

**Quarantine evaluation of *Eucryptorrhynchus brandti* (Harold)
(Coleoptera: Curculionidae), a potential biological control
agent of tree-of-heaven, *Ailanthus altissima* in Virginia, USA**

Nathan Jon Herrick

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State
University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Entomology

APPROVED:

Scott M. Salom

Loke T. Kok

Richard C. Reardon

Roger R. Youngman

Shepard M. Zedaker

4 February 2011

Blacksburg, Virginia

Keywords: *Eucryptorrhynchus brandti*, *Ailanthus altissima*, *Leitneria floridana*,
biological control, invasive species, insect rearing, host range testing

© 2011, Nathan Jon Herrick

Quarantine evaluation of *Eucryptorrhynchus brandti* (Harold) (Coleoptera: Curculionidae), a potential biological control agent of tree-of-heaven, *Ailanthus altissima* in Virginia, USA

Nathan Jon Herrick

Scott M. Salom and Loke T. Kok, Co-chairs

Department of Entomology

(ABSTRACT)

Ailanthus altissima (Mill.) Swingle is a tree native to Asia that was intentionally introduced into the United States in the late eighteenth century. *Ailanthus altissima* has become an invasive species that has spread throughout most of North America. Lack of effective management tactics for suppression of *A. altissima* has led to alternate control methods. Investigations into using biological control with the weevil *Eucryptorrhynchus brandti* were initiated in 2004. Studies were conducted to understand the general biology of *E. brandti*, rearing efficacy, and host specificity. *Eucryptorrhynchus brandti* is univoltine, has a life cycle similar to the closely related species *Cryptorrhynchus lapathi* (L.), with 6 instars, and completes development in 126 ± 6.5 d at 25°C. Efficient egg to adult rearing was accomplished by caging 12 m and 12 f for 7 days on 23 – 92 cm long billets. Males and females can be differentiated by the structure of the metathoracic sternite and 1st abdominal segment. Host specificity experiments show that *E. brandti* preferentially feeds on North American *A. altissima* when tested against 29 species from 14 families. Larval development in the rare species *Leitneria floridana* Chapm. was apparent. Additional studies show that *A. altissima* does not occur across *L. floridana* distribution but may have the potential to invade *L. floridana* sites.

This dissertation is dedicated to my loving daughter

Alexis Moon Herrick

Acknowledgments

I would like to thank my major advisors, L. Kok and S. Salom for their unyielding support as scientists and mentors; D. Mullins, R. Reardon, R. Youngman, and S. Zedaker for their support and helpful advice towards this research; D. Yu-Zhou, H. Ji, and Y. Bo for collection and shipment of weevils; T. McAvoy, A. Snyder, S. Bolling, J. Helvey, S. Hight, S. Reitz, J. Mass, K. Kirkman, M. Kaeser, L. Anderson, L. Brown for assistance maintaining plant materials, locating research sites, providing research advice, manufacturing materials, and/or supporting this research; and the USDA Forest Service for funding.

Table of Contents

List of Figures.....	vi
List of Tables.....	vii
Chapter 1 Introduction.....	1
1.1 Origin, biology, and history of <i>Ailanthus altissima</i> (Sapindales: Simaroubaceae).....	1
1.2 Distribution and Control of <i>Ailanthus altissima</i> in the United States.....	5
1.3 Biological Control and its potential in suppressing <i>Ailanthus altissima</i>	9
1.4 Research rationale and objectives.....	11
Chapter 2 Biology, Development, and Rearing of <i>Eucryptorrhynchus brandti</i> (Coleoptera: Curculionidae) in Quarantine.....	14
2.1 Introduction.....	15
2.2 Materials and methods.....	17
2.3 Results	23
2.4 Discussion.....	24
Chapter 3 Host specificity testing of the weevil, <i>Eucryptorrhynchus brandti</i> (Coleoptera: Curculionidae), in quarantine for biological control of the tree of heaven, <i>Ailanthus altissima</i>	34
3.1 Introduction.....	35
3.2 Materials and methods.....	36
3.3 Results	39
3.4 Discussion.....	40
Chapter 4 A description of <i>Leitneria floridana</i> (Sapindales: Simaroubaceae) throughout its native range with special reference to the invasive tree-of-heaven, <i>Ailanthus altissima</i>	47
4.1 Introduction.....	48
4.2 Materials and methods.....	50
4.3 Results	52
4.4 Discussion.....	53
Chapter 5 Conclusions.....	59
References cited.....	61

List of Figures

Fig. 1.1. Cladogram depicting hypothesized relationships in the Order Sapindales (Judd et al. 2002).....	1
Fig. 2.1. (A) Ventral male metathoracic sternite and 1 st abdominal segment of <i>E. brandti</i> (concave/flat) and (B) ventral female metathoracic sternite and 1 st abdominal segment of <i>E. brandti</i> (convex).....	32
Fig. 2.2. Cumulative mean eggs oviposited per female <i>E. brandti</i> and associated male/female mortality (n = 30) at 25°C, 60% R.H., and 14:10 L:D.....	33
Fig. 4.1. County distribution of <i>L. floridana</i> , <i>A. altissima</i> , and sample site locations (☆) in the south eastern and south central United States.....	58

List of Tables

Table 1.1. Native North American, Mexican, and Caribbean species currently and formerly in Simaroubaceae.....	2
Table 2.1. Comparison of mean emergence and mean female and male weight of adult <i>E. brandti</i> reared on billets of different length, age, and exposed to different densities of adult weevils.....	31
Table 2.2. Mean (\pm sem) head capsule widths and ratios (head capsule width (mm) at instar $i-1$ /head capsule width (mm) at instar i) of <i>E. brandti</i> larvae reared in the laboratory.....	32
Table 3.1. Comparison of no-choice foliage feeding tests of adult <i>E. brandti</i>	43
Table 3.2. Comparison of choice foliage feeding tests of adult <i>E. brandi</i>	45
Table 4.1. Comparison of the morphology (mean \pm sem) and site characteristics of <i>L. floridana</i> sampled at sites in Georgia, Florida (2 sites), Texas, Arkansas, and Missouri.....	56
Table 4.2. Proportion (%) of woody species growing in association with <i>L. floridana</i> at sampling sites in Georgia, Florida (2 sites), Texas, Arkansas, and Missouri.....	57

Chapter 1. Introduction

1.1 Origin, biology, and history of *Ailanthus altissima* (Sapindales: Simaroubaceae)

Simaroubaceae are trees and shrubs, occasionally thorny, with scattered secretory cells that are often present in the leaves and bark. The family has conspicuous pith with quassinoid type bitter triterpenoid compounds (Judd et al. 2002). Worldwide approximately 30 genera and 200 species are currently in Simaroubaceae (Watson and Dallwitz 1992, Fernando et al. 1995, Judd et al. 2002, Stevens 2005, USDA-ARS 2006, USDA-NRCS 2007). Members of Simaroubaceae are found in southern North America, Mesoamerica, South America, Caribbean, Asia, Australia, and Africa. Simaroubaceae is placed in the order Sapindales in the Eurosides II clade (Fig. 1.1) (Fernando and Quinn 1995, Judd et al. 2002, Jacobs 2003). Sapindales are woody, with pinnately compound

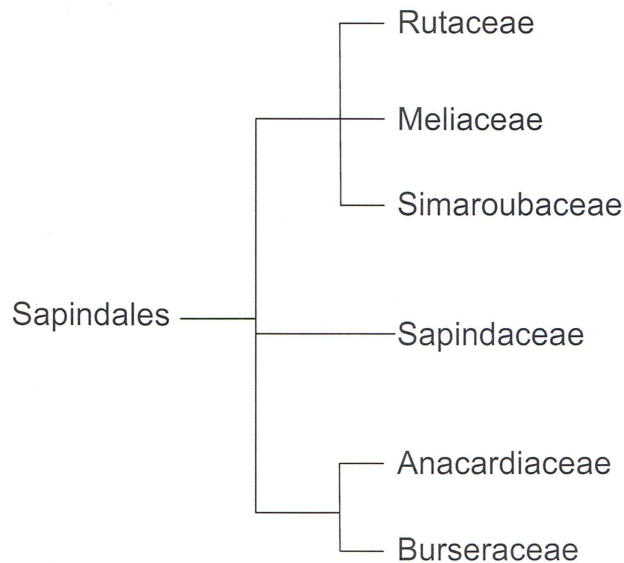


Fig 1.1. Cladogram depicting hypothesized relationships in the Order Sapindales (Judd et al. 2002).

leaves, and flowers have a distinct nectar disk. The closest related families to Simaroubaceae (Quassia or Tree-of-Heaven Family) are Rutaceae (Citrus or Rue Family) and Meliaceae (Mahogany Family) (Fig. 1.1). Ten Simaroubaceae species are native to North America, Mexico, and the Caribbean (Table 1.1). Recently, *Leitneria* formerly in

Table 1.1. Native North American, Mexican, and Caribbean species currently and formerly in Simaroubaceae.

Family	Genus	Species	Distribution
Simaroubaceae	<i>Castela</i>	<i>emoryi</i> (Gray) Moran & Felger	California, Arizona, and Mexico
		<i>erecta</i> (Turp.)	Texas
		<i>polyandra</i> (Moran & Felger)	Mexico
	<i>Holacantha</i>	<i>stewartii</i> C.H. Muell.	Texas
	<i>Leitneria</i>	<i>floridana</i> Chapman	Arkansas, Florida, Georgia, Missouri, and Texas
	<i>Picrasma</i>	<i>antillana</i> (Eggers) Urban	Caribbean
		<i>excelsa</i> (Sw.) Planch.	Caribbean and Mesoamerica
	<i>Quassia</i>	<i>amara</i> L.	Caribbean
	<i>Simarouba</i>	<i>glauc</i> DC	Florida
		<i>tulae</i> Urban	Caribbean
Picramniaceae	<i>Alvaradoa</i>	<i>amorphoides</i> Liebm.	Florida and Mexico
	<i>Picramnia</i>	<i>pentandra</i> Sw.	Florida and Caribbean

Leitneriaceae was placed in Simaroubaceae as a sister to *Ailanthus* based on serological affinity with *Ailanthus* and *Picrasma* (Petersen and Fairbrothers 1983, Fernando and Quinn 1995). The two genera *Alvaradoa* and *Picramnia*, formerly in Simaroubaceae, have been placed in the new family, Picramniaceae based on the structures of fatty acids and anthraquinones and sugar-linked anthracenone derivatives (Fernando and Quinn 1995, Judd et al. 2002, Jacobs 2003). Several resources still place *Alvaradoa* and

Picramnia in Simaroubaceae and do not include *Leitneria* (Stevens 2005, IT-IS 2006, USDA-NRCS 2007).

An important member of Simaroubaceae is *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae), a dioecious, rapid growing (1.5 m/yr) medium to large tree (25 – 35 m) that produces up to 300,000 seeds/year (Sheppard et al. 2006). Leaves reach up to 0.9 m long and are pubescent, alternate, and pinnate. Leaves have 11 – 41 leaflets and are not toothed except for a pair of gland-tipped teeth near their bases. Leaflets are sub-opposite with one to several glandular teeth near the base. Twigs are hairless, yellow-brown, and stout, with continuous yellowish pith. Leaf scars are large, triangular, with numerous bundle scars. The bark is grey-brown, smooth, or with narrow light-colored grooves. Flowers are polygamous and are green-yellow-purple. The calyx is regular, 5-parted with imbricate lobes. Staminate flowers have 10 stamens, perfect flowers have 2-3 stamens, and pistillate flowers have none. The disk is 10 lobed. Ovaries are 2-5 parted and rudimentary in male flowers. The ovary becomes fruit with 1-5 narrowly oblong 1- seeded samaras (Robinson and Fernald 1908, Petrides 1988, Ansari and Ali 1999).

Tree-of-heaven is the accepted common name of *Ailanthus altissima*; however, it has many vernacular names and synonyms (Ansari and Ali 1999, Kowarik and Säumel 2007). Furthermore, genetic research by Dallas et al. (2005) isolated three varieties of *A. altissima* in the Mediterranean Region: *Ailanthus altissima* var. *erythrocarpa*, *A. altissima* var. *sutchuenensis*, and *A. altissima* var. *tanakai*. The common name is said to

have derived from the genus and the native *Ailanthus intergrifolia*. The genus is a Moluccan name ‘Aylanto’ and means ‘tree reaching for the sky’ in reference to its height, canopy, and lack of lateral branching (Robinson and Fernald 1908, Engler 1931, Hu 1979). The tree-of-heaven (TOH) is considered native to China (Udvardy 1998, Ding et al. 2006), Japan, Northern India (Ansari and Ali 1999), Pakistan (Ashraf and Rehman 2001), eastern Asia, and Malaysia (Ballero et al. 2003). Tree-of-heaven often is regarded as an important ornamental species in many of these countries because of its aesthetic value and ability to withstand environmental pollutants and water stress in environment’s congested by anthropogenic activities (Sargent, 1888, Ding et al. 2006). Traditional Chinese culture has used TOH for its anti-tumor properties (Ammirante et al. 2006). Tree-of-heaven also has been used to treat diarrhea, dysentery, heart ailments, asthma, epilepsy, emmenagogue, ophthalmic diseases, seborrhea, and scabies (Ansari and Ali 1999). The ground-bark of TOH is classified as a narcotic because it expresses similar depressive effects on the nervous system as tobacco (Ansari and Ali 1999). It is also a known allergen (Ballero et al. 2003). Tree-of-heaven has been used in reforestation to prevent soil erosion (Ashraf and Rehman 2001), to isolate herbicidal compounds and insecticides (Ding et al. 2006), and in research to isolate anti-HIV agents to combat AIDS (Chang et al. 2003). In the nineteenth century, TOH was widely cultivated and used for breeding *Samia cynthia* (Drury) (Lepidoptera: Saturniidae), a replacement for the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) (Ballero et al. 2003) for production of silk and subsequent textiles. Hu (1979) and Kowarik and Säumel (2007) provide exhaustive reviews of the genus and species.

1.2 Distribution and Control of *Ailanthus altissima* in the United States

Tree-of-heaven is an introduced species in Europe (Ballero et al. 2003, Lenzin et al. 2004), Africa, South America, and North America (Ding et al. 2006). Seed was introduced from China to Paris between 1740 and 1750 (Hu 1979, Tellman 2002) and into North America as an ornamental shade tree during the late eighteenth century from Europe into Philadelphia, Pennsylvania (Feret 1985, Tellman 1997). Multiple introductions into New York occurred during the early nineteenth century (Davies 1942, Dame and Brooks 1972, Hu 1979). The last historical record of TOH introduction(s) into the United States occurred during the gold rush in the mid-nineteenth century when Chinese laborers brought the tree into California from Chinese seed stock for its medicinal and cultural value (Tellman 2002). China is regarded as the country of origin of the TOH because of its history of introduction into the U.S. via Chinese seed stock from Europe and China (Tellman 2002).

The TOH is established throughout most of the United States from Washington to New England and south to northern Florida, Texas, and southern California (Kartesz and Meacham 1999, Howard 2004, USDA-NRCS 2007). However, its relative abundance varies throughout its distribution. It is most common near its centers of initial introduction and less common in areas distant from its introduction. It is established in oak-hickory ecosystems and ecosystems as different as freshwater tidal estuaries (Garrison et al. 1977, Kiviat 2004). Research suggests that the spread of TOH can be aided by foraging deer (Matrai et al. 2004), but dispersal is most common via anthropogenic activities, wind dispersal, and/or water (Kowarik and Säumel 2007). The

diversity or varieties of TOH genetic isolates occurring in the U.S. is not well-studied. However, Feret et al. (1974) found that seed width and biomass are greatest in northern populations. Furthermore, they found that trees from California grow taller than eastern populations and this difference was not related to climatic or soil conditions. Feret and Bryant (1974) compared North American and Chinese populations and found significant differences in 11 of 14 growth traits. They suggested that alterations in gene pool have occurred since its first introduction and this may have been influenced through selection of plants with desirable traits for propagation. Nevertheless, TOH is an incredibly resilient species, as can be seen by the wide range of habitats it infests (Garrison et al. 1977, Kiviat 2004, Marler 2000). The resilience of TOH was apparent over 120 years ago when Sargent (1888) recognized its ability to adapt to “the dirt and smoke, the dust and drought of cities, for the ability to thrive in the poorest soils.” More recent research by Orwig and Foster (1998) and Kowarik (1995) suggested that death of an individual tree within a mature forest can allow for the intrusion, establishment, and propagation of TOH. Conversely, research by Huebner (2003) suggested that mature forest ecosystems are less susceptible to invasion by TOH. The latter is likely the case; however, the TOH is capable of invading mature forest ecosystems. In Virginia, TOH is a dominant species along roadsides and occupies hundreds of acres in the Shenandoah National Park (Marler 2000). The TOH is tolerant of urban conditions, poor soils, air pollution, and is common in dusty, smoggy areas; such as inner cities, where other species are not viable. In urban areas it often is found sprouting in alleys, sidewalks, parking lots and streets. In rural areas its is commonly found in fields, along roadsides, fencerows, woodland edges and forest openings (Patterson 1976, Kowarik 1995, Knapp and Canham 2000, Kostel-

Hughes et al. 2005). It also occurs as seedlings in recently planted fields or as persistent thickets in rocky, non-tillable areas (Swearingen and Pannill 2004).

