APPLICATION OF THE F₁ STERILE INSECT TECHNIQUE (F₁SIT) FOR FIELD HOST RANGE TESTING OF *Episimus utilis* ZIMMERMAN (LEPIDOPTERA: TORTRICIDAE), A CANDIDATE FOR BIOLOGICAL CONTROL OF BRAZILIAN PEPPERTREE

By

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To my parents
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# TABLE OF CONTENTS

ACKNOWLEDGMENTS ............................................................................................................... 4

LIST OF FIGURES ....................................................................................................................... 7

ABSTRACT ..................................................................................................................................... 8

CHAPTER

1 INTRODUCTION .................................................................................................................. 10

2 LITERATURE REVIEW ....................................................................................................... 14

   The F₁ Sterile Insect Technique (F₁SIT) ................................................................................ 14
      Description ...................................................................................................................... 14
      History ........................................................................................................................... 14
      Field Application of F₁SIT ............................................................................................ 16
   Host Range Testing Protocols ............................................................................................... 19
      No-Choice Tests ............................................................................................................. 19
      Choice Tests .................................................................................................................. 20
   Open Field Testing ............................................................................................................. 20
   *Schinus terebinthifolius* Raddi .......................................................................................... 22
      Taxonomy ........................................................................................................................ 22
      Common Names ............................................................................................................... 23
      Description ...................................................................................................................... 23
      Distribution .................................................................................................................... 23
   Environmental Impacts ......................................................................................................... 24
      Ecosystem ........................................................................................................................ 24
      Human and Animal Health ............................................................................................... 25
      Beneficial Uses ................................................................................................................ 25
   Controlling Brazilian Peppertree .......................................................................................... 26
      Mechanical Control ........................................................................................................ 26
      Physical Control .............................................................................................................. 26
      Chemical Control ............................................................................................................ 27
      Biological Control .......................................................................................................... 27
   *Episimus utilis* Zimmerman ............................................................................................... 28
      Taxonomy ........................................................................................................................ 28
      Biology ............................................................................................................................. 28
      History of Introduction of *E. utilis* ............................................................................... 29
3 USE OF THE F₁ STERILE INSECT TECHNIQUE (F₁SIT) AS A TOOL FOR FIELD HOST RANGE TESTING OF *Epismus utilis* ZIMMERMAN (LEPIDOPTERA: TORTRICIDAE), A CANDIDATE BIOLOGICAL CONTROL AGENT OF BRAZILIAN PEPPERTREE.................................................................38

Introduction .............................................................................................................................38
Materials and Methods ...........................................................................................................41
  Colony Rearing ................................................................................................................41
  Radiation Biology Study .................................................................................................42
  Statistical Analysis for Radiation Biology Study ............................................................44
  Inherited Sterility Methodology ......................................................................................45
  Statistical Analysis for Inherited Sterility .......................................................................46
Results .....................................................................................................................................46
  Radiation Biology Study .................................................................................................46
  Inherited Sterility .............................................................................................................46
Discussion ...............................................................................................................................47

4 CONCLUSIONS ....................................................................................................................59

LIST OF REFERENCES ...............................................................................................................62

BIOGRAPHICAL SKETCH .........................................................................................................71
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Dense stands of <em>Schinus terebinthifolius</em> in Florida.</td>
<td>31</td>
</tr>
<tr>
<td>2-2</td>
<td>Morphology of <em>Schinus terebinthifolius</em>.</td>
<td>32</td>
</tr>
<tr>
<td>2-3</td>
<td>Native distribution of Brazilian peppertree in South America by country.</td>
<td>33</td>
</tr>
<tr>
<td>2-4</td>
<td>Worldwide distribution of Brazilian peppertree.</td>
<td>34</td>
</tr>
<tr>
<td>2-5</td>
<td>Distribution of Brazilian peppertree in Florida.</td>
<td>35</td>
</tr>
<tr>
<td>2-6</td>
<td>A-F. Life cycle of <em>E. utilis</em>.</td>
<td>36</td>
</tr>
<tr>
<td>2-7</td>
<td>Effect of <em>E. utilis</em> on Brazilian peppertree.</td>
<td>37</td>
</tr>
<tr>
<td>3-1</td>
<td>Results of laboratory no-choice tests performed with <em>E. utilis</em>.</td>
<td>50</td>
</tr>
<tr>
<td>3-2</td>
<td>Potted BP plant covered by a clear acrylic cylinder.</td>
<td>51</td>
</tr>
<tr>
<td>3-3</td>
<td>Materials used for irradiation of <em>E. utilis</em> moths.</td>
<td>52</td>
</tr>
<tr>
<td>3-4</td>
<td>Cesium-137 Gammacell® 1000 irradiator (F.A.S.T. Gainesville, FL).</td>
<td>52</td>
</tr>
<tr>
<td>3-5</td>
<td>Waxed paper oviposition chamber for mating and ovipositing by <em>E. utilis</em> moths.</td>
<td>53</td>
</tr>
<tr>
<td>3-6</td>
<td>Fecundity (mean number of eggs laid) per mated female of <em>E. utilis</em> adults for three crosses.</td>
<td>54</td>
</tr>
<tr>
<td>3-7</td>
<td>Fertility (mean percentage of eggs that hatched) of <em>E. utilis</em> adults for three crosses treated with increasing dose of gamma radiation.</td>
<td>55</td>
</tr>
<tr>
<td>3-8</td>
<td>Fecundity (mean number of eggs laid) of F1 crosses of <em>E. utilis</em> adults as a result of radiation administered to parental males.</td>
<td>56</td>
</tr>
<tr>
<td>3-9</td>
<td>Fertility (mean percentage of eggs that hatched) of F1 crosses of <em>E. utilis</em> adults as a result of radiation administered to parental males.</td>
<td>57</td>
</tr>
<tr>
<td>3-10</td>
<td>Percentage of F1 <em>E. utilis</em> adult males as a result of radiation administered to <em>E. utilis</em> parental males.</td>
<td>58</td>
</tr>
</tbody>
</table>
APPLICATION OF THE F₁ STERILE INSECT TECHNIQUE (F₁SIT) FOR FIELD HOST RANGE TESTING OF *Episimus utilis* ZIMMERMAN (LEPIDOPTERA: TORRICIDAE), A CANDIDATE FOR BIOLOGICAL CONTROL OF BRAZILIAN PEPPERTREE

