

EVALUATION OF TWO POTENTIAL BIOLOGICAL CONTROL AGENTS OF BRAZILIAN
PEPPERTREE (SCHINUS TEREBINTHIFOLIUS) IN FLORIDA

By

VERONICA MANRIQUE

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To my life companion, Rodrigo Diaz, for always being by my side

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Abstract of Dissertation Presented to the Graduate School
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By

Veronica Manrique

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Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), introduced from South America, invades a variety of habitats in south and central Florida. The leaflet-roller moth *Episimus utilis* Zimmerman (Tortricidae) and the thrips *Pseudophilothrips ichini* Hood (Phlaeothripidae) were selected as potential biocontrol agents of Brazilian peppertree. The objective of this study was to examine the factors influencing the effectiveness of these two biocontrol agents against Brazilian peppertree in Florida. Results revealed that Brazilian peppertree genotypes did not affect the performance of *E. utilis*, but had a strong effect on the performance of two populations of *P. ichini*. The thrips haplotypes 2 and 3 were well adapted to all Florida genotypes and should be considered as biocontrol agent of Brazilian peppertree.

Episimus utilis performed well when reared on plants grown in fresh-water environments, and also when fed on high-nutrient treatments. Therefore, Brazilian peppertrees growing in upland habitats and high-fertility soils (e.g., abandoned farms) will provide high-quality hosts for *E. utilis* establishment. In addition, temperature-dependent models and GIS mapping predicted a range of 5.8 to 9.7 generations per year for *E. utilis* throughout Florida. Based on the isothermal

lines of the pupal stage (lethal exposure times), establishment of *E. utilis* may occur throughout Florida, Hawaii, and southern parts of California, Texas and Arizona.

Brazilian peppertree seedlings exposed to high levels of defoliation (30 larvae/plant) suffered a significant reduction in number of leaflets, plant height, foliar biomass, shoot: root ratio, and relative growth rate compared to control plants (no herbivory). In addition, these plants were not able to compensate for insect damage two months after herbivory. However, low levels of defoliation (15 larvae/plant) did not affect Brazilian peppertree growth and biomass allocation in the laboratory. Therefore, the effectiveness of *E. utilis* to suppress this weed will vary in relation to insect densities present in the field.

The results obtained in this Dissertation provide a better understanding of the interactions between two biocontrol agents and Brazilian peppertree. This information will be used to select suitable sites for field releases, and assist in developing an effective long-term control of Brazilian peppertree in different habitats in Florida.

CHAPTER 1 LITERATURE REVIEW

Introduction

Biological invasions are widely recognized as a serious threat to many natural and agricultural ecosystems worldwide (e.g., Simberloff 1996, Parker et al. 1999, Mack et al. 2000, Myers and Bazely 2003, Inderjit 2005). People have been moving plants and animals to different places for many centuries, but it was not until the mid 1900s that scientists began to study the environmental risk associated with nonindigenous species (Inderjit 2005). Since then, there have been a number of studies on the biology and ecology of nonindigenous species aimed at improving the understanding of the processes and dynamics of invasions (e.g., Elton 1958, Williamson 1996, Mooney and Hobbs 2000, Inderjit et al. 2005, Richardson and Pysek 2008). Many hypotheses were developed in order to explain the establishment and spread of species into new areas (e.g., Elton 1958, Tilman 1982, Davis and Thompson 2000, Blossey and Notzold 1995, Ellstrand and Schierenbeck 2000, Keane and Crawley 2002). The Enemy Release hypothesis (ERH) predicts the rapid increase in abundance and distribution of plant species when released from co-adapted natural enemies in the introduced range (Williams 1954, Williamson 1996, Keane and Crawley 2002). A variant of this hypothesis is the Evolution of Increased Competitive Ability (EICA) hypothesis, which predicts that an invader can reallocate resources from defense to growth and reproduction when released from their specialized natural enemies in the introduced range (Blossey and Notzold 1995). Thus, there is an increase in the competitive ability of introduced individuals compared to native species. Evidence both for and against ERH and EICA can be found in the literature (e.g. Willis et al. 2000, Siemann and Rogers 2001, Agraval and Kotanen 2003, Blair and Wolfe 2004, Cappuccino and Carpenter 2005, Genton et al. 2005, Joshi and Vrieling 2005).

Invasive species are an increasing problem in many ecosystems, and introduced species are estimated to cause annual economic losses of \$120 billion in the USA (Pimentel et al. 2005). Different management strategies have been employed against invasive plant species (e.g., Hobbs and Humphries 1995, Myers and Bazely 2003, Baker and Wilson 2004, Emery and Gross 2005, Hulme 2006). Classical biological control of weeds involves the intentional introduction of host-specific natural enemies (e.g., insect herbivores, pathogens) from the native range to suppress the target weed (Harris 1991, van Driesche and Bellows 1996, Nordlund 1996, DeLoach 1997, Cuda 2004). The reunion of co-adapted natural enemies with their host plants may result in a reduction of weed population density in the introduced range in accordance with the ERH. The steps in a typical weed biological control programs according to Harley and Forno (1992) are: 1) selection of the target weed, 2) literature review (taxonomy, biology, ecology of the target weed), 3) foreign explorations (search for potential agents in the native range), 4) ecological studies of the weed and natural enemies, 5) preparation of import permits to transport candidates to quarantine facilities, 6) host-specificity testing (no-choice and choice testing), 7) mass rearing and release, 8) evaluation and monitoring of agent establishment and subsequent control of the target weed, and 9) distribution of agents to new sites. Even though it often takes 10 years or longer to achieve, biological control is an environmentally friendly and sustainable approach which may be the best alternative for controlling the spread of many invasive plant species (Strong and Pemberton 2000, Myers and Bazely 2003). Classical biological control has a long history of success in both terrestrial and aquatic ecosystems (e.g., Hartley 1990, Julien and Griffith 1998, Grevstad 2006, Barton et al. 2007). Well-known examples include suppression of *Opuntia* cacti by *Cactoblastis cactorum* Berg. in Australia in 1926 (Dodd 1940, Hosking et al. 1988, Julien and

Griffiths 1998), and the control of *Hypericum perforatum* L. by *Chrysolina* beetles in the western USA (Huffaker and Kennett 1959, Julien and Griffiths 1998).

Study Overview

Hawaii and Florida often are cited as the two states most vulnerable to invasion in the USA, with over 4000 and 925 introduced species, respectively (Cox 1999). The establishment and spread of invasive plant species poses a serious threat to many ecosystems in Florida. Prominent examples include paperbark tree *Melaleuca quinquenervia* Cavanilles (Myrtaceae) invading Florida wetlands (Bodle et al. 1994, Turner et al. 1998), Australian pine *Casuarina equisetifolia* L. (Casuarinaceae) growing along coastal dunes (Morton 1980, Gordon 1998), Old World climbing fern *Lygodium microphyllum* (Cav.) (Lygodiaceae) that forms large mats covering native species (Nauman and Austin 1978, Pemberton and Ferriter 1998), and Brazilian peppertree *Schinus terebinthifolius* Raddi (Anacardiaceae) that is one of the most widespread invaders, especially in the Everglades (Cuda et al. 2006).

Brazilian peppertree is an introduced perennial plant that has become widely established throughout central and south Florida (Fig. 1-1) (Cuda et al. 2006). This species is native to Argentina, Brazil and Paraguay (Barkley 1944, 1957), and was brought to Florida as an ornamental in the 1840s (Mack 1991, Cuda et al. 2006). In the USA, Brazilian peppertree occurs in Hawaii, California, Arizona, Texas and Florida (Habeck et al. 1994, Cuda et al. 2006). Although Brazilian peppertree is still grown as an ornamental in some states (e.g., California, Texas, and Arizona), this plant has been recognized as an invasive species in Hawaii (Morton 1978, Randall 1993), California (Randall 2000), and Texas (Gonzalez and Christoffersen 2006). In Florida, Brazilian peppertree is considered to be one of the worst invasive species by the Florida Exotic Pest Council, and is recognized as one of the most widespread exotic plants in the state (Cuda et al. 2006).

Although herbicides and mechanical methods (e.g., cutting, burning and flooding) are routinely used for controlling existing Brazilian peppertree stands (Gioeli and Langeland 1997, Cuda et al. 2006), these methods are extremely labor intensive and can be very expensive, especially for large infestations. In addition, chemical and mechanical controls are unsuitable for some natural areas (e.g., mangrove forests) because they may have negative effects on non-target species (Doren and Jones 1997) and may increase water pollution. Therefore, alternative methods for long-term control of Brazilian peppertree are urgently needed in Florida.

Brazilian peppertree has been recognized as a target for classical biological control since the 1980s. Several phytophagous insects and a fungal pathogen were identified from exploratory surveys conducted in northern Argentina and southeastern Brazil as potential biological control agents because they clearly damaged the plant and appeared to be host specific in their native range (Bennett et al. 1990, Bennett and Habeck 1991, Habeck et al. 1994, Cuda et al. 2004). The following are two promising natural enemies of Brazilian peppertree that are currently being studied in Florida quarantine laboratories: 1) the leaflet roller moth *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae), and 2) the shoot and flower attacking thrips *Pseudophilothrips ichini* (= *Liothrips ichini*) Hood (Thysanoptera: Phlaeothripidae).

Taxonomy and Molecular Phylogenetics of *Schinus terebinthifolius* Raddi

The taxonomy of Brazilian peppertree follows the higher classification scheme published by Mabberley (1997):

- Kingdom Plantae
- Division Magnoliophyta
- Class Dicotyledonae (Magnoliopsida)
- Subclass Rosidae
- Order Sapindales
- Family Anacardiaceae
- Tribe Rhoeeae
- Genus *Schinus* L.
- Subgenus *Euschinus*
- Species *Schinus terebinthifolius* Raddi 1820

The genus *Schinus* is native to South America and includes approximately 28 species (Barkley 1944, 1957). Four *Schinus* species have been introduced into the continental United States: *S. longifolius* (Lindl.) Spreg. in Texas, *S. molle* L. (type species) and *S. polygamus* (Cav.) Cabrera in California, and Brazilian peppertree in California, Florida, Arizona and Texas. Although *S. molle* is still a popular ornamental, the California Exotic Pest Plant Council has listed this species as a category B invasive species (Cal-IPC 2006). Barkley (1944) described five varieties of Brazilian peppertree in South America based solely on morphological characters. Of the five recognized varieties, three have been introduced into the United States: *S. terebinthifolius* var. *acutifolius* Engl. in California; *S. terebinthifolius* var. *terebinthifolius* Raddi in California, Florida, Hawaii and Puerto Rico; and *S. terebinthifolius* var. *raddianus* Engl. in Florida and Puerto Rico (Barkley 1944). However, many of the morphological characteristics used to distinguish these different varieties in Florida are broadly overlapping and it is unclear how well differentiated these varieties are in the native range (Cuda et al. 2006).

In order to determine the origin and patterns of introduction of Brazilian peppertree in Florida, Williams et al. (2005, 2007) collected Brazilian peppertree samples from the introduced range in the USA (Florida, Hawaii, Texas, US Virgin Islands, California) and from the native range (Brazil, Argentina, Paraguay). These samples were used to characterize six polymorphic nuclear microsatellite loci in Brazilian peppertree, and a region of the chloroplast DNA (cpDNA) was sequenced (Williams et al. 2002, 2005). Two cpDNA haplotypes were found in Florida; haplotype A is more common on the west coast while haplotype B is more common on the east coast (Williams et al. 2005, 2007). Nuclear microsatellite DNA analyses revealed that extensive hybridization has occurred between these two types of plants since arriving in Florida (Williams et al. 2005, 2007). In addition, genetic studies in the native range identified eleven cpDNA

haplotypes (A-K) of Brazilian peppertree in Brazil, and haplotype D is the most common and widespread (Williams et al. 2005, unpub. data). The source location of haplotype A has been found along the coast of southeastern Brazil, and the haplotype B has recently been located in the coast of Bahia in northeastern Brazil (Williams et al. 2005, unpub. data).

Biology and Ecology of *Schinus terebinthifolius* Raddi

The invasion and displacement of native species by Brazilian peppertree poses a serious threat to biodiversity in many ecosystems of Florida (Morton 1978). Brazilian peppertree is found mainly in disturbed sites such as highway rights-of-way, canals, fallow farmlands, but it also invades natural communities including pinelands, hardwood hammocks, and mangrove forests (Cuda et al. 2006). Recent estimates based on aerial surveys indicate that approximately 2833 km² of all terrestrial ecosystems in central and south Florida have been invaded by this weed (Cuda et al. 2006). Several attributes of this plant contribute to its invasiveness, including a large number of fruits produced per female plant, an effective mechanism of dispersal by birds (Panetta and McKee 1997), tolerance to shade (Ewel 1978), fire (Doren et al. 1991), and drought (Nilson and Muller 1980b), allelopathic effects on neighboring plants (Gogue et al. 1974, Nilson and Muller 1980a, Morgan and Overholt 2005), and tolerance to saline conditions (Ewe 2001, Ewe and Sternberg 2002).

Brazilian peppertree is dioecious, and the main flowering period in Florida occurs from September to October with a much-reduced bloom occurring from March to May (Ewel et al. 1982). Numerous small, white flowers occur in dense axillary panicles near the end of branches. Flowers produce abundant amounts of pollen and nectar, and are primarily insect-pollinated. A massive number of bright red fruits are typically produced on the plants from November to February. The fruits are eaten and dispersed primarily by birds and mammals although some dispersal occurs by gravity or water (Ewel et al. 1982). Brazilian peppertree also is capable of

resprouting from above-ground stems and crowns after damage from cutting, fire, or herbicide treatment. Resprouting and suckering are often profuse and the growth rates of the sprouts are high, which contribute to the formation of dense clumps (Woodall 1979, Cuda et al. 2006).

The reproductive potential of Brazilian peppertree is enormous with female trees producing thousands of seeds every year. Seed germination occurs from November to April, but mainly from January to February, and seed viability ranges from 30-60% (Ewel et al. 1982). Seedlings are able to tolerate a broad range of extreme soil moisture conditions (Ewel 1978), and survival of established seedlings ranges from 66-100% (Ewel et al. 1982). Although the leaflets are present on Brazilian peppertree plants year round, vegetative growth becomes dormant in winter (October to December), corresponding to the flowering period. Under optimal growing conditions, Brazilian peppertree is capable of producing seeds three years after germination (Ewel et al. 1982).

Several studies have shown that the success of Brazilian peppertree as an invader in a variety of habitats in south Florida is a consequence of its capacity to tolerate and persist during adverse conditions. Greenhouse studies have shown that the growth rates and morphology of Brazilian peppertree are less affected by salinity than white mangrove (*Laguncularia racemosa* L.) or some other native freshwater species (Ewe 2001). Brazilian peppertree also was less affected by seasonality and more tolerant to root flooding than some native plant species growing in the Everglades National Park (Ewe and Sternberg 2002). In addition, total concentration of nutrients (N, P, Zn, and Cu) was higher in disturbed soils (e.g., previous farmed land) compared to undisturbed soils in the Everglades (Li and Nordland 2001). The high correlations between Brazilian peppertree leaflet P and plant-available P in soils suggest that P enrichment in farmed soils facilitated the invasion of this area by Brazilian peppertree (Li and Nordland 2001).

Biology and Ecology of Biological Control Candidates

The leaflet roller moth *E. utilis* is native to southeastern Brazil (Zimmerman 1978). The larval stage usually has five instars but occasionally six; larvae feed on Brazilian peppertree leaflets and can completely defoliate small plants (Martin et al. 2004). First to third instars tie together young leaflets with silk to feed, while older instars feed inside a cylindrical rolled leaflet (Fig. 1-2a). The dimorphic adults are small, grayish brown moths with distinctive wing patterns that readily separate males from females (Fig. 1-2b) (Zimmerman 1978). Females oviposit up to 172 eggs during their lifetime (Martin et al. 2004). Adults lived on average 7 days, and one generation was completed in 43 days at 22°C (Martin et al. 2004). Because *E. utilis* completes its entire life cycle in the canopy of the host plant, this agent may be an appropriate biological control agent against Brazilian peppertree growing in sites exposed to seasonal flooding in Florida. In the 1950s, *E. utilis* was released and established in Hawaii but successful control of Brazilian peppertree populations was not achieved (Goeden 1977, Yoshioka and Markin 1991, Julien and Griffiths 1998). Factors such as unfavorable abiotic and biotic conditions present in Hawaii may explain this outcome. For example, high larval mortality of *E. utilis* caused by introduced and native parasitoids and predators has been recorded (Davis 1959, Krauss 1963), which probably reduced the effectiveness of this biocontrol agent.

Host specificity studies for *E. utilis* were conducted in Brazil and in the Entomology and Nematology Department's containment facilities, Gainesville, FL. In total 61 plants species belonging to 23 plant families were tested (J. P. Cuda, unpubl. data). No-choice tests showed that larval feeding damage was restricted to plants in the family Anacardiaceae, and Brazilian peppertree suffered the greatest damage. Some development to the adult stage also occurred on European smoketree (*Cotinus coggygria* Scop.), Chinese pistache (*Pistacia chinensis* Bunge), cultivated pistachio (*Pistacia vera* L.), fragrant sumac (*Rhus aromatica* Ait.), winged sumac

(*Rhus copallinum* L.), poison sumac (*Toxicodendron vernix* L.), poisonwood (*Metopium toxiferum* L.), and cashew (*Anacardium occidentale* L.), which demonstrated that these plant species are in the physiological host range of the insect. However, choice tests showed that *E. utilis* clearly preferred Brazilian peppertree over the aforementioned non-target species. Even though *E. utilis* attacks non-target plant species in the laboratory, this has not been observed in the native range or in Hawaii (Cuda et al. 2006). A petition for the release *E. utilis* in Florida for biological control of Brazilian peppertree is in preparation.

The thrips *P. ichini* is native to southeastern Brazil (Hood 1949), and has been found feeding only on Brazilian peppertree in its native range (Garcia 1977). Thrips feeds by punching their food substrate with the solitary mandibule (left mandibule only developed) and sucking the cell contents through the maxillary stylets (Mound 2005). Both larvae and adult stages of *P. ichini* severely damage the host plant; larval feeding usually kills the growing shoot tips and adult feeding may cause flower abortion (Fig. 1-3) (Garcia 1977). Immature thrips feed on plants during two larval stages, and the remainder of the immature life cycle occurs in the soil. Thrips belonging to the family Phlaeothripidae are characterized by having three non-feeding pupal instars (the propupa, pupa I and pupa II) instead of two seen in other families of thrips (Mound and Marullo 1996). The adults are black and winged, and the larvae are mostly red and wingless. Females require a pre-oviposition period (5-15 days), and can lay on average 220 eggs during their lifetime (Garcia 1977). They exhibit arrhenotokous reproduction whereby unmated females produce only male progeny (Mound and Marullo 1996). The entire life cycle from egg to egg can be completed in 38 days at 24°C in controlled laboratory conditions (Garcia 1977). In Brazil, *P. ichini* is polyvoltine and can produce up to four generation per year (Garcia 1977).

Studies of the biology and host specificity of *P. ichini* were conducted in Brazil and in Florida quarantine; 37 plant species belonging to 10 families were tested (Garcia 1977, Cuda et al. 2002). No-choice tests showed that thrips larvae fed and completed their development only on Brazilian peppertree and California peppertree (*Schinus molle* L.). California peppertree is a popular ornamental in California, but the California Invasive Plant Council recently listed this tree as invasive (Cal-IPC 2006). Choice tests showed that females oviposited on Brazilian and California peppertrees, and a very few eggs were laid on poisonwood and American smoketree. Larvae that hatched on poisonwood survived only for few days, and all eggs laid on American smoketree were non viable. The thrips *P. ichini* was recommended for field release by the federal interagency Technical Advisory Group for Biological Control Agents of Weeds (TAG) in 2007.

The use of tortricid moths and thrips as biocontrol agents has shown to be effective against many noxious weeds. For example, galling by immature stages of the moth *Epiblema strenuana* Walk. at early stages of plant growth significantly reduced plant height, stem height, flower production, and biomass of *Parthenium hysterophorus* L. (Asteraceae) in Australia (McFadyen 1992, Dhileepan and McFadyen 2001). In addition, biological control of *Rubus argutus* Link by the moth *Croesia zimmermani* Clark has been effective in pastures and open areas in Hawaii (Julien 1992). Examples of thrips as biocontrol agents includes *Amyothrips andersoni* O'Neill on alligatorweed *Alternanthera philoxeroides* (Martius) Grisebach (Maddox 1973), *Liothrips mikaniae* Priesner on mile-a-minute weed *Mikania micrantha* Kunth (Cock 1982), *L. urichi* Karny on Koster's curse *Clidemia hirta* L. (Julien and Griffiths 1998), and *Sericothrips staphylinus* Haliday on gorse *Ulex europaeus* L. (Hill et al. 2001, Davies et al. 2005).

