Cascading Ecological Impacts of Emerald Ash Borer: Tritrophic Interactions Between Prickly Ash, Giant Swallowtail Butterfly Larvae, and Larval Predators

Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Kevin Barry Rice, M.S.

Graduate Program in Entomology

The Ohio State University

2013

Dissertation Committee:

Daniel A. Herms, Advisor

John Cardina

Charles Goebel

P. Larry Phelan
Copyright by

Kevin Barry Rice

2013
Abstract

The introduction of emerald ash borer, *Agrilus planipennis* (EAB), into forests of Midwestern US and Southeastern Ontario forests has resulted in the death of millions of ash trees, *Fraxinus* spp. Large-scale canopy gaps created by ash mortality increases light reaching understory plants. The native understory shrub prickly ash, *Zanthoxylum americanum*, contains furanocoumarins, highly biocidal secondary metabolites, that deter most insect herbivores. Furanocoumarins are photoactivated, becoming more toxic when exposed to UV light (Berenbaum 1978). Furthermore, furanocoumarin biosynthesis is energy intensive, and their concentration increases when photosynthesis is enhanced by increased light availability. Theory predicts that female plants invest more resources in defense, while males allocate more towards growth. Therefore, male and female prickly ash growing in canopy gaps may differ in their furanocoumarin concentrations, growth rates, and reproductive effort.

Giant swallowtail butterfly larvae, *Papilio cresphontes*, are specialist herbivores on prickly ash capable of detoxifying furanocoumarins. Energetic costs of furanocoumarin detoxification can slow larval development, and thus increase exposure to natural enemies. In a series of field and lab experiments, we examined the effects of EAB-induced canopy gaps on resource allocation of male and female prickly ash, and growth and survival of giant swallowtail larvae.
Prickly ash growing in canopy gaps had lower specific leaf area (SLA) compared to shaded plants. Male plants grew more in sun compared to males in shade. However, females located in sun grew less than females in shade. Females produced more flowers than males, and canopy gaps increased total flower and fruit production. Therefore, male plants invested proportionally more resources towards growth, whereas females invested more in reproduction. Canopy gaps significantly affected physical defenses of prickly ash. Thorn length, thorn area, and width of thorns increased in gaps, and females produced higher thorns than males, but these patterns varied by year.

We quantified furanocoumarins, total phenolics, and nitrogen concentration in sun and shaded foliage of male and female prickly ash using high performance liquid chromatography. Foliar total furanocoumarin concentrations were higher in sun plants than shade. Leaf nitrogen concentration was greater in male plants. In addition, we examined the effects of prickly ash foliage collected from understory and canopy gaps on the development of *P. cresphontes* larvae. Larvae that consumed foliage from plants growing in sun had lower relative growth rates compared to larvae feeding on foliage from shaded plants. Our results suggest *P. cresphontes* larval development is negatively affected by widespread canopy gap formation. There was no difference in survival of larvae placed on plants growing in sun or shade, with mortality over 48 hours close to 70% in both habitats. We predict, however, that giant swallowtail larval survival will be lower in EAB infested forests because gaps increase the toxicity of prickly ash resulting in decreased growth rates that will increase exposure time of larvae to natural enemies.
This study represents an example of one of many indirect ecological impacts of the emerald ash borer induced ash mortality likely to cascade through invaded forests.
Acknowledgements

I thank my advisor Dan Herms for continual support and guidance. He encouraged me to “think big” and focus on questions I wanted to answer when writing my proposal and designing experiments. I also thank my committee members John Cardina, Charles Goebel, Larry Phelan and Mark Scriber for suggestions, comments, and guidance. I thank the members of the Herms and Cardina labs (Cathy Herms, Diane Hartzler, Bryant Chamber, Wendy Klooster, Larry Long, Kayla Perry, Vanessa Muilenburg, Bill Barrington, Sarah Rudawsky) for their support and help with data collection. I like to thank Priya Rajarapu for helping with lab work. Thank you Bruce Birr for analysis of C:N. I thank Vic Mastro, John Lelito, Dave Denlinger, and Mike Rietz for lab space while conducting feeding bioassays. I thank Peter Curtis for Li-Cor training. I thank Mary Gardiner and Kathleen Knight for guidance. I thank Lori Jones for her continual administrative help. Finally, I thank my wife Cara, who supported my dream and provided endless encouragement.
Vita

2004................................. B.S. Biology, University of North Carolina at Asheville

2007................................. M.S. Entomology, Auburn University

2007 to 2008......................... Area Extension Agent

University of Arizona

2008 to 2009......................... Graduate Teaching Associate,

Department of Entomology,

The Ohio State University

2008 to 2013......................... Graduate Research Associate,

Department of Entomology,

The Ohio State University

Publications


Field of Study

Major Field: Entomology
Table of Contents

Abstract .......................................................................................................................... ii
Acknowledgments........................................................................................................... v
Vita.................................................................................................................................. vi
Table of Contents ......................................................................................................... vii
List of Tables .................................................................................................................. viii
List of Figures ............................................................................................................... ix

Chapter 1: Direct and indirect effects of invasive species on native ecosystems ......................... 1
  Introduction .................................................................................................................. 1
  Direct effects of invasive arthropods .......................................................................... 1
  Indirect effects of invasive arthropods ..................................................................... 5
  Ecology and biology of prickly ash ......................................................................... 7
  Ecology and biology of giant swallowtail butterflies .............................................. 10
  Introduction to plant defense theory ....................................................................... 11
  Hypotheses and objectives ..................................................................................... 13
  References ............................................................................................................... 15

Chapter 2: Differential allocation tradeoffs in male and female prickly ash growing in canopy gaps and the understory .......................................................... 22
  Abstract .................................................................................................................... 22
  Introduction ............................................................................................................. 24
  Methods .................................................................................................................... 26
  Results ..................................................................................................................... 32
  Discussion ............................................................................................................... 57
  References ............................................................................................................... 86

Chapter 3: Indirect effects of an alien insect herbivore on native plant photosynthesis, gas exchange, and water use efficiency ........................................... 99
  Abstract ................................................................................................................. 99
  Introduction .......................................................................................................... 101
  Methods ................................................................................................................. 103
  Results ................................................................................................................... 104
  Discussion ............................................................................................................. 108
  References ............................................................................................................. 111

Chapter 4: Summary and suggestions for future work ......................................................... 114
  References ............................................................................................................. 121

Literature Cited ....................................................................................................... 124
List of Tables

Table 1.1. Herbivores of prickly ash…………………………………………………….. 19

Table 2.1. Growth of male and female prickly ash growing in sun and shade by year………………………………………………………………………………….. 39

Table 2.2. Effect of sun and shade on thorn length, base length, and thorn area in 2010 and 2011……………………………………………………………………… 47

Table 2.3. Effect of plants sex on thorn length, base length, and thorn area in 2010 and 2011…………………………………………………………………………… 47

Table 2.4. Identification of furanocoumarins including m/z and retention time (RT) in prickly ash foliage…………………………………………………………… 49
List of Figures

Figure 2.1. Size of canopy gaps above prickly ash plants growing in sun and shade treatments in A 2010 and B 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$)................................................................. 33

Figure 2.2. Specific leaf area of prickly ash plants growing in sun and shade in A 2010 and B 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).................................................................................................. 35

Figure 2.3. Comparison of male and female prickly ash in sun and shade during 2010......................................................................................................................... 37

Figure 2.4. Growth of male A and female B prickly ash in sun and shade during 2010. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).................................................................................................................. 38

Figure 2.5. Comparison of male and female prickly ash growth in sun and shade during 2011......................................................................................................................... 39

Figure 2.6. Growth of male A and female B prickly ash in sun and shade during 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).................................................................................................................. 40

Figure 2.7. Female prickly fruit production in sun and shade in 2010. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).................................................................................................................. 42

Figure 2.8. Female prickly fruit production in sun and shade in 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).................................................................................................................. 42

Figure 2.9. Comparison of male and female prickly ash flower production in 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).................................................................................................................. 43
Figure 2.10. Comparison of prickly ash flower production in sun and shade in 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$). ................................................................. 44

Figure 2.11. Comparison of prickly ash thorn density on plants in sun and shade in 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$). ................................................................. 48

Figure 2.12. Total foliar furanocoumarin concentrations from prickly ash growing in sun and shade. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$). ......................................................................... 50

Figure 2.13. Total foliar phenolics concentration from prickly ash growing in sun and shade. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$). ......................................................................... 51

Figure 2.14. Comparison of foliar nitrogen concentration between male and female prickly ash. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$). ......................................................................... 52

Figure 2.15. Comparison of relative growth rates of fourth instar larvae consuming sunny and shaded prickly ash foliage in A 2010 and B 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$). ............................................. 54

Figure 2.16. Comparison of relative growth rates of first instar larvae consuming A sunny and shaded prickly ash foliage and B male and female foliage. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$). ....................... 55

Figure 2.17. Giant swallowtail mortality on prickly ash growing in sun and shade. Standard errors are depicted........................................................................... 56

Figure 2.18. Conceptual model showing cascading direct and indirect effects of EAB on prickly ash, giant swallowtail butterfly larvae, and larvae predators. Solid lines represent direct effects and dashed lines represent indirect effects. Positive effects are indicated with (+) and negative effects are shown as (-). ........................................... 58

Figure 3.1. Percent canopy gap was greater in gap treatments compared to control treatment. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$). ........................................................................... 105

Figure 3.2. Prickly ash plants growing in canopy gaps had greater maximum photosynthesis than plants in the shade. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$). ........................................................................... 106
Figure 3.3. Canopy gaps increase prickly ash stomatal conductance. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$)........ 107

Figure 3.4. Shaded prickly ash had greater instantaneous water use efficiency than plants in canopy gaps. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$)................................................................. 108
Chapter 1

Direct and Indirect Effects of Invasive Species on Native Ecosystems

Introduction

Introductions of non-native species have accelerated recently due to increased human travel and trade (Di Castri 1989, Liebhold and Tobin 2008). Although most non-natives fail to establish and spread (Williamson and Fitter 1996), some establish populations, expand their geographic range, resulting in ecological and environmental impacts, and thus are defined as invasive (Richardson et al. 2000). The direct impacts of invasive species are well documented. Invasive species are the second greatest threat to biodiversity following habitat loss (Wilcove et al. 1998), and are the leading cause native species decline in forest systems (Vitousek 1997, Lovett et al. 2006). Surprisingly few studies, however, have quantified the potential indirect effects invasive species have on native ecosystems.

Direct Effects

Invasive predators can have rapid direct effects on native species. For example, the invasive ladybird beetle, *Harmonia axyridis*, arrived in Britain and Belgium in the early 2000’s. Within five years native ladybird beetle species richness had declined by 30%
and 44% in Belgium and Britain, respectively (Roy et al. 2012). Several recent studies suggest that *H. axyridis* preys on native ladybeetle eggs, thereby directly reducing their abundance (Gardiner et al. 2011, Katsanis et al. 2013).

Invasive generalist predators can directly impact entire communities. The brown tree snake, *Bogiga irregulari*, was unintentionally introduced to the forested island of Guam in the 1940’s (Lockwood et al. 2007). Guam has no native snakes and its native species lack a coevolutionary history with snakes, thus lack antipredator snake adaptations. The brown tree snake is responsible for driving 10 out of 13 of Guam’s forest birds species to extinction (Fritts and Rodda 1998). Invasive predators can affect native predators through competition for resources and interguild predation. The invasive red imported fire ant, *Solenopsis invicta*, is a generalist predator responsible for the decline in native ant abundance in the United States. In areas where invasive fire ants have established, native ant species richness has declined by 50% (Porter and Savignano 1990). Invasive ants directly impact and displace native ants through aggressive interguild predation (Holway and Suarez 1999). Invasive ant predation has direct negative effects on native arthropods, birds, reptiles and mammals (Allen et al. 2000, Allen et al. 2001, Eubanks et al. 2002, Morrison 2002, Orrock and Danielson 2004).

Invasive herbivores can have intense direct effects on native ecosystems. At least 400 insect herbivores have invaded North American forests (Mattson et al. 2007) and several have resulted in intense ecological damage (Gandhi and Herms 2010a). Hemlock woolly adelgid, *Adelges tsugae*, (HWA) is an invasive insect unintentionally introduced to Western North America from Asia during the early 20th century. In its native range
HWA rarely results in tree mortality (McClure and Cheah 1999). HWA was discovered in the eastern United States in the 1950’s on susceptible eastern hemlock, *Tsuga canadensis*, and is responsible for wide spread hemlock decline in the eastern United States (McClure 1989). Tree mortality can occur within 4 years after infestation (McClure 1991, Cobb et al. 2006). Eastern hemlock composes up to 55% of coniferous forests volume in the Northeastern United States and HWA has the potential to extirpate the entire species of eastern hemlock from the North American continent (Orwig and Foster 1998, Smith and Sheffield 2000).

Gypsy moth, *Lymantria dispar*, was accidently released in Massachusetts in 1869 (Liebhold et al. 1989) and currently occupies most of northeastern North America (Liebhold et al. 1992). Gypsy moths have defoliated more than 34 million hectares of North American forests in the last 90 years. The European elm bark beetle, *Scolytus multistriatus*, was accidently introduced to North America from Europe in the 1920’s (May 1934) and with its associated fungal symbiont is responsible for complete removal of the dominant American elm, *Ulmus Americana*, tree from forest canopies across North America (Barnes 1976).

Asian longhorned beetle, *Anoplophora glabripennis* (ALB), is one of the world’s most notorious invasive species because it is difficult to detect, is polyphagous, and has the potential to cause massive ecologic and economic damage (Simberloff and Rejmánek 2011). ALB is native to Asia and has been detected in five US states and one Canadian province (Haack et al. 2010). ALB colonizes at least 13 genera of North American trees (e.g. *Acer, Aesculus, Salix, Plantanus, Ulmus, Betula, and Populus*) and most of eastern
North American forests are extremely vulnerable to ALB (Townsend Peterson and Scachetti-Pereira 2004, Hu et al. 2009). Intensive eradication programs have been implemented in areas where ALB has been detected. However, if ALB manages to escape control efforts, the impact on North American forests will be devastating.

Emerald ash borer, *Agrilus planipennis* (EAB), is a recently discovered invasive wood borer. EAB was unintentionally introduced to the midwestern United States from Asia during the 1990’s, although only discovered in 2002 (Haack et al. 2002, Cappaert et al. 2005). As of April 2013, EAB had been detected in 20 US states and 2 Canadian provinces (http://www.emeraldashborer.info). EAB larvae voraciously attack ash trees, *Fraxinus spp.*, feeding on the vascular phloem, eventually girdling the tree resulting in tree mortality. Recent estimates suggest that over 20 million ash trees have died as a result of EAB in Michigan alone and up to eight billion trees are at risk (Poland 2007, Lindell et al. 2008). All North American ash trees that EAB has encountered are extremely susceptible and EAB is predicted to functionally extirpate the entire *Fraxinus* genus from the North American continent (Gandhi and Herms 2010b).

Gandhi and Herms (2010b) report there are at least 282 arthropods that utilize North American ash as a food source or shelter and 43 native arthropods that feed exclusively on ash. EAB can have a direct negative effect on these arthropods. These species are at high risk of extinction as EAB threatens to eliminate their entire food source.

Invasive herbivores continue to directly impact North American forested landscapes. Direct effects such as defoliation and tree mortality alter ecosystem
processes (Liebhold et al. 1995). Furthermore herbivores have the potential to alter successional trajectories (Connell and Slatyer 1977). Therefore, the direct impacts associated with invasive species will undoubtedly cascade throughout the ecosystems and indirectly affect multiple processes and interactions.