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Host range testing is used in weed biological control to demonstrate the safety of potential biological control agents. However, these laboratory tests may overestimate host range leading to “false positives” where the insect accepts plant species that it would not normally accept in nature. As cage testing may inhibit the behavior of the potential biological control agent, open-field studies may provide a more realistic setting of environmental and ecological conditions that the biological control agent will encounter upon release in the proposed areas of introduction. Open-field studies, however, are prohibited in the area of introduction. Reproductively inactivated potential biological control agents produced as a result of the application of the F₁ sterile insect technique (F₁SIT) could be used to conduct field testing in a safe and temporary manner. A situation where this could be used is in the biological control of Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), an invasive, exotic species distributed widely throughout central and southern Florida. A leaf-rolling moth, *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae) is a potential biological control agent of Brazilian peppertree. Traditional laboratory no-choice and choice tests performed with *E. utilis* produced ambiguous results where selected non-target species including fragrant sumac (*Rhus aromatica* L.), winged
sumac (*Rhus copallinum* L.), poison sumac (*Toxicodendron vernix* (L.) Kuntze), pistachio (*Pistacia vera* L.) and cashew (*Anacardium occidentale* L.) were unexpectedly accepted as host species. Therefore, the use of F1SIT for field host range testing was investigated as a new approach for risk assessment of potential biological control agents. Male and Female virgin *E. utilis* adults were treated with increasing doses of radiation and either inbred or outcrossed to nontreated *E. utilis* adults. Five pairs of adults were placed in triangular waxed paper oviposition cages and allowed to mate and oviposit for two intervals of 5 days. The number of eggs laid (fecundity) and the number of eggs that hatched (fertility) were counted for each egg sheet per dose. As the dose of radiation increased, there were no significant changes in the fecundity of nontreated females mated with treated males, yet fewer eggs were laid by treated females. Fertility for both treated males and females decreased with increasing doses of radiation. The dose at which treated females were found to be 100% sterile was 200 Gy. There were no significant changes in the fecundity for F1 females and males resulting from treated parental males with increasing dose of radiation. Fertility for F1 females and males resulting from treated parental males declined as the dose of radiation increased. There was a moderate positive correlation for the F1 sex ratio of males to females with increasing dose of radiation. The dose at which F1 females and males were found to be 100% sterile was 225 Gy. Results from this study were similar to results found in other tortricid moths including the codling moth, *Cydia pomonella* (L.), and the false codling moth, *Thaumatotibia leucotreta* (Meyrick). As the dose of radiation increased, there was an increase in sterility, a decrease in fecundity for both treated female crosses, and a higher ratio of F1 males to females. This novel approach could be used to safely and temporarily test potential biological control agents in the field.
CHAPTER 1
INTRODUCTION

Host-specificity tests are used in weed biological control to determine whether or not a potential biological control candidate is safe to release in the field. Potential candidates are routinely subjected to caged laboratory tests that are either no-choice (subjected to non-target plants only) or choice (subjected to host plant and selected non-target plants) (Marohasy 1998; Withers et al. 1999). The laboratory physiological host range is measured by larval development (i.e. survival, length of time to complete development) and adult reproduction, which are used to predict the field ecological host range (Julien and White 1997). Some biologists believe that host-specificity tests often overestimate host range, which leads to the rejection of acceptable candidates (Sands and Van Driesche 2000). For example, in no-choice starvation tests (entire lifetime of an insect) the range of plant species on which larvae can complete their development is often broader than the range of species that females accept for oviposition (Schoonhoven et al. 1998). A cage environment where oviposition tests take place also may yield false-positive results because it creates a restrictive environment where the pre-alighting cues of a female such as visual and olfactory cues may be inhibited causing an increase in the number of test plants accepted leading to a false positive result (Marohasy 1998).