Selecting Effective Biological Control Agents

Biological control programs often have been criticized for their lack of predictability in terms of agent establishment and success (Ehler 1990, Harris 1998). Several factors are usually

considered during the process of agent selection, such as host specificity (Follett and Duan 1999, van Driesche et al. 2000, Sheppard et al. 2003), climatic adaptability (Wapshere 1983, Senaratne et al. 2006), and more recently, impact on the target weed (McEvoy and Coombs 1999, Briese et al. 2002, van Klinken and Raghu 2006). Host specificity testing has received the most attention by biological control practitioners (Briese and Walker 2002, Sheppard et al. 2003), but effectiveness of biocontrol agents has received much less consideration (van Klinken and Raghu 2006). There often is a wide range of potential agents available in the native range, but usually only a few of those agents effectively suppress the target weed (McFadyen 2003, van Klinken and Raghu 2006). For example, of the 21 insects released against prickly pear in Australia, only four contributed to the control of this weed (Hosking et al. 1988, Julien and Griffiths 1998). Therefore, more effort should be given to studying the effect of candidate agents on plant performance early in the process of agent selection in order to improve the outcome of biological control programs (McClay and Balciunas 2005).

There is general agreement among biological control practitioners that highly specialized natural enemies that share an evolutionary history with their hosts are likely to be the most effective agents for controlling invasive species (Strong and Pemberton 2000, Myers and Bazely 2003, Williams et al. 2005). Although host specificity has usually been considered at the species level, there is increasing evidence that natural enemy populations may be locally adapted to specific genotypes of their host (Nissen et al. 1995, Hufbauer and Roderick 2005). Local host or ‘fine-tuned’ adaptation refers to a process whereby locally occurring herbivores with shorter generation times than their hosts and poor dispersal capabilities adapt rapidly to a specific host genotype compared to other hosts to which they have no regular exposure (Edmunds and Alstad 1978, Ebert 1994, Gandon and Van Zandt 1998). Several studies have shown that the dispersal

capacity of thrips adults is typically limited, and movements occur mostly between neighboring host-plants, which result in low gene flow and strong local adaptation (Karban and Strauss 1994, Rhainds and Shipp 2003, Rhainds et al. 2005). For example, thrips populations of *Apterothrips secticornis* Trybom were specifically adapted to individual clones of their host *Erigeron glaucus* Ker. (Karban 1989). In the case of the Brazilian peppertree, two cpDNA haplotypes are found in Florida (A and B), and the majority of individuals are intraspecific hybrids between the two original introductions (Williams et al. 2005, 2007). Therefore, the performance of biological control agents should be tested against all plant genotypes present in the area of introduction.

Host-plant quality (e.g., nitrogen content) often influences the performance of insect herbivores (Wheeler and Center 1996, Price 2000, Hunter 2001, Wheeler 2001, Schwab and Raghu 2006). As mentioned before, Brazilian peppertree invades different habitats in Florida (e.g. mangrove forests, pinelands, abandoned farms) and environmental conditions encountered in different habitats may affect survival and effectiveness of potential biocontrol agents. In addition to host-plant quality, climate (e.g., temperature) present in the introduced range also may affect the establishment of biocontrol agents (Sutherst 2000, Byrne et al. 2002).

Developmental biology studies coupled with degree-day calculations have been used to predict many aspects of insect population dynamics, such as temperature requirements for a species and number of generations expected at specific locations (Logan et al. 1976, Briere et al. 1999, Herrera et al. 2005). In addition, the potential distribution of a particular insect also is associated with its ability to tolerate cold temperatures, which is essential for successful over-wintering and permanent population establishment (Chen and Kang 2005, Coetzee et al. 2007, Lapointe et al. 2007). Therefore, understanding the relationship between development and temperature is useful for predicting the outcome of classical biological control programs.

Many authors have emphasized the importance of measuring herbivore ability to suppress the target weed in order to select the most effective biocontrol agent (McEvoy and Coombs 1999, Balciunas 2000, Pratt et al. 2005, van Klinken and Raghu 2006). However, few pre-release studies have been conducted to measure the effect of herbivore damage on growth, survival, and reproduction of the target weed (McClay and Balciunas 2005). Of the 38 weed biocontrol projects examined by McFadyen (2003), only 54 out of 132 agents released (41%) contributed to the successful control of those weeds. Therefore, greater effort should be directed towards selecting agents on the basis of their potential efficacy (Sheppard 2003, McClay and Balciunas 2005). For instance, Treadwell and Cuda (2007) observed that multiple artificial defoliation events significantly affected growth and fruit production of Brazilian peppertree. Although artificial defoliation has been frequently used to measure plant responses to herbivory (Dhileepan et al. 2000, Broughton 2003, Wirf 2006), plants may respond differently to actual insect damage (Lehtila and Boalt 2004, Schat and Blossey 2005). Therefore, the effectiveness of biological control candidates of Brazilian peppertree should be evaluated before field releases are undertaken (Balciunas 2000).

Goals and Hypotheses

The overall goal of this research was to determine the potential effectiveness of the leaflet roller moth *E. utilis* and the thrips *P. ichini* as biological control agents of Brazilian peppertree in Florida. More specifically, insect performance was evaluated on different Brazilian peppertree genotypes found in Florida. In addition, Brazilian peppertree growing under different environmental conditions (e.g., saline environments, rich nutrient soil, etc.) may affect herbivore survival. Thus, laboratory experiments examined the effect of host-plant quality on the development and survival of *E. utilis*. Climate (e.g., temperature) may also affect permanent

establishment of biocontrol agents (Pilkington and Hoddle 2006, Lapointe et al. 2007).

Therefore, the temperature requirements of *E. utilis* were evaluated in the laboratory. Finally, the effect of *E. utilis* herbivory on growth and biomass allocation of Brazilian peppertree seedlings was evaluated. The results obtained in this study will be used to select suitable sites for field releases, and assist in developing an effective long-term control strategy for Brazilian peppertree in different habitats in Florida.

The specific hypotheses that were tested in this study are:

Component 1: Insect performance on different Brazilian peppertree genotypes

- **Hypothesis 1a:** The survival and development of *E. utilis* are similar when feeding on different Brazilian peppertree genotypes.
- **Hypothesis 1b:** The survival and development of *P. ichini* are similar when reared on different Brazilian peppertree genotypes.

Host specificity studies have shown that *E. utilis* is oligophagous and feeds on Brazilian peppertree and several plant species in the Anacardiaceae family in the laboratory (Cuda, unpubl. data). In contrast, *P. ichini* has a narrow host range, and can only develop on Brazilian peppertree and the congener *S. molle* (Cuda et al. 2002). Therefore, I hypothesized that *E. utilis* and *P. ichini* would develop on all Florida genotypes.

Component 2: The effect of host-plant quality on the performance of *Episimus utilis*

- **Hypothesis 2a:** The survival and development of *E. utilis* are greater on Brazilian peppertree plants grown in high nutrient environments.
- **Hypothesis 2b:** The survival and development of *E. utilis* are similar on Brazilian peppertree exposed to different salinity levels.

Brazilian peppertree invades different habitats in Florida (e.g., mangrove forests, pinelands, abandoned farms) and environmental conditions encountered in these habitats may affect the development of potential biocontrol agents. The performance of insect herbivores is usually affected by the nutrient contents of their host-plants, and greater survival occurs on high-

nutritional hosts (e.g., high N contents) (Mattson 1980, Wheeler and Center 1996, Stiling and Moon 2005). Therefore, I hypothesized that *E. utilis* would perform better when reared on Brazilian peppertree exposed to high fertility treatments in the laboratory. In addition, *E. utilis* is found in coastal locations in its native range (Brazil) (Martin et al. 2004), which suggests that this insect could tolerate saline environments.

Component 3: Temperature requirements for development of *Episimus utilis*

- **Hypothesis 3a:** The developmental rate of *E. utilis* will allow multiple generations per year to be completed in Florida, and the upper developmental threshold will be sufficiently high to not preclude its establishment.
- **Hypothesis 3b:** The ability of *E. utilis* to tolerate cold temperatures varies among insect stages, and lethal times at cold temperatures can be used to predict favorable regions for insect establishment.

Climate (e.g., temperature) is known to influence insect distribution and population growth, and therefore, the ability of a species to establish in new areas (Sutherst 2000, Byrne et al. 2002). Temperature is an important factor that influences insect survival and developmental rate (Logan et al. 1976, Briere et al. 1999, Herrera et al. 2005). Therefore, I hypothesized that *E. utilis* developmental rates will increase with temperature within the bounds of certain lower or upper developmental thresholds. Geographic Information System (GIS) models will be used to predict the number of generations per year that *E. utilis* may have at different locations post-release (Pilkington and Hoddle 2006). In addition, the potential distribution of a particular insect is associated with its ability to tolerate cold temperatures (Chen and Kang 2003, Lapointe et al. 2007). Thus, lethal times (LT₅₀ and LT₉₀) at low temperatures could be used to predict the northern limit of the distribution of *E. utilis* in the continental USA.

Component 4: Impact of *E. utilis* on growth and biomass allocation of Brazilian peppertree seedlings

- **Hypothesis 4a:** Growth and biomass allocation of Brazilian peppertree are negatively affected by insect defoliation.
- **Hypothesis 4b:** Brazilian peppertree seedlings cannot compensate for high levels of herbivory.

The plant's aboveground architecture (e.g., number of leaflets) is important for determining the photosynthetic capacity and growth rates of plants (Pearcy et al. 1987). Therefore, I hypothesized that defoliation by the biocontrol agent *E. utilis* will affect the growth and biomass allocation of Brazilian peppertree seedlings. In addition, plant compensatory growth varies with the amount and timing of insect damage (Thomson et al. 2003, Schooler and McEvoy 2006). Young plants are usually more vulnerable to insect damage, and therefore Brazilian peppertree seedlings exposed to high levels of herbivory will suffer a reduction in growth and biomass, and seedlings will not be able to compensate following insect damage.



A



B

Figure 1-1. Brazilian peppertree in Florida. A) large stands, B) detail of plant leaflet and fruits.



Figure 1-2. *Episimus utilis*. A) larval feeding damage, B) female (right) and male (left) adults.



A



B



C

Figure 1-3. *Pseudophilothrips ichini*. A) larvae, B) pupae, C) adult.

CHAPTER 2
EFFECT OF HOST-PLANT GENOTYPES ON THE PERFORMANCE OF TWO
BIOLOGICAL CONTROL AGENTS OF BRAZILIAN PEPPERTREE

Introduction

Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), a woody perennial plant native to South America (Barkley 1944, 1957), was introduced into Florida, USA, as an ornamental between ca. 1898 - 1900 (Morton 1978). Brazilian peppertree is recognized as one of the most widespread exotic plants in Florida (Cuda et al. 2006), and aerial surveys estimate that approximately 2833 km² of all terrestrial ecosystems in central and south Florida have been invaded by this noxious weed (Cuda et al. 2006). Chloroplast DNA (cpDNA) and nuclear microsatellite analyses indicated that two different populations of Brazilian peppertree were introduced separately on the east and west coasts of Florida (Williams et al. 2005, 2007). Haplotype A is more common on the west coast whereas haplotype B is more common on the east coast (Fig. 2-1). Microsatellite DNA analyses revealed that extensive hybridization has occurred between these two types of plants since arriving in Florida (Williams et al. 2005, 2007). In addition, genetic studies in the native range have identified eleven cpDNA haplotypes (A-K) in Brazil, and haplotype D is the most common and widespread (Williams et al. 2005, unpubl. data).

A classical biological control program was initiated in the 1980s against Brazilian peppertree in Florida. Several phytophagous insects were identified as potential biological control agents from exploratory surveys conducted in southeastern Brazil, including the leaflet rolling moth *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae), and the shoot and flower attacking thrips *Pseudophilothrips ichini* Hood (Thysanoptera: Phlaeothripidae) (Bennett et al. 1990, Bennett and Habeck 1991, Habeck et al. 1994, Cuda et al. 1999, Cuda et al. 2006). The larval stages of *E. utilis* feed on Brazilian peppertree leaflets and can completely defoliate small

plants (Martin et al. 2004). Because *E. utilis* completes its entire life cycle in the canopy of its host plant, this herbivore may be an appropriate agent for Brazilian peppertree growing on sites exposed to seasonal flooding. In the case of *P. ichini*, both larval and adult stages damage the host plant by feeding on the growing shoot tips and flowers causing flower abortion (Garcia 1977). Immature thrips undergo two larval stages on the plant, and three non-feeding pupal stages occur in the soil (Garcia 1977, Cuda et al. in press). Studies of the biology and host specificity of *E. utilis* and *P. ichini* were conducted in Brazil and Florida quarantine (Garcia 1977, Harmuch et al. 2001, Martin et al. 2004, Cuda et al. 2006). The thrips *P. ichini* was recommended for field release by the federal interagency Technical Advisory Group for Biological Control Agents of Weeds (TAG) and a release petition for *E. utilis* is in preparation.

There is general agreement among ecologists and weed biological control practitioners that highly specialized natural enemies that share an evolutionary history with their hosts are likely to be the most effective agents for controlling invasive plants (Zwolfer and Preiss 1983, Strong and Pemberton 2000, Myers and Bazely 2003, Williams et al. 2005). However, hybridization between plants from different source populations in the introduced range may compromise 'old associations'. Therefore, the objective of this study was to examine the performance of the leaflet roller *E. utilis* and the thrips *P. ichini* on all Brazilian peppertree genotypes found in Florida. In addition, the performance of two populations of *P. ichini* were evaluated on different Brazilian peppertree genotypes found in Florida and in the native range.

Materials and Methods

Insects and Plants

This study was conducted at the Biological Control Research and Containment Laboratory (BCRCL) located at the Indian River Research and Education Center (IRREC) of the University of Florida, Fort Pierce, FL, USA. A colony of *E. utilis* was initiated in August 2006 at the

BCRCL, and insects were reared on potted Brazilian peppertree plants (Florida genotypes) (pots: 18 cm height, 17 cm diameter) inside environmental growth chambers (25 ± 2 °C, 60-70% RH, 14L: 10D photoperiod) (for specific details on rearing methods, see Martin et al., 2004).

Episimus utilis individuals were originally collected in 2003 from Brazilian peppertree haplotypes C and D found in the vicinity of Curitiba, located in Parana Province of southeastern Brazil. Two different populations of *P. ichini* were imported to the BCRCL in Florida. Both populations were collected in Brazil; the first population was found feeding on Brazilian peppertree Brazil haplotypes C and D (Curitiba), and the second was found on Brazilian peppertree Brazil haplotype A (Vicosa). Preliminary genetic studies using mitochondrial DNA revealed that these two thrips populations are genetically distinct; the first population (Curitiba) was categorized as haplotype 5, and the second (Vicosa) as haplotypes 2 and 3 (D. Williams, unpub. data). A colony of the thrips haplotype 5 was initiated in January 2007 at the BCRCL. Since poor survival was obtained when thrips 5 were reared on Brazilian peppertree (Florida genotypes), the colony was maintained on *Schinus molle* L. (Anacardiaceae) potted plants. After all experiments were completed using this thrips population, the colony was terminated. A colony of the thrips haplotypes 2 and 3 was initiated in November 2007 at the BCRCL, and insects were reared on Brazilian peppertree potted plants (Brazilian haplotype A). Voucher specimens of *E. utilis* and *P. ichini* were deposited in the Florida State Collection of Arthropods, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA.

Brazilian peppertree plants representing four Florida genotypes were grown from cuttings that were collected in the field from west (haplotype A), east (haplotype B), and hybrids (haplotypes A or B) plants previously confirmed by genetic analysis (Williams et al. 2002, Williams et al. 2005). Individuals were classified as “pure bred” eastern (haplotype B) or western

(haplotype A) if they had the ancestry coefficient value (q) > 0.90 in a respective cluster, and as hybrids (haplotypes A or B) if they contained a maximum of $q = 0.40$ for either western or eastern ancestry. The cut ends from different Florida Brazilian peppertree plants (~10 plants per genotype) were coated in rooting hormone powder (Schultz TakeRoot® Rooting Hormone, Bridgeton, MO) and planted in pots (18 cm height, 17 cm diameter) using sterilized soil mix (Fafard® germination mix, Agawam, MA). Pots were placed in the shade and misted every 10 minutes. After 3 months, cuttings with attached roots were transplanted to new pots (18 cm height, 17 cm diameter) containing soil mixture (Fafard® #3B mix), and all plants were placed in the greenhouse under ambient conditions and watered as needed. Brazilian peppertree seeds from Brazil (~5 plants per genotype) were collected in Blumenau (haplotype A) and in Cutitiba (haplotype D), and grown inside a quarantine greenhouse at the BCRCL. Genetic analyses were conducted on all plants to confirm plant genotypes (Williams et al. 2002, 2005). *Schinus molle* seedlings were purchased from a nursery in California (Sherman Growers, San Marcos, CA), and grown inside a greenhouse at the BCRCL. All plants were fertilized once with 15 g of Osmocote® (a slow release fertilizer 15-9-12, N-P-K), and 400 ml per pot of liquid fertilizer (Miracle Grow® 24-8-16) monthly.

Life History Parameters of *E. utilis* on Different Brazilian Peppertree Genotypes

Experiments were conducted inside an environmental growth chamber (25 ± 2 °C, 60-70 % RH, 14L: 10D photoperiod) at the BCRCL. Four treatments were established (six to eight replicates per treatment): 1) Brazilian peppertree Florida haplotype B (BP FL-B), 2) Brazilian peppertree Florida haplotype A (BP FL-A), 3) Brazilian peppertree Florida hybrid A (BP FL-HA), and 4) Brazilian peppertree Florida hybrid B (BP FL-HB). Ten neonate larvae of *E. utilis* were caged on each potted plant inside a clear acrylic cylinder (45 cm height, 15 cm diameter) with six holes (6 cm diameter) and tops covered by a fine mesh to allow air circulation. After 20

days, the cylinders were removed and all plant foliage was cut. Last instars or pupae were placed individually inside plastic vials (29.5 ml, Bio-Serv, Frenchtown, NJ) containing moist filter paper and one plant leaflet. Upon adult emergence, individual pairs from each treatment (4-6 pairs per treatment) were placed inside oviposition wax paper cages containing a cotton wick soaked in Gatorade® (limon-lime flavor) and one Brazilian peppertree leaflet. Rectangles of wax paper (19 × 30 cm) were stapled together to form a cage for oviposition (Moeri 2007). These cages were placed inside Ziploc® freezer bags (22.5 × 18 cm) and kept in the environmental growth chamber. After all adults died, the numbers of hatched and unhatched eggs were counted under a dissecting microscope. Several insect parameters were recorded: 1) pupal weight (mg), 2) developmental time to adult (days), 3) survival to adult (%), 4) adult longevity (days), 5) fecundity (total eggs laid), and 6) fertility (% eggs hatched).

In a separate experiment, *E. utilis* development and survival was evaluated on two Brazilian peppertree genotypes: BP FL-HB, and Brazil haplotype D (BP BZ-D). The *E. utilis* colony was originally collected from haplotypes D and C present in Curitiba, Brazil, and therefore, BP BZ-D was included in the test. Individual neonate larvae were placed inside vials (29.57 ml, Bio-Serv, Frenchtown, NJ) containing moist filter paper and a plant leaflet, and 10 vials were used for each replicate (five replicates total). Several insect parameters were recorded: 1) developmental time to adult (days), 2) survival to adult (%), and 3) adult longevity (days).

Life History Parameters of *P. ichini* on Different Brazilian Peppertree Genotypes and *Schinus molle*

The experiments described below were conducted separately for each thrips population (haplotype 5, haplotypes 2-3) inside an environmental growth chamber (28 ± 2 °C, 60-70 % RH, 14L: 10D photoperiod) at the BCRCL in Fort Pierce, FL. Seven treatments were established (eight replicates per treatment): 1) BP FL-B, 2) BP FL-A, 3) BP FL-HA, 4) BP FL-HB, 5) BP

BZ-A, 6) BP BZ-D, and 7) *S. molle*. Ten neonates of *P. ichini* were placed inside a plastic vial (11 cm height, 5 cm diameter) containing a plant shoot and moist filter paper. Vials were checked every other day, and moisture and food was added as needed. The insect parameters recorded were: 1) survival to adult (%), and 2) developmental time to adult (days).