**Indirect Effects**

Indirect effects can be more complex and less obvious than direct impacts and therefore harder to quantify (Parker et al. 1999). Until recently, relatively few studies have attempted to quantify indirect effects of invasive species. Indirect effects of previous North American forest invasions such as Dutch elm disease and chestnut blight were largely ignored. Invasive organisms can indirectly impact native species by changing forest structure, altering nutrient cycling, disrupting native foraging behavior, diluting genotypes by hybridizations, interfering with biotic interactions such as mutualisms, and facilitating the establishment and spread of other invasive species (Krueger and May 1991, Orwig and Foster 1998, DiGregorio et al. 1999, Scriber 2004, Traveset and Richardson 2006, Lockwood et al. 2007, Orwig et al. 2008, Dodds et al. 2010, Gandhi and Herms 2010a, b, Mattos and Orrock 2010). Indirect effects can cascade throughout the ecosystem, affecting multiple trophic levels in unpredictable ways.

For example, the yellow crazy ant, *Anoplolepis gracilipes*, was unintentionally introduced to Christmas Island 80 years ago. Ants have a direct negative impact on the native red crabs, because ants attack and consume crabs (O'Dowd et al. 2003). Native
crabs are voracious omnivore on the forest floor, and its feeding reduces understory plant abundance. The absence of the green crab due to ant predation has lead to accelerated forest succession. Seedling establishment increased by 300% in ant invaded habitats. Changes in forest structure have resulted in changed nutrient cycling, decomposition, community diversity and reduced the islands biotic resistance resulting in increased invasive species establishment (O’Dowd et al. 2003). EAB is likely to exert numerous indirect effects on native species. Twenty-six different North American forest types are composed of ash trees and ash is a dominant tree in some eastern North American forests (Burns and Honkala 1990, Rumble and Gobeille 1998). EAB may increase competition for resources on arthropods that utilize ash as an alternate host plant. Gandhi and Herms (2010b) noted 30 arthropods in North America use *Fraxinus* as a secondary or occasional food source. As ash trees die, these arthropods will be forced to remain on alternative host plants. Arthropods that use these plants as their primary host may experience increased crowding and resource competition

Redman and Scriber (2000) observed indirect negative effects of invasive gypsy moths on native tiger swallowtail butterflies, *Papilio glaucus*. Tiger swallowtail and gypsy moth share a common host plant, quacking aspen (Redman and Scriber 2000). Tiger swallowtail larvae consuming foliage that was previously fed on by gypsy moths experienced reduced growth rates, most likely due to changes in host plant chemistry. In field experiments tiger swallowtails experienced apparent competition (increased parasitism) when sharing a host plant with gypsy moths. Classical biological control purposefully introduces natural enemies in order to manage invasive species populations.
Biological control agents can be classified as invasive species because they establish populations, spread, and often have non-target indirect effects (Parry 2009, Gandhi and Herms 2010a). Hawkins and Marino (1997) reported 51 species of parasitoids released for classical biological control in North America attacking native arthropods.

Ecology and Biology of Prickly ash

Prickly ash, *Zanthoxylum americanum*, is a dioecious native North American citrus shrub (Family Rutacea). Prickly ash is reported to occur in 36 US states and two Canadian provinces (USDA 2013), although due to habitat destruction prickly ash is listed as an endangered species in Florida (Coile and Garland 1998). Prickly ash can be a dominant shrub in the forest landscape (Henderson et al. 1985, Barnes and Dibble 1988, Munger and Karasov 1994, Knutson and Klass 1997, Harrington and Kathol 2009), and it commonly grows along riversides, rocky uplands, swamps, and opened meadows often forming dense fencelike thickets (Henderson et al. 1985, Reinartz and Popp 1987). Prickly ash is a long lived plant that can persist for several decades (Barnes and Dibble 1988). For example, prickly ash planted in a botanical garden in 1773 was still present 192 years later (Belden 1965). Individual shrubs live for over 30 years (Reinartz and Popp 1987) but the clonal sprouts persist. Prickly ash is fast growing and outcompetes native trees (Barnes and Dibble 1988).

Prickly ash foliage contains highly biocidal defensive compounds, furanocoumarins. Furanocoumarins are photoactivated and increase in concentration
when stimulated by UV radiation (Berenbaum 1978, Murray et al. 1982).

Furanocoumarins are photogenotoxins that form crosslinks with DNA and interfere with DNA replication (Joshi and Pathak 1983, Arnason et al. 1992, Lee and Berenbaum 1993, Bissonnette et al. 2008). Furanocoumarins are also photosensitizers that can react with oxygen to form reactive singlet oxygen (Lee and Berenbaum 1992, Berenbaum et al. 1995). UV light can result in an excited furanocoumarin that accepts a photon (Arnason et al. 1992). The excited furanocoumarin donates an electron to ground state oxygen thereby creating a singlet oxygen (Larson 1986).


In addition to toxic leaves, prickly ash contains physical defenses (thorns) that further deter herbivory. Although prickly ash is widely distributed and is often a dominant shrub, few herbivores consume the foliage due to the furanocoumarins and physical defenses. Several studies have reported the complete absence of mammalian browsing on prickly ash (Barnes and Dibble 1988, van der Hoek et al. 2002).

A few insect herbivores have evolved specialized furanocoumarin detoxification enzymes (P450’s) that allow them to consume plants containing
furanocoumarins (Berenbaum 1983, Berenbaum et al. 1992). P450 detoxification of furanocoumarins begins by opening the furan ring, making the compound more polar, thus more hyrophillic and easier to expel quickly (Bull et al. 1986, Nitao 1990). P450s are common in all organisms and can have broad substrate specificity (Berenbaum et al. 1992, Scott and Wen 2001).

At least six insect herbivores consume prickly ash foliage (Table 1.1). Several of these are specialists with no alternative host plants. At least one insect herbivore feeds on prickly ash phloem. Furanocoumarins are not translocated through the vascular tissues of prickly ash (Berenbaum 1981b), thus xylem and phloem feeders are not exposed to furanocoumarins.

Table 1.1 Herbivores of prickly ash

<table>
<thead>
<tr>
<th>Herbivore</th>
<th>Feeding Guild</th>
<th>Order</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Papilio Cresphontes</em></td>
<td>Leaf chewer</td>
<td>Lepidoptera</td>
<td>(Robinson 2002)</td>
</tr>
<tr>
<td><em>Papilio glaucus</em></td>
<td>Leaf chewer</td>
<td>Lepidoptera</td>
<td>(Robinson 2002)</td>
</tr>
<tr>
<td><em>Papilio troilus</em></td>
<td>Leaf chewer</td>
<td>Lepidoptera</td>
<td>(Robinson 2002)</td>
</tr>
<tr>
<td><em>Derospidea brevicollis</em></td>
<td>Leaf chewer</td>
<td>Coleoptera</td>
<td>(Wilcox 1954)</td>
</tr>
<tr>
<td><em>Dichomeris citrifoliella</em></td>
<td>Bud chewer</td>
<td>Lepidoptera</td>
<td>(Robinson 2002)</td>
</tr>
<tr>
<td><em>Agronopterix nigrinotella</em></td>
<td>Leaf chewer</td>
<td>Lepidopteran</td>
<td>(Robinson 2002)</td>
</tr>
<tr>
<td><em>Hyphantria cuna</em></td>
<td>Leaf chewer</td>
<td>Lepidoptera</td>
<td>(Robinson 2002)</td>
</tr>
<tr>
<td><em>Aphis rumicis</em></td>
<td>Phloem feeder</td>
<td>Hemiptera</td>
<td>(Mertins et al. 1973)</td>
</tr>
</tbody>
</table>
Although extremely toxic, prickly ash does provide ecological benefits to several invertebrate and vertebrate animals. Prickly ash is a common nesting site for gray catbirds, *Dumetella carolinensis*, (Johnson and Best 1980), and several other bird species (personal observation). Dense thickets and physical defenses of prickly ash may deter avian egg and fledgling predators. Prickly ash provides overwintering habitat for beneficial biocontrol agents in New York vineyards (Williams and Martinson 2000), and prickly ash fruits are consumed by multiple birds and mammals (USDA 2013).

Native Americans were the first to discover the numbing effect when the bark or foliage of prickly ash is chewed, and thus called it the toothache tree (Moerman 1998). More recently the phenolics and essential oils in prickly ash have been examined as possible drug treatments for a wide array of diseases including sexually transmitted diseases, cancer, fungal infections, bacterial infections (Bafi-Yebo et al. 2005, USDA 2013) and as a possible organic pesticide (Bowers et al. 1993, Ge and Weston 1995).

**Ecology and Biology of Giant Swallowtail Caterpillar**

The giant swallowtail butterfly, *Papilio cresphontes*, is the largest North American butterfly, with a wing span up to 6.9 cm (McAuslane 2009). Giant swallowtail is distributed from northern New England to southern Florida, and westward to California (Minno et al. 2005). Recent studies have suggested that giant swallowtail is currently expanding their northern range as climate change decreases the occurrence of fall frosting events (Finkbeiner et al. 2011). Giant swallowtail is multivoltine in southern states and
bivoltine in northern states. Adults feed on nectar and males gain additional nutrients through puddling. Larvae consume several plants in the Rutaceae family such as prickly ash, lime prickly ash, *Zanthoxylum fagara*; Hop tree, *Ptelea trifoliata*; and several cultivars of agricultural citrus plants *Citrus spp.*, however they rarely cause economic damage (Scriber and Dowell 1991).

Gravid females lay single eggs on the upper side of leaves. Five instars occur and larval development is completed in three weeks (Bullock and Pelosi 1992). Larvae mimic bird droppings and have a pair of eversible projections, osmeteria that are expelled as an antipredator response when larvae are disturbed. Osmeterial glands release a pungent odor, isobuteric acid, which deters arthropod predators (McAuslane 2009).

**Introduction to Plant Defense Theory**

The growth-differentiation balance hypothesis (Herms and Mattson 1992) states that plant allocation patterns exhibit adaptive phenotypic plasticity in response to variation in resource availability, and because resources are limited, tradeoffs occur between growth, reproduction, and secondary metabolism. For example, plants in resource-rich environments are predicted to allocate proportionally more resources towards growth, whereas plants in resource-limited environments are expected to allocate proportionally more resources towards defense. In dioecious plants, male and females are expected to differentially allocate resources due to different selection pressures (Montesinos et al. 2006). Male fitness is determined, in part, by quantity of pollen produced and males are
predicted to increase quantities of mates (Leigh et al. 2006). In addition to flowers, females produce fruits and seeds and are predicted to increase the quality of offspring (Bawa 1980). Fruit production increases female reproductive investments at a cost to growth (Lloyd and Webb 1977, Ashman 2002); therefore, females grow slower than males and are predicted to have increased defenses (Herms and Mattson 1992, Cipollini and Whigham 1994, Massei et al. 2006).


UV irradiation induces the transcriptional activation of the enzyme phenylalanine ammonia lyase (PAL) (Jones 1984), an important precursor to furanocoumarin biosynthesis (Kolb et al. 2001, Babu et al. 2003). Therefore, prickly ash growing in canopy gaps caused by EAB may have increased growth and defenses compared to shaded prickly ash, and male and female plants may allocate resources to these processes differently.

Increased furanocoumarins reduce herbivore performance, and specialist herbivores consuming diets with increased levels of toxins increase resource allocations towards detoxification mechanisms (Berenbaum 1978, Pritsos et al. 1988, Berenbaum et al. 1989, Berenbaum and Zangerl 1993, Diawara et al. 1993, Zangerl and Berenbaum
detoxification enzymes diverts energy from growth, resulting in reduced growth rates
(Berenbaum and Zangerl 1993, Berenbaum and Zangerl 1994). The slow growth-high
mortality hypothesis predicts that slower growing individuals will experience increased
exposure time to natural enemies (Price et al. 1980, Clancy and Price 1987). Therefore,
giant swallowtail consuming prickly ash growing in canopy gaps may experience reduced
growth rates as they increased allocations towards detoxification and, thus may
experience greater mortality from predation.

The objective of this research was to quantify the indirect effects of EAB on a
native plant-insect interaction. The central hypothesis is that canopy gaps created by ash
mortality caused by EAB will increase the amount of UV light reaching the forest
understory. Increased light will induce multiple physiological changes within prickly
ash, including increase concentrations of furanocoumarins. Male and female prickly ash
will allocate resources differently. Increased furanocoumarin production will decrease
giant swallowtail larval growth rates and thereby increase their exposure to natural
enemies. These hypotheses were tested using field and laboratory studies.

Indirect competition among herbivores can occur when one herbivore induces
chemical defenses within host plant that negatively affects another herbivore sharing that
host (Niemelä et al. 1984). Herbivore population dynamics also can be affected by
chemical induced changes in host plants (Redman and Scriber 2000, Kaplan and Denno
2007). Giant swallowtail and EAB, however, do not share host plants and do not
compete for resources. EAB could have indirect negative effect on giant swallowtail
larvae by inducing chemical changes in prickly ash. This study is one of the first to quantify indirect effects on induced chemical defenses by an invasive herbivore on a native herbivore that does not share a common host plant. However, since invasive herbivores have such wide-scale effects on native ecosystems, they can disrupt nutrient cycling, decomposition, and successional trajectories, future studies may find this to be a common phenomenon (Gandhi and Herms 2010a).
References


Chapter 2

Differential Allocation Tradeoffs in Male and Female Prickly Ash Growing in Canopy Gaps and the Shaded Understory

Abstract

Emerald ash borer, *Agrilus planipennis* (EAB), was unintentionally introduced to Midwestern forests in the 1990’s. EAB attacks and kills healthy North American ash trees (*Fraxinus spp.*), and is responsible for death of millions of ash trees in Midwestern forests. Canopy gaps created by dying ash trees increases light availability to understory plants. Prickly ash, *Zanthoxylum americanum*, is a native understory shrub that contains furanocoumarins, highly toxic secondary metabolites. Furanocoumarin biosynthesis is enhanced with UV radiation. Chemical defense theory predicts tradeoffs occur in resource allocations between male and female plants, with males investing more resource in growth and females investing more in reproduction. Therefore, male and female prickly ash growing in canopy gaps may differ in their furanocoumarin concentrations, growth rates, and reproductive effort.
Giant swallowtail butterfly larvae, *Papilio cresphontes*, are specialist herbivores on prickly ash that detoxify furanocoumarins using cytochrome P450s. However, furanocoumarin detoxification is energetically costly and slows larval development. The slow growth-high mortality hypothesis predicts slower growing individuals will experience increased mortality due to increased exposure to natural enemies.

We quantified the affects of EAB-induced canopy gaps on resource allocation of male and female prickly ash growing in canopy gaps and the shade. Prickly ash growing in gaps had greater photosynthetic capability than shade plants. Male plants invested proportionally more resources towards growth, whereas females invested more in reproduction. Prickly ash growing in gaps had higher concentrations of furanocoumarins, and increased thorn production.

We examined the affects of prickly ash foliage collected from plants growing in sun and shade on *P. cresphontes* development. Larvae grew slower when consuming foliage from plants growing in sun compared to shade. Our results suggest *P. cresphontes* larvae development is negatively affected by canopy gap formation. There was no difference in survival of larvae placed on plants in gaps compared to understory plants, with mortality over 48 hours close to 70% in both habitats. We predict that giant swallowtail larval survival will be lower in EAB infested forests because gaps increase the toxicity of prickly ash resulting in decreased growth rates and increased exposure time to natural enemies.
Introduction

Emerald ash borer, *Agrilus planipennis* (EAB), is an invasive buprestid beetle unintentionally introduced from Asia to North America during the 1990’s (Cappaert et al. 2005). As of March 2013, EAB has spread to 19 US states and two Canadian provinces. EAB is restricted to ash trees in the genus *Fraxinus*. In its native Asian range (China, Korea, Japan, Mongolia, eastern Russia, and Taiwan) EAB only attacks stressed ash trees (*F. chinensis, F. mandshurica*, and *F. rynchophylla*) and rarely kills healthy trees (Akiyama and Ohmono 2000, Wei et al. 2004). However, North American ash trees lack a coevolutionary history with EAB, and healthy trees are susceptible to attack. EAB has killed millions of ash trees in the midwestern United States and has the potential to functionally extirpate the entire *Fraxinus* genus from North American. There are at least eight billion ash trees in North America and ash trees are an important component of over two dozen North American forest types (Burns and Honkala 1990, Poland 2007). Simultaneous wide-scale ash mortality will likely have many indirect cascading effects on native organisms (Gandhi and Herms 2010a).