Because cage testing may inhibit normal behavior, open-field studies can provide a more realistic setting in which insects can display an array of behaviors (Clement and Cristofaro 1995). However, field testing can pose environmental risks in the geographical area of introduction and is therefore prohibited. Although some field testing can occur in the country of origin of the weed (Zwölfer and Harris 1971), all test plant species in the area of introduction may not be represented in the natural enemy’s native range. More importantly, it may not be
possible to import all the required test plants into the countries of origin of the target weed because of quarantine restrictions.

The use of reproductively inactivated or limited biological control agents may be a possible solution to field testing in proposed areas of introduction. According to LaChance (1985), there are three genetic methods of control that have been developed and field tested against Lepidoptera. These include the sterile insect technique (SIT), the $F_1$ sterile insect technique ($F_1$SIT), and backcross sterility. Both SIT and $F_1$SIT involve the release of insects that have been genetically altered by radiation, providing either full or partial sterility, and will introduce sterility into the wild population. Backcross sterility is different from SIT and $F_1$SIT because there is no use of radiation and it introduces sterility factors into the population that can persist indefinitely because of production of multiple generations (LaChance 1985). Both backcross sterility and $F_1$SIT may offer a better level of suppression because the suppressive effects may persist for one or more generations (LaChance 1985). Through the application of $F_1$ sterility, however, candidate insect weed biological control agents could be safely released for field host range testing because they will produce only a single generation of insects, which are sterile. Advantages of this approach include exposure of the potential biological control candidate to the actual environmental conditions it would experience post-release, prediction of true field host range in the area of introduction, and the ability to suspend releases of irradiated insects if non-target species are being attacked, without risk of permanent establishment (Carpenter et al. 2001a). This novel approach could possibly be applied to exotic lepidopterans that are potential weed biological control agents for field host range testing (Dunn 1978; Cullen 1990; Greany and Carpenter 2000). This would allow the insect to be observed under actual
conditions that it would be subjected to upon release and observation of insect behavior including oviposition, host-finding, and larval feeding preferences (Carpenter et al. 2001a).

An example of a situation where F_{1}SIT could be used for field host range testing is in the biological control of Brazilian peppertree *Schinus terebinthifolius* Raddi (Anacardiaceae), a dioecious evergreen tree native to Brazil, Paraguay, and Argentina that was introduced into Florida in 1898 as an ornamental (Austin 1978; Ewel et al. 1982). Currently, Brazilian peppertree is distributed widely throughout central and southern Florida, and is listed as a prohibited plant by the Florida Department of Environmental Protection (FLDEP 1993), as a noxious weed by the Florida Department of Agriculture and Consumer Services (FLDACS 1999) and as a Category 1 invasive exotic species by the Florida Exotic Pest Plant Council (FLEPPC 2007) because it is drastically altering native plant communities. It also has exhibited invasive behavior in California (Randall 2000), Hawaii (Hight et al. 2002), and Texas (Gonzalez and Christoffersen 2006) as well as in subtropical regions of at least 20 different countries (Ewel et al. 1982).

In 1994, several natural enemies of Brazilian peppertree were imported into a quarantine facility in Florida as candidates for classical biological control (Cuda et al. 1999). One of these was a South American leaf-rolling moth, *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae). Larvae of *E. utilis* feed by scraping the surface of the Brazilian peppertree leaflets. As they mature, the developing larvae are capable of completely defoliating the plant (Martin et al. 2004). Laboratory host range testing of *E. utilis* produced ambiguous results; non-target plant species were unexpectedly accepted as developmental hosts in both no-choice and choice tests (J.P. Cuda unpubl. data). The behavior of the insects may have been inhibited by the caged laboratory environment, producing “false positive” results where the insects accept plants that
they would not normally accept in a natural environment (Withers et al. 1999). Therefore, we believe that the broad physiological host range exhibited by *E. utilis* is not indicative of the behavior in a natural environment (ecological host range). For these cases where laboratory tests do not produce definitive results, F1SIT could be an additional tool to complement laboratory host specificity testing.

The F1 sterile insect technique is an approach that involves using radiation to partially sterilize male insects, resulting in the production of sterile progeny. Some of the effects of F1SIT include reduced egg hatch and a single generation of offspring that are highly sterile and mostly male (North 1975). Compared to SIT, F1SIT uses a lower dose of radiation resulting in increased quality and competitiveness of the moths (North 1975). Studies also have shown that using partially sterilizing doses of radiation may be more effective than using fully sterilizing doses of radiation to suppress pest populations (Toba et al. 1972; Proverbs et al. 1978).