In its native range, TOH is maintained under natural control by more than 40 phytophagous arthropod species and nearly 20 pathogens (Ding et al. 2006). In the U.S. the ailanthus webworm, *Atteva punctella* (Cramer) (Lepidoptera: Yponomeutidae), is the only insect present that causes significant defoliation to young seedlings of TOH (Kok et al. 2008), however suppression is not apparent. Of the nearly 20 pathogens known to attack TOH, few occur in the U.S. (Ding et al. 2006, Schall and Davis 2009). Research by Schall and Davis (2009) indicated significant mortality of TOH by the fungus *Verticillium albo-atrum* in areas of Pennsylvania. This fungus may aid in suppressing TOH populations. Tree-of-heaven produces allelopathic compounds capable of inhibiting the growth of nearly 90 tree species (Mergen 1959). The lack of natural enemies of TOH and its ability to suppress alternate plant growth over a wide range of habitats are perhaps the most significant attributes of this species that allow it to out-compete native flora in North America, within or outside of mature forests. As a result, dense clonal stands form and impede new growth of native plant species. Furthermore, evidence suggests that secondary compounds produced by invasive plant species such as TOH, can be detrimental to native fauna (e.g. amphibians) (Maerz et al. 2005).

Conventional control of the TOH is difficult and costly. Mechanical control of TOH is labor intensive and expensive because it requires the removal of the entire tree and root system before any attempt to completely eradicate the plant from an area is likely to be

successful. Simply felling a tree can stimulate stump and root sprouting and increase stand density (Burch and Zedaker 2003). Mechanical removal is not a common practice because of this; nevertheless, it remains in practice because there are few options available for TOH control. Chemical control of TOH is most common. Some chemicals registered for TOH control are: dicamba, glyphosate, imazapyr, metasulfuron methyl, and triclopyr. They are applied as foliar sprays, basal-bark treatments, injection, or applied to cut stumps. Unfortunately, chemical control of TOH is a reoccurring practice because it can leave treated sites barren of any plant life resulting in the re-intrusion of invasive species like TOH. There is concern that repeated large-scale herbicide applications may become detrimental to the environment and/or promote resistance, in addition to being labor intensive and costly (ISHRW 2005).

Tree-of-heaven has become a serious threat to habitats throughout its North American distribution. Nationally, the tree has become an economical, ecological, and agricultural pest. It is registered as a noxious weed in New Hampshire, Vermont, Connecticut, and California (USDA-NRCS 2007). A significant amount of funds are applied annually to control the TOH along interstates, agricultural areas, and in National/State Parks. Estimates to control the tree are as much as 8750 USD/ha (Kok et al. 2008). Recognition of the competitiveness of this species and attempts to control it have lead to costly, labor intensive, and environmentally detrimental control practices. Increasing documentation of plant resistance to herbicides also is a serious concern (ISHRW 2005). Therefore, alternate control measures have been sought.

1.3 Biological Control and its potential in suppressing *Ailanthus altissima*

Biological control is the intentional deployment of insects, mites, nematodes, and/or pathogens to suppress a pest population below economic and/or environmental damaging levels. Target pests are most commonly insects or weeds; however, there have been several attempts to control amphibians, reptiles, and/or mammals with biological control throughout various regions of the world (Julien and Griffiths 1998). Zimdahl (1999) provided several philosophical differences of what constitutes a weed. The traits of what qualify a weed for control are not well-defined (Schooler et al. 2004). However, a legal definition aids in protecting the environment and facilitating the practice. The National Invasive Species Council, Executive Order 13112, and the Executive Summary in the National Invasive Species Management Plan define a weed as a plant species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic and/or environmental harm and/or harm to human health (Federal Registrar 1999). Common long-term tactics for dealing with weed invasions; such as the TOH, is the intentional deployment of insect herbivores; although the use of plant pathogens is increasing in importance for invasive weed suppression. Species representing several specific insect orders constitute the vast majority of beneficial organisms used in classical biological control programs. Hymenopterans have been widely used in biological control of arthropods, while coleopterans have been prominent in the biological control of weeds (Julien et al. 1984, Julien and Griffiths 1998, Schooler et al. 2004). Many species of moths (Lepidoptera) and beetles (Coleoptera) are known to be destructive plant pests and thus it is not surprising that members of these two orders are generally more efficacious weed biological control agents than representatives from

other orders (van Driesche et al. 2008, Herrick and Kok 2010). Desirable traits of the potential agent are better defined than for the traits of the target pest. In the case of biological control of weeds, the most effective agents: 1) destroy the vascular tissues of the plant; 2) vector a virulent pathogen; 3) attack the plant throughout its growing season; 4) are multivoltine; 5) have high reproductive rates; 6) are protected from generalist predators; 7) feed gregariously; and 8) cover the full geographic range of the target plant (Schooler et al. 2004)

Classical biological control of weeds for long term weed suppression is a multi-step process and generally involves taxonomic identification of the suspected weed, environmental and economic-cost benefit analysis, foreign exploration to locate, identify, and import potential insect control agents from their place of origin, quarantine testing to evaluate host specificity, rearing and/or mass production of the agent, assessment for the best methodology for release if approved by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine for field release, and post-release evaluations (Schooler et al. 2004). Perhaps the most important step in the process of biological control is host specificity testing. This procedure aims to minimize the possibility of the agent having an impact on plant species other than the target weed (Briese 2005). The centrifugal phylogenetic testing method is the foundation of host-specificity testing. The method involves exposing a series of non-target organisms that have the closest phylogenetic relationship to the target species then progressively exposing more distantly related species until the host range of the agent has been adequately assessed (Wapshere 1974). The Technical Advisory Group for the Biological

Control Agents of Weeds is a group within the United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine that, in addition to monitoring the permit process for the importation of biological control agents, also facilitates biological control of weeds by providing guidance to researchers based on potential non-target impacts and conflicts of interest and may suggest additional species to test (APHIS 2007). Another important procedure in biological control is the rearing method of the agent once in quarantine. This is especially a concern if the biology of the agent is not known. Experimentation can be severely limited and/or produce erroneous data without the ability to successfully and efficiently rear the agent. Several studies have found that rearing density, sex ratio, food quality/quantity, and/or growth environment (e.g. temperature, relative humidity, and/or photoperiod) can negatively impact mating behavior, fecundity/fertility, insect fitness, development, and/or health of offspring (Chen et al. 2006, Esperk et al. 2007, Faccoli 2009, Akbulut 2009, Dmitriew et al. 2010, Rauter et al. 2010, Li et al. 2010, Kuriwada et al. 2009, 2010, Woreta and Sukovata 2010). Therefore, it is important to optimize rearing strategies to produce the fittest individuals. This also can facilitate greater quantities of the agent for field release.

1.4 Research rationale and objectives

Ailanthus altissima fits well under the National Invasive Species Council, Executive Order 13112, and the Executive Summary in the National Invasive Species Management Plan, in that it is an economical and environmental threat to the North American habitats it infests (Federal Registrar 1999). Due to this, the lack of natural enemies of tree-of-heaven in the United States, and lack of sustainable control methods, a biological control

program was initiated in 2004. Foreign exploration in China revealed several potential candidate agents (Ding et al. 2006). The weevil, *Eucryptorrhynchus brandti* Harold (Coleoptera: Curculionidae) was among one of the agents showing the greatest potential and was shipped into quarantine for testing. *Eucryptorrhynchus brandti* is a univoltine species native to China where it is considered a pest. In some areas of China, 80 to 100% of tree-of-heaven trees were attacked by *E. brandti* and the closely related co-habiting species *E. chinensis* causing 12 to 37% mortality (Ge 2000, Ding et al. 2006). Both species are common in China (Xiao 1992). The cryptorrhynchinae weevils are commonly associated feeding on dead or dying plant material; however, some feed on live plants (Reichart et al. 2010). The general biology of *E. brandti* is not well-known. Adults feed on leaves, buds and petioles. Larvae develop under the bark, feeding on and destroying the cambial tissues (Ding et al. 2006). Larvae overwinter under the bark and adults overwinter in the soil near host trees (Ge 2000). Ding et al. (2006) provided a review of these species and their potential as biological control agents of TOH.

Implementing a sustainable control program is essential for suppressing tree-of-heaven populations. The principal objective in this study was to evaluate *E. brandti* as a potential suppressive agent of tree-of-heaven. Studies were performed to evaluate the biology, development, and most efficacious rearing method for *E. brandti* (Chapter 2). These studies facilitated studies to determine the specificity of *E. brandti* for tree-of-heaven and its potential to have non-target impacts (Chapter 3). Finally, based on the host-specificity results and *E. brandti* affinity for the non-target *Leitneria floridana* Chapman, a field

study was conducted to evaluate the potential for tree-of-heaven to cohabit with *L. floridana* and the potential for *E. brandti* to survive in *L. floridana* habitats (Chapter 4).

Chapter 2. **Biology, Development, and Rearing of** *Eucryptorrhynchus brandti* (Coleoptera: Curculionidae) in **Quarantine**

Abstract

Eucryptorrhynchus brandti (Harold) was identified in China and imported into the United States as a potential suppressive agent for the invasive weed, tree-of-heaven, *Ailanthus altissima*. Limited published information exists on *E. brandti*. Studies on its general biology, development, and most efficacious rearing method for *E. brandti* under quarantine were conducted. *Eucryptorrhynchus brandti* is univoltine. The metathoracic sternite and 1st abdominal segment are convex in females and concave or flat in males. Females are larger than males. Female and male sagittal measurements averaged 11.50 ± 1.35 and 10.35 ± 0.91 mm, respectively. Transversal measurements averaged 4.22 ± 0.59 and 3.73 ± 0.53 mm for females and males, respectively. Mean weight of females and males were 0.10 ± 0.03 g and 0.07 ± 0.02 g, respectively. Females lived longer than males, averaging 66.3 ± 37.3 d and 46.5 ± 39.7 d, respectively. Egg production was low with a mean of 3.4 ± 3.5 eggs (range, 0 -14 eggs). Egg stage averaged 5.0 ± 0.9 d, the larval stage had 6 instars and developed in 110 ± 15.0 d, the pupal stage was 16 ± 7.0 d long at 25°C. Its life cycle is similar to a closely related species, *Cryptorrhynchus lapathi* (L.). A method for rearing *E. brandti* on cut *A. altissima* billets under quarantine conditions was developed. The most efficient egg to adult rearing method involved caging 12 m and 12 f for 7 days on 23 – 92 cm long billets. Length of time after cutting

the billet up to three weeks did not affect adult emergence. This information will facilitate conducting host-specificity tests to determine its impact on non-target species.

Keywords: *Eucryptorrhynchus brandti*, classical biological control, insect rearing, insect development, *Ailanthus altissima*, tree-of-heaven.

2.1 Introduction

Ailanthus altissima (Mill.) Swingle (Sapindales: Simaroubaceae), tree-of-heaven, is a tree species native to Asia (Ansari and Ali 1999, Ashraf and Rehman 2001, Ballero et al. 2003, Ding et al. 2006). It was first introduced into the United States in the 1700's and is now distributed and invasive throughout much of North America where it out-competes native vegetation (Davies 1942, Feret 1985, Tellman 1997, USDA-NRCS 2007). The invasiveness of tree-of-heaven is primarily attributed to its: (1) tolerance of extreme environmental conditions; (2) allelopathic properties reducing competition; (3) high seed production; (4) copious sprouting after cutting; and (5) lack of herbivores capable of suppressing this plant (Sargent 1888, Mergen 1959, Garrison et al. 1977, Kowarik 1995, Orwig and Foster 1998, Marler 2000, Kiviat 2004, Ding et al. 2006, Sheppard et al. 2006). Mechanical and chemical controls are current tactics used for suppression, but implementation is costly, and can be as much as 8,750 USD/ha (Kok et al. 2008).

In 2004, investigations into the use of biological control for suppression of *A. altissima* were initiated. A weevil, *Eucryptorrhynchus brandti* (Harold) (Coleoptera: Curculionidae), was identified in China and imported to Virginia Tech Beneficial Insect

Quarantine Facility for testing as a possible biological control agent (Ding et al. 2006). A review of the literature indicates few publications regarding *E. brandti*. Information that does exist focuses on controlling *E. brandti* because it is considered a pest of *A. altissima* in China (Dong et al. 1993, Jian et al. 2004). *Eucryptorrhynchus brandti* is a univoltine species. In some areas of China, 80 to 100% of tree-of-heaven trees were attacked by *E. brandti* and the closely related co-habiting species *E. chinensis* causing 12 to 37% mortality (Ge 2000, Ding et al. 2006). Both species are common in China (Xiao 1992). The general biology of *E. brandti* is not well-known. Adults feed on leaves, buds, and petioles. Larvae develop under the bark, feeding on and destroying the cambial tissues, resulting in more damage to trees than adult feeding (Ding et al. 2006). Larvae overwinter under the bark and adults overwinter in the soil near host trees (Ge 2000).

An important component of any classical biological control of weeds program (i.e. the intentional importation of insects, mites, and/or pathogens to suppress a weed population) is to understand the biology and development of an agent under evaluation for potential release (Delfosse 2004, Purcell et al. 2004). Additionally, rearing of the agent is critical for studying the organism's biology, development, and for devising the most efficient method of mass production. An efficient rearing program can facilitate the production of the appropriate quantity of organisms required for deployment, establishment, and aids in optimizing organism fitness (Mackauer 1976, King and Leppla 1984, Center and Pratt 2004, Hansen 2004, Kuriwada et al. 2009, 2010, Li et al. 2010, Rauter et al. 2010, Woreta and Sukovata 2010).

This study focused on the general biology, longevity, fecundity, ovipositional duration, development, and most efficient method for rearing *E. brandti* to gain an understanding of the life habits of this potential agent and to develop an effective rearing method. This information will assist in conducting ongoing host specificity tests.

2.2 Materials and methods

Basic Morphology

Adults of *Eucryptorrhynchus brandti* were obtained from Dr. Du Yuzhou, Yangzhou University, the People's Republic of China (PRC) in May of 2006 and reared on *A. altissima* billets 92 cm in length and 17.8 cm diam. This initial shipment of insects was reared in a cage 45 x 114 x 66 (w x l x d) cm with fiberglass window screening (mesh count/in 18 x 16, wire diam. 0.279 mm) for ventilation. F₁ progeny was collected from May 2007 through June 2007. One-hundred F₁ adult male and 100 female *E. brandti* were measured sagittally from their head to the most posterior abdominal segment and transversally from the most anterior section of the thorax to obtain size estimates. Weevils also were weighed to obtain wet weight estimates. The same weevils were observed under the microscope to make a determination if gender can be differentiated using the method developed by Thompson (1932) where insects were allowed to copulate and then microscopic examination for differences in the metathoracic sternite and 1st abdominal segment were observed. Once identified to gender, individuals were placed in 0.5 L cups and observed for mating to confirm gender.