In a separate experiment, adult longevity of each population of *P. ichini* was measured using the same host plants mentioned above, including vials with no food (control). Ten newly emerged adults of *P. ichini* (five females: five males) were placed in each vial containing a plant shoot and moist filter paper, and seven to eight replicates per treatment were used. Vials were checked every other day, and survival and pre-oviposition period were recorded. Experiments were terminated when all adults died.

Feeding Preference of *P. ichini* Adults on Different Brazilian Peppertree Genotypes and *Schinus molle*

Newly emerged females (1-2 days old) of each *P. ichini* population, with no previous feeding experience, were used in the experiments (25 ± 2 °C). Six females of *P. ichini* were released in the center of each Petri dish (15 cm diameter) containing six leaflet disks (2 cm diameter) of the different Brazilian peppertree genotypes (BP FL-A, BP FL-B, BP FL-HA, BP BZ-A, BP BZ-D) and *S. molle* arranged in a circle (Fig. 2-2). A total of 40 Petri dishes (replicates) were used for each thrips population, and observations were conducted every half hour for a total of 4 hours. The observations consisted of recording the number of thrips on each leaflet disk. Thrips were considered feeding on the plant when found standing on the disk.

Data Analysis

Life-history parameters (averaged values per replicate) for *E. utilis* and *P. ichini* were compared between host plants using one-way analysis of variance (ANOVA) (SAS Institute 1999). Two-way ANOVA was used to compare pupal weight of *E. utilis* between genders and

plant treatments (SAS Institute 1999). Data expressed as percentages (e.g., survival and eggs hatched) were transformed using the arcsine square root-transformation (Zar 1999). For the feeding preference experiment, the total number of thrips in each plant treatment was analyzed over time using non-parametric Friedman's analysis of variance by ranks for each thrips population (Zar 1999). Statistically different means were separated using the Student-Neuman-Keuls (SNK) test (SAS Institute 1999). A significance level of $\alpha = 0.05$ was used for all statistical analyses.

Results

Life History Parameters of *E. utilis* on Different Brazilian Peppertree Genotypes

No differences were detected for survival (~54%), developmental time (~32 days) and adult longevity (~9 days) of *E. utilis* among the Florida Brazilian peppertree genotypes (survival: $F_{3,26} = 0.11, P = 0.93$; developmental time: $F_{3,29} = 0.12, P = 0.94$; longevity: $F_{3,23} = 0.9, P = 0.46$; Table 2-1). Pupal weight did not differ among plant genotypes (17.6 ± 0.5 mg), but female pupae (18.27 ± 0.8 mg) were larger than male pupae (17.01 ± 0.5 mg) (genotypes: $F_{3,59} = 2.11, P = 0.11$; sex: $F_{1,59} = 5.48, P = 0.02$; genotype \times sex: $F_{3,59} = 0.33, P = 0.8$). In addition, no differences were detected in fecundity (84.6 ± 10.5 eggs laid) or fertility ($67.7 \pm 9.5\%$ eggs hatched) of *E. utilis* females reared on the Florida genotypes (fecundity: $F_{3,17} = 0.87, P = 0.47$; fertility: $F_{3,17} = 0.11, P = 0.95$).

When *E. utilis* was reared either on Brazilian peppertree Florida hybrid B (BP FL-HB) or Brazilian haplotype D (BP BZ-D), similar results were obtained. No differences between plant genotypes were detected for survival to adult ($33 \pm 7\%$), developmental time to adult (34.5 ± 0.8 days), or adult longevity (5 ± 0.5 days) (survival: $F_{1,8} = 1.0, P = 0.34$; developmental time: $F_{1,31} = 0.07, P = 0.78$; longevity: $F_{1,21} = 0.94, P = 0.34$).

Life History Parameters of *P. ichini* on Different Brazilian Peppertree Genotypes and *Schinus molle*

Survival to the adult stage of *P. ichini* haplotype 5 differed among host-plants tested ($F_{6,51} = 29.95$, $P < 0.0001$; Table 2-2). The highest survival was observed on *S. molle* (75%), followed by Brazil haplotype D (44%) and Brazil haplotype A (24%) (Table 2-2). The lowest survival was obtained on all Brazilian peppertree Florida genotypes tested (0 to 4%) (Table 2-2). No differences were detected among host plants for developmental time to adult ($F_{3,22} = 1.39$, $P = 0.27$; Table 2-2). In contrast, the thrips haplotypes 2-3 had similar survival on all host plants tested except for Brazil haplotype D (6%) ($F_{6,53} = 6.6$, $P < 0.0001$; Table 2-2). Similarly, developmental time to the adult stage did not differ among host plants tested except for BP BZ-D (20 days) ($F_{6,51} = 3.03$, $P < 0.01$; Table 2-2).

Adult longevity of *P. ichini* haplotype 5 differed among the host plants tested ($F_{4,39} = 60.75$, $P < 0.0001$; Table 2-2). Adults survived longer on *S. molle* followed by Brazil haplotype D and Brazil haplotype A (Table 2-2). Longevity of <10 days was recorded for those adults exposed to either Brazilian peppertree Florida A or no food (control) (Table 2-2). Adults laid eggs on *S. molle* and all Brazilian peppertree Brazil genotypes, but no eggs were laid on BP-FL A. The pre-oviposition period was similar for all the three host plants that received eggs (12 ± 1 days) ($F_{2,23} = 2.45$, $P = 0.11$). In contrast, *P. ichini* haplotypes 2-3 had similar adult longevity in all host plants tested except for BP BZ-D (6 days) and control treatments (5 days) (Table 2-2). In addition, thrips haplotypes 2-3 laid eggs on all host plant tested except for BP BZ-D on which no eggs were found. The pre-oviposition period (days) of thrips haplotypes 2-3 varied among host plants ($F_{4,33} = 3.2$, $P = 0.027$), being shorter on BP BZ-A (6.5 ± 0.5) and BP FL-A (7.7 ± 1.2) compared to *S. molle* (8.5 ± 1.3), BP FL-B (10.5 ± 1.7), and BP FL-HA (13 ± 1.6).

Feeding Preference of *P. ichini* Adults on Different Brazilian Peppertree Genotypes and *Schinus molle*

Statistical differences were detected in the number of adults of *P. ichini* haplotype 5 feeding on different host plants over time, and the interaction of host plant and time was also significant (treatment: $F_{5,1919} = 42.8$, $P < 0.0001$; time: $F_{7,1919} = 4.3$, $P = 0.0001$; treatment \times time: $F_{35,1919} = 1.9$, $P = 0.0007$). After a 4-hour trial, females preferred to feed on all Brazil genotypes and *S. molle*, compared to Florida genotypes ($F_{5,239} = 4.6$, $P = 0.0005$; Fig. 2-3). Different results were obtained when thrips haplotypes 2-3 were used. Statistical differences were observed of the number of adults feeding on different host plants over time, and its interaction was also significant (treatment: $F_{5,1919} = 141$, $P < 0.0001$; observation: $F_{234,1919} = 9.9$, $P < 0.0001$; time: $F_{7,1919} = 30.2$, $P < 0.0001$; treatment \times time: $F_{35,1919} = 3.6$, $P < 0.0001$). After a 4-hour trial, females preferred to feed on BP-BZ A and *S. molle*, compared to Florida genotypes and BP-BZ D ($F_{5,239} = 10.4$, $P < 0.0001$; Fig. 2-3).

Discussion

Classical biological control has provided sustainable and long-term control of many invasive weeds in both terrestrial and aquatic ecosystems (e.g., Hartley 1990, Julien and Griffiths 1998, Grevstad 2006, Barton et al. 2007). Therefore, the introduction of host specific natural enemies against Brazilian peppertree may contribute to the control of this invasive species in Florida and elsewhere. Although host specificity has usually been considered at the species level, there is increasing evidence that natural enemy populations may be locally adapted to specific genotypes of their host (Nissen et al. 1995, Hufbauer and Roderick 2005). For instance, the performance of multiple haplotypes of the mite *Floracarus perrepae* Knihinicki differed between haplotypes of their plant host *Lygodium microphyllum* Cav. (Goolsby et al. 2006). Thus,

the mite haplotype that is best adapted to the particular fern haplotype found in the introduced range (Florida) offers the greatest prospect for control (Goolsby et al. 2006).

In the case of the Brazilian peppertree, the majority of individuals in Florida are intraspecific hybrids between the two original introductions (Williams et al. 2005, 2007). In the native range, cpDNA haplotypes are geographically structured with only one or two closely related haplotypes occurring at a given location (Williams et al. 2005, unpub. data). The source location of haplotype A has been found along the coast of southeastern Brazil, whereas the origin of haplotype B is not yet known, but is believed to be along the northeast coast of Brazil (Williams et al. 2005, unpub. data). Therefore, haplotypes A and B are distantly separated and it is unlikely that hybridization occurs naturally in the native range (Williams et al. 2005, 2007). These hybrid genotypes in the introduced range will not have locally adapted natural enemies in the native range, and so insect performance will need to be tested against all plant genotypes present in the area of introduction. Results from this study revealed that Brazilian peppertree genotypes did not affect the performance of the leaflet roller *E. utilis*, but had a strong effect on the performance of two populations of the thrips *P. ichini*. Although *E. utilis* seems to be a biological control agent well adapted to Brazilian peppertree *sensu lato*, this insect may not be sufficiently host specific to release in Florida. Host specificity tests in the laboratory found that *E. utilis* also feeds on other related plant species in the Anacardiaceae family (J.P. Cuda, unpublished data), but these non-target effects were not observed in the native range or in Hawaii (Cuda et al., 2006). Further studies are being conducted in Florida quarantine to determine whether *E. utilis* is safe to release in Florida.

This study showed that two populations of *P. ichini* differed significantly in their ability to utilize different genotypes of their host plant. Poor survival to adulthood (0-4%) and short adult

longevity (<10 days) were obtained for the haplotype 5 thrips on all Florida genotypes, while higher survival (~50%) and longevity (~30 days) were observed for the haplotypes 2-3 thrips on these Florida genotypes. In addition, thrips haplotype 5 did not lay eggs on Florida genotypes, whereas thrips haplotypes 2-3 laid eggs on all plants tested except for Brazil haplotype D. Therefore, the thrips haplotypes 2-3 that were originally collected from Brazil haplotype A are well adapted to Florida genotypes and should be considered as potential biocontrol agent of Brazilian peppertree in Florida. The high performance of *P. ichini* observed on *S. molle*, a close relative of Brazilian peppertree not present in Florida, was not unexpected. Although *P. ichini* is not found feeding on this plant in its native range, previous laboratory tests showed that *S. molle* is in the physiological host range of the thrips (Cuda et al. 2006). *Schinus molle* is a popular ornamental in California, but the California Exotic Pest Plant Council recently listed this species as a Category B invasive species (Cal-IPC, 2006).

Local host or ‘fine-tuned’ adaptation refers to a process whereby locally occurring herbivores with shorter generation times than their hosts and poor dispersal capabilities adapt rapidly to a specific host genotype compared to other hosts with which they have no regular exposure (Edmunds and Alstad 1978, Hartley and Forno 1992, Ebert 1994, Gandon and Van Zandt 1998, Goolsby et al. 2006). In this study, each thrips population of *P. ichini* performed better on the plant genotype from where it was originally collected. Preliminary genetic studies showed that thrips haplotype 5 is only associated with BP BZ-D and C, while haplotypes 2-3 were found on haplotypes A, K, N, and M in the native range (D. Williams unpubl. data). Brazilian peppertree haplotype K is close related to haplotype B (Williams et al. 2005, unpubl. data), which may explain the high performance of thrips 2-3 on BP FL-B. Overall, these results suggest that different populations of the thrips *P. ichini* are adapted to particular genotypes of

Brazilian peppertree in the native range. Similarly, the thrips populations of *Apterothrips secticornis* Trybom were specifically adapted to individual clones of their host *Erigeron glaucus* Ker. (Karban 1989). Several studies have shown that the dispersal capacity of thrips adults is typically limited, and movements occur mostly between neighboring host-plants, which result in low gene flow and strong local adaptation (Karban and Strauss 1994, Rhainds and Shipp 2003, Rhainds et al. 2005). In contrast, lepidopteran species such as *E. utilis* are capable of flying over larger distances (Suckling et al. 1994, Showers et al. 2001), and this may explain in part the differences in diet breadth between *E. utilis* and *P. ichini*. Another important trait usually associated with locally-adapted herbivores is parthenogenic reproduction or haplodiploidy (Rice 1983, Boecklen and Mopper 1998), which occurs in *P. ichini* (Garcia 1977).

Biological control programs have been criticized for their lack of predictability in terms of agent establishment and success (Ehler 1990, Harris 1998). In order to improve the predictability of biocontrol, the effectiveness of the natural enemies should be examined on all host genotypes found in the introduced range. In addition, genetic studies may help to identify co-adapted natural enemies in the native range that may be the most effective against a particular genotype present in the introduced range (Williams et al. 2005, Goolsby et al. 2006). This study showed that two candidate biological control agents differed in their ability to utilize different genotypes of their host-plant. The leaflet roller *E. utilis* performed well on all Brazilian peppertree genotypes tested (Florida and Brazil), whereas two populations of *P. ichini* differed in their ability to utilize different host plant genotypes. Poor survival was recorded for the thrips haplotype 5 when reared on the Florida genotypes, while higher performance on all host plants tested, except for Brazil D, was obtained for the thrips haplotypes 2-3. These results suggest that different populations of *P. ichini* are adapted to different Brazilian peppertree genotypes in the

native range, and highlight the importance of testing biological control agents on different genotypes of the weed from both the native and introduced range. Future studies should examine the chemical composition (e.g., secondary compounds) of the different Brazilian peppertree genotypes, which may provide greater insight into the interaction between these herbivores and their host-plants.

Table 2-1. Life history parameters (mean \pm SE) of *E. utilis* on different Brazilian peppertree Florida genotypes. BP FL-A=Brazilian peppertree Florida haplotype A, BP FL-B=Brazilian peppertree Florida haplotype B, BP FL-HB= Brazilian peppertree Florida hybrid B, BP FL-HA=Brazilian peppertree Florida hybrid A.

	Survival to adult (%)	Developmental time to adult (days)	Adult longevity (days)
BP FL-A	55.0 \pm 8.86	31.9 \pm 0.64	8.42 \pm 0.78
BP FL-B	55.0 \pm 9.44	32.0 \pm 0.52	8.16 \pm 0.75
BP FL-HA	56.25 \pm 8.22	31.52 \pm 0.32	9.58 \pm 1.14
BP FL-HB	48.33 \pm 11.9	31.89 \pm 0.63	9.56 \pm 0.2

Table 2-2. Life history parameters (means \pm SE) of two populations of *P. ichini* reared on different host plants. BP FL-A = Brazilian peppertree Florida haplotype A, BP FL- B = Brazilian peppertree Florida haplotype B, BP FL-HA = Brazilian peppertree Florida hybrid A, BP FL-HB = Brazilian peppertree Florida hybrid B, BP BZ-A = Brazilian peppertree Brazil haplotype A, BP BZ-D = Brazilian peppertree Brazil haplotype D, control = no food. Different letters in the same column indicate statistical differences between plant treatments ($P < 0.05$).

Host plants	Survival to adult (%)		Development to adult (days)		Adult longevity (days)	
	Thrips 5	Thrips 2-3	Thrips 5	Thrips 2-3	Thrips 5	Thrips 2-3
BP FL-A	3.8 \pm 2.6 d	51.2 \pm 8.9 a	18.7 \pm 1.33 a	15.8 \pm 0.5 b	9.09 \pm 0.3 c	34.8 \pm 1.6 a
BP FL-B	0 d	57.6 \pm 11.2 a	-	16.2 \pm 0.5 b	-	27.8 \pm 1.3 b
BP FL-HA	0 d	68.7 \pm 10.4 a	-	16.4 \pm 0.6 b	-	25.8 \pm 1.9 b
BP FL-HB	0 d	52.5 \pm 4.5 a	-	17.4 \pm 1 b	-	-
BP BZ-A	24.4 \pm 11.5 c	41.2 \pm 5.8 a	18.8 \pm 0.9 a	16.4 \pm 0.5 b	18.4 \pm 2.2 b	34.8 \pm 1.4 a
BP BZ-D	43.7 \pm 6.6 b	6.6 \pm 2.1 b	17.7 \pm 0.3 a	20 \pm 1 a	21.4 \pm 1.1 b	5.7 \pm 0.4 c
<i>S. molle</i>	75.5 \pm 7 a	42.5 \pm 3.6 a	17.3 \pm 0.5 a	17.1 \pm 0.5 b	33.1 \pm 1.4 a	25.8 \pm 2.2 b
Control	-	-	-	-	8.4 \pm 0.4 c	5.4 \pm 0.5 c

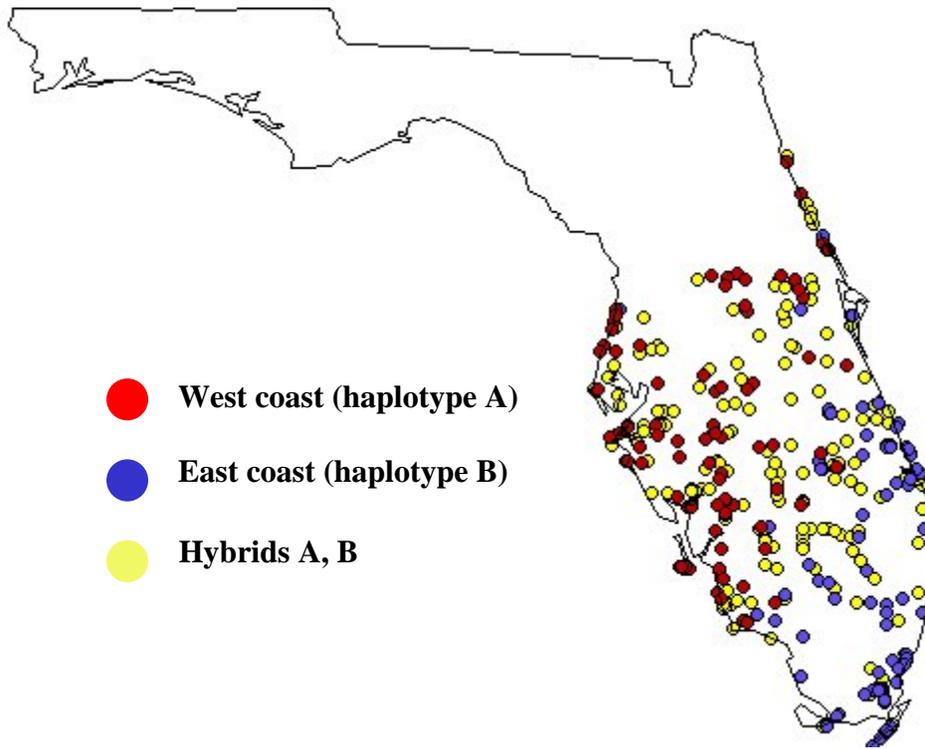


Fig. 2-1. Brazilian peppertree genotypes present in Florida

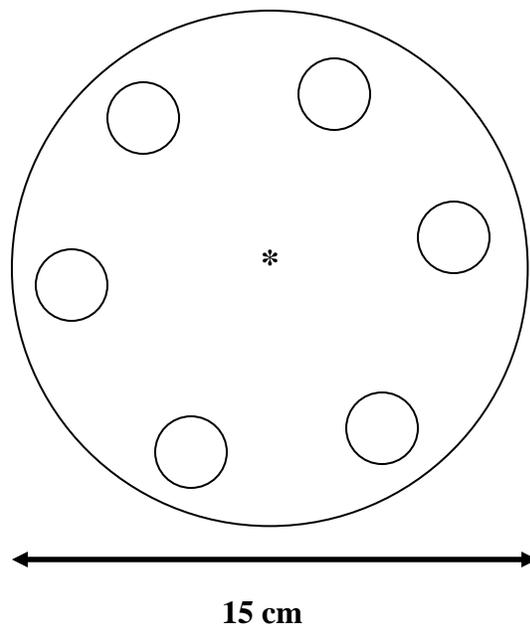


Fig. 2-2. Diagram showing the set-up used for the feeding preference trial of *P. ichini*. * Release point of thrips adults.