In order to use F1SIT for field host range testing of potential biological control agents, the dose of radiation used to provide sterility in the F1 generation must be determined along with the effects of radiation on the F1 generation. The performance of the irradiated insects also must be comparable to the non-irradiated insects. Therefore oviposition rate, survival rates for F1 larvae, larval development, and host-finding behavior need to be examined in order for field host range testing to be complimentary to laboratory testing in terms of insect competence. The objective of the current study was to determine the dose of radiation that would ensure full sterility in the F1 generation and verify the effects of F1 sterility in *E. utilis*.
CHAPTER 2
LITERATURE REVIEW

The F₁ Sterile Insect Technique (F₁SIT)

Description

The F₁ Sterile Insect Technique (F₁SIT) is an autocidal method of pest control whereby a minimum dose of radiation is applied to male insects causing the insects to be partially sterile. Upon mating with nontreated females, there is a reduction of progeny in the filial or F₁ generation. Alternative terms for F₁SIT include inherited sterility, inherited partial sterility, partial sterility, semi-sterility, delayed sterility, and F₁ sterility (LaChance 1985; Carpenter et al. 2005). In the case of Lepidoptera, chromosomes are considered to be holokinetic (no primary site of microtubule attachment) due to a lack of centromeres which result in sister chromatids separating by parallel disjunction during mitotic metaphase. Each chromosome also has a large, localized kinetochore plate (centromere) where the spindle microtubules attach during cell division. The plate covers most of the chromosome ensuring that a majority of radiation-induced breaks will not lead to a loss of chromosome fragments. Due to reduced fragment loss, there is a reduction in the amount of sterility in the parental generation. The F₁ generation, however, inherits a greater degree of sterility as a result of rearrangement of chromosomes and production of “genetically unbalanced gametes” (LaChance et al. 1970; Squire 1973; Carpenter et al. 2005).

History

The idea of “sterilizing” insects was conceived in the early 20th century by three researchers working independently. These scientists (A.S. Serebrovskii, F.L. Vanderplank and E. F. Knipling) each played a role in developing the sterile insect technique (SIT) (Klassen and Curtis 2005). Through their efforts, SIT would become an important tool in pest management strategies.
The first major success using SIT in an insect eradication program occurred in the late 1950s against New World screwworm *Cochliomyia hominivorax* (Coquerel), a parasite of warm-blooded animals that significantly affects livestock health and production (Wyss 2000; Klassen and Curtis 2005). The goal of the program was to release sterile male flies to mate with wild females for the purpose of reducing screwworm populations. The first successful release was in 1951 on Sanibel Island, Florida. Sterile male flies were released from airplanes as part of a release-recapture experiment that resulted in the reduction of wild populations of the New World screwworm on Sanibel Island, but not complete eradication. Later releases of the sterilized flies on the island of Curacao, however, resulted in complete eradication of the flies (Klassen and Curtis 2005). In 1958, an eradication program began in Florida with airplane releases of up to 1,160 flies per km² per week at certain “hot spots” (Klassen and Curtis 2005). The releases ended in 1959 with complete eradication of the flies (Klassen and Curtis 2005). The successful outcome of the pilot program resulted in a global campaign that led to the eradication of the New World screwworm in the USA, Mexico, Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Panama north of the canal, some Caribbean islands, and Libya (Vargas-Teran et al. 2005). Additionally, there are many other examples of successful programs using sterile insects to control pest populations including the codling moth (*Cydia pomonella* (L.)), fruit flies including the Caribbean fruit fly (*Anastrepha suspensa* (Loew)); Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)); melon fly (*Bactrocera cucurbitae* (Coquillett)); Mexican fruit fly (*Anastrepha ludens* (Loew)); oriental fruit fly (*Bactrocera dorsalis* Hendel); Queensland fruit fly (*Bactrocera tryoni* (Froglatt)); West Indian fruit fly (*Anastrepha obliqua* (Macquart)), the onion maggot (*Delia antiqua* (Meigen)), pink bollworm (*Pectinophora gossypiella* (Saunders)), and several species of tsetse flies (*Glossina* spp.) (Klassen and Curtis 2005).
Although SIT has been used to control lepidopteran pests, the quality and competitiveness of the released insects is reduced due to the high doses of radiation required to achieve complete sterility. Lepidoptera are highly radioresistant and therefore require high doses of radiation (LaChance et al. 1985). To solve this problem, partial sterility was investigated (Proverbs 1962). Instead of a dose of radiation that would completely sterilize males, a lower dose of radiation could be applied, resulting in partially sterile males that when outcrossed to fertile females produce a single generation of offspring. This generation is characterized by reduced egg hatch. In their study of radiation-induced genetic anomalies in the silkworm moth, \textit{(Bombyx mori (L.))} Astaurov and Frolova (1935), first reported inherited sterility in the mid-1930s in the Soviet Union (Carpenter et al. 2005). Soon after this first report of inherited sterility, Ostriakova-Varshaver (1937) confirmed inherited sterility in the greater wax moth, \textit{Galleria mellonella (L.)} (Carpenter et al. 2005). Almost 30 years later, Proverbs (1962) demonstrated the effects of partial sterility in the codling moth in Canada. The significant findings of these early studies led to further research on partial sterility and its use in pest suppression (Astaurov and Frolova 1935; Ostriakova-Varshaver 1937; Proverbs 1962).