Larval Rearing

From 14 May 2007 through 26 November 2007 and 9 May 2008 through 24 November 2008, cut billets of *A. altissima* of varying lengths and time intervals after cutting were exposed to varying densities of *E. brandti* adults. This duration of experimentation encompassed adult emergence in the laboratory. Billet lengths were ca. 92, 46, and 23 cm. These billet lengths were chosen to proportionately reduce the size of billets required for rearing, ease handling of billets, and reduce the space required for rearing. The cut billets age was classified as ‘fresh’ (< 24 hours post-cutting), 1 week, 2 weeks, or 3 weeks old. These age classes were chosen to minimize the effort required to fell a tree in the field and the cryptorhynchinae weevils are commonly associated feeding on dead or dying plant material. Experiments were replicated 48 times. *Ailanthus altissima* trees were felled monthly at a site 37° 08’ 6” N, -80° 32’ 13” W, and 533 m alt. in Radford, Virginia, cut to the appropriate lengths, and transported back to a greenhouse at the Virginia Tech Beneficial Insects Quarantine Laboratory, Price’s Fork Research Station. Billets were placed into an aluminum watering trough approximately 35 x 120 x 2.54 (w x l x d) cm. The tops of each billet were waxed with paraffin, the bottom was left unwaxed, and water was added to the watering trough to prevent desiccation. The diameter, time after cutting, and length were recorded for each billet, and then each billet was assigned a unique number for subsequent identification. Trees being cut were ca. 22 years old and had a diam. of ca. 18 cm. Billets were randomly assigned to one of three cages 45 x 114 x 66 (w x l x d) cm with window screen, and were exposed to adult *E. brandti* of mixed ages, at three densities: 12:12 (female: male), 24:24, and 48:48. Four – 5 *Ailanthus* leaves were placed in each cage for weevils to feed on. Cages were inspected

weekly to replace individuals if they died and to replace leaves. Billets also were replaced weekly with a new billet of random length and post-cutting age.

After billets were exposed to *E. brandti* adults they were placed in a separate room at 25°C, 60% R.H., under a photoperiod of 14:10 (light:dark), using natural light. These conditions were chosen because they were conditions that were successful in rearing the initial shipment of weevils from China and similar to local ambient conditions. Also, Esperk et al. (2007) reported that extreme temperatures (low 20°C, high 30°C), low humidity (40%), and short day length (12h light) can prolong development in some Coleoptera. Billets were then placed in a watering trough similar to that previously described. Water was replaced bi-weekly. Billets were loosely wrapped with window screen which was stapled closed creating a sleeve around the billet. The bottom of the screen was secured around the base of the billet with a bungee cord and the top of the screen was secured with a plastic-coated aluminum wire-tie to allow for collection of emerged adults. Billets were inspected daily for adult emergence. When an adult was found, it was identified to gender, weighed, and the billet it emerged from was recorded. Experiments were conducted as a split-plot design with age post-cutting (0, 1, 2, 3 weeks), length (23, 46, or 92 cm), and density (24, 48, or 96 insects) as the main factors, using insect weight and number of adult's emerged/dm² as an indicator of rearing efficacy. The number of adult's emerged/dm² was calculated by dividing the total number of adults emerged by the surface area of an individual billet. Data were analyzed using analysis of variance and treatment differences were separated using least square means Tukey-Kramer HSD test at $\alpha = 0.05$.

Adult and Egg Longevity and Oviposition

A pair of newly emerged (< 24 h post-emergence) *E. brandti* male and female collected from the larval rearing study was placed into a 0.5 L container with 2 - 3 *A. altissima* leaf clusters (i.e. a leaf section containing 4 leaflets) wrapped with cheese cloth inserted into a hole in the container lid to determine adult longevity, egg production and adult feeding rate. The containers were placed, lid side down on a 100 ml cup to provide the leaves with water. Two holes (5 cm diam.) covered with window screen were made on each side of the cup for ventilation. These cages were placed in Percival[®] environmental chambers at 25°C, 60% R.H., with a photoperiod of 14:10 (light:dark), and each cup was inspected every three days for mortality, oviposition, and to replace leaf clusters. Feeding on leaves was measured as described by Kok et al. (1992, 2008) using a transparent millimeter square grid. Leaves, cups, lids, and gauze were inspected for eggs. Experiments were replicated 30 times. All eggs recovered were carefully placed on filter paper (5.5 cm diam.) using a soft tipped paintbrush, and placed into Petri dishes (2 cm h x 5.5 cm diam.). Two drops of a 0.17% methyl paraben and 0.17% sorbic acid solution were placed onto the edge of the filter paper using an eyedropper to provide moisture and inhibit mold and fungal growth. Petri dishes were placed in environmental chambers at 25°C, 60% R.H., with a photoperiod of 14:10 (light:dark), and inspected every daily for emergence of neonates and/or to add 2 additional drops of methyl paraben-sorbic acid solution. Neonates were preserved in vials containing 75% ETOH for head capsule measurements. Data from these first instar head capsule measurements were used in the larval and pupal development study described below.

Larval and Pupal Development

To determine the number of larval instars or groupings and larval duration four male and 4 female *E. brandti* were fed *A. altissima* leaves in a similar fashion as described above, however, they were allowed to feed and mate for 15 d. Following the feeding/mating interval, insects were placed in plastic 15 x 27 x 36 (h x w x l) cm cages with a lid with a 7 x 18 cm hole covered with window screen. A 12 cm long x 14 cm diam. billet (with the top waxed) was provided as an oviposition substrate and *A. altissima* leaves were provided as food. Billets were set on top of a folded-moist paper towel to prevent desiccation. Billets, food, and any dead insects were replaced weekly. Billets that were removed from the cages were set in watering troughs as previously described in chambers at 25°C, 60% RH, with a photoperiod of 14:10 (light:dark). Billets were dissected at 20, 35, 50, 65, 80, 95, 110, and 125 d after exposure to adults to detect individual instars. Each dissection interval was replicated 10 times. Recovered larvae were preserved in 75% ETOH for head capsule measurements. When a pupa was found it was placed into a Petri dish 2 cm h x 5.5 cm diam., at 25°C, 60% RH, 14:10 (light:dark) until adult eclosion. Dyar's rule is the ratio of the head capsule width (mm) at instar $i - 1$ /head capsule width (mm) at instar i and was used to determine the number of instars (Dyar 1890).

Adult Overwintering

To determine adult overwintering survival, weevils collected in China in November and shipped into quarantine in the United States, were randomly selected and placed in 4 clear plastic containers (25 h x 13 d x 14 w cm) in groups of 60 adults/container.

Containers had one hole on each side (10 cm diam.) covered with window screen. Before placing the insects in the containers, the bottom of the container was filled with Sta-Green[®] Nursery Blend Tree and Shrub Planting Mix approximately 8 cm deep and 5 ml distilled water was added for moisture. An *A. altissima* billet section (12 cm long x 6 cm diam.) was placed on top of the soil. Weevils were fed *A. altissima* leaf clusters for one week at the start of the experiment. The containers were placed in growth chambers at an initial temperature of 8°C, 60% R.H., and a photoperiod of 10.5L:13.5D (light:dark). The initial temperature and photoperiod corresponded to the local monthly average ambient temperature in Blacksburg, VA in November. They were changed to simulate mean local temperature and photoperiod in subsequent months based on data collected between 1999 and 2006. The exposure temperature and photoperiod for each month were as follows: November, 8.1°C, 10.5L:13.5D; December, 1.6°C, 10L:14D; January, 1.4°C, 10L:14D; February, 3.0°C, 11L:13D; March, 7.4°C, 12L:12D. One container was maintained at constant rearing temperatures (25°C) and served as the control. Because weevils in the laboratory display an initial peak emergence in March, the surviving insects were removed mid-March and placed on *A. altissima* billets (46 cm long x 17 cm diam.) to determine if they successfully overwintered and could reproduce by counting the number of adult progeny produced.

2.3 Results

Basic Morphology

Sagittal measurements of males averaged 10.35 ± 0.91 mm (mean \pm SD) and transversal measurements averaged 3.73 ± 0.53 mm ($n = 100$). Mean male weight was 0.07 ± 0.02 g. Female sagittal measurements averaged 11.50 ± 1.35 mm and transversal measurements averaged 4.22 ± 0.59 mm ($n = 100$). Mean female weight was 0.10 ± 0.03 g. There was 100% ($n = 100$ pairs) success in males mounting females when using the gender identification method described by Thompson (1932). Ventrally, the metathoracic sternite and 1st abdominal segment is concave or flat in males and convex in females (Fig. 2.1 A, B).

Larval Rearing

Billet age and length did not influence the number of adult *E. brandti* emerged (Table 2.1). However, a density of 48:48 (male:female) significantly reduced the number of *E. brandti* emerged. Similarly, billet age, length, and density did not influence male weight. However, female weight was significantly reduced at a density of 48:48 (Table 2.1). Weevils emerged with a sex ratio of 1:1 for all treatments.

Adult and Egg Longevity and Oviposition

Male *E. brandti* survived 46.5 ± 39.7 days ($n = 30$) and females survived 66.3 ± 37.3 days ($n = 30$). The mean number of eggs oviposited per ovipositing female over its lifetime was 3.4 ± 3.5 eggs ($n = 58$), 43% ($n = 13$) of the females did not lay eggs. The maximum number of eggs oviposited/individual was 14 eggs. Oviposition began on day 28, peaked

from day 33 – 39, and ceased on day 84. Mortality was gradual during their lifespan (Fig. 2.2). Adults consumed an average $21.8 \pm 1.61 \text{ cm}^2/\text{leaf material/individual}$ over their lifetime. At 25°C , duration of the egg stage averaged 5.0 ± 0.9 days ($n = 42$). Egg width was 0.64 ± 0.03 mm and length was 0.91 ± 0.05 mm. Egg hatch was 69%.

Larval and Pupal Development

The mean growth ratio in head capsule width was 0.67 ± 0.06 . Using Dyar's rule, it appears that 6 instars occur for development of *E. brandti* larvae (Table 2.2). This is similar to the closely related species *Cryptorhynchus lapathi* (L.) (Coleoptera: Curculionidae) which had a mean growth ratio of 0.73 (Hannon et. al 2008). Duration of the larval stage was 110 ± 15.0 d. The duration of each individual instar was not able to be determined due to the limitations of sampling for larvae under the bark of the billets.

Adult Overwintering

All insects in the control died because there was not a sufficient source of food. Only 12.8% ($n = 23$) died in the experimental replicates exposed to the overwintering regime from mid-November till the end of March the following year. The surviving individuals successfully reproduced. Thirty four females and 50 males developed from eggs oviposited by approximately 78 females that successfully overwintered.

2.4 Discussion

Eucryptorhynchus brandti is a univoltine species. The entire life cycle from egg to adult averaged 126.5 ± 6.5 days (mean \pm sem) at 25°C . This species can be identified to gender

by using the descriptions identified by Thompson (1932). The metathoracic sternite and 1st abdominal segment are convex in females and concave or flat in males. This is helpful in both rearing efforts and for setting up experiments. The data indicate that females are generally larger than males.

Intraspecific competitive interactions during the larval or adult stages can severely hinder production and fitness during the rearing process. Several studies have found that rearing density, sex ratio, food quality, quantity, and abiotic factors can negatively impact mating behavior, fecundity, insect fitness, development, and offspring health (Chen et al. 2006, Esperk et al. 2007, Faccoli 2009, Akbulut 2009, Dmitriew et al. 2010, Rauter et al. 2010, Li et al. 2010, Kuriwada et al. 2009, 2010, Woreta and Sukovata 2010). The data indicate that density of *E. brandti* adults is a primary factor influencing productivity and female fitness. No differences were found between the use of 24 and 48 adults, but there was a decrease in adult productivity of about 50% and a significant decrease in female weight when density was increased from 48 to 96 adults. Thus, the optimal density of adults used to rear *E. brandti* on a 92, 46, or 23 cm long x 18 cm diam. billet, should be 12 males and 12 females with a billet up to 3 weeks post-cutting because female fitness as determined by weight is greatest. Also, a 46 cm billet is easier to handle than a 92 cm billet and decreases the space required for rearing. The 23 cm billet tends to desiccate faster than the other lengths. The decrease in productivity at the highest density of ovipositing adults may be due to a lack of adequate nutrients to support reproduction. The low emergence may also be due to an excessive larval density resulting in a reduced amount of food to complete development to the adult stage. Similar results were found

by Faccoli (2009) who studied the impact of density on the bark beetle *Tomicus destruens* (Wollaston) (Coleoptera: Curculionidae: Scolytinae). Faccoli (2009) found a significant decrease in the number of eggs, larvae, pupae and adults per female when density was increased from 0.25 to 1.50 females/dm². Faccoli (2009) concluded that high breeding densities decrease female fecundity and resulted in high mortality of offspring. Rauter et al. (2010) found a decrease in female body mass of the burying beetle, *Nicrophorus pustulatus* Herschel (Coleoptera: Silphidae), when reared under high densities; but in contrast, the smaller females produced larger broods than larger females. This study did not investigate the relation of female size and fecundity and the possibility that smaller females may be as fecund as larger females. Future studies should focus on determining the effects of female size on oviposition rate.

Females of *E. brandti* live approximately 20 days longer than males in the laboratory. This may be due to their mating behavior. When a male weevil locates a suitable female for mating he mounts the female, rarely dismounts unless disturbed, while the female continues to feed. Cessation of male feeding when mounted on the female may result in lower food consumption reducing its longevity.

The estimates of oviposition by *E. brandti* are much lower than expected and likely inaccurate. Reichert et al. (2010) found that the closely related *Cryptorhynchus melastomae* Champion (Coleoptera: Curculionidae) produces an average of 150 eggs/female. Low fecundity in this study suggests that a behavioral component of the insects may be influencing fecundity or a lack of quality nutrients to support

reproduction. This also could have been caused by other factors that include female/male incompatibility, difficulty in locating eggs, food quality, or the artificial rearing environment. For example, Woreta and Sukovata (2010) found that adult longevity, adult weight, and fecundity of the forest cockchafer *Melolontha hippocastani* F. (Coleoptera: Melolonthidae) were significantly reduced when reared among four different tree species indicating that food quality can significantly impact insect fecundity. Chen et al. (2006) found improved flight performance in well-nourished individuals of the plum curculio, *Conotrachelus nenuphar* (Herbst) Coleoptera: Curculionidae) when compared with undernourished beetles. Well-nourished individuals may not be produced by the use of cut billets under quarantine where space is limited. Such rearing conditions are not similar to naturally growing trees and could explain the low number of eggs laid by the females in this study. An additional cause may be the effect of multiple matings of males with females. Research by Campbell (2005) on the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), found that continuous exposure to one male increased the lifetime fecundity of females by increasing the period of time that eggs were produced when compared with mating with one male for a specific mating interval. Progeny size, however, was reduced. Campbell (2005) also found that exposure of one female to five males significantly decreased the number and size of progeny produced. Gay et al. (2009) reported that females of *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae) kept continuously with males have reduced lifetime egg production compared with females mated once and then isolated from males. They suggested that reduced fitness may be caused by damage induced by male genitalia that bear spines puncturing the female reproductive tract and/or toxic compounds in the ejaculate. They also suggested that the

behavioral component of male harassment results in reduced fecundity. In contrast, Wilson et al. (1999) found an increase in lifetime fecundity after multiple matings in *Callosobruchus analis* (Fab.) (Coleoptera: Bruchidae) and *C. maculatus*. When oviposition sites were abundant, fecundity increased after a second but not a third copulation for *C. maculatus* and after a second and third copulation for *C. analis*. When oviposition sites were limited to *C. maculatus* fecundity increased after a third mating. Experiments were conducted using leaf material as an oviposition substrate since eggs of *E. brandti* are extremely difficult to find and collect undamaged in their natural substrate just below the bark of TOH. Leaf material likely is an inadequate oviposition substrate and may have caused a reduction in fecundity in these experiments. Future studies should focus on evaluating mating time the effect of multiple matings on female *E. brandti*, and the impact of oviposition substrate and availability on fecundity. Since oviposition peaked between day 33 and 39 in this experiment, removal of males before this peak might aid in increasing fecundity/fertility. Studies also should focus on dissections of females to determine optimization of fecundity, as influenced by food type (i.e. leaves versus stems versus cambial tissue), and number of males required for successful reproduction with an individual female.

The number of instars in Curculionidae is more variable than those in some insect families such as the Carabidae (Coleoptera) and Culicidae (Diptera) (Salas and Frank 2001). In fact, many insect species within many families have considerable variation in the number of instars during their lifecycle (Esperk et al. 2007). For example, Pittendrigh et al. (1997) found 4 – 5 instars in the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera:

Curculionidae), Gold et al. (1999) found 5 – 8 instars in the banana root borer, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), and Wen et al. (2004) found 5 – 7 instars in *Hylobitelus xiaoi* Zhang (Coleoptera: Curculionidae). Variation in the number of instars of all three species occurred under standardized rearing conditions. Plasticity in the number of instars is primarily due to temperature, photoperiod, food quality, quantity, rearing density, injury, and humidity (Esperk et al. 2007). The data indicate that *E. brandti* had 6 instars or ‘larval groupings’. Studies by Hannon et al. (2008), on the closely related poplar-and-willow borer [*Cryptorhynchus lapathi* (L.) (Coleoptera: Curculionidae)], found similar results in field collected beetles and identified 6 larval groupings. The entire time required for development from 1st instar to pupation was approximately 110.0 ± 15.0 days. Pupal duration was 16.0 ± 7.0 days.