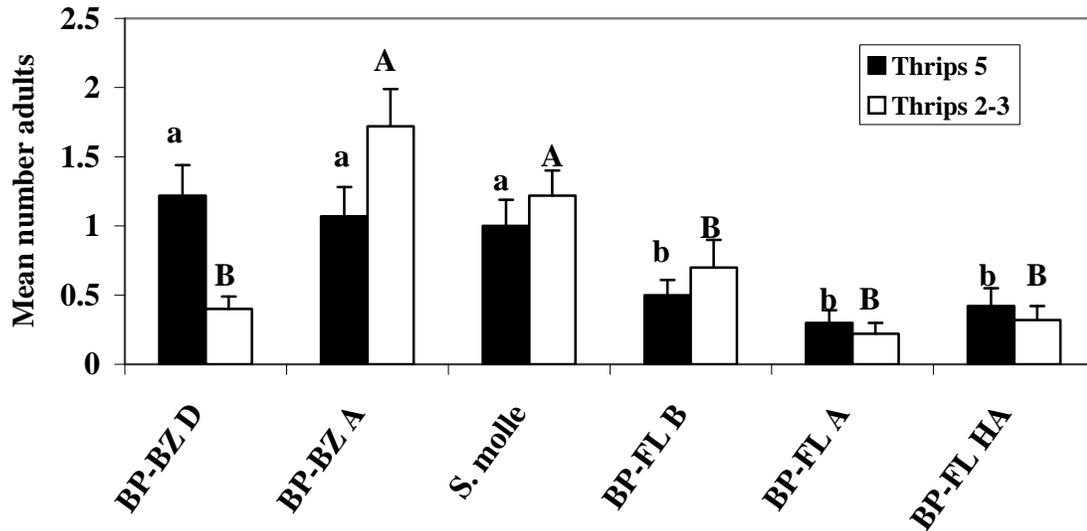


Figure 2-3. Number of adults (mean \pm SE) of *P. ichini* feeding on each host plant after a 4-hour trial. Different letters indicate statistical differences between host plants ($P < 0.05$); lower case letter shows differences for thrips 5, upper case letter shows differences for thrips 2-3.

CHAPTER 3
INFLUENCE OF HOST-PLANT QUALITY ON THE PERFORMANCE OF THE
BIOCONTROL AGENT EPISIMUS UTILIS

Introduction

Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), is an introduced perennial plant that has become widely established throughout central and south Florida (Cuda et al. 2006). This species is native to Argentina, Brazil and Paraguay (Barkley 1944, 1957), and was brought to Florida as an ornamental in the 1840s (Mack 1991). Brazilian peppertree not only colonizes disturbed sites such as highway rights-of-way, canals, fallow farmlands, and drained wetlands, but also invades natural communities, including pinelands, hardwood hammocks and mangrove forests in Florida (Myrtinger and Williamson 1986, Ewe 2001, Cuda et al. 2006). The invasion and displacement of native species by Brazilian peppertree poses a serious threat to biodiversity in many ecosystems of Florida (Morton 1978). Several attributes of this plant contribute to its invasiveness, including a large number of fruits produced per female plant, an effective mechanism of dispersal by birds (Panetta and McKee 1997), tolerance to shade (Ewel 1978), fire (Doren et al. 1991), and drought (Nilson and Muller 1980b), allelopathic effect on neighboring plants (Gogue et al. 1974, Nilson and Muller 1980a, Morgan and Overholt 2006), and tolerance to saline conditions (Ewe 2001, Ewe and Sternberg 2002).

Although herbicides and mechanical methods (e.g., cutting, burning and flooding) are routinely used for controlling existing Brazilian peppertree stands (Gioeli and Langeland 1997, Cuda et al. 2006), these methods are extremely labor intensive and can be very expensive, especially for large infestations. In addition, chemical and mechanical controls are unsuitable for some natural areas (e.g., mangrove forests) because they may have negative effects on non-target species (Doren and Jones 1997) and may increase water pollution. Therefore, alternative methods for long-term control of Brazilian peppertree are urgently needed. A classical biological

control program was initiated in 1980s, and the leaflet-roller moth *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae) was selected as a potential biocontrol agent of Brazilian peppertree because it clearly damaged the plant and appeared to be host specific in its native range (Martin et al. 2004, Cuda et al. 2006). The larvae of *E. utilis* feed on Brazilian peppertree leaflets and can completely defoliate small plants (Martin et al. 2004). Early instars tie young leaflets together to feed, whereas older instars feed and pupate inside a cylindrical rolled leaflet. The dimorphic adults are small, grayish brown in color with distinct wing patterns that separate the sexes (Zimmerman 1978). Because the entire life cycle of *E. utilis* occurs in the canopy of the host plant, this insect may be well adapted to areas subjected to seasonal flooding in south Florida. In the 1950s, *E. utilis* was released and established in Hawaii but successful control of Brazilian peppertree populations was not achieved (Goeden 1977, Yoshioka and Markin 1991, Julien and Griffiths 1998). However, its inability to control the plant in Hawaii should not preclude its release into other areas infested with Brazilian peppertree. Unfavorable abiotic or biotic factors may have contributed to its failure in Hawaii. For example, high larval mortality of *E. utilis* by introduced and native parasitoids and predators of agricultural pests was documented following its release (Davis 1959, Krauss 1963).

Because Brazilian peppertree invades different habitats in Florida (e.g., mangrove forests, pinelands, abandoned farms), the environmental conditions encountered in different habitats may influence the survival and effectiveness of potential biocontrol agents. Therefore, the objective of this study was to evaluate the performance of *E. utilis* reared on Brazilian peppertree exposed to different salinity and nutrient levels in the laboratory. Results from this study will provide a better understanding of the interaction between Brazilian peppertree and *E. utilis*, one of its

principal herbivores. This information will improve the selection of release sites favorable for establishing the insect in Florida following its release from quarantine.

Materials and Methods

Insect Rearing

Laboratory experiments were conducted at the Biological Control Research and Containment Laboratory (BCRCL) located at the Indian River Research and Education Center (IRREC) of the University of Florida, Fort Pierce, FL. A colony of *E. utilis* was initiated in August of 2006 at the BCRCL, and insects were reared on individual Brazilian peppertree plants (Florida genotypes) grown in nursery pots (18 cm height, 17 cm diameter) inside environmental growth chambers (25 ± 2 °C, 60-70 % RH, 14L: 10D photoperiod) (see Martin et al. (2004) for rearing procedures). Insects were originally collected in 2003 in the vicinity of Curitiba located in the Parana Province of southern Brazil, and imported to the quarantine facility in Gainesville, FL. Voucher specimens of *E. utilis* were deposited in the Florida State Collection of Arthropods, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA.

Life History Parameters of *E. utilis* on Brazilian Peppertree Exposed to Different Salinity Levels

Brazilian peppertree plants (24) were grown from seeds collected from Florida's east coast saline environment (Fort Pierce, FL) to assure plant adaptability to those conditions and maintained in the same size nursery pots (18 cm height, 17 cm diameter pots). Plants were grown using a mixture of soil (Fafard® #3B mix) and sand (1:1); all plants were placed in the greenhouse at BCRCL and watered as needed. Plants were fertilized once with 15 g of Osmocote® slow release fertilizer (15-9-12, N-K-P), and monthly with 400 ml per pot of liquid fertilizer (Miracle Grow 24-8-16). Forty-five days prior to initiating the experiments, three levels of soil salinity were established as follows (eight replicates each): 1) fresh water environment (0

parts per thousand salinity), 2) low saline environment (6 ppt salinity), and 3) high saline environment (12 ppt salinity). Plants were either irrigated with tap water (fresh water environment) or with water supplemented with seawater (36 ppt) to obtain 6 or 12 ppt salinity. In order to prevent plant stress, a stepwise increase in salt concentration (1 ppt every 3 days) was used. Plants were irrigated with each salinity level for the duration of the experiment (~2 months).

Several plant parameters were recorded at the beginning of the experiment (four to six replicates per treatment): 1) leaflet nutrient (N, P, K) and sodium contents (Na), 2) leaflet toughness, 3) specific leaflet area (SLA=leaf area / leaf dry weight), and 4) percent leaflet moisture content (LMC=(fresh - dried leaf weight) x 100 / fresh weight). Leaflet toughness was measured using a modified 300 g Pesola (Forestry Suppliers Inc., Jackson, MS) with a probe that estimates the pressure required to puncture leaflet tissues (average of four punctures per leaflet). Leaflet toughness, SLA and LMC were determined for one newly expanded leaflet (2nd from top of the stem), and one mature leaflet (3rd from bottom of the stem) from each treatment plant. For the nutrient and sodium analyses, leaflet samples from each plant treatment were harvested, oven-dried at 70°C for 1 week and ground; all samples were sent to the Agricultural Analytical Services Laboratory, Pennsylvania State University, PA.

Experiments were conducted inside an environmental growth chamber (25 ± 2 °C, 60-70 % RH, 14L: 10D photoperiod) starting on December 15, 2006. Ten neonate larvae of *E. utilis* were caged on each potted plant inside a clear acrylic cylinder (45 cm height, 15 cm diameter) with six holes (6 cm diameter) and tops covered by a fine mesh to allow air circulation. After 15 days, plant foliage was carefully examined and removed; mature larvae were placed individually inside plastic vials (29.57 ml, Bio-Serv, Frenchtown, NJ) containing moist filter paper and one

plant leaflet. To measure oviposition, newly emerged adult pairs from each treatment (4-5 pairs per treatment) were placed inside wax paper cages (rectangles of 19 x 30 cm) containing one Brazilian peppertree leaflet and a cotton wick with Gatorade® for food (Moeri 2007). The oviposition cages were placed inside Ziploc® freezer plastic bags and kept in the environmental growth chamber (same as before). After all adults died, the numbers of hatched and unhatched eggs were counted using a microscope. Several insect parameters were recorded: 1) pupal weight (mg), 2) developmental time to adult (days), 3) survival to adult (%), 4) adult longevity (days), 5) fecundity (total eggs laid), and 6) fertility (% eggs hatched).

Life History Parameters of *E. utilis* on Brazilian Peppertree Exposed to Different Nutrient Levels

Brazilian peppertrees (24 plants) were grown from seeds collected in Fort Pierce, FL using a mixture of potting soil (Fafard® #3B mix) and sand (1:1), and all plants were placed in a greenhouse at BCRCL and watered as needed. Plants were fertilized monthly using 400 ml per pot of liquid fertilizer (Miracle Grow 24-8-16, N-P-K) during the first 4 months to assure adequate plant growth. Two months before starting the experiment, three nutrient levels were established as follows (eight replicates each): 1) low nutrient-level (no fertilizer), 2) medium nutrient-level (fertilized once per month using 4 mg of fertilizer (Miracle Grow 24-8-16) per liter of water), and 3) high nutrient-level (fertilized twice per month using Miracle Grow 24-8-16).

Experiments were conducted inside an environmental growth chamber (25 ± 2 °C, 60-70 % RH, 14L: 10D photoperiod) starting on August 15, 2007. Several plant parameters described previously were recorded at the beginning of the experiment (six to eight replicates per treatment): 1) leaflet nutrient content (N, P, K), 2) leaflet toughness, 3) SLA, and 4) LMC. Ten neonate larvae of *E. utilis* were caged on each potted plant inside a clear acrylic cylinder. After

15 days, plant foliage was removed and examined for insects; mature larvae were placed individually inside plastic vials (29.57 ml, Bio-Serv, Frenchtown, NJ) containing moist filter paper and one plant leaflet. The same insect parameters were recorded as in the salinity study: 1) pupal weight (g), 2) developmental time to adult (days), 3) adult survival, 4) adult longevity (days), 5) fecundity (total eggs laid), and 6) fertility (% eggs hatched).

Data Analyses

Insect parameters (% survival to adult, adult longevity, fecundity, and fertility) and plant parameters (leaflet toughness, SLA, LMC, and nutrient content) were compared among treatments (different salinity levels or nutrient levels) using one-way analysis of variances (ANOVA) (SAS Institute 1999). The proportion of individuals surviving and the proportion of eggs hatched were arcsine square root transformed prior to analysis (Zar 1999). Pupal weight was compared between plant treatment and gender using two-way ANOVA (SAS Institute 1999). Means were separated using the post-hoc Student-Neuman-Keuls (SNK) test (SAS Institute 1999). A significance level of $\alpha = 0.05$ was used for all statistical analyses.

Results

Life History Parameters of *E. utilis* on Brazilian Peppertree Exposed to Different Salinity Levels

Higher survival to the adult stage was obtained when insects were exposed to Brazilian peppertree plant grown in fresh (55%) and low salinity-environments (36%), while survival was significantly lower under high salinity conditions (6%) (arcsine transformation survival: $F_{2,23} = 18.6$, $P < 0.0001$; Table 3-1). Time of development to the adult stage also differed between treatments ($F_{2,19} = 24.23$, $P < 0.0001$) with the longest time (44 days) observed for those larvae reared at the highest salinity level (12 ppt) (Table 3-1); however, adult longevity was similar between treatments ($F_{2,15} = 0.17$, $P = 0.84$; Table 3-1). In addition, pupal weight differed

between treatments and adult gender, but the interaction was not significant (treatment: $F_{1,28} = 45.11$, $P < 0.0001$; gender: $F_{1,28} = 4.92$, $P = 0.03$; treatment \times gender: $F_{1,28} = 0.03$, $P = 0.85$). Higher pupal weight was obtained for *E. utilis* reared on Brazilian peppertree plants grown in the fresh-water environment (18.93 ± 0.9 mg) compared to low salinity-environments (13.73 ± 1 mg), and female pupae (17.58 ± 0.9 mg) were heavier than males (15.68 ± 0.8 mg). However, no differences were detected in fecundity (88.9 ± 18 eggs laid) or fertility ($81.3 \pm 6.4\%$ eggs hatched) between fresh and low saline environments (fecundity: $F_{1,8} = 0.03$, $P = 0.87$; fertility: $F_{1,8} = 0.03$, $P = 0.86$). Pupal weight and reproductive parameters were not examined for *E. utilis* reared under high-salinity conditions (12 ppt) due to low numbers of pupae and adults obtained.

Leaflet nutrient and sodium contents also differed between salinity treatments (Table 3-2). Higher N and P were obtained on leaflets from fresh-water environments compared to saline environments, whereas, higher K and Na levels were recorded from low and high-saline environments (Table 3-2). In addition, toughness of mature leaflets (ML) was greater in the high-salinity treatments (12 ppt), whereas specific leaflet area (SLA) of new expanded leaflets (NEL) was greater in the fresh-water treatment (Table 3-3). Percent leaflet moisture content (LMC) was higher in the fresh-water compared to saline treatments (Table 3-3).

Life History Parameters of *E. utilis* on Brazilian Peppertree Exposed to Different Nutrient Levels

Higher percent survival of *E. utilis* to the adult stage occurred on Brazilian peppertree plants exposed to medium (25%) and high (40%) nutrient levels compared to the low level (1%) ($F_{2,23} = 12.8$, $P = 0.0002$; Table 3-4). Time of development to the adult stage and adult longevity was similar on medium and high nutrient treatments (time of development: $F_{1,12} = 1.57$, $P = 0.24$; longevity: $F_{1,11} = 0.97$, $P = 0.35$; Table 3-4). Although pupal weight did not differ between medium and high nutrient treatments, female pupae (17.9 ± 0.5 mg) weighed more than male

pupae (16.36 ± 1.0 mg) (treatment: $F_{1,19} = 0.42$, $P = 0.52$; gender: $F_{1,19} = 5.93$, $P = 0.02$; treatment \times gender: $F_{1,19} = 0.00$, $P = 0.96$). No differences were detected in fecundity (~ 133 total eggs laid) of *E. utilis* females reared on Brazilian peppertree plants grown in the medium or high nutrient treatments, whereas higher fertility (88% eggs hatched) was obtained in the high nutrient treatment (fecundity: $F_{1,9} = 1.13$, $P = 0.31$; fertility: $F_{1,9} = 6.25$, $P = 0.03$; Table 3-4). Development and reproductive parameters were not examined for *E. utilis* reared on low nutrient levels due to low numbers of adults obtained.

The leaflet nutrient contents likewise differed between fertilization treatments (Table 3-5). Higher N, P, and K levels were detected in Brazilian peppertree leaflets exposed to high nutrient treatment compared to the medium or low treatments (Table 3-5), confirming that the application of fertilizer resulted in nutritional differences in leaves. In addition, lower toughness of NEL and higher leaflet moisture content of ML were recorded on high nutrient plants compared to medium or low treatments (Table 3-6). Specific leaflet area (SLA) was not influenced by nutrient treatments (Table 3-6).

Discussion

Several factors influence the establishment of biological control agents, such as climate, natural enemies present in the introduced range and host-plant quality (Sutherst and Maywald 1985, Wheeler and Center 1996, Hunter 2001, Wheeler 2001, Byrne et al. 2003, Senaratne et al. 2006). Furthermore, the nutritional status of many weeds has played an important role in successful biological control programs (Myers 1987, Julien 1992). For example, initial applications of nitrogen fertilizer helped in the establishment of *Cactoblastis* moth on prickly pear cactus (Dodd 1940) and the *Cyrtobagous* weevil on the *Salvinia* aquatic fern (Thomas and Room 1986). In addition, plant palatability varies across salinity gradients (Levine et al. 1998, Moon and Stiling 2000, Goranson and Ho 2004), and high salinity levels may be detrimental to

some insect herbivores (Hemminga and van Soelen 1988, Moon and Stiling 2002, Schile and Mopper 2006). Results from this study showed that the performance of the candidate biocontrol agent *E. utilis* was influenced by its host-plant quality. Survival to adult was similar when insects were reared either on Brazilian peppertree irrigated with freshwater (55%) or low salinity-environments (36%), whereas lower survival was obtained for in the high salinity-environment (6%). In addition to high mortality, time of development to adult for *E. utilis* was longest in the high-salinity environment, which indicates poor host-quality for these plants. Differences in insect performance also were detected on Brazilian peppertree plants exposed to different nutrient levels. Increased survival of *E. utilis* was observed under medium (25%) and high (40%) nutrient levels compared to low (1.25%). Higher fertility was obtained when *E. utilis* was reared on plants in the high nutrient treatments. Therefore, the increase of leaflet nutrient content (N, P, K) improved insect performance overall.

Brazilian peppertree invades different habitats in Florida, including transitional mangrove forests where salinity levels range between 0 to 25 ppt depending on the time of the year (rainy vs. dry season) (Ewe 2001, Cuda et al. 2006). Thus, the salinity levels used in this study (6 and 12 ppt) are within the range of values encountered in the field. Brazilian peppertrees exposed to fresh-water environments were higher-quality host plants, as evidenced by the higher survivorship of *E. utilis*. Differences in plant parameters (e.g., leaflet nutrient contents, toughness, etc.) may explain the changes in insect survival observed among salinity-treatments. For example, N and P leaflet content were higher in fresh-water environments compared to saline environments, whereas K and Na were higher in low and high-saline environments. Several studies have shown that nitrogen is a key factor limiting the performance of insect herbivores, and an increase of N availability usually improves larval survival, growth rate and

reproduction of many insect species (Slansky and Feeny 1977, Wheeler and Center 1996, Bowdish and Stiling 1998, Hinz and Muller-Scharer 2000, Stiling and Moon 2005). In contrast, large amounts of sodium (Na) may disrupt metabolic function and could be detrimental to some insect herbivores (Wang et al. 2001, Wang 2002, Schile and Mopper 2006). Increased leaflet toughness also may reduce herbivore feeding and therefore influence insect performance (Feeny 1970, Raupp 1985, Wheeler and Center 1996). In this study, Brazilian peppertree plants exposed to high salinity conditions had tougher mature leaflets and lower moisture content, which negatively affected *E. utilis* survival. In contrast, Brazilian peppertrees irrigated only with freshwater had a higher specific leaflet area (SLA), which translates into thinner leaflets and lower toughness. Similarly, high salinity levels have been shown to adversely affect the performance of coleopteran stem-borers (Hemminga and van Soelen 1988), and were detrimental to leaf-mining insects (Schile and Mopper 2006). In addition, increased salinity and decreased N availability negatively affected the gall density of *Asphondylia borrichiae* Rossi and Strong (Diptera: Cecidomyiidae) (Moon and Stiling 2002).