**Field Application of F\textsubscript{1}SIT**

As with the use of SIT, the F\textsubscript{1}SIT approach also is environmentally friendly because it, too, minimizes the use of insecticides to suppress pests. This is achieved by introducing sterility into the wild populations through the release of partially sterile males and fully sterile females. F\textsubscript{1}SIT is particularly useful for controlling many economically important species of Lepidoptera. Multiple studies involving inherited sterility and pest lepidopteran species have been conducted, including those on fall armyworm \textit{(Spodoptera frugiperda (J.E. Smith))} (Carpenter et al. 1985, 1997), corn earworm \textit{(Helicoverpa zea (Boddie))} (Carpenter et al. 1986, 1987, 1989), cabbage looper \textit{(Trichoplusia ni (Hubner))} (North and Holt 1969), Mediterranean flour moth
(Ephestia kuehniella (Zeller)) (Ayvaz and Tunçbilek 2006), potato tuberworm (Phthorimaea operculella (Zeller)) (Makee and Saour 1997), codling moth (Bloem et al. 1999a,b), false coding moth (Thaumatotibia leucotreta (Meyrick)) (Bloem et al. 2003; Hofmeyr et al. 2005), and cactus moth (Cactoblastis cactorum (Berg)) (Carpenter et al. 2001a,b). Each of these studies produced similar results including an F$_1$ generation that was more sterile than the parental generation yet was competitive with the native population. This suggested greater potential for pest suppression compared with using a fully sterilizing dose of radiation (North 1975; LaChance 1985).

The codling moth is a serious pest of apples and pears that developed resistance to the insecticides once used to control it. Autocidal control methods such as SIT and F$_1$SIT have now been developed and incorporated into an area-wide integrated management plan to control this pest (Bloem et al. 2005). Proverbs (1962) believed that control of the codling moth could be achieved by releasing sterile male moths. Field tests were conducted in Canada, the United States, Europe, the former Soviet Union, and Switzerland, confirming the effectiveness of the SIT method (Proverbs 1982; LaChance 1985). Because the release of sterile males in the SIT program had been so effective, Knipling (1970) proposed that the release of partially sterile codling moths may complement the sterile releases thereby achieving a greater level of success. Studies by Proverbs (1971) and Proverbs et al. (1973) compared the results of releasing fully sterile codling moths with those of partially sterile moths in laboratory and field cage studies conducted in Canada. They found that partially sterile moths were more competitive in terms of sperm quality and suppression of reproduction. Although the results of partial sterility were promising, potential damage from F$_1$ larvae on the fruit was considered to be too much of a risk (Proverbs et al. 1978). Bloem et al. (1999a,b) found that releasing partially sterile male moths resulted in a higher level of competitiveness than in males treated with higher doses of radiation.
Despite concerns about economic damage by $F_1$ larvae, it was concluded that suppression would occur more quickly with $F_1$ males mating with wild females in addition to continued releases of partially sterile male moths (Bloem et al. 1999a).

Another example of the utility of the $F_1$SIT approach in terms of field application is the suppression of pest introductions. For example, the cactus moth, *Cactoblastis cactorum* (Berg), which is native to South America, was released in Australia as a biological control agent of several invasive species of *Opuntia* cacti (Zimmerman et al. 2000). The moth was so successful in controlling the invasive *Opuntia* species that it was later released in Africa, New Caledonia, Hawaii, Mauritius, the Caribbean Islands, the Cayman Islands, St Helena, Ascension Island, and Pakistan (Zimmerman et al. 2000). In 1989, the cactus moth arrived in Florida following the release of the insect in the Caribbean for biological control of several native *Opuntia* species (Habeck and Bennett 1990). The moth currently threatens native *Opuntia* species throughout the southern United States and Mexico (Zimmerman et al. 2000; Hight et al. 2005). It was suggested by Carpenter et al. (2001a,b) that $F_1$SIT could be used to control the spread of the cactus moth. The effects of $F_1$ sterility on fecundity, fertility, and competitiveness in the cactus moth demonstrated that 200 Gy would be the optimum dose of radiation to ensure survival and $F_1$ sterility (Carpenter et al. 2001a,b). Field studies also have been conducted to determine mating behavior and overflooding ratios (Hight et al. 2003, 2005). Overall, $F_1$SIT has been shown to be an effective method for controlling a variety of lepidopteran pests. Because this technique has been shown to predictably control reproduction in Lepidoptera, a novel application of $F_1$SIT could be used to safely test potential weed biological control candidates *in the field* for risk assessment if results of traditional laboratory host range testing were ambiguous (Bloem and Carpenter 2001; Carpenter et al. 2001a,b; Tate et al. 2007).
Host Range Testing Protocols