Few progeny were produced from the 157 individuals (ca. 78 females and 79 males) that survived in the overwintering experiment under the "simulated winter" temperature regime. The individuals in this experiment were all combined into one rearing cage. The high density (i.e. >24 males and 24 females per cage) resulted in reduced productivity as was found in the larval rearing study. This is likely due to high rearing density or sex ratio but may also be affected by food quality, quantity, and abiotic factors (Chen et al. 2006, Esperk et al. 2007, Faccoli 2009, Akbulut 2009, Dmitriew et al. 2010, Rauter et al. 2010, Li et al. 2010, Kuriwada et al. 2009, 2010, Woreta and Sukovata 2010). Despite the low number of progeny produced, it demonstrates that adults from the previous year when overwintered can become reproductively active again the following spring and allows continuous rearing without continued reliance on importation. Future experiments

should examine the maximum, minimum, and optimal conditions required for overwintering in eggs, larvae, and adults to maximize productivity.

From these studies a method for rearing *E. brandti* in quarantine has been developed and the ability to maintain a healthy colony for multiple generations is now possible. There are several areas that should be addressed to further refine the rearing process. In particular, the reproductive potential of females should be quantified by dissection of adults, the amount of time required for successful mating should be assessed to minimize stress on females, and adult feeding preference for various parts of *A. altissima* (i.e. leaves, stems, and cambial tissues) should be determined to aid in maximizing reproductive output. These rearing techniques will facilitate the ability to conduct ongoing host-specificity tests to determine its impact on non-target species.

Table 2.1. Comparison of mean emergence and mean* female and male weight of adult *E. brandti* reared on billets of different length, age, and exposed to different densities of adult weevils.

Factor	no. of billets	Mean (\pm sem) adults emerged/dm²	Mean (\pm sem) male weight (mg)	Mean (\pm sem) female weight (mg)
Billet Length (cm)				
23	48	17.5 \pm 1.9	61.0 \pm 2.0	76.0 \pm 3.0
46	48	21.3 \pm 1.9	58.0 \pm 2.0	81.0 \pm 2.0
92	48	20.2 \pm 1.9	59.0 \pm 2.0	78.0 \pm 2.0
Billet Age (wk)				
0	36	19.2 \pm 2.7	63.0 \pm 5.0	82.0 \pm 4.0
1	36	16.6 \pm 2.7	60.0 \pm 5.0	80.0 \pm 4.0
2	36	20.5 \pm 2.7	57.0 \pm 5.0	78.0 \pm 4.0
3	36	22.4 \pm 2.7	58.0 \pm 5.0	77.0 \pm 3.0
Insect Density (M:F)				
12:12	48	21.8 \pm 2.4a	60.0 \pm 4.0a	83.0 \pm 3.0a
24:24	48	24.6 \pm 2.4a	61.0 \pm 4.0a	79.0 \pm 3.0ab
48:48	48	12.6 \pm 2.4b	55.0 \pm 4.0a	73.0 \pm 3.0b

*Means (\pm sem) within a column followed by the same letter within each treatment are not significantly different at $P \leq 0.05$, least square means Tukey-Kramer HSD test.

Table 2.2. Mean (\pm sem) head capsule widths and ratios (head capsule width (mm) at instar $i - 1$ /head capsule width at instar $_i$) of *E. brandti* larvae reared in the laboratory.

Larval grouping	no.	mm	Range	Growth ratio
I	135	0.31 \pm 0.02	0.25 - 0.39	{0.67
II	46	0.46 \pm 0.01	0.40 - 0.69	
III	26	0.82 \pm 0.02	0.70 - 0.99	{0.73
IV	33	1.13 \pm 0.02	1.00 - 1.39	{0.67
V	50	1.68 \pm 0.03	1.40 - 1.99	
VI	51	2.39 \pm 0.02	2.00 - 2.50	{0.70

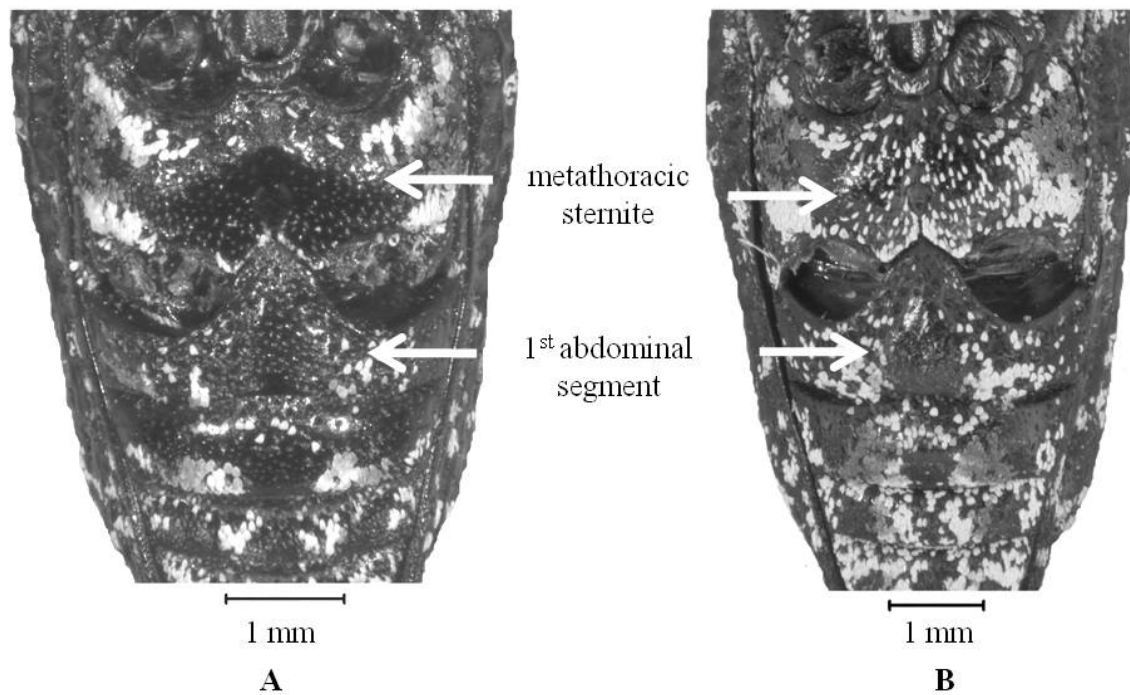


Figure 2.1. (A) Ventral male metathoracic sternite and 1st abdominal segment of *E. brandti* (concave/flat) and (B) ventral female metathoracic sternite and 1st abdominal segment of *E. brandti* (convex).

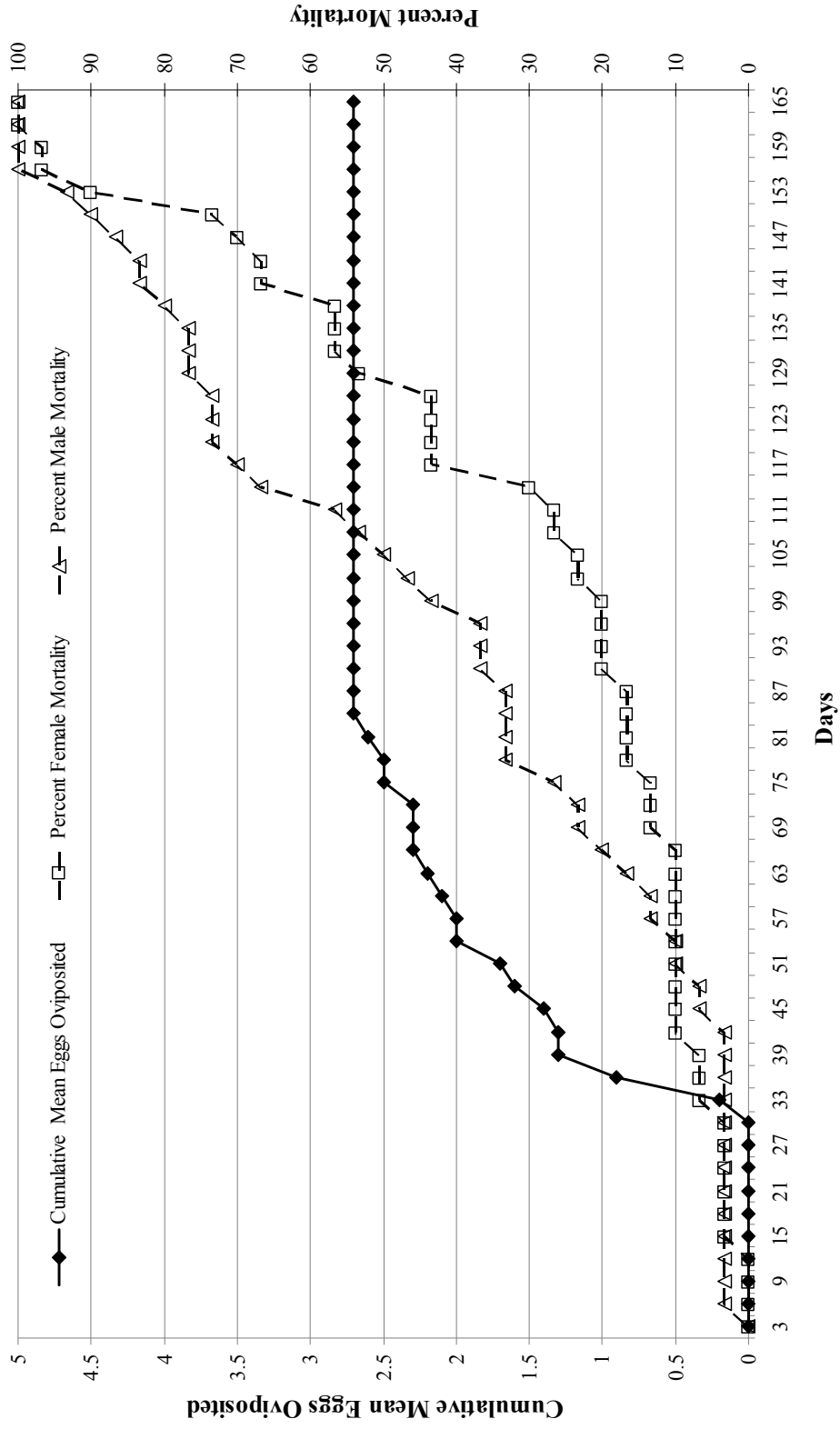


Figure 2.2. Cumulative mean eggs oviposited per female *E. brandti* and associated male/female mortality (n = 30) at 25°C, 60% R.H., and 14:10 L:D.

Chapter 3. Host specificity testing of the weevil, *Eucryptorhynchus brandti* (Coleoptera: Curculionidae), in quarantine for biological control of the tree of heaven, *Ailanthus altissima*

Abstract

Ailanthus altissima (Mill.) Swingle, tree-of-heaven, is a species native to Asia. It was first introduced into the United States in the 1700s and is now distributed and invasive throughout much of North America. Mechanical and chemical controls are current tactics used for suppression but implementation is costly. Biological control was initiated in 2004 as a potentially sustainable tactic for tree-of-heaven suppression. *Eucryptorhynchus brandti* (Harold) was identified in China and imported for quarantine testing as a potential biological control agent. Adult choice and no-choice feeding tests on foliage of North American tree-of-heaven and 30 test species, no-choice larval development tests, and no-choice oviposition tests in 2007 and 2008 were conducted to determine the specificity of this weevil for the tree-of-heaven. *Eucryptorhynchus brandti* adults fed significantly more on tree-of-heaven foliage when compared with all test species. Range of means for feeding on North American *Ailanthus altissima* was 32.5 - 106.5 mm²/adult/day in no-choice tests, with significantly reduced feeding on *Simarouba glauca* DC, paradise tree (7.7 ± 6.7 mm²/adult/day), and *Leitneria floridana* Chapman, corkwood (47.6 ± 20.8 mm²/adult/day). The mean range of feeding by *E. brandti* on all other test species was 0.0 to 3.3 ± 5.0 mm²/adult/day. Larval development only occurred in seedlings of the species *Leitneria floridiana* (20%, n = 10) and tree-of-heaven controls (70%, n = 10). Adults were unable to successfully reproduce on seedlings of *L. floridiana* (0%, n = 10) when compared with tree-of-heaven controls (70%, n = 10). *Eucryptorhynchus brandti* is highly

host specific for tree-of-heaven. Development testing of *E. brandti* on larger specimens of *L. floridiana* as well as understanding the ecology of *L. floridiana* and its association, or lack thereof, with tree-of-heaven in the field is required.

Keywords: classical biological control, *Eucryptorrhynchus brandti*, *Ailanthus altissima*, tree-of-heaven, invasive species, host specificity.

3.1 Introduction

Ailanthus altissima (Mill.) Swingle (Sapindales: Simaroubaceae), tree-of-heaven, is a tree species native to Asia (Ansari and Ali 1999, Ashraf and Rehman 2001, Ballero et al. 2003, Ding et al. 2006). It was first introduced into the United States in the 1700's and is now distributed and invasive throughout much of North America where it out-competes native vegetation (Davies 1942, Feret 1985, Tellman 1997, USDA-NRCS 2007). The invasiveness of tree-of-heaven is primarily attributed to its: (1) tolerance of extreme environmental conditions; (2) allelopathic properties reducing competition; (3) high seed production; (4) copious sprouting after cutting; and (5) lack of herbivores capable of suppressing this plant (Sargent 1888, Mergen 1959, Garrison et al. 1977, Kowarik 1995, Orwig and Foster 1998, Marler 2000, Kiviat 2004, Ding et al. 2006, Sheppard et al. 2006, Kok et al. 2008). Mechanical and chemical controls are current tactics used for suppression, but implementation is costly, and can be as much as 8,750 USD/ha (Kok et al. 2008).

In 2004, investigations into the use of biological control for suppression of *A. altissima* were initiated. *Eucryptorrhynchus brandti* (Harold) (Coleoptera: Curculionidae), was identified in China and imported to Virginia Tech Beneficial Insect Quarantine Facility for testing as a

potential biological control agent (Ding et al. 2006). A review of the literature indicates few publications regarding *E. brandti*. Information that does exist focuses on controlling *E. brandti* because it is considered a pest of *A. altissima* in China (Dong et al. 1993, Jian et al. 2004). *Eucryptorrhynchus brandti* is a univoltine species. In some areas of China, 80 to 100% of tree-of-heaven trees were attacked by *E. brandti* and the closely related co-habiting species *E. chinensis* causing 12 to 37% mortality (Ge 2000, Ding et al. 2006). Both species are common in China (Xiao 1992). The general biology of *E. brandti* is not well-known. Adults feed on leaves, buds, and petioles. Larvae develop under the bark, feeding on and destroying the cambial tissues, resulting in more damage to trees than adult feeding (Ding et al. 2006). Larvae overwinter under the bark and adults overwinter in the soil near host trees (Ge 2000).

Adult foliage feeding tests, larval development tests, and oviposition tests were conducted to determine if *E. brandti* is a host specific herbivore of the tree-of-heaven. If *E. brandti* is a host specific herbivore of the tree-of-heaven, recommendations will be made to the United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Technical Advisory Group for Biological Control Agents of Weeds to release *E. brandti* from quarantine for tree-of-heaven suppression.

3.2 Materials and methods

Adult foliage feeding tests

In 2007 and 2008, 30 plant species (including *A. altissima* grown from seed obtained directly from Chinese trees) that are taxonomically, ecologically, and/or economically related to *A. altissima* were exposed to 1 newly emerged (< 24 h post-emergence) male and 1 female *E.*

brandti for 3 days to measure the amount of foliage consumed. Experiments were conducted in a choice and no-choice fashion. Choice experiments consisted of one *A. altissima* leaf cluster (i.e. a leaf containing 4 leaflets) and one leaf cluster of a test species. One no-choice experiment consisted of two leaf clusters of a test species and a second no-choice control consisted of two *A. altissima* leaf clusters. Plant species were replicated 10 times. Experiments were conducted in 0.5 L containers. Leaf clusters were wrapped with cheese cloth and inserted into a hole in the container lid. The containers were placed, lid side down on a 100 ml cup to provide the leaf clusters with water. Two holes (5 cm diam.) covered with aluminum window screen were made on each side of the cup for ventilation. These cages were placed in Percival® environmental chambers at 25°C, 60% R.H., with a photoperiod of 14:10 (light:dark). These conditions were chosen because they were conditions that were successful in rearing the initial shipment of weevils from China and similar to local ambient conditions. Also, Esperk et al. (2007) reported that extreme temperatures (low 20°C, high 30°C), low humidity (40%), and short day length (12h light) can prolong development in some Coleoptera. Feeding on leaves was measured as described by Kok et al. (1992, 2008) using a transparent millimeter square grid. After the 3 d feeding interval, all adults were removed and placed in alternate containers with new leaf clusters as described above to insure that adults were feeding in all tests. No mortality occurred during the experiments. Data were analyzed using the Tukey-Kramer HSD test at $\alpha = 0.05$.