Ash mortality resulting from EAB attack creates canopy gaps that are faster forming, larger, and more widespread than natural gaps resulting from single tree falls (Gandhi and Herms 2010a). Canopy gaps increase light penetration to the forest floor and this increased light may benefit native understory plants by increasing photosynthesis (Gehring and Delph 2006). Increased photosynthate can result in increased growth and reproductive outputs. Northern prickly ash, *Zanthoxylum americanum*, is a native
dioecious understory shrub. Prickly ash belongs to the citrus family (Rutacea) and therefore is not attacked by EAB. The foliage of prickly ash contains chemical defenses such as furanocoumarins, which are photoactivated secondary metabolites that become more toxic in the presence of UV light (Waterman 1975, Murray et al. 1982, Bafi-Yeboa et al. 2005). Furanocoumarins are highly biocidal and intercalate with DNA. Since furanocoumarins disrupt DNA they are extremely toxic to most organisms including insects, mammals, birds, and reptiles (Murray et al. 1982). Prickly ash also contains physical defenses (thorns) that further deter mammalian browsing (Cooper and Owen-Smith 1986, Pellissier 2013, Takei et al. 2013).

The growth-differentiation balance hypothesis (GDBH) predicts that male and female plants differentially allocate resources, with males investing more in growth and females investing more in reproduction and defenses (Herms and Mattson 1992). The GDBH suggests that resource allocations devoted to reproductive output occurs at a cost to growth, and slower growing plants invest more resources towards chemical defenses. If prickly ash conforms to the predictions of the GDBH, then males should grow faster than females and females should have higher reproductive outputs and defenses. Male and female plants may respond to increased light in canopy gaps differently. Canopy gaps can be considered resource-rich environments for prickly ash providing increased light, higher temperatures, and increased nutrients from fallen and decaying trees (Orwig et al. 2008). Males should invest increased resources (photosynthate) in growth while females should increase flower and fruit production.
Although furanocoumarins deter most herbivores from consuming prickly ash foliage, there are several specialist herbivores that have evolved furanocoumarin detoxification mechanisms, which allow them to consume prickly ash leaves. For example, the giant swallowtail caterpillar, *Papilio cresphontes*, is able to ingest prickly ash foliage by quickly detoxifying furanocoumarins with cytochrome P450s. However, P450 metabolism is energetically costly and energy required for detoxification is diverted from developmental processes, often resulting in a reduced growth rates (Berenbaum and Zangerl 1994). The slow growth-high mortality hypothesis predicts that reduced growth rates increase exposure time to natural enemies; and consequently slow growing individuals may experience increased mortality (Price et al. 1980, Clancy and Price 1987).

Therefore, prickly ash plants situated under canopy gaps created by EAB-induced ash mortality are likely to contain higher levels of furanocoumarins and thus be more toxic than shaded prickly ash. Giant swallowtail larvae feeding on plants with increased toxicity will have to devote more resources to detoxification at the expense of growth, and slower growing larvae may experience increased predation due to longer exposure time to natural enemies.

**Methods**

During the summer of 2009, prickly ash plants growing under EAB-induced canopy gaps (15-20% gap) were located in forests of Oakland County Michigan. These sites are
located at the epicenter of EAB invasion (Smith 2006) and represent true aftermath forests with close to 100% ash tree mortality. Plant height, length, width, and main stem diameter at breast height were recorded. Each plant growing in a canopy gap (sun treatment) was paired with a nearby plant growing in the understory (gap ≤ 5%) (shade treatment). All plants were taller than two m because prickly ash does not flower until five years of age (Reinartz and Popp 1987). Plant sex was determined by the presence of stamens or pistils on flowers and later verified by fruit production. Paired plants were the same sex, size, and had similar architecture. Percent canopy gap above plants was quantified using handheld densitometers. Plants were tagged and then GPS coordinates recorded. Seven male and seven female pairs (total = 28 plants) were used in 2010 field experiments. In 2011, six male and six female pairs (total =24 plants) were used. Percent canopy gap was assessed each year and if gap conditions changed between years, new plant pairs were located that fit the assigned sun and shade treatments. Field and laboratory experiments were conducted in 2010 and repeated in 2011.

Prickly ash growth and specific leaf area

New shoot growth was measured on four randomly selected branches of each plant at the end of each growing season. Five fully expanded leaflets were haphazardly collected from the upper plant. Leaf area was quantified using a Li-Cor leaf area meter. Leaflets were oven dried for 48 hrs and specific leaf area (cm² g⁻¹) calculated.
Prickly ash reproductive effort

In 2010 and 2011, fruit production was measured by counting total fruit yield on each female plant. In addition to fruit production reproductive effort was also quantified by counting the total number of flowers on male and female plants in 2011.

Prickly ash physical defenses

Thorn size was quantified by measuring the length and base width of ten thorns located on new shoot growth. Measurements were recorded using microcallipers. Thorn area was calculated using the area of a triangle formula \( A = \frac{1}{2} (\text{Base} \times \text{Height}) \). During 2011 thorn density was quantified by recording the number of thorns on new shoot growth on four random branches on each plant.

Leaf chemistry

Three fully expanded leaves (with approximately 4-6 leaflets) from the top of each plant were collected for furanocoumarin and total phenolics concentration quantification. Leaves were immediately placed in a cooler with dry ice and transported to a laboratory in Wooster, OH where they were briefly submerged in liquid nitrogen and placed in an 80° freezer. Foliar compounds were analyzed using high performance liquid chromatography (HPLC). Leaves were homogenized in 100% methanol using a 5:1
solvent to leaf ratio. Four-hydroxybenzoic acid was used as the internal standard (IS) in a 12:5 IS to solvent ratio. IS was placed on each sample leaf before homogenization. Each leaf sample and solute was placed in a 1.5-ml microcentrifuge tube and leaves ground to a fine power using a hand pestle. After homogenization samples were placed in a centrifuge for two minutes. Supernatants were syringe filtered, placed in a fresh 1.5-ml microcentrifuge tube, and stored at 20°C until analyzed with HPLC. Separation of compounds was achieved using 0.1% formic acid buffer (formic acid & H2O) and 0.1% methanol as solvents at a flow rate of 1ml/min. An additional four leaflets were collected from each plant for nitrogen analysis. Leaf samples were placed in a drying oven at 15° for 48 hrs. Leaves were ground and nitrogen concentration was analyzed using a Thermo Electron Corporation (CE Instruments) Flash EA-1112 C-N Analyzer with an AS 3000 Autosampler.

Swallowtail feeding bioassays

Giant swallowtail butterflies were captured from MI forests. Butterflies were transported to a laboratory and released in an outside cage (2m X 2m) constructed from PVC pipe framing and mesh netting. Each butterfly was removed from the cage daily and hand fed a 20% honey solution. Five potted prickly ash plants were placed in the cage and plants were scouted twice daily for freshly laid eggs. Leaflets with eggs were removed from plants and placed into individual Petri dishes containing filter paper and a layer of plaster of Paris to hold water and maintain humidity. Petri dishes were placed in
growth chambers and maintained at 25° with a 16 hr day length. Petri dishes were monitored twice a day for egg hatch. Newly hatched larvae were supplied with fresh field-collected male prickly ash foliage from shaded understory plants daily.

Fourth instar larvae emerging on the same day were randomly assigned to one of four feeding treatments (male sunny foliage, male shaded foliage, female sunny foliage, and female shaded foliage). Fourth instar larvae consume the most foliage and grow the most; therefore, performance calculations on these instars should represent the entire larval life stages (Scriber and Slansky 1981, Slansky and Scriber 1985). Fresh foliage from field-tagged prickly ash plants (see above) was collected for feeding bioassays. Larvae mass and prickly ash leaf area offered to each larva were recorded prior to feeding experiments. Fresh foliage was provided every 24 hrs. After 48 hours larvae mass, leaf area consumed, and dry frass mass were calculated. Relative growth rate (RGR) (mg biomass gained per day per mg larval biomass) for each larva was calculated according to Farrar et al. (1989) (RGR = growth / (initial mass X Time). In 2011, feeding bioassays were also conducted on first instars.

*Swallowtail mortality/predation bioassays*

During 2011, fourth and first instar larvae were placed on prickly ash plants growing in sun and shade. A single larva was placed on the upper side of fully expanded leaves on the upper portion of the plant. Larvae were monitored for five minutes after transfer to confirm they were anchored on the leaf. Each plant was scouted
daily for two days to monitor larvae survival. Plants used for predation bioassays were not touching other plants. Therefore, if a larvae was not present on the plant, we assumed a predation evident had occurred.

**Statistical analysis**

A full factorial designed experiment was conducted to analyze the effects of EAB-induced canopy gaps on prickly ash physiology. The two independent variables in the study are plant sex (male, female) and canopy light condition (shade, sun). Both factors were considered fixed. Percent canopy gaps were quantified using handheld densitometers. The dependent variables recorded from prickly ash were new shoot growth, flower production, fruit production, thorn length, thorn base length, thorn area, specific leaf area, thorn density, and leaf chemistry. A two-way Analysis of Variance (ANOVA) ($\alpha = 0.05$) was used to test the effects of sun and shade treatments and their interactions using SAS 9.3 (SAS Institute 1999). If the interaction between sex and light was significant, main effects were ignored and simple effects were examined followed by an effect size calculation using the appropriate error terms. To control for Type I error rate across the simple effects, alpha was set at 0.025 ($\alpha/2 = 0.05/2$). A One-way ANOVA was used to analyze fruit production. Canopy gap data was arc sine transformed for both years to meet the normality assumption. In 2010, new growth and thorn area did not meet the assumptions of normality and were log-transformed. In 2011, new growth, thorn density, and flower production violated the assumption of normality.
New growth and flowers were log-transformed, while thorn density was square root-transformed.

A separate experiment examined the effect of canopy gaps on host plant quality and survival of giant swallowtail butterfly larvae. The dependent variables were swallowtail relative growth rate and larvae mortality. The independent variables were plant sex (male, female) and canopy light condition (shade, sun). Both factors are considered fixed. Data were analyzed by two-factor (2 X 2) Analysis of Variance (ANOVA) (\( \alpha = 0.05 \)) using SAS 9.3 (SAS Institute. 1999). If the interaction between sex and light was significant, simple effects were examined followed by an effect size calculation using the appropriate error terms.

In 2010, swallowtail relative growth rates violated the normality assumption and were square root-transformed. In 2011, first instar relative growth rates were square root transformed to meet the normality assumption. Percent mortality was calculated for each instar group at each field site and paired t-tests were used to compare larvae survival in sun and shade.

**Results**

*Canopy gaps*

In 2010, canopy gaps between shade treatments (5 ± 0.37%) and sun treatments (18 ± 0.78%) treatments were significant (\( F_{1,28}=226.01, \ p<0.0001 \)) (**Fig. 2.1A**), but
percent canopy gap did not differ between male and female plants (F_{1,28}=0.76, p=0.39).

In 2011, canopy gaps were significant between shade (4 ± 0.29%) and sun (24 ± 1.9%) treatments (F_{1,24}=334.44, p<0.0001) (Fig. 2.1B), but gaps between male and female plants did not differ (F_{1,24}=2.14, p=0.16).

**Figure 2.1.** Size of canopy gaps above prickly ash plants growing in sun and shade treatments in A 2010 and B 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different (α = 0.05).
In 2010, shaded foliage had higher specific leaf area than sun foliage (368 ± 11.4 and 268 ± 14.3 cm$^2$ g$^{-1}$, respectively) ($F_{1,28}=29.43$ $p<0.0001$) (Fig. 2.2A). No difference was observed between male (308.1 ± 15.87 cm$^2$ g$^{-1}$) and female foliage (328.7 ± 19 cm$^2$ g$^{-1}$) ($F_{1,28}=1.26$, $p=0.26$) and no significant interaction occurred between sex and light ($F_{1,28}=0.85$, $p=0.36$).

Similar trends were observed in 2011, with significant effects between shade and sun foliage (362.3 ± 11.8 and 234 ± 17.8 cm$^2$ g$^{-1}$ respectively) ($F_{1,24}=39.72$, $p<0.0001$) (Fig. 2.2B), and no differences between male (305.3 ± 19.9 cm$^2$ g$^{-1}$) and female leaves.
(281.1 ± 30.4 cm² g⁻¹) (F₁,₂₄=1.28, p=0.27). No interaction occurred between sex and light (F₁,₂₄=1.93, p=0.17).

**Figure 2.2.** Specific leaf area of prickly ash plants growing in sun and shade in A 2010 and B 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different (α = 0.05).
In 2010, no difference in growth was observed between males and female plants (6.9 ± 1.3 and 5.9 ± 0.9 cm/year, respectively) ($F_{1,28}=0.01$, $p=0.97$). Growth of shade and sun plants was not significantly different (4.4 ± 0.5 and 8.5 ± 1.4 cm/year, respectively) ($F_{1,28}=3.55$, $p=0.06$). However, a significant interaction between sex and light ($F_{1,28}=4.61$, $p=0.039$) (Fig. 2.3) was observed, indicating differences between growth in light conditions were dependent on the sex of plants. Therefore, simple effects were examined.
Figure 2.3. Comparison of male and female prickly ash in sun and shade during 2010.

Approximately 9% ($\omega^2 = 0.0898$) of the total variance of new growth is due to the interaction of sex and light. Male plants grew more in sun than shade ($F_{1,28}=8.14$, $p=0.0077$) (Fig. 2.4A). Females in sun and shade did not differ in growth ($F_{1,28}=0.02$, $p=0.88$) (Fig. 2.4B).
Figure 2.4. Growth of male A and female B prickly ash in sun and shade during 2010. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).
Overall plant growth was higher in 2011 compared to 2010 and the effects of plant sex and canopy light on differed (Table 2.1). In 2011, male plants ($47.5 \pm 10.3$ cm/year) grew more than females ($16.8 \pm 2.9$ cm/year) ($F_{1,24}=6.34, p=0.021$) and plants in the sun ($51.6 \pm 9.8$ cm/year) grew more than those in the shade ($47.5 \pm 10.3$ cm/year) ($F_{1,24}=17.13, p<0.005$). Additionally, the interaction between sex and light was significant ($F_{1,24}=7.27, p=0.014$) (Fig.2.5) indicating differences between growth in light conditions were sex dependent.

Table 2.1. Shoot elongation (cm) of male and female prickly ash growing in sun and shade by year.

<table>
<thead>
<tr>
<th>Year</th>
<th>Shade</th>
<th>Sun</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>4.4 ± 0.5</td>
<td>8.5 ± 1.4</td>
<td>6.9 ± 1.3</td>
<td>5.9 ± 0.9</td>
</tr>
<tr>
<td>2011</td>
<td>47.5 ±10.3</td>
<td>51.6 ± 9.8</td>
<td>47.5 ± 10.3</td>
<td>16.8 ± 2.9</td>
</tr>
</tbody>
</table>

Figure 2.5. Comparison of male and female prickly ash growth in sun and shade during 2011.
Therefore, main effects were ignored and simple effects were examined. Approximately 16% ($\omega^2 = 0.163$) of the total variation is due to the interaction between sex and light. In the sun, sex of plants had significant effects ($F_{1,24}=13.59, P=0.0017$). Males in the sun grew more than males in the shade ($F_{1,24}=25.7, p<0.0001$) (Fig. 2.6A), whereas female growth was unaffected by light ($F_{1,24}= 0.95, p=0.34$) (Fig. 2.6B). In the shade, sex of plant did not significantly affect growth ($F_{1,24}=0.02, p=0.90$).

