forister ML (2011) Use of exotic hosts

by *Lepidoptera*: Widespread species colonize more novel hosts. Evolution :2719

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17:164

Lankau RA, Rogers WE, Siemann E (2004) Constraints on the utilisation of the invasive Chinese tallow tree *Sapium sebiferum* by generalist native herbivores in coastal prairies. Ecol Entomol 29:66-75

Lieurance D, Cipollini D (2013a) Exotic *Lonicera* species both escape and resist specialist and generalist herbivores in the introduced range in North America. Biol Invasions 15:1713-1724.

Lieurance D, Cipollini D (2013b) Environmental influences on growth and defence responses of the invasive shrub, *Lonicera maackii*, to simulated and real herbivory in the juvenile stage. Annals of Botany 112:741-749

Lieurance D (2012) Mechanisms of success: plant-herbivore interactions and the invasion of non-native *Lonicera* species in North America. , Wright State University / OhioLINK

Loewy KJ, Flansburg AL, Grenis K, Kjeldgaard MK, McCarty J, Montesano L, Vernick J, Murphy SM (2013a) Life history traits and rearing techniques for fall webworms (*Hyphantria cunea* Drury) in Colorado. J Lepid Soc 67:196-205

Luken JO, McKnight B (1993) Prioritizing patches for control of invasive plant species: a case study with Amur honeysuckle. Biological pollution: the control and impact of invasive exotic species.Indiana Academy of Sciences, Indianapolis :211-214

Luken JO, Thieret JW (1996) Amur honeysuckle, its fall from grace. Bioscience :18

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl :689

Mason PA, Wilkes SR, Lill JT, Singer MS (2011) Abundance trumps quality: bi-trophic performance and parasitism risk fail to explain host use in the fall webworm. Oikos 120:1509-1518

McCall AC, Fordyce JA (2010) Can optimal defence theory be used to predict the distribution of plant chemical defences? J Ecol :985

McEwan RW, Birchfield MK, Schoergendorfer A, Arthur MA (2009a) Leaf phenology and freeze tolerance of the invasive shrub Amur honeysuckle and potential native competitors. Journal of the Torrey Botanical Society 2: 212

McEwan RW, Rieske LK, Arthur MA (2009b) Potential interactions between invasive woody shrubs and the gypsy moth (*Lymantria dispar*), an invasive insect herbivore. Biol Invasions 11:1053-1058

McNeish RE, Moore EM, Benbow ME, McEwan RW (2015) Removal of the invasive shrub, *Lonicera maackii*, from riparian forests influences headwater stream biota and ecosystem function. River Research & Applications 31:1131-1139

Munger GT (2002) *Lonicera japonica*. In: Anonymous . U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. http://www.fs.fed.us/database/feis/.

Murphy SM, Loewy KJ (2015) Trade-offs in host choice of an herbivorous insect based on parasitism and larval performance. Oecologia 179: 741-751

Myers JH, Post BJ (1981) Plant nitrogen and fluctuations of insect populations: a test

with the cinnabar moth: tansy ragwort system. Oecologia 48:151-156

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ 52:273-288

Price, PW, Denno RF, Eubancks MD, Finke DL, Kaplan I (2011) Insect ecology: behavior, populations and communities. Cambridge University Press, New York

Rhoades DF (1983) Responses of alder and willow to attack by tent caterpillars and webworms: evidence for pheromonal sensitivity of willows. In: Hedin P (ed) Plant resistance to insects. American Chemical Society, Washington DC, 55-68

Rhoades DF, Cates RG (1976) Toward a general theory of plant antiherbivore chemistry. Recent advances in phytochemistry 10:168-213

Sadler KC (2006) Exotic invasive species: The guests that won't go home. Green Teacher :6-9

Saul L (1999) Importance of insects and their arthropod relatives.

Smith DR, Gibson GA (1984) Filacus, a new genus for four species of sawflies previously placed in *Macrophya* or *Zaschizonyx* (Hymenoptera: Tenthredinidae). Pan-Pac Entomol 60:101-113

Sourakov A, Paris T (2014) Fall webworm, *Hyphantria cunea* (Drury) (Insecta: Lepiodoptera: Arctiidae: Arctiinae). In: Anonymous . IFAS Extension University of Florida.

Strauss SY, Agrawal AA (1999) Review: The ecology and evolution of plant tolerance to herbivory. Trends in Ecology & Evolution 14:179-185.

VanLaerhoven S, McGregor RR, Gillespie DR (2000) Leaf damage and prey type

determine search effort in *Orius tristicolor*. Entomol Exp Appl 97:167-174

Vitousek PM, DAntonio CM, Loope LL, Rejmanek M, Westbrooks R (1997) Introduced

species: A significant component of human-caused global change. N Z J Ecol

21:1-16

Warrington S, Whittaker JB (1985) An experimental field study of different levels of

insect herbivory induced by *Formica rufa* predation on sycamore (*Acer*

pseudoplatanus) II. J Appl Ecol :787

Warren O, Tadic M (1970) The fall webworm, *Hyphantria cunea* (Durry). Ark Agr Exp

Sta Bull: 106

Williamson M, Fitter A (1996) The varying success of invaders. Ecology :1661

2. PERFORMANCE OF THE SPECIALIST HONEYSUCKLE SAWFLIES (*ABIA AMERICANA* AND *ABIA AMERICANA*) ON NATIVE AND NON-NATIVE LONICERA SPECIES WITH VARYING DEGREES OF DAMAGE

2.1 INTRODUCTION

Many invasive plant species in their native ranges are often poor competitors with limited distribution (Keane and Crawley 2002; Lieurance 2012). However, in non-native ranges, these non-native plants can become dominant and extremely invasive (Keane and Crawley 2002; Lieurance 2012). The Enemy Release Hypothesis is one of the leading hypotheses to explain this phenomenon. This hypothesis suggests that release from natural enemies, especially from co-evolved specialist herbivores, is one major reason invasive plants are so successful in their novel environments (Keane and Crawley 2002;Colautti et al. 2004; Lieurance 2012). This release results in the rapid increase in abundance, distribution, and vigor of invasive plant species (Keane and Crawley 2002; Colautti et al. 2004; Lieurance 2012). Without enemies to keep non-native plants "in check" the plants can easily outcompete native plants and start spreading rapidly.

As invasive plant species enter a new habitat, they interact with native fauna, especially insect communities. These novel-plant insect interactions can be positive, negative, or neutral. A positive interaction could occur in which an insect benefits by successfully recognizing and using the invasive plant to increase in population size

19

(Davis and Cipollini 2014). A negative interaction could occur in which insects have lower fitness and reproductive success on the novel plant because one or all life stages cannot utilize the host (Davis and Cipollini 2014). Lastly, a neutral interaction could occur because the native insect does not recognize the novel plant as a food source and does not use it (Davis and Cipollini 2014).

Since specialist herbivores feed on relatively few, closely related species, they are particularly vulnerable to non-native plant invasions (Ali and Agrawal 2012). Invasive plants can outcompete and greatly reduce native plant populations used by the specialists. Monophagous herbivores and specialist herbivores on rarer plants are especially threatened because if the herbivore consumes all of its host plant it may not be able to find another. Adult specialist herbivores may be more vulnerable to enemy attack due to increased time searching for an oviposition site in a rare host. Specialist herbivores may not be able to utilize invasive hosts due to novel defenses, elevated chemical defenses, or the lack of specific oviposition cues (Callaway and Ridenour 2004; Cappuccino and Arnason 2006; Jahner et al. 2011). Through time, specialists will need to evolve mechanisms to utilize or avoid new invasive plant species if they are to persist in heavily invaded habitats.

There are approximately 200 species in the genus *Lonicera* (Caprifoliaceae), with 18 native and 16 introduced in North America (Zheng et al. 2005; Lieurance and Cipollini 2013a). The three most invasive *Lonicera* species in North America are *Lonicera maackii, Lonicera japonica,* and *Lonicera tatarica* (Schierenbeck et al. 1994; Luken and Thieret 1996; Hutchinson and Vankat 1997). These three invasive species have established themselves and become dominant in some habitats, especially in the

20

eastern United States. One of the most prevalent non-native plant species in southwestern Ohio, *L. maackii*, has many invasive traits such as a long growing season (McEwan et al. 2009), allelopathic suppression of other plants (Dorning and Cipollini 2006; Cipollini et al. 2008b), high fruit production (Ingold and Craycraft 1983), and anti-herbivore defenses (Cipollini et al. 2008a). In the field, *L. maackii* receives about 3% herbivory damage, which is significantly lower than herbivory rates on native congeners (Lieurance and Cipollini 2012; Lieurance and Cipollini 2013a). Native *Lonicera* species, such as *L. reticulata,* are not very abundant or common in their native ranges (http://plants.usda.gov).

In this study, the specialist honeysuckle sawflies (*Abia americana* (Cimbicidae) and *Abia inflata* (Cimbicidae)) were used to investigate the impact of non-native honeysuckle species and quality of foliage on the performance and life history traits of native specialist insects. Both *Abia* species are native to North America and are one of a few specialists that feed on native honeysuckle and its relatives (Lieurance and Cipollini 2013a). Adult *Abia* emerge in late April to early May (Figure 2.1, 2.2). The female uses her ovipositor to insert eggs into the edges of leaves. The larvae will hatch from the egg in 5-8 days and begin feeding on the edges of leaves. After about 3-4 weeks of development, the larvae pre-pupate in a fibrous cocoon in the soil and leaf litter. *Abia americana* overwinter in this pre-pupal form until emerging the next spring.

The larvae of both *A. americana* and *A. inflata* commonly feed on the leaves of native honeysuckle, causing significant defoliation. We have found *A. americana* feeding on snowberry (*Symphoricarpos albus*) leaves, a close relative to native honeysuckle (personal observation). In the laboratory, *A. inflata* larvae can also feed and survive on

non-native *L. maackii* relatively well, but are very rarely found feeding on them in the field (Lieurance and Cipollini 2012; Lieurance and Cipollini 2013a; Stireman personal observation). These larvae often cause significant foliage damage on *L. reticulata.* Since native *L. reticulata* is not very abundant, the larvae may be forced to eat damaged foliage or switch to a new host such as the non-native *L. maackii* which has invaded habitats with *L. reticulata*. Damaged leaves are typically not an ideal food source for *A. inflata* because they have a lower leaf area, lower water and nutrient content, higher toughness, an increase in defensive chemicals, (Feeny 1970; Myers and Post 1981; Rhoades 1983) and are a visual and odor cue for predators (Anthony 1998; VanLaerhoven et al. 2000). The cost of switching to a non-native host or consuming damaged leaves were determined in this study. Oviposition and eggs survival were examined on native (*L. reticulata* and *S. albus*) and non-native (*L. maackii* and *L. japonica*) plant species. Very little is known about the specialist honeysuckle sawfly and very little research has been done with them.

The primary objective of these experiments were to determine if all stages of *A. americana* could utilize non-native, invasive honeysuckle species. The secondary goal was to compare the performance of *A. americana* larvae on those non-native species to native honeysuckle species. The final objective was to determine the potential costs to *A. inflata* larvae consuming damaged leaves of a native host or switching to an alternative host.

In our oviposition studies with *A. americana,* we predicted that *A. americana* adults not given a choice will oviposit more eggs in native honeysuckle plants compared to non-native honeysuckle plants due to a lack of oviposition cues in non-native hosts. The eggs laid in these native honeysuckle species will have higher egg survival and take

less time to hatch because non-native plants can have novel defenses that may lower egg survival. We predicted that newly emerged *A. americana* larvae will be able to consume and complete their lifecycle on native (*L. reticulata* and *S. albus*) and non-native (*L. maackii* and *L. japonica*) foliage because all species are closely related. However, we believed *A. americana* larvae will grow faster, grow larger, have higher survivorship, and reach pre-pupation faster if the larvae consume native *L. reticulata* foliage due to *A. americana* larvae being adapted to consume and develop on native foliage.

In our no-choice feeding bioassays with newly emerged *A. inflata* larvae given damaged and undamaged native *L. reticulata* and non-native *L. maackii* foliage, we predicted larvae will grow faster, grow larger, have higher survivorship, and reach pre-pupation faster if larvae consume undamaged, native honeysuckle leaves. Damaged foliage have higher secondary defenses which may lead to lower performance and *A. inflata* larvae have evolved to deal with defenses found in native foliage. We hypothesize that a reduction in larval mass and relative growth rate will also be seen if we switch two week old *A. inflata* from undamaged *L. reticulata* to damaged *L. reticulata*.

2.2 METHODS

2.2.1 Abia americana Oviposition 2015

No-choice oviposition bioassays were conducted with adult *A. americana* collected from *S. albus* with sweep nets outside of the library located on Wright State University campus in Dayton, OH. Collection began April 13, 2015 and ended April 18 2015. A total of 26 adults were collected. All adults were placed separately into clear plastic deli container (4 x 6 x 6 cm). Each container contained a twig (10-15 cm) placed in a 7.6 cm water pick filled with DI water to keep the twig fresh [*S. albus* (N=2), *L. reticulata* (N=8), *L. maackii* (N=6), and *L. japonica* (N=6)]. Twigs of *L. reticulata, L. maackii,* and *L. japonica* were collected from a common garden located on Wright State University's campus. *Symphoricarpos albus* twigs were collected outside the library at Wright State University. Twigs were cut at an angle and immediately placed in DI water for transport to the laboratory on the days adult sawflies were collected. A cotton ball moistened with a 10% sucrose solution was placed at the bottom of each container as food for the adults. The containers were placed into an incubator at 25°C (16:8 L:D).

For approximately three hours every day the oviposition containers were placed under a 150 watt soft white incandescent lamp mounted 1 meter above the containers. The lamp simulated natural light which seemed to induce some oviposition behavior. Before being placed under the lamp, each twig was checked for eggs. If an egg was found, it was recorded and given a label with a unique number. Twigs with eggs were moved into their own deli container with a new water pick. Twigs were replaced in the oviposition containers as needed. All adults were kept on their original host for the entirety of the experiment. The total number of eggs for each female was determined.

24

2.2.2 Abia americana Egg Survival 2015

Twigs with *A. americana* eggs collected from the oviposition studies were kept individually in deli containers in an incubator at 25°C (16:8 L:D). Each egg was checked daily for hatching or changes in egg appearance. Percentage hatched and average number of days to hatch were determined for eggs in each host.

2.2.3 Feeding Bioassays with Newly Emerged Abia americana Larvae on Native and Non-native Hosts 2015

Newly hatched larvae from the oviposition trials were used in no-choice bioassays. Once a larva hatched, it was weighed and transferred to a fresh twig of the host plant from which it hatched. Larvae were placed individually into deli containers. Larvae were placed on *S. albus* (N=1), *L. reticulata* (N=2), *L. maackii* (N=3), or *L. japonica* (N=14). (Note: many eggs did not hatch which lead to small sample sizes) Larvae were haphazardly placed in an incubator at 25°C (16:8 L:D). The larvae were weighed and checked for survivorship and pupation every 2-4 days. New leaves and water were added as needed. Leaves remained in containers for no longer than a week. Once a week, the containers were cleaned of frass. The experiment ended after all larvae reached the pre-pupal stage or died.

2.2.4 Abia inflata Feeding Bioassays on Damaged and Undamaged Leaves

  (A) <u>Newly Emerged *Abia inflata* Larvae on Native and Non-native *Lonicera* 2015</u>

No-choice bioassays were set-up the same way as the host treatment above.

Newly hatched *A. inflata* larvae were collected from *L. reticulata* leaves at Kiser Lake

State Park in Conover, Ohio on May 4, 2015. The larvae were transferred to the lab in a

deli container containing *L. reticulata* leaves. A week prior to collecting larvae, *L.*

reticulata and *L. maackii* leaves were damaged in the common garden on Wright State

University. Leaves were cut in half with a pair of scissors to simulate herbivory. The

leaves were left for a week to give the plant time to react to the damage. Once in the

laboratory, larvae were weighed and placed individually into new deli containers. Larvae

were reared on undamaged *L. reticulata* (N=15), damaged *L. reticulata* (N=15),

undamaged *L. maackii* (N=15), or damaged *L. maackii* (N=15).

(B) Early Instar *Abia inflata* Larvae on *L. reticulata* 2014

Two-three week old *A. inflata* larvae were collected from *L. reticulata* leaves

from Kiser Lake State Park on May 22, 2014. Larvae were pooled into a container with

L. reticulata leaves for transport. Extra *L. reticulata* leaves were collected for the

bioassays. We collected undamaged *L. reticulata* leaves that had no herbivore damage

and we also collected damaged *L. reticulata* leaves that had approximately 50%

herbivore damage. Leaves were transported in a gallon size Ziploc bag. Leaves were kept

in a refrigerator during the experiment. Larvae were initially weighed before being placed

individually in a deli container with a few leaves placed in a moistened kim wipe to keep

them fresh. (The very smallest and very largest larvae were not used, because our goal

was to get larvae close in starting size and age.) The deli containers were placed

haphazardly in an incubator at 25°C (16:8 L:D). Larvae were weighed every 2-3 days and

leaves were added as needed. Once a week new kim wipes were added and frass was removed. The bioassay ended after 9 days due to limited supply of damaged *L. reticulata* leaves.

2.2.5 Data Analysis

Repeated measures ANOVA were used to compare larval masses with plant species as the between subjects effect and time as the within subjects effect. Eggs per female, days to eclosion, pupal mass, days to pre-pupation, and relative growth rates were compared among plant species using ANOVA. Comparisons of means were made using Tukey post-hoc tests. Percent hatching, percent survival, and percent pre-pupating compared using chi-square tests. All percentages were converted to arcsine values. Survivorship was compared among plant species using the non-parametric Mantel-Cox test. All statistical analyses were performed using GraphPad Prism (Version 6.07, GraphPad Software, Inc., La Jolla, California).

2.2.6 Calculations

Relative Growth Rate

Relative growth rate is calculated by subtracting the natural logarithm of initial mass from the natural logarithm of mass at the second time point and dividing by difference in time between weighing.

Relative Growth Rate (RGR) = $\dfrac{\ln(W_2) - \ln(W_1)}{T_2 - T_1}$

T_1 = time of initial weight (days) W_1 = initial mass (grams)
T_2 = time of second weight (days) W_2 = second mass (grams)

2.3 RESULTS

2.3.1 *Abia americana Oviposition 2015*

In the no-choice oviposition assays, adult *A. americana* laid eggs in native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. japonica*) species. The mean number of eggs per female was not significantly different between native and non-native species (Table 2.1, Fig 2.3a). All females offered *S. albus* and 63% of females offered *L. reticulata* oviposited (Fig 2.3b). Whereas, 43% and 33% of females offered *L. maackii* and *L. japonica,* respectively, oviposited (Fig 2.3b).

2.3.2 *Abia americana Egg Survival 2015*

Eggs successfully hatched on all native and non-native species. Eggs laid in *L. japonica* (non-native) had the very highest hatching success at 94% (Fig 2.4a). Eggs oviposited in *L. reticulata* (native) and *L. maackii* (non-native) had the lowest percentage hatching at 9% and 29%, respectively (Fig 2.4a). Eggs laid in *S. albus* had intermediate hatching success and 57% (Fig 2.4a). All *A. americana* larvae hatched in 5-8 days. Eggs took significantly longer to hatch when laid in *S. albus* compared to *L. maackii* (non-native) (Table 2.2, Fig 2.4b). The average time to hatch for eggs laid in *L. reticulata* (native) and *L. japonica* (non-native) did not differ significantly from the other two species.

2.3.3 *Feeding Bioassays with Newly Emerged Abia americana Larvae on Native and Non-native Hosts 2015*

In the no-choice bioassays with *Abia americana* reared from eggs, repeated measures ANOVA only showed differences in larval mass changes through time (Table

28

2.3, Figure 2.5a). There was no difference in larval mass by species or an interaction of time and species (Table 2.3, Figure 2.5a). *Abia americana* larvae feeding on *L. japonica* had 100% mortality by day 2. There was no difference in relative growth rate between *S. albus, L. reticulata,* and *L. maackii* (Figure 2.5b). There were significant differences in survivorship (X=264.00, df =3, P<0.0001) of larvae reared on the four different species. Larvae reared on *L. reticulata* and *L. maackii* had 100% survival (Figure 2.5c). Larvae reared on *S. albus* had 50% survival, whereas no larvae survived on *L. japonica* (Figure 2.5c). Larvae on all species except for *L. japonica* successfully reach the pre-pupal stage, and days to pupation did not differ between species (Table 2.4, Figure 2.5d).

2.3.4 *Abia inflata Feeding Bioassays on Damaged and Undamaged Leaves*

  (A) <u>Newly Emerged Larvae on Native and Non-native *Lonicera* 2015</u>

In the no-choice bioassays with newly hatched *Abia inflata* collected from Kiser Lake State Park, repeated measures ANOVA revealed differences in changes in larval mass through time (Table 2.5, Figure 2.6a). However, there was no significant difference found between species nor an interaction between time and species (Table. 2.5). All larvae pupated on *L. reticulata* by day 18. There was no difference in relative growth rate and larval survival between native and non-native species or damaged and undamaged treatments (Figure 2.6 b-d). Larvae reared on undamaged *L. reticulata* pupated faster than *A. americana* larvae reared on damaged *L. maackii* (Figure 2.7a). However, the time it took larvae feeding on damaged *L. reticulata* and undamaged *L. maackii* to pupate was not different than the other two treatments. Larvae successfully reached pre-pupation on native and non-native honeysuckle as well as on damaged leaves. The percentage

reaching pre-pupation on all species and damage level did not differ significantly (Figure 2.7b).

(B) Early Instar *Abia inflata* Larvae on *L. reticulata* 2014

In the no-choice bioassays with two week old *Abia americana* collected from *L. reticulata* from Kiser Lake State Park, repeated measures ANOVA revealed differences in changes in larval mass through time and an interaction between time and damage level (Table 2.7, Figure 2.8a). There was no significant difference in larval mass found between damaged and undamaged *L. reticulata* (Table. 2.7). The repeated measures ANOVA for relative growth rate over the 7 days found differences in RGR through time, but no interaction or treatment effect was found (Table 2.8). On day 5, larvae feeding on undamaged *L. reticulata* had a significantly higher relative growth rate compared to damaged foliage (Figure 2.8b).

2.4 DISCUSSION

2.4.1 *Abia americana Oviposition 2015*

In the no-choice oviposition studies, *A. americana,* females successfully oviposited in native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. japonica*) hosts. Contrary to our prediction, *A. americana* females did not oviposit more eggs in native hosts compared to non-native hosts. When looking at the percentage of females laying eggs on each host, higher percentages of females laid eggs on the native hosts compared to the non-native hosts. This oviposition study was very important, because in the face of invasion by *L. maackii* and *L. japonica,* adult *A. americana* sawflies are able to oviposit in non-native *L. maackii* and *L. japonica*.

2.4.2 *Abia americana Egg Survival 2015*

Although we did not find a difference between the average number of eggs laid in each host, we did find a significant difference in the percentage of eggs hatching and the time to eclosion. Eggs laid in non-native *L. japonica* had very high egg survival compared to all other native and non-native hosts. Eggs laid in *S. albus*, a close relative to native honeysuckle, had over 50% of eggs hatch, but eggs took significantly longer to hatch on this host compared to non-native *L. maackii*. Both non-native *L. maackii* and native *L. reticulata* had very low hatching success. Our results did not support our hypothesis that eggs laid in native hosts will have higher egg survival and faster time to eclosion. Native *L. reticulata* had the lowest egg survivorship of all hosts. This was surprising because we often find *A. americana* using *L. reticulata* in the field. The low egg survival in both *L. reticulata* (Figure 2.10) and *L. maackii* (Figure 2.11) was due to the production of secondary chemicals in theses leaves attacking the eggs (Hilker and

Fatouros 2015). After 24 hours, small brown circles started to form around the eggs, after a week many of the eggs turned black and were surrounded by a large ring of dead leaf tissue. This defensive response was not seen in the *S. albus* or *L. japonica* leaves, which lead to much higher hatching rates in these two hosts.

In the field, we also observed this defensive response on native *L. reticulata* leaves at attempted oviposition sites and around eggs, but not nearly as great as some of the responses we saw in the lab. The females in our study were only given a few leaves to oviposit, this often lead to multiple eggs being laid in each leaf. In the most extreme example, we had 7 eggs oviposited in one *L. reticulata* leaf (Figure 2.10). The defensive response in the leaf was amplified by how many eggs are laid in the leaf. The more eggs laid in a leaf, the stronger the defensive response, and the higher the egg mortality. As *L. maackii* is slowly taking over and outcompeting native *L. reticulata*, adult *A. americana* may be forced to oviposit multiple eggs in *L. reticulata* leaves. This may greatly reduce the number of eggs hatching due to this defensive response. Alternatively, reduction in *L. reticulata* populations may lead to a host shift of *A. americana* to *L. maackii* in habitats with both honeysuckle species. Even though *L. maackii* has a similar defensive response to *A. americana* eggs, it is much more abundant than *L. reticulata* leading to greater dispersal of eggs throughout the plant and possible higher egg survival due to this dispersal. In the most extreme case where *L. maackii* is all that remains in a habitat, *A. americana* females will be able to use *L. maackii* as an alternative host if they can recognize it in the field. The native *A. americana* may be able to capitalize on the abundant early foliage of *L. maackii* and maybe help keep the early foliage in check.

2.4.3 *Feeding Bioassays with Newly Emerged Abia americana Larvae on Native and Non-native Hosts 2015*

Newly emerged *A. americana* larvae on native *L. reticulata,* native *S. albus*, and non-native *L. maackii* had similar larval mass through time, relative growth rates, survival, and time to pre-pupation. Although hatching rates on non-native *L. japonica* were the highest, no larvae survived more than 2 days on the *L. japonica* foliage. Some larvae refused to eat the foliage and starved to death, others died while feeding on the foliage. This was not surprising because previous studies done with these honeysuckle sawflies found that early instar larvae switched onto *L. japonica* could not survive due to the leaves being highly toxic to this species (Lieurance and Cipollini 2013a). Our predictions were partially correct, newly emerged larvae were able to consume non-native *L. maackii* foliage but were not able to consume non-native foliage of *L. japonica*. Also, *A. americana* larvae did not have higher performance on all native hosts compared to non-native hosts as predicted.

This study was important to determine if *A. americana* larvae can reach pre-pupation when reared from eggs on native and non-native hosts. It was found that *A. americana* larvae can survive and perform just as well on non-native *L. maackii* as on native host they are found on in the field. However, Lieurance and Cipollini (2013) found that larvae given a choice between *L. reticulata* and *L. maackii* strongly preferred to consume the native host. Even though honeysuckle sawfly larvae can perform well on both native and non-native hosts, they may consume all the native host before switching to a novel host.

2.4.4 *Abia inflata Feeding Bioassays on Damaged and Undamaged Leaves*

Both native *L. reticulata* and *S. albus* have relatively low abundances in the field compared to non-native, invasive species like *L. maackii* and *L. japonica.* These invasives continue to spread and outcompete native species preferred by *A. inflata,* greatly reducing the populations of *L. reticulata* and *S. albus*. Since we have only found *A. inflata* feeding on native *L. reticulata* and *S. albus* in the field, these native plants may receive greater herbivore damage from *A. inflata* in invaded areas. Larvae may be forced to consume damaged leaves which have lower quality due to lower water and nutrient content, higher toughness, and higher quantities of defensive chemicals (Feeny 1970; Myers and Post 1981; Rhoades 1983). Damaged leaves are not only a lower quality food source they are also a visual and odor cue for predators (Anthony 1998; VanLaerhoven et al. 2000). An alternative to consuming damaged leaves is to switch to a novel host.

(A) Newly Emerged *Abia inflata* Larvae on Native and Non-native *Lonicera* 2015

Abia inflata larvae reared for the entirety of their life cycle on damaged and undamaged *L. reticulata* and *L. maackii* had no difference in larval mass through time, relative growth rate, or larval survivorship. These results did not support our prediction that honeysuckle larvae would grow larger, grow faster, and have higher survivorship on undamaged foliage. However, larvae reared on undamaged *L. reticulata* foliage reached pre-pupation significantly faster than larvae reared on damaged, non-native *L. maackii*. This did support our hypothesis that larvae reared on undamaged, native foliage would reach pre-pupation faster than larvae on damaged, non-native foliage.