Brazilian peppertree also is found in high-fertility soils such as disturbed sites or former farmlands in south and central Florida (Li and Norland 2001, Cuda et al. 2006). For example, the invasion of the Everglades National Park by Brazilian peppertree was facilitated by nutrient enrichment in abandoned farm soils in the Hole-in-the-Donut (Li and Norland 2001). Results from this study showed that Brazilian peppertree nutrient levels influenced the development and survival of *E. utilis* in the laboratory. Higher survival (40%) and fertility (88% eggs-hatched) occurred in the high nutrient treatments, which contained higher leaflet nutrient content (N, P, K), lower toughness and higher moisture content of leaflets. There often is a positive relationship between nitrogen and insect performance (Slansky and Feeny 1977, Mattson 1980, Wheeler and

Center 1996, Stiling and Moon 2005). In addition, dietary water has a nutritional value for most insect herbivores (Scriber 1979, Waring and Cobb 1992, Huberty and Denno 2004), and increase in water content has a positive effect on the growth performance of many lepidopteran species (Scriber 1979, Huberty and Denno 2004, Schoonhoven et al. 2005). Moreover, tougher foliage due to water stress can reduce nitrogen availability and adversely affect performance of chewing insects (Huberty and Denno 2004). Another important factor to consider is plant allelochemicals or secondary compounds that may affect insect development (Karban and Baldwin 1997). For example, an increase of nutrient uptake by plants may result in higher allocation to growth and reduce production of carbon-based allelochemicals (Bryant et al. 1983, Herms and Mattson 1992). This study indicates that Brazilian peppertrees growing in high-fertility soils will provide high-quality hosts for *E. utilis* development and survival, and insect establishment may occur more rapidly at those sites compared to low-fertility soils.

Overall, this study provides a better understanding of the interaction between Brazilian peppertree and *E. utilis*, a candidate biological control agent of this noxious weed in Florida. Brazilian peppertree plants growing in fresh water environments (e.g., upland pinelands, hammocks forests, along canals) and in high or medium-fertility soils (e.g., abandoned farmlands, disturbed sites) will provide high-quality hosts for *E. utilis* development and survival. In addition, coastal environments with saline levels of ~6 ppt. also will provide suitable sites for insect establishment. This information will help to select the most suitable sites for field releases and successful establishment of *E. utilis* in Florida. Further studies should evaluate population densities of *E. utilis* in different habitats in the native range in order to understand better how environmental conditions affect population dynamics of this species in the field.

Table 3-1. Life history parameters of *E. utilis* (means \pm SE) reared on Brazilian peppertree exposed to different salinity levels. Different letters in the same column indicate statistical differences between treatments ($P < 0.05$).

Salinity levels	Survival to adult (%)	Time of development (days)	Adult longevity (days)
0 ppt.	55 \pm 8.86 a	32.3 \pm 0.8 c	8.5 \pm 0.6
6 ppt.	36.25 \pm 6.25 a	39.37 \pm 1 b	8.94 \pm 0.6
12 ppt.	6.25 \pm 3.24 b	44.37 \pm 2.32 a	8.75 \pm 0.9

Table 3-2. Leaflet nutrient contents (means \pm SE) of Brazilian peppertree exposed to different salinity levels. Different letter in the same column indicate statistical differences between treatments ($P < 0.05$), $df = 2, 13$.

Salinity levels	N (%)	P (%)	K (%)	Na (ug/g)
0 ppt.	2.2 \pm 0.07 a	0.42 \pm 0.07	1.69 \pm 0.19 b	4700 \pm 465 b
6 ppt.	1.8 \pm 0.09 b	0.27 \pm 0.01	2.71 \pm 0.02 a	10906 \pm 1185 a
12 ppt.	1.8 \pm 0.09 b	0.21 \pm 0.006	2.89 \pm 0.11 a	12452 \pm 923 a
<i>F</i>	6.07	3.97	18.77	28.11
<i>P</i>	0.01	0.05	0.0003	<0.0001

Table 3-3. Plant parameters (means \pm SE) of Brazilian peppertree exposed to different salinity levels. NEL=new expanded leaflet, ML=mature leaflet. Different letters in the same column indicate statistical differences between salinity treatments ($P < 0.05$), df = 2, 11.

Salinity levels	Leaflet toughness (g/mm ²)		Specific leaflet area (cm ² / g)		Leaflet moisture content (%)	
	NEL	ML	NEL	ML	NEL	ML
0 ppt.	22.3 \pm 2.7	49.3 \pm 4.6 b	135.4 \pm 6.5 a	100.3 \pm 8.3	75 \pm 4.6 a	67.2 \pm 0.5 a
6 ppt.	36.9 \pm 6.4	61.6 \pm 6.4 b	97.6 \pm 11.2 b	84.1 \pm 7.7	53.5 \pm 3.2 b	56.2 \pm 1 c
12 ppt.	50 \pm 9.9	77.8 \pm 3.2 a	86 \pm 7.1 b	88.5 \pm 8.6	52.8 \pm 4.2 b	59.1 \pm 0.9 b
<i>F</i>	3.9	8.45	9.01	1.03	9.7	45.6
<i>P</i>	0.06	0.008	0.007	0.39	0.005	<0.0001

Table 3-4. Life history parameters of *E. utilis* (means \pm SE) reared on Brazilian peppertree exposed to different nutrient levels. Different letters in the same column indicate statistical differences between treatments ($P < 0.05$).

Nutrient levels	Survival to adult (%)	Time of development (days)	Adult longevity (days)	Fecundity (total number eggs)	Fertility (% egg hatched)
Low	1.25 \pm 1.25 b	-	-	-	-
Medium	25 \pm 8.23 a	35.76 \pm 1.1	11.93 \pm 0.5	119.5 \pm 16.5	78.9 \pm 3.03 b
High	40 \pm 6.54 a	34.1 \pm 0.8	11.28 \pm 0.5	148 \pm 21.7	88.7 \pm 1.1 a

Table 3-5. Leaflet nutrient contents (means \pm SE) of Brazilian peppertree exposed to different nutrient levels. Different letters in the same column indicate statistical differences between treatments ($P < 0.05$), $df = 2,17$.

Nutrient levels	N (%)	P (%)	K (%)
Low	0.95 \pm 0.06 c	0.14 \pm 0.01 c	1.89 \pm 0.05 b
Medium	1.54 \pm 0.06 b	0.31 \pm 0.02 b	1.72 \pm 0.07 c
High	2.01 \pm 0.05 a	0.45 \pm 0.02 a	2.22 \pm 0.05 a
<i>F</i>	77.11	50.36	20.33
<i>P</i>	<0.0001	<0.0001	<0.0001

Table 3-6. Plant parameters (means \pm SE) of Brazilian pepperrtee exposed to different nutrient levels. NEL=new expanded leaflet, ML=mature leaflet. Different letters in the same column indicate statistical differences between treatments ($P < 0.05$), df = 2, 23.

Nutrient levels	Leaflet toughness (g)		Specific leaflet area (cm ² / g)		Leaflet moisture content (%)	
	NEL	ML	NEL	ML	NEL	ML
Low	26.39 \pm 2.77 a	56.29 \pm 3.97	145 \pm 14.6	124.5 \pm 6.8	66.21 \pm 1.55	67.24 \pm 1.1 b
Medium	19.05 \pm 2.67 ab	57.94 \pm 2.26	145.6 \pm 9.1	132.6 \pm 14.2	69.1 \pm 1.25	70.13 \pm 0.9 a
High	14.98 \pm 2.04 b	56.85 \pm 3.36	169.8 \pm 7.5	130.7 \pm 10.4	70.04 \pm 0.53	70.9 \pm 0.7 a
<i>F</i>	5.25	0.07	1.7	0.15	2.77	4.31
<i>P</i>	0.01	0.93	0.21	0.86	0.08	0.027

CHAPTER 4
TEMPERATURE-DEPENDENT DEVELOPMENT AND POTENTIAL DISTRIBUTION OF
THE BIOCONTROL AGENT *EPISIMUS UTILIS*

Introduction

Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), is an introduced perennial plant that has become widely established throughout central and south Florida (Cuda et al. 2006). This species is native to Argentina, Brazil and Paraguay (Barkley 1944, 1957), and was brought to Florida as an ornamental in the 1840s (Mack 1991, Cuda et al. 2006). In the USA, Brazilian peppertree occurs in Hawaii, California, Arizona, Texas and Florida (Habeck et al. 1994, Cuda et al. 2006). In Florida, Brazilian peppertree is recognized as one of the worst invasive species by the Florida Exotic Pest Council, and is one of the most widespread exotic plants in the state (Cuda et al. 2006). The invasion and displacement of native species by Brazilian peppertree poses a serious threat to biodiversity in many ecosystems of Florida (Morton 1978).

Brazilian peppertree has been a target for classical biological control in Florida since the 1980s (Habeck et al. 1994, Cuda et al. 2006). During exploratory surveys conducted in southeastern Brazil, the leaflet roller *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae) was found to be commonly associated with Brazilian peppertree (Bennett et al. 1990, Bennett and Habeck 1991, Martin et al. 2004). The larvae feed on Brazilian peppertree leaflets and can completely defoliate small plants (Martin et al. 2004). First to third instars tie together young leaflets with silk to provide a cryptic feeding site, while older instars feed inside a cylindrical rolled leaflet. Adults are small, grayish brown moths with distinct wing patterns that separate males from females (Zimmerman 1978). The entire life cycle of *E. utilis* occurs in the canopy of Brazilian peppertree, which may be a favorable characteristic to allow establishment of this agent in areas subjected to seasonal flooding in south Florida. *Episimus utilis* was released and

established in Hawaii in the 1950s, but successful control of Brazilian peppertree populations has not been achieved (Goeden 1977, Yoshioka and Markin 1991, Julien and Griffiths 1998). Several factors may explain this outcome, such as unfavorable abiotic and biotic conditions for *E. utilis* in Hawaii. For example, high larval mortality by introduced and native parasitoids and predators has been documented (Davis 1959, Krauss 1963).

An understanding of temperature-dependent development of biocontrol candidates such as *E. utilis* is important when predicting potential geographic distribution and population dynamics in introduced areas. Developmental biology studies coupled with degree-day calculations have been used to predict many aspects of insect population dynamics, such as temperature requirements for a species and number of generations expected at specific locations (Logan et al. 1976, Briere et al. 1999, Lewis et al. 2003, Herrera et al. 2005). For example, Geographic Information System (GIS) models were used to predict favorable areas for permanent establishment of the egg parasitoid *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae), which is the major biocontrol agent of *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae) in California and Hawaii (Pilkington and Hoddle 2006). In addition, the potential distribution of a particular insect also is associated with its ability to tolerate cold temperatures, which is essential for successful over-wintering and permanent population establishment (MacDonald et al. 1999, Bale et al. 2002, Chen and Kang 2003, Coetzee et al. 2007, Lapointe et al. 2007).

Therefore, understanding the relationship between development and temperature is useful for predicting the outcomes of classical biological control programs. The objectives of this study were to determine temperature-dependent development and survival of *E. utilis*, and use this

information to generate a map to predict the number of generations per year of this biocontrol candidate across the full range of Brazilian peppertree in the USA.

Materials and methods

Plants and Insects

Studies were conducted at the Biological Control Research and Containment Laboratory (BCRCL) located at the Indian River Research and Education Center (IRREC) of the University of Florida, Fort Pierce, FL. A colony of *E. utilis* was initiated in August of 2006 from a culture maintained in Gainesville, Florida originally collected in 2003. Insects were reared on Brazilian peppertree potted plants inside environmental growth chambers ($25 \pm 2^\circ\text{C}$, 60-70% RH, 14L: 10D) (for specific details on rearing methods, see Martin et al. 2004). Insects were originally collected in 2003 from the vicinity of Curitiba located in the Parana province of southern Brazil. Brazilian peppertree leaflets were collected from the field in Fort Pierce, washed and placed inside plastic bags with a moist paper towel inside a refrigerator set at 5°C . All leaflets used in the experiments were no more than 2 days old. Voucher specimens of *E. utilis* were deposited in the Florida State Collection of Arthropods, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA.

Survival and Developmental Times Calculations

Temperature-dependent development studies at seven constant temperatures (10, 15, 20, 25, 30, 33, $35 \pm 0.5^\circ\text{C}$) were conducted inside environmental growth chambers (60-70% RH, 14L: 10D). HOBO® data loggers were placed inside each environmental growth chamber to confirm the experimental conditions. Neonate larvae were placed individually inside plastic vials (29.57 ml, Bio-Serv, Frenchtown, NJ) containing moist filter paper and a Brazilian peppertree leaflet. A total of 50 replicates (one vial = one replicate) per temperature was used. Vials were checked every other day, and water was added to maintain humidity and food was added as

needed. Survival and insect stages were recorded until adult emergence. In addition, single pairs of *E. utilis* adults (> 2 days old) were placed inside oviposition wax paper cages containing one Brazilian peppertree leaflet and a cotton wick with Gatorade® (lemon-lime) as diet (Moeri 2007). Oviposition cages were made using rectangles of wax paper (19 x 30 cm) that were stapled together to form a cage (Moeri 2007). Each oviposition cage was placed inside a Ziploc® freezer bag to maintain high humidity ($25 \pm 2^\circ\text{C}$, RH=60-70 %, photoperiod 14L: 10D). The wax paper cages with fresh eggs (<1 d old) were placed inside environmental growth chambers at each of the aforementioned seven temperatures (170-244 eggs from at least four females were used per temperature). Eggs were checked daily under the microscope to determine the percentage of eggs hatched and duration of egg development. The general linear model procedure (PROC GLM, SAS Institute 1999) was used to analyze the developmental time of each insect stage and all immature stages combined (egg-adult) for each temperature, and means were separated using the Student-Neuman-Keuls (SNK) test ($P < 0.05$).

Temperature Thresholds and Degree-days Calculations

The linear portion of the developmental rate curve [$R(T) = a + bT$] for each insect stage and all immature stages combined was modeled using the least squares linear regression (PROC GLM, SAS Institute 1999); T refers to temperature, and a and b are the intercept and slope estimates, respectively. The lower temperature threshold was estimated by the intersection of the regression line at $R(T) = 0$, $T_0 = -a/b$. Degree-day requirements for each stage and all immature stages combined were calculated using the inverse slope ($1/b$) of the appropriate fitted linear regression line (Campbell et al. 1974).

The non-linear relationship between developmental rate $R(T)$ and temperature T for each insect stage and all immature stages combined was fitted using the Logan 6 model which allows the estimation of the upper developmental threshold (Logan 1976, Roy et al. 2002). For the

Logan model equation $R(T) = \psi [e^{(\rho T)} - e^{(\rho T_L - (T_L - T)/\Delta T)}]$; $R(T)$ refers to the developmental rate at temperature T , ψ is the maximum developmental rate, ρ is a constant defining the rate at optimal temperature, T_L is the lethal maximum temperature, and ΔT is the temperature range at which physiological breakdown becomes the overriding influence (Logan 1976, Roy et al. 2002). The developmental rate of *E. utilis* was modeled using the Marquardt algorithm of PROC NLIN that determines parameter estimates through partial derivations (SAS Institute 1999). The range of temperatures used for the linear and non-linear models (15 to 30°C) was selected based on those temperatures where *E. utilis* completed its development.

Cold Tolerance of *E. utilis*

Cold tolerance studies were conducted using egg, fifth instar, pupa, and adult *E. utilis* stages, and the same methodology was employed for each insect stage. Four insects (larvae, pupae or adults) were placed inside a vial (29.57 ml, Bio-Serv, Frenchtown, NJ) containing moist filter paper and a Brazilian peppertree leaflet or Gatorade®, and a total of twenty vials per insect stage were used for each temperature. In addition, adult pairs (>2 days old) were placed inside oviposition wax paper cages containing one Brazilian peppertree leaflet and a cotton wick with Gatorade®. Wax paper cages (20 for each temperature) with fresh eggs (< 1 day) were placed inside Ziplocs freezer bags and used for the experiments (1,200-1,800 eggs per temperature).

All insect stages of *E. utilis* were acclimated to low temperatures by exposing them gradually from 15°C to the final temperature in intervals of 5°C per day. Each insect stage (eggs, fifth instars, pupae, and adults) was exposed to three constant temperatures (10, 5, and 0°C) for 0.5, 1, 2, 4, and 8 days (four replicates per exposure time). Since pupae were still alive after 8 days, a longer exposure time (ranging from 10 to 24 days) was used until all pupae had died at 0 and 5°C. After each exposure time, insects were placed at room temperature (25 ± 1°C) and survival was assessed 24 h later. Egg mortality was measured by examining egg-hatching daily

during 14 days after exposure to cold temperatures. Percentage survival (arcsine square root transformation) of each insect stage was analyzed by two-way analysis of variance (ANOVA) with exposure time and temperature as factors (SAS Institute 1999). The pupal stage was the most tolerant to cold temperatures; therefore, this stage was considered the most plausible overwintering stage and was used for further analysis. Effect of temperature and exposure time on percent pupal survival was analyzed using the logistic regression (SAS Institute 1999). The time required to 50 and 90% mortality ($L_{time_{50}}$ and $L_{time_{90}}$) of the population at a specific temperature was estimated using PROC PROBIT (SAS Institute 1999). The $L_{time_{50}}$ and $L_{time_{90}}$ for pupae at 5 and 0°C were used to develop models to predict isothermal lines with regions unfavorable for *E. utilis* establishment. The NAPPFAST database stores daily climate information from 1,879 weather stations across North America (Borchert and Magarey 2005), and probability maps were generated using the last 10 years of weather data with the above conditions. The maps were imported to ArcGis 9.0 and a line delimiting the frequency of occurrence for 5 out of 10 years was created (Lapointe et al. 2007).

GIS Maps to Predict Number of Generations of *E. utilis* in the USA

The methodology developed by Pilkington and Hoddle (2006) to generate prediction grids using ArcGis® was used in this study. GIS maps were generated from all US states where Brazilian peppertree is present. Daily temperatures from Florida (98 stations), Texas (396 stations), Arizona (61 stations), California (188 stations) and Hawaii (55 stations) were obtained from weather stations recorded by the Applied Climate Information System (CLIMOD, Southeast Regional Climate Center, <http://acis.dnr.sc.gov/Climod/>). Values for minimum and maximum temperatures were averaged separately for the last 5 to 11 years depending on the availability of data. A Microsoft Excel application developed by University of California-Davis (DegDay v.1.01, <http://biomet.ucdavis.edu/>) was used to obtain the accumulated degree-days for

E. utilis. This application calculates the accumulated degree-days using the single sine method, which employs the upper and lower temperature developmental thresholds of an organism, and daily average of minimum and maximum temperatures (Baskerville and Emin 1969). The number of generations per year was predicted by dividing the cumulative degree-days per station by K, the degree-day requirement for egg to adult development.

A spreadsheet was generated with the weather station name, latitude, longitude and number of *E. utilis* generations per year for each state (Florida, Texas, Arizona, California and Hawaii), saved as IV dBase file and imported into ArcGis 9.0 (ESRI Inc., Redlands, CA, USA). The imported file was converted into a shapefile using the ADD X-Y DATA function. A shapefile of each state was obtained from the Continental USA database of AWhere® (AWHERE, Inc., Denver, CO, USA) to project the geographic range of predictions. The ArcGis Geostatistical Analyst function (ESRI Inc., Redlands, CA, USA) was used to generate prediction grids of *E. utilis* generations per year. The Inverse Distance Weighted (IDW) deterministic method was used to generate weighted averages of nearby known values, and predict values at unsampled locations (Pilkington and Hoddle 2006). One of the advantages of the IDW methods is that it assigns closer values more weight to the predicted value than those that are further away.

Results

Survival and Developmental Time Calculations

Survival of neonate larvae to the adult stage of *E. utilis* was obtained at 15, 20, 25, and 30°C, but all larvae died at 10, 33, and 35°C (Fig. 4-1). The highest percent survival (62%) was recorded at 20°C followed by 25 and 30°C (44%), and the lowest was obtained at 15°C (2%) (Fig.4-1). Time of development of each insect stage decreased with increasing temperatures from 15 to 30°C (eggs: $F_{4,1083} = 9625.2$, $P < 0.0001$; larvae: $F_{3,96} = 2289.9$, $P < 0.0001$; pupae: $F_{3,77} =$

268.5, $P < 0.0001$; Table 4-1), and duration of development to adult was longest (136.6 days) at 15°C and shortest (30.94 days) at 30°C.

Temperature Thresholds and Degree-day Calculations

The linear regression parameters for each insect stage and all immature stages combined for *E. utilis* are shown in Table 4-2. The lower temperature thresholds varied from 8.17°C for eggs to 11.46°C for pupae, and for all stages combined (egg-adult) was 9.6°C (Table 4-2). The degree-day requirements for *E. utilis* varied from 94.34 for eggs to 384.61 for all larval stages combined, and the degree-day requirement from egg to adult was 588.23 (Table 4-2).

The parameter estimates for the Logan nonlinear model for each insect stage and all immature stages combined of *E. utilis* are shown in Table 4-3. The upper temperature threshold varied from 32°C for the larval stage and 35°C for the egg stage; the upper threshold for complete development was predicted to be 33°C (Table 4-3). The rate of development increased with temperature until the curve reached an optimum between 28 and 31°C, followed by a rapid decrease as temperatures reached the upper temperature threshold (Fig. 4-2).