**Figure 2.6.** Growth of male A and female B prickly ash in sun and shade during 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$)
Prickly ash reproductive effort

In 2010, female prickly ash reproductive effort was analyzed by recording total fruit production on each plant. Females in the sun produced more fruit (630 ± 152) than females in shade (79 ± 78) (F_{1,14}=8.20, p=0.035) (Fig. 2.7). In 2011, the same trend was observed with females in the sun (306 ± 13) producing more fruit compared to females in the shade (58 ± 8) (F_{1,12}=52.99, p=0.0008) (Fig. 2.8).
**Figure 2.7.** Female prickly fruit production in sun and shade in 2010. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).

**Figure 2.8.** Female prickly fruit production in sun and shade in 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).
Males produced fewer flowers (29 ± 3) compared to females (224 ± 21) 
(F1,24=28.29, p=0.0001) (Fig. 2.9) and plants in the sun (175 ± 18) produced more 
flowers compared to shaded plants (34 ± 3) (F1,24=7.34, p=0.014) (Fig. 2.10). There was 
no interaction between sex and light (F1,24=0.91, p=0.35).

Figure 2.9. Comparison of male and female prickly ash flower production in 2011. 
Standard errors are depicted and bars with an asterisk (*) are significantly different (α = 
0.05).
Figure 2.10. Comparison of prickly ash flower production in sun and shade in 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).

Prickly ash physical defenses

In 2010, thorn length was greater in sun plants ($5.77 \pm 0.27$ mm) than shaded plants ($4.78 \pm 0.28$ mm) ($F_{1,28}=6.07$ $p=0.019$) (Table 2.2). Thorn length did not differ between male and female plants ($5.4 \pm 0.39$ and $5.13 \pm 0.19$ mm, respectively) ($F_{1,28}=0.44$, $p=0.51$) (Table 2.3) and no significant interaction occurred between sex and light ($F_{1,28}=0.02$, $p=0.89$). Thorn area among sun plants ($21.59 \pm 2.2$ mm) was greater than shade plants ($15.89 \pm 1.92$ mm) ($F_{1,28}=4.47$ $p=0.042$). Thorn area did not differ between males ($19.73 \pm 2.57$ mm) and females ($17.65 \pm 1.71$ mm) ($F_{1,28}=0.1$ $p=0.75$) and no significant interaction was observed between sex and light ($F_{1,28}=0.01$, $p=0.97$).
Thorn base did not differ between sun and shade (7.35 ± 0.57 and 6.4 ± 0.47 mm, respectively) \((F_{1,18}=1.56, p=0.22)\), males and females (6.97 ± 0.55 and 6.76 ± 0.51 mm) \((F_{1,28}=0.08, p=0.78)\) or the interaction between sex and light \((F_{1,28}=0.01, p=0.9854)\).

In 2011, thorn length was greater in sun (8.72 ± 0.47 mm) than shade plants (5.89 ± 0.82 mm) \((F_{1,24}=14.06, p=0.0015)\). Thorn length was greater in males (8.37 ± 0.62 mm) than females (6.03 ± 0.84 mm) \((F_{1,24}=9.5, p=0.0064)\). A significant interaction between sex and light occurred for thorn length \((F_{1,24}=4.67, p=0.044)\) \((\text{Fig. 2.13})\), indicating differences between thorn length in light conditions were dependent on the sex of plants.

Therefore, main effects were ignored and simple effects were examined. Approximately 7% \((\omega^2 = 0.077)\) of the total variation is due to the interaction between sex and light. Light had a significant effect on female thorn length \((F_{1,24}=17.01, p=0.0006)\). Females in the sun (8.34 ± 0.68 mm) produced thorns with greater length compared to females in the shade (3.73 ± 0.27 mm). Light had no effect on male thorn length \((F_{1,24}=1.72, p=0.2059)\) \((\text{Fig 2.10})\). In the shade, sex of plant significantly affected thorn length \((F_{1,24}=13.75, p=0.0016)\). Males in the shade (7.70 ± 1.0 mm) produced thorns with greater length than females in the shade (3.73 ± 0.27 mm). In the sun sex of plants has no significant effects on thorn length \((F_{1,24}=0.42, P=0.52)\).

Thorn base was greater in sun (5.82 ± 0.19 mm) than shaded plants (4.15 ± 0.34 mm) \((F_{1,24}=24.67, p<0.0001)\) in 2011. No difference occurred between male and females (5.24 ± 0.24 and 4.68 ± 0.5 mm, respectively) \((F_{1,24}=2.77, p=0.11)\). There was a
significant interaction between sex and light ($F_{1,24}=6.49$, $p=0.02$), indicating differences between thorn base in light conditions were dependent on the sex of plants.

Therefore, main effects were ignored and simple effects were examined. Approximately 10% ($\omega^2 = 0.103$) of the total variation was due to the interaction between sex and light. In the shade, sex of plants significantly affected thorn base ($F_{1,24}=8.86$, $p=0.0081$). Males in the shade ($4.8 \pm 0.36$ mm) produced thorns with greater bases compared to shaded females ($3.38 \pm 0.40$ mm).

In the sun, sex of plants had no effect on size of thorn base ($F_{1,24}=0.39$, $P=0.53$). Light had no significant effect on the thorn base of male plants ($F_{1,24}=3.81$, $p=0.06$). Light had a significant effect on the size of female thorn bases ($F_{1,24}=27.35$, $p<0.0001$)

Females in the sun ($5.98 \pm 0.33$ mm) produced thorns with larger bases compared to females in the shade ($3.38 \pm 0.40$ mm). Thorn area was greater in sun ($26.41 \pm 1.93$ mm) plants than shade plants ($14.03 \pm 2.88$ mm) ($F_{1,24}=17.83$, $p=0.0005$). Males had greater thorn area ($23.54 \pm 2.43$ mm) compared to females ($16.24 \pm 3.49$ mm) ($F_{1,24}=6.16$, $p=0.023$). No significant sex X light interaction occurred for thorn area ($F_{1,24}=3.91$, $p=0.063$).
Table 2.2. Effect of sun and shade on thorn length, base length, and thorn area in 2010 and 2011.

<table>
<thead>
<tr>
<th></th>
<th>Sun Mean ± SE (mm)</th>
<th>Shade Mean ± SE (mm)</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorn Length 2010</td>
<td>5.77 ± 0.27</td>
<td>4.78 ± 0.28</td>
<td>6.07</td>
<td>0.019</td>
</tr>
<tr>
<td>Thorn Length 2011</td>
<td>8.72 ± 0.47</td>
<td>5.89 ± 0.82</td>
<td>14.06</td>
<td>0.0015</td>
</tr>
<tr>
<td>Thorn Base Length 2010</td>
<td>7.35 ± 0.57</td>
<td>6.4 ± 0.47</td>
<td>1.56</td>
<td>0.22</td>
</tr>
<tr>
<td>Thorn Base Length 2011</td>
<td>5.82 ± 0.19</td>
<td>4.15 ± 0.34</td>
<td>24.67</td>
<td>0.0001</td>
</tr>
<tr>
<td>Thorn Area 2010</td>
<td>21.59 ± 2.2</td>
<td>15.89 ± 1.92</td>
<td>4.47</td>
<td>0.042</td>
</tr>
<tr>
<td>Thorn Area 2011</td>
<td>26.41 ± 1.93</td>
<td>14.03 ± 2.88</td>
<td>17.83</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Table 2.3. Effect of plants sex on thorn length, base length, and thorn area in 2010 and 2011.

<table>
<thead>
<tr>
<th></th>
<th>Male Mean ± SE (mm)</th>
<th>Female Mean ± SE (mm)</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorn Length 2010</td>
<td>5.4 ± 0.39</td>
<td>5.13 ± 0.19</td>
<td>0.44</td>
<td>0.51</td>
</tr>
<tr>
<td>Thorn Length 2011</td>
<td>8.37 ± 0.62</td>
<td>6.03 ± 0.84</td>
<td>9.5</td>
<td>0.0064</td>
</tr>
<tr>
<td>Thorn Base Length 2010</td>
<td>6.97 ± 0.55</td>
<td>6.67 ± 0.51</td>
<td>0.08</td>
<td>0.78</td>
</tr>
<tr>
<td>Thorn Base Length 2011</td>
<td>5.24 ± 0.24</td>
<td>4.68 ± 0.5</td>
<td>2.77</td>
<td>0.11</td>
</tr>
<tr>
<td>Thorn Area 2010</td>
<td>19.73 ± 2.5</td>
<td>17.65 ± 1.71</td>
<td>0.1</td>
<td>0.75</td>
</tr>
<tr>
<td>Thorn Area 2011</td>
<td>23.54 ± 2.4</td>
<td>16.24 ± 3.49</td>
<td>6.16</td>
<td>0.023</td>
</tr>
</tbody>
</table>
In 2011, thorn density was recorded on new growth. Plants in sun (7 ± 1.001) produced more thorns on average compared to shade plants (3 ± 1) (F_{1,24}=9.6, p=0.0062) (Fig. 2.11). Male and female plants did not differ in thorn density (6 ± 1 and 4 ± 1, respectively) (F_{1,24}=1.64, p= 0.21), and no interaction occurred between sex and light (F_{1,24}=0.11, p= 0.75).

**Figure 2.11.** Comparison of prickly ash thorn density on plants in sun and shade in 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different (α = 0.05).

---

*Leaf chemistry*

Three furanocoumarins, xanthotoxin, bergapten, and psoralen, were identified from prickly ash foliage based on relative retention time, mass fragmentation, and UV
absorption from previously published data (Table 2.2). Peak 1 had a retention time of 23.37 minutes and a mass-to-charge ratio (m/z) at 186. These characteristics are consistent with psoralen. Peak 2 had a retention time of 24.02 minutes and an m/z at 217.1, consistent with the compound bergapten. Peak 3 had a retention time of 26.7 minutes and an m/z at 217.2 suggesting this compound is xanthotoxin.

Table 2.4 Identification of furanocoumarins including M/z and retention time (RT) in prickly ash foliage.

<table>
<thead>
<tr>
<th>Compound</th>
<th>m/z</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoralen</td>
<td>186</td>
<td>23.37</td>
</tr>
<tr>
<td>Bergapten</td>
<td>217.1</td>
<td>24.02</td>
</tr>
<tr>
<td>Xanthotoxin</td>
<td>217.2</td>
<td>26.7</td>
</tr>
</tbody>
</table>

Prickly ash foliage in sun had 2.2 times higher total furanocoumarins concentration compared to shade leaves ($F_{1,24}=8.95$, $p=0.0082$) (Fig.2.12). Male and female foliage did not differ in total furanocoumarin concentration ($F_{1,24}=0.36$, $p=0.5584$) and no interaction between light and sex occurred ($F_{1,24}=0.08$, $p=0.7768$).
**Figure 2.12** Total foliar furanocoumarin concentrations from prickly ash growing in sun and shade. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).

Foliage from plants in the sun contained 1.2 times higher concentrations of total phenolics than shaded leaves ($F_{1,24} = 9.15, p=0.0076$) ([Fig 2.13](#)). Male and female leaves ($F_{1,24}=0.01, p=0.9261$) did not differ in total phenolics and no interaction occurred between sun and shade ($F_{1,24}=0.01, p=0.9238$).
Figure 2.13 Total foliar phenolics concentration from prickly ash growing in sun and shade. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).

Psoralen concentration did not differ between sun and shaded leaves ($F_{1,24}=3.43$, $p=0.081$) or male and female foliage ($F_{1,24}=4.38$, $p=0.051$). No interaction between sex and light occurred ($F_{1,24}=0.57$, $p=0.4602$). Bergapten concentration did not differ between sun and shade ($U=121$, $p=0.433$) leaves or male and female (foliage ($U=122$, $p=0.0.0996$). Xanthotoxin did not differ between sun and shade leaves ($U=112$, $p=0.8775$) or male and female ($U=93$, $p=0.69$).

Male prickly ash foliage ($3.05 \pm 0.108\%$) contained a higher percent nitrogen concentration than female foliage ($2.61 \% \pm 0.069\%$) ($F_{1,24}=10.3$, $p=0.0055$) (Fig. 2.14). No difference in nitrogen occurred between shaded ($2.87 \pm 0.063\%$) and sunny plants.
(2.82 ± 0.177%) (F_{1,24}=0.17, p=0.68) and no interaction occurred between sex and light (F_{1,24}=3.24, p=0.090).

**Figure 2.14.** Comparison of foliar nitrogen concentration between male and female prickly ash. Standard errors are depicted and bars with an asterisk (*) are significantly different (α = 0.05).

**Swallowtail feeding bioassays**

In 2010, larvae consuming foliage from plants growing in sun had lower relative growth rates (0.33 ± 0.01 g g^{-1}/day^{-1}) compared to larvae consuming shaded foliage (0.40 ± 0.02 g g^{-1}/day^{-1}) (F_{1,32}=4.35, p=0.046) (Fig. 2.15A). No difference in larvae RGR was observed between those feeding on male plants (0.37 ± 0.02 g g^{-1}/day^{-1}) versus female plants (0.37 ± 0.02 g g^{-1}/day^{-1}) (F_{1,32}=0.01, p=0.95), and no interaction between sex and light occurred (F_{1,32}=0.03, p=0.87).
In 2011, RGR did not differ between first instars consuming sun (0.53 ± 0.04 g g\(^{-1}\)/day\(^{-1}\)) or shaded foliage (0.57 ± 0.04 g g\(^{-1}\)/day\(^{-1}\)) (F\(_{1,64}=0.46, p=0.49\)) (Fig. 2.16A) or between larvae feeding on male (0.56 ± 0.04 g g\(^{-1}\)/day\(^{-1}\)) versus female plants (0.54 ± 0.04 g g\(^{-1}\)/day\(^{-1}\)) (F\(_{1,64}=0.05, p=0.81\)) (Fig. 2.16B). No interaction between sex and light was observed (F\(_{1,64}=0.27, p=0.60\)).

In 2011, fourth instar feeding bioassays were repeated and the same trend was observed. Swallowtail larvae feeding on foliage from plants in the sun had lower RGR (0.1 ± 0.83 g g\(^{-1}\)/day\(^{-1}\)) compared to larvae feeding on shade foliage (0.12 ± 0.62 g g\(^{-1}\)/day\(^{-1}\)) (F\(_{1,36}=4.51, p=0.041\)) (Fig. 2.15B). RGR did not differ between larvae feeding on male plants (0.12 ± 0.69 g g\(^{-1}\)/day\(^{-1}\)) and female plants (0.10 ± 0.84 g g\(^{-1}\)/day\(^{-1}\)) (F\(_{1,36}=2.08, p=0.15\)) and no interaction between sex and light was observed (F\(_{1,36}=0.19, p=0.66\)).
Figure 2.15. Comparison of relative growth rates of fourth instar larvae consuming sunny and shaded prickly ash foliage in A 2010 and B 2011. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).
Figure 2.16. Comparison of relative growth rates of first instar larvae consuming A sunny and shaded prickly ash foliage and B male and female foliage. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).
Swallowtail mortality/predation bioassays

In 2011, giant swallowtail caterpillar survival (first and fourth instars) was compared on prickly ash plants growing in canopy gaps versus shade at four separate sites. First instar mortality did not differ between those placed on plants in sun (0.31 ± 0.10%) compared to larvae on shaded plants (0.15 ± 0.085%) (t=1.14, p=0.29) (Fig 2.17). Likewise fourth instar survival was not different in sun (0.48 ± 0.21%) or shade (0.33± 0.15%) (t=0.6, p=0.57) (Fig. 2.17).