34

Although our results did not show a cost to consuming damaged leaves in the laboratory setting, there are many other factors we did not include in our study. First, our larvae were not exposed to any predators. Predators often use damaged leaves as a visual and odor cue to find herbivores (Anthony 1998; VanLaerhoven et al. 2000). Second, our larvae were fed artificially damaged leaves, cut a week prior with scissors in the field. Simulated herbivory and natural herbivory can evoke different plant response in the leaves, and often simulated herbivory is a poor substitute for actual damage done by an herbivore (Strauss and Agrawal 1999). When a herbivore consumes foliage, their saliva and the chewing of the leaf can trigger different plant response compared to the clipping of foliage with relatively clean scissors (Strauss and Agrawal 1999; Musser et al. 2005). However, Lieurance and Cipollini (2013) found the plant chemistry did not differ between simulated and real herbivory in *L. maackii*.

(B) Early Instar *Abia inflata* Larvae on *L. reticulata* 2014

Abia inflata larvae switched onto damaged *L. reticulata* foliage had significantly lower larval mass after 7 days and a lower relative growth rate at day 5 compared to larvae feeding on undamaged foliage. This supported our hypothesis that larvae switched onto foliage damaged by *A. inflata* will have lower larval weight and relative growth rates. As *L. maackii* and *L. japonica* spread into native habitats, *L. reticulata* populations are likely to decline. These honeysuckle sawflies cause high levels of defoliation early in the season on native *L. reticulata*, which may cause a reduction in the growth and reproductive success of *L. reticulata* later in the season (Warrington and Whittaker 1985; Crawley 1989; Lieurance and Cipollini 2013b). This may lead to the further decline of

this native species. Not only will damaged foliage reduce *L. reticulata's* growth and reproductive success, but could also reduce the fitness of adult sawflies due to lower larval mass and growth of larvae feeding on damaged foliage. If there are large populations of *A. inflata*, damaged *L. reticulata* foliage may be the only foliage left for these specialists to consume. This is especially true in the face of invasions by non-natives such as *L. maackii* and *L. japonica* into native habitats. This specialist may need to evolve to recognize and utilize other native or non-native hosts in order to persist.

2.5 CONCLUSION

In the field, the interaction of *A. americana* with novel, non-native *L. maackii* and *L. japonica* appears to be neutral, where *A. americana* does not recognize these novel hosts as a suitable host and does not use it. However, from our laboratory studies, *A. americana* and *L. japonica* have a clearly negative relationship. Adult sawflies can oviposit successfully on *L. japonica* but all larvae quickly die from eating *L. japonica* foliage. If *A. americana* are ovipositing on *L. japonica* in the field, this could be a potential egg sink for this sawfly species, which could lead to its decline. An investigation into oviposition preference is key to fully determine this interaction. On the other hand, our studies show *A. americana* and *L. maackii* have a positive interaction, in which *A. americana* adults and larvae can utilize this novel host and potentially increase in population size. Adult *A. americana* can successfully oviposit in *L. maackii* and newly emerged larvae can perform just as well on *L. maackii* as its native host.

In the field, *A. inflata* has not been found utilizing *L. maackii* and *L. japonica*. This suggests *A. inflata* adults do not recognize these hosts as suitable and do not use them, a neutral interaction. Lieurance and Cipollini (2013a) found *A. inflata* larvae have a negative relationship with *L. japonica,* because all larvae switched onto this host cannot survive. On the other hand, they found *A. inflata* larvae can perform on non-native *L. maackii* just as well as their native host *L. reticulata*, indicating a potential positive interaction with this novel host (Lieurance and Cipollini 2013a). *Abia inflata* can utilize novel hosts when no other options are available, but when given a choice, *A. inflata* larvae strongly prefer to consume native *L. reticulata,* suggesting a neutral interaction (Lieurance and Cipollini 2013a). During the early growing season, larvae cause

significant defoliation of their native host, which leads many larvae faced to choose between consuming damaged leaves or switching to a novel host. *Abia inflata* that remain on the damaged foliage have lower larval mass and growth rates, which may reduce *A. inflata* populations if they do not utilize the abundant novel hosts around them, in particular *L. maackii*.

In the face of *L. maackii* invasion, *A. americana* and *A. inflata* may be forced utilize this very abundant novel host, especially if there are few native honeysuckles remaining in a habitat. Adult sawflies that recognize *L. maackii* as a suitable hosts will have reduced searching time to find an oviposition site due to the high abundance of this non-native plant. This reduced searching time will increase adult sawfly survivorship and potential reproductive fitness. As time passes, *A. americana* and *A. inflata* may be able to fully utilize novel hosts, especially *L. maackii*. Both of these *Abia* species could potentially become native specialists that can help keep *L. maackii* populations "in-check" especially early on in the growing season.

2.6 REFERENCES

Ali JG, Agrawal AA (2012) Review: Specialist versus generalist insect herbivores and plant defense. Trends Plant Sci 17:293-302

Anthony R (1998) The third trophic level of plant defence: neotropical social wasps' use of odours of freshly damaged leaves when hunting. Rev Bras Zool :1075

Callaway RM, Ridenour WM (2004) Novel weapons: Invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment :436

Cappuccino N, Arnason JT (2006) Novel chemistry of invasive exotic plants. Biology Letters 2:189-193

Cipollini D, Stevenson R, Enright S, Eyles A, Bonello P (2008a) Phenolic metabolites in leaves of the invasive shrub, *Lonicera maackii*, and their potential phytotoxic and anti-herbivore effects. J Chem Ecol 34:144-152

Cipollini D, Stevenson R, Cipollini K (2008b) Contrasting effects of allelochemicals from two invasive plants on the performance of a nonmycorrhizal plant. Int J Plant Sci :371

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ, Rejmanek M (2004) Is invasion success explained by the enemy release hypothesis? Ecol Lett 7:721-733

Crawley MJ (1989) Insect herbivores and plant-population dynamics. Annu Rev Entomol 34:531-564

Davis S, Cipollini D (2014) Do mothers always know best? Oviposition mistakes and resulting larval failure of *Pieris virginiensis* on *Alliaria petiolata*, a novel, toxic host. Biol Invasions 16:1941-1950

Dorning M, Cipollini D (2006) Leaf and root extracts of the invasive shrub, *Lonicera maackii*, inhibit seed germination of three herbs with no autotoxic effects. Plant Ecol :287

Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology :565. Annual review of entomology 60: 493-515

Hilker M, Fatouros NE (2015) Plant responses to insect egg depostion

Hutchinson TF, Vankat JL (1997) Invasibility and effects of Amur honeysuckle in Southwestern Ohio forests. Conserv Biol :1117

Ingold JL, Craycraft MJ (1983) Avian frugivory on honeysuckle (*Lonicera*) in soutwestern Ohio in fall. Ohio Journal of Science 83:256-258

Jahner JP, Bonilla MM, Badik KJ, Shapiro AM, Forister ML (2011) Use of exotic hosts by *Lepidoptera*: Widespread species colonize more novel hosts. Evolution :2719

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17:164

Lieurance D, Cipollini D (2013a) Exotic *Lonicera* species both escape and resist specialist and generalist herbivores in the introduced range in North America. Biol Invasions 15:1713-1724

Lieurance D, Cipollini D (2013b) Environmental influences on growth and defence responses of the invasive shrub, *Lonicera maackii*, to simulated and real herbivory in the juvenile stage. Annals of Botany 112:741-749

Lieurance D (2012) Mechanisms of success: plant-herbivore interactions and the invasion

of non-native *Lonicera* species in North America. , Wright State University / OhioLINK

Lieurance D, Cipollini D (2012) Damage levels from arthropod herbivores on *Lonicera maackii* suggest enemy release in its introduced range. Biol Invasions 14:863-873

Luken JO, Thieret JW (1996) Amur honeysuckle, its fall from grace. Bioscience :18

McEwan RW, Birchfield MK, Schoergendorfer A, Arthur MA (2009) Leaf phenology and freeze tolerance of the invasive shrub Amur honeysuckle and potential native competitors. The Journal of the Torrey Botanical Society :212

Musser RO, Cipollini DF, Hum-Musser S, Williams SA, Brown JK, Felton GW (2005) Evidence that the caterpillar salivary enzyme glucose oxidase provides herbivore offense in solanaceous plants. Arch Insect Biochem Physiol 58:128-137

Myers JH, Post BJ (1981) Plant nitrogen and fluctuations of insect populations: a test with the cinnabar moth: tansy ragwort system. Oecologia :151

Rhoades DF (1983) Responses of alder and willow to attack by tent caterpillars and webworms: evidence for pheromonal sensitivity of willows. In: Hedin P (ed) Plant resistance to insects. American Chemical Society, Washington DC, pp 55-68

Schierenbeck KA, Mack RN, Sharitz RR (1994) Effects of herbivory on growth and biomass allocation in native and introduced species of Lonicera. Ecology :1661

Strauss SY, Agrawal AA (1999) Review: The ecology and evolution of plant tolerance to herbivory. Trends in Ecology & Evolution 14:179-185

VanLaerhoven S, McGregor RR, Gillespie DR (2000) Leaf damage and prey type determine search effort in *Orius tristicolor*. Entomol Exp Appl 97:167-174

41

Warrington S, Whittaker JB (1985) An experimental field study of different levels of

    insect herbivory induced by *Formica rufa* predation on sycamore (*Acer*

    pseudoplatanus) II. J Appl Ecol :787

Zheng H, Wu Y, Ding J, Binion D, Fu W, Reardon R (2005) Invasive plants established

    in the United States that are found in Asia and their associated natural enemies vol

    1. Chinese Academy of Sciences 1

Table 2.1 ANOVA tables for average number of *A. americana* eggs laid per female on native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. japonica*) hosts in 2015.

	DF	MS	F	P value	Significant
Treatment	4	12.8800	0.65	P = 0.6317	No
Error	24	19.7400			

Table 2.2 ANOVA table for mean number of days for *A. americana* larvae to hatch on native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. japonica*) hosts in 2015.

	DF	MS	F	P value	Significant
Treatment	3	1.1310	4.06	P = 0.0209	Yes
Error	20	0.2783			

Table 2.3 ANOVA table for newly emerged *A. americana* larval mass through time when reared on 2 native (*S. albus* and *L. reticulata*) and 1 non-native (*L. maackii*) in 2015.

	DF	MS	F	P value	Significant
Time	4	0.0814	24.42	P < 0.0001	Yes
Treatment	2	0.0098	4.67	P = 0.1198	No
Time x Treatment	8	0.0041	1.22	P = 0.3650	No
Error	12	0.0033			

Table 2.4 ANOVA table for mean number of days for newly emerged *A. americana* larvae to reach pre-pupation on 2 native (*S. albus* and *L. reticulata*) and 1 non-native (*L. maackii*) species in 2015.

	DF	MS	F	P value	Significant
Treatment	2	5.095	3.06	P = 0.1564	No
Error	4	1.6670			

Table 2.5 ANOVA table for *A. inflata* larval mass through time when reared on damaged or undamaged *L. reticulata* and *L. maackii* leaves collected from Kiser Lake State Park in 2015.

	DF	MS	F	P value	Significant
Time	4	0.4540	97.46	P < 0.0001	Yes
Treatment	3	0.0129	2.71	P = 0.0623	No
Time x Treatment	12	0.0052	1.12	P = 0.3469	No
Error	124	0.0047			

Table 2.6 ANOVA table for mean number of days for *A. inflata* to reach the pre-pupal stage on native *L. reticulata* and non-native *L. maackii* with varying degrees of damage.

	DF	MS	F	P value	Significant
Treatment	3	19.2500	4.37	P = 0.0112	Yes
Error	31	4.4080			

Table 2.7 ANOVA table for *A. inflata* larval mass through time when reared on damaged or undamaged *L. reticulata* leaves collected from Kiser Lake State Park in 2014.

	DF	MS	F	P value	Significant
Time	3	0.3266	336.20	P < 0.0001	Yes
Treatment	1	0.0171	2.03	P = 0.1685	No
Time x Treatment	3	0.0035	3.61	P = 0.0180	Yes
Error	63	0.0010			

Table 2.8 ANOVA table for the relative growth rate (RGR) of *A. inflata* larvae reared on damaged or undamaged *L. reticulata* leaves collected from Kiser Lake State Park in 2014.

	DF	MS	F	P value	Significant
Time	2	0.0674	89.83	P < 0.0001	Yes
Treatment	1	0.0190	3.05	P = 0.0953	No
Time x Treatment	2	0.0003	0.42	P = 0.6574	No
Error	42	0.0007			

Adult

Pre-pupa
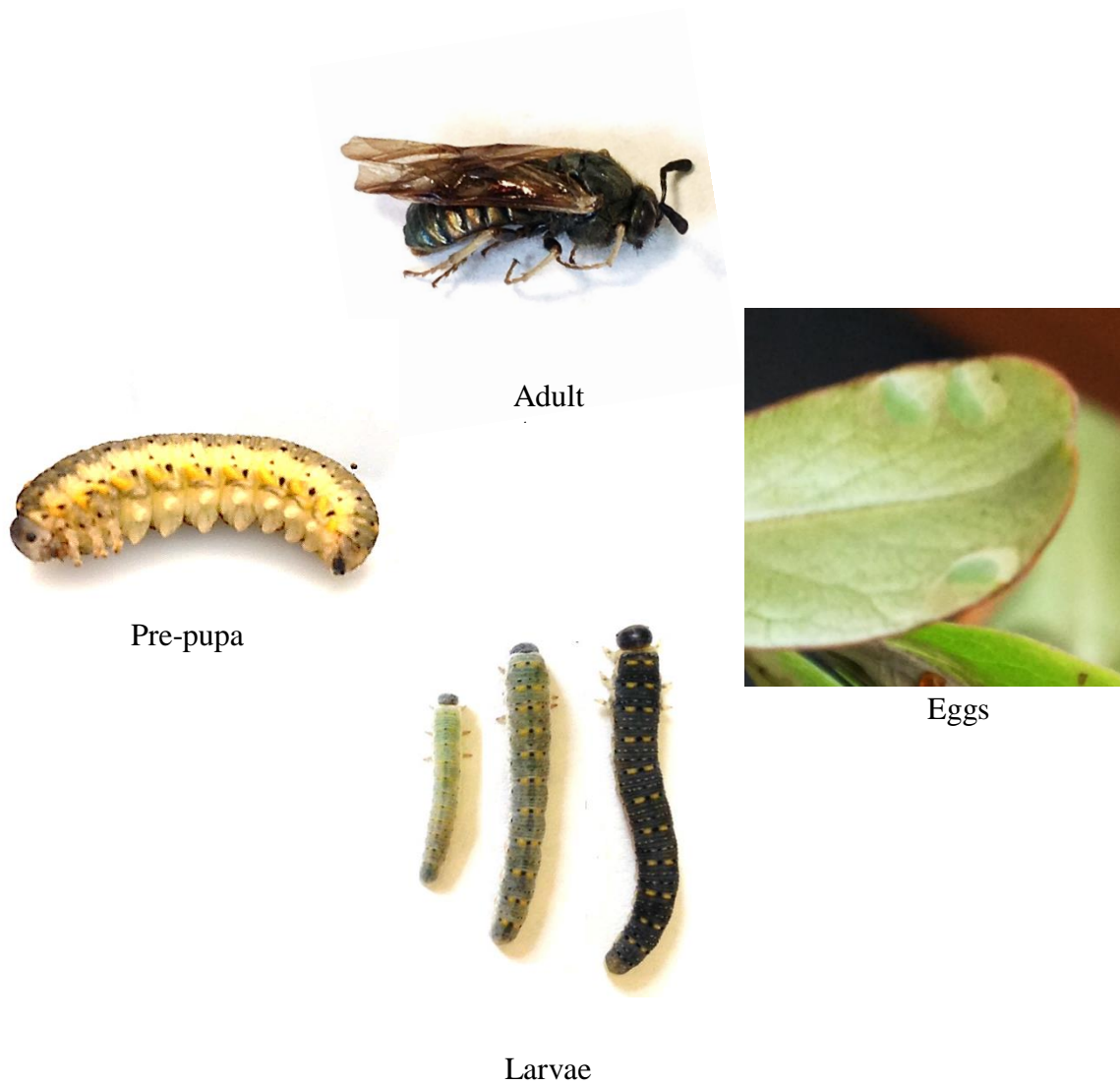
Eggs

Larvae

Figure 2.1 Life cycle of *Abia americana*. Adult females lay eggs in leaves using her saw-like ovipositor. The eggs hatch in 5-8 days. The larvae start off with few spots and markings and as they get older, the larvae get darker with more distinct markings. After 18-25 days, the larvae spin a protective cocoon and pre-pupate. *Abia americana* overwinters as a pre-pupa and in early April, pupates and emerges.
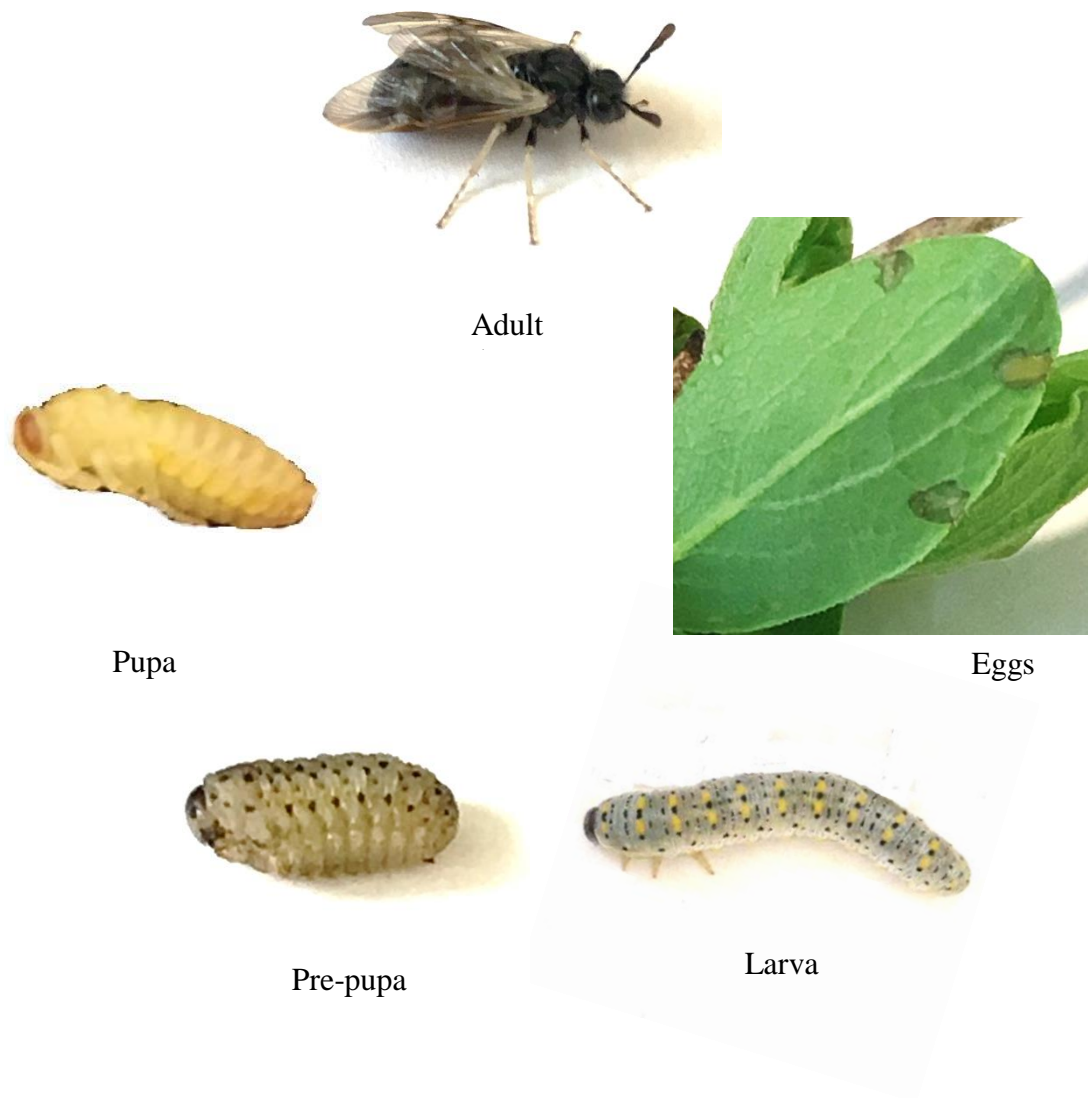
Figure 2.2 Life cycle of *Abia inflata*. Adult females lay eggs in leaves using her saw-like ovipositor. The eggs hatch in 5-8 days. The larvae start off with few spots and markings and as they get older, the larvae get darker with more distinct markings. After 18-25 days, the larvae spin a protective cocoon and pre-pupate. *Abia inflata* overwinters as a pre-pupa and in early April, pupates and emerges.
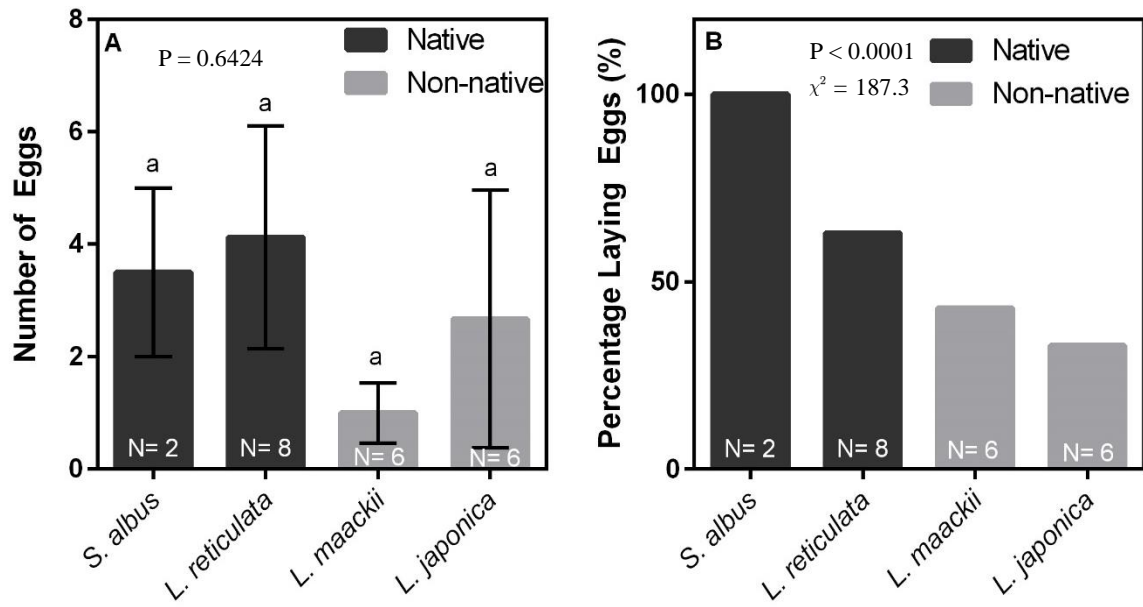
Figure 2.3 Mean number of eggs per female *A. americana* (A) and percentage of *A. americana* females laying eggs (B) in a no-choice bioassay with two native (*S. albus* and *L. reticulata*) and two non-native (*L. maackii* and *L. japonica*) species.
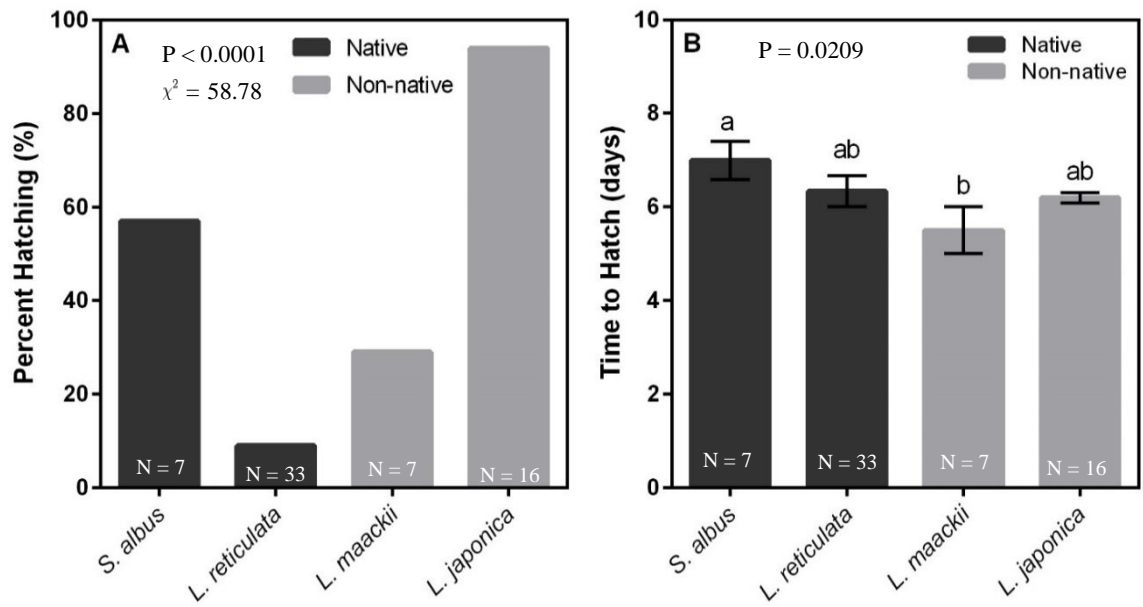
Figure 2.4 Percentage of *A. americana* larvae hatching from oviposition trials in native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. japonica*) species (A), and the average time to hatch (B). Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05).
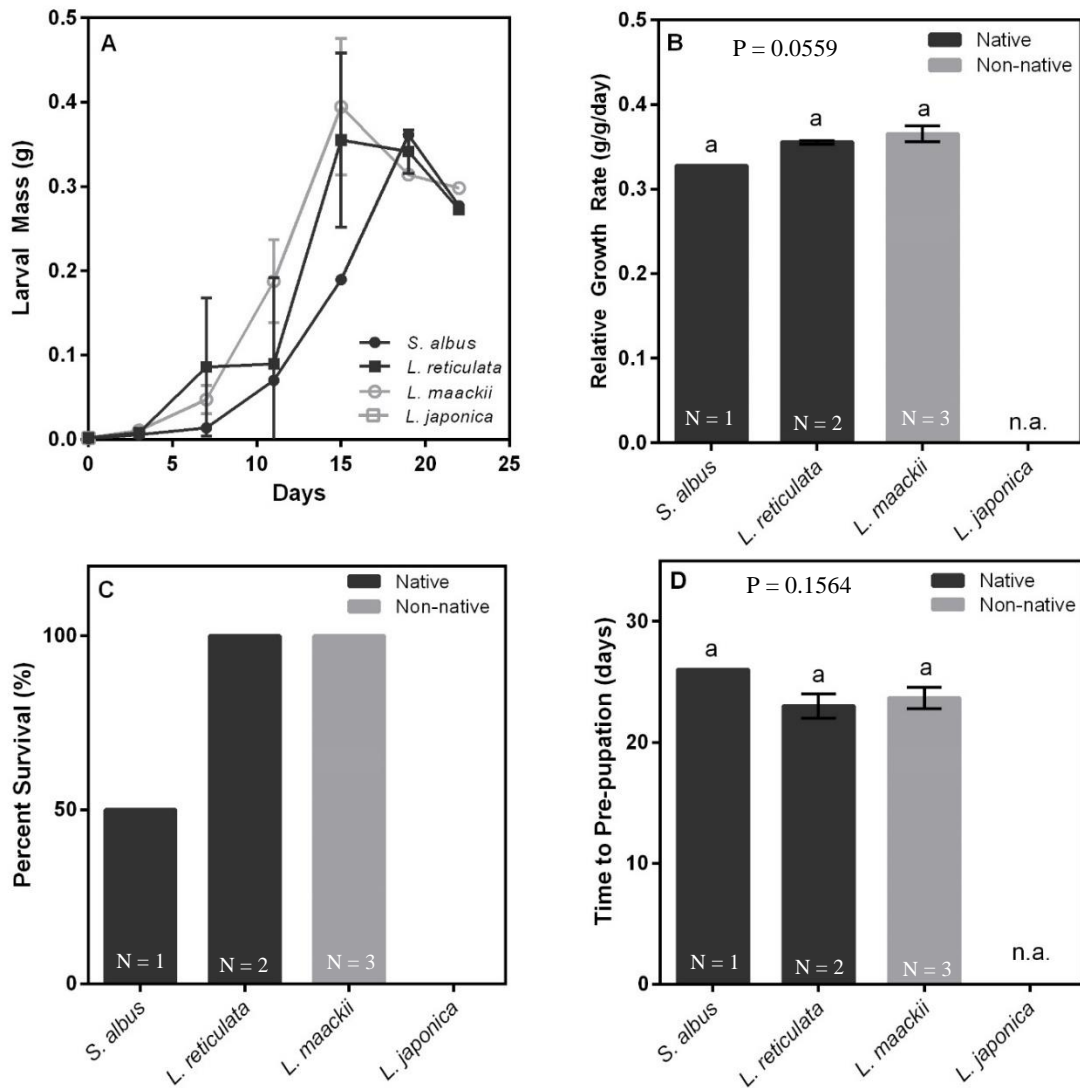
Figure 2.5 Mean larval mass of the specialist *Abia americana* feeding on the foliage of two native and two non-native species (A), relative growth rate (RGR) measured at peak larval weight on day 15 (B), final larval survivorship (C), and time to pre-pupation (D) in 2015. Species not included in the statistical analysis are denoted as "n.a.".
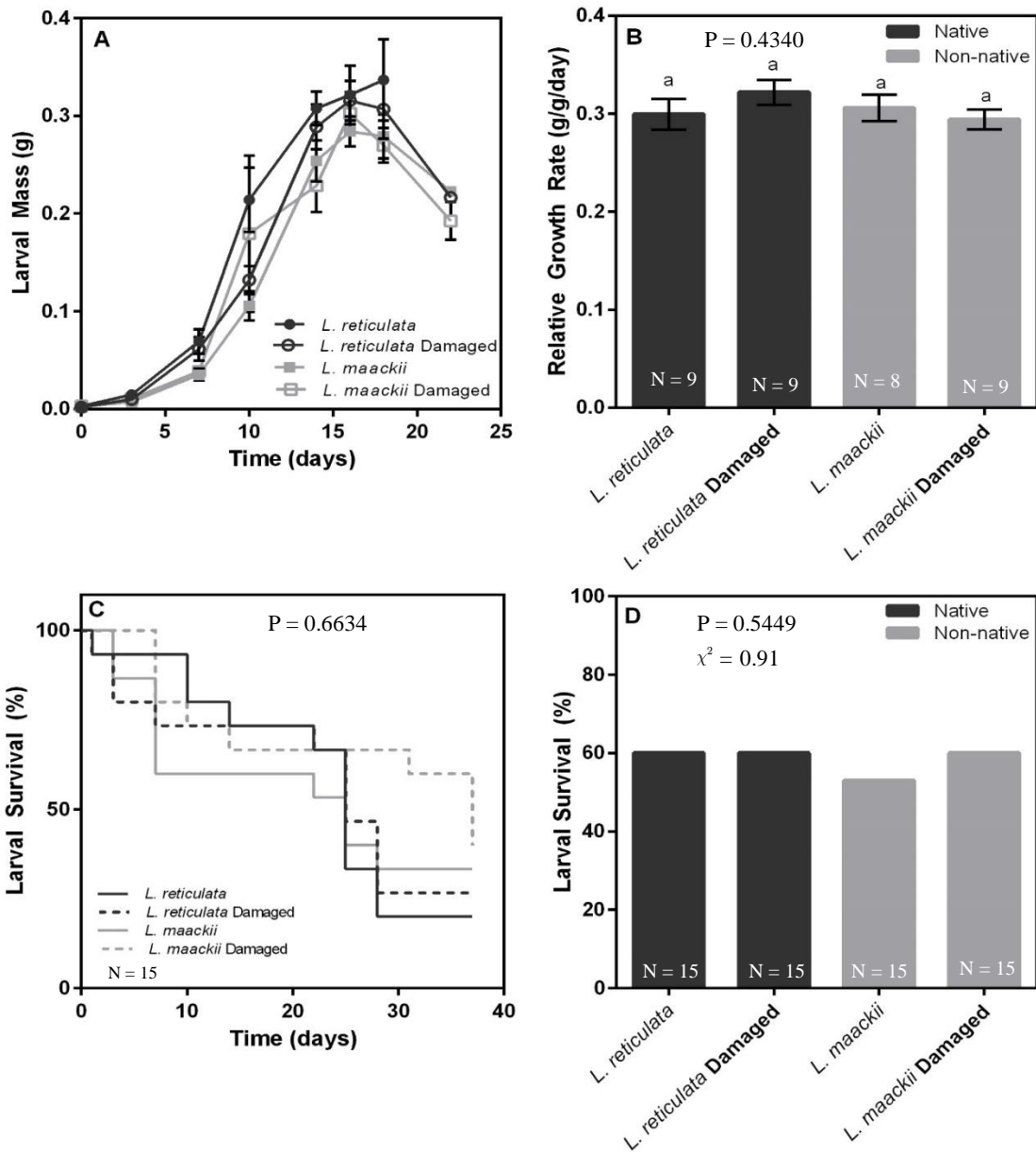
Figure 2.6 Mean larval mass of the specialist *Abia inflata* feeding on damaged and undamaged foliage of one native and one non-native *Lonicera* species (A), relative growth rate (RGR) measured at peak larval weight on day 16 (B), larval survivorship to pre-pupation (C), and larval survival up to the pre-pupal stage (D). Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05).
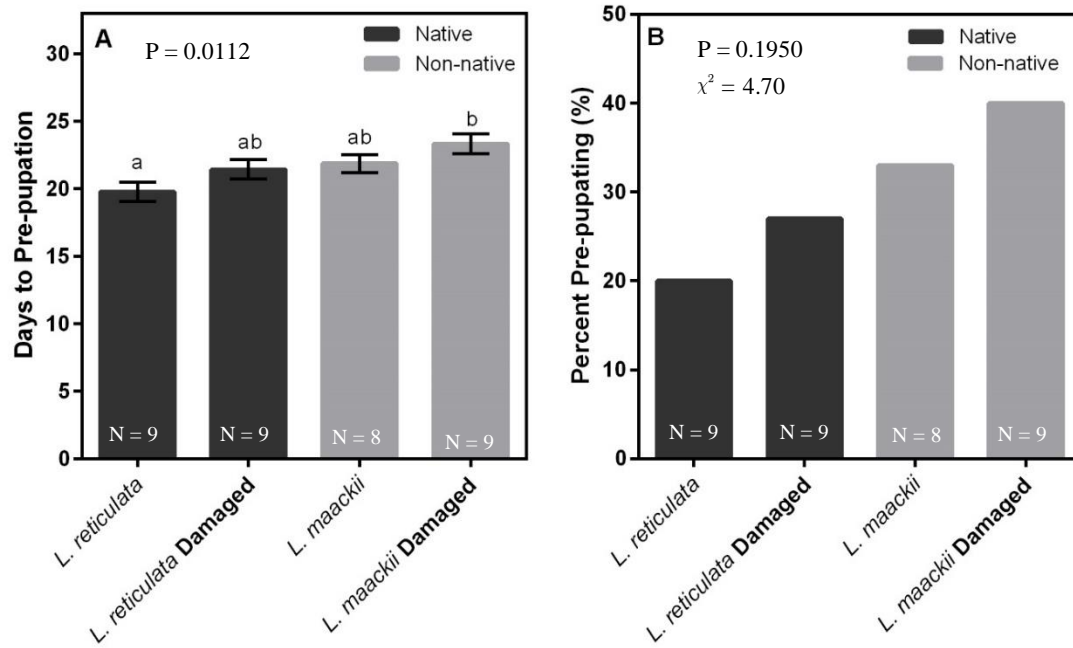
Figure 2.7 Mean days for *Abia inflata* larvae to reach pre-pupation feeding on damaged and undamaged foliage of one native and one non-native *Lonicera* species (A), and percentage reaching pre-pupation (B). Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05).
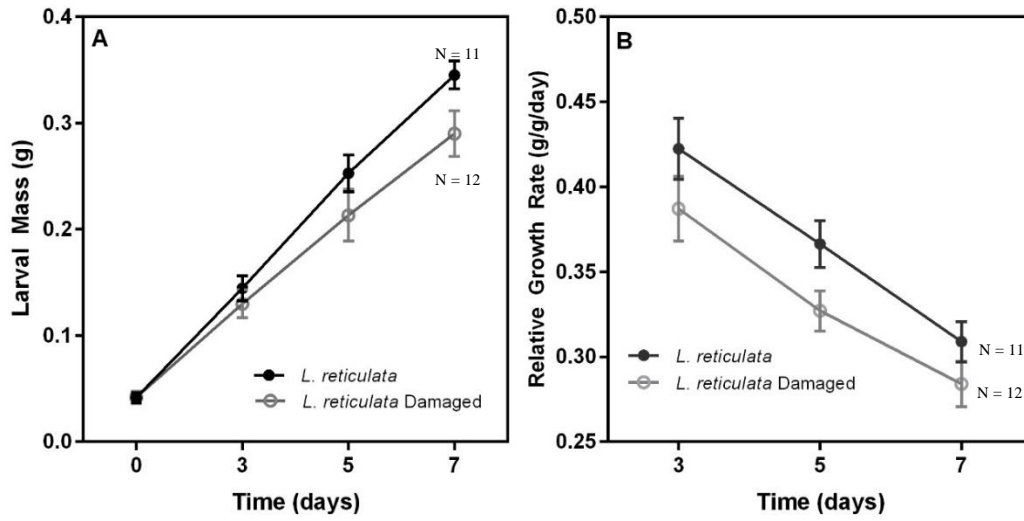
Figure 2.8 Mean larval mass of the specialist *Abia inflata* feeding on damaged and undamaged foliage of native *L. reticulata* for 7 days (A), and relative growth rate (RGR) through time (B) in 2014.