One of the key steps in weed biological control programs is the evaluation of the host range of candidate biological control agents of invasive, exotic plants. Promising natural enemies (typically insects) are identified and further studied in their native ranges to determine their potential for controlling an invasive, exotic plant. Candidate biological control agents are typically subjected to rigorous caged laboratory trials involving no-choice and choice tests (Marohasy 1998; Withers et al. 1999). The host range (or host specificity) of an insect is used to evaluate any risks to potential non-target species in the proposed area of introduction (Littlefield and Buckingham 2004). Therefore, a wide range of more than 50 potential host plant species from those most closely related to more distantly related families are tested, with emphasis on native, endangered, and economically important species (Wapshere 1974; McEvoy 1996; Schaffner 2001). These tests involve exposing the potential host plant species to the larval and/or adult stages of the insect (Schaffner 2001). Results are used to determine whether the potential candidate is safe to release.

No-Choice Tests

Initially, a potential biological control agent (neonate and naïve adult) is exposed to a series of test plant species and the target species separately (Marohasy 1998; Sands and Van Driesche 1999). The purpose of the no-choice or starvation tests is to determine whether the insect is “restricted to a single host plant species” (Schaffner 2001). The results of the no-choice test identify the range of plant species that can support complete insect development and reproduction in the absence of the target species. A starvation test is the most conservative type of host range test because it will invoke more of a response from the insect compared with choice tests because of the “starved” state of the insect (McEvoy 1996).
One of the problems with testing taxonomically or chemically-related plants in no-choice tests is that many of these species will support some development in a confined laboratory setting. A Canadian study revealed that only 4% of Canadian biological control agents which fed on non-target plants in no-choice laboratory tests actually fed on these plants in the field (Harris 2004). In most cases, the range of plants determined by the no-choice test was 70% broader than the field host range (Harris 2004). Selection in laboratory no-choice tests is based primarily on larval development, whereas in field host-range tests, the focus is more on the behavior of the adult (Harris 2004).

Choice Tests

When test plant species are accepted in a laboratory no-choice test, these species need to be further tested to rule out any risk of attack in the field (Harris 2004). Choice tests can be designed to include test plants either with or without the target plant and involve mobile stages of the potential biological control agent, usually the adult stage (Schaffner 2001). The main objective is to retest plant species on which the insect fed, oviposited, or completed development in the no-choice tests (Schaffner 2001). In a choice test, it is important to demonstrate which plants the biological control agent will select when given multiple choices, including the host plant, as this is more representative of the natural environment where the insect will be introduced (Cullen 1990; Littlefield and Buckingham 2004). Although related species may have been attacked, if there is greater attack on the host plant, this may indicate “specific” behavior of the biological control agent (Harris 1964).

Open Field Testing

Results from the laboratory host specificity tests are used to determine whether a candidate biological control agent is “safe” to release in the field. The main point of these tests is to identify the laboratory (or physiological) host range which is then used to estimate or
predict the field (or ecological) host range of the candidate biological control agent (Julien and White 1997). Some biologists believe that these tests overestimate the true host range, consequently leading to the rejection of otherwise acceptable biological control candidates (Dunn 1978; Marohasy 1998; Cullen 1990). One such example involves no-choice starvation tests (entire lifetime of an insect) where the range of plant species on which the immature stages of a candidate biological control agent completes its development is often broader than the range of plant species a female biological control agent will accept for oviposition (Schoonhoven et al. 1998; Schaffner 2001). Likewise, a cage environment for testing the female’s oviposition behavior also may yield “false-positive” results when a female accepts a test plant that would not normally be selected in the field (Marohasy 1998). Therefore laboratory host specificity tests by their nature often produce ambiguous results (Dunn 1978; Cullen 1990; Marohasy 1998).

Because cage testing may inhibit normal behavior, open-field studies can provide a more realistic setting where insects can display an array of behaviors (Clement and Cristofaro 1995; Littlefield and Buckingham 2004). Field tests can serve as another tool to supplement laboratory tests by focusing on host finding and oviposition behavior of the candidate biological control agent when exposed to an array of potential host plants. The goal of the field tests is to reexamine non-target hosts in a more natural setting that were attacked in laboratory tests. Among the first researchers to use open-field testing were Andrés and Angalet (1963) in Italy. They examined the safety of the weevil, *Microlarinus lypriformis* (Wollaston) as a proposed biological control agent of puncturevine (*Tribulus terrestris* L.). The importance of a free-choice environment without the restriction of a laboratory cage was proposed by Dunn (1978). He suggested using partially opened field cages to allow insects to leave if they came into contact with an unacceptable plant (Dunn 1978). Other suggestions by Dunn (1978) and later by Cullen
(1990) were to release sterile insects in the area of introduction or only unmated females or males, to exterminate insects after use, or to rely on natural die off of the insects.