Figure 2.17. Giant swallowtail mortality on prickly ash growing in sun and shade. Standard errors are depicted.
Discussion

This research provides evidence that EAB has direct and indirect effects on North American forest ecosystems (Fig. 2.18). All North American ash trees that EAB has encountered are extremely susceptible and die as a result of EAB infestation (Prasad et al. 2010). Therefore, EAB has a direct negative effect on ash trees. In canopy gaps, prickly ash males increase in growth and females increase reproductive effort. Both male and females increase furanocoumarin levels in canopy gaps. In forests without EAB, ash trees typically have an indirect negative effect on prickly ash by reducing light resources. Giant swallowtail larvae consume prickly ash foliage. Therefore, prickly ash provides resources to the caterpillar and has a direct positive effect on giant swallowtails, whereas swallowtails have a direct negative impact on prickly ash by consuming foliage. Giant swallowtail larvae provide predators (birds and arthropods) with nutritional resources. Therefore, swallowtail larvae provide a direct positive effect on predators and predators directly negatively affect larvae. EAB has an indirect positive effect on prickly ash by creating canopy gaps. Gaps increase growth, reproduction, and defenses in prickly ash. Thus, EAB also has an indirect negative effect on giant swallowtail larvae by increasing the toxicity of prickly ash, thereby increasing developmental time and exposure to swallowtail predators. Host plant quality can affect the performance of higher trophic levels (Scriber and Slansky 1981, McGovern et al. 2006, Lampert et al. 2008). Therefore, EAB has an indirect positive effect on swallowtail predators by increasing the availability (longer duration) of their prey (swallowtail larvae).
**Figure 2.18** Conceptual model showing cascading direct and indirect effects of EAB on prickly ash, giant swallowtail butterfly larvae, and larvae predators. Solid lines represent direct effects and dashed lines represent indirect effects. Positive effects are indicated with (+) and negative effects are shown as (-).
The growth-differentiation balance hypothesis (GDBH) predicts that tradeoffs occur in resource allocations in dioecious plants, with males investing more resources towards growth while females invest more in reproduction and defense (Herms and Mattson 1992). In accordance to the predictions of the GDBH, male prickly ash plants invested more resources in growth and females invested more in reproduction. Male prickly ash plants grew faster than female plants in both years of the study. In 2011, male plants grew 282% more than females. Previous studies quantifying growth among female and male prickly ash observed similar patterns, with male prickly ash investing in growth and females allocated resources towards fruit production (Popp and Reinartz 1988). Niesenbaum (1992) examined growth and reproduction in the dioecious shrub *Lindera benzoin* and noted that males allocated more resources into shoot growth while females invested more in reproduction. In 2011, female prickly ash produced 760% more flowers than males. A recent meta-analysis on resource allocations of dioecious plants concluded that male plants produce fewer flowers than females (Cornelissen and Stiling 2005). Females have additional reproductive costs compared to males due to fruit and seed maturation, which require additional nutrient investments (Bullock 1984, Antos and Allen 1990, Cipollini and Stiles 1991). In both years, female prickly ash produced large quantities of fruit. Females produced an average of 630 fruits in 2010 and 306 fruits in 2011. In the Midwestern United States, prickly ash begins fruiting in early May. However, fruits do not ripen until late August and remain on the plant until late September (Reinartz and Popp 1987). In addition to production costs, long-lived fruits such as prickly ash require more resources for maintenance and maturation (Stiles 1980).
Therefore, tradeoffs between growth and reproduction in these females are more intense (Niesenbaum 1992, Dawson and Geber 1999).

Photosynthesis is predicted to increase with increasing light availability (Herms and Mattson 1992). Therefore, increased light availability is expected to increase plant growth (Richards and Coley 2008). Canopy gaps created by tree mortality increase light and nutrient availability and may stimulate understory plant growth (Denslow 1987, Richards and Coley 2006). In dioecious plants, males and females growing under canopy gaps are predicted to allocate increased photosynthate towards different physiological processes. Male and female prickly ash responded differently to increased light availability in EAB-induced canopy gaps. Male prickly ash invested increased carbon gains into growth whereas females invest more in reproductive output. Male plants in the sun did not increase flower production compared to shaded males, and female plants in the sun did not increase growth compared to shaded females.

Forest canopy gaps can affect the physiology of understory plants, including sexual reproduction and growth (Vandepitte et al. 2009). Gaps created by EAB induced several physiological changes within prickly ash. Female plants in the sun produced more flowers and fruits compared to shaded females. Plants in the sun produced 503% more flowers compared to shaded plants. In 2010, the average number of fruits per plant increased 790% in the sun compared to shade. Likewise, in 2011, females in the sun produced 522% more fruit compared to shaded females. Gehring and Delph (2006) found similar results in the dioecious plant Silene latifolia. When artificially shaded, females dramatically reduced fruit production but invested more in leaf production,
perhaps as a mechanism to increase limited light. Previous studies have observed increased reproductive effort in forest plants growing in canopy gaps (Routhier and Lapointe 2002, Kanno and Seiwa 2004, Pagés and Michalet 2006).

Female prickly ash growing in the shade likely allocated resources away from sexual reproduction and increased resource allocations towards clonal reproduction. A previous study on prickly ash observed that females increased clonal root production in shaded forest conditions compared to females growing in sunny habitats (Popp and Reinartz 1988). Vandepitte et al. (2009) observed similar trends in the dioecious plant *Mercurialis perennis*; females growing in canopy gaps reduced clonal propagation and increased sexual reproductive effort.

In addition to chemical defenses, plants have evolved numerous physical defenses to combat herbivory (Styrsky et al. 2006). The growth-differentiation balance hypothesis predicts that tradeoffs will occur between plant growth and physical defense production; however, physical defenses such as thorns may be more common in resource-rich habitats because production of thorns is dependent on plant growth (Herms and Mattson 1992). Most studies examining the effects of light on plant defense have focused on a few chemical traits, and very few studies have examined the effects of light on multiple defensive traits such as physical and chemical defenses (Yamawo and Hada 2010). Gibson et al. (1993) observed increased prickle production on *Rubus vestitus* in resource-rich environments and reduced thorns when plants were growing in nutrient poor environments. Canopy gaps affected physical defenses in prickly ash. In both 2010 and 2011, prickly ash growing in canopy gaps had greater thorn length and thorn area
compared to shaded plants. Several studies suggested that longer thorns are sharper and thereby reduce browsing, especially by large mammalian herbivores (Abrahamson 1975, Young 1987, Takei et al. 2013). Increased thorn density can also reduce herbivory. Several studies have observed reduced browsing on heavily prickled or thorny plants, suggesting that increased thorn density can reduce herbivory (Cooper and Owen-Smith 1986, Takada et al. 2003).

Thorn length and thorn area increased in canopy gaps. Since length is correlated with sharpness, plants in sun were more defended by shaper thorns than prickly ash in the shade. Prickly ash thorn density also increased in canopy gaps. Therefore, canopy gaps created by EAB increased the qualitative (sharpness) and quantitative (thorn density) physical defenses in prickly ash. Males and females allocated resources to thorn length differently. In the shade, male plants had taller thorns than females. However, males in the sun did not increase thorn length. Females in the sun increased thorn length compared to females in the shade. The same trend occurred for thorn base in 2011. In the shade, males had larger thorn base compared to females, but light did not affect male thorn base. Female thorn base increased in canopy gaps compared to shaded females. Prickly ash thorn data conforms to predictions made by the growth-differentiation balance hypothesis. In canopy gaps with increased light resources females invested more in physical defenses by increasing the length and sharpness of thorns, whereas males did not increase physical defenses when light resource increased.

Insect herbivores, however, are not likely affected by thorns (Takada et al. 2003). Rather, these physical defenses likely deter larger browsing herbivores (Pellissier 2013).
No browsing activity was observed on prickly ash during two years of field studies. A previous study reported zero browsing on prickly ash during a three month field study (van der Hoek et al. 2002). Although mammalian browsing does not appear to be common on prickly ash, large mammalian browsers could have been more abundant in the past. The cost of thorns is likely relatively low, especially if they are produced in resource-rich habitats. Therefore, thorns may continue to be produced in the absence of browsing herbivores. It is also possible that browsing on prickly ash occurs in early fall. Prickly ash foliage is present until late October.

Secondary metabolites, including furanocoumarins, are often less concentrated in older foliage (Scriber and Slansky 1981, Trumble et al. 1992). This can result from a dilution effect as leaves expand or as secondary metabolites decrease as they are reabsorbed (Herms and Mattson 1992). The optimal defense theory predicts that plant parts are defended in direct proportion of their value (Rhoades and Cates 1976). Therefore, younger leaves have a higher value than mature leaves because they have a high future reinvestment potential (photosynthate) compared to mature leaves (Harper 1989). Additionally, younger leaves have increased nitrogen concentrations and are preferred by herbivores (Coley 1980, Mattson 1980, Kursar and Coley 1991, Kaplan et al. 2011). Multiple studies have confirmed higher furanocoumarin levels in younger leaves compared to mature leaves (Zobel and Brown 1990, Heath-Pagliuso et al. 1992, McCloud et al. 1992). Zobel and Brown (1990) estimated that furanocoumarin concentrations can be 100 times higher in new foliage compared to mature leaves. Therefore, it is possible that browsing occurs on less toxic leaves later in the season on
less chemically defended shaded prickly ash and thorns may deter herbivory during this time of year.

By investment more in reproduction female plants have reduced growth rates, and slower growing plants are predicted to have greater allocations towards defenses (Herms and Mattson 1992). However, both males and females growing in sun increased total phenolics and total furanocoumarin concentrations. The growth-differentiation hypothesis predicts a nonlinear relationship between tradeoffs between growth and secondary defenses, with both growth and defenses increasing when resources increase from low to moderate levels (Herms and Mattson 1992). Therefore, a positive correlation between growth and secondary metabolites is possible even when tradeoffs occur between common precursors. Thus, both fast growing males and slow growing female prickly ash increased total furanocoumarin and total phenolics concentrations in canopy gaps. Furanocoumarins are produced from the phenylpropanoid pathway (Hahlbrock and Scheel 1989). An important precursor enzyme in the phenylpropanoid pathway is phenylalanine ammonia-lyase (PAL). UV light transcriptionally activates the production of PAL and 4-comarate: COA lyase, the final enzyme in the production of furanocoumarins (Chappell and Hahlbrock 1984, Hahlbrock and Scheel 1989, Logemann et al. 1995). Therefore, furanocoumarin biosynthesis is induced by ultraviolet radiation (UV) and furanocoumarins increase in concentration in the presence of UV light. Increased carbon allocation to the phenylpropanoid pathway result in increased furanocoumarin production (Hahlbrock and Scheel 1989). Therefore, prickly ash
growing in canopy gaps are predicted to increase furanocoumarin production due to increased photosynthesis.

Male investment in furanocoumarins in canopy gaps may not function only as an antiherbivore defensive mechanism. Defensive compounds may serve multiple plant functions in addition to reducing herbivory (Herms and Mattson 1992, Karban and Baldwin 1997), including protection from UV light (Rhoades 1977, Lee and Lowry 1980, Les and Sheridan 1990, Zobel and Brown 1993, Caldwell et al. 2006). Plants experience photoinhibition at high light levels. Photoinhibition results from high UV radiation and damages photosystem II, thus reducing photosynthetic efficiency (Asada et al. 1987, Barber and Andersson 1992). Plants experiencing UV stress in high light environments increase phenolics in leaf epidermal tissue to absorb excess light and therefore prevent damage to lower mesophyll cells and photosystem II (Larcher 1995, Stapleton 1992). Solecka (1997) suggested that furanocoumarins protect plants from UV stress in high light environments. Several studies have observed photoprotective properties of flavonoids in high light environments (Havaux and Kloppstech 2001, Jordan 2002, Bassman 2004, Burchard et al. 2008) in addition to their role as antiherbivore defensive compounds (Gould et al. 2006, Treutter 2006). Flavonoids and furanocoumarins are produced by the phenylpropanoid pathway and require the same precursor (PAL), which is up regulated in the presence of UV. Therefore, furanocoumarin toxicity may have arisen as an exaptation with the primary role being photoprotection. Plants with photoprotective compounds such as furanocoumarins could experience reduced competition for nutrients if they could persist in high light environments where other
trees and shrubs have reduced fitness due to increased photoinhibition. Therefore, furanocoumarin up-regulation in canopy gaps may first act to shield sensitive plant tissues from extreme and harmful UV radiation. Reduced photosynthesis and photodamage would negatively affect both male and female plants. Therefore, it is not surprising that both males and females increase furanocoumarin concentrations in canopy gaps.

Male prickly ash foliage had higher concentrations of nitrogen than female plants. The increased nutrients required for female fruit production probably resulted in less nitrogen availability in female foliage (Herms and Mattson 1992). Within leaves nitrogen concentration is positively correlated with photosynthetic capacity and most of nitrogen within a leaf is devoted to photosynthesis (Field and Mooney 1986, Hirose and Werger 1987, Makino and Osmond 1991, Walcroft et al. 1997). Nitrogen deficient plants have reduced photosynthetic rates (Boussadia et al. 2010). Increased photosynthetic capability associated with increased leaf nitrogen concentration can increase photosynthate accumulations that can be invested in growth. Fruit production by females requires more nutrient investment in reproduction; therefore, females have less nitrogen available for leaves (Stiles 1980, Cipollini and Stiles 1991). Leigh et al. (2006) observed similar results in a dioecious shrub, with males investing more in leaf nitrogen compared to females.

Leaf nitrogen concentration is typically positively correlated with furanocoumarin concentration and furanocoumarin concentrations are positively associated with UV light (Berenbaum 1978, Berenbaum 1981b). Sunny prickly ash leaves contained more total
furanocoumarins compared to shaded leaves. However, light did not affect leaf nitrogen concentration. A few studies have observed a negative correlation of nitrogen and xanthotoxin (Berenbaum et al. 1989, Dercks et al. 1990), whereas others studies have failed to find these correlations (Reitz et al. 1997). Xanthotoxin concentrations in prickly ash leaves were intermediate between lower levels of psoralen and higher levels of bergapten. Therefore, the negative correlation of xanthotoxin with nitrogen may cancel out the positive correlations of bergapten and psoralen. Alternatively, leaves used for nitrogen analysis during this study where fully mature and collected when second generation giant swallowtail larvae were actively feeding in the field. Young leaves have higher concentrations of furanocoumarins and nitrogen (Coley 1980, Mattson 1980, Sribner and Slansky 1981, Zobel and Brown 1990, Heath-Pagliuso et al. 1992, McCloud et al. 1992). Nitrogen levels in mature prickly ash leaves might be diluted at a faster rate than furanocoumarins. Male prickly ash may have maintained high levels of nitrogen in order to continue gaining carbon and growing. The related species Chinese prickly ash, *Zanthoxylum ailanthoides*, continues to grow and shoots continue to elongate until September (Watanabe 1979). Males could store photosynthate received later in the season to allocate towards growth the following spring.

Plant tissue contains about 2% nitrogen, yet insect herbivore bodies are composed of 40% nitrogen; thus, nitrogen is often a limiting factor affecting insect herbivore growth, and insect growth is determined by the ability to metabolize and convert nitrogen efficiently (Southwood 1973, Sribner 1984). Therefore, increases in leaf nitrogen concentration often lead to increased herbivory, and small differences in nitrogen
concentration can affect herbivore performance (Mattson 1980, Scriber and Slansky 1981, Scriber 1984, Mattson and Scriber 1987, Huberty and Denno 2006). Since male prickly ash had higher foliar nitrogen concentrations and shaded prickly ash had lower total furanocoumarin concentrations, swallowtail larvae consuming shaded male prickly ash would be predicted to have higher growth rates. However, no interaction between light and plant sex occurred in the larvae feeding bioassays. This may be explained by the quality of nitrogen within the leaf. Cockfield (1988) suggested that herbivores can metabolize amino acids quickly whereas complex proteins require breakdown before assimilation. Total nitrogen concentration was analyzed in prickly ash leaves and the quality of nitrogen was not quantified. If increased nitrogen concentration in male prickly ash leaves was composed of complex protein molecules, increased nitrogen may not increase the performance of giant swallowtail larvae because the metabolic costs of digesting the proteins was high. Alternatively, although leaf nitrogen concentration is positively correlated with herbivore growth, it may not be the most limited essential nutrient (Odum 1971). Therefore, if swallowtail growth on prickly ash is limited by a nutrient other than nitrogen, additional nitrogen resources would not increase larval growth.