Cold Tolerance of *E. utilis*

Survival of all insect stages of *E. utilis* was reduced when exposed to cold temperatures (Table 4-4; Fig. 4-3). Temperature and exposure time, and their interaction, had a significant effect on survival of eggs, pupae and adults (eggs: temperature $F_{2,45} = 489.7$, $P < 0.0001$; exposure time $F_{4,45} = 44.1$, $P < 0.0001$; temperature x time $F_{8,45} = 11.6$, $P < 0.0001$; pupae: $F_{2,79} = 47.4$, $P < 0.0001$; $F_{9,79} = 48.3$, $P < 0.0001$; $F_{8,79} = 5.9$, $P < 0.0001$; adults: $F_{2,45} = 105.2$, $P < 0.0001$; $F_{4,45} = 46.8$, $P < 0.0001$; $F_{8,45} = 19.1$, $P < 0.0001$ respectively; Table 4-4; Fig. 4-3). Survival of larvae decreased with exposure time but no differences were detected between temperatures (temperature $F_{2,45} = 1.7$, $P = 0.2$; exposure time $F_{4,45} = 65.6$, $P < 0.0001$; temperature x time $F_{8,45} = 2.3$, $P = 0.03$; Table 4-4). The egg stage was the most sensitive to cold

temperatures, since all eggs died after 2 days exposure to 0°C (Table 4-4). The pupal stage was the most tolerant to cold temperatures; therefore, this stage was considered the most plausible over-wintering stage and was used for further analysis. Pupal survival was high after 8 days of exposure at 5 and 10°C, with an average of 68.7 and 87.5% respectively (Fig. 4-3). In contrast, survival at 0°C decreased rapidly after 4 days with an average survival of 18.7% after 8 days (Fig. 4-3). The pupal lethal times at $L_{time_{50}}$ at 0°C was 5.38 days and the $L_{time_{90}}$ was 8.82 days, while at 5°C, the $L_{time_{50}}$ and $L_{time_{90}}$ were 10.44 and 27.65 days, respectively (Fig. 4-3).

GIS Maps to Predict Number of Generations of *E. utilis* in the USA

GIS maps were generated to predict the number of generations per year of *E. utilis* in Florida, Texas, Arizona, California and Hawaii (Fig. 4-4). Because Brazilian peppertree is present only in southern parts of Texas, Arizona, and California, we focused on these areas for further analysis. The number of generations followed a temperature gradient with 9 to 6 generations per year in warmer areas (e.g., southern Florida, Texas, and Arizona) compared to 1 to 4 generations in colder areas (e.g., northern Florida, south-east Arizona, and south-central California) (Fig. 4-4). The predicted number of generations per year of *E. utilis* varied from 0.5 to 9.8 in the Hawaiian Islands, where the insect has been released and established (Fig. 4-4). Permanent establishment will not occur in areas with < 1 generation per year (Fig. 4-4). According to the isothermal lines ($L_{time_{50}}$ and $L_{time_{90}}$ at 0 and 5°C), potential establishment may occur throughout Florida and southern California, and in southern parts of Texas and Arizona (Fig. 4-5). No limitations due to cold temperatures occur in Hawaii since all the isothermal lines are north of the Islands.

Discussion

Climate (e.g., temperature, humidity) is known to influence insect distribution and population growth, and therefore, the ability of a species to establish in new areas (Crawley

1987, Sutherst 2000, Byrne et al. 2002). Thus, understanding how temperature ranges affect insect development and survival is important for predicting areas of establishment and potential distribution of biological control agents in proposed areas of introduction. In addition, the mapping of the predicted distribution may help to assess the proximity to native species that may be at risk of non-target effects. This study showed that temperature affected the development of *E. utilis*, a candidate biocontrol agent of Brazilian peppertree in Florida. Developmental times decreased with increasing temperatures, ranging from 136.6 days at 15°C to 30.9 at 30°C, and no survival to adulthood was observed at 10, 33, and 35°C. Previous studies reported that time of development from egg to adult of *E. utilis* was 43.6 days at 22°C (Martin et al. 2004), which is close to the 51.2 days recorded here at 20°C. However, this is the first study to evaluate development of *E. utilis* at different temperatures, providing a better understanding of its environmental requirements.

Insect development may be different at fluctuating temperatures found in natural environments (Worner 1992, Brakefield and Mazzotta 1995). However, biological interpretations obtained from linear and non-linear models using constant temperatures have been shown to approximate fluctuating temperature conditions (Worner 1992, Doerr et al. 2002, Liu et al. 2002). Linear and non-linear models were used to determine lower (9.6°C) and upper (33°C) temperature limits for *E. utilis*, which may be close to the true critical temperatures thresholds for this insect. The lower threshold predicted by the linear model may be underestimated since no survival was obtained at 10°C. If we consider both laboratory data and model predictions together, the true lower threshold may range between 9.6 and 15°C, and the upper threshold between 30 and 33°C. Temperatures such as 9.6 or 33°C are present only for several hours a day in Florida (depending on the location), which most likely will allow *E. utilis* to survive at these

extremes. Furthermore, during times with unfavorable weather conditions, insects can locate microclimates that are suitable for survival (Ferro and Southwick 1984, Wilhoit et al. 1991, Chown and Crafford 1992). Similar studies have been conducted on other lepidopteran species (e.g., Kim et al. 2001, Doerr et al. 2002, Liu et al. 2002, Nabeta et al. 2005). For example, the lower (8.4°C) and upper thresholds (34°C) for development and degree-days from egg to adult (500) were calculated for the tortricid moth *Endopiza viteana* (Clemens) in the laboratory (Tobin et al. 2001).

GIS models that employ both laboratory developmental data and long-term climate records can be useful for predicting favorable areas for permanent establishment of biological control agents (Pilkington and Hoddle 2006, Diaz et al. in press). In this study, the number of generations per year for *E. utilis* varied from 0.5 to 9.8 depending on the location. Clearly, in areas where <1 generation per year is possible, permanent establishment is precluded. Brazilian peppertree is mainly present in south and central Florida, but recent reports indicate that this plant is now found in more northern parts of the state (northern peninsula near Georgia border and Florida Panhandle) (Meisenberg 2007). The models predicted a range of 5.8 to 9.7 generations per year for *E. utilis* throughout Florida, which indicates the existence of favorable conditions for establishment of this agent. If a release permit is eventually issued by APHIS, the potential dispersal and spread of *E. utilis* to states other than Florida should be considered. Although Brazilian peppertree is still grown as an ornamental in other states (e.g., California, Texas, and Arizona), this plant has been recognized as an invasive species in Hawaii (Morton 1978, Randall 1993), California (Randall 2000), and Texas (Gonzalez and Christoffersen 2006). Therefore, the establishment of *E. utilis* in other states may help to control Brazilian peppertree in these locations. During periods of unfavorable conditions (e.g., winter), many lepidopteran

species enter diapause (Nagarkatti et al. 2001, Goehring and Oberhauser 2002, Sims 2007). Therefore, if *E. utilis* does diapause, the predicted numbers of generations obtained here may be overestimated. However, there is no evidence of diapause in Hawaii or the native range. In addition, the pre-oviposition period of *E. utilis* at different temperatures was not evaluated, and including this variable in the model may improve the prediction. According to Martin et al. (2004), the peak of egg production for *E. utilis* at 22°C occurred 2 days after adult emergence; thus, the pre-oviposition period for this species is quite short. Nevertheless, the calculation of number of generations provides an indicator of potential efficacy of the biocontrol agent at a specific location (Hart et al. 2002, Mills 2005, Pilkington and Hoddle 2006). Therefore, those locations capable of producing many generations of *E. utilis* (e.g., south Florida, south Texas) may experience greater control of the target weed compared to those areas with fewer generations (e.g., south-east Arizona, south-central California). In Hawaii, the predicted number of generations of *E. utilis* ranged from 0.5 to 9.8, depending primarily on altitude. According to Yoshioka and Markin (1991), this insect species is widely distributed on Brazilian peppertree in the Hawaiian Islands, but the population dynamics (e.g., generations per year) have not been evaluated.

In addition to temperature-dependent development studies, the ability of an insect to tolerate unfavorable conditions such as low temperatures also should be considered when predicting establishment into new areas (MacDonald et al. 1999, Bale et al. 2002, Chen and Kang 2005, Coetzee et al. 2007, Lapointe et al. 2007). Exposure to low temperatures (5 and 0°C) affected the survival of all life stages of *E. utilis*, and relative susceptibility was eggs > adults > larvae > pupae. Eggs were the most sensitive stage with 100% mortality after 2 days at 0°C, while pupae were the most cold-tolerant stage with 100% mortality occurring after 12 days at

0°C. Therefore, we considered pupae as the over-wintering stage of *E. utilis*, and used the pupal lethal times ($L_{time_{50}}$ and $L_{time_{90}}$) to predict the northern limit of the species distribution. The $L_{time_{50}}$ was 5 days and $L_{time_{90}}$ was 9 days at 0°C, while at 5°C $L_{time_{50}}$ and $L_{time_{90}}$ were 10 and 28 days, respectively. Based on the isothermal lines, potential establishment of *E. utilis* may occur throughout Florida and southern parts of California, Texas and Arizona. In addition, favorable conditions are present throughout the Hawaiian archipelago, which permitted the establishment of *E. utilis*. The result for Hawaii has been validated since the insect is known to be widely established there (Goeden 1977, Yoshioka and Markin 1991, Julien and Griffiths 1998).

Climate matching between the region of origin and region of introduction is important when selecting candidate agents for biological control (Sutherst and Maywald 1985, Byrne et al. 2003, Senaratne et al. 2006). Florida is located between the latitudes 25 and 30°N, and, therefore, may provide suitable conditions for *E. utilis* establishment since this insect was collected between the same range of latitudes in the southern hemisphere (Martin et al. 2004). However, models (e.g., CLIMEX) that employ solely a combination of climatic variables to predict potential distributions will identify climatic homologues without reference to a particular species (Davis et al. 1998a, 1998b, Baker et al. 2000). Therefore, more accurate predictions may be achieved by selecting climatic variables (e.g., temperature) and their thresholds according to biological responses of a particular species determined by experimentation (Sharpe and deMichele 1977, Baker et al. 2000, Pilkington and Hoddle 2006). Results from this study provide a better understanding of the temperature requirements of *E. utilis*. In addition, GIS mapping is useful for predicting the population dynamics and performance of this species throughout the introduced range of Brazilian peppertree in the USA. However, complementary field studies

should be conducted to determine other key environmental factors (e.g., relative humidity) that may affect survival of *E. utilis*, and incorporate this information into the GIS mapping program to improve the prediction for establishment of this species in the USA.

Table 4-1. Mean (\pm SE) developmental time (days) of immature stages of *E. utilis* at five constant temperatures. Different letters in the same row indicate statistical differences $P < 0.05$.

	15°C	20°C	25°C	30°C	35°C
Eggs	16.55 \pm 0.09 a N=220	7.93 \pm 0.04 b N=244	4.95 \pm 0.03 c N=222	4.09 \pm 0.01 d N=232	4.18 \pm 0.03 d N=170
Larvae (1-5)	85.57 \pm 1.34 a N=7	26.85 \pm 0.37 b N=35	20.00 \pm 0.28 c N=31	19.40 \pm 0.29 c N=27	-
Pupae	34.50 \pm 0.50 a N=2	16.41 \pm 0.33 b N=31	9.34 \pm 0.37 c N=26	7.45 \pm 0.21 d N=22	-
Egg to adult	136.62	51.19	34.29	30.94	-

Table 4-2. Linear regression parameters estimates describing the relationship between temperature and developmental rate (1/D) of *E. utilis* stages

Stage	Intercept	Slope	R ²	N	Lower Threshold (°C)	Degree Days
Eggs	-0.0866	0.0106	0.94	1087	8.17	94.34
Larvae (1-5)	-0.0219	0.0026	0.85	99	8.42	384.61
Pupae	-0.0871	0.0076	0.99	80	11.46	131.58
Egg to adult	-0.0164	0.0017	0.95	80	9.6	588.23

Table 4-3. Logan non-linear model parameters for developmental rate of *E. utilis*. ψ = maximum developmental rate, ρ = empirical constant, TL= upper temperature threshold, ΔT = temperature range at which physiological breakdown becomes the overriding influence (Logan 1976).

Stage	Parameter	Estimate (\pm SE)	R ²
Egg	ψ	0.021 (0.011)	0.979
	ρ	0.086 (0.022)	
	TL	35.005 (0.650)	
	ΔT	1.716 (0.619)	
Larvae	Ψ	0.002 (0.003)	0.966
	P	0.133 (0.088)	
	TL	32.005 (0.200)	
	ΔT	2.497 (1.919)	
Pupae	Ψ	0.002 (0.002)	0.988
	P	0.1702 (0.071)	
	TL	33.002 (0.098)	
	Δ	2.869 (1.491)	
Egg to adult	Ψ	0.0008 (0.0006)	0.989
	P	0.169 (0.178)	
	TL	33.003 (0.142)	
	ΔT	3.895 (4.425)	

Table 4-4. Mean (\pm SE) percentage survival of eggs, larvae, and adults of *E. utilis* at different temperatures and exposure times (days)

Exposure time (days)	Eggs			Larvae V				Adults		
	10°C	5°C	0°C	10°C	5°C	0°C	10°C	5°C	0°C	
0.5	94.6 \pm 2.0	57.3 \pm 8.6	34.2 \pm 4.1	93.7 \pm 6.2	93.7 \pm 6.2	93.7 \pm 6.2	100 \pm 0	100 \pm 0	87.5 \pm 12.5	
1	89.5 \pm 3.4	61.8 \pm 3.1	9.0 \pm 3.2	75.0 \pm 10.2	75.0 \pm 10.2	93.7 \pm 6.2	100 \pm 0	100 \pm 0	81.2 \pm 6.2	
2	93.2 \pm 2.9	21.9 \pm 9.1	0 \pm 0	75.0 \pm 0	68.7 \pm 6.2	68.7 \pm 6.2	100 \pm 0	93.7 \pm 6.2	68.7 \pm 6.2	
4	77.4 \pm 4.7	2.1 \pm 2.1	0 \pm 0	68.7 \pm 11.9	68.7 \pm 6.2	56.2 \pm 6.2	93.7 \pm 6.2	93.7 \pm 6.2	0 \pm 0	
8	81.6 \pm 3.4	0 \pm 0	0 \pm 0	31.2 \pm 6.2	0 \pm 0	0 \pm 0	100 \pm 0	31.2 \pm 11.9	0 \pm 0	

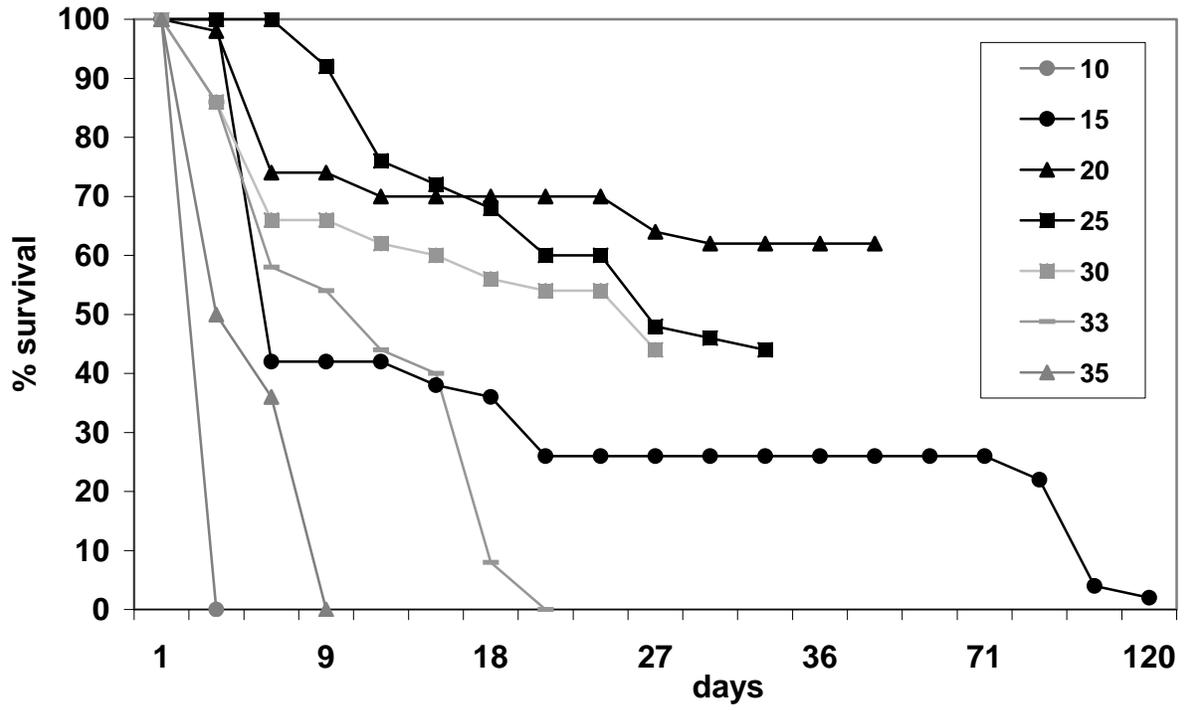


Fig. 4-1. Percentage survival from larvae to adult of *E. utilis* at seven constant temperatures.

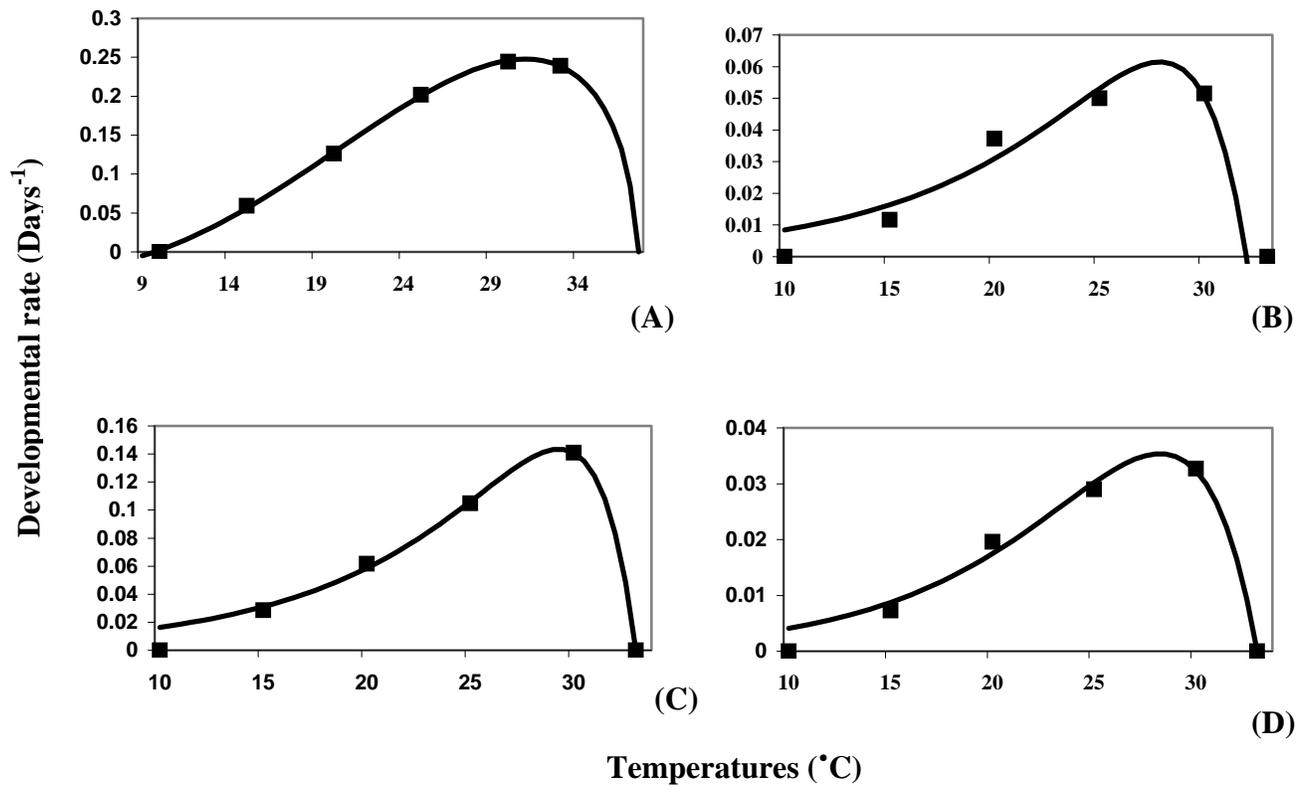


Figure 4-2. Developmental rates (Days⁻¹) of each immature stage of *E. utilis*. A) eggs, B) larvae, C) pupae, (D) total immature stages. Single dots are observed values and solid lines are Logan non-linear model predictions.