A

B
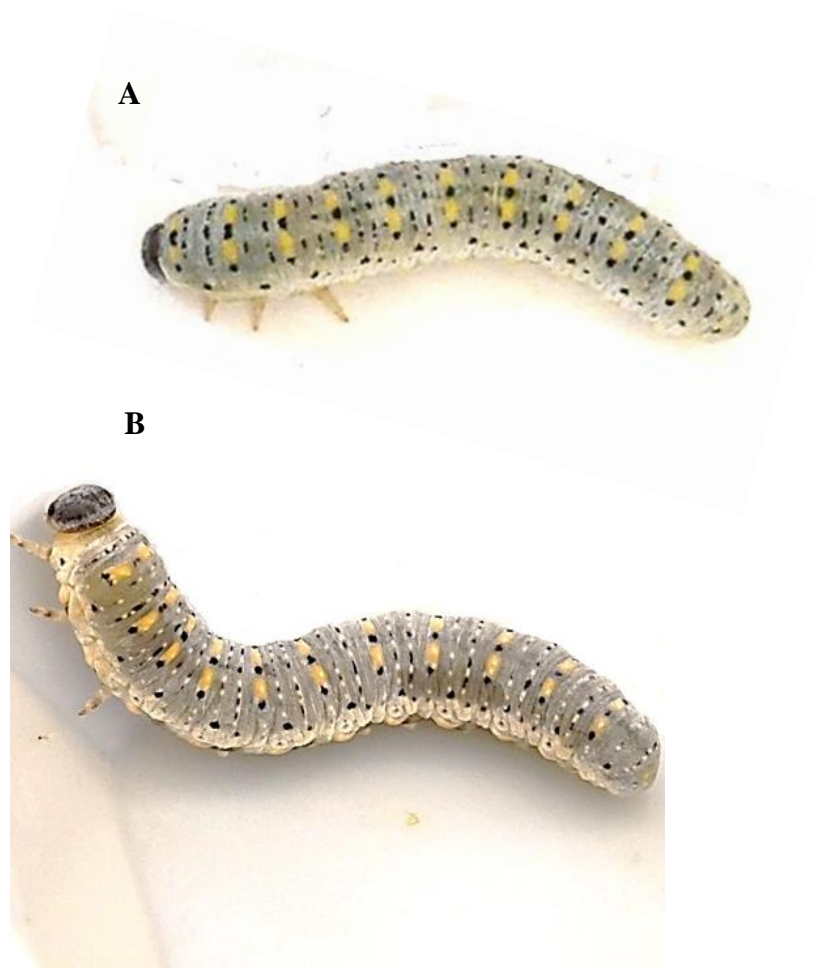
Figure 2.9 Larval comparison between *A. inflata* **(A) and** *A. americana* **(B) after 12-14 days of development. Both have very similar markings and coloration. However,** *A. americana* **has white dots between markings.**
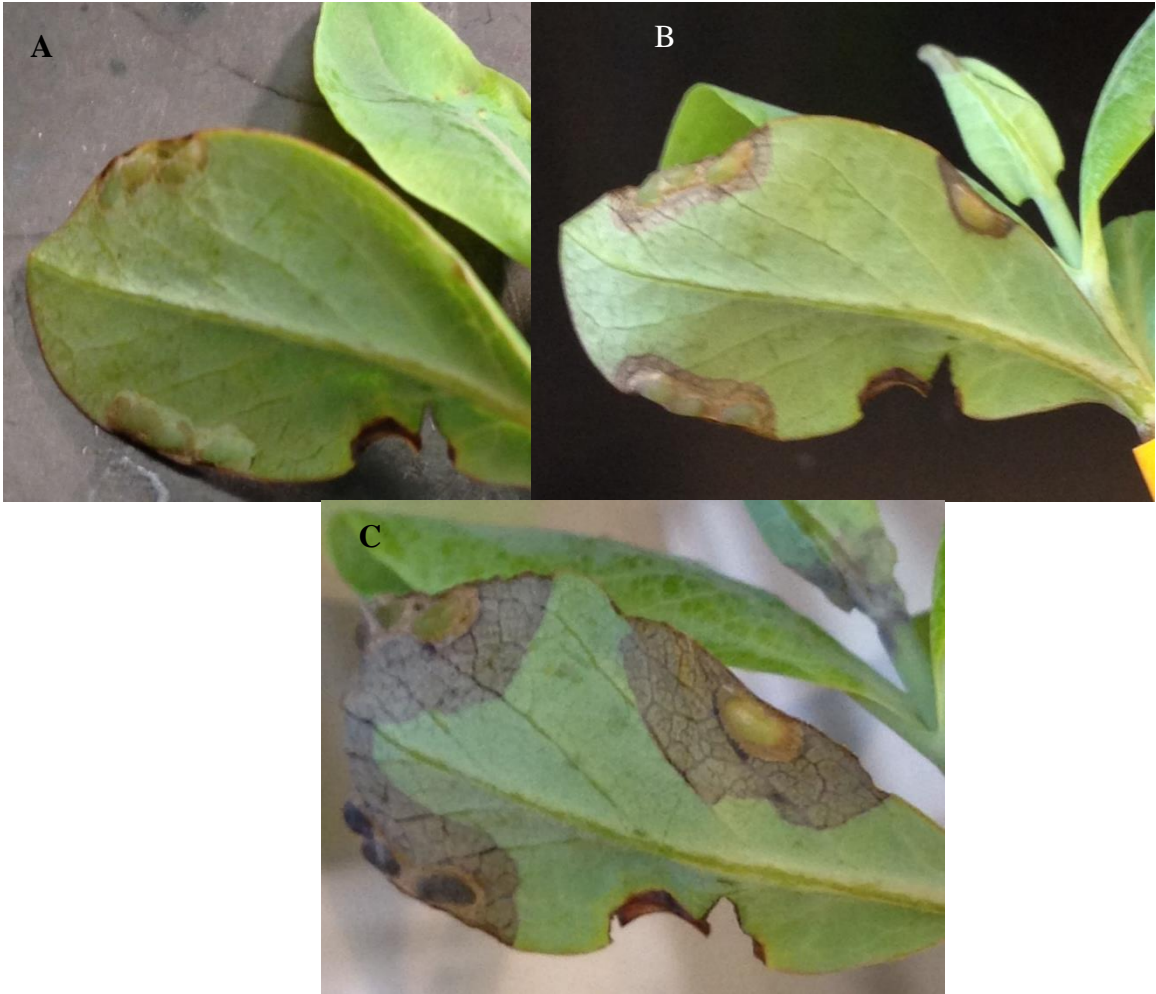
Figure 2.10 *Lonicera reticulata* **leaf response to seven** *Abia americana* **eggs laid by Female I on April 18, 2015. The plant response is shown above 1 day (A), 3 days (B), and 6 days (C) following oviposition. As you can see, the** *L. reticulata* **leaves were responding to the eggs laid by the** *A. americana* **females and this response led to the death of the eggs.**
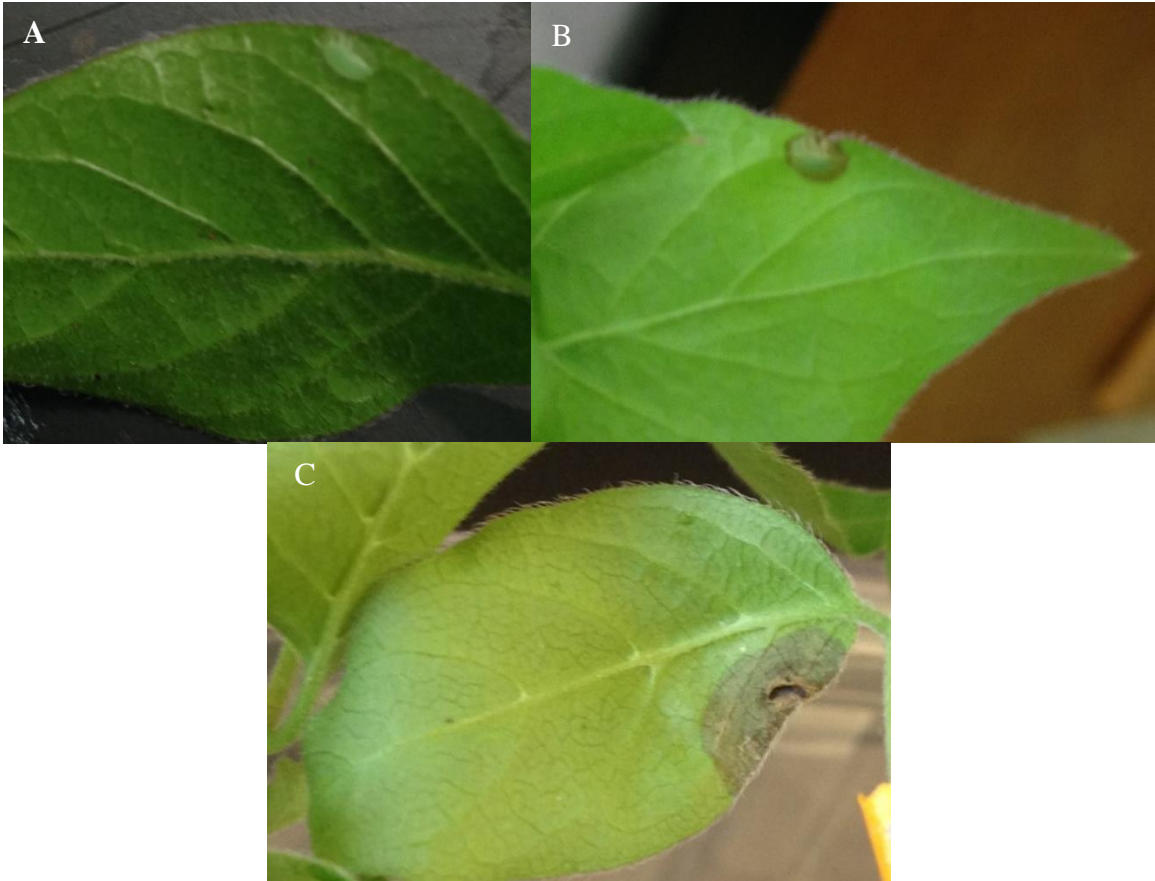
Figure 2.11 *Abia americana* **egg oviposited into non-native** *L. maackii* **leaf 0 days (A), 1 day (B), and 8 days (C) after oviposition. As you can see the** *L. maackii* **leaves were responding to the eggs laid by the** *A. americana* **females and this response led to the death of some of the eggs.**

3. PERFORMANCE OF THE SPECIALIST SNOWBERRY SAWFLY

(*ZASCHIZONYX MONTANA*) ON NATIVE AND NON-NATIVE SPECIES WITH

VARYING DEGREES OF DAMAGE

3.1 INTRODUCTION

3.1.1 Background

Many invasive plant species in their native ranges are often poor competitors with limited distribution (Keane and Crawley 2002; Lieurance 2012). However, in non-native ranges, these non-native plants can become dominant and extremely invasive (Keane and Crawley 2002; Lieurance 2012). The Enemy Release Hypothesis is one of the leading hypotheses to explain this phenomenon. This hypothesis suggests that release from natural enemies, especially from co-evolved specialist herbivores, is one major reason invasive plants are so successful in their novel environments (Keane and Crawley 2002; Colautti et al. 2004;Lieurance 2012). This release results in the rapid increase in abundance, distribution, and vigor of invasive plant species (Keane and Crawley 2002; Colautti et al. 2004; Lieurance 2012). Without enemies to keep the non-native host "in check" the plant can easily outcompete native plants and spread rapidly.

As invasive plant species enter a new habitat, they start interact with native fauna, especially insect communities. These novel-plant insect interactions can be positive,

negative, or neutral. A positive interaction could occur in which an insect benefits by successfully recognizing and using the invasive plant to increase in population size (Davis and Cipollini 2014). A negative interaction could occur in which insects have lower fitness and reproductive success on the novel plant because one or all life stages cannot utilize the host (Davis and Cipollini 2014). Lastly, a neutral interaction could occur because the native insect does not recognize the novel plant as a food source and does not use it (Davis and Cipollini 2014).

Since specialist herbivores feed on relatively few, closely related species, they are particularly vulnerable to and threatened by invasive plant invasions (Ali and Agrawal 2012). Invasive plants can outcompete and greatly reduce native plant populations used by the specialists. Monophagous herbivores and specialist herbivores on rarer plants are especially threatened because if the herbivore consumes all of its host plant it may not be able to find another. Specialist herbivores may not be able to utilize invasive hosts due to novel defenses, elevated chemical defenses, or the lack of specific oviposition cues (Callaway and Ridenour 2004; Cappuccino and Arnason 2006; Jahner et al. 2011). Through time, specialists will need to evolve mechanisms to utilize or avoid new invasive plant species if they are to persist in extremely invaded habitats.

There are approximately 200 species in the genus *Lonicera* (Caprifoliaceae), with 18 native and 16 introduced in North America (Zheng et al. 2005; Lieurance and Cipollini 2013). The three most invasive *Lonicera* species in North America are *Lonicera maackii, Lonicera japonica,* and *Lonicera tatarica* (Schierenbeck et al. 1994; Luken and Thieret 1996; Hutchinson and Vankat 1997). The most important invader in southwestern Ohio, *L. maackii*, has many invasive traits such as a long growing season (McEwan et al.

2009), allelopathic suppression of other plants (Dorning and Cipollini 2006; Cipollini et al. 2008b), high fruit production (Ingold and Craycraft 1983), and anti-herbivore defenses (Cipollini et al. 2008a). In the field, *L. maackii* receives about 3% herbivory damage, which is significantly lower than herbivory rates on native congeners (Lieurance and Cipollini 2012; Lieurance and Cipollini 2013). Along with this, native *Lonicera* species, such as *L. reticulata,* are not very abundant or common in their native ranges (http://plants.usda.gov). In Kentucky and Tennessee, *L. reticulata* is on the endangered or "possibly" endangered species lists (http://plants.usda.gov).

There are 13 native species in the genus *Symphoricarpos* (Caprifoliaceae) in North America. *Symphoricarpos* is a close relative to the genus *Lonicera.* Most of these species are only found in a few states, most in western states. *Symphoricarpos albus,* common snowberry, is found throughout most of the United States and Canada. In Kentucky, *S. albus* is considered endangered and it is on the threatened species list in Maryland. Most *S. albus* is planted ornamentally but it can be found along moist clearings, along stream banks, in swamp thickets, and in open forests (Makarevich et al. 2009; http://plants.usda.gov).

In this study, the specialist snowberry sawfly (*Zaschizonyx montana* (Tenthredinidae)) was used to investigate the impact of non-native honeysuckle species and quality of foliage on the performance and life history traits of this native insect. *Zaschizonyx montana* is native to North America and is only known to feed on native *Symphoricarpos* species (Smith and Gibson 1984). Adult *Z. montana* emerge in late April to early May (Fig 3.1). Adult *Z. montana* feed on the *Symphoricarpos* leaves. Female *Z. montana* will oviposit eggs with her ovipositor into the edges of leaves. The larvae will

hatch from eggs in 5-8 days and begin feeding on the edges of leaves. After about 2-3 weeks of development, the larvae start to pre-pupate and turn lime green. Once the larvae hit this stage they do not feed anymore and remained curled underneath foliage. It is still not known if the larvae overwinter in their larval stage, pre-pupal stage, or pupal stage.

The larvae and adults of *Z. montana* commonly feed on the leaves of native snowberry, *Symphoricarpos albus,* causing significant defoliation (personal observation). Since native *S. albus* is not very abundant, areas with large populations of *Z. montana* larvae may lead to depletion of native *S. albus.* If all undamaged foliage is consumed, sawfly larvae may be forced to eat damaged foliage or switch to a new host such as the non-native *L. maackii* which has invaded habitats with *S. albus*. Damaged leaves may not be an ideal for *Z. montana* because they have a lower leaf area, lower water and nutrient content, higher toughness, an increase in defensive chemicals, (Feeny 1970; Myers and Post 1981; Rhoades 1983) and are a visual and odor cue for predators (Anthony 1998; VanLaerhoven et al. 2000). The cost of switching to a non-native (*L. tatarica, L. maackii,* and *L. sempervirens*) host, native host (*L. reticulata*), or consuming damaged *S. albus* leaves will be determined in this study. Larval performance of newly emerged *Z. montana* larvae on native (*L. reticulata* and *S.* albus) and non-native (*L. maackii* and *L. japonica*) will be evaluated. Lastly, larval performance will be compared between larvae reared on damaged and undamaged *L. maackii* and *L. reticulata.* This study is important because little is known about the specialist snowberry sawfly and no research has been done with them.

The main objective of these experiments were to determine if larvae of *Z. montana* could utilize native and non-native honeysuckle species. The secondary goal was to

compare the performance of *Z. montana* larvae on native and non-native honeysuckle species compared to its preferred host, *S. albus.* The final objective was to determine the potential costs of consuming damaged *S. albus, L. reticulata,* and *L. maackii* leaves or switching to an alternative host.

We predicted *Z. montana* larvae will be able to complete their life cycle on native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. japonica*) because all these species are closely related. However, we predicted *Z. montana* larvae will grow faster, grow larger, have higher survivorship, and reach pre-pupation faster if the larvae consume their preferred host, *S. albus,* because they have evolved to use this native host. Lastly, we predicted that two week old *Z. montana* larvae switched from *S. albus* to a new native or non-native honeysuckle species, then the larvae switched to the non-native foliage will grow more slowly, have smaller mass, lower survival, and take longer to reach pre-pupation compared to larvae that remain on their preferred host, *S. albus* due to increased defensive or novel leaf chemistry in non-native hosts.

We predicted *Z. montana* larvae will grow faster, grow larger, have higher survivorship, and reach pre-pupation faster if the larvae consume undamaged, native foliage compared to larvae reared on damaged, non-native foliage. We predicted that two week old *Z. montana* switched from undamaged *S. albus* to damaged foliage will decrease will grow more slowly, have smaller mass, lower survival, and take longer to reach pre-pupation compared to larvae that remain on undamaged *S. albus* foliage. Damaged foliage has higher secondary defenses which may lead to lower *Z. montana* larval performance.