Larval development tests

Plant species that were fed on substantially ($> 7 \text{ mm}^2/\text{adult}/\text{day}$) in no-choice tests of the previous experiment (i.e. *Simarouba glauca* and *Leitneria floridana*) were inoculated with 3 first instar *E. brandti* that were collected from the rearing colony. Two of the following *Citrus* spp.

were included in the experiment because of their economic importance: *C. aurantifolia* (Christm.) Swingle, *C. aurantium* L., *C. limon* (L.) Burm. F., *C. paradisi* Mcfad., *C. reticulata* Blanco, and *C. sinensis* Osbeck. *Ailanthus altissima* seedlings served as the control. All plants were ca. 2 years old, ca. 1.5 cm diam, grown under natural light, at ca. 25°C, using 26.5 L pots, with Sta-Green® Nursery Blend Tree and Shrub Planting Mix and 0.09:0.06:0.05% N:P:K. This diameter was used because larger specimens and quantities of *Simarouba glauca* and *Leitneria floridiana* were not accessible. Three 0.20 cm holes were drilled 0.4 cm deep into the base, middle, and top of each plant. Larvae were transferred directly into the holes from dissected *A. altissima* billets using a soft tipped brush and sealed with masking tape. Care was taken to assure that larvae would not stick to the adhesive covering the hole by placing an additional piece of tape that did not have adhesive over the area covering the hole. Experiments were replicated 10 times. Plants were dissected after 4 months and the presence or absence of larval development was recorded.

Adult oviposition tests

Plant species that showed development of *E. brandti* in the previous experiments (i.e. *L. floridana*) were challenged by caging 5 newly emerged (< 24 h post-emergence) male and 5 female *E. brandti* until all females died. Plants were screened with 7 X 6 holes/cm² mesh screen and wrapped with a bungee cord around the base of the screen to secure the screen to the pot. The top of the screen was sealed with an aluminum tie. PVC stakes were used to support the screen away from the plant. Plants were grown and were the same age and approximate diameter as those in the experiments above. *Ailanthus altissima* seedlings served as the control. Experiments were inspected weekly for weevil mortality and replicated 10 times. When a male

died it was replaced but only to maintain a 1:1 (male:female). Experiments ended when all females died. Plants were dissected 4 months after female mortality and it was assumed that oviposition and developmental success occurred if a larva and/or feeding were found. Data were recorded as presence or absence of development.

3.3 Results

Adult foliage feeding tests

In no-choice tests, *E. brandti* consumed significantly more North American *A. altissima* foliage than any of the test species (Table 3.1). However, substantial feeding did occur on *S. glauca* and *L. floridiana*. In addition, *E. brandti* consumed significantly more Chinese *A. altissima* than North American *A. altissima*. In choice tests, similar results were found. *Eucryptorrhynchus brandti* consumed significantly more *A. altissima* than any of the test species (Table 3.2). Feeding on *S. glauca* and *L. floridiana* was substantially reduced when in the presence of *A. altissima* compared with the no-choice test.

Larval development tests

Development past the first instar occurred in 70% (n = 10) of *A. altissima* control plants. No development occurred in *S. glauca* or any *Citrus* spp. (n = 10). However, development in *L. floridana* was 20% (n = 10). Development of larvae to pupation was only observed in 1 *A. altissima* seedling. All of the control seedlings and the two *L. floridana* seedlings where development occurred were girdled and resulted in seedling mortality before completion of larval development.

Adult oviposition tests

Adults successfully oviposited on *A. altissima* control plants resulting in 70% (n = 10) of seedlings containing developing larvae while 0% (n=10) of *L. floridana* seedlings contained developing larvae.

3.4 Discussion

Of the 29 test plant species, *E. brandti* adults fed significantly more on foliage of *A. altissima* in no-choice and choice tests. While adults used in these tests were all newly emerged, the variability in feeding was likely caused by various levels of adult feeding required by individuals to bore a hole for emergence from host billets. This behavior acts as an initial nutrient source for emerging adults. In adult no-choice tests the greatest amount of feeding occurred on the test species *S. glauca* and *L. floridana*, both of which are in the same family as *A. altissima*. However, when given the choice between *A. altissima* and *S. glauca* or *L. floridana*, adults preferentially fed on the target plant with substantially reduced feeding on *S. glauca* and *L. floridana* compared with no-choice tests. There was significantly more adult feeding on Chinese *A. altissima* foliage than on North American *A. altissima* foliage. Feret et al. (1974) found genetic differences between eastern and western North American *A. altissima*. The original introduction of *A. altissima* was into the north eastern United States from Europe whereas a separate introduction occurred in California from plants imported directly from China (Hu 1979, Tellman 2002). These routes of *A. altissima* introduction into the United States help to explain these genetic differences and preferential feeding of *E. brandti* on Chinese *A. altissima*. Future studies should focus on determining if Chinese strains of *A. altissima* are more susceptible to damage by *E. brandti* than North American strains.

In the no-choice larval development tests, larvae developed from first instars through late instars in the *A. altissima* controls as well as *L. floridana* seedlings, but with reduced success. It is suspected that larvae were unable to complete development because of the small (1.5 cm diam.) size of the seedlings tested resulting in girdling and subsequent larval mortality. *Leitneria floridana* is a threatened species indigenous to Georgia, Florida, Texas, Arkansas, and Missouri. The species primarily occurs in shaded marsh habitats, often inundated with brackish water (Koller 1997). *Ailanthus altissima* occurs in stony and sterile soils to habitats with rich alluvial bottoms, is most common in its original areas of introduction and anthropogenically disturbed sites, and is not as common in areas most distant from these centers of introduction and natural sites (Kowarik and Säumel 2007). These habitat differences may limit *A. altissima* invasion into *L. floridana* habitats. While large specimens of *L. floridana* are difficult to obtain, future studies need to focus on the possibility of *E. brandti* being able to sustain populations on *L. floridana*. Larger specimens will be required for testing.

The no-choice oviposition tests reveal that newly emerged *E. brandti* are capable of sustaining additional generations on *A. altissima* seedlings but not on *L. floridana*. Antibiosis is common in many plant-insect interactions (Franco et al. 2002, Broberg and Borden 2005, Ulmer and Dosdall 2006, Kiggundu et al. 2007, Cortez-Rocha et al. 2009). *Leitneria floridana* may have evolved a physiological barrier to oviposition and/or emerging neonates of species like *E. brandti* that attempt to penetrate cambial tissues. In retrospect, inoculating larvae into plants as was done in the larval development tests may have prohibited *L. floridana* physiological response to egg eclosion and/or neonate penetration. Studies by Broberg and Borden (2005) on the closely related poplar-and-willow borer, *Cryptorhynchus lapathi* (L.) (Coleoptera: Curculionidae), found

decreased levels of oviposition and cessation of larval development in clones of *Populus maximowiczii* Henry (Salicales: Salicaceae) caused by antixenosis. Franco et al. (2002) provided a review of several α -amylase inhibitors and their importance in plant defense mechanisms to herbivores. Furthermore, Kiggundu et al. (2007) found that sap production produced by *Musa* L. spp. (Zingiberales: Musaceae) when exposed to ovipositing banana weevils, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), reduced egg eclosion rates on some resistant cultivars. Sap production, antibiosis, antixenosis, and/or plant α -amylase inhibitors may contribute to the reduction in successful egg eclosion and/or neonate development of ovipositing *E. brandti* on *L. floridana*. The previous experiments were conducted with newly emerged adults. Studies should be conducted with adults fed *A. altissima* until peak oviposition and then placed on *L. floridana*. Perhaps newly emerged adults placed directly on *L. floridana* lack the nutritional requirements for egg production.

Future studies should focus on assessing the potential for *A. altissima* to invade *L. floridana* habitats and if that threat is more significant than the potential non-target impacts that may be caused by the release of *E. brandti* for *A. altissima* suppression. Until studies are conducted to fully understand the potential impact of *E. brandti* on *L. floridana*, I recommend that this agent remain in quarantine.

Table 3.1. Comparison of no-choice foliage feeding tests of adult *E. brandti*.

Family	Test species	Common name	no.	Foliage consumed	
				Test species mean \pm SD (mm ² /adult/day)	North American <i>A.</i> <i>altissima</i> mean \pm SD (mm ² /adult/day)
Simaroubaceae	<i>Ailanthus altissima</i> (Mill.) Swingle	Chinese tree-of-heaven	10	95.6 \pm 16.2	57.3 \pm 27.2 ^a
	<i>Castela emoryi</i> (A. Gray) Moran & Felger	crucifixion thorn	10	2.4 \pm 2.0	75.8 \pm 14.6 ^a
	<i>Simarouba glauca</i> DC	paradise tree	10	7.7 \pm 6.7	76.9 \pm 31.1 ^a
	<i>Leitneria floridana</i> Chapman	corkwood	10	47.6 \pm 20.8	97.4 \pm 31.9 ^a
Meliaceae	<i>Swietenia mahogoni</i> (L.)	mahogany	10	0	58.5 \pm 26.6 ^a
Rutaceae	<i>Citrus aurantifolia</i> (Christm.) Swingle	lime	10	0.4 \pm 1.0	98.5 \pm 40.2 ^a
	<i>Citrus aurantium</i> L.	sour orange	10	1.1 \pm 2.1	75.3 \pm 23.1 ^a
	<i>Citrus limon</i> (L.) Burm. F.	lemon	10	3.3 \pm 5.0	32.5 \pm 22.2 ^a
	<i>Citrus paradisi</i> Macfad.	grapefruit	10	2.2 \pm 3.9	49.7 \pm 21.8 ^a
	<i>Citrus reticulata</i> Blanco	tangerine	10	0.1 \pm 0.3	78.6 \pm 41.0 ^a
	<i>Citrus sinensis</i> Osbeck	sweet orange	10	3.1 \pm 4.6	46.8 \pm 27.3 ^a
	<i>Ptelea trifoliata</i> L.	common hoptree	10	1.1 \pm 1.2	67.9 \pm 21.6 ^a
	<i>Zanthoxylum americanum</i> Mill.	northern prickly-ash	10	0.9 \pm 1.7	57.2 \pm 27.2 ^a
Aceraceae	<i>Casimiroa edulis</i> La Llave	white sapote	10	0	102.8 \pm 18.7 ^a
	<i>Acer rubrum</i> L.	red maple	10	1.3 \pm 3.4	54.2 \pm 39.5 ^a
Fagaceae	<i>Quercus alba</i> L.	white oak	10	0.5 \pm 1.7	66.6 \pm 27.6 ^a
	<i>Quercus rubra</i> L.	red oak	10	0.9 \pm 1.9	57.4 \pm 38.3 ^a

Juglandaceae	<i>Carya glabra</i> (Mill.) Sweet	pignut hickory	10	0.2 ± 0.4	65.5 ± 38.9 ^a
	<i>Juglans nigra</i> L.	black walnut	10	0	45.3 ± 27.6 ^a
Magnoliaceae	<i>Liriodendron tulipifera</i> L.	tulip poplar	10	0	50.4 ± 23.0 ^a
Burseraceae	<i>Bursera simaruba</i> (L.) Sarg.	gumbo limbo	10	0.9 ± 2.0	46.6 ± 15.3 ^a
Pinaceae	<i>Pinus taeda</i> L.	loblolly pine	10	0	73.7 ± 36.2 ^a
Anacardiaceae	<i>Rhus typhina</i> L.	staghorn sumac	10	0.4 ± 1.4	47.9 ± 27.7 ^a
	<i>Mangifera indica</i> L.	mango	10	0.4 ± 0.9	80.1 ± 18.8 ^a
Cupressaceae	<i>Juniperus virginiana</i> L.	eastern redcedar	10	0	42.9 ± 24.5 ^a
Fabaceae	<i>Robinia pseudoacacia</i> L.	black locust	10	0.1 ± 0.2	58.6 ± 26.6 ^a
	<i>Glycine max</i> (L.) Merr.	soybean	10	0	94.8 ± 26.6 ^a
Rosaceae	<i>Crataegus</i> spp.	hawthorne	10	0	58.9 ± 24.3 ^a
	<i>Prunus serotina</i> Ehrh.	black cherry	10	0	77.0 ± 19.5 ^a
Poaceae	<i>Zea mays</i> L.	corn	10	2.6 ± 5.7	68.0 ± 23.7 ^a

^a Denotes significant differences ($P < 0.05$) across a row between North America *A. altissima* and the test species (Tukey-Kramer HSD).

Table 3.2. Comparison of choice foliage feeding tests of adult *E. brandtii*.

Family	Test species	Common name	no.	Foliage consumed	
				Test species mean \pm SD (mm ² /adult/day)	North American <i>A.</i> <i>altissima</i> mean \pm SD (mm ² /adult/day)
Simaroubaceae	<i>Ailanthus altissima</i> (Mill.) Swingle	Chinese tree-of-heaven	10	83.8 \pm 33.0	41.1 \pm 36.2 ^a
	<i>Castela emoryi</i> (A. Gray) Moran & Felger	crucifixion thorn	10	0	75.2 \pm 27.6 ^a
	<i>Simarouba glauca</i> DC	paradise tree	10	1.1 \pm 1.2	68.3 \pm 26.1 ^a
	<i>Leitneria floridana</i> Chapman	corkwood	10	8.0 \pm 8.1	84.4 \pm 26.8 ^a
Meliaceae	<i>Swietenia mahogoni</i> (L.)	mahogany	10	0	90.5 \pm 31.1 ^a
Rutaceae	<i>Citrus aurantifolia</i> (Christm.) Swingle	lime	10	0	115.7 \pm 6.8 ^a
	<i>Citrus aurantium</i> L.	sour orange	10	0.3 \pm 0.7	65.6 \pm 29.8 ^a
	<i>Citrus limon</i> (L.) Burm. F.	gemon	10	1.2 \pm 3.7	24.6 \pm 15.2 ^a
	<i>Citrus paradisi</i> Macfad.	grapefruit	10	0.1 \pm 0.2	53.4 \pm 18.2 ^a
	<i>Citrus reticulata</i> Blanco	tangerine	10	0	73.3 \pm 31.1 ^a
	<i>Citrus sinensis</i> Osbeck	sweet orange	10	0	43.6 \pm 23.8 ^a
	<i>Ptelea trifoliata</i> L.	common hoptree	10	0.2 \pm 0.4	76.9 \pm 29.4 ^a
	<i>Zanthoxylum americanum</i> Mill.	northern prickly-ash	10	0	55.7 \pm 18.9 ^a
	<i>Casimiroa edulis</i> La Llave	white sapote	10	0	77.1 \pm 37.1 ^a
Aceraceae	<i>Acer rubrum</i> L.	red maple	10	0	62.2 \pm 29.2 ^a
Fagaceae	<i>Quercus alba</i> L.	white oak	10	0	72.3 \pm 29.0 ^a

Juglandaceae	<i>Quercus rubra</i> L.	red oak	10	0	49.8 ± 34.8 ^a
	<i>Carya glabra</i> (Mill.) Sweet	pignut hickory	10	0.0 ± 0.1	59.3 ± 33.1 ^a
	<i>Juglans nigra</i> L.	black walnut	10	0	58.0 ± 16.5 ^a
Magnoliaceae	<i>Liriodendron tulipifera</i> L.	tulip poplar	10	0	54.1 ± 35.9 ^a
Burseraceae	<i>Bursera simaruba</i> (L.) Sarg.	gumbo limbo	10	0.3 ± 1.0	48.1 ± 20.8 ^a
Pinaceae	<i>Pinus taeda</i> L.	loblolly pine	10	0	73.8 ± 34.5 ^a
Anacardiaceae	<i>Rhus typhina</i> L.	staghorn sumac	10	0	47.7 ± 29.1 ^a
	<i>Mangifera indica</i> L.	mango	10	0	71.6 ± 29.3 ^a
Cupressaceae	<i>Juniperus virginiana</i> L.	eastern redcedar	10	0	49.0 ± 27.7 ^a
Fabaceae	<i>Robinia pseudoacacia</i> L.	black locust	10	0.1 ± 0.2	58.6 ± 26.6 ^a
	<i>Glycine max</i> (L.) Merr.	soybean	10	0	50.1 ± 25.5 ^a
Rosaceae	<i>Crataegus</i> spp.	hawthorne	10	0	45.4 ± 22.9 ^a
	<i>Prunus serotina</i> Ehrh.	black cherry	10	0	77.0 ± 19.5 ^a
Poaceae	<i>Zea mays</i> L.	corn	10	0.1 ± 0.2	69.9 ± 18.0 ^a

^a Denotes significant differences ($P < 0.05$) across a row between North America *A. altissima* and the test species (Tukey-Kramer HSD).