Specialist herbivores are extremely efficient at metabolizing toxic furanocoumarins. For example, the black swallowtail, *Papilio polyxenes*, which consumes plants containing furanocoumarins, detoxified 50% of consumed compounds within 1.5 hrs and completely detoxified the compounds with 24 hr (Bull et al. 1986). Although giant swallowtail caterpillars are specialists on furanocoumarin containing
plants, in both years of this study fourth instar larvae consuming prickly ash leaves from plants growing in gaps had reduced relative growth rates compared to larvae consuming foliage from shaded plants. Foliage from prickly ash in canopy gaps had significantly higher concentrations of total furanocoumarins. However, no individual furanocoumarins differed in concentration among treatments. This suggests that there is a synergistic interaction between furanocoumarins. Increased total furanocoumarin concentration in sunny foliage is the likely mechanism for reduced growth rates in swallowtail larvae. This is likely due to a single specific P450 that is responsible for detoxifying all furanocoumarins. Therefore, competition for the active site of the P450 increases as total furanocoumarins increase. Increased furanocoumarins could overwhelm P450 metabolism. Swallowtail larvae may have reduced consumption rates in order to alleviate the increased toxicity. Reduced consumption rates likely caused the result in reduced growth rates.

Previous studies have suggested that specialized P450’s detoxify multiple furanocoumarins. For example, cytochrome CYP6B1 from the black swallowtail can metabolize xanthotoxin and other furanocoumarins (Cohen et al. 1992). Ma et al. (1994) suggested that detoxification of xanthotoxin and bergapten inhibit each other in the black swallowtail, perhaps due to completion for an active site on the P450. Furanocoumarin biosynthesis is extremely energetically costly to plants, requiring extensive resource allocations. Gershenzon (1994) estimated that to produced 1 g of psoralen, 3.39 g of glucose were required. Psoralen, bergapten, and xanthotoxin were identified in prickly ash leaves. Both xanthotoxin and bergapten are derivates of psoralen and therefore
require more energy to produce due to biosynthetic modifications (Zangerl and Berenbaum 1997). Zangerl and Berenbaum (1997) suggested that furanocoumarin biosynthesis results in direct tradeoffs to plant fitness. They estimated that for each milligram per gram production of furanocoumarins, seed biomass was reduced by 37 mg. Single furanocoumarins have lethal effects and deter generalist herbivores. For example, an LC$_{50}$ of 0.0004% was observed when xanthotoxin was added to artificial diet of cabbage loppers (Ahmad et al. 1987). Likewise, when xanthotoxin was added to armyworm diets, larvae failed to develop beyond second instars resulting in 100% mortality of larvae (Berenbaum 1978). Since single furanocoumarins have such lethal effects on generalist herbivores, there should be no selection pressure to produce additional furanocoumarins, especially when considering the cost of production.

The investment in additional furanocoumarins likely arises from the evolution of specialist ability to detoxify specific furanocoumarins. In fact, different furanocoumarins have different toxic effects. For example, black swallowtails and parsnip webworms, specialist herbivores that consume plants containing furanocoumarins, metabolized angular furanocoumarins more efficiently than linear furanocoumarins (Berenbaum and Zangerl 1993). Berenbaum and Feeny (1981) suggested that host plants evolved angular furanocoumarins to combat herbivores that previously evolved the ability to detoxify linear furanocoumarins. The three furanocoumarins identified in prickly ash are linear furanocoumarins. However, linear furanocoumarins can have differential toxicity. For instance, xanthotoxin is more toxic to beet armyworms than psoralen and bergapten (Diawara et al. 1993). In addition to increased photogenotoxicity, xanthotoxin has
increased photooxidant properties. Blan and Grossweiner (1987) estimated that xanthotoxin can produce three times more singlet oxygens than bergapten. Likewise, specialist herbivores have different detoxification abilities for different furanocoumarins. For example, xanthotoxin has no effect on webworm growth whereas bergapten slows growth as webworms are not as efficient at detoxifying this compound (Berenbaum et al. 1989).

Several studies have suggested that multiple linear furanocoumarins can have synergistic effects on herbivores. The combination of xanthotoxin, psoralen, and bergapten in beet armyworm diets increased larvae mortality compared to when larvae consumed individual furanocoumarins (Diawara et al. 1993). Likewise, a mixed furanocoumarin diet produced higher mortality than a xanthotoxin only diet in Heliothis zea (Berenbaum et al. 1991). Xanthotoxin detoxification in parsnip webworms was reduced by 60% when bergapten and sphondin were added in equal amounts compared to xanthotoxin only treatments (Zangerl and Berenbaum 1993). Multiple furanocoumarins within the same host plant that act synergistically against herbivores, suggesting they evolved in response to specialist detoxification of singular furanocoumarins and provide further evidence for stepwise covevolutionary arms races between herbivores and host plants. Phototoxic plants and specialist herbivore that feed on them are more likely to conform to stepwise evolution due to the reduced number of herbivore that feed on these plants (Arnason et al. 1992).

Negative impacts on growth have been demonstrated on other specialist herbivores consuming diets with increased secondary metabolites. Detoxification of
secondary metabolites by P450s can be energetically costly to herbivores (Brattsten et al. 1977, Scriber and Slansky 1981). For example, P450 detoxification in southern armyworm, *Spodoptera eridania*, consuming a diet with 0.5% nicotine significantly reduced larvae growth rates (Cresswell et al. 1992). The parsnip webworm regularly feed on plants containing furanocoumarins; however, webworm relative growth rates were reduced when bergapten was added to their diet (Berenbaum et al. 1989). P450s are inducible; therefore, herbivores on host plants containing no or low levels of secondary metabolites do not expend energy maintaining energetically expensive P450s (Nitao 1990). However, there are physiological limits on the ability of specialists to up regulate P450s and detoxify plant allelochemicals (Zangerl and Berenbaum 1993). As defensive compounds increase in host plants, herbivore P450s are induced and up regulated. This requires extensive energetic resources. Resources required to up regulate P450 and detoxify metabolites are diverted from resources that would be allocated to herbivore growth. Berenbaum and Zangerl (1994) suggest that detoxification of xanthotoxin by specialist webworms results in a cost to larvae growth. Therefore, as host plants become more toxic, herbivore growth is slowed as they divert resources from growth to detoxification.

Fourth instar giant swallowtails experienced reduced growth rates when consuming sunny prickly ash compared to larvae consuming shaded leaves. Sunny foliage had higher levels of total furanocoumarins. Therefore, larvae consuming sunny foliage needed to detoxify more furanocoumarins and divert resources from growth to detoxification. As predicted by Scriber and Slansky (1981), first instars had higher
relative growth rates compared to fourth instars, perhaps because fourth instars are less selective feeders than early instars. Furthermore, the decreases in gut surface area to body volume and the size of leaf material consumed by later instars may reduce digestibility (Scriber and Slansky 1981). Additionally, later instars consume a greater proportion of fiber that can result in lower digestibility (Slansky and Scriber 1985).

No differences in growth rates were observed between first instar larvae consuming sunny and shaded prickly ash. First instars are typically more sensitive to defensive compounds compared to older instars, possibly due to higher consumption rates resulting in increased encounters of toxins relative to their body weight (Scriber and Slansky 1981, Slansky and Scriber 1985). First instars typically experience greater reductions in growth rates or mortality when consuming diets high in secondary metabolites compared to older instars.

Numerous studies have observed increased negative effects of toxins on younger larvae. Lahtinen et al. (2004) observed no effects on growth rates of fifth instar geometrid larvae consuming flavonoids, but first instars suffered reduced growth and mortality consuming the same concentrations of flavonoids. Early instars of gypsy moths were more susceptible to BT than older instars (Herms 2003), and phenolic glycosides reduced growth rates of first instars but fourth instars were unaffected by the same concentrations (Lindroth and Hemming 1990). Hedin et al. (1988) found that cotton, *Gossypium hirsutum*, allelochemicals were toxic to neonates but had no effect on older instars in the tobacco budworm, *Heliothis virescens*. Neonate larvae of specialist herbivores are more susceptible to host plant secondary metabolites. First instar parsnip
webworms consuming plants with high furanocoumarin concentrations died, whereas fifth instars experienced reduced growth but survived (Zangerl and Berenbaum 1993). Likewise, Erickson (1973) observed no effects on growth of late instar specialist monarch caterpillars consuming toxin cardiac glycosides. However, the development time and mortality of first instar monarch, *Danaus plexippus*, larvae increased with increasing concentrations of cardiac glycosides (Zalucki and Brower 1992, Zalucki and Malcolm 1999, Zalucki et al. 2001).

A possible explanation for the lack of increased susceptibility of first instar giant swallowtail larvae consuming foliage with greater furanocoumarin content could be preprogrammed increased P450 activity in first instars. Lepidopteran larvae and adult female swallowtails are unable to access furanocoumarins in host plants (Berenbaum and Zangerl 1992, Zangerl and Berenbaum 1993). Since gravid female swallowtails preferentially oviposit on sunny foliage, first instar larvae have a high probability of encountering high levels of furanocoumarins. Neonate caterpillars must begin consuming food immediately or risk mortality. Therefore, they cannot wait to up-regulate P450’s when contacting furanocoumarins.

Harrison et al. (2001) quantified P450 activity in different life stages of the black swallowtail and found that P450s were down regulated or had no activity in life stages that did not consume furanocoumarins such as eggs, pupa, and adults. Other studies have noted that P450 activity is greatest in larval instars that consume the most toxins because of increased feeding activity (Ahmad 1986, Stevens et al. 2000). Reduced P450 activity in life stages with decreased contact with allelochemicals likely evolved as a cost saving
mechanism (Cianfroagna et al. 2002a). When detoxification enzymes are not required, energy can be diverted to growth instead of detoxification.

Other studies have failed to detect reduced performance and survival of larvae consuming increased toxins. For example, a recent study quantifying P450 activity in different larval stages of emerald ash borers found no difference in P450 levels in neonates and late instar larvae (Personal Communication Priya Rajarapuswapna), suggesting that neonate detoxification enzymes are up regulated before larvae begin consuming host plant metabolites. Likewise, increased xanthotoxin had no effects on black swallowtail or two-tailed swallowtail growth rates (Berenbaum 1981a, Mao et al. 2006). Likewise, Herms et al. (1997) found that three-day-old Karner blue, *Lycaeides melissa samuelis*, instar survival was higher than later instars on foliage containing low levels of BT. Manuwoto and Scriber (1986) found no difference in the growth and survival of promethean silkmoth, *Callosamia promethean*, first instar larvae consuming diets with increased tannic acid or condensed tannins. Black swallowtail may benefit from increased furanocoumarin concentrations (Berenbaum 1981a). Black swallowtails consuming diets with xanthotoxin had increased growth rates and attained larger masses than larvae without xanthotoxin in their diets, suggesting that furanocoumarins may act as a feeding stimulant.

Although no differences were observed in first instar giant swallowtail growth rates, fourth instar giant swallowtails feeding on foliage with higher furanocoumarins levels experienced slower growth rates. Prior to initiation of bioassays, fourth instar larvae were maintained on shaded prickly ash. Since shaded prickly ash leaves contain
lower total furanocoumarins, larvae likely down regulated P450 activity and diverted
energy from detoxification metabolism towards growth. When feeding bioassays began,
fourth instars consuming sunny foliage may have needed to up regulate their P450
activity to detoxify furanocoumarins. This could have resulted in reduced growth rates.
In addition to up regulating P450s when encountering high concentrations of toxins,
insects can down regulate P450 activity, perhaps as an energetic cost saving mechanism
(Davies et al. 2006). Neonate larvae on sunny plants would maintain high P450 activity
throughout all instars because of high furanocoumarin levels and thus have lower growth
rates compared to swallowtails in forests without EAB.

Switching giant swallowtail larvae diets from shaded prickly ash foliage to sunny
foliage may be analogous to switching host plants. Sunny prickly ash foliage contained
different secondary chemistry and possibly primary metabolites. Although diet mixing
may benefit generalist species by reducing the effects of specific qualitative defenses,
some studies have observed detrimental effects of host plant switching on insect
herbivores (Dethier 1988, Bernays and Minkenberg 1997, Karban and Baldwin 1997, van
Dam et al. 2000). Polyphagous lepidopteran larvae typically remain on a single host
plant throughout larval development (Scriber 1975, Mattson 1977, Barbosa et al. 1986).
When generalist larvae are switched to a different alternative host plant mid way though
larvae development, they usually experience reduced growth rates (Scriber 1979).
Reduced growth rates would not occur if the larvae started and remained feeding on the
alternative host. All third instar tiger swallowtails switched to spicebush, *Lindera
benzoin*, from black cherry, *Prunus serotina*, foliage died before reaching pupation.
(Scriber 1979). However, tiger swallowtails are able to survive on spicebush when hatching on this host plant. Scriber (1979) observed reduced larvae growth rates, for both a polyphagous and specialist swallowtails when host plants were switched on third instars, and suggested that up-regulation of detoxification metabolism was the mechanism resulting in reduced growth.

Insect herbivores must avoid predators (top down) while obtaining adequate nutrients (bottom up) for growth, development, and reproduction (Lawton and McNeill 1979). Therefore, laboratory feeding bioassays should be coupled with field predation bioassays if addressing ecological questions (Scriber and Slansky 1981). Light availability can impact host plant quality and predator abundance (Richards and Coley 2008) and adjacent sunny and shaded patches can have different predation pressures and nutrient availability for herbivorous insects (Maiorana 2008). Furthermore, increased productivity leads to top down control, whereas reduced productivity results in bottom up control (Richards and Coley 2006). Multiple studies have suggested that phytophagous insects prefer to feed on and are more abundant on sunny foliage (Lincoln and Mooney 1984, Shure and Phillips 1991, Louda and Rodman 1996, Jokimäki et al. 1998, Sipura and Tahvanainen 2003, Richards and Coley 2006) and herbivore performance is often enhanced on sunny plants. For example, the *Zunacetha annulata* caterpillar experienced higher growth rates and greater pupae weights on shrubs growing in canopy gaps compared to larvae growing on shaded plants (Harrison 1987). Lepidopteran pupae weight was correlated with adult fecundity, with larger pupae producing more eggs (Hespenheide 1973, Slansky and Scriber 1985, Honěk 1993). Richards and Coley (2008)
conducted feeding bioassays on the same species and observed higher growth rates and increased pupae weights from caterpillars consuming sunny foliage compared to shaded foliage.

Increased herbivore abundance in canopy gaps can result in increased insect predator abundance in gaps (Richards and Coley 2006). The enemies hypothesis predicts increased predator abundance in complex habitats, which in turn decreases herbivores in these habitats (Root 1973). Canopy gap formation in forested landscapes can be considered increasing complexity of the habitat. Richards and Coley (2006) observed a 40% increase in predation in canopy gaps compared to the understory. However, Hanks and Denno (1993) observed increased predation of herbivores in forested habitats compared to opened sunny habitats.