3.2 METHODS

3.2.1 Feeding Bioassays on Native and Non-native Hosts

  (A) <u>Newly Emerged Larvae 2015</u>

      Stems of *S. albus* were collected outside of the Dunbar Library on the campus of Wright State University in Dayton, OH from April 15, 2015- April 28, 2015. Stems were cut at an angle and placed immediately into DI water. The stems were transported into the laboratory and checked for eggs. Once an egg was found, it was given a unique label. The stems with eggs were then placed individually in a water pick with DI water and placed into a clear plastic deli container (4 x 6 x 6 cm). The deli container had air holes poked into the lid. Each deli container contained 1-3 stems. The deli containers were placed upright in an incubator at 25°C (16:8 L:D). Eggs were checked daily for newly hatched larvae.

      Once a larva hatched, it was weighed and transferred with a paintbrush to a fresh stem. [See section 3.2.3 Leaf Collection below for more details on how the stems were obtained.] Larvae were placed on *S. albus* (N=16), *L. reticulata* (N=15), *L. maackii* (N=18), or *L. japonica* (N=9). Larvae were placed individually into deli containers. Larvae were haphazardly placed in an incubator at 25°C (16:8 L:D). The larvae were weighed, checked for survivorship and pre-pupation every 2-4 days. New leaves and water were added as needed. Leaves remained in containers for no longer than a week. Once a week, the containers were cleaned of frass. The experiment ended after all larvae reached the pre-pupal stage or died.

(B) <u>Early Instar Larvae 2014</u>

No-choice bioassays were set-up with approximately 1-2 week old larvae collected directly off of *S. albus* plants on April 15, 2014. The largest and smallest larvae were excluded from the study. Larvae were weighed and placed individually into deli containers with leaves of *S. albus* (N=10), *L. reticulata* (N=10), *L. sempervirens* (N=10), *L. maackii* (N=9), or *L. tatarica* (N=7). Instead of stems, larvae were given multiple leaves placed on moistened kim wipes. Extra leaves were stored in Ziploc gallon bags in a fridge at 4 °C. The larvae were weighed, checked for survivorship and pre-pupation every 2-4 days. The experiment ended after all larvae reached the pre-pupal stage or died.

3.2.2 Feeding Bioassays on Damaged and Undamaged Leaves

(A) <u>Newly Emerged Larvae on Damaged *L. reticulata* and *L. maackii* 2015</u>

No-choice bioassays were set-up as described above in section 3.2.1 (A). The only change was newly emerged *Z. montana* larvae were placed on 50% damaged foliage of *L. reticulata* (native) and *L. maackii* (non-native). A week prior to larvae hatching, *L. reticulata* and *L. maackii* leaves were damaged in the common garden on Wright State University. Leaves were cut in half with a pair of scissors to simulate herbivory. The branches with damaged leaves were marked and left for a week to give the plant time to react to the damage. After a week, the stems were collected for the no-choice bioassay. Larvae were placed on one of the following: undamaged *L. reticulata* (N=15), damaged *L. reticulata* (N=14), undamaged *L. maackii* (N=18), or damaged *L. maackii* (N=19). The larvae were weighed, checked for survivorship and pre-pupation every 2-4 days. The

experiment ended after all larvae reached the pre-pupal stage or died. [Note: the larvae reared on the undamaged foliage are the same larvae for the host bioassay]

(B) <u>Early Instar Larvae on Damaged *S. albus* 2014</u>

No-choice bioassays were set-up as described above in section 3.2.1 (B). The only modification was using 50% damaged *S. albus* leaves. Leaves of *S. albus* with approximately 50% herbivore damage from *Z. montana* larvae were collected outside of the Dunbar Library on the campus of Wright State University in Dayton, OH. Two week-old larvae were reared individually on undamaged *S. albus* (N=10) or damaged *S. albus* (N=10). The larvae were weighed, checked for survivorship and pre-pupation every 2-4 days. The experiment ended after all larvae reached the pre-pupal stage or died. [Note: the larvae reared on the undamaged foliage are the same larvae for the host bioassay]

3.2.3 Leaf Collection

All *Lonicera* species were collected from a common garden on the campus of Wright State University. The *Lonicera* plants in this garden were grown 1.5 m apart, in full sunlight, and received a high fertilizer treatment. *Symphoricarpos albus* leaves were collected on Wright State University campus.

Small twigs were taken from the outer 15-20 cm of the branch. The twigs were cut at an angle and immediately placed into a beaker of DI water. The twigs were transferred quickly back to the laboratory and kept at room temperature and in ambient light for no more than a week.

3.2.4 *Statistical Analysis*

Repeated measures ANOVA were used to compare larval masses with time as the within subjects effect and plant species as the between subjects effect. Pupal mass, days to pupation, and relative growth rates were compared among plant species using one-way ANOVA. Comparisons of means were made using Tukey post-hoc tests. Survivorship was compared among plant species using the non-parametric Mantel-Cox test. Percent larval survival and percent pre-pupating were compared using chi-square tests. All percentages were converted to arcsine values. All statistical analyses were performed using GraphPad Prism (Version 6.07, GraphPad Software, Inc., La Jolla, California).

3.2.5 *Calculations*

Relative Growth Rate

Relative growth rate is calculated by subtracting the natural logarithm of initial mass from the natural logarithm of mass at the second time point and dividing by difference in time between weighing.

Relative Growth Rate (RGR) = $\dfrac{\ln(W_2) - \ln(W_1)}{T_2 - T_1}$

T_1 = time of initial weight (days) W_1 = initial mass (grams)
T_2 = time of second weight (days) W_2 = second mass (grams)

3.3 RESULTS

3.3.1 Feeding Bioassays on Native and Non-native Hosts

   (A) Newly Emerged Larvae 2015

   In the no-choice bioassays with *Z. montana* reared from eggs, repeated measures ANOVA revealed differences in larval mass by species, through time, and an interaction between time and species (Table 3.1, Figure 3.2a). By day 3, all larvae feeding on *L. japonica* died. At peak larval weight, there was no difference between relative growth rate of larvae reared on *S. albus, L. reticulata,* and *L. maackii* (Table 3.2, Figure 3.2b). There was a significant difference in larval survival among the different species (Figure 3.2c). Larvae fed *L. reticulata* had the lowest final larval survival at 21%, those on *L. maackii* had intermediate larval survival at 65%, and larval survival on *S. albus* was the highest at 86% (Figure 3.2d). Larvae on all species except *L. japonica* were able to successfully reach the pre-pupal development stage and there was no significant difference in the amount of time it took larvae to reach that stage (Table 3.3, Figure 3.3a). The percentage reaching the pre-pupal stage differed significantly due to the high larval mortality on *L. reticulata* and *L. japonica* (Figure 3.3b)

   (B) Early Instar Larvae 2014

   In the no-choice bioassays with two week old *Z. montana* collected from *S. albus* from Wright State University, repeated measures ANOVA revealed differences in larval mass through time and between plant species (Table 3.4, Figure 3.4a). However, there was a marginally significant interaction detected between species and time (Table 3.4). After four days, larvae on *L. tatarica* had a significantly higher relative growth rate compared to both native species (*S. albus* and *L. reticulata*), but not significantly

67

different than *L. maackii* (Figure 3.4b). Larval survival was significantly different among all species (Figure 3.4c). All larvae fed on *L. sempervirens* died by day 10 (Figure 3.4c). There was 100% larval survival on *S. albus* (Figure 3.4d). The final larval survivorship was higher on native *S. albus* and *L. reticulata* compared to non-native *L. maackii* and *L. tatarica* (Figure 3.4d). Larvae on all species except *L. sempervirens* were able to successfully reach the pre-pupal development stage and there was no significant difference in the amount of time it took larvae to reach that stage (Table 3.6, Figure 3.5a). The percentage reaching the pre-pupal stage differed significantly, larvae reared on *S. albus* had the highest success rate (Figure 3.3b). Larvae reared on non-native *L. maackii* and *L. tatarica* had similar percentage reaching pre-pupation at 67% and 71%, respectively (Figure 3.5b).

3.3.2 Feeding Bioassays on Damaged and Undamaged Leaves
(A) <u>Newly Emerged Larvae on Damaged *L. reticulata* and *L. maackii* 2015</u>

In the no-choice bioassays with *Z. montana* reared from eggs, repeated measures ANOVA revealed differences in larval mass between treatments, through time, and an interaction between time and treatment (Table 3.7, Figure 3.6a). At peak larval weight, larvae feeding on damaged *L. reticulata* had a significantly lower relative growth rate compared to larvae feeding on damaged and undamaged *L. maackii*, but not significantly different than undamaged *L. reticulata* (Table 3.8, Figure 3.6b). Survivorship differed significantly through time between the treatments (Figure 3.6c). Larvae feeding on damaged and undamaged *L. reticulata* had high mortality in the first five days of the bioassay (Figure 3.6c). Undamaged *L. maackii* had the highest larval survival through

68

time followed by damaged *L. maackii* with final larval survival at 65% and 47%, respectively (Figure 3.6d). Larvae on damaged and undamaged *L. reticulata* had low larval survival at 7% and 21%, respectively (Figure 3.6d). All larvae successfully reached the pre-pupal stage and the time to reach pre-pupation differed significantly between treatments (Table 3.9, Figure 3.7a). It took larvae on damaged *L. reticulata* significantly less time to reach the pre-pupal stage compared to undamaged *L. reticulata* and damage *L. maackii* (Figure 3.7a). The percentage of larvae reaching the pre-pupal stage differed significantly among treatments (Figure 3.7b). More larvae were successful at pre-pupating on undamaged leaves compared to damaged leaves. More larvae on the non-native *L. maackii* reached the pre-pupation stage compared to native *L. reticulata* due to high larval mortality on the native.

(B) Early Instar Larvae on Damaged *S. albus* 2014

In the no-choice bioassays with two week old *Z. montana* collected off of *S. albus* from Wright State University, repeated measures ANOVA revealed no differences in larval mass through time, between treatments, or an interaction between time and treatment (Table 3.10, Figure 3.8a). There was no difference in relative growth rate, final larval survival, or time to reach the pre-pupal stage between larvae reared on damaged and undamaged *L. reticulata* foliage (Figure 3.8b-d).

3.4 DISCUSSION

3.4.1 *Zaschizonyx montana Performance on Native and Non-native Hosts*

Newly emerged and two week old *Z. montana* larvae were able to complete their life cycle and had similar larval mass through time, relative growth rate, and days to pre-pupation on their preferred host *S. albus*, native *L. reticulata*, and non-native *L. maackii* and *L. tatarica*. Newly hatched larvae had a difficult time adhering to and consuming the waxy *L. reticulata* foliage, which lead to high larval mortality during the first four days after larval eclosion. This supported our prediction that *Z. montana* would be able to complete their life cycle on *S. albus, L. reticulata,* and *L. maackii*, but our prediction of higher performance on native species was shown to be incorrect. Surprisingly, we found larvae switched onto non-native *L. tatarica* had higher larval mass through time and relative growth rate after four days compared to all native and non-native species including *Z. montana'*s preferred host *S. albus.* In the face of *L. maackii* invasion, *Z. montana* larvae will be able to use *L. maackii* as a potential host. However, the other non-native in this study, *L. japonica*, and the native *L. sempervirens* were not a suitable larval host because all larvae died within a few days on these species foliage. This shows that not all native and non-native hosts can be used by this specialist herbivore.

3.4.2 *Zaschizonyx montana Performance on Damaged and Undamaged Foliage*

Symphoricarpos albus has relatively low abundances in the field compared to non-native, invasive species like *L. maackii*, *L. tatarica,* and *L. japonica* (http://plants.usda.gov). As these invasives continue to spread, they will compete and reduced native *Symphoricarpos* species populations. Since *Z. americana* is only known to

feed on native *Symphoricarpos*, these native plants may start receive greater herbivore

damage from *Z. americana* in invaded areas (Smith and Gibson 1984). This is especially

true, because adult *Z. americana* feed on foliage before they lay eggs, which can cause

significant early season defoliation on snowberry even before larval herbivore damage.

This ultimately will lead to larvae being forced to consume damaged leaves which may

have lower quality due to lower water and nutrient content, higher toughness, and higher

quantities of defensive chemicals (Feeny 1970; Myers and Post 1981; Rhoades 1983). An

alternative to consuming damaged leaves is to switch to a novel host which may come at

a cost or may lead to finding a new suitable host for *Z. montana*.

　　As predicted, *Z. montana* larvae reared on damaged and undamaged *L. reticulata*

and *L. maackii* were able to reach the pre-pupal stage. In general, larvae on undamaged

foliage had higher larval mass, relative growth rate, larval survival, and higher percentage

reach-pupation even though some values were not significantly higher. This supported

our hypothesis that *Z. montana* larvae would perform better on undamaged foliage.

However, larvae consuming damaged *L. reticulata* did reach pre-pupation faster than

larvae on undamaged *L. reticulata* and damaged *L. maackii*. This could have been due to

the low sample size of larvae feeding on damaged *L. reticulata* foliage after the large

larval mortality early in development. Newly hatched larvae had a difficult time adhering

to and consuming the waxy *L. reticulata* foliage, which lead to high larval mortality

during the first four days after larval eclosion on both damaged and undamaged foliage.

When comparing native and non-native hosts, *Z. montana* larvae on non-native *L.

maackii* foliage had higher relative growth rates, larval survival, and percentage reaching

pre-pupation compared to native *L. reticulata*. This did not support our prediction that *Z. montana* larvae would have the highest performance on undamaged, native foliage.

Although we found some evidence for a decrease in larval performance on damaged foliage, this cost may be greater in the field with natural leaf herbivory compared to the simulated herbivory in our experiment. Simulated herbivory and natural herbivory can evoke different plant response in the leaves, and often simulated herbivory is a poor substitute for actual damage done by a herbivore (Strauss and Agrawal 1999). When a herbivore consumes foliage, their saliva and the chewing of the leaf can trigger different plant response compared to the clipping of foliage with relatively clean scissors (Strauss and Agrawal 1999; Musser et al. 2005). However, Lieurance and Cipollini (2013) found that the plant chemistry did not differ between simulated and real herbivory in *L. maackii*. Another major factor not in our experiment was exposure to predators. Larvae feeding on damaged leaves could be more easily spotted and attacked by a predator. Lastly, *L. maackii* does not typically receive 50% herbivore damage in the field as was tested in this study and has abundant foliage (Lieurance and Cipollini 2012). Larvae in areas with high abundances of *L. maackii* will always have undamaged foliage to consume.

Two week old *Z. montana* larvae switched onto damaged *S. albus* foliage had similar larval mass through time, relative growth rates, larval survival, and time reaching pre-pupation as larvae feeding on undamaged foliage. This did not support our prediction that *Z. montana* larvae feeding on damaged foliage would have lower performance. This was surprising because damaged leaves often have lower quality and higher chemical defenses (Feeny 1970; Myers and Post 1981; Rhoades 1983). However, since *Z. montana*

is a specialist on *Symphoricarpos* species, it may have evolved mechanisms to tolerate the secondary defensive compounds produced by *S. albus* which allowed it to perform just as well on damaged and undamaged foliage. In the face of invasion when undamaged *Symphoricarpos* foliage is unavailable, *Z. montana* larvae could be able to consume damaged leaves instead of switching to a novel host. However, our study was done in a laboratory setting which does not fully mimic the conditions of *Z. montana* larvae in the field. For example, predators are one of the most important factors our larvae were not exposed in this study. Predators often use damaged leaves as a visual and odor cue to find herbivores (Anthony 1998; VanLaerhoven et al. 2000). Larvae feeding on damaged leaves could be more easily spotted and attacked by a predator. Switching to a novel host may be beneficial if predator populations are high to reduce to risk of being spotted.

3.5 CONCLUSION

This was the first study to look at host performance of the specialists *Z. montana* on its preferred host *S. albus* along with close native and non-native *Lonicera* relatives. These performance assays are the first step in determining the potential interactions of *Z. montana* with novel host plants. It was found that this specialist sawfly is able to perform equally well on its preferred native host, *S. albus,* and the non-native host, *L. maackii*. This could indicate a positive interaction in which *Z. montana* can utilize this non-native host and increase in population size. This positive interaction is possible if adult *Z. montana* are able to recognize *L. maackii* as a suitable host and oviposit eggs on this novel host. The eggs must be able to successfully hatch and larvae must be able to feed on foliage. If adult or larval *Z. montana* do not recognize *L. maackii* as a suitable host, this interaction will be neutral. On the other hand, if eggs cannot hatch in *L. maackii* then this interaction will be negative.

High larval mortality on native *L. sempervirens,* native *L. reticulata*, and non-native *L. japonica* indicate a potential negative interaction between *Z. montana* and these hosts in the laboratory. However, *Z. montana* larvae that switched onto *L. reticulata* after two weeks on *S. albus* had similar performance to larvae that remained on *S. albus*, possibly indicating *L. reticulata* as a suitable host for later development. Adult interaction with these *Lonicera* hosts will play a major role in determining these interactions in the field. If adults do not recognize or utilize a host, then the resulting interaction is neutral. However, if an adult does oviposit in these hosts and the eggs cannot hatch, then all of these host could have negative interactions. Oviposition studies will need to be done in the future to help determine how *Z. montana* interacts with native and non-native *Lonicera* species.

3.6 REFERENCES

Ali JG, Agrawal AA (2012) Review: Specialist versus generalist insect herbivores and plant defense. Trends Plant Sci 17:293-302

Anthony R (1998) The third trophic level of plant defence: neotropical social wasps' use of odours of freshly damaged leaves when hunting. Rev Bras Zool :1075

Callaway RM, Ridenour WM (2004) Novel weapons: Invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment :436

Cappuccino N, Arnason JT (2006) Novel chemistry of invasive exotic plants. Biology Letters 2:189-193

Cipollini D, Stevenson R, Enright S, Eyles A, Bonello P (2008a) Phenolic metabolites in leaves of the invasive shrub, *Lonicera maackii*, and their potential phytotoxic and anti-herbivore effects. J Chem Ecol 34:144-152

Cipollini D, Stevenson R, Cipollini K (2008b) Contrasting effects of allelochemicals from two invasive plants on the performance of a nonmycorrhizal plant. Int J Plant Sci :371

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ, Rejmanek M (2004) Is invasion success explained by the enemy release hypothesis? Ecol Lett 7:721-733

Davis S, Cipollini D (2014) Do mothers always know best? Oviposition mistakes and resulting larval failure of *Pieris virginiensis* on *Alliaria petiolata*, a novel, toxic host. Biol Invasions 16:1941-1950

Dorning M, Cipollini D (2006) Leaf and root extracts of the invasive shrub, *Lonicera maackii*, inhibit seed germination of three herbs with no autotoxic effects. Plant Ecol :287

Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring

feeding by winter moth caterpillars. Ecology :565

Hutchinson TF, Vankat JL (1997) Invasibility and effects of Amur honeysuckle in

Southwestern Ohio forests. Conserv Biol :1117

Ingold JL, Craycraft MJ (1983) Avian frugivory on honeysuckle (*Lonicera*) in

soutwestern Ohio in fall. Ohio Journal of Science 83:256-258

Jahner JP, Bonilla MM, Badik KJ, Shapiro AM, Forister ML (2011) Use of exotic hosts

by *Lepidoptera*: Widespread species colonize more novel hosts. Evolution :2719

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis.

Trends in Ecology & Evolution 17:164

Lieurance D, Cipollini D (2013) Exotic *Lonicera* species both escape and resist specialist

and generalist herbivores in the introduced range in North America. Biol

Invasions 15:1713-1724.

Lieurance D (2012) Mechanisms of success: plant-herbivore interactions and the invasion

of non-native *Lonicera* species in North America. , Wright State University /

OhioLINK

Lieurance D, Cipollini D (2012) Damage levels from arthropod herbivores on *Lonicera*

maackii suggest enemy release in its introduced range. Biol Invasions 14:863-873

Luken JO, Thieret JW (1996) Amur honeysuckle, its fall from grace. Bioscience :18

Makarevich I, Kovalenko S, Gusarova T, Gubin Y (2009) Iridoids from *Symphoricarpos*

albus. Chemistry of Natural Compounds 45:40-44

McEwan RW, Birchfield MK, Schoergendorfer A, Arthur MA (2009) Leaf phenology and freeze tolerance of the invasive shrub Amur honeysuckle and potential native competitors. The Journal of the Torrey Botanical Society :212

Musser RO, Cipollini DF, Hum-Musser S, Williams SA, Brown JK, Felton GW (2005) Evidence that the caterpillar salivary enzyme glucose oxidase provides herbivore offense in solanaceous plants. Arch Insect Biochem Physiol 58:128-137

Myers JH, Post BJ (1981) Plant nitrogen and fluctuations of insect populations: a test with the cinnabar moth: tansy ragwort system. Oecologia :151

Rhoades DF (1983) Responses of alder and willow to attack by tent caterpillars and webworms: evidence for pheromonal sensitivity of willows. In: Hedin P (ed) Plant resistance to insects. American Chemical Society, Washington DC, pp 55-68

Schierenbeck KA, Mack RN, Sharitz RR (1994) Effects of herbivory on growth and biomass allocation in native and introduced species of Lonicera. Ecology :1661

Smith DR, Gibson GA (1984) Filacus, a new genus for four species of sawflies previously placed in *Macrophya* or *Zaschizonyx* (Hymenoptera: Tenthredinidae). Pan-Pac Entomol 60:101-113

Strauss SY, Agrawal AA (1999) Review: The ecology and evolution of plant tolerance to herbivory. Trends in Ecology & Evolution 14:179-185

VanLaerhoven S, McGregor RR, Gillespie DR (2000) Leaf damage and prey type determine search effort in *Orius tristicolor*. Entomol Exp Appl 97:167-174

Zheng H, Wu Y, Ding J, Binion D, Fu W, Reardon R (2005) Invasive plants established in the United States that are found in Asia and their associated natural enemies vol 1. Chinese Academy of Sciences 1

Table 3.1 ANOVA table for *Z. montana* larval mass of survivors through time when reared on native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. japonica*) leaves collected from Wright State University in 2015.

	DF	MS	F	P value	Significant
Time	7	0.0087	144.10	P < 0.0001	Yes
Treatment	2	0.0011	5.95	P = 0.0086	Yes
Time x Treatment	14	0.0002	3.87	P < 0.0001	Yes
Error	72	0.0000			

Table 3.2 ANOVA table for mean relative growth rate of surviving *Z. montana* larvae on native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. japonica*) leaves in 2015.

	DF	MS	F	P value	Significant
Treatment	2	0.0050	1.65	P = 0.2156	No
Error	22	0.0030			

Table 3.3 ANOVA table average number of days for *Z. montana* larvae to reach the pre-pupal stage on native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. japonica*) leaves collected from Wright State University in 2015.

	DF	MS	F	P value	Significant
Treatment	2	10.1000	2.81	P = 0.0793	No
Error	25	3.5960			

Table 3.4 ANOVA table for *Z. montana* larval mass of survivors through time when reared on native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. tatarica*) leaves collected from Wright State University in 2014.

	DF	MS	F	P value	Significant
Time	3	0.0011	17.12	P < 0.0001	Yes
Treatment	3	0.0006	5.75	P = 0.0041	Yes
Time x Treatment	9	0.0001	1.99	P = 0.0528	No
Error	72	0.0000			

Table 3.5 ANOVA table for mean relative growth rate of surviving *Z. montana* larvae after four days on native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. tatarica*) leaves in 2014.

	DF	MS	F	P value	Significant
Treatment	3	0.0260	3.48	P = 0.0313	Yes
Error	24	0.0074			

Table 3.6 ANOVA table average number of days for *Z. montana* larvae to reach the pre-pupal stage on native (*S. albus, L. reticulata,* and *L. sempervirens*) and non-native (*L. maackii* and *L. tatarica*) leaves collected from Wright State University in 2014.

	DF	MS	F	P value	Significant
Treatment	4	62.9700	18.05	P < 0.0001	Yes
Error	29	3.4880			

Table 3.7 ANOVA table for *Z. montana* larval mass of survivors through time when reared on undamaged and damaged *L. reticulata* and *L. maackii* in 2015.

	DF	MS	F	P value	Significant
Time	7	0.0024	48.92	P < 0.0001	Yes
Treatment	2	0.0010	6.98	P = 0.0098	Yes
Time x Treatment	14	0.0002	4.87	P < 0.0001	Yes
Error	84	0.0000			

Table 3.8 ANOVA table for mean relative growth rate of surviving *Z. montana* larvae at peak larval mass when reared on damaged and undamaged *L. reticulata* and *L. maackii* in 2015.

	DF	MS	F	P value	Significant
Treatment	3	0.0162	7.54	P = 0.0016	Yes
Error	19	0.0022			

Table 3.9 ANOVA table average number of days for *Z. montana* larvae to reach the pre-pupal stage on damaged and undamaged *L. reticulata* and *L. maackii* in 2015.

	DF	MS	F	P value	Significant
Treatment	3	15.7700	4.80	P = 0.0101	Yes
Error	22	3.2830			

Table 3.10 ANOVA table for *Z. montana* larval mass of survivors through time when reared on damaged and undamaged *S. albus* leaves collected from Wright State University in 2014.