Chapter 4. A description of *Leitneria floridana* (Sapindales: Simaroubaceae) throughout its native range with special reference to the invasive tree-of-heaven, *Ailanthus altissima*.

Abstract

Leitneria floridana, corkwood, is an uncommon tree native to the southeastern and south central United States. Field studies were conducted to assess the morphology and site characteristics of *L. floridana* throughout its native range and to determine if *Ailanthus altissima* has invaded *L. floridana* populations. Six sites were visited in Georgia (1 site), Florida (2 sites), Texas (1 site), Arkansas (1 site), and Missouri (1 site). Plants at the Florida1 site were significantly taller and had a larger trunk diameter than any other location throughout *L. floridana* distribution. Plant density was greatest at the Florida1 site; however, the amount of *L. floridana* was greatest at the Florida2 site. Water depth varied at the time of sampling from 0 – 31 cm across its distribution. *Atteva punctella* (Cramer) (Lepidoptera: Yponomeutidae) was the only herbivore found feeding on *L. floridana*. Across all sites, woody species in the family Arecaceae (2.1%), Euphorbiaceae (1.5%), Fabaceae (0.8%), Fagaceae (7.2%), Hamamelidaceae (0.3%), Juglandaceae (9.6%), Magnoliaceae (0.3%), Oleaceae (0.5%), Rosaceae (0.3%), Rubiaceae (0.8%), Taxodiaceae (6.1%), and Ulmaceae (3.0%) were found growing in association with *L. floridana* (Simaroubaceae, 67.5%) (n = 372). *A. altissima* was not found in the immediate vicinity of *L. floridana* populations. Management of invasive weed species for the protection of *L. floridana* is discussed.

4.1 Introduction

Corkwood, *Leitneria floridana* Chapm. (Sapindales: Simaroubaceae), formerly misidentified as *Myrica floridana* (Chapm.) Alph. Wood (Wood 1870), is a rare tree native to Georgia, Florida, Texas, Arkansas, and Missouri with at least one known cultivated population in Illinois (Sharma and Graves 2004, USDA-NRCS 2007). Specimens also have been collected by the Parks Department in Rochester, New York in 1925, 1927, and 1968 but there is no evidence if the species occurs there (Koller 1997). *Leitneria floridana* occurs in brackish and fresh water marshes, wet woodlands, swampy prairies, and estuarine shores (Koller 1997, Sharma et al. 2008). The classification of *L. floridana* has endured substantial debate since its first discovery by Dr. E. T. Leitner in 1835 in the salt water marshes of Florida's Apalachicola River (Koller 1997). Formerly, the species was placed in the monotypic family Leitneriaceae within Sapindales. Most recently it has been placed in Simaroubaceae as a sister to the invasive tree-of-heaven, *Ailanthus*, based on serological affinity with *Ailanthus* and *Picrasma* (Petersen and Fairbrothers 1983, Fernando and Quinn 1995).

NatureServe scientists, its collaborators, with input from taxonomic experts, use a conservation ranking system based on three letters G, N, and S (i.e. global, national, and subnational) depending on the geographic area under consideration as well as 5 numbers that are the same under each letter where 1 is critically imperiled, 2 is imperiled, 3 is vulnerable, 4 is apparently secure, and 5 is secure (NatureServe 2009). Ten factors are used to assign a conservation ranking that NatureServe combines into three major groups: rarity, trends, and threats (NatureServe 2009). The rarity group accounts for population size, range extent, area of occupancy, number of occurrences (i.e. distinct populations), number of occurrences or percent area having

viable/ecological integrity, and environmental specificity. The trends group considers long and short-term trends in population size or area of a particular species. The threats group accounts for the overall threat impact, which is determined by considering the magnitude and severity of major threats, and intrinsic vulnerability (NatureServe 2009). *Leitneria floridana* has a global conservation rank of G3 (vulnerable; either very rare and local or found locally in a restricted range). It has a state rank of S1 (critically imperiled because of extreme rarity) in Georgia and Texas, S2 (imperiled because of rarity) in Missouri, and S3 (very rare and local or found locally in a restricted range) in Arkansas and Florida (Sharma et al. 2008, NatureServe 2009, CPC 2010). It is generally accepted that *L. floridana* rarity is due to habitat destruction and land management practices for recreational purposes (Koller 1997, Rosen et al. 2006).

Tree-of-heaven, *Ailanthus altissima* (Mill.) Swingle (Sapindales: Siamaroubaceae), is a dioecious, rapid growing (1.5 m a year) medium to large tree (25 – 35 m) that produces up to 300,000 seeds a year (Sheppard et al. 2006). *Ailanthus altissima* is an introduced species in North America (Ding et al. 2006). Seed was introduced from China to Paris between 1740 and 1750 (Hu 1979, Tellman 2002) and into North America as an ornamental shade tree during the late eighteenth century from Europe into Philadelphia, Pennsylvania (Ferret 1985, Tellman 1997). Multiple introductions into New York occurred during the early nineteenth century (Davies 1942, Dame and Brooks 1972, Hu 1979). The last historical record of *A. altissima* introduction(s) into the United States occurred during the gold rush in the mid-19th century when Chinese laborers brought the tree into California from Chinese seed stock for its medicinal and cultural value (Tellman 2002). Tree-of-heaven is established throughout most of the United States from Washington to New England and south to northern Florida, Texas, and southern

California (Kartesz and Meacham 1999, Howard 2004, USDA-NRCS 2007). However, its relative abundance varies throughout its distribution. It is most common near its centers of initial introduction and less common in areas distant from its introduction. It is established in a wide variety of ecosystems from oak-hickory to freshwater tidal estuaries (Garrison et al. 1977, Kiviat 2004). Tree-of-heaven has become a serious threat to habitats throughout its North American distribution. Nationally, the tree has become an economical, ecological, and agricultural pest. It is registered as a noxious weed in New Hampshire, Vermont, Connecticut, and California (USDA-NRCS 2007).

Native plant community structure and diversity can be negatively altered by the invasion of non-indigenous species like *Ailanthus altissima* (Westbrooks 2001, Woods 1993, Zimdahl 1995, Olden and Poff 2003, Heady et al. 1992, Rosen and Faden 2005, Rosen et al. 2006). *Ailanthus altissima* occurs in every state that *L. floridana* occurs; however, it is only known to overlap in Jefferson and Franklin counties in Florida; Brazoria County, Texas; Jefferson, Craighead, and Mississippi counties, Arkansas; and Ripley County, Missouri (USDA-NRCS 2007). Therefore, a field survey was conducted of *L. floridana* throughout its native range to gain a better understanding of *L. floridana* in its native habitat and to determine if *A. altissima* has invaded or has the potential to invade *L. floridana* habitats. This information will allow us to better address future control tactics for *A. altissima* suppression.

4.2 Materials and methods

From the 9 September through 5 October 2009 native sites of *L. floridana* were visited in the Joseph W. Jones Ecological Research Center at Ichauway, Dougherty County, Georgia (1 site,

31° 42' 48" N; -84° 44' 44" W, 52.1 m alt.), Big Bend Wildlife Management Area, Snipe Island, Taylor County, Florida (2 sites, locale1: 30° 12' 72" N; -83° 96' 92" W, 7.3 m alt.; locale2: 30° 12' 50" N; -83° 97' 03" W, 14.6 m alt.), San Bernard National Wildlife Management Area, Bird Pond Unit, Brazoria County, Texas (1 site, 29° 08' 18" N; -95° 12' 33" W, 14.5 m alt.), Bayou Meto Wildlife Management Area, Cox Cyprus Lake, Arkansas County, Arkansas (1 site, 34° 28' 67" N; -91° 63' 14" W, 53.3 m alt.), and Sand Pond Conservation Area, Ripley County, Missouri (1 site, 36° 50' 35" N; -90° 60' 00" W, 92.7 m alt.) (Fig. 4.1). These sites were chosen because they were the most easily accessible or were previously known by herbarium specimens and/or recommended by scientists in the area. At each site, a 10 m² quadrat was established around *L. floridana* stands to estimate the density of the stand. This quadrat size was chosen because *L. floridana* colonies were small and permitted sampling the entire population at most sites. Within the quadrates plant height, trunk diameter, trunk taper, alternate woody species, water depth and number of herbivores per plant were calculated or recorded. Trunk diameter was measured at dbh (diameter at breast height) at the Florida1 site. Trunk diameter was measured half way from the base to the top of the plants at the remaining sites because they were shorter than breast height. From the trunk taper calculation the trunk shape was classified as described by Brack (1999) and Socha and Kulej (2007): 0.25 cm/m is neiloid, 0.33 cm/m is conoid, 0.50 cm/m is quadratic paraboloid, 0.60 cm/m is cubic paraboloid, and 1.00 cm/m is cylindrical. From the north, south, east, and west edge of each quadrat, 100 m transects were established to determine if additional *L. floridana* stands and/or *A. altissima* were present. Individual sites were treated as replicates and all plants within a quadrat were measured. Plant height, trunk diameter, trunk taper, and water depth were analyzed with analysis of variance and least square means Tukey-

Kramer HSD at $\alpha = 0.05$. All other measures were not replicated, so they were not compared statistically among the sites.

4.3 Results

Leitneria floridana at the Florida1 site was significantly taller and had greater trunk diameter than at all other sites suggesting that the site was older and established before the other sites that were sampled (Table 4.1). Also, the Florida1 site was not shaded by overstory vegetation while all other sites were, suggesting that *L. floridana* is a poor competitor in shaded environments. Plant height was similar at Florida2, Georgia, Texas, and Missouri sites; however, plants were significantly taller at the Florida2 site than at the Arkansas site. Trunk diameter at the Florida2 site was significantly greater than *L. floridana* sampled at the Georgia, Texas, Arkansas, and Missouri sites (Table 4.1). Trunk taper at the Florida1 and Florida2 differed significantly from *L. floridana* at the Georgia, Texas, and Arkansas sites but not the Missouri site. Using the breast height form factor (trunk taper), the data indicate that plants at Florida1, Florida2, and Missouri have a cylindrical trunk, Georgia and Texas plants have a quadratic paraboloid taper, and Arkansas plants have a cubic paraboloid taper.

Leitneria floridana density was at least 3 times greater at the Florida1 site than at the Florida2 and Georgia sites, again suggesting that it is an older site. Texas, Arkansas, and Missouri sites had the lowest density (Table 4.1). Across all 100 m transects, the Florida2 site had the greatest amount of *L. floridana* from the main sampling quadrat followed by Arkansas then Texas. Plants were sparse/absent within 100 m from the main sampling quadrates at the Florida1, Georgia, and Missouri sites (Table 4.1). Minimum water depth was greatest at the Florida1 site

followed by the Georgia site. Maximum water depth was greatest at the Florida1 and Georgia site followed by the Florida2 site. Standing water was absent in some locations at the Florida2 site and completely absent at the Texas, Arkansas, and Missouri sites. Soil was saturated at sites where standing water was absent. Mean water depth was similar at the Florida1 and Georgia sites. Water depth was significantly greater at the Florida1 site than at the Florida2, Texas, Arkansas, and Missouri sites (Table 4.1). The only herbivore found (larvae and pupae) was *Atteva punctella* (Cramer) (Lepidoptera: Yponomeutidae) at the Florida2 (n = 48), Texas (n = 10), and Arkansas sites (n = 7).

Among the research sites, *L. floridana* was generally common; however, Missouri had substantially more *Quercus lyrata* than other locations and species growing in association with *L. floridana* (Table 4.2). Across all sites, *Carya aquatica* was commonly found growing in association with *L. floridana*, excluding Georgia which had *Taxodium distichum* growing as the next most common species. *Ailanthus altissima* was not found at any of the sites. However, the invasive tree *Triadica sebifera* was found in close association with *L. floridana* in Texas (Table 4.2).

4.4 Discussion

The data confirm the clonal growth of *L. floridana*. The species grows in small isolated populations and is often inundated by standing water or found growing in previously inundated and moist soils. The majority of locations investigated contain short, wispy plants that are small in diameter. Sharma et al. (2008) conducted a similar study on *L. floridana* and suggested that the Missouri population is the most unique phylogenetically; however, the results suggest that

the Florida population is the most unique. For example, the results indicate that plants in Florida can grow more than three times taller than plants in Missouri. Sharma et al. (2008) reported that plants in Missouri grow taller than plants in Florida. Furthermore, plants at the Florida1 site grew two times taller and had a diameter approximately two times larger than the Florida plants reported by Sharma et al. (2008). Trunk taper, or the breast height form factor, is used by foresters to aid in determining trunk volume and yield (Brack 1999). This measurement also is used as a morphological measure to determine phylogenetic relatedness (Socha and Kulej 2007). Regarding trunk taper, the only similarity that was found with Sharma et al. (2008) was in the cylindrical trunk shape of the Florida plants. These differences in results are most likely due to differences in site location, measurements, and/or age of the sites sampled. Future studies need to encompass several sites across the distribution *L. floridana* in each state and during different times of the year to accurately assess the species ecological role within the community it grows in and across its distribution. Genetic analysis would be useful in determining the relatedness of *L. floridana* provenances.

Native plant community structure and diversity can be negatively altered by the invasion of non-indigenous species (Rosen et al. 2006). For example, at a site location similar to ours in Brazoria Co., Texas, Rosen et al. (2006) found decreased species richness within plots containing *Cyperus entriarianus* Boeckeler (Cyperales: Cyperaceae). Many invasive plant species produce allelopathic compounds that are capable of suppressing the growth of alternate plant species, reducing competition. The invasive tree *Ailanthus altissima* produces the compound ailathone that is capable of suppressing more than 90 species (Mergen 1959). *Ailanthus altissima* is encroaching on *L. floridana* habitats (Fig. 4.1). *Ailanthus altissima* was not found growing in

association with *L. floridana*, but the site conditions might support the growth of *A. altissima* in communities adjacent to *L. floridana* colonies. Many of the sites that were visited were inundated with water. It is not known if *A. altissima* can grow in inundated conditions, however it is known that *A. altissima* can invade estuarine habitats (Kiviat 2004), woodland communities, and thrives in sites influenced by anthropogenic activities (Kowarik 1995). All of the sites that were visited were generally isolated; however, every site had evidence of anthropogenic activity in the vicinity of *L. floridana* stands. The Georgia, Arkansas, and Missouri sites maintain access roads for recreational purposes, the Florida sites maintain access roads for housing and recreation, and the Texas site manages the understory vegetation for hunting access and quality. Most sites in this study were selected partially for ease of accessibility based on herbarium specimens and local botanists in the areas owing to the presence of anthropogenic activities. The Texas site was the least accessible and did not have any access roads. Anthropogenic activities support the invasion of invasive species (Byers 2002). The presence of the invasive tree *Triadica sebifera* in the Texas site supports the potential invasion by woody invasive species like *A. altissima* into *L. floridana* habitats. While conservation efforts are common for *L. floridana* management (CPC 2010), managers of invasive plant species also need to be aware of such sensitive species and incorporate management tactics to have as little impact on such species. Any management tactic of *A. altissima* should consider its potential impact on *L. floridana*.

Table 4.1. Comparison of the morphology (mean \pm sem)* and site characteristics of *L. floridana* sampled at sites in Georgia, Florida (2 sites), Texas, Arkansas, and Missouri.

Measurement	State Sites					
	Georgia	Florida1	Florida2	Texas	Arkansas	Missouri
Plant height (m)	1.14 \pm 0.14bc	2.59 \pm 0.09a	1.20 \pm 0.13b	0.86 \pm 0.16bc	0.79 \pm 0.15c	0.67 \pm 0.24bc
Trunk diameter (cm)	0.67 \pm 0.17c	2.57 \pm 0.11a	1.30 \pm 0.15b	0.45 \pm 0.20c	0.39 \pm 0.18c	0.59 \pm 0.29c
Trunk taper (cm/m)	0.54 \pm 0.07c	1.00 \pm 0.05a	1.06 \pm 0.07a	0.53 \pm 0.08c	0.69 \pm 0.08bc	0.90 \pm 0.12ab
Quadrat density (plants/m ²)	4.30	12.00	4.80	1.50	1.70	0.70
North transect density (plants/m ²)	0.00	0.00	1.00	0.00	0.00	0.00
South transect density (plants/m ²)	0.00	0.00	4.50	0.00	1.70	0.00
East transect density (plants/m ²)	0.00	0.00	4.80	0.00	1.60	0.00
West transect density (plants/m ²)	0.00	0.00	0.00	1.55	0.00	0.00
Min. water depth (cm)	5.08	30.48	0.00	0.00	0.00	0.00
Max. water depth (cm)	30.48	30.48	16.19	0.00	0.00	0.00

*Means followed by the same letter across each row are not significantly different at $P \leq 0.05$, Least Square Means Tukey-Kramer HSD test.