Forsman et al. (2010) observed increased bird abundance in forest canopy gaps. Likewise, canopy gaps resulting from EAB-induced ash mortality have resulted in increased bird diversity and species richness (Larry Long, personal communication). Avian predators have dramatic impacts on lepidopteran larvae in forest systems. Bird predators are typically responsible for late instar caterpillar mortality whereas arthropods are responsible for early instar mortality (Pollard 1979, Feeny et al. 1985, Stefanescu 2000, Nixon and Roland 2012). Bird predators inflicted 55% mortality per day on forest tent caterpillars (Nixon and Roland 2012) Birds are reported as the number one predator of cabbage butterfly larvae and can be responsible for 80% mortality of late instars per day (Baker 1970) and Stefanescu (2000) suggests birds are the main predators of swallowtail larvae.
Arthropod predators may be more abundant in canopy gaps. For example Lichtenberg and Lichtenberg (2003) observed increased caterpillar mortality due to vespid attack in open canopies compared to understory. Ants, an important predator of lepidopteran larvae, are also more abundant on plants in sunny habitats compared to shade (Montllor and Bernays 1993, Yamawo and Hada 2010). Ant foraging activity on prickly ash is high especially in canopy gaps (personal observation) suggesting prickly ash may possess extrafloral nectaries. Ants attracted to extrafloral nectarines for carbohydrates will remove neonate larvae and eggs return them to the next as a protein source (Barton 1986).

Previous field studies suggested that first instars swallowtails suffered greater predation than later instars. Feeny et al. (1985) observed greater predation on first instar black swallowtails compared to later instars. Likewise first instar pipevine swallowtails, *Battus philenor*, experienced more predation compared to older instars (Fordyce and Shapiro 2003, Fordyce 2006). Zalucki et al. (2002) estimated that early instar lepidopteran larvae predation can be as high as 96%. Field studies quantifying monarch butterfly larvae survival estimated that 88-97% of first instars suffer from mortality (Zalucki and Brower 1992). First instar lepidopteran larvae are also more prone to mortality from abiotic forces such as drowning, desiccation, and high and low temperatures (Harcourt 1966, Blau 1980, Qayyum and Zalucki 1987, Annamalai et al. 1988, Fordyce and Shapiro 2003). In this study, light availability and instar stage had no significant effect on giant swallowtail larvae survival. However, more predation occurred in canopy gaps. Both first and fourth instars experienced high predation on
shaded and sunny prickly ash. Within 48 hours 39.5% of larvae in sun and 25% of larvae in the shade were preyed upon. Although predation events were not directly observed by researchers, they are the most likely factor leading to larvae disappearance. Larvae were placed on prickly ash and allowed to acclimate before researchers left the plant. Caterpillars were observed until they began actively moving on foliage, indicating they had obtained adequate grip. Prickly ash plants used in predation bioassays were not touching other plants. Therefore, larvae were restricted to the plant they were placed on. Furthermore, swallowtail larvae do not typically wander off host plants (Scriber 1975, Feeny et al. 1985). Other field studies conducted on lepidopteran larvae predation have quantified and confirmed larvae disappearance with predation events (Dempster 1971, Feeny et al. 1985).

Predation is the leading cause of swallowtail mortality (Feeny et al. 1985). Feeny et al. (1985) estimated that female black swallowtails must produce 60 eggs in order to replace themselves once in the next generation. Different predators likely exist in different habitats. For example, Shrewsbury and Raupp (2006) suggested that generalist predators are more abundant in complex habitats whereas specialists are more abundant in simple habitats. Other studies observed increased predation in shaded environments compared to sunny habitats (Hanks and Denno 1993, Trumbule et al. 1995). Slansky (1982) suggested that insects in sunlight experience greater predation and parasitism compared to those remaining in shade. Therefore, different predators could be responsible for predation of swallowtail larvae in sun versus shade.
The eggs, larvae and pupae of swallowtails are associated with numerous parasitoids (Feeny et al. 1985). Our study did not quantify the effects of parasitoids in the predation study. It is possible that parasitism rates differ among habitats. Parasitoids use more reliable physical and chemical signals (Price et al. 1980) and therefore may locate swallowtail easier in a specific habitat. Therefore, parasitism of eggs, larvae, and pupae may be different in sun and shaded prickly ash.

Fitness parameters of parasitoids such as clutch size and sex ratio are often correlated with host body size, and parasitoids select optimal hosts (Brault 1991). Swallowtail larvae consuming shaded prickly ash may result in larger final pupae mass due to faster developmental rates. This may be especially true for second-generation giant swallowtail larvae that must enter pupal diapause before leaves senesce and temperatures decline. Parasitoid fitness is positively correlated with host pupae mass (Harvey et al. 2003). If giant swallowtail pupae are larger in shaded habitats they may be preferred by and suffer greater mortality by pupal parasitoids. Such interactions would be consistent with the high performance high mortality hypothesis proposed by Singer et al. (2012). There are at least four pupae parasitoids that attack giant swallowtails (McAuslane 2009) and multiple Trichogramma parasitoids attack swallowtail eggs. Parasitoid host selection factors such as host size or habitat preference may interact to differentially affect swallowtail survival. Therefore, if longer predation bioassays were conducted, differences in first and fourth instar survival may become evident.

Furanocoumarins are rapidly detoxified by giant swallowtail larvae, and larvae do not sequester the compounds. Therefore, consuming foliage with high levels of
furanocoumarins is not predicted to deter larval predation. However, studies on the
parsnip webworm, another herbivore that does not sequester but detoxifies
furanocoumarins from its host plant, found low levels of unmetabolized furanocoumarins
in the hemolymph of webworms (Ode 2006). McGovern et al. (2006) suggested that
parsnip webworm parasitoids likely encountered furanocoumarins in webworm
hemolymph. Furthermore the larval parasitoid of webworm, *Campoletis sonorensis*, had
reduced survivability and reduced clutch sizes when reared on webworms that consumed
foliage with high concentrations of furanocoumarins (Ode et al. 2004). Most
swallowtails, including giant swallowtails, prefer to oviposit in sunny habitats. If
parasitoid pressure is high and parasitoids are negatively affected by plant secondary
metabolites, selection pressure might favor ovipositing in sunny habitats. Parasitoid
pressure may be greater than general predation pressure. Therefore, the reduced growth
rates observed from feeding on sunny foliage may outweigh the risks associated with
uncheck parasitism in shaded habitats. Faeth et al. (1981) suggested that top down
effects could be more important than plant quality. Egg and larvae parasitism is a major
mortality factor for swallowtails (Feeny et al. 1985). Egg and larvae development is
faster on sunny plants, requiring fewer accumulated degree days to pupation and several
studies suggest insects favor ovipositing in sunny patches to reduce development time
(Grossmueller and Lederhouse 1985, Slansky and Scriber 1985, Cappuccino and Root
2008). In general, swallowtail larvae on sunny plants developed with 35% fewer growth-
degree-hours than larvae on shaded plants (Grossmueller and Lederhouse 1985).
Therefore, if parasitism pressure is high, selection will favor egg laying in sunny habitats.
Scriber and Slansky (1981) suggested that for some lepidopteran, temperature could be more important than host plant quality for development. Lastly, ovipositing in sunny patches may reduce fungal or bacterial infections of eggs and larvae. Furanocoumarins have strong antifungal and antimicrobial effects (Murray et al. 1982, Desjardins et al. 1989, Baï-Yeboa et al. 2005). Lepidopteran eggs and larvae are often attacked by viruses and bacteria that eventually result in swallowtail mortality (Carter and Feeny 1985). If sunny prickly ash foliage reduced these mortality factors due to the high levels of furanocoumarins, selection may favor ovipositing in sunny patches.

Results from giant swallowtail feeding and predation bioassays are consistent with the slow-growth high mortality hypothesis that predicts slower growth rates will increase the exposure time of natural enemies, thereby increasing the probability of predation. (Price et al. 1980, Clancy and Price 1987). The slow-growth high mortality hypothesis has been supported by numerous studies. For instance, Häggström and Larsson (1995) found that when the leaf beetle, Galerucella lineola, was reared on low quality host plants growth was delayed. As a result, predation on these beetles was more intense than on beetles developing faster on better host plants. Likewise, cabbage white butterfly, Pieris rapae, larvae feeding on plants with low nitrogen content experienced reduced growth rates and suffered greater mortality from predators and parasitoids than fast growing larvae on nitrogen rich plants (Loader and Damman 1991). Fordyce (2006) observed that slower growing swallowtails experienced higher mortality. Williams (1999) suggested that the slow growth-high mortality hypothesis is most relevant for surface feeding herbivores, such as swallowtail larvae that are more prone to predators.
The predation bioassays confirm that giant swallowtail larvae are under intense predation pressure. Within 48 hours, 25% of giant swallowtail larvae suffered mortality. Giant swallowtail larvae required at least 20 days to complete larval development (Bullock and Pelosi 1992). Intense predation occurred on both early and late instar larvae. Therefore, it is likely that most giant swallowtail larvae suffered mortality before developing into sexually reproducing adults. Fourth instars had reduced growth rates when they consumed foliage from canopy gaps that had higher concentrations of furanocoumarins those that consumed shaded leaves. This growth reduction can be explained by increased P450 detoxification that diverts energy away from larvae growth. Since giant swallowtail larvae experience heavy predation, delayed growth rates will increase the probability of predation. Therefore, giant swallowtail larvae consuming prickly ash plants located in forests with EAB will experience greater predation and mortality. In addition to increases predator exposure, delays in larvae growth can affect adult fecundity. Delays in larvae development will likely delay adult emergence, especially for first generation giant swallowtails that do not enter diapause. Delayed adult emergence will increase the age of first reproduction in adults. Small increases in age of first reproduction can lead to large effects on fitness, especially for short lived organisms such as swallowtails (Snell 1978, Blau 1981). Therefore, giant swallowtails developing in forests with EAB may produce less offspring. In addition to increased predation, this could have serious impacts for giant swallowtail butterflies at the population level. First generation giant swallowtails do not enter diapause and overwinter; therefore, these adults are typically larger and more fecund than the second
generation (Kimura and Tsubaki 1986). Increased predation paired with reduced adult fecundity could negatively affect the population dynamics of this rare butterfly species.

An overwhelming majority of studies investigating the impacts of invasive herbivores have focused on direct effects or indirect competitive effects on host plant quality. This study provides evidence that an invasive herbivore can also affect native host plant quality in plants that the invasive herbivore does not consume. EAB does not directly interact with prickly ash. However, it indirectly affects the quality of prickly ash foliage by producing large canopy gaps that induce physiological changes within prickly ash. Changes in prickly ash chemistry resulting from canopy gap formation indirectly negatively affect native giant swallowtail larvae by delaying development and increasing exposure to natural enemies interactions.
References


Smith, A. 2006. Effects of community structure on forest susceptibility and response to the emerald ash borer invasion of the Huron River watershed in southeastern Michigan. Entomology. The Ohio State University, Columbus, OH.


Chapter 3

Indirect Effects of an Alien Insect Herbivore on Native Plant Photosynthesis, Gas Exchange and Water Use Efficiency

Abstract:
Emerald ash borer, *Agrilus planipennis* (EAB), is an invasive herbivorous insect that attacks and kills healthy North American ash *Fraxinus* spp. trees. In EAB infested forests, large canopy gaps form as ash trees die. Canopy gaps increase light availability to understory plants, which can induce several physiological changes such as increased photosynthesis. Gaps also increase temperature and water vapor deficit, which can result in increased plant water loss due to increased evapotranspiration.

Trade-offs occur in resource allocations between male and female plants. Male plants are predicted to invest in growth whereas females are predicted to invest in reproduction and defense. Differential investment in resources can result in different physiological responses such as photosynthetic capacity, water use efficiency, and stomatal conductance. This study quantified differential physiological responses between male and female prickly ash, *Zanthoxylum americanum*, growing in EAB-induced canopy gaps and the shaded understory.
Prickly ash growing in canopy gaps had higher rates of photosynthesis and stomatal conductance and lower instantaneous water use efficiency. No differences were observed in male and female photosynthetic rates, water use efficiency or stomatal conductance.
Introduction

The invasive emerald ash borer, *Agrilus planipennis* (EAB), was unintentionally introduced to the Midwestern United States approximately 20 years ago (Cappaert et al. 2005). Since its arrival this invasive insect has had a dramatic effect on Midwestern North American forests, killing millions of ash trees (Poland 2007). Wide-scale canopy gaps created by invasive herbivores such as EAB do not mimic natural canopy gap formation. Invasive herbivores are often able to attack and kill native trees due to a lack of coevolutionary histories, whereas native herbivore tree mortality is usually restricted to stressed plants. Therefore, canopy gaps created by invasive herbivores are often larger, more wide spread, and faster forming than canopy gaps created by natural successional events (Gandhi and Herms 2010). Large-scale canopy gaps created by EAB may positively affect understory plants. Gap formation increases light availability to the forest floor and understory plants. Light availability is positively correlated with photosynthesis and plant growth, and reproductive output increases with increasing photosynthesis (Herms and Mattson 1992, Richards and Coley 2008). Therefore, native plants may indirectly benefit from EAB-induced canopy gaps.

Prickly ash, *Zanthoxylum americanum*, is a ubiquitous native Midwestern dioecious shrub. Numerous studies have observed tradeoffs in resource allocations among dioecious plants. For instance, males often invest more resources towards growth, resulting in greater above ground biomass, while females invest more in reproduction (Bullock 1984, Gehring and Linhart 1993, Hemborg 1998, Nicotra 1999, Obeso 2002).
This trend was apparent in previous studies conducted on prickly ash. Popp and Reinartz (1988) found that male prickly ash plants allocated more biomass to vegetative growth and less to reproduction compared to female plants.

Differential allocation of resources between male and female plants often results in different physiological responses. For instance Dudley and Galen (2007) suggest females should have decreased water use efficiency compared to males because they need to maintain greater carbon assimilation rates to compensate for their larger investments in reproduction. Therefore, females should maximize photosynthetic efforts regardless of associated transpirational water loss. Fruit production requires high photosynthetic rates (Wardlaw 1990) and female plants are predicted to have higher photosynthetic rates than males (Obeso 2002). Multiple studies have observed increased rates of photosynthesis in female plants (Dawson and Bliss 1989, Dawson and Ehleringer 1993, Lokker et al. 1994, Laporte and Delph 1996). Several studies have observed increased WUE in male plants (Dawson and Ehleringer 1993, Leigh and Nicotra 2003). Furthermore, differences in WUE can be exacerbated by environmental conditions. Guangxiu et al. (2009) suggested that abiotic factors can drive the physiological differences observed in dioecious plants. For instance, male boxelder trees tolerate drought conditions better than females (Dawson and Ehleringer 1993). Likewise, Dawson and Bliss (1989) observed that male willows exhibited greater osmotic adjustments compared to females under drought conditions. A few studies have observed greater WUE in female plants (Gehring and Monson 1994, Retuerto et al. 2008).
Male and female plants can display different morphological characteristics that affect WUE. For example, male *Hippophate rhamnoides* plants tended to have higher stomatal densities (Guangxiu et al. 2009). However, other studies have observed opposite trends. For example, female *Acer negundo* were found to have higher stomatal densities (Dawson and Ehleringer 1993). Therefore, different species likely exhibit different physiological responses with varying environmental conditions. In addition to drought, increased UV radiation can affect WUE. Robson et al. (2009) observed that seedlings in canopy gaps had greater WUE than understory plants even under drought conditions. Gap plants in reduced water treatments also exhibited greater WUE than shaded plants despite increased UV stress. The authors suggested that gap seedlings compensated for increased transpirational losses with increased osmotic adjustment and stomatal closing. The objectives of this research were to quantify physiological responses (photosynthetic capacity, water use efficiency and stomatal conductance) between male and female prickly ash growing in canopy gaps and the shaded understory.

**Methods**

Six male and six female prickly ash plants were growing in EAB-induced canopy gaps (15-20% gap) in Michigan forests. Each plant in a canopy gap was paired with a nearby prickly ash plant of the same sex, size, and architecture growing in the shaded understory (gap ≤ 5%). Plant locations were recorded using GPS. On 25 August, 2011, light saturated photosynthesis and stomatal conductance was measured on each plant using a portable photosynthesis meter (Li-6400, LI-COR, Inc. Lincoln, NE). Instantaneous WUE
(WUEi = photosynthesis/conductance) was calculated by dividing photosynthesis by conductance. Measurements were made in the morning from 8:30-10:30 AM.