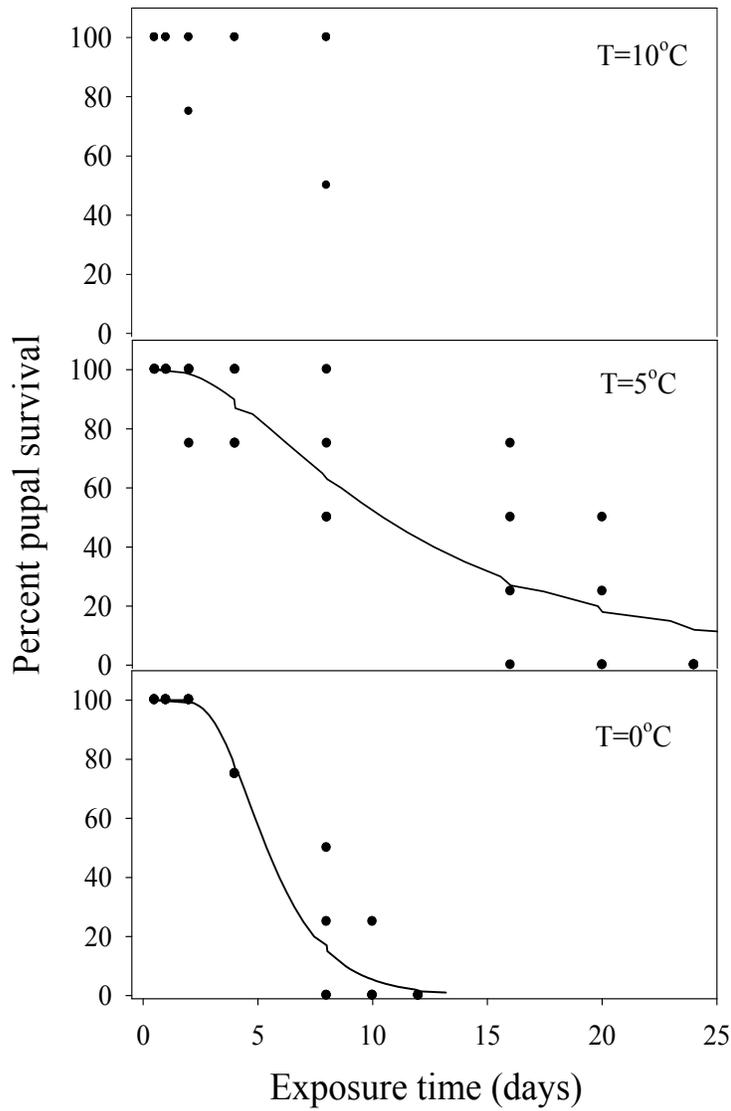


Figure 4-3. Pupal survival of *E. utilis* at different exposure times and temperatures (10, 5 and 0°C). Single dots are observed values and lines are the expected value of the logistic regression. The data obtained at 10°C did not follow a logistic regression so no line was plotted.

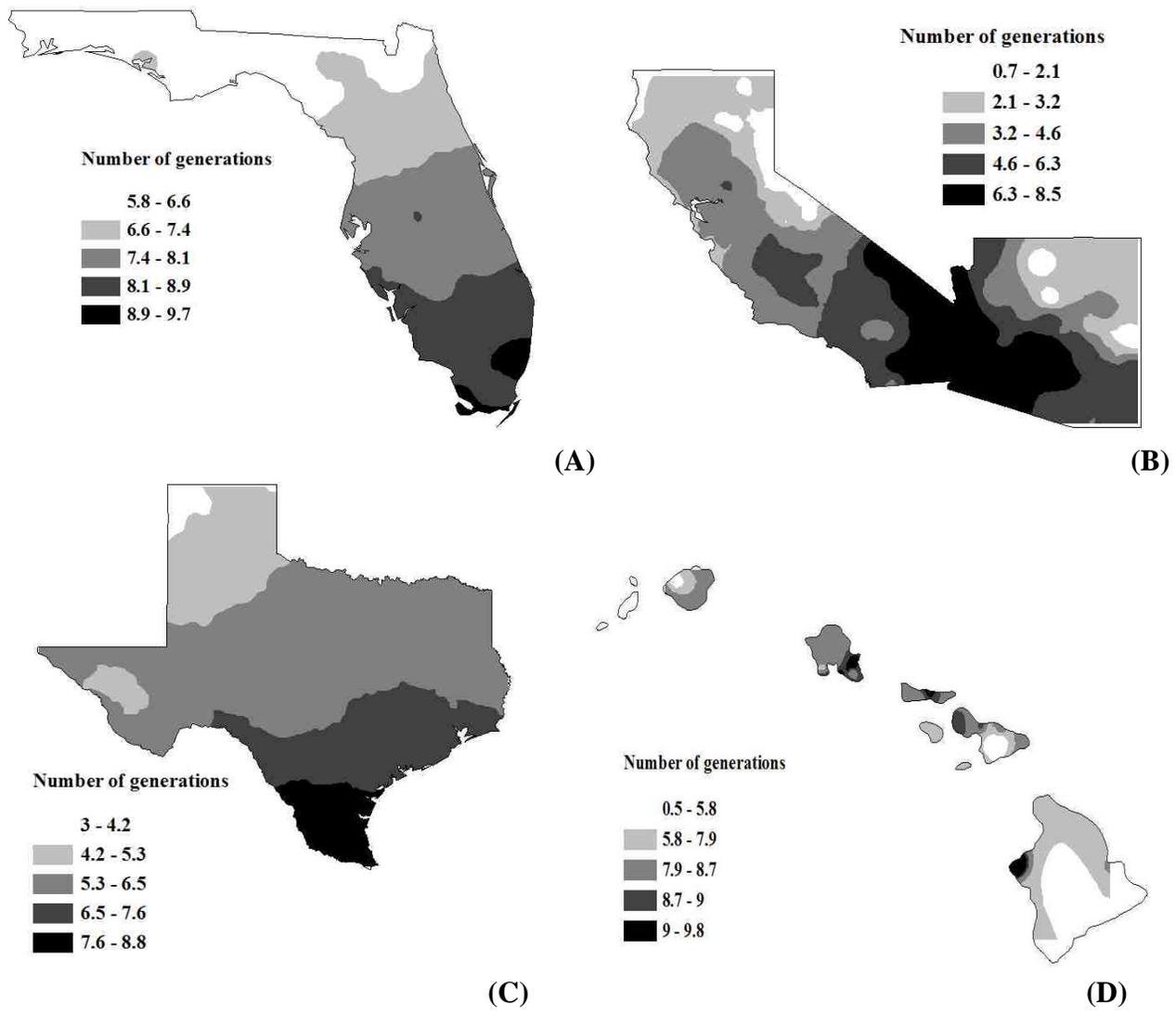


Figure 4-4. Predicted number of generations per year for *E. utilis* in the USA. A) Florida, B) California-Arizona, C) Texas, D) Hawaii.

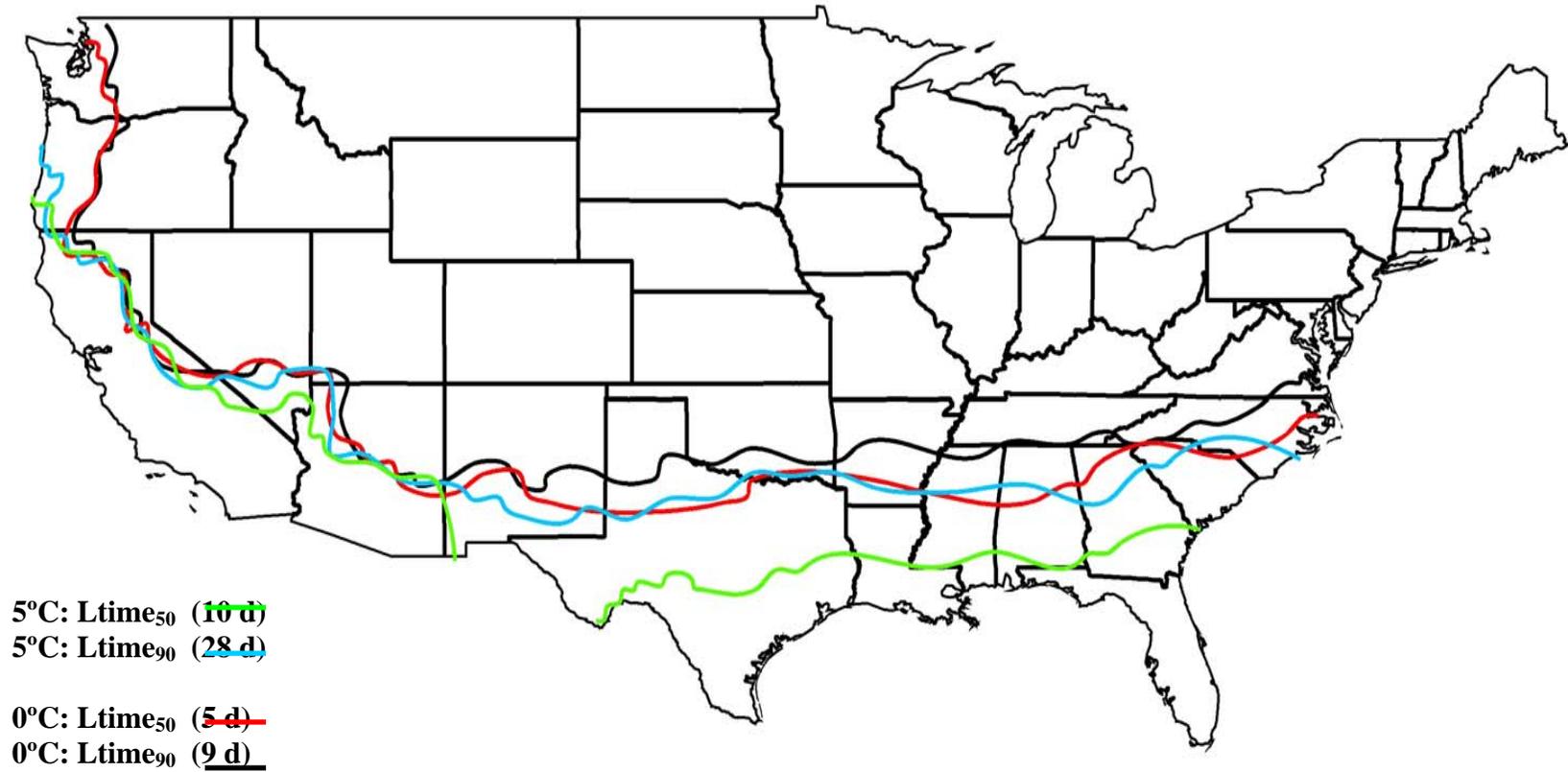


Figure 4-5. Map showing the isothermal lines ($Ltime_{50}$ and $Ltime_{90}$) at 0 and 5°C for *E. utilis* in the USA.

CHAPTER 5
EFFECT OF HERBIVORY ON GROWTH AND BIOMASS OF BRAZILIAN PEPPERTREE
SEEDLINGS

Introduction

Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), is native to South America (Barkley 1944, 1957), and was introduced into Florida as an ornamental plant in the 1840s (Mack 1991, Cuda et al. 2006). In the USA, Brazilian peppertree occurs in Hawaii, California, Arizona, Texas and Florida (Habeck et al. 1994, Cuda et al. 2006). This noxious weed invades a variety of habitat types in Florida, including disturbed sites (e.g., canals, fallow farmlands) and natural communities (e.g., pinelands, hardwood hammocks and mangrove forests) (Morton 1978, Cuda et al. 2006). Brazilian peppertree is characterized by having a large numbers of fruit per female plant, an effective dispersal mechanism by birds (Panetta and McKee 1997), tolerance to shade (Ewel 1978), fire (Doren et al. 1991), saline conditions (Ewe 2001, Ewe and Sternberg 2002) and drought (Nilson and Muller 1980b), and allelopathic effect on neighboring plants (Gogue et al. 1974, Nilson and Muller 1980a, Morgan and Overholt 2006).

Herbicides and mechanical methods (e.g., cutting, burning and flooding) are routinely used for controlling existing Brazilian peppertree stands (Gioeli and Langeland 1997, Cuda et al. 2006). However, these methods are labor intensive and not cost effective especially for large infestations. A classical biological control program was initiated in the 1980s, and the leaflet roller moth *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae) was selected as a potential biocontrol agent (Martin et al. 2004, Cuda et al. 2006). The larval stage of *E. utilis* has five instars but occasionally six; larvae feed on plant leaflets and can completely defoliate small plants (Martin et al. 2004). Adults are small, grayish brown in color and have distinct wing patterns that separate males from females (Zimmerman 1978). The entire life cycle of *E. utilis* occurs in the canopy of Brazilian peppertree, which may be a favorable characteristic for

establishment of the insect in areas subjected to regular flooding in south Florida. Although *E. utilis* was released and established in Hawaii in the 1950s, control of Brazilian peppertree was not achieved (Goeden 1977, Yoshioka and Markin 1991). Several biotic and perhaps abiotic factors may explain this outcome. For example, high levels of larval parasitism and predation were reported in the field soon after the release of *E. utilis* in Hawaii, which undoubtedly affected its performance (Davis 1959, Krauss 1963, Goeden 1977, Julien and Griffiths 1998).

During the process of agent selection, several factors are usually considered such as host specificity (Follett and Duan 1999, van Driesche et al. 2000, Sheppard et al. 2003), climatic adaptability (Wapshere 1983, Byrne et al. 2003, Senaratne et al. 2006), and impact on the target weed (McEvoy and Coombs 1999, Briese et al. 2002, van Klinken and Raghu 2006). More recently, several authors have emphasized the importance of measuring the herbivore's ability to suppress the target weed in order to select the most effective biocontrol agent (McEvoy and Coombs 1999, Balciyanus 2000, Pratt et al. 2005, van Klinken and Raghu 2006). According to Treadwell and Cuda (2007), multiple artificial defoliations significantly affected growth and fruit production of Brazilian peppertree. Artificial defoliation has been frequently used to measure plant responses to herbivory (Dhileepan et al. 2000, Broughton 2003, Wirf 2006, Treadwell and Cuda 2007). However, plants may respond differently to actual insect damage (Lehtila and Boalt 2004, Schat and Blossey 2005).

The objective of this study was to examine the effect of different levels of herbivore damage on growth and biomass allocation of Brazilian peppertree seedlings in the laboratory. In addition, plant responses to herbivory were measured immediately following insect damage and after a period of 2 months. This information provides a better understanding of the interaction

between *E. utilis* and its host plant, especially its potential effectiveness as a biocontrol agent of Brazilian peppertree in Florida.

Materials and Methods

Plants and Insects

Laboratory experiments were conducted at the Biological Control Research and Containment Laboratory (BCRCL) located at the Indian River Research and Education Center (IRREC) of the University of Florida, Fort Pierce, FL, USA. Brazilian peppertree plants were grown from seeds collected from the west coast of Florida (haplotype A and hybrid A genotypes). Plants were grown in nursery pots (18 cm height, 17 cm diameter) using potting soil (Fafard® #3B mix), and all plants were placed in the greenhouse at BCRCL and watered as needed. Plants were fertilized once with 15 g of Osmocote® (a slow release fertilizer 15-9-12, N-K-P), and 400 ml per pot of liquid fertilizer (Miracle Grow® 24-8-16) monthly.

A colony of *E. utilis* was initiated in August 2006 at the BCRCL, and insects were reared on Brazilian peppertree potted plants (all Florida genotypes) inside environmental growth chambers (25 ± 2°C, 60-70% RH, 14L: 10D photoperiod) (for more details on rearing see Martin et al. 2004). Insects were originally collected in 2003 in the vicinity of Curitiba located in Parana Province, southern Brazil, and maintained at the quarantine facility in Gainesville, FL. Voucher specimens of *E. utilis* were deposited in the Florida State Collection of Arthropods, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA.

Experimental Procedure

Brazilian peppertree seedlings (5 months old) were used in the experiment. Six plants were harvested, oven-dried at 70°C for 1 week, and weighed to measure initial aboveground biomass (leaflets, stems). Three treatments were established (12 replicates per treatment): 1) control (no herbivory), 2) low herbivory (15 larvae/plant) and 3) high herbivory (30

larvae/plant). Neonate larvae (0, 15, or 30) of *E. utilis* were placed on each treatment plant, and covered by a clear acrylic cylinder (45 cm height, 15 cm diameter) with six holes (6 cm diameter) and tops covered by a fine mesh to allow air circulation. Plants were assigned randomly to each treatment. Experiments were conducted inside an environmental growth chamber ($25 \pm 2^\circ\text{C}$, 60-70% RH, 14L: 10D photoperiod) at the BCRCL. Upon adult emergence, all insects were removed from the cylinders and six plants from each treatment were transplanted to larger pots (22 cm height, 22 cm diameter pots) to allow continued growth. The total number of adults emerged from each treatment was recorded in order to corroborate final insect densities per treatment.

Plant parameters were measured at two different times during the experiment: 1) immediately after adults emerged (six plants per treatment), and 2) two months following exposure to herbivory (six plants per treatment). The following plant parameters were measured: 1) number of leaflets, 2) height (cm), 3) basal stem diameter (cm), 4) biomass (g) (roots, stems, and leaflets), and 5) shoot: root ratio (g g^{-1}). For the biomass parameter, plants were harvested, oven-dried at 70°C for 1 week, and weighed (separately for total leaflets, stems and roots) at each time interval. Relative plant growth rate (RGR) was separately calculated for leaflets and stems using the following index (Wirf 2006):

$$\text{RGR} = [\log \text{ final biomass} - \log \text{ initial biomass}] / \text{ months}$$

Data Analysis

Plant parameters (e.g., height, biomass, etc) were compared among herbivory treatments (control, low, and high) using one-way analysis of variance (ANOVA) (SAS Institute 1999). The shoot: root ratio was log transformed prior to the statistical analysis (Zar 1999). The relative growth rate (RGR) was compared between herbivory treatment and time interval (immediately after herbivory and 2-months following herbivory) using two-way ANOVA (SAS Institute

1999). Since there was a significant interaction between treatment and time ($P < 0.05$), one-way ANOVA was used to compare plant treatments separately for each time interval (immediately herbivory and 2-months following herbivory). The total number of *E. utilis* adults emerging from low and high herbivory treatments was analyzed using one-way ANOVA (SAS Institute 1999). Means were separated using the post-hoc Student-Neuman-Keuls (SNK) test (SAS Institute 1999). A significance level of $\alpha = 0.05$ was used for all statistical analyses.

Results

The total number of adults recovered at the end of the experiment differed between herbivory treatments ($F_{1,23} = 94.91$, $P < 0.0001$). Whereas an average of 3.2 ± 0.7 adults emerged in the low herbivory treatment, 12.1 ± 0.6 adult moths were recovered from the high herbivory treatment. These data corroborate that two different herbivory levels were established. Brazilian peppertree plants exposed to the high herbivory treatments had fewer leaflets compared to the low herbivory and control treatment immediately following the herbivory event (Table 5-1). In addition, plant height and number of leaflets were significantly lower on low and high herbivory treatments than on the control two months following insect damage (Table 5-1). Basal stem diameter, however, was not affected by herbivory (Table 5-1).

Biomass allocation of Brazilian peppertree also differed between herbivory treatments (Table 5-2). Plants exposed to high herbivory treatments had a lower leaflet biomass and a lower shoot: root ratio compared to the low herbivory and control treatments (Table 5-2). However, stem biomass was not affected by herbivory (Table 5-2). Two months after insect damage occurred, plant biomass also differed between treatments; leaflet biomass and shoot: root ratio were lower in the high herbivory treatment compared to the control (Table 5-2).

In addition, the relative growth rate (RGR) of Brazilian peppertree was negatively affected by herbivory (Fig. 5-1). Differences were detected for leaflet RGR between treatment and time,

and also its interaction (treatment: $F_{2,35} = 16.88$, $P < 0.0001$; time: $F_{1,35} = 9.15$, $P = 0.005$; treatment x time: $F_{2,35} = 8.29$, $P = 0.0014$). High levels of defoliation resulted in negative values of RGR for both leaflets (-0.5 ± 0.05 g/month) and stems (-0.02 ± 0.04 g/month) immediately after herbivory occurred (Fig. 5-1). Moreover, Brazilian peppertree plants exposed to high levels of defoliation had lower leaflet RGR compared to control plants (no herbivory) immediately after herbivory and 2 months following insect damage (Fig. 5-1a). Even though no differences were detected in the stem RGR between treatment and time (treatment: $F_{2,35} = 2.95$, $P = 0.06$; time: $F_{1,35} = 0.06$, $P = 0.8$; treatment x time: $F_{2,35} = 0.99$, $P = 0.38$), slightly higher RGR was detected on plants exposed to low herbivory immediately following insect damage (Fig. 5-1b).