Table 4.2. Proportion (%) of woody species growing in association with *L. floridana* at sampling sites in Georgia, Florida (2 sites), Texas, Arkansas, and Missouri.

Family	Species	Common name	State Sites					
			Georgia	Florida1	Florida2	Texas	Arkansas	Missouri
Arecaceae	<i>Sabal palmetto</i> (Walter) Lodd. Ex Schult.	cabbage palmetto	0.0	2.2	7.9	0.0	0.0	0.0
Euphorbiaceae	<i>Triadica sebifera</i> (L.) Small	Chinese tallow	0.0	0.0	0.0	18.7	0.0	0.0
Fabaceae	<i>Sesbania drummondii</i> (Rydb.) Cory	poisonbean	0.0	0.0	0.0	9.4	0.0	0.0
Fagaceae	<i>Quercus lyrata</i> Walter	overcup oak	3.9	0.0	0.0	0.0	19.4	51.4
Hamamelidaceae	<i>Liquidambar styraciflua</i> L.	sweetgum	1.3	0.0	0.0	0.0	0.0	0.0
Juglandaceae	<i>Carya aquatica</i> (Michx. f.) Nutt.	water hickory	0.0	8.2	14.3	18.7	12.9	17.1
Magnoliaceae	<i>Magnolia virginiana</i> L.	sweetbay	1.3	0.0	0.0	0.0	0.0	0.0
Oleaceae	<i>Fraxinus pennsylvanica</i> Marshall	green ash	0.0	0.0	0.0	6.3	0.0	0.0
Rosaceae	<i>Prunus serotina</i> Ehrh.	black cherry	1.3	0.0	0.0	0.0	0.0	0.0
Rubiaceae	<i>Cephalanthus occidentalis</i> L.	common buttonbush	3.9	0.0	0.0	0.0	0.0	0.0
Simaroubaceae	<i>Ailanthus altissima</i> (Mill.) Swingle	tree-of-heaven	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Leitneria floridana</i> Chapm.	corkwood	55.8	89.6	76.2	46.9	54.8	20.0
Taxodiaceae	<i>Taxodium distichum</i> (L.) Rich.	bald cypress	29.9	0.0	0.0	0.0	0.0	0.0
Ulmaceae	<i>Ulmus americana</i> L.	American elm	2.6	0.0	1.6	0.0	12.9	11.4
		N	77	134	63	32	31	35

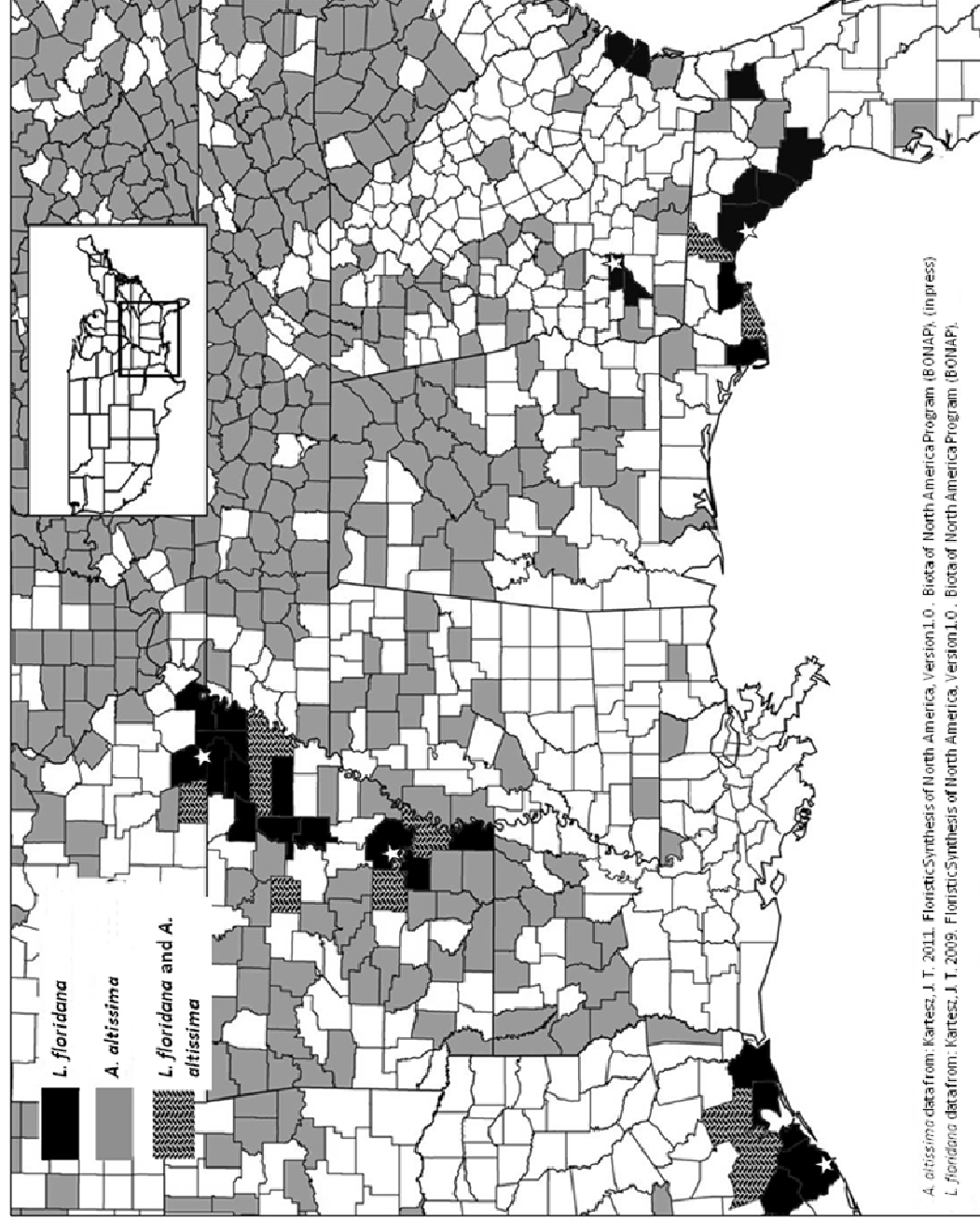


Figure 4.1. County distribution of *L. floridana*, *A. altissima*, and sample site locations (☆) in the southeastern and south central United States.

Chapter 5 Conclusions

Ailanthus altissima, tree-of-heaven (TOH), is an incredibly resilient species capable of invading many different habitats. Current control methods are not sustainable and expensive. Biological control of the TOH with *Eucryptorrhynchus brandti* is a promising management tactic for its suppression. Knowledge of the general biology of the potential agent *E. brandti* and the ability to efficiently rear it will aid in future studies pertaining to the species and its mass production. Understanding the reproductive potential of females, the amount of time required for successful mating, and adult feeding preference for various parts of *A. altissima* (i.e. leaves, stems, and cambial tissues) will aid in maximizing reproductive output. While *E. brandti* is highly specific for the TOH, concern of larval development on the rare *Leitneria floridana* is apparent. However, it does not appear that *E. brandti* can develop on *L. floridana* from ovipositing females. While large specimens of *L. floridana* are difficult to obtain, future studies should focus on the possibility of *E. brandti* being able to sustain populations on *L. floridana*. Choice tests between *A. altissima* and *L. floridana* plants with adult *E. brandti* would provide further information regarding female selection for oviposition substrates between these two species in the field. Sharma et al. (2008) suggested that soil moisture is the most important environmental feature influencing the occurrence of *L. floridana*, with soil moisture and density positively correlated. It is unlikely that *E. brandti* adults would be able to overwinter in soil inundated with water. The TOH has not been found in association with *L. floridana* to date but may invade areas in the vicinity of *L. floridana* colonies. Future studies should investigate the potential of the TOH to impede growth of *L. floridana* through allelopathic effects and its ability to grow in conditions inundated with water. The invasion of TOH into *L. floridana* habitats potentially poses a greater threat to *L. floridana* populations than *E. brandti*, especially since *L. floridana*

distribution is partially hindered by poor competitive ability. The results of this research indicate that *Eucryptorhynchus brandti* is an ideal agent for TOH suppression. In addition to that previously discussed, future studies should focus on mass production and release strategies. However, I do not recommend that *E. brandti* be released from quarantine until future studies confirm that the agent cannot successfully complete development or sustain populations on *L. floridana* or in *L. floridana* habitats.

References cited

- Akbulut, S. 2009. Comparison of the reproductive potential of *Monochamus galloprovincialis* on two pine species under laboratory conditions. *Phytoparasitica* 37: 125-135.
- Ammirante, M., R. D. Giacomo, L. D. Martino, A. Rosati, M. Festa, A. Gentilella, M. C. Pascale, M. A. Belisario, A. Leone, M. C. Turco, and V. D. Feo. 2006. 1-methoxy-canthin-6-one induces c-jun NH₂-terminal kinase-dependent apoptosis and synergizes with tumor necrosis factor-related apoptosis-inducing ligand activity in human neoplastic cells of hematopoietic or endodermal origin. *Cancer Res.* 66: 4385-4393.
- Ansari, S. H. and M. Ali. 1999. Recent developments in the chemistry of *Ailanthus altissima*. *Hamdard Medicus* 42: 58-63.
- APHIS. 2007. Plant health permits. TAG. Available: http://www.aphis.usda.gov/plant_health/permits/tag/charter.shtml [2007, April, 1].
- Ashraf, M. and Sham-ur-Reman. 2001. A short note on the vegetative propagation of *Ailanthus* and *Prosopis*. *Pak. J. Forest.* 51: 85.
- Ballerio, M., A. Ariu, and G. Piu. 2003. Allergy to *Ailanthus altissima* (tree of heaven) pollen. *Allergy* 58: 532-533.
- Brack, C. 1999. Forest measurement and modeling. Tree shape. Department of Forestry, Australian National University. Available: <http://www.fennerschool-associated.anu.edu.au/measuration/shape.htm> [2011, January 9].
- Briese, D. T. 2005. Translating host-specificity test results into the real world: the need to harmonize the yin and yang of current testing procedures. *Biol. Control* 35: 208-214.
- Broberg, C.L. and J.H. Borden. 2005. Hybrid poplar clones with *Populus maximowiczii* parentage demonstrate postoviposition antibiosis to *Cryptorhynchus lapathi* (Coleoptera: Curculionidae). *J. Econ. Entomol.* 98: 2254-2259.
- Burch, P. L. and S. M. Zedaker. 2003. Removing the invasive tree *Ailanthus altissima* and restoring natural cover. *J. Arbor.* 29: 18-24.
- Byers, J. E. Impact of non-indigenous on natives enhanced by anthropogenic alteration of selection regimes. *Oikos* 97: 449-458.
- Campbell, J.F. 2005. Fitness consequences of multiple mating on female *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *Environ. Entomol.* 34: 833-843.
- Center, T.D., and P.D. Pratt. 2004. Post-release procedures for biological control agents of aquatic and wetland weeds. Pp. 71-84, *In* E. M. Coombs, J. K. Clark, G. L. Piper, and A. F.

- Cofrancesco, Jr. (eds.), Biological control of invasive plants in the United States. Oregon State University Press, Corvallis, OR.
- Chang, Y-S., Y-H. Moon and E-R. Woo. 2003. Virus-cell fusion inhibitory compounds from *Ailanthus altissima* Swingle. Korean J. Pharmacogenetics 34: 28-32.
- Chen, H., C. Kaufmann, and H. Scherm. 2006. Laboratory evaluation of flight performance of the plum curculio (Coleoptera: Curculionidae). J. Econ. Entomol. 99: 2065-2071.
- Cortez-Rocha, M.O., J.L. Ríos-Soto, R.I. Sánchez-Mariñez, F.J. Wong-Corral, A. Burgos-Hernández, J. Borboa-Flores, and J. Leos-Martínez. 2009. Relationship between chemical and physical parameters of maize varieties and susceptibility to *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Southwest. Entomol. 34: 159-166.
- CPC-Center for Plant Conservation. 2010. CPC National Collection Plant Profile: *Leitneria floridana*. Available:
http://www.centerforplantconservation.org/collection/cpc_viewprofile.asp?CPCNum=2466
[2011 January, 15].
- Dallas, J.F., M.J.B. Leitch, and P.E. Hulme. 2005. Microsatellites for tree of heaven. (*Ailanthus altissima*). Mol. Ecol. Notes 5: 340-342.
- Dame, L. L. and H. Brooks. 1972. Handbook of the trees of New England. Dover Publications, New York, New York.
- Davies, P. A. 1942. The history, distribution, and value of *Ailanthus* in North America. Transactions, Kentucky Academy of Science. 9: 12-14.
- Delfosse, E.S. 2004. Introduction. Pp. 1-11, In E. M. Coombs, J. K. Clark, G. L. Piper, and A. F. Cofrancesco, Jr. (eds.), Biological control of invasive plants in the United States. Oregon State University Press, Corvallis, OR.
- Ding, J., Y. Wu, H. Zheng, W. Fu, R. Reardon and M. Liu. 2006. Assessing potential biological control of tree-of-heaven, *Ailanthus altissima* in North America. Biocontrol Sci. Tech. 16: 547-566.
- Dmitriew, C., M.W. Blows, and L. Rowe. 2010. Ontogenetic change in genetic variance in size depends on growth environment. Am. Nat. 175: 640-649.
- Dong, Z. L., W C. Gao, Q. Cao, J. G. Shan, Q. S. Qi, W.X. Wang, J.W. Lei, G. Zheng, and L.H. Zhang. 1993. Control of weevils damaging *Ailanthus* trees in Beijing with steinernematid nematodes. Chinese J. Biol. Ctrl. 9: 173-175.
- Dyar, H.G. 1890. The number of molts of lepidopterous larvae. Psyche 5: 420-422.