However, these types of studies are prohibited in the area of introduction due to federal and state regulatory restrictions. Therefore, field tests can be done only in the country of origin or native range of the insect. The main difficulty with field testing in the country of origin or native range of the insect is that it may not be possible to export critical test plant species from the proposed release area to the country of origin for testing. Other problems associated with open field testing include availability of insects in the native range where they are subjected to parasitism or predation and discrimination of damage associated with other insect species in the area. Despite these limitations, at least 19 species of insects and one mite species have been released based on incorporation of open field tests in the insect’s native region (Clement and Cristofaro 1995, Bredow et al. 2007, Gandolfo et al. 2007). The use of laboratory and field host-range testing may not be required for all candidate biological control agents, but for some that are in fact safe and actually pose very little risk, it could mean the difference between being accepted or rejected by regulatory agencies.

**Schinus terebinthifolius** Raddi

**Taxonomy**

Brazilian peppertree is a member of the Family Anacardiaceae. The family consists of 60 to 80 genera worldwide comprising around 600 species (Cronquist 1981). Most species are tropical and include trees, shrubs, and woody vines. Some of the well-known genera include mango (*Mangifera*), sumac (*Rhus*), pistachio (*Pistacia*), cashew (*Anacardium*), and poison ivy (*Toxicodendron*) (Cronquist 1981). The genus *Schinus* includes 29 species and is native to parts of South America including Argentina, southern Brazil, Uruguay, Paraguay, Chile, Bolivia, and Peru (Barkley 1944, 1957).
Common Names

Brazilian peppertree has many different common names as a result of its spread around the world. In Hawaii, it is known as wilelaiki and nani-o-hilo. Christmas berry is also a common name in Hawaii as well as in Guam. False pepper or faux poivrier are common names in the French Riviera whereas warui is used in Fiji. In native Brazil and Argentina, common names include aroeira, aroeira negra, aroeira vermelha, aroeira de Minas, aroeira de praia, corneiba and chichita (Argentina). In Cuba, Brazilian peppertree is known as copal, whereas in Puerto Rico it is known as pimienta de Brasil. Within the United States, names include Brazilian pepper, pink pepper and peppertree (Morton 1978, Cuda et. al 2006).

Description

Brazilian peppertree is a large evergreen shrub or small tree that can grow up to 7.5 m in height and has compound leaves with 3-11 (usually 7-9) leaflets that produce a peppery or turpentine-like smell when crushed (Fig 2-1). The flowers are small, white, unisexual and found in short-branched clusters. The fruits appear in bunches as glossy green drupes ripening to a bright red color from October to December in the northern hemisphere. The bright red berries and shiny green leaves contribute to the popularity of Brazilian peppertree as an ornamental during the holiday season (Fig 2-2) (Morton 1978; Ewel et al. 1982).

Distribution

Brazilian peppertree is native to Brazil, Paraguay, and Argentina (Fig 2-3). The mid to late 1800s marked the beginning of the distribution of Brazilian peppertree throughout the world as an ornamental (Barkley 1944, 1957). Brazilian peppertree is considered an invasive weed in the subtropical regions of at least 20 different countries (Ewel et. al 1982), including Bermuda, Bahamas, Australia, American Samoa, Fiji, Island of Mauritius, Micronesia, New Caledonia, Reunion Island, South Africa, and Tahiti (Habeck et. al 1994; USDA NRCS 2007) (Fig 2-4).
Brazilian peppertree has also been introduced into other South American countries, parts of Central America, Mediterranean Europe, North Africa, and southern Asia (Morton 1978). Because of its attractive green leaves and red berries, Brazilian peppertree is a favorite ornamental in Florida, Texas, Hawaii, Arizona, and California as well as parts of the Caribbean including the Bahamas, Commonwealth of Puerto Rico and US Virgin Islands (Habeck et. al 1994; USDA NRCS 2007). However, Brazilian peppertree is now considered an invasive, exotic weed in Florida, Texas, California, and Hawaii because it is altering native plant communities by displacing native species and changing community structure (Hosaka and Thistle 1954; Yoshioka and Markin 1991; Randall 2000; Hight et al. 2003; FLEPPC 2007). Currently, Brazilian peppertree is distributed widely throughout central and southern Florida. According to Wunderlin and Hansen (2004), voucher specimens have been received by statewide herbaria from 34 different Florida counties (Fig 2-5).