	DF	MS	F	P value	Significant
Time	3	0.0003	4.88	P = 0.0045	Yes
Treatment	1	0.0000	0.06	P = 0.8134	No
Time x Treatment	3	0.0000	0.88	P = 0.4549	No
Error	54	0.0000			

Adult

Pre-pupa
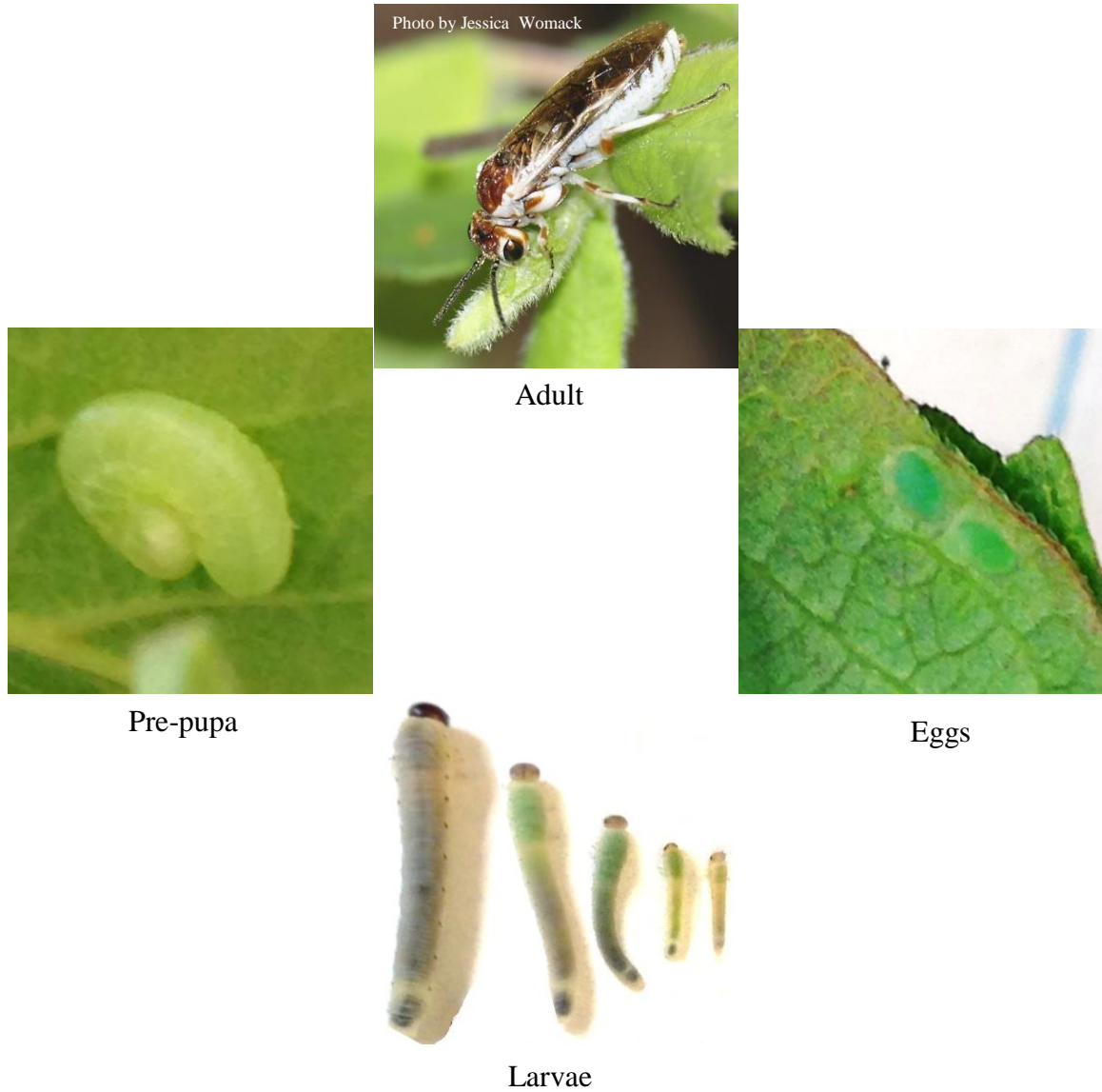
Eggs

Larvae

Figure 3.1 Life cycle of _Z. montana_. Adult females lay eggs in leaves using her saw-like ovipositor. The eggs hatch in 5-8 days. The larvae start off with no spots and markings and as they get older, the larvae get darker with visible spots on the sides of their bodies. After 2-3 weeks, the larvae turn lime green and lose their spots. They remain curled underneath foliage for weeks. It is unsure if _Z. montana_ overwinters as a larva or pre-pupa. Adults emerge in late April to early May.
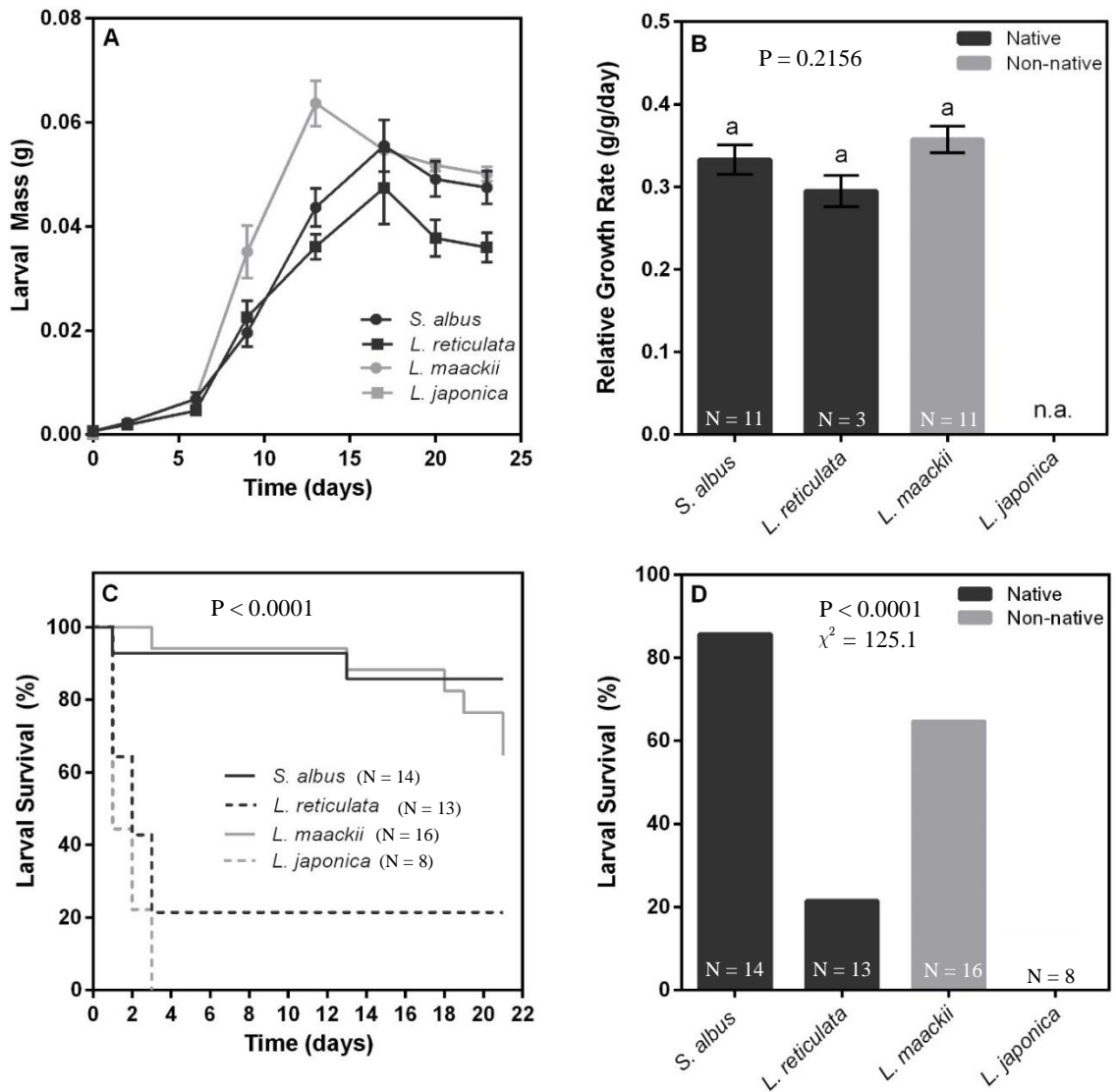
Figure 3.2 Mean larval mass of the specialist *Zaschizonyx montana* feeding on the foliage of two native (*S. albus* and *L. reticulata*) and two non-native species (*L. maackii* and *L. japonica*) (A), relative growth rate (RGR) measured at peak larval weight on day 13 (B), larval survivorship (C), and final larval survivorship (D). Species not included in the statistical analysis are denoted as "n.a.". Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05).
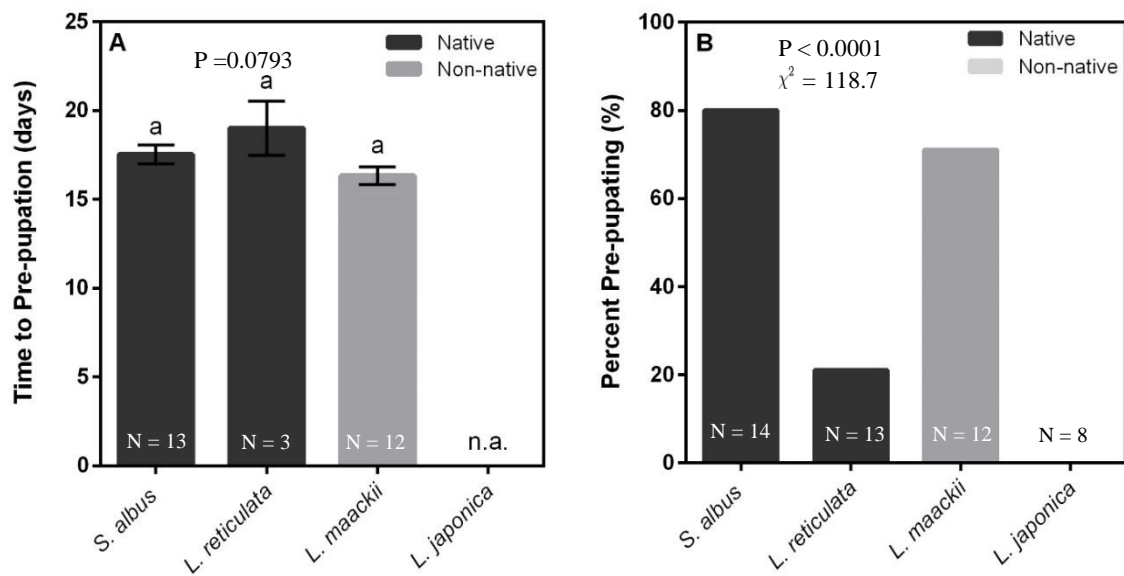
Figure 3.3 Mean days for *Zaschizonyx montana* larvae to reach pre-pupation feeding on foliage of 2 native and 2 non-native species (A), and percentage reaching pre-pupation (B). Species not included in the statistical analysis are denoted as "n.a.". Letters represent a difference in means determined through Tukey post hoc tests ($P < 0.05$).
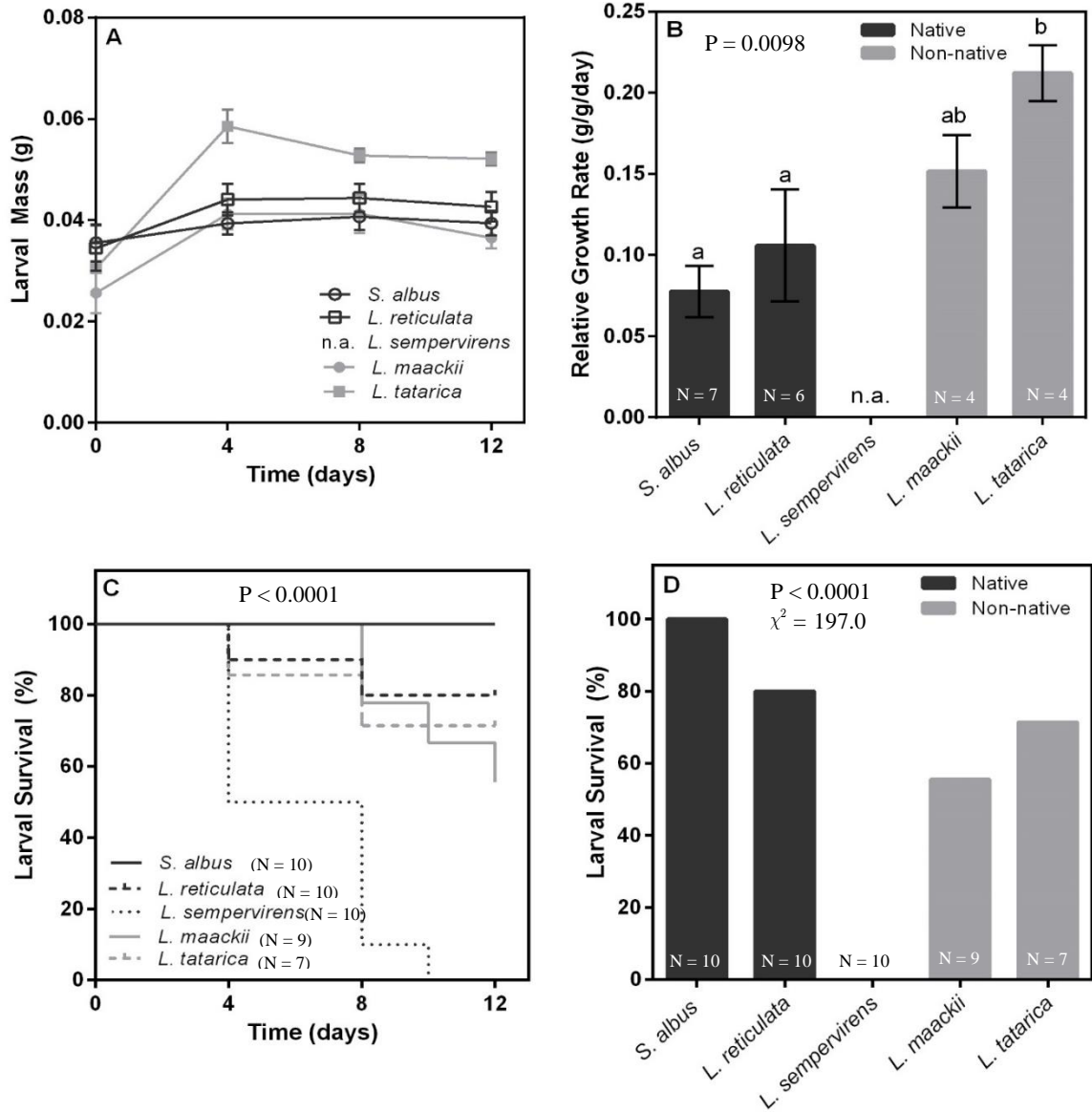
Figure 3.4 Mean larval mass of two week old *Z. montana* feeding on foliage of three native and two non-native species (A), relative growth rate (RGR) measured at peak larval weight on day four (B), larval survivorship (C), and final larval survivorship (D). Species not included in the statistical analysis are denoted as "n.a.". Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05).
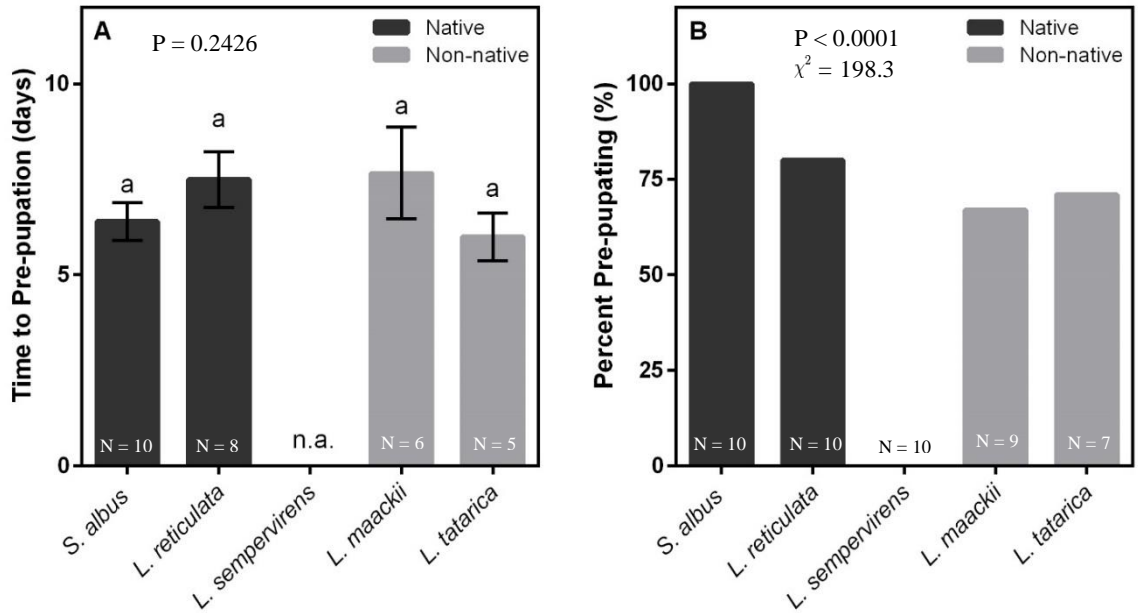
Figure 3.5 Mean days for *Z. montana* larvae to reach pre-pupation feeding on foliage of three native and two non-native species (A), and percentage reaching pre-pupation (B). Species not included in the statistical analysis are denoted as "n.a.". Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05).
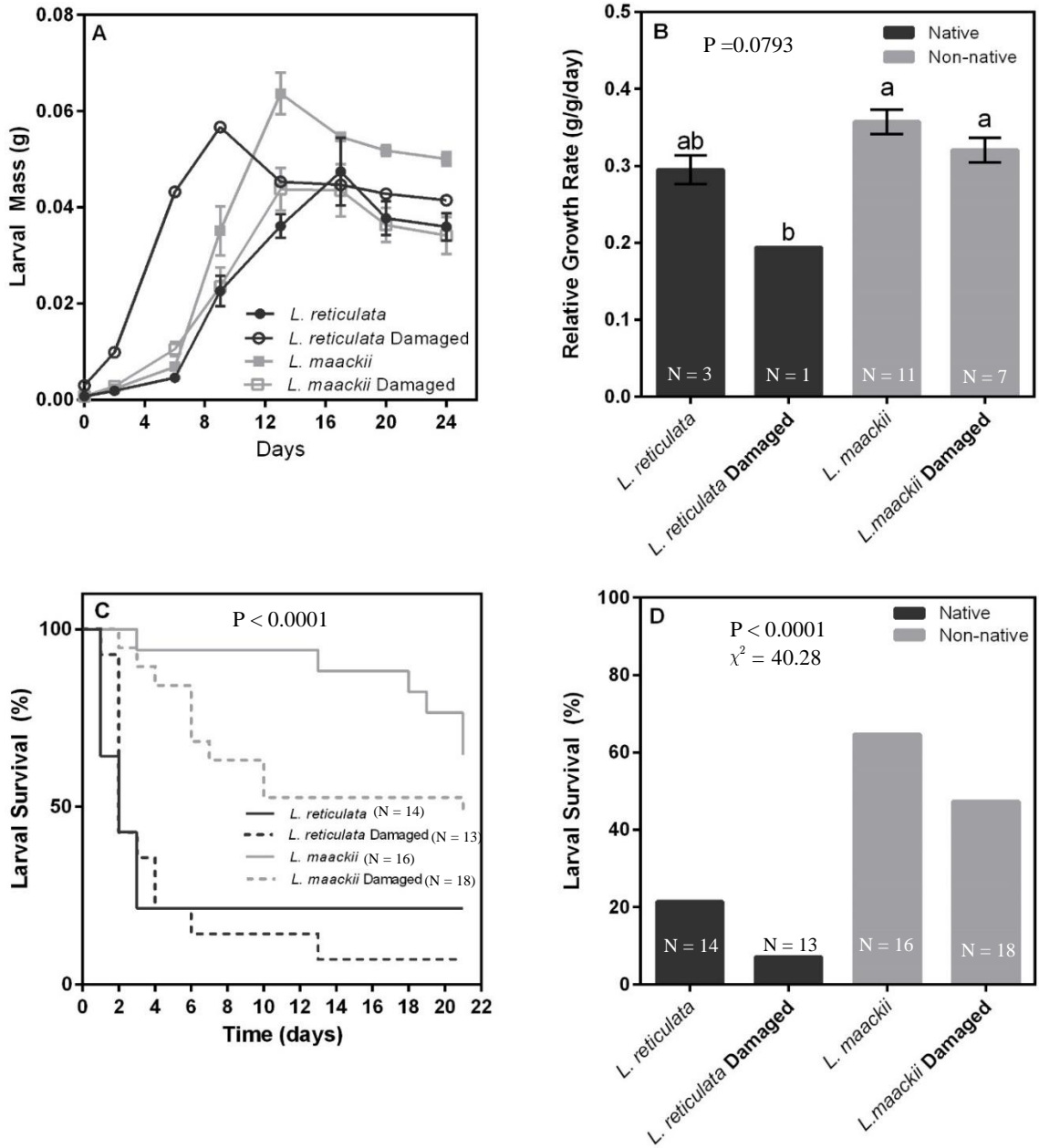
Figure 3.6 Mean larval mass of the specialist *Z. montana* feeding on damaged and undamaged foliage of native *L. reticulata* and non-native *L. maackii* (A), relative growth rate (RGR) measured at peak larval weight on day 13 (B), larval survivorship (C), and final larval survivorship (D). Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05).
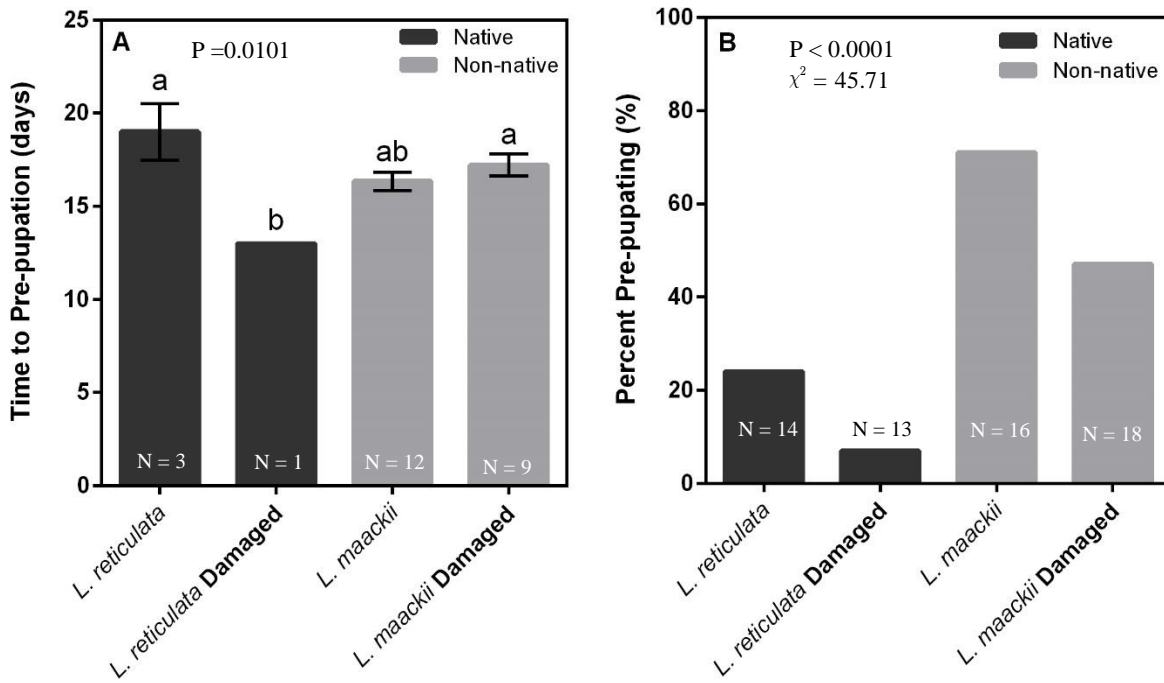
Figure 3.7 Mean days for *Z. montana* larvae to reach pre-pupation feeding on damaged and undamaged foliage of native *L. reticulata* and non-native *L. maackii* (A), and percentage reaching pre-pupation (B). Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05).
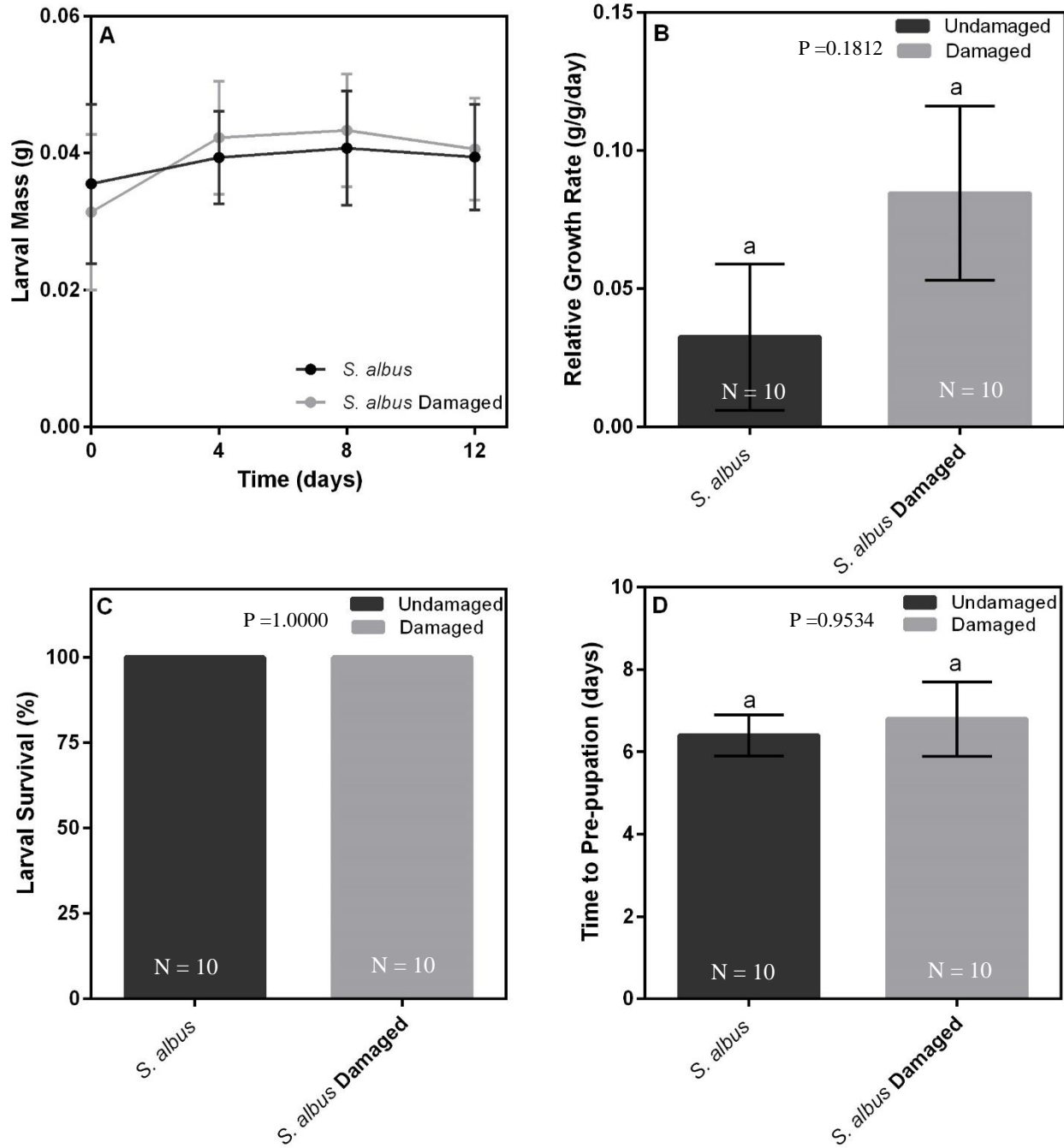
Figure 3.8 Mean larval mass of two week old *Z. montana* feeding on foliage of undamaged and damaged *S. albus* (A), relative growth rate (RGR) measured at peak larval weight on day four (B), final larval survivorship (C), and mean time to reach pre-pupation (D). Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05).

4. PERFORMANCE AND PREFERENCE OF THE GENERALIST FALL WEBWORM (*HYPHANTRIA CUNEA*) ON NATIVE AND NON-NATIVE SPECIES

4.1 INTRODUCTION

As invasive plant species enter a new habitat, they interact with native fauna, especially insect communities. These novel-plant insect interactions can be positive, negative, or neutral. A positive interaction could occur in which an insect benefits by successfully recognizing and using the invasive plant to increase in population size (Davis and Cipollini 2014). A negative interaction could occur in which insects have lower fitness and reproductive success on the novel plant because one or all life stages cannot utilize the host (Davis and Cipollini 2014). Lastly, a neutral interaction could occur because the native insect does not recognize the novel plant as a food source and does not use it (Davis and Cipollini 2014).

Generalist herbivores can consume a wide range of plant species that are not closely related to one another. Generalist herbivores can tolerate a wide variety of toxins, but are not specialized to deal with any particular plant defense (Bernays and Minkenberg 1997; Ali and Agrawal 2012). Being able to consume a wide range of hosts reduces the herbivores exposure to high levels of allelochemicals, but producing a wide range of detoxification enzymes can be metabolically costly, (Bernays and Minkenberg 1997; Ali and Agrawal 2012). Generalist herbivores, just like specialists, are threatened by non-

native plant invasions. Invasive plants often outcompete native plants and could potentially reduce the abundance of ideal foliage for the generalist herbivore. The generalist may be left eating lower quality food, which reduces the overall fitness and health of the organism. The generalist may be able to consume the invasive host but may not choose to consume it because it does not recognize the new host as a food source (Lankau et al. 2004). However, being able to eat a large range of species ensures the generalists will not become stranded if they run out of food on their original host, they can just move onto the next native or even invasive species (Bernays and Minkenberg 1997).

The generalist herbivore chosen for this study is the fall webworm (*Hyphantria cunea*). *Hyphantria cunea* is a generalist herbivore native to North America that can feed on over 630 different plant species (Warren and Tadic 1970). *Hyphantria cunea* has become an invasive pest in Europe, China, and North Korea (Sourakov and Paris 2014).There are two races of fall webworm, the black and the red race (Loewy et al. 2013a). The red race is more common in southern areas, whereas the black race is more common in more northern areas including Ohio (Loewy et al. 2013a). In Ohio, adults emerge in late May to early July (Fig 4.1). Females lay egg masses of 400-1000 eggs on the underside of leaves (Sourakov and Paris 2014). Fall webworm caterpillars have 5-8 instars. The caterpillars of *H. cunea* stay in large groups and build webs on the outer branches of their host (Mason et al. 2011). The red race caterpillars feed together their whole entire lives, the black race feed together for most of their lives until they reach later instars. Then the caterpillars will disperse, mature, and pupate in the soil. Fall

webworms in more northern areas, like Ohio, only have one to two generation per year, but more southern areas can have as many as four generations in one year (Gordon 1976).

Hyphantria cunea is known to feed on honeysuckle, and we have found it feeding on invasive *Lonicera maackii* and native *Lonicera reticulata* locally in the field (personal observation). Caterpillars of *H. cunea* have also been seen on other invasive hosts such as *L. japonica* and *P. calleryana* (personal observations). Many bioassays have been conducted using *H. cunea.* However, none of these studies have examined *H. cunea* feeding preferences and performance on invasive species, in particular, various invasive honeysuckle species, compared to native host plants. This study evaluated the performance of *H. cunea* on an artificial diet, native (*L. reticulata* and *P. serotina*) and non-native (*L. maackii, L. japonica, E. umbellata, E. alatus,* and *P. calleryana*) species. Another performance assay was conducted to look at the impact of switching four week old *H. cunea* caterpillars from native *P. serotina* to native (*L. reticulata* and *L. sempervirens*) and non-native (*L. maackii, L. japonica,* and *L. tatarica*). Lastly, choice studies were conducted with caterpillars to determine host preference when given a choice between various combinations of native and non-native foliage.

The primary objective of these experiments were to determine if all stages of *H. cunea* could utilize non-native, invasive honeysuckle species along with other known invasive species. The secondary goal was to compare the performance of *H. cunea* caterpillars on native and non-native species. The final objective was to determine the feeding preference of *H. cunea* caterpillars when given a choice between consuming a native or non-native host.

We predicted *H. cunea* caterpillars reared on native (*L. reticulata* and *P. serotina*) foliage from eggs in groups will have higher survivorship and larval mass after four weeks compared to caterpillars reared on non-native (*L. maackii, L. japonica, P. calleryana, E. umbellata,* and *E. alatus*) foliage. *Hyphantria cunea* have used native foliage much longer in their evolutionary history compared to non-native hosts which should lead to faster development. When these *H. cunea* are reared individually on native and non-native foliage, we hypothesized caterpillars feeding on native foliage will grow faster, grow larger, have higher survival, reach pupation faster, have higher pupal mass, and have a higher percent emergence.

We predicted that 4 week old *H. cunea* caterpillars switched onto native (*L. reticulata* and *L. sempervirens*) and non-native (*L. maackii, L. tatarica,* and *L.* japonica) honeysuckle foliage will be able to complete their development on these new hosts because most of their life cycle has been completed on native foliage and generalist caterpillars are able to tolerate a wide range of toxins. However, we predicted *H. cunea* feeding on native *Lonicera* foliage will grow faster, grow larger, have higher survival, reach pupation faster, have higher pupal mass, and have a higher percent emergence.

If *H. cunea* caterpillars are given a choice between native and non-native foliage, we predicted caterpillars will consume more native foliage no matter if they were reared on a native or non-native host before given the choice. Due to the long evolutionary history between native foliage and *H. cunea,* caterpillars may have mechanisms to recognize high quality, native foliage. If given a choice between two non-native hosts, we predict the caterpillars will have no preference because non-native foliage may not have recognizable volatiles *H. cunea* uses to find high quality, native hosts.

4.2 METHODS

4.2.1 No-choice Feeding Bioassay in Groups on Native and Non-native Hosts 2015

Eggs were collected from *H. cunea* adults that emerged in 2015 from pupae in the 2014 trials. Pupae were placed individual into 60 x 15 mm petri dishes with moistened vermiculite. Multiple petri dishes with pupae were placed together into plastic deli container (4 x 6 x 6 cm). Pupae were grouped based on larval host. The containers were placed in the fridge (4° C) on September 9, 2014 and transferred to an incubator at 25°C (16:8 L:D) on May 20, 2015. Every month, I would check the vermiculite and add more DI water as needed to keep it moistened. Containers were checked daily for adults.

One male and one female were placed together for 1-3 days for mating to occur. There were a total of 15 female and male pairs. Once mating was observed or three days had elapsed, the females were placed into a 6 quart, clear plastic storage container. In each container, there was a stem of *L. reticulata* and *L. maackii* inserted into separate water picks filled with DI water. Females were left in the oviposition containers until they died. All eggs were counted and removed from the containers. Eggs from four females were placed in groups of 50 on parafilm. The parafilm was placed with eggs facing upwards on a leaf and the leaf was placed into a 100 x 20 mm petri dishes. Eggs were placed on an artificial diet (Southland products Inc.), native foliage (*L. reticulata* and *P. serotina*), and non-native foliage (*L. maackii*, *L. japonica*, *P. calleryana*, *E. umbellata,* and *E. alatus*). Eggs from each female were haphazardly divided between host species. The petri dishes with eggs were placed in the incubator at 25°C (16:8 L:D). Eggs were checked daily for hatching. The total number hatched for each treatment was recorded. The newly hatched larvae were reared on their host in a group for 4 weeks.

New leaves were added as needed. The number surviving was checked every 2-5 days. Larvae were not weighed until the end of the 4 weeks to prevent larval mortality by handling the early instar caterpillars.

4.2.2 No-choice Feeding Bioassays on Native and Non-native Hosts

(A) Newly Emerged Larvae on Artificial Diet, Native, and Non-native Hosts 2015

The four week old *H. cunea* reared in groups were weighed and separated into individual containers (Genpak Clear Hinged Deli Container, Plastic, 24 oz, 7-1/4 x 6-2/5 x 2-1/4). The plastic deli containers had air holes perforating the lid and small push pins inserted into the side of the container to allow each container to stand freely. The sharp metal point inside the container was covered in hot glue to prevent the pins from falling off and to prevent caterpillars from impaling themselves on the sharp point. A 7.62 cm floral water pick with a cap was taped to the bottom of the container and filled with DI water. A 12-16 cm stem of foliage was placed into each water pick that corresponded with the original host of each *H. cunea* caterpillar. The number of caterpillar reared on each host was dependent on the group survivorship, ideally 20 replicates were set-up. [*L. reticulata* (N=20), *P. serotina* (N=20), *L. maackii* (N=20), *L. japonica* (N=9), *P. calleryana* (N=20), *E. umbellate* (N=3), and *E. alatus* (N=10)]. The artificial diet (N=20) was not placed in a water pick, but rather was poured into a 60 x 15 mm petri dish that was glued to the deli container at the same height as the foliage stems.

After the caterpillars were transferred to their new containers, they were haphazardly placed under grow lights. The grow lights were suspended in groups of three above a 1.5 m long folding table. Each group of lights was suspended above the table at a

96

height at which they were emitting 50-60 nm of light. The lights were placed on timers to be turned on between 6 am and 10 pm (16:8 L:D). The temperature under the lights was 25-27 °C. Containers were placed into two rows that were centered underneath grow lights. The caterpillars were weighed, checked for survivorship, and pupation every 3-5 days. Relative growth rate was calculated by subtracting the natural logarithm of initial mass from the natural logarithm of mass at the second time point and dividing by difference in time between weighing. Relative Growth Rate (RGR) = $\dfrac{\ln(W_2)- \ln(W_1)}{T_2 - T_1}$ [T_1 = time of initial weight (days), T_2 = time of second weight (days), W_1 = initial mass (grams) and, W_2 = second mass (grams) New leaves and water were added as needed. Leaves remained in containers for no longer than a week. Once a week, the containers were cleaned of frass. Once caterpillars pupated, the pupa was weighed and placed back under the lights for several weeks to allow for adult emergence. The experiment ended after all larvae died, reach pupation, or emerged.

(B) Four-week Old Larvae Switched onto Native and Non-native *Lonicera* 2014

A no-choice bioassay was set-up using *H. cunea* caterpillars collected from one web found on *P. serotina* from Hocking County, OH on August 1, 2014. The caterpillars were approximately 3-4 weeks old. On August 4, 2014 the caterpillars were separated individually into a clear plastic deli container (4 x 6 x 6 cm) with a 1.5 mL centrifuge tube filled with DI water. The centrifuge tube was used as a water pick to support a 12-16 cm stem of foliage of one of the following *Lonicera* species: *L. reticulata* (N=14), *L. sempervirens* (N=9), *L. maackii* (N=14), *L. japonica* (N=13), and *L. tatarica* (N=13). The

containers were haphazardly placed in an incubator at 25°C (16:8 L:D). Larvae were checked and reared as described above in section A.

4.2.3 Choice Bioassays on Native and Non-native Hosts

  (A) Native and Non-native Hosts 2015

      Choice bioassays were conducted using 4.5 week old *H. cunea* caterpillars reared from eggs on *L. maackii*, *P. serotina,* and *P. calleryana*. Caterpillars were placed individually into a deli container and given equally sized leaves of two hosts. One of the hosts was the species the caterpillar was reared on and the other host was a native or non-native species. The different combinations of original host/new choice host were as follows: *L. maackii/L. reticulata* (N=10), *L. maackii/P. serotina* (N=10), *L. maackii/P. calleryana* (N=7), *P. calleryana/L. reticulata* (N=7), *P. calleryana/P. serotina* (N=7), *P. serotina/L. maackii* (N=7). The caterpillars were placed in an incubator at 25°C (16:8 L:D). The caterpillars were given three days to consume foliage. After three days, the total leaf area eaten was measured to the nearest cm^2. A preference index was calculated using this equation:

Choice Index = $\dfrac{\text{Leaf area removed }_{\text{Choice 1}} - \text{Leaf area removed }_{\text{choice 2}}}{\text{Total leaf area removed}}$ x 100

The total leaf area removed from one host was subtracted by the total leaf area removed from host two. The difference is divided by the total leaf area and multiplied by 100. A zero indicated no choice, a positive number indicated choice for the first host, and a negative number indicated choice for second host. A mean choice index was found to determine if there was a preference for one leaf species over another.

(B) <u>Native and Non-native *Lonicera* 2014</u>

Choice bioassays were performed the same way as described above. The only difference was *H. cunea* caterpillars were first reared for approximately 4-5 weeks on slippery elm, *Ulmus rubra,* and then given a choice between *L. reticulata/L. maackii* (N=10), *L. reticulata/L. tatarica* (N=10), and *L. maackii/L. tatarica* (N=10). The caterpillars were allowed to feed for four days.

4.2.4 Data Analysis

Repeated measures ANOVA were used to compare larval masses with plant species as the between subjects effect and time as the within subjects effect. Mean larval mass in groups, relative growth rates, pupal mass, days to pupation, and days to emergence were compared among plant species using ANOVA. Comparisons of means were made using Tukey post-hoc tests. Percent survival, percent pupating, and percent emergence were compared using chi-square tests. All percentages were converted to arcsine values. Survivorship was compared among plant species using the non-parametric Mantel-Cox test. Sample T-test were used to determine if the choice feeding index differed from zero. A zero indicated no choice, a positive number indicated choice for initial host, and a negative number indicated choice for new host. All statistical analyses were performed using GraphPad Prism (Version 6.07, GraphPad Software, Inc., La Jolla, California).

4.3 RESULTS

4.3.1 No-choice Feeding Bioassay in Groups on Native and Non-native Hosts 2015

In the no-choice bioassays with *Hyphantria cunea* reared from eggs in groups, larval survivorship varied significantly between hosts (Figure 4.2a). Larval survivorship was highest on non-native *L. maackii* and *P. calleryana* at 96% and 97%, respectively. All native species ranged between 45-60% larval survival. Non-native *E. alatus, E. umbellata,* and *L. japonica* had the lowest larval survivorship between 10-25%. Larvae feeding on the artificial diet, *L. reticulata,* and *L. maackii* were significantly heavier after four weeks than larvae on the other species (Figure 4.2b). Larvae feeding on *L. japonica* were the smallest and significantly smaller than larvae feeding on native *P. serotina* (Figure 4.2b). Larvae reared on *P. serotina*, *P. calleryana, E. alatus,* and *E. umbellata* had similar larval masses (Figure 4.2b)

4.3.2 No-choice Feeding Bioassays on Native and Non-native Hosts

(A) Newly Emerged Larvae on Artificial Diet, Native, and Non-native Hosts 2015

In the no-choice bioassays with *Hyphantria cunea* reared from eggs, repeated measures ANOVA revealed differences in larval mass between species, through time, and an interaction between time and species (Table 4.3, Figure 4.3a). There was a statistically significant difference between relative growth rate (RGR) between all species on day 14 before any *H. cunea* pupated (P=0.0014, Figure 4.3b). Caterpillars feeding on native *P. serotina* had the highest RGR, which was significantly higher than caterpillars feeding on the artificial diet and *P. calleryana* (Figure 4.3b). RGR of *H. cunea* feeding on the native species did not differ significantly from one another, nor did the RGR of

caterpillars feeding on non-native species (Figure 4.3b). There was 100% mortality of caterpillars feeding on *L. japonica* and *E. umbellata* by day 7 and day 2, respectively (Figure 4.3a). Larval survival was significantly different between species (P < 0.0001, x^2 = 366.3, Figure 4.3c). Caterpillars had 100% survival on *L. maackii* followed by 90% survival on *L. reticulata, P. serotina,* and *E. alatus*. Overall, *H. cunea* had pretty high survivorship on most species, except for non-native *L. japonica* and *E. umbellata* (Figure 4.3c).

Female pupal mass of *H. cunea* reared on all species were significantly heavier than male pupal mass (P = 0.0003, Table 4.3). Repeated measures ANOVA also revealed differences in pupal mass between species, but no interaction between host and sex (Table 4.3). Comparing mean female pupal mass between species, caterpillars reared on the artificial diet had significantly lower mass than caterpillars reared on *L. maackii* (Figure 4.4a). However, female pupal masses between all other species did not differ significantly. Male pupal mass was significantly higher on *L. reticulata* and *L. maackii* compared to *E. alatus* (Figure 4.4a). The ratio of males and females reaching pupation on each species was significantly different (P<0.0001, x^2 = 50.27, Figure 4.4b). The caterpillars reared on the artificial diet had the highest percentage of females reaching pupation at 86%. The *H. cunea* caterpillars reared on the natives had more females reach pupation than males, compared to more males reaching pupation on the non-native *P. calleryana* and *E. alatus* (Figure 4.4b). Similar to the native species, *L. maackii* had more females reach pupation. Caterpillars reared on the artificial diet, *L. reticulata*, *P. serotina,* and *L. maackii* reached pupation significantly faster than caterpillars reared on *P. calleryana* and *E. alatus* (Table 4.4, Figure 4.5a). The percentage pupating on all species

was very high, and was significantly different between species (P<0.0001, $x^2 = 55.07$).

Caterpillars reared on the artificial diet and *P. calleryana* had the lowest percentages

pupating at 65% and 70%, respectively (Figure 4.5b).

The mean time to emergence did not differ between species (P=0.6912, Table 4.5,

Figure 4.5c). However, there was a significant difference in the percentage emergence

between treatments (P<0.0001, $x^2 = 137.0$, Figure 4.5d). Over 50% of pupae reared on *E.*

alatus and 25% of pupae on *P. serotina* emerged (Figure 4.5d). Overall, a low

percentage of *H. cunea* emerged in 2015, no adults emerged from pupae reared on the

artificial diet (Figure 4.5d).

(B) Four-week Old Larvae Switched onto Native and Non-native *Lonicera* 2014

In the no-choice bioassays with four-week old *Hyphantria cunea*, repeated

measures ANOVA revealed differences in larval mass through time, between species,

and an interaction between time and species (Table 4.6, Figure 4.6a). However, relative

growth rate at peak caterpillar mass was not significantly different between species

(P=0.0800, Figure 4.6b). All *H. cunea* had high survival which differed significantly

between species (P<0.0001, $x^2 = 36.82$, Figure 4.6c). Caterpillars reared on native *L.*

sempervirens and *L. reticulata* had the highest larval survival at 100% and 92% (Figure

4.6c). Caterpillars reared on non-native *L. tatarica* had high survival at 92%. However,

caterpillars reared other non-natives, *L. maackii* and *L. japonica,* had survival just under

80% (Figure 4.6c).

Mean female pupal mass of *H. cunea* reared on all species was significantly

higher than male pupal mass (P = 0.0015, Table 4.8). Repeated measures ANOVA also

revealed differences in pupal mass between species, and an interaction between host and sex (Table 4.8). Pupal mass of males did not differ between species treatment (Figure 4.7a). Female pupal mass did vary between species. Females reared on *L. tatarica* had significantly heavier pupal masses compared to both native species and *L. japonica* (Figure 4.7a). The smallest female pupae were reared on *L. sempervirens* and *L. japonica* (Figure 4.7a). The ratio of males and females reaching pupation on each species was not significantly different between species, but there is a highly suggestive trend than species impacts sex ratios (P=0.0596, $x^2 = 9.061$, Figure 4.7b). For all species, except for *L. reticulata*, there was a higher percentage of males reaching pupation compared to females (Figure 4.7b). The time to pupation did not differ between species (P = 0.4652, Table 4.9, Figure 4.8c). The percentage pupating on all species was very high, and was significantly different between species (P<0.0001, $x^2 = 35.75$). *Hyphantria cunea* had the highest pupation success on *L. sempervirens* and *L. tatarica* at 100% and 92%, respectively (Figure 4.8b). Caterpillars reared on *L. reticulata*, *L. maackii,* and *l. japonica* had pupation success right around 80% (Figure 4.8b).

No adults emerged in 2014, however many did emerge in 2015. The mean time to emerge after being removed from the cold storage, did not different between species (P=0.1450, Table 4.10, Figure 4.8c). There was a significant difference in the percentage emergence between treatments (P = 0.0009, $x^2 = 18.62$, Figure 4.8d). The pupae reared on native *L. sempervirens* and *L. reticulata* had the highest percent emergence at 78% and 67% respectively (Figure 4.8d). The pupae reared on non-native species had under 60% emergence (Figure 4.8d). When looking at percentage emergence by sex, 74% of all female pupae emerged whereas, 47% of male pupae emerged.

4.3.3 Choice Bioassays on Native and Non-native Hosts

(A) Native and Non-native Hosts 2015

In the choice bioassays with 27 day old *H. cunea* reared on *L. maackii*, larvae showed a significant preference for non-native *L. maackii* foliage over non-native *P. calleryana* (P = 0.0010, t = 6.000, Figure 4.9a). Larvae consumed more native *L. reticulata* and *P. serotina* foliage when given a choice between non-native *L. maackii,* but this was not significant (P = 0.2415, t = 1.254; P = 0.1372, t = 1.632; Figure 4.9a). In the choice bioassays with *H. cunea* reared on *P. serotina*, larvae showed a significant preference for native *P. serotina* over non-native *L. maackii* foliage (P = 0.0127, t = 3.505, Figure 4.9b). In the choice bioassays with *H. cunea* reared on *P. calleryana*, larvae showed a significant preference for native *P. serotina* over non-native *P. calleryana* (P = 0.0005, t = 6.873, Figure 4.9c). Larvae consumed more native *L. reticulata* foliage when given a choice of *P. calleryana,* but this was not significant (P = 0.2367, t = 1.315; Figure 4.9c).

(B) Native and Non-native *Lonicera* 2014

In the choice bioassays with four-week old *Hyphantria cunea* reared on *P. serotina*, larvae showed a significant preference for native *L. reticulata* over *L. maackii* (P = 0.0130, t = 3.086, Figure 4.10). Larvae consumed more *L. reticulata* foliage when given a choice between native *L. reticulata* and non-native *L. tatarica,* but this was not significant (P = 0.0683, t = 2.070, Figure 4.10). When given a choice between two non-native species, *H. cunea* larvae had no preference (P = 0.5710, t = 0.5879, Figure 4.10).

4.4 DISCUSSION

4.4.1 *Hyphantria cunea Performance on Native and Non-native Hosts*

  (A) <u>Newly Emerged Larvae in groups</u>

      Larval survivorship in groups varied greatly between the artificial diet, native species, and non-native species. *Hyphantria cunea* reared on non-native *L. maackii* and *P. calleryana* had very high larval survival compared to the artificial diet, the two native species (*L. reticulata* and *P. serotina)*, and the three other non-native species (*E. alatus, E. umbellata,* and *L. japonica*). This was surprising and did not support our hypothesis that *H. cunea* larvae would have higher survivorship when reared in groups on native species. Newly emerged larvae can survive at least 4 weeks in a group on all native and non-native hosts tested in this experiment in the absence of predators and disease.

      Similar to larval survivorship, mean larval mass after four weeks in groups varied on the different hosts. The *H. cunea* caterpillars reared on native *L. reticulata* had significantly higher larval mass than four of the non-native species (*P. calleryana, E. alatus, E. umbellata,* and *L. japonica*). Many of the non-native hosts tested had extremely low larval mass after four weeks, especially *H. cunea* reared on *L. japonica.* This provides some support for our prediction that *H. cunea* would have higher larval mass reared on native species compared to non-native species. However, larvae reared on non-native *L. maackii* had similar larval mass compared to *L. reticulata* and significantly higher larval mass compared to native *P. serotina.* This was surprising because *H. cunea* caterpillars are often found on and prefer *P. serotina* in the field, so I predicted *H. cunea* would perform well on this preferred host (Barbosa and Greenblatt 1979; Travis 2005)*.*

Larvae reared on *P. serotina* had larval mass significantly lower than non-native *L. maackii*, similar to the non-native *P. calleryana*, and higher than non-native *L. japonica*.

(B) Four-week Old Larvae

In the no-choice bioassays, larval survival was relatively high on the artificial diet and all native and non-native species except for larvae feeding on non-native *E. umbellata* and *L. japonica*, which experienced 100% larval mortality after being separated individually. These larvae were extremely small after four weeks, and may not have been developmentally ready to survive and feed alone. However, *H. cunea* reared on *E. alatus* started off smaller than larvae reared on *E. umbellata,* but they were able to survive relatively well individually.

The typical pattern for larval growth is a steady increase in larval weight until a maximum mass, after which there is a slight decline due to decreased feeding before pupation. This pattern can be clearly seen for all hosts, except for *P. serotina, E. umbellata,* and *L. japonica.* There were some differences in the shape of the larval mass curves between hosts. *Hyphantria cunea* reared on non-native *P. calleryana* and non-native *E. alatus* took longer to reach their peak mass and longer to pupate compared to caterpillar reared on the artificial diet, *L. reticulata* (native)*,* and *L. maackii* (non-native). Larvae reared on native *P. serotina* foliage had two peak larval masses, one with the artificial diet, *L. reticulata,* and *L. maackii*, and the other peak with *E. umbellata* and *L. japonica.* The two peaks were caused by differences in growth rate of larvae. Most of the larvae grew quickly and gained mass, whereas, some larvae grew more slowly and took much longer to reach peak mass. The mean days to pupation for *H. cunea* on native *P.*

serotina was significantly faster than non-native *P. calleryana* and *E. alatus*. This provides some support that *H. cunea* will grow faster and reach pupation faster on native foliage compared to some non-native species (*P. calleryana, E. alatus, E. umbellata,* and *L. japonica*), but not compared to some non-native hosts (*L. maackii*). Prolonged development time can negatively impact *H. cunea* larval survival, because they are exposed longer to parasitoids (Morris 1976; Jang et al. 2015), invertebrates (Morris 1972a), and birds (Morris 1972b).

Female *H. cunea* pupae were larger than male pupae in all treatments except for the larvae reared on the artificial diet. Female *H. cunea* reached the highest average female pupal mass on native *L. reticulata,* non-native *L maackii*, and non-native *P. calleryana*. This did not support our prediction of *H. cunea* having higher pupal masses on native hosts. Larvae were able to reach similar female and male pupal masses on *P. calleryana* despite taking significantly longer to reach pupation compared to the artificial diet, the two natives, and non-native *L. maackii*. However, larvae reared on non-native *E. alatus* had lower female and male pupal masses than *H. cunea* reared on other hosts. Female pupal mass is positively correlated with female potential fecundity, the potential number of offspring the female can produce (Morris and Fulton 1970; Awmack and Leather 2002; Loewy et al. 2013b). Loewy et al. (2013b) found that every 1 mg of pupal mass was equal to 2.35 eggs. Male pupal mass is also important, because the larger the male the more energy he will have to fly to and copulate with as many females as possible, thus increasing his fitness as well (Rossiter 1991; Engels and Sauer 2007).

Sex ratios are very important to maintaining *H. cunea* population. Typically *H. cunea* have a female biased sex ratio when reared on an artificial diet and equal when

reared on native foliage from Colorado (Yearian et al. 1966; Loewy 2013). In sawflies, sex ratios were dependent on plant quality, where male-biased ratios were a result of larvae feeding on slow-growing low quality plants and female-biased ratios were found when larvae consumed fast-growing high quality foliage (Craig et al. 1992). This male-biased sex ratios on low quality plants has also been seen in other sawflies, leafhoppers, and aphids (Awmack and Leather 2002). More females reached pupation on the artificial diet, *L. reticulata, P. serotina*, and *L. maackii*. However, more males reached pupation on non-native *P. calleryana* and *E. alatus*. This may suggest that some poor quality hosts or non-native hosts may cause higher female larval mortality compared to males. Female *H. cunea* pupae are larger than males, so female larvae must be on a host that allows them to gain enough mass to pupate. If the host quality is extremely low, females may die before they can gain enough mass to pupate. This could potential lead to reduction in overall *H. cunea* populations.

Many of the *H. cunea* larvae in this study went into diapause instead of emerging. However, pupae did eclose on all native and non-native hosts except for *E. umbellata, L. japonica,* and the artificial diet. The time to eclosion did not different between hosts. However, almost 50% of pupae emerged on non-native *E. alatus* which was much higher than all other non-native and native hosts. This was especially surprising because *E. alatus* was a low quality host in which larvae took significantly longer to pupate and were smaller than most other species. Previous studies have shown that *H. cunea* feeding on low quality host often enter diapause compared to larvae feeding on high quality hosts (Gomi et al. 2005), This did not support my prediction that *H. cunea* pupae reared on native foliage would have higher percentage emerging compared to non-native foliage.

(C) <u>Four-week Old Larvae Switched onto *Lonicera*</u>

All *H. cunea* larvae switched onto native and non-native *Lonicera* were able to complete their life cycle on these hosts and had very similar larval mass curves which reached peak larval mass between day 16 and 18. Larvae reared on non-native *L. maackii* and non-native *L. tatarica* had higher larval mass compared to the two natives (*L. reticulata* and *L. sempervirens*) and *L. japonica.* Larval survival and percentage reaching pupation were relatively high for *H. cunea* on the 2 native and 3 non-native *Lonicera,* ranging between 75-100%. The highest survivorship and percent pupated was on native *L. sempervirens*. Relative growth rates and time to pupation did not differ between all hosts. Male pupal mass were not different between hosts and were smaller than female pupae reared on *L. tatarica, L. maackii,* and *L. reticulata*. Female pupal mass did differ significantly between treatments. Larvae reared on non-native *L. maackii* and *L. tatarica* had the highest female pupal mass. Whereas, females reared on *L. japonica* had the lowest pupal mass. *Hyphantria cunea* reared on *L. reticulata* and *L. sempervirens* had intermediate female pupal mass that was significantly lower than larvae reared on *L. tatarica.* Female pupal mass is important for future female reproductive fitness, the larger the female the more eggs she can lay (Morris and Fulton 1970; Loewy et al. 2013b). This suggests that female larvae reared on low quality hosts, could not gain as much mass as larvae reared on higher quality hosts. This will lead to lower fitness of females reared on non-native *L. japonica.*

The ratio of female to male pupae on *L. reticulata* was equal, but on all other hosts it was male biased. As mentioned above, fall webworms typically have a female biased sex ratio on the artificial diet, or an equal ratio when reared on foliage (Yearian et

al. 1966; Loewy 2013). In this study it is unclear if the foliage caused the shift in the sex ratios due to female larval mortality or if female larvae were preferentially parasitized by parasitoids in the field. A small percentage of larvae, removed from this study, were attacked by parasitoids leading to their death. Lastly, there was no difference in the length of time for adult emergence on the native and non-native *Lonicera* species, however, there was a difference in the percentage emergence. *Hyphantria cunea* larvae that were reared on native foliage had higher percentage emerging compared to all non-native hosts. In all host, a higher percentage of female pupae emerged than male pupae, this was interesting because more males reached pupation. You would expect equal numbers of male and female pupae to emerge when reared under the same environmental conditions. Overall, *H. cunea* performed very well when switched onto *L. reticulata, L. sempervirens, L. maackii, L. tatarica,* and *L. japonica*. There was no support for our prediction that *H. cunea* larvae would perform better on native hosts over non-native hosts, in fact, larvae reared on non-native *L. maackii* and *L. tatarica* performed extremely well sometimes even better than on closely related native species. The ability for *H. cunea* to be able to switch to a new non-native host will benefit this species in the face of invasion by *L. maackii, L. tatarica,* and *L. japonica*.

4.4.3 *Choice Bioassays on Native and Non-native Hosts*

In the choice bioassays with *H. cunea* larvae reared on black cherry for 4 weeks and given a choice between native *L. reticulata* and non-native *L. maackii,* larvae significantly preferred and consumed more native foliage than non-native foliage. When given a choice between native *L. reticulata* and non-native *L. tatarica*, larvae consumed

more native foliage but this was not a significant preference. When given a choice between the two invasive species, *H. cunea* larvae had no preference and consumed similar amounts of each non-native host. This supports our prediction that caterpillars will consume and prefer native foliage over non-native foliage and larvae will have no preference when given a choice between two non-native hosts.

Hyphantria cunea caterpillars reared on non-native *L. maackii* for 27 days had a significant preference for *L. maackii* over non-native *P. calleryana*. Larvae also preferred to feed on native *L. reticulata* and native *P. serotina* over *L. maackii* but this was not significant. Larvae reared on native *P. serotina* strongly preferred *P. serotina* over non-native *L. maackii* when given a choice. Lastly, larvae reared on non-native *P. calleryana* preferred native *L. reticulata* and *P. serotina* over *P. calleryana* when given a choice. Overall, no matter what foliage the *H. cunea* larvae were reared on, they would almost always choose to consume a native host over a non-native host. When given the option between two non-natives, *H. cunea* in this case chose the natal host. This supports our prediction that caterpillars will consume and prefer native foliage over non-native foliage. However, it did not support our prediction that caterpillars will have no preference when given a choice between two non-native hosts.

4.5 CONCLUSION

Overall, some non-native species (*L. maackii*, *L. tatarica* and *P. calleryana*) appear to be suitable host for *H. cunea* and other non-native species (*L. japonica, E. alatus,* and *E. umbellata*) are unsuitable hosts for early larval development by reducing the larval survival and preventing larval growth. There appears to be a range in the leaf quality of novel hosts which corresponds with *H. cunea* performance. The high leaf quality (e.g. *L. maackii*) leads to high larval performance, the intermediate leaf quality (e.g. *P. calleryana*) leads to intermediate larval performance, and the low leaf quality (*L. japonica*) leads to low larval performance. This same leaf quality associated with larval performance can be seen in native hosts as well, where better quality hosts lead to higher performance than lower quality foliage (Loewy 2013).

The larval interactions between *H. cunea* and novel hosts is dependent on the novel host and the time of interaction. For example, newly emerged larvae perform very poorly on *L. japonica, E. umbellata,* and *E. alatus* indicating a negative interaction between *H. cunea* and the novel hosts. On the other hand, newly emerged larvae feeding on non-native *L. maackii* perform very well, indicating a positive interaction with some novel hosts. The life stage of *H. cunea* at the interaction plays a big role. Later instar larvae which fed on *L. japonica* were able to survive and develop just as well as on native hosts, indicating a positive interaction between *H. cunea* larvae and this novel host. Later instar *H. cunea* larvae are very mobile and often switch hosts as they continue to develop outside of their natal nest. Later instar *H. cunea* were able to successfully switch hosts onto all native and non-native *Lonicera* tested. However, when given a choice, *H. cunea*

larvae will prefer to consume native foliage, suggesting a neutral interaction in which *H. cunea* larvae will avoid using non-native hosts.

The novel plant insect interaction between adult *H. cunea* and novel hosts was not a focus of this study, but we did observe *H. cunea* webs on non-native *L. maackii, L. japonica,* and *P. calleryana* in the field. This indicates that female *H. cunea* are recognizing and ovipositing eggs on novel hosts in the field. This is supported by a study done by Mason et al (2011), looking at female *H. cunea* oviposition host choice. They found females were choosing host based on the abundance of the potential hosts rather than larval performance or avoidance of enemies (Mason et al. 2011). This suggest *H. cunea* females have high selective pressure to reduce oviposition search time to avoid potential predation (Loewy 2013). Since invasive, non-native hosts are often very abundant in invaded sites, *H. cunea* may oviposit on non-native host more often than previously thought. Since, adult *H. cunea* do not select host based on larval performance, this may lead to negative impacts of laying on eggs on novel hosts that do not support the development of early instar larvae like *L. japonica* and *E. umbellata.*

The generalist *H. cunea* appears to already be interacting with novel hosts as they invade habitats throughout Ohio and the United States. On native hosts, *H. cunea* can cause significant defoliation (Cranshaw et al. 2000) and reduce plant growth. *Hyphantria cunea* may start increasing herbivory rates on these non-native hosts, which typically receive minimal herbivore damage, in particular *L. maackii* (Lieurance and Cipollini 2012). *Hyphantria cunea* could potentially become a native generalist that can help keep *L. maackii, L. japonica, L. tatarica,* and *P. calleryana* populations "in-check" especially in the summer and fall.

113

4.6 REFERENCES

Ali JG, Agrawal AA (2012) Review: Specialist versus generalist insect herbivores and
     plant defense. Trends Plant Sci 17:293-302

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects.
     Annual Rev Entomol 47 (1):817

Barbosa P, Greenblatt J (1979) Effects of leaf age and position on larval preferences of
     the fall webworm, *Hyphantria cunea* (Lepidoptera: *Arctiidae*). Can Entomol
     111:381-383

Bernays EA, Minkenberg OPJM (1997) Insect herbivores: Different reasons for being a
     generalist. Ecology :1157

Craig TP, Price PW, Itami JK (1992) Facultative sex ratio shifts by a herbivorous insect
     in response to variation in host plant quality. Oecologia 920 (2):153-161

Cranshaw W, Leatherman D, Jacobi WR, Mannix L (2000) Insects and diseases of
     woody plants of the central Rockies. Colorado State University Cooperative
     Extension, Fort Collins, CO

Davis S, Cipollini D (2014) Do mothers always know best? Oviposition mistakes and
     resulting larval failure of *Pieris virginiensis* on *Alliaria petiolata*, a novel, toxic
     host. Biol Invasions 16:1941-1950

Engels S, Sauer KP (2007) Energy beyond the pupal stage: Larval nutrition and its long-
     time consequences for male mating performance in a scorpionfly. J Insect Physiol
     53:633-638

Gomi T, Hirochika M, Nagasaka M, Fukuda T (2005) Effects of diet on life-history traits

in a trivoltine Kobe population of *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae). Appl Entomol Zool 40(3): 475-482

Gordon SW (1976) Population studies of the fall webworm in Ohio with the development of life tables in relation to temperature. Dissertation Master, Ohio State University

Jang T, Rho MS, Koh SH, Lee KP (2015) Host-plant quality alters herbivore responses to temperature: a case study using the generalist *Hyphantria cunea*. Entomol Exp Appl 154:120-130

Lankau RA, Rogers WE, Siemann E (2004) Constraints on the utilisation of the invasive Chinese tallow tree *Sapium sebiferum* by generalist native herbivores in coastal prairies. Ecol Entomol 29:66-75

Lieurance D, Cipollini D (2012) Damage levels from arthropod herbivores on *Lonicera maackii* suggest enemy release in its introduced range. Biol Invasions 14:863-873

Loewy KJ (2013a) Host use and geographic variation in fall webworms (*Hyphantria cunea*). Dissertation Master, University of Denver

Loewy KJ, Flansburg AL, Grenis K, Kjeldgaard MK, McCarty J, Montesano L, Vernick J, Murphy SM (2013b) Life history traits and rearing techniques for fall webworms (*Hyphantria cunea* Drury) in Colorado. J Lepid Soc 67:196-205

Mason PA, Wilkes SR, Lill JT, Singer MS (2011) Abundance trumps quality: bi-trophic performance and parasitism risk fail to explain host use in the fall webworm. Oikos 120:1509-1518

Morris RF (1976) Relation of mortality caused by parasites to the population density of *Hyphantria cunea*. Can Entomol 108 (11):1291-1294

Morris RF (1972a) Predation by insects and spiders inhabiting colonial webs of

Hyphantria cunea. Can Entomol 104 (8):1197-1207

Morris RF (1972b) Predation by wasps, birds, and mammals on *Hyphantria cunea*. Can

Entomol 104(10):1581-1591

Morris RF, Fulton WC (1970) Models for the development and survival of *Hyphantria*

cunea in relation to temperature and humidity. Mem Entomol Soc Can 70:1-312

Rossiter MC (1991) Environmentally-based maternal effects: A hidden force in insect

population dynamics? Oecologia 87 (2):288-294

Sourakov A, Paris T (2014) Fall webworm, *Hyphantria cunea* (Drury) (Insecta:

Lepiodoptera: Arctiidae: Arctiinae). In: Anonymous . IFAS Extension University

of Florida.

Warren O, Tadic M (1970) The fall webworm, *Hyphantria cunea* (Durry). Ark Agr Exp

Sta Bull: 106

Table 4.1 ANOVA table for mean larval mass of the generalist *H. cunea* feeding in groups for four weeks in a no-choice bioassay on the artificial diet, native (*L. reticulata* and *P. serotina*), or non-native (*L. maackii, P. calleryana, E. alatus, E. umbellata,* and *L. japonica*) species.

	DF	MS	F	P value	Significant
Treatment	7	0.0044	29.76	P < 0.0001	Yes
Error	172	0.0001			

Table 4.2 ANOVA table for *H. cunea* caterpillar mass of survivors through time when reared individually on an artificial diet, native (*L. reticulata* and *P. serotina*), and non-native (*L. maackii, P. calleryana, E. alatus, E. umbellata,* and *L. japonica*) species for two weeks before pupation began.

	DF	MS	F	P value	Significant
Time	3	0.0976	177.70	P < 0.0001	Yes
Treatment	5	0.0676	16.53	P < 0.0001	Yes
Time x Treatment	15	0.0049	8.98	P < 0.0001	Yes
Error	252	0.0005			

Table 4.3 ANOVA table for *H. cunea* pupal mass of female and male reared in a no-choice bioassay on an artificial diet, native (*L. reticulata* and *P. serotina*), and non-native (*L. maackii, P. calleryana, E. alatus*) species.

	DF	MS	F	P value	Significant
Sex	1	0.0062	14.43	P = 0.0003	Yes
Host	5	0.0020	4.56	P = 0.0012	Yes
Sex x Host	5	0.0006	1.28	P = 0.2808	No
Error	68	0.0004			

Table 4.4 ANOVA table for the mean number of days for H. cunea to reach pupation after being reared in a no-choice bioassay on an artificial diet, native (*L. reticulata* and *P. serotina*), and non-native (*L. maackii, P. calleryana, E. alatus*) hosts.

	DF	MS	F	P value	Significant
Treatment	5	1400.0000	24.83	P < 0.0001	Yes
Error	86	56.3900			

Table 4.5 ANOVA table for the mean number of days after pupation for *H. cunea* to emerge in 2015 after being reared in a no-choice bioassay native (*L. reticulata* and *P. serotina*), and non-native (*L. maackii, P. calleryana, E. alatus*) species.

	DF	MS	F	P value	Significant
Treatment	4	54.3800	0.57	P = 0.6912	No
Error	11	95.7200			

Table 4.6 ANOVA table for *H. cunea* caterpillar weight of survivors through time when reared on native (*L. reticulata* and *L. sempervirens*) and non-native (*L. maackii, L. tatarica,* and *L. japonica*) honeysuckle species in 2014.

	DF	MS	F	P value	Significant
Time	5	0.0998	153.80	P < 0.0001	Yes
Treatment	4	0.0153	2.92	P = 0.0300	Yes
Time x Treatment	20	0.0015	2.24	P = 0.0022	Yes
Error	255	0.0006			

Table 4.7 ANOVA table for mean relative growth rate of surviving *H. cunea* after being placed individually for 14 days on native (*L. reticulata* and *L. sempervirens*) and non-native (*L. maackii, L. tatarica,* and *L. japonica*) honeysuckle species.

	DF	MS	F	P value	Significant
Treatment	4	0.0024	2.22	P = 0.0800	No
Error	51	0.0011			

Table 4.8 ANOVA table for *H. cunea* mean pupal mass of female and male *H. cunea* caterpillars switched after four weeks onto native (*L. reticulata* and *L. sempervirens*) and non-native (*L. maackii, L. tatarica,* and *L. japonica*) honeysuckle species.

	DF	MS	F	P value	Significant
Sex	1	0.0032	11.62	P = 0.0015	Yes
Host	4	0.0034	12.27	P < 0.0001	Yes
Sex x Host	4	0.0016	5.73	P = 0.0009	Yes
Error	41	0.0003			

Table 4.9 ANOVA table for the mean number of days for *H. cunea* to reach pupation after being reared in a no-choice bioassay on native (*L. reticulata* and *L. sempervirens*) and non-native (*L. maackii, L. tatarica,* and *L. japonica*) honeysuckle species.

	DF	MS	F	P value	Significant
Treatment	4	71.5500	2.88	P = 0.0323	Yes
Error	49	24.8800			

Table 4.10 ANOVA table for the mean number of days for adults to emerge after being removed from 4 °C to 25 °C incubator to emerge on native (*L. reticulata* and *L. sempervirens*) and non-native (*L. maackii, L. tatarica,* and *L. japonica*) honeysuckle species.

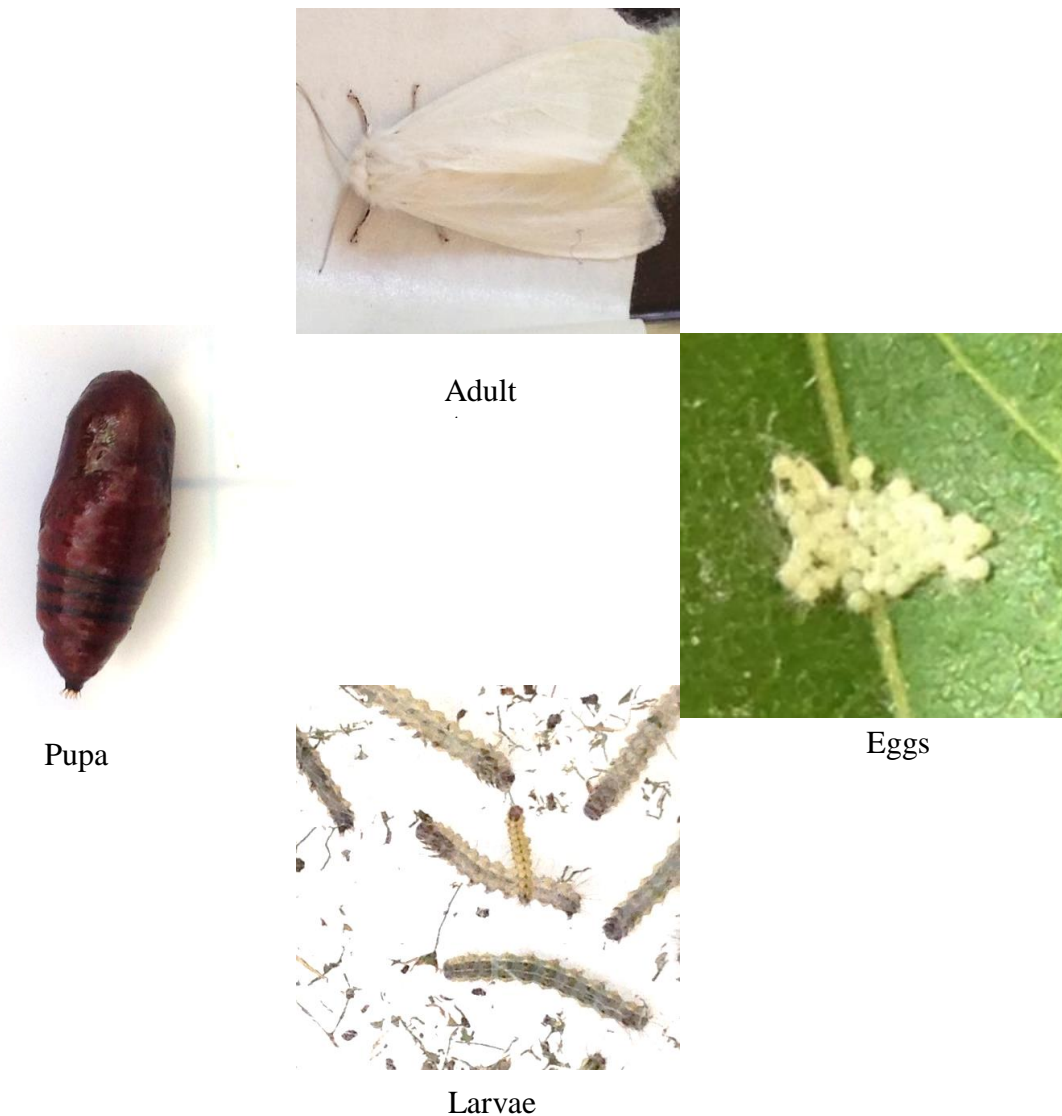|	DF	MS	F	P value	Significant
Treatment	4	5.1630	0.15	P = 0.9635	No
Error	24	35.6100			

Adult

Pupa

Eggs

Larvae

Figure 4.1 Life cycle of *H. cunea*. Adult females lay eggs on leaves in large groups covered with here scales. The eggs hatch in 9-13 days. Larvae typically feed in groups for at least 4 weeks, after four weeks the larvae continue development individual. Larvae begin to pupate around 40 days after hatching. Some pupa go into diapause until the next year and others will emerge the same year after about 18-22 days.
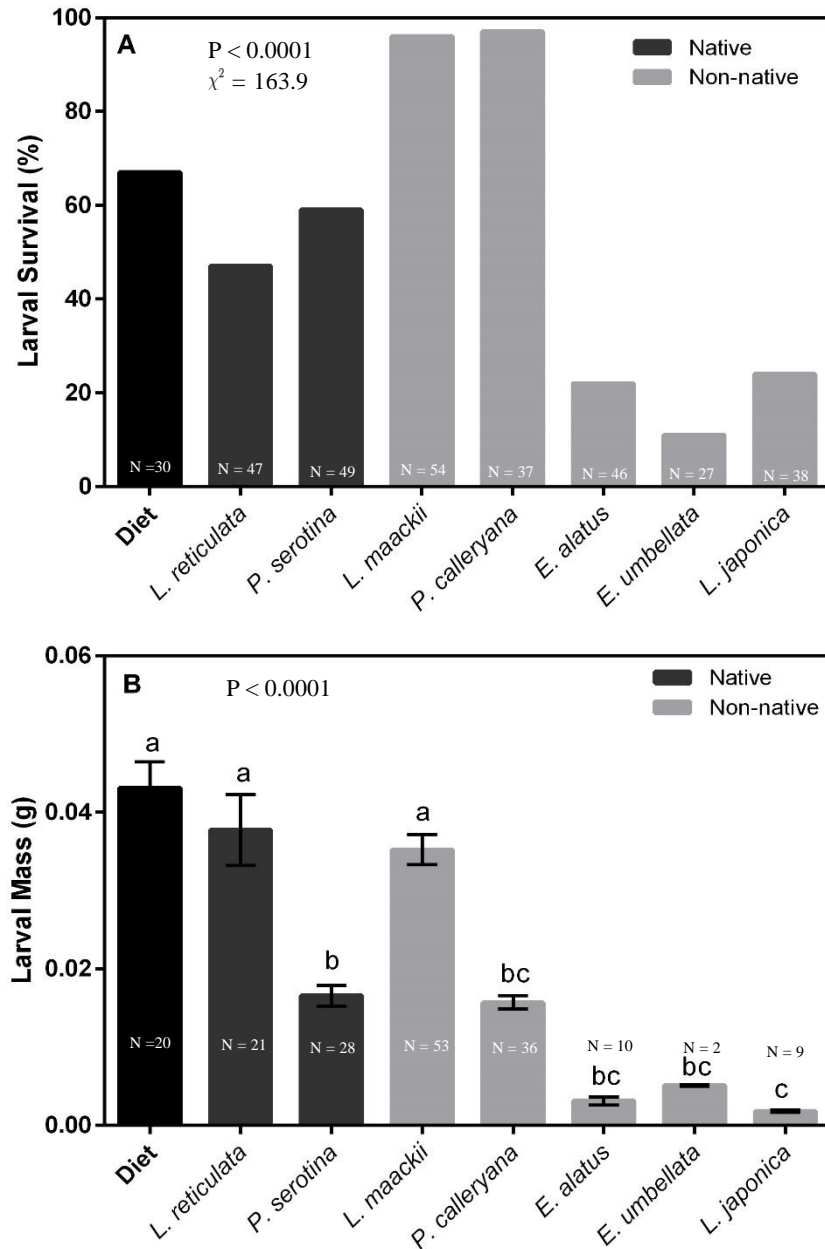
Figure 4.2   Larval survivorship (A) and larval mass (B) of the generalist *H. cunea* feeding in groups for four weeks in a no-choice bioassay on artificial diet, native (*L. reticulata* and *P. serotina*), or non-native (*L. maackii, P. calleryana, E. alatus, E. umbellata,* and *L. japonica*) species. Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05).
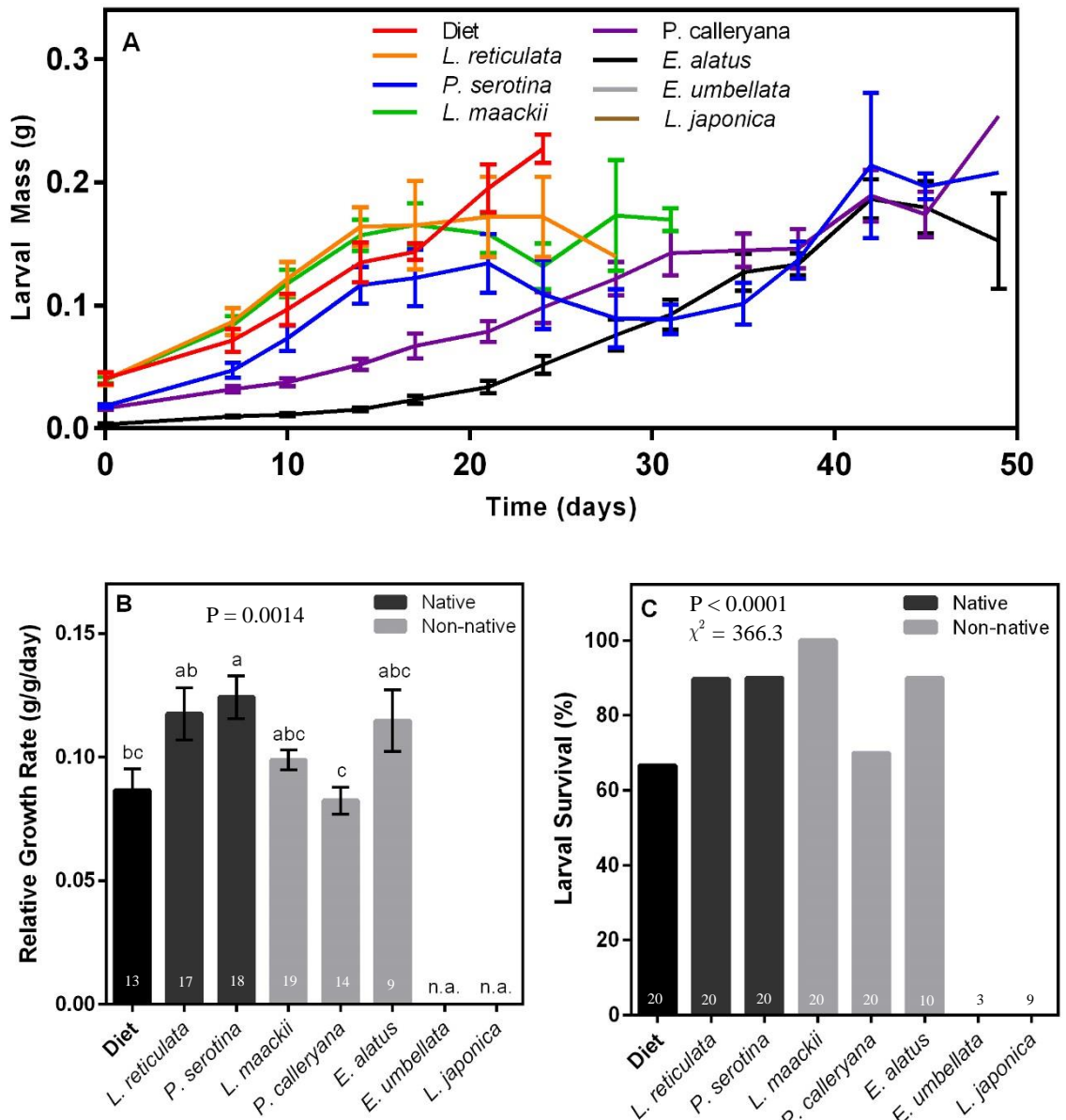
Figure 4.3   Mean larval mass of surviving *H. cunea* feeding on an artificial diet, and the foliage of two native and five non-native species for 49 days (A), relative growth rate (RGR) (B), and larval survival (C). RGR was calculated on day 14, before any larvae pupated. Species not included in the statistical analysis are denoted as "n.a.". Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05). Numbers in the bars represent sample size.
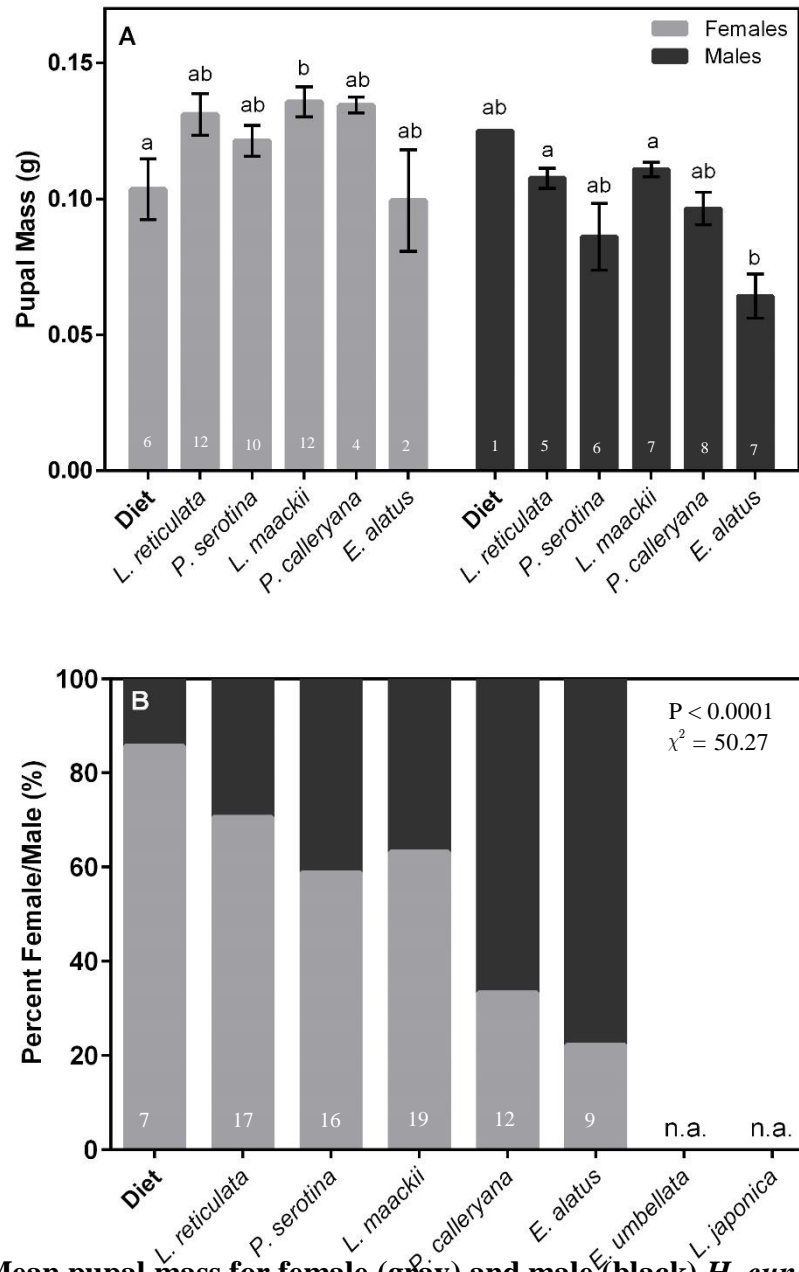
Figure 4.4 Mean pupal mass for female (gray) and male (black) *H. cunea*
caterpillars feeding on an artificial diet, and foliage of two native and five non-
native species (A), and ratio of female and male pupae (B). Species not included in
the statistical analysis are denoted as "n.a.". Letters represent a difference in means
determined through Tukey post hoc tests (P < 0.05). Numbers in the bars represent
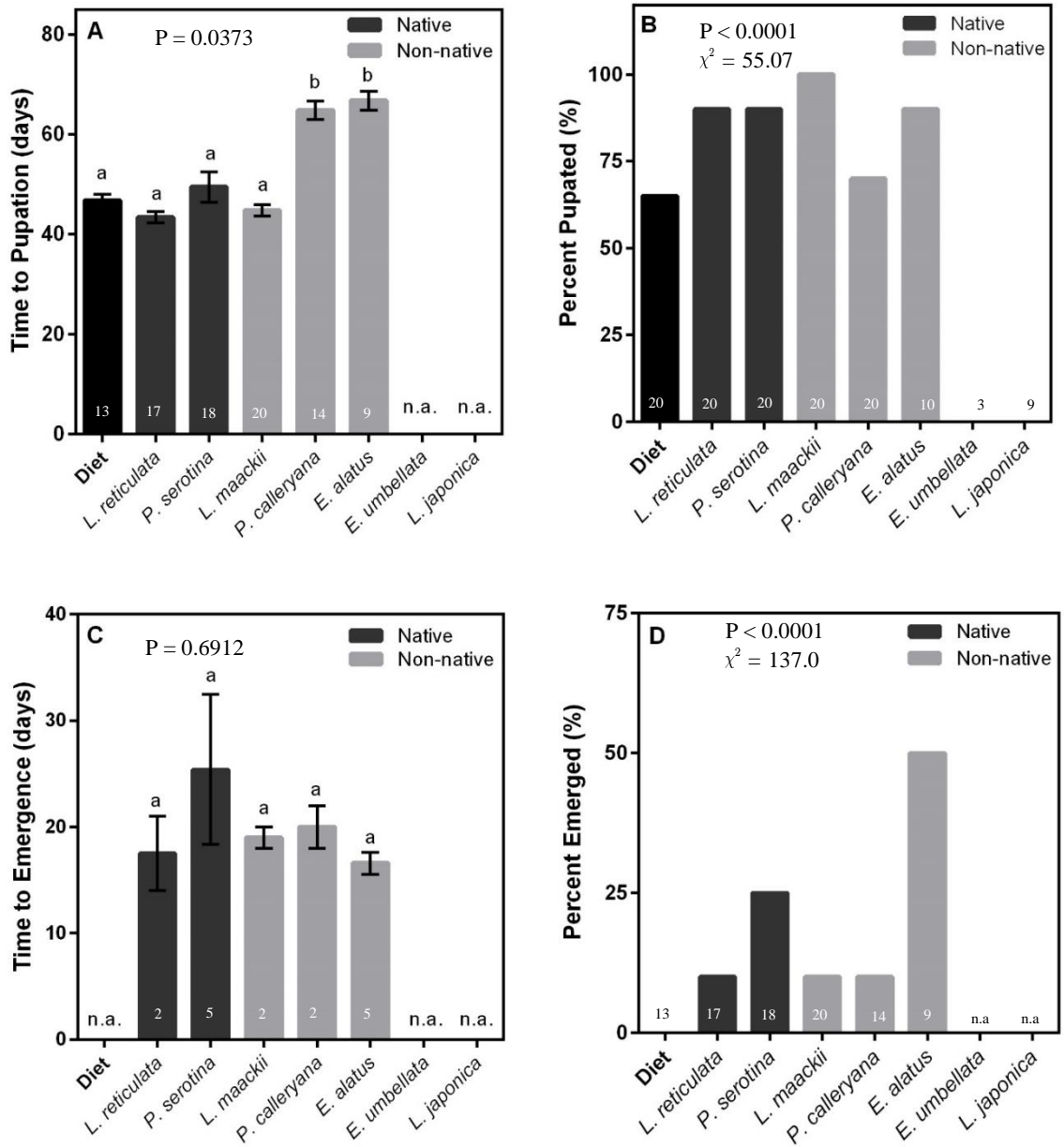**sample size.**