- Engler, A. 1931. Die Natürlichen Pflanzenfamilien, vol. 19a, 2nd ed. Wilhelm Engelmann, Leipzig.
- Esperk, T., T. Tammaru, and S. Nylin. 2007. Intraspecific variability in number of larval instars in insects. *J. Econ. Entomol.* 100: 627-645.
- Faccoli, M. 2009. Breeding performance of *Tomicus destruens* at different densities: the effect of intraspecific competition. *Entomol. Exp. Appl.* 132: 191-199.
- Federal Register. 1999. Executive order 13112. Presidential Documents. 64: 6183-6186.
- Feret, P.P. and R. L. Bryant. 1974. Genetic differences between American and Chinese *Ailanthus* seedlings. *Silvae Genet.* 23: 144-148.
- Feret, P.P., R. L. Bryant, and J.A. Ramsey. 1974. Genetic variation among seed sources of *Ailanthus altissima* (Mill.) Swingle. *Sci. Hortic.* 2: 405-411.
- Feret, P. P. 1985. *Ailanthus*: variation, cultivation, and frustration. *J. Arbor.* 11: 361-368.
- Fernando, E.S., and C.J. Quinn. 1995. Picramniaceae, a new family, and recircumscription of Simaroubaceae. *Taxon* 44: 177-181.
- Fernando, E.S., P.A Gadek, and C.J. Quinn. 1995. Simaroubaceae, an artificial construct: evidence from *RBCL* sequence variation. *Am. J. Bot.* 82: 92-103.
- Franco, O.L., D.J. Rigden, F.R. Melo, and M.F. Grossi-de-Sá. 2002. Plant α -amylase inhibitors and their interaction with insect α -amylases: structure, function, and potential for crop protection. *Eur. J. Biochem.* 269: 397-412.
- Garrison, G. A., A. J. Bjugstad, D. A. Duncan, M. E. Lewis and D. R. Smith. 1977. Vegetation and environmental features of forest and range ecosystems. Agricultural Handbook 475. Washington, D. C., U.S. Department of Agriculture, Forest Service. 68 pp.
- Gay, L., P.E. Eady, R. Vasudev, D.J. Hosken, and T. Tregenza. 2009. Costly sexual harassment in a beetle. *Physiol. Entomol.* 34: 86-92.
- Ge, T. 2000. Preliminary study on the biology of *Eucryptorrhynchus brandti*. Newsletter of Forest Pests 2: 17-18.
- Hannon, E.R., N.T. Kittelson, J.A. Eaton, and J.J. Brown. 2008. Screening hybrid poplar clones for susceptibility to *Cryptorrhynchus lapathi* (Coleoptera: Curculionidae). *J. Econ. Entomol.* 101: 199-205.
- Hansen, R.W. 2004. Handling insects for use as terrestrial biological control agents. Pp. 59-70, *In* E. M. Coombs, J. K. Clark, G. L. Piper, and A. F. Cofrancesco, Jr. (eds.), Biological

- control of invasive plants in the United States. Oregon State University Press, Corvallis, OR.
- Herrick, N.J. and L.T. Kok. 2010. Classical biological control of weeds with Curculionidae. Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 5: 1-11.
- Heady, H.F., J.W. Bartolome, M.D. Pitt, G.D. Savelle, and M.C. Stroud. 1992. California prairie. Pp. 313-335, *In* R.T. Coupland (ed.). Ecosystems of the world 8A. Natural grasslands: introduction and western hemisphere. Elsevier, Amsterdam, Holland. 469 pp.
- Howard, J.L. 2004. *Ailanthus altissima*. *In* J.L. Howard (ed.), Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: <http://www.fs.fed.us/database/feis/> [2005, August 25].
- Hu, S. Y. 1979. *Ailanthus*. *Arnoldia* 39: 29-50.
- Huebner, C. D. 2003. Vulnerability of oak-dominated forests in West Virginia to invasive exotic plants: temporal and spatial patterns of nine exotic species using herbarium records and land classification data. *Castanea* 68: 1-14.
- ISHRW – International Survey of Herbicide Resistant Weeds. 2005. <http://www.weedscience.org/in.asp>.
- IT-IS. 2006. Taxonomic Information System on-line database, <http://www.itis.usda.gov>. [September 2006].
- Jacobs, H. 2003. Comparative phytochemistry of *Picramnia* and *Alvaradoa*, genera of the newly established family Picramniaceae. *Biochem. Syst. Ecol.* 31: 773-783.
- Jianguang, L., H. Zhao, and Y. Jie. 2004. Use of ZXX-65 vacuum circulatory fumigation equipment against *Eucryptorrhynchus brandti* (Harold). *Forest Pests and Disease* 1: 2004.
- Judd, W.S., C.S. Cambell, E.A. Kellogg, P.E. Stevens, and M.J. Donoghue. 2002. Plant systematics a phylogenic approach. Sinauer Associates, Inc. Sunderland, MA. 576 pp.
- Julien, M.H., J.D. Kerr, and R.R. Chan. 1984. Biological control of weeds: and evaluation. *Prot. Ecol.* 7: 3-25.
- Julien M.H., M.W. Griffiths. 1998. Biological control of weeds: a world catalogue of agents and their target weeds. CABI Publishing, New York, NY. 223 pp.
- Kartesz, J.T. and C.A. Meacham. 1999. Synthesis of the North American flora (Windows Version 1.0), [CD-ROM]. Available: North Carolina Botanical Garden. In cooperation with the Nature Conservancy, Natural Resources Conservation Service, and U.S. Fish and Wildlife Service [2001, January 16].

- Kartesz, J. T. 2009. Floristic Synthesis of North America, Version 1.0 . Biota of North America Program (BONAP). Available: <http://www.pollenlibrary.com/Specie/Leitneria+floridana/>, [February, 2011].
- Kartesz, J.T. 2011. Floristic synthesis of North America, version 1.0, Biota of North America Program (BONAP). (in press).
- Kiggundu, A., C.S. Gold, M.T. Labuschange, D. Vuylsteke, and S. Louw. 2007. Components of resistance to banana weevil (*Cosmopolites sordidus*) in *Musa* gerplasm in Uganda. Entomol. Exp. Appl. 122: 27-35.
- King, E.G., and N.C. Leppla. 1984. Advances and Challenges in Insect Rearing. Agric. Res. Serv., USDA, U.S. Government Printing Office. Washington, DC. 306 p.
- Kiviat, E. 2004. Occurrence of *Ailanthus altissima* in a Maryland freshwater tidal estuary. Castanea 69: 139-142.
- Knapp, L.B. and C.D. Canham. 2000. Invasion of an old-growth forest in New York by *Ailanthus altissima*: sapling growth and recruitment in canopy gaps. J. Torrey. Bot. Soc. 127: 307-315.
- Kok, L.T., T. J. McAvoy, R.A. Malecki, S.D. Hight, J.J. Drea, and J.R. Coulson. 1992. Host specificity tests of *Galerucella californiensis* (L.) and *G. pusilla* (Duft) (Coleoptera: Chrysomelidae), potential biological control agents of purple loosestrife, *Lythrum salicaria* L. (Lythraceae). Biol. Control 2: 282-290.
- Kok, L. T., S. M. Salom, S. Yan, N. J. Herrick, and T. J. McAvoy. 2008. Quarantine evaluation of *Eucryptorrhynchus brandti* (Harold) (Coleoptera: Curculionidae), a potential biological control agent of tree of heaven, *Ailanthus altissima*, in Virginia, USA. Pp. 292-300, In Julien, M.H., Sforza, R., Bon, M.C., Evans, H.C., Hatcher, P.E., Hinz, H.L. and Rector, B.G. (eds.), Proceedings of the XII International Symposium on Biological Control of Weeds. CAB International Wallingford, UK.
- Koller, G.L. 1997. *Leitneria floridana*: a shrub for wet woodland conditions. Arnoldia. Spring: 14-20.
- Kostel-Hughes, F., T.P. Young, and J.D. Wehr. 2005. Effects of leaf litter depth on the emergence and seedling growth of deciduous forest tree species in relation to seed size. J. of the Torrey Bot. Soc. 132: 50-61.
- Kowarik, I. 1995. Clonal growth in *Ailanthus altissima* on a natural site in West Virginia. J. Veg. Sci. 6: 853-856.
- Kowarik, I. and I. Säumel. 2007. Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. Perspect. Plant Ecol. 8: 207-237.

- Kuriwada, T., N. Kumano, K. Shiromoto, and D. Haraguchi. 2009. High population density and egg cannibalism reduces the efficiency of mass-rearing in *Euscepes postfasciatus* (Coleoptera: Curculionidae). *Fla. Entomol.* 92: 221-228.
- Kuriwada, T., N. Kumano, K. Shiromoto, and D. Haraguchi. 2010. The effect of mass-rearing on death-feigning behavior in the sweet potato weevil (Coleoptera: Brentidae). *J. Appl. Entomol.* 134: 652-658.
- Lenzin, H., C. Erismann, M. Kissling, A. K. Gilgen and P. Nagel. 2004. Abundance and ecology of selected neotypes in the city of Basel (Switzerland). *Tuexenia* 24: 359-371.
- Li, L., W. Qin, Z. Ma, W. Yan, S. Huang, and Z. Peng. 2010. Effect of temperature on the population growth of *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) on sugarcane. *Environ. Entomol.* 39: 999-1003.
- Maerz, J. C., C. J. Brown, C. T. Chapin and B. Blossey. 2005. Can secondary compounds of an invasive plant affect larval amphibians? *Funct. Ecol.* 19: 970-975.
- Mackauer, M. 1976. Genetic problems in the production of biological control agents. *Annu. Rev. Entomol.* 21: 369-385.
- Marler, M. 2000. A survey of exotic plants in federal wilderness areas. Pp. 318-327, *In* Cole, David N.; McCool, Stephen F.; Borrie, William T.; O'Loughlin, Jennifer, comps. *Wilderness science in a time of change conference--Volume 5: wilderness ecosystems, threats, and management; 1999 May 23-27; Missoula, MT. Proceedings RMRS-P-15-VOL-5. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.*
- Matrai, K., L. Szemethy, P. Toth, K. Katona and J. Szekely. 2004. Resource use by red deer in lowland nonnative forests, Hungary. *J. Wildlife Manage.* 68: 879-888.
- Mergen, F. 1959. A toxic principle in the leaves of *Ailanthus*. *Bot. Gaz.* 121: 32-36.
- NatureServe. 2009. Conservation status. NatureServe version 7.1. Available: <http://www.natureserve.org/explorer/ranking.htm#globalstatus> [2009, February 2].
- Olden, J.D. and N.L. Poff. 2003. Toward a mechanistic understanding and prediction of biotic homogenization. *Am. Nat.* 162: 442-460.
- Orwig, D. A. and D. R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, USA. *J. of the Torrey Bot. Soc.* 125: 60-73.
- Patterson, D.T. 1976. The history and distribution of five exotic weeds in North Carolina. *Castanea* 41:177-180.

- Petersen, D.E. and D.E. Fairbrothers. 1983. A serotaxonomic appraisal of *Amphipterygium* and *Leitneria* – two amentiferous taxa of Rutiflorae (Rosidae). *Syst. Bot.* 8: 134-148.
- Petrides, G.A. 1988. Peterson field guides to eastern trees. Houghton Mifflin Co., Boston and New York. 272 pp.
- Pittendrigh, B.R., J.E. Huesing, R.E. Shade, and L.L. Murdock. 1997. Monitoring of rice weevil, *Sitophilus oryzae*, feeding behavior in maize seeds and the occurrence of supernumerary moults in low humidity conditions. *Entomol. Exp. Appl.* 83: 225-231.
- Purcell, M.F., J.A. Goolsby, and W. Forno. 2004. Foreign Exploration. Pp. 27-31, *In* E. M. Coombs, J. K. Clark, G. L. Piper, and A. F. Cofrancesco, Jr. (eds.), *Biological control of invasive plants in the United States*. Oregon State University Press, Corvallis, OR.
- Rauter, C.M., M.J. McGuire, M.M. Gwartney, and J. E. Space. 2010. Effect of population density and female body size on number and size of offspring in a species with size-dependent contests over resources. *Ethology* 116: 120-128.
- Reichert, E., M.T. Johnson, E. Chacón, R.S. Anderson, and T.A. Wheeler. Biology and host preferences of *Cryptorhynchus melastomae* (Coleoptera: Curculionidae), a possible biocontrol agent for *Miconia calvescens* (Melastomataceae) in Hawaii. *Environ. Ent.* 39: 1848-1857.
- Robinson, B.L. and M.L. Fernald. 1908. Gray's new manual of botany: A handbook of the flowering plants and ferns of the Central and Northeastern United States and adjacent Canada, 7th ed. American Book Company, New York. 926 pp.
- Rosen, D.J. and R.B. Faden. 2005. *Gibasis pellucid* (Commelinaceae), a new and potentially weedy genus and species for Texas. *Sida* 21: 1931-1934.
- Rosen, D.J., R. Carter, and C.T. Bryson. 2006. The recent spread of *Cyperus entrerianus* (Cyperaceae) in the southeastern United States and its invasive potential in bottomland hardwood forests. *Southeast. Nat.* 5: 333-344.
- Salas, J. and J.H. Frank. 2001. Development of *Metamasius callizona* (Coleoptera: Curculionidae) on pineapple stems. *Fla. Entomol.* 84: 123-126.
- Sargent, C. S. 1888. The *Ailanthus*. *Garden and Forest* 1888: 1385-1386.
- Schall, M.J. and D.D. Davis. 2009. Verticillium wilt of *Ailanthus altissima*: susceptibility of associated tree species. *Plant Dis.* 93: 1158-1162.
- Schooler, S.S., P.B. McEvoy, and E. M. Coombs. 2004. The ecology of biological control. Pp. 15-26, *In* Coombs, E.M., J.K. Clark, G. L. Piper, A. F. Cofrancesco, (eds.), *Biological control of invasive plants in the United States*. Oregon State University Press, Corvallis, OR.

- Sharma, J. and W.R. Graves. 2004. Midwinter cold hardiness of *Leitneria floridana* from three provenances. *J. Environ. Hort.* 22: 88-92.
- Sharma, J., J.A. Schrader, and W.R. Graves. 2008. Ecology and phenotypic variation of *Leitneria floridana* (Leitneriaceae) in disjunct native habitats. *Castanea* 73: 94-105.
- Sheppard, A. W., R. H. Shaw and R. Sforza. 2006. Top 20 environmental weeds for classical biological control in Europe: A review of opportunities, regulations and other barriers to adoption. *Weed Res.* 46: 93-177.
- Socha, J. and M. Kulej. 2007. Variation of the tree form factor and taper in European larch of Polish provenances tested under conditions of the Beskid Sądecki mountain range (southern Poland). *J. Forest Sci.* 53: 538-547.
- Stevens, P.F. 2005. Angiosperm Phylogeny Website. Version 6, May 2005. <http://www.mobot.org/MOBOT/research/APweb/>.
- Swearingen, J.M. and P. Pannill. 2004. PCA Alien plant working group. www.nps.gov/plants/alien/fact/aial1.htm (Sept. 2006).
- Tellman, B. 1997. Exotic pest plant introduction in the American Southwest. *Desert Plants* 13: 3-10.
- Tellman, B. 2002. Human introduction of exotic species in the Sonoran Region, pp. 25-46 in *Invasive exotic species in the Sonoran Region*. University of Arizona Press, Tucson, Arizona. 424 pp.
- Thompson, J.R., Jr. 1932. Sex differentiation of adults of *Conotrachelus nenuphar*. *J. Econ. Entomol.* 25: 807-810.
- Udvardy, L. 1998. Spreading and coenological circumstances of the tree of heaven (*Ailanthus altissima*) in Hungary. *Acta Bot. Hung.* 41: 299-314.
- Ulmer, B.J. and L.M. Dossall. 2006. Glucosinolate profile and oviposition behavior in relation to the susceptibilities of Brassicaceae to the cabbage seedpod weevil. *Entomol. Exp. Appl.* 121: 203-213.
- USDA, ARS. 2006. National Genetic Resources Program. *Germplasm Resources Information Network - (GRIN)* [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland. URL: http://www.ars-grin.gov2/cgi-bin/npgs/html/tax_search.pl?ailanthus+altissima (August 2006).
- USDA, NRCS. 2007. The PLANTS Database, Version 3.5 (<http://plants.usda.gov>). National Plant Data Center, Baton Rouge, LA 70874-4490 USA (August 2006).

- Van Driesche, R.V., M. Hoddle, and T. Center. 2008. Control of pests and weeds by natural enemies: an introduction to biological control. Blackwell Publishing, Malden, MA 473 pp.
- Wapshere, A. J. 1974. A strategy for evaluating the safety of organisms for biological weed control. *Ann. Appl. Biol.* 77: 201-211.
- Watson, L. and M.J. Dallwitz. 1992 onwards. The families of flowering plants: Descriptions, illustrations. identification, information retrieval. Version: 13th January 2005. <http://delta-intkey.com>.
- Wen, X., Y. Kuang, M. Shi, H. Li, Y. Luo, and R. Deng. 2004. Biology of *Hylobitelus xiaoi* (Coleoptera: Curculionidae), a new pest of slash pine, *Pinus elliottii*. *J. Econ. Entomol.* 97: 1958-1964.
- Westbrooks, R.G. 2001. Invasive species, coming to America: New strategies for biological protection through prescreening, early warning, and rapid response. *Wildland Weeds* 4: 5-11.
- Wilson, N., T.J. Tufton, and P.E. Eady. 1999. The effect of single, double, and triple matings on the lifetime fecundity of *Callosobruchus analis* and *Callosobruchus maculatus* (Coleoptera: Bruchidae). *J. Insect Behav.* 12: 295-306.
- Wood, A. 1870. *Myrica floridana* (Chapm.) Alph. Wood. *Amer. Bot. Fl.* 1: 309.
- Woods, K.D. 1993. Effects of invasion by *Lonicera tatarica* L. on herbs and tree seedlings in four New England forests. *Am. Midl. Nat.* 130: 62-74.
- Woreta, D., and L. Sukovata. 2010. Effect of food on development of the *Melolonta hippocastani* F. beetles (Coleoptera: Melolonthidae). Forest Research Institute, Warszawa, Poland, *Leśne Prace Badawcze* 71: 195-199.
- Xiao, G.R. 1992. Forest insects of China. Beijing: China Forestry Publishing House.
- Zimdahl, R.L. 1995. Introduction. Pp. 1-18, *In* A.E. Smith (Ed.). Handbook of weed management systems. Marcel Decker, Inc., New York, NY.
- Zimdahl R.L. Fundamentals of weed science. 1999. Academic Press, New York, NY. p. 1-13.