**Environmental Impacts**

**Ecosystem**

Brazilian peppertree was not popular when first introduced into Florida as an ornamental in 1898, but eventually was commonly cultivated, and ultimately invaded natural areas (Alexander and Crook 1974; Austin 1978). Invasion occurs in disturbed as well as undisturbed areas, including hammocks, pinelands, mangrove forests, canal banks, roadsides, and abandoned pastures where Brazilian peppertree produces dense monospecific stands (Loope and Dunevitz 1981; Ewel et. al 1982; Bennett et. al 1990). Seed dispersal of Brazilian peppertree occurs by wildlife; raccoons (*Procyon lotor* L.) and opossums (*Didelphus virginianus* Kerr) aid in local dispersal whereas frugivorous birds such as robins (*Turdus migratorius* L.) are responsible for long-distance dispersal (Ewel et. al 1982; Panetta and McKee 1997). Because of dispersal by wildlife along with its tolerance of extreme moisture conditions and ability to grow in shady
environments, Brazilian peppertree rapidly became a serious threat to the biodiversity of Florida (Ewel 1979). By the early 1990s, the southern and central portions of the state were heavily infested, comprising an estimated > 280,000 ha (1 million acres) of Brazilian peppertree (Habeck 1995). Based on recent estimates from aerial surveys of Florida, approximately 300,000 ha of terrestrial ecosystems were infested as of 1997 (Ferriter 1997).

**Human and Animal Health**

Sap and volatiles produced by flowers of Brazilian peppertree can cause allergic reactions in humans (Ewel et al. 1982). Allergenic properties of the plant are due in part to the fact that Brazilian peppertree is closely related to the more familiar poison ivy (*Toxicodendron radicans* L. Kuntz), poisonwood (*Metopium toxiferum* L. Krug), and poison oak (*Toxicodendron toxicarium* Salib. Gillis) (Ewel et al. 1982). Reactions to Brazilian peppertree include dermatitis, eye inflammation, facial swelling, severe itching, rash, respiratory irritation, sneezing, sinus congestion, and headaches (Morton 1969, 1978). Severity of the symptoms depends on the individual. Animals such as horses (*Equus caballus* L.) and cattle (*Bos taurus* L.) also are susceptible to allergenic properties of Brazilian peppertree. Reactions of the animals to Brazilian peppertree may include dermatitis, fatal colic, eye swelling, and enteritis. The fruit of Brazilian peppertree also has been found to produce a narcotic or toxic effect on birds (Morton 1978).

**Beneficial Uses**

In Brazil, Brazilian peppertree has various economic uses. For instance, the wood is used for construction. The bark produces a resinous extract, which is used to preserve fishing lines and nets. Brazilian peppertree also has some medicinal value used in homeopathy as a remedy for gout, muscular atony, pain associated with arthritis, strain of tendons, intestinal weakness, inertia of the reproductive organs, skin complaints, chills, tumors, lymphatic swellings, diarrhea, and hemoptysis (Morton 1978). Brazilian peppertree serves as an important nectar source for
commercial honey bees in both Florida and Hawaii (Sanford 1988; Yoshioka and Markin 1991). According to Sanford (1978), Brazilian peppertree is one of Florida’s best nectar producing plants resulting in honey that has a “peppery” taste and is popular locally. The fruit of Brazilian peppertree also is used as a spice and sold in gourmet stores in a dried form in addition to decorative uses in wreaths made in Hawaii (Morton 1978; Yoshioka and Markin 1991; Habeck et al. 1994).

Controlling Brazilian Peppertree

Mechanical Control

Hand pulling seedlings and small plants is feasible until the plant reaches several feet in height. It is extremely important to remove the root system so that resprouting will not occur. Larger plants require the use of bulldozers, front-end loaders, root rakes and other specialized heavy equipment (Langeland 1990). Removal may be successful when used in combination with other control methods such as physical or chemical control. If mechanical methods are used alone, soil disturbance often can lead to plant re-growth (Langeland 1990).

Physical Control

The use of fire, soil removal, and flooding are physical tactics that may be used to stress plants. This stress can leave the plant in a weakened condition possibly leading to die-off. The use of fire has had mixed results and may prove to be a liability in some areas due to property damage and human injury (Langeland 1990; Randall 2000). Soil removal is costly and labor intensive. Because established Brazilian peppertree plants can withstand extended hydroperiods (Langeland 1990; Ewe 2004) flooding may interfere with water conservation efforts. Therefore in order to be effective, these techniques should be used in combination with other methods of control.
Chemical Control

Currently, the most widely used method for managing Brazilian peppertree is chemical control. According to Ewel et al. (1982), several herbicides have been shown to be effective for controlling Brazilian peppertree. In their study, the effectiveness of five different herbicides was tested: a dicamba + 2,4-D combination, granular dicamba, an isopropylamine salt of glyphosate, a triazine compound