Discussion

Classical biological control has been successful in controlling many invasive weeds in both aquatic and terrestrial ecosystems (Harley 1990, Julien and Griffith 1998, Grevstad 2006, Barton et al. 2007). However, biological control programs often have been criticized for their lack of predictability in terms of agent establishment and success (Ehler 1990, Harris 1998). Therefore, evaluating the effectiveness of biocontrol agents against the target weed is important during the process of agent selection to maximize the success of biological control programs (Balciunas 2000, Raghu and Dhileepan 2005, van Klinken and Raghu 2006). This study showed that growth and biomass allocation of Brazilian peppertree seedlings were negatively affected by herbivory caused by *E. utilis*. Plants exposed to high levels of defoliation (30 larvae/plant) suffered a significant reduction in number of leaflets, plant height, foliar biomass, and relative growth rate (RGR) compared to control plants (no herbivory). Because aboveground architecture (e.g., number of leaflets) is important for determining the photosynthetic capacity and growth rates of plants (Percy et al. 1987), defoliation by the biocontrol agent *E. utilis* clearly affected the performance of Brazilian peppertree in the laboratory. The decreased plant growth may translate

into a reduced competitive ability of Brazilian peppertree after herbivory, which may allow re-establishment of native plant species in the field.

Terrestrial plants have evolved different strategies to cope with herbivory (Rosenthal and Kotanen 1994, Strauss and Agrawal 1999). Plants may compensate for the removal of plant tissue by changing biomass allocation patterns following herbivore damage (Belsky 1986, Schierenbeck et al. 1994, Gavloski and Lamb 2000). For example, *Melaleuca quinquenervia* (Cav.) S.T. Blake partially compensated for herbivory by producing new stems and replacing foliage, but reproduction was still reduced (Pratt et al. 2005). There is usually a balance between plant biomass invested in the shoots and that invested in the roots (Pearsall 1927, Wilson 1988), and many plants can restore the shoot: root ratio after herbivory (Chapin 1980, Paige and Whitman 1987). Results obtained here showed that the shoot: root ratio of the invasive Brazilian peppertree was reduced after exposed to high levels of defoliation, and this ratio was not restored after 2 months. In addition, foliar RGR also decreased in the high herbivory treatment compared to control plants (no herbivory). Even though plant reproduction was not measured here, the reduced growth of Brazilian peppertree seedlings may translate into lower resource allocation to fruit production and decreased plant fitness. This indirect effect of ‘herbivory’ on Brazilian peppertree reproduction was observed by Treadwell and Cuda (2007).

Plant compensatory growth may vary with the amount and timing of insect damage (Maschinski and Whitman 1989, Thomson et al. 2003, Schooler and McEvoy 2006). In this case, low levels of defoliation (15 larvae/plant) did not affect Brazilian peppertree growth and biomass allocation in the laboratory. Therefore, the effectiveness of *E. utilis* in suppressing this weed will vary in relation to insect densities present in the field. Similarly, the damage on *Lythrum salicaria* L. was proportional to densities of the biocontrol agent *Galerucella pusilla* Duftschmid

(Schooler and McEvoy 2006). According to Treadwell and Cuda (2007), mature Brazilian peppertree plants were able to compensate for a single artificial defoliation, but multiple defoliations significantly reduced fruit production in the field. Young plants are usually more vulnerable to insect damage, and this may be the case for Brazilian peppertree seedlings that suffer a reduction of growth and biomass following one defoliation event (high herbivory treatments). However, the environmental conditions (biotic and abiotic) present in the introduced range also will influence the plant's ability to tolerate herbivory (Cox and McEvoy 1983, Belsky 1986, Osier and Lindroth 2004). For example, Brazilian peppertree plants growing in high fertility soils (e.g., abandoned farmlands in the Everglades) may be able to recover faster from defoliation by increasing resource allocation towards aboveground biomass.

In summary, Brazilian peppertree seedlings were negatively affected by high levels of defoliation in the laboratory. Plant parameters such as height, foliar biomass, RGR, and shoot: root ratio were reduced immediately after herbivory occurred. In addition, Brazilian peppertree seedlings were not able to compensate for insect damage 2 months following herbivory. The ability of plants to compensate for herbivory has direct implications for biological control, and should be considered when examining effectiveness of potential agents. The leaflet roller moth *E. utilis* may be an effective biocontrol agent against Brazilian peppertree in Florida. Further studies are needed to provide a better understanding of the effect of this agent on its host-plant, in particular the potential to reduce plant populations in the field (Halpern and Underwood 2006). Thus, not only individual plant parameters such as those measured in this study, but also population parameters (e.g., fruit production, seedling recruitment) should be evaluated in order to help predict the outcome of biocontrol programs.

Table 5-1. Morphometric plant parameters (mean \pm SE) of Brazilian peppertree exposed to different levels of herbivory. Control=no herbivory, low=15 larvae/plant, high=30 larvae/plant. Different letters in the same row indicate statistical differences, $df = 2, 17$ ($P < 0.05$).

	Control	Low	High	<i>F</i>	<i>P</i>
After herbivory					
Number of leaflets	66.16 \pm 2.9 a	55.83 \pm 6.2 a	20.83 \pm 4.3 b	25.7	<0.0001
Plant height (cm)	55.5 \pm 2.85	57.33 \pm 1.62	51.5 \pm 2.48	1.59	0.24
Basal stem diameter (cm)	0.64 \pm 0.07	0.62 \pm 0.02	0.49 \pm 0.03	3.15	0.07
Two-months after herbivory					
Number of leaflets	119 \pm 7.15 a	89.83 \pm 12.1 b	73.16 \pm 7.82 b	6.25	0.01
Plant height (cm)	75.67 \pm 2.58 a	67.67 \pm 1.67 b	66.5 \pm 3.22 b	3.77	0.04
Basal stem diameter (cm)	0.68 \pm 0.03	0.65 \pm 0.04	0.59 \pm 0.03	1.14	0.34

Table 5-2. Biomass allocation (g) (means \pm SE) of Brazilian peppertree exposed to different levels of herbivory. Control=no herbivory, low=15 larvae/plant, high=30 larvae/plant. Different letters in the same row indicate statistical differences, $df = 2, 17$ ($P < 0.05$).

	Control	Low	High	<i>F</i>	<i>P</i>
After herbivory					
Total leaflets	3.02 \pm 0.61 a	3.35 \pm 0.43 a	1.01 \pm 0.17 b	8.26	0.003
Total stems	2.25 \pm 0.45	2.52 \pm 0.21	1.79 \pm 0.18	1.46	0.26
Shoot: root ratio	3.35 \pm 0.35 a	3.05 \pm 0.26 a	2.31 \pm 0.14 b	4.11	0.03
Two-months after herbivory					
Total leaflets	5.23 \pm 0.7 a	3.32 \pm 0.9 ab	2.11 \pm 0.49 b	4.82	0.02
Total stems	4.19 \pm 0.64	3.28 \pm 0.63	2.13 \pm 0.42	3.24	0.06
Shoot: root ratio	6.44 \pm 0.78 a	4.69 \pm 0.47 ab	3.77 \pm 0.44 b	5.15	0.01

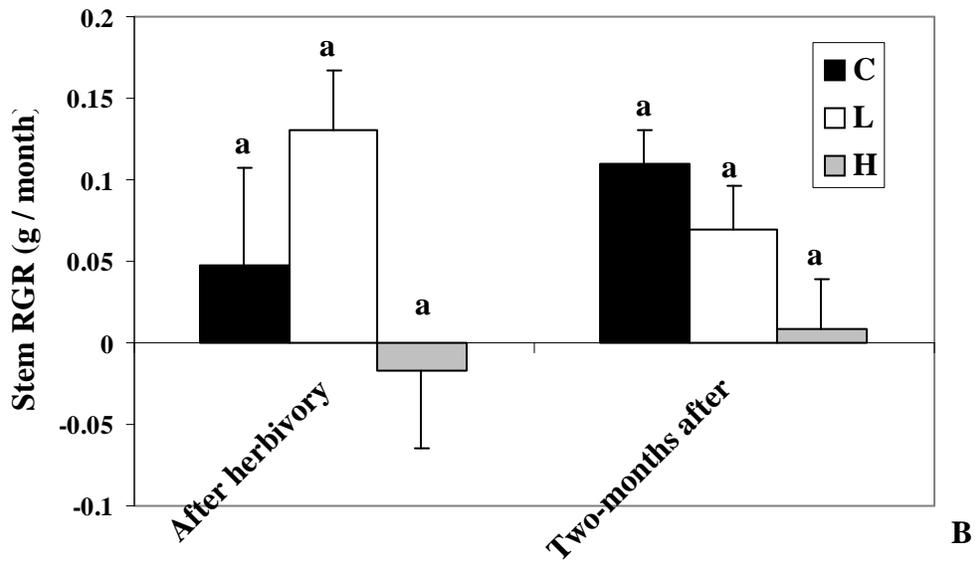
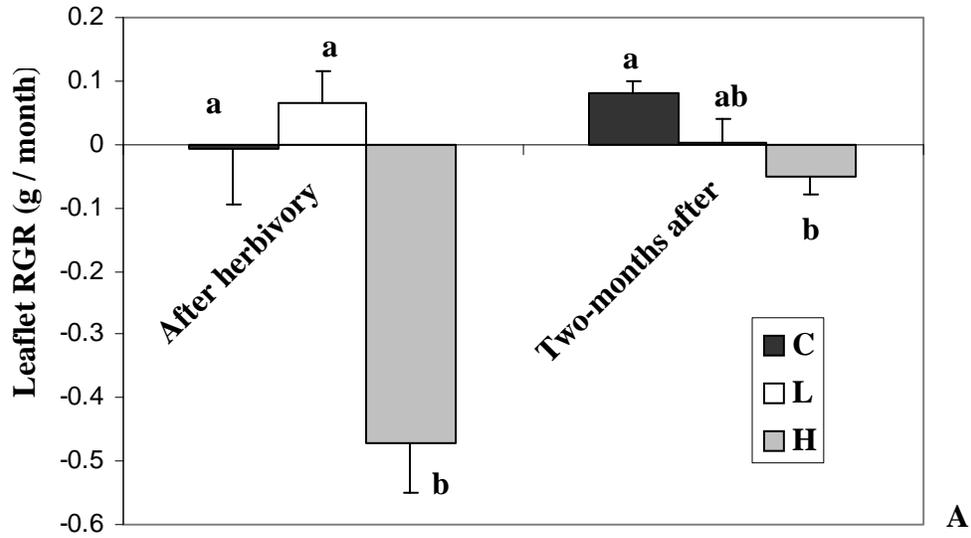


Figure 5-1. Relative growth rate (RGR) (means \pm SE) of leaflets and stems of Brazilian peppertree exposed to different levels of herbivory. A) leaflets RGR, B) stems RGR. C=control (no herbivory), L=low herbivory (15 larvae/plant), H=high herbivory (30 larvae/plant). Different letters indicate statistical differences between treatments ($P < 0.05$).

CHAPTER 6 CONCLUDING REMARKS

Brazilian peppertree (*Schinus terebinthifolius*) is recognized as one of the most widespread exotic plants in Florida (Cuda et al. 2006). This noxious weed forms large monocultures and displaces native vegetation in a variety of habitats throughout central and south Florida (Cuda et al. 2006). Classical biological control has been successful in suppressing many invasive weeds in both terrestrial and aquatic ecosystems (e.g., Julien and Griffith 1998, Grevstad 2006, Barton et al. 2007). However, many authors have emphasized the importance of selecting effective biocontrol agents to avoid the release of herbivores that will fail to suppress the target weed in the introduced range (McEvoy and Coombs 1999, Balciunas 2000, Pratt et al. 2005, van Klinken and Raghu 2006). The purpose of this study was to examine the performance of two candidate biocontrol agents of Brazilian peppertree, more specifically, a) to determine life-history parameters of the leaflet roller moth *Episimus utilis* and the thrips *Pseudophilothrips ichini* on different plant genotypes (chapter 2), b) to evaluate the effect of host-plant quality on the development of *E. utilis* (chapter 3), c) to examine the temperature requirements and potential distribution of *E. utilis* in Florida (chapter 4), and d) to measure the impact of *E. utilis* on growth and biomass allocation of Brazilian peppertree seedlings (chapter 5).

Chloroplast DNA (cpDNA) and nuclear microsatellite analyses indicated that two different populations of Brazilian peppertree were introduced separately on the east and west coasts of Florida (Williams et al. 2005, 2007). Haplotype A is more common on the west coast whereas haplotype B is more common on the east coast, and extensive hybridization has occurred between these two types of plants since arriving in Florida (Williams et al. 2005, 2007). Results from this study revealed that Brazilian peppertree genotype did not affect the performance of the leaflet roller *E. utilis*, but had a strong effect on the performance of two

populations of the thrips *P. ichini* (chapter 2). Whereas poor survival to adulthood (0-4%) was obtained for the haplotype 5 thrips on all Florida genotypes, higher survival (~50%) was observed when the haplotype 2-3 thrips was fed these Florida genotypes. Moreover, thrips haplotype 5 were unable to lay eggs on Florida genotypes, whereas thrips haplotype 2-3 laid eggs on all plants tested except for Brazil haplotype D. Therefore, the thrips haplotype 2-3 that was originally collected from Brazil haplotype A are well adapted to Florida genotypes and should be considered as a potential biocontrol agent of Brazilian peppertree in Florida. (Note: *P. ichini* was recently recommended for release by the federal interagency Technical Advisory Group (TAG) for Biological Control Agents of Weeds (J.P. Cuda, unpubl. rept.)).

Because Brazilian peppertree invades different habitats in Florida (e.g., mangrove forests, pinelands, abandoned farms), the environmental conditions encountered in these different habitats may influence the survival and effectiveness of potential biocontrol agents. Results from this study showed that the performance of *E. utilis* was influenced by host-plant quality (chapter 3). Survival to adulthood (36-55%) was similar when insects were reared either on Brazilian peppertree irrigated with freshwater (0 ppt) or with water with a low salinity (6 ppt), but lower survival (6%) was obtained in high salinity-environment (12 ppt). Therefore, Brazilian peppertree plants growing in high-salinity environments (e.g., mangrove forests) may not support high populations of *E. utilis*. Differences in insect performance also were detected on Brazilian peppertree plants exposed to different nutrient levels (chapter 3). Increased survival to adulthood was observed under medium (25%) and high (40%) nutrient levels compared to low nutrient levels (1.25%). Moreover, faster development (34 d) and higher fertility (88% egg hatch) occurred in the high nutrient treatments, which contained higher leaflet nutrient content (N, P, K), lower toughness and higher moisture content of newly expanded leaflets. This study

indicates that Brazilian peppertrees growing in high-fertility soils (e.g., disturbed sites, abandoned farmlands; Li and Norland 2001) will provide high-quality hosts for *E. utilis* development and survival, and insect establishment may occur more rapidly at those sites compared to low-fertility soils.

Climate (e.g., temperature) is known to influence insect distribution and population growth, and therefore, the ability of a species to establish in new areas (Crawley 1987, Sutherst 2000, Byrne et al. 2002). According to this study, the number of generations per year for *E. utilis* varied from 0.5 to 9.8 across the entire range of Brazilian peppertree in USA (chapter 4). The models predicted a range of 5.8 to 9.7 generations per year for *E. utilis* in Florida, which suggests the existence of favorable temperature conditions for establishment of this agent. Those locations capable of producing many generations of *E. utilis* (e.g., south Florida, south Texas) may experience greater control of the target weed compared to those areas with fewer generations (e.g., south-east Arizona, south-central California). In addition, exposure to low temperatures (5 and 0°C) affected the survival of all life stages of *E. utilis*, and pupae were the most cold-tolerant stage (chapter 4). Based on the isothermal lines of the pupal lethal exposure times for 50 and 90% of the population, establishment of *E. utilis* may occur throughout Florida and southern California, but only in the southern parts of Texas and Arizona. Moreover, favorable conditions are present throughout the Hawaiian archipelago, which has been validated since the insect is known to be widely established there (Yoshioka and Markin 1991, Julien and Griffiths 1998).

During the process of agent selection, evaluating the ability of biocontrol agents to suppress the target weed is important to maximize the success of biological control programs (Raghu and Dhileepan 2005, van Klinken and Raghu 2006). This study showed that growth and

biomass allocation of Brazilian peppertree seedlings were negatively affected by herbivory (chapter 5). Plants exposed to high levels of defoliation as a result of *E. utilis* feeding damage (30 larvae/plant) suffered a significant reduction in number of leaflets, plant height, foliar biomass, shoot: root ratio, and relative growth rate compared to control plants (no herbivory). In addition, Brazilian peppertree seedlings were not able to compensate for insect damage 2 months after herbivory. However, low levels of defoliation (15 larvae/plant) did not affect Brazilian peppertree growth and biomass allocation in the laboratory. Therefore, the effectiveness of *E. utilis* to suppress this weed will vary in relation to insect densities present in the field.

Overall, this dissertation (chapters 2-5) contributes to the body of knowledge on weed biological control by providing a better understanding of the interactions between two biocontrol agents and Brazilian peppertree. However, many questions are still unanswered and should be evaluated in the near future. For example, differences in chemical profiles between Brazilian peppertree genotypes may help explain the population genetic structure of *P. ichini* found in the native range. Even though *P. ichini* has been approved to release by TAG, host-specificity of the thrips haplotypes 2-3 may differ from the first population and should be evaluated. In addition, further studies are needed to determine whether the leaflet roller *E. utilis* is safe to release in Florida. Long-term field studies should be conducted if these agents are released in Florida in order to monitor insect establishment and impact on Brazilian peppertree populations and changes in plant community structure over time.

The major findings of this study are summarized below:

1. Two candidate biological control agents differed in their ability to develop on different Brazilian peppertree genotypes. The leaflet roller *E. utilis* performed well on all host plant genotypes, whereas two populations of the thrips *P. ichini* varied in their ability to utilize their host-plant.
2. Different populations of the thrips *P. ichini* are locally adapted to different Brazilian peppertree genotypes in the native range.

3. The thrips haplotype 2-3, originally collected from Brazilian peppertree haplotype A, is well adapted to all Florida genotypes and should be considered as a potential biocontrol agent of Brazilian peppertree in Florida.
4. Brazilian peppertrees growing in fresh or low saline environments, and also plants present in high-fertility soils, will provide high quality hosts for *E. utilis* establishment.
5. The best sites for field releases of *E. utilis* in Florida are fresh upland communities (e.g., pineland forests, along canals), and high-nutrient environments (e.g. disturbed sites, abandoned farmlands that occur in the Everglades).
6. The predicted number of generations of *E. utilis* will follow a temperature gradient, with more generations per year in warmer areas.
7. The existence of favorable temperature conditions in Florida for *E. utilis* development suggests that this agent could successfully establish throughout the state.
8. High levels of defoliation caused by *E. utilis* feeding damage negatively affected growth and biomass allocation of Brazilian peppertree seedlings.
9. The leaflet roller *E. utilis* may be an effective biological control agent of Brazilian peppertree in Florida if the results of host range testing indicate that it is safe to release.

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BIOGRAPHICAL SKETCH

Veronica Manrique was born in Buenos Aires, Argentina in 1972. She obtained her B. S. in Biology in May 2000 at the University of Buenos Aires, Argentina. Her initial experience working in entomology was at the USDA-ARS South American Biological Control Laboratory, where she conducted exploratory surveys of biocontrol agents of crop pests in northeast Argentina, and was responsible of the maintenance of several insect colonies. Since then, she has developed a deep interest in classical biological control, plant-insect interactions, and management of invasive species. In 2000, Veronica worked as a foreign research associate for six months at the USDA-ARS Southern Insect Management Research Unit in Stoneville, MS. After that, she started her graduate studies at Texas A&M University, and she obtained her Master degree in Entomology in December 2003. Her master's thesis was entitled "Host habitat location mediated by olfactory stimuli in *Anaphes iole* (Hymenoptera: Mymaridae), an egg parasitoid of *Lygus hesperus* (Hemiptera: Miridae)". Veronica entered the graduate program at the Department of Entomology & Nematology of the University of Florida in January 2004. During her research, she conducted several studies to evaluate the effectiveness of two biocontrol agents of the invasive Brazilian peppertree in Florida. She completed her Dissertation and was granted the degree of Doctor of Philosophy in May 2008.