Figure 4.5 Mean days for *H. cunea* caterpillars to reach pupation feeding on a artificial diet, and foliage of two native and five non-native species (A), percentage reaching pupation (B), days to emergence (C), and percentage emerged (D). Species not included in the statistical analysis are denoted as "n.a.". Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05). Numbers in the bars represent sample size.
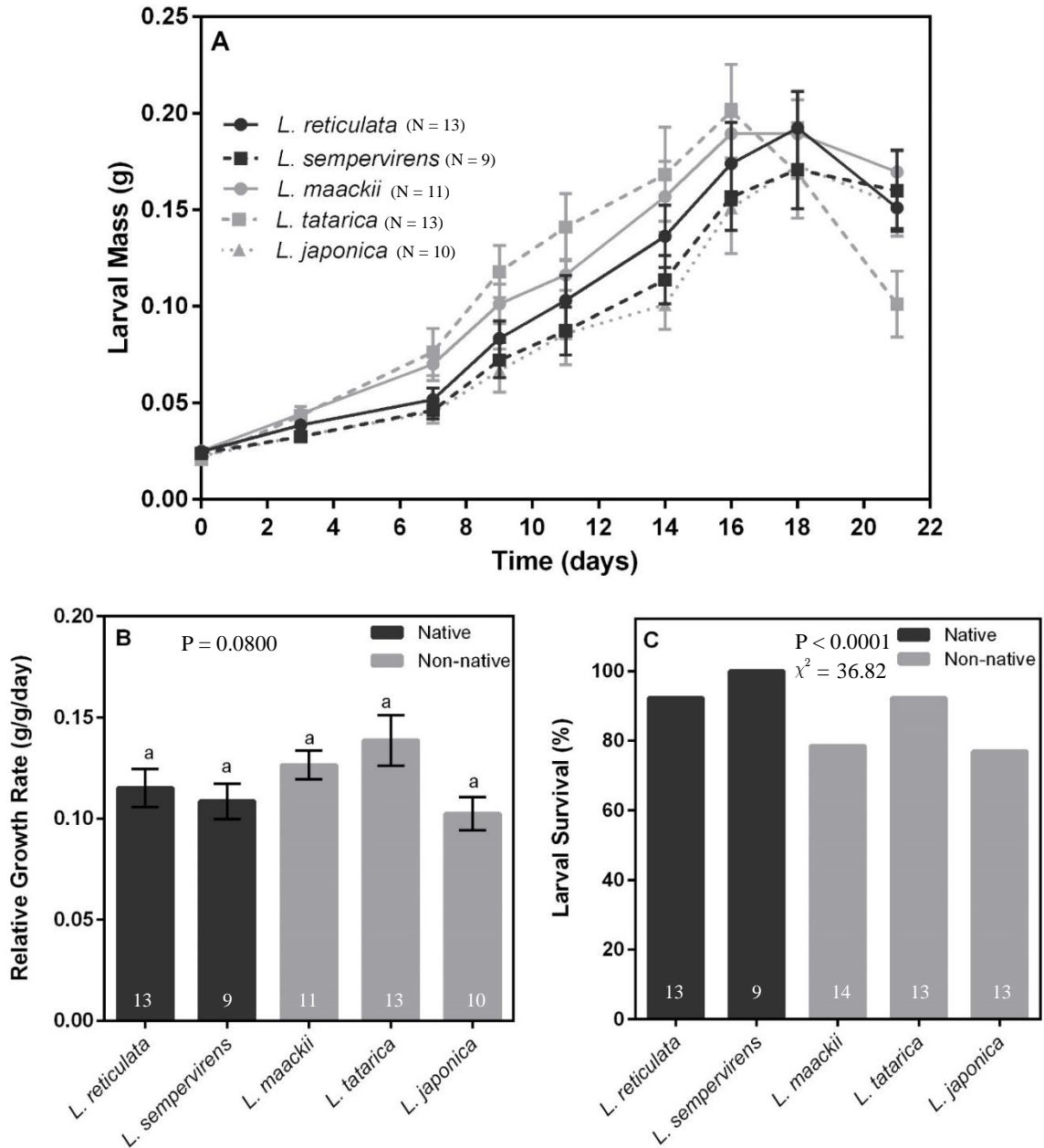
Figure 4.6 Mean larval mass of surviving *H. cunea* feeding on foliage of two native and three non-native *Lonicera* species (A), relative growth rate (RGR) (B), and final larval survival (C). RGR was calculated on day 14, before any larvae pupated. Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05). Numbers in the bars represent sample size.
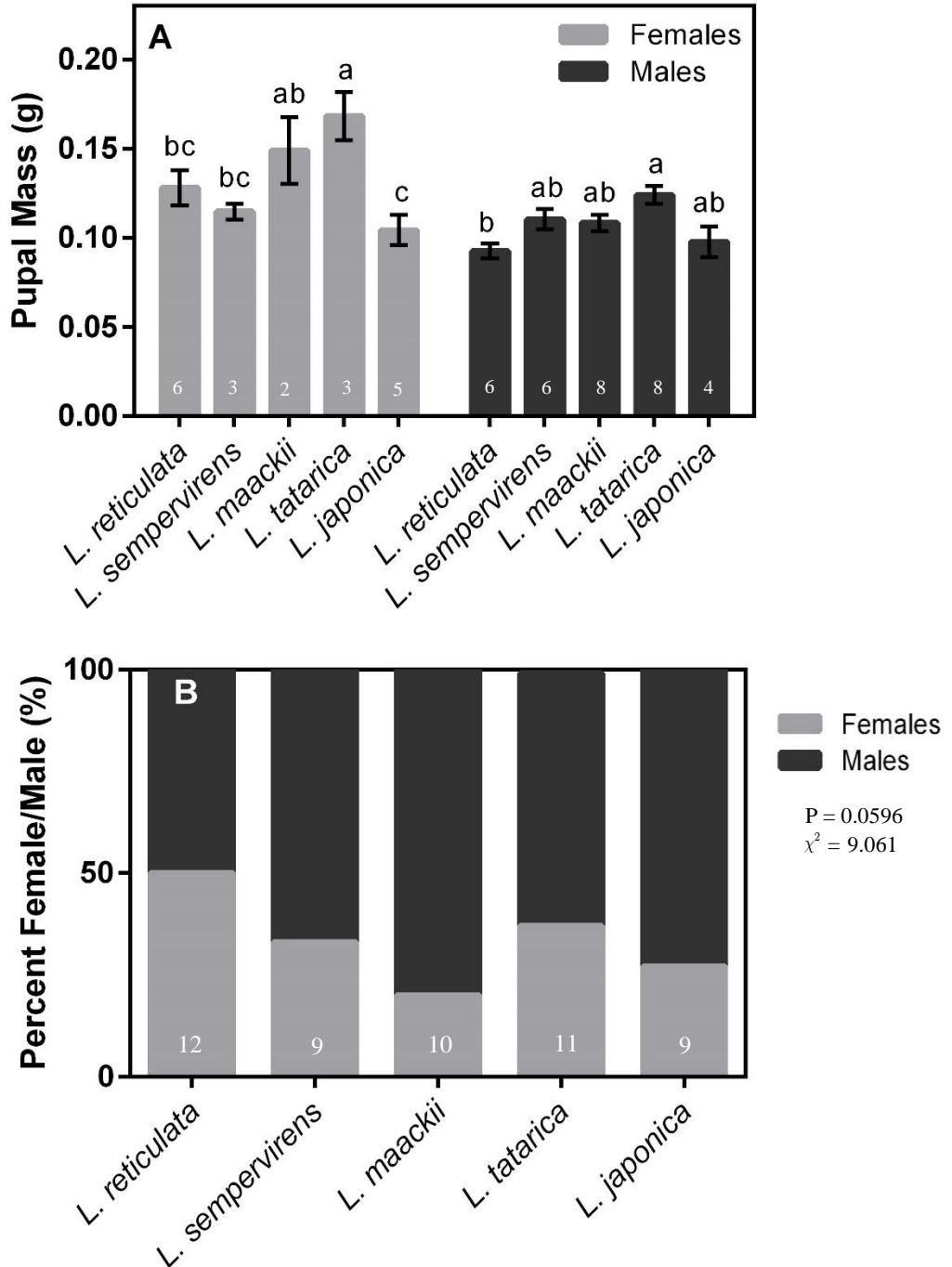
Figure 4.7 Mean pupal mass for female (gray) and male (black) *H. cunea*

caterpillars feeding on foliage of two native and three non-native *Lonicera* **species**

(A), and ratio of female and male pupae (B). Letters represent a difference in means

determined through Tukey post hoc tests (P < 0.05). Numbers in the bars represent
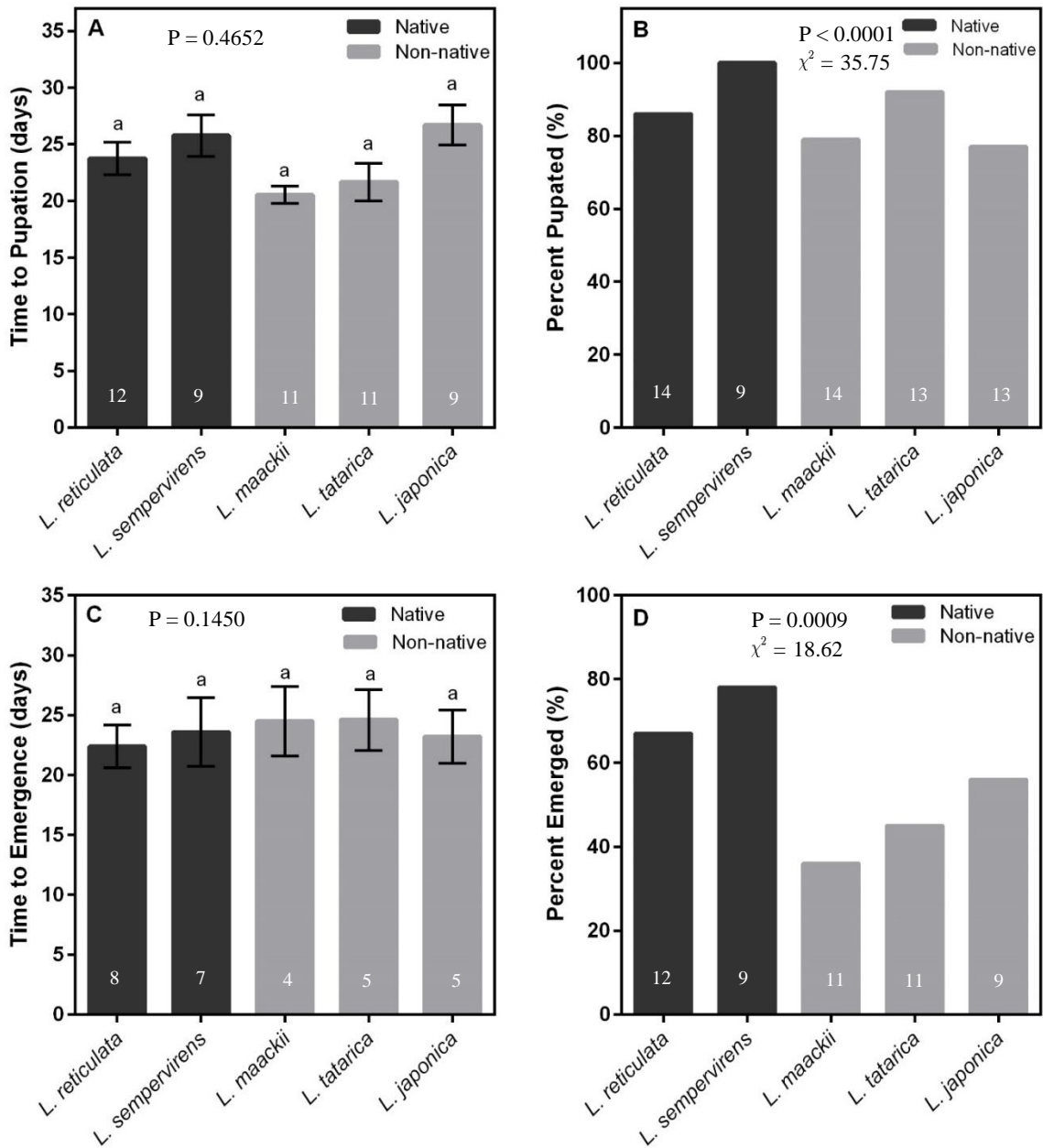
sample size.

Figure 4.8 Mean days for *H. cunea* caterpillars to reach pupation feeding on foliage of two native and three non-native *Lonicera* species (A), percentage reaching pupation (B), days to emergence after a cold period of 4 °C for 8 months (C), and percentage emerged (D). Letters represent a difference in means determined through Tukey post hoc tests (P < 0.05). Numbers in the bars represent sample size.
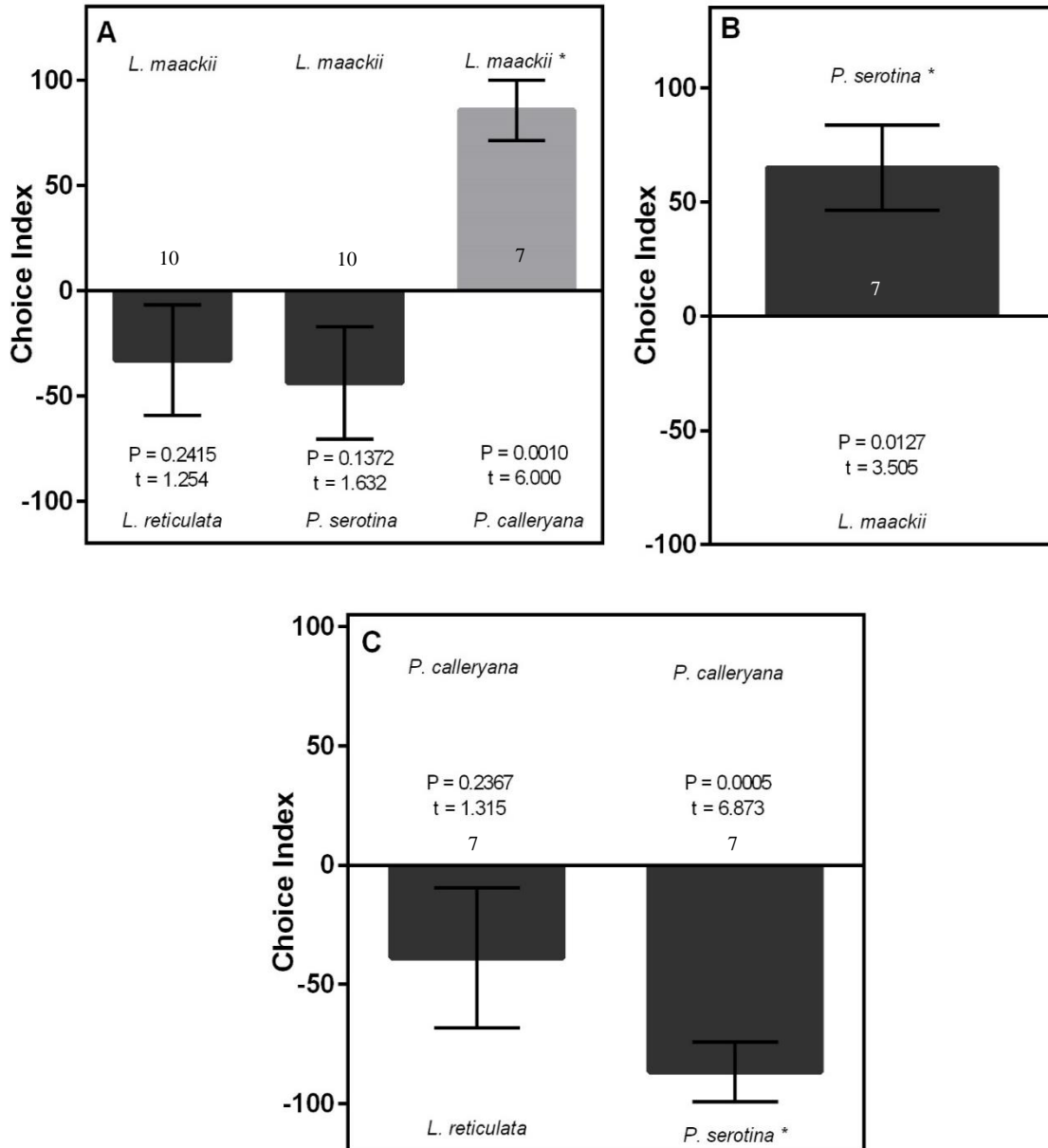
Figure 4.9   Mean larval choice preference of the generalist *H. cunea* when reared on (A) *L. maackii,* (B) *P. serotina,* and (C) *P. calleryana* for 27 days and then given native and non-native host choices. The choice index was calculated as (Leaf area removed Choice 1 - Leaf area removed choice 2/ Total leaf area removed) x 100. The mean choice index is shown above. One sampled T-tests were performed to see if choice index differed from zero (no preference). * = significant preference. Numbers in the bars represent sample size.
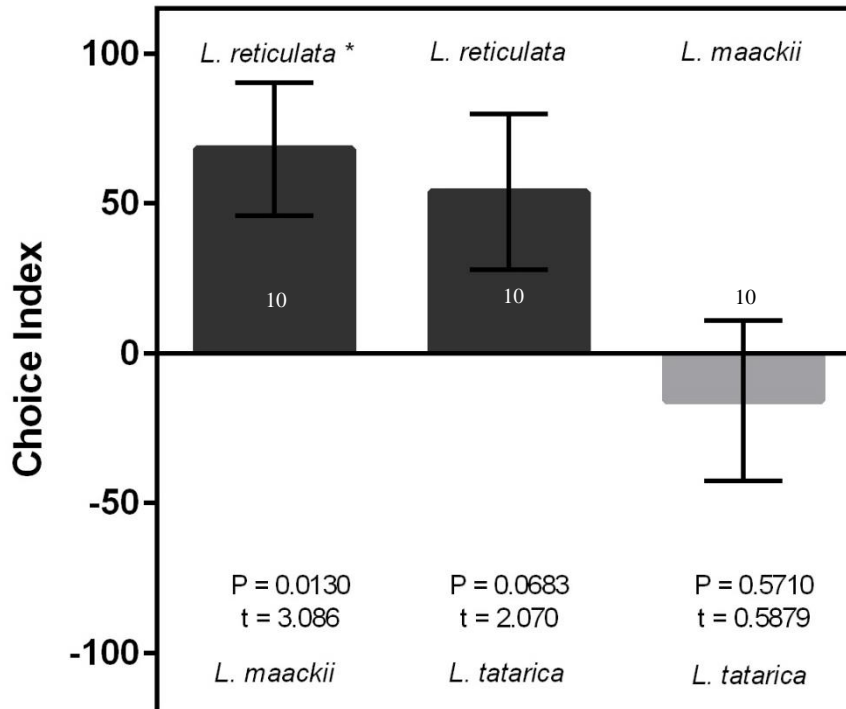
Figure 4.10   Mean larval choice preference of the generalist *H. cunea* when given a choice between *L. reticulata* and *L. maackii*, *L. reticulata* and *L. tatarica*, and *L. maackii* and *L. tatarica*. *Hyphantria cunea* caterpillars were reared on black cherry for approximately 4 weeks before the choice trials were run in 2014. The choice index was calculated as (Leaf area removed Choice 1 - Leaf area removed choice 2/ Total leaf area removed) x 100. The mean choice index is shown above. One sampled T-tests were performed to see if choice index differed from zero (no preference). * = significant preference. Numbers in the bars represent sample size.

5. CONCLUSIONS, FUTURE DIRECTIONS, AND APPLICATIONS

In this study, I found that the specialist sawfly *A. americana* can lay eggs and the eggs can successfully develop in native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. japonica*) foliage. Newly emerged *A. americana* and *Z. montana* larvae can reach pre-pupation on native *L. reticulata,* native *S. albus,* and non-native *L. maackii*, but have 100% larval mortality on *L. japonica*. When *Z. montana* larvae were switched onto non-native *Lonicera* species after 2 weeks, larvae reached pre-pupation on native (*S. albus* and *L. reticulata*) and non-native (*L. maackii* and *L. tatarica*) foliage. *Abia inflata* and *Z. montana* larvae performed equally well on undamaged and artificially damaged foliage. However, *A. inflata* had a reduction in larval mass on herbivore damaged foliage, whereas, *Z. montana* larvae had no reduction in mass on herbivore damaged foliage.

The generalist *H. cunea* caterpillars reared from eggs on native and non-native host had a wide range of performance. Some non-native species (*L. maackii*, *L. tatarica* and *P. calleryana*) appear to be suitable host for *H. cunea,* whereas other non-native species (*L. japonica, E. alatus,* and *E. umbellata*) are unsuitable hosts for early larval development. When four week old *H. cunea* caterpillars are switched onto native and non-native *Lonicera* species caterpillars could reach pupation, even on *L. japonica.* When *H. cunea* are given a choice between native and non-native foliage, caterpillars preferred native foliage. However, when given a choice between two non-native species,

caterpillars had no preference when the non-native species were closely related, and strongly preferred *L. maackii* over *P. calleryana.*

Overall, both specialist and generalist herbivores can perform equally well on most native hosts and some non-native invasive plants. All species in this study can perform well and complete their whole life cycle on *L. maackii.* This was surprising to find, because in the field, *L. maackii* has relatively low herbivory rates. For specialists, this low herbivory suggests a neutral interaction in which *A. americana, A. inflata,* and *Z. montana* do not use or do not recognize *L. maackii* as a suitable, novel host. If no native foliage is present, the specialist *A. americana* will lay eggs in *L. maackii* and *L. japonica.* On the other hand, the generalist *H. cunea* appears to have a positive interaction with *L. maackii*, because adults will oviposit on *L. maackii* in the field and the larvae can perform well on this novel host. Other novel hosts, especially *L. japonica*, are not suitable hosts for the specialist or generalist herbivores in this study. If adults preferentially oviposit on *L. japonica*, this could lead to population declines of some our specialist and generalist herbivores. More oviposition studies are needed to fully investigate the interactions of these specialist and generalist herbivores with these novel host and additional non-native hosts. Also, choice oviposition studies would help determine if adults prefer to oviposit in native or non-native foliage.

As *L. maackii* continues to spread and outcompete the preferred, native hosts of these specialists and generalist, they may be forced to interact with this novel host. Adults that recognize *L. maackii* as a suitable hosts will have reduced searching time to find an oviposition site due to the high abundance of this non-native plant. This reduced searching time will increase adult survivorship and potential reproductive fitness. From

our studies, *A. americana, A. inflata, Z. montana,* and *H. cunea* larvae can perform just as well on *L. maackii* as their preferred native hosts. Non-native *L. maackii* is very abundant compared to native hosts, which would provide an ample food supply to herbivores that can recognize it as a host. *Hyphantria cunea* adults are already starting to recognize *L. maackii* and other non-native species as suitable host due to selection pressure on adults to quickly find oviposition sites. If selection pressure to find an oviposition site is high enough due to a reduction in native host populations, then adult *A. americana, A. inflata,* and *Z. montana* may begin to evolve mechanisms to fully utilize and recognize novel hosts, especially *L. maackii*. The specialists *A. americana, A. inflata, Z. montana* along with the generalist *H. cunea* could potentially become native herbivores that can help keep *L. maackii* populations "in-check". Futures studies determining factors that influence host choices of adults and larvae could benefit efforts at using these native insects as biocontrol agents for *L. maackii* or other non-native, invaders.