Statistical Analysis.

A full factorial designed experiment was conducted to analyze the effects of EAB-induced canopy gaps on prickly ash physiology. Percent canopy gaps were quantified using handheld densitometers. A two way Analysis of Variance (ANOVA) was used to compare sun and shade on photosynthesis, stomatal conductance, and WUEi between male and female plants.

Results

Percent canopy gap differed between opened (24.59 ± 1.9) and closed (4.39 ± 0.29) gap treatments (F₁,₂₄=334.44, p<0.0001) (Fig.3.1). Canopy gaps did not differ between male and female prickly ash (F₁,₂₄=2.14, p=0.16) and no significant interaction occurred between sex and light (F₁,₂₄=2.08, p=0.17).
**Figure 3.1.** Percent canopy gap was greater in gap treatments compared to control treatment. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).

Light saturated photosynthesis was higher ($10.6725 \pm 0.8896 \, \mu\text{mol CO}_2 \, \text{m}^{-2} \text{s}^{-1}$) in prickly ash plants growing in canopy gaps than shaded plants ($5.5438 \pm 0.4233 \, \mu\text{mol CO}_2 \, \text{m}^{-2} \text{s}^{-1}$) ($F_{1,24}=27.01$, $p=0.0002$) (Fig 3.2). Male ($8.61 \pm 1.32 \, \mu\text{mol CO}_2 \, \text{m}^{-2} \text{s}^{-1}$) and female ($7.6061 \pm 1.01 \, \mu\text{mol CO}_2 \, \text{m}^{-2} \text{s}^{-1}$) plants did not differ in photosynthetic rates ($F_{1,24}=1.03$, $p=0.3291$) and no interaction occurred between sex and light ($F_{1,24}=0.92$, $p=0.3565$).
Figure 3.2. Prickly ash plants growing in canopy gaps had greater maximum photosynthesis than plants in the shade. Standard errors are depicted and bars with an asterisk (*) are significantly different (α = 0.05).

Prickly ash in the sun (0.3041 ± 0.0307 g, mol H$_2$O m$^{-2}$ s$^{-1}$) had higher stomatal conductance than those in the shade (0.1191 ± 0.0217 g, mol H$_2$O m$^{-2}$ s$^{-1}$) (F$_{1,24}$=23.76, p=0.0004) (Fig. 3.3). Stomatal conductance did not differ between male (0.2158 ± 0.0508 g, mol H$_2$O m$^{-2}$ s$^{-1}$) and female plants (0.2074 ± 0.0357 g, mol H$_2$O m$^{-2}$ s$^{-1}$) (F$_{1,24}$=0.05, p=0.8288) and no interaction between sex and light occurred (F$_{1,24}$=1.69, p=0.2178).
Figure 3.3. Canopy gaps increase prickly ash stomatal conductance. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).

Shaded prickly ash plants had greater instantaneous water use efficiency (55.4938 ± 7.3063 µmol/mmol) than plants in canopy gaps (36.2251 ± 2.756 µmol/mmol) ($F_{1,24}=5.73$, $p=0.0339$) (Fig. 3.4). Male (49.0846 ± 6.6077 µmol/mmol) and female (42.6344 ± 6.3926 µmol/mmol) plants did not differ in water use efficiency ($F_{1,24}=0.64$, $p=0.4385$), and no interaction between light and sex occurred ($F_{1,24}=0.53$, $p=0.4788$).
Figure 3.4. Shaded prickly ash had greater instantaneous water use efficiency than plants in canopy gaps. Standard errors are depicted and bars with an asterisk (*) are significantly different ($\alpha = 0.05$).

Discussion

Prickly ash growing in canopy gaps produced by EAB-induced ash mortality experienced increased photosynthetic capacity. Therefore, EAB indirectly benefits prickly ash. Increased photosynthate can increase prickly ash growth and reproductive outputs (fruits and flowers). Canopy gaps also increased prickly ash stomatal conductance. Sun leaves are thicker than shade leaves (Walters 2005) which results in increased cell abundance and cell differentiation (Herms and Mattson 1992). Therefore, sun leaves have greater
numbers of chloroplasts per leaf area, which increases photosynthetic capacity (Lambers et al. 1998).

Plant life history traits are confined by differential abiotic and biotic selection pressures and specific combinations of these pressures will reduced fitness (Grime 1977). Physiological tradeoffs exist among species that determine their survival in low versus high light conditions. Shade tolerant plants have lower respiration rates, lower stomatal density, and lower investments in photosynthetic machinery than high light tolerant plants and thus experience reduced growth rates compared to sun adapted plants (Walters 2005). Sun adapted plants are fast growers but unable to survive in heavily shaded habitats (Kobe et al. 1995, Pacala et al. 1996). Several plants have the ability to survive in shade and sun by acclimating their photosynthetic machinery (Thompson et al. 1992).

Prickly ash plants are able to survive in both shaded and sunny habitats. Results from this study suggest they do this by adjusting photosynthesis and stomatal conductance. Guard cells control stomatal openness in plants and can react to different environmental pressures extremely quickly (Nilson and Assmann 2007). Therefore, stomata control CO₂ assimilation and transpiration. Stomatal conductance is the rate of CO₂ uptake and water vapor expulsion through leaf stomata (Lombardozzi et al. 2012). Low stomatal conductance can reduce photosynthesis by decreasing carbon availability. Since CO₂ uptake is correlated with water loss, plants must balance the benefits of carbon gain with the detrimental effects of water loss. Canopy gaps in forests increase radiation and vapor pressure deficit, both of which increase transpirational water loss in plants.
(Robson et al. 2009). Therefore, prickly ash in canopy gaps have increased carbon assimilation but reduced water use efficiency due to greater stomatal conductance. Benefits from carbon gain likely outweigh the costs of water loss for prickly ash plants growing in gaps. Furthermore, plants in sun can decrease the negative impacts of water loss through osmotic adjustment. Sun plants have increased carbon gains and can actively increase carbon based solutes into cells, thereby resulting in lowered osmotic potential (Sanders and Arndt 2012).

Tradeoffs occur in resource allocations between male and female plants (Herms and Mattson 1992) and numerous studies have observed differences in photosynthetic capacity (Dawson and Bliss 1989, Nicotra et al. 2003, Schultz 2009) and water use efficiencies (Dawson and Ehleringer 1993, Leigh and Nicotra 2003, Dudley and Galen 2007) between male and female plants. However, male and female prickly ash did not differ in photosynthesis or water use efficiency. A few studies have suggested that differences observed in photosynthesis rates between male and female plants are only evident in extreme environments. For example, Jones et al. (1999) observed that male and female Salix arctica had similar photosynthetic rates at ambient temperatures, but males had higher rates when temperatures increased. Therefore, differential photosynthetic rates and water use efficiency in prickly ash may only be evident with increased stress.
References


Chapter 4

Summary and Future Work

The unintentional introduction of EAB to North American forests has resulted in the formation of large, wide-scale canopy gaps as EAB attacks and kills North American ash trees (Poland 2007). In 2004, prior wide spread ash decline resulting from EAB, canopy gaps in southeastern Michigan forests averaged less than 10% openness (Smith 2006). Dying ash trees caused canopy gaps to increase in size by 100% by 2006 (Smith 2006). Large gaps increased light availability to understory plants. Increased light can positively affect understory plants by increasing carbon gained from increased photosynthesis (Herms and Mattson 1992). Increased carbon resources can be invested in increased growth, reproductive outputs, and plant defenses (Richards and Coley 2008).

The growth-differentiation balance hypothesis predicts that male and female plants will allocate resources to growth and reproduction differently, with males investing more in growth and females investing more in reproduction and secondary metabolites (Herms and Mattson 1992). Female investment in reproduction comes at a cost to growth. Therefore, female plants typically grow slower than males. Slower growing plants are predicted to have increased secondary metabolites compared to fast growers.
Therefore, slower growing female plants may also contain higher concentrations of chemical defenses.

Prickly ash, *Zanthoxylum americanum*, is a native dioecious understory shrub (Family Rutacea) that is abundant in Midwestern forests. Prickly ash is moderately shade tolerant, but can induce physiological changes that enable it to survive in high light environments. We hypothesized that male and female prickly ash allocated resources to different physiological process. Furthermore, we predicted that males and females would respond to increased light availability resulting from dying ash differentially. We designed a field experiment to examine resource allocations between male and female prickly ash in canopy gaps created by EAB and the shaded understory. Plants chosen for gap treatments were growing under dead ash trees with a gap opening ranging between 15 and 20%. Plants in the shaded treatment, that were designed to mimic the forest canopy prior to EAB invasion, were located in the understory with less than 5% opened canopy. Gap openness was assessed using handheld densitometer above each prickly ash. Each densitometer reading was conducted by the same researcher in order to reduce variation and error.

EAB canopy gaps increased light reaching understory plants. Prickly ash situated under canopy gaps had increased photosynthetic rates compared to shaded plants, but sex of plant had no affect on photosynthesis. Canopy gaps had a positive effect on male prickly ash growth and female reproductive effort in both years of the study. In 2010, males in the sun grew 196% more than shaded males. Likewise, in 2011, males in the sun grew 177% more than shaded males. Male foliage contained higher nitrogen
concentrations than females. Female prickly ash did not grow more in sun than shade in both years. Both males and females increased flower production in canopy gaps. Additionally, females increased fruit production in canopy gaps. Females in the sun produced 797% more fruits than those in shade in 2010, and 527% more fruits in 2011. Both male and females had more total phenolics, total furanocoumarins, and prickle density in the sun. Results from this research support the predictions of the growth-differentiation balance hypothesis. Male and female plants in gaps had increased photosynthetic capabilities and therefore had increased carbon resources. Male prickly ash invested these resources in growth whereas females invested them in reproductive efforts.

Furanocoumarins are photoactivated defense compounds that increase in toxicity and concentration when exposed to light (Berenbaum 1978), and prickly ash foliage contains furanocoumarins (Bafi-Yeboa et al. 2005). We hypothesized that prickly ash growing in gaps would have higher concentrations of furanocoumarins than those in shade. We identified three linear furanocoumarins (psoralen, bergapten, and xanthotoxin) in prickly ash foliage, and sunny foliage had higher total concentrations of furanocoumarins.

Canopy gaps induced changes in prickly ash secondary chemistry, growth, physical defenses, and reproductive investments. Host plant quality affects herbivore performance (Mattson 1980, Slansky and Scriber 1985). Therefore, we predicted that giant swallowtail larvae consuming sunny prickly ash foliage with higher concentrations of furanocoumarins would have reduced growth rates. Fourth instar giant swallowtails
had reduced growth when feeding on sunny leaves in both years. Early instars are often more susceptible to toxins than later instars (Scriber and Slansky 1981, Slansky and Scriber 1985, Hedin et al. 1988, Lindroth and Hemming 1990, Zangerl and Berenbaum 1993, Lahtinen et al. 2004). Therefore, we hypothesized that first instar giant caterpillars would experience greater growth reductions or mortality when consuming sunny prickly ash foliage compared to fourth instar larvae. Surprisingly, no reduction in growth was observed for first instars consuming sunny foliage. We hypothesize that first instar larvae have preprogrammed up regulated detoxification enzymes (P450’s) upon hatching, perhaps due to swallowtail preference to oviposit on sunny foliage that contains high levels of furanocoumarins. Future studies should examine and compare P450 activity in giant swallowtail instars and quantify possible tradeoffs that occur with up-regulation of detoxification enzymes.

The slow growth-high mortality hypothesis predicts that slower growing individuals will experience greater predation due to increased exposure to natural enemies (Price et al. 1980, Clancy and Price 1987). We hypothesized that slower developing giant swallowtails would experience increased predation. We also hypothesized that larvae located in canopy gaps would experience increased predation due to increased visibility to avian predators. Different predators attack different larval instars of swallowtail larvae (Pollard 1979, Feeny et al. 1985, Stefanescu 2000, Nixon and Roland 2012). For instance, birds consume older larvae whereas younger instars are consumed by arthropod predators. Therefore, we hypothesized that first and fourth instar larvae would experience different predation pressure. We found no difference in
mortality between first and fourth instars, and mortality was not different in sun or shade. However, giant swallowtail larvae experienced intense predation pressure; larvae experienced over 30% mortality within 48hrs. Swallowtail larvae are under intense predation pressure and longer larvae lifespan will increase the exposure time to natural enemies.

Increased giant swallowtail mortality due to increased developmental time could affect the population dynamics of this rare butterfly. Future studies should compare giant swallowtail butterfly counts in EAB aftermath and EAB unaffected forests to examine if there will be long term effects of EAB on giant swallowtail populations. Interestingly, giant swallowtails are reported to be increasing their northern range due to increased autumn temperatures (Finkbeiner et al. 2011). Future studies should compare prickly ash chemistry, growth, and reproductive tradeoffs in northern areas that have no coevolutionary history with giant swallowtails. Prickly ash growing in areas without swallowtail larvae may have reduced secondary metabolites, in order to increase growth and reproduction.

Prickly ash is a fast growing native plant that outcompetes most native trees (Barnes and Dibble 1988). Prickly ash could be used in EAB forest restoration projects. Large canopy gaps created by EAB result in an unnatural disturbance where invasive weeds can establish. Invasive plants free from natural enemies often have increased competitive abilities and therefore outcompete native plants (Blossey and Notzold 1995, Keane and Crawley 2002). However, some fast growing native plants are able to outcompete invasive plants (Martin et al. 2010). Prickly ash is a good candidate to
compare growth rates among invasive plants. Male prickly ash could be planted in forested areas with high ash composition before EAB invades. Prickly ash is fast growing native and produces clonal sprouts. Additionally, under closed canopy conditions prickly ash dies. Therefore, as canopy gaps close, prickly ash will be reduced and native tree seedling can sprout.

Invasive species have a long history of invading and disrupting North American forests (Niemela and Mattson 1996, Mattson et al. 2007). Invasive herbivores alter nutrient cycling, succession, decomposition, and also interfere with native species foraging and biotic interactions (Krueger and May 1991, Orwig and Foster 1998, DiGregorio et al. 1999, Scriber 2004, Traveset and Richardson 2006, Lockwood et al. 2007, Orwig et al. 2008, Dodds et al. 2010, Mattos and Orrock 2010). Invasive herbivores can directly and indirectly compete with native arthropods for food resources, thereby affecting native arthropod performance (Redman and Scriber 2000, Gandhi and Herms 2010b). An overwhelming majority of studies investigating the impacts of invasive herbivores have focused on direct effects or indirect competitive effects on host plant quality. This study provides strong support that an invasive herbivore can also affect native host plant quality in plants that the invasive herbivore does not consume. EAB does not directly interact with prickly ash. However, it indirectly affects the quality of prickly ash foliage by producing large canopy gaps that induce physiological changes within prickly ash. Changes in prickly ash chemistry resulting from canopy gap formation indirectly negatively affect native giant swallowtail larvae by delaying development and increasing exposure to natural enemies. Non competitive indirect
effects of invasive species are probably a common phenomenon and future studies should aim to investigate these interactions.
References


121


Literature Cited


Mattson, W. J., P. Niemelä, I. Millers, and Y. Inguanzo. 1994. Immigrant phytophagous insects on woody plants in the United States and Canada: an annotated list.27


Smith, A. 2006 Effects of community structure on forest susceptibility and response to the emerald ash borer invasion of the Huron River watershed in southeastern Michigan. Entomology. The Ohio State University, Columbus, OH.


Southwood, T. 1973. The insect/plant relationship—an evolutionary perspective. in Symposia of the Royal Entomological Society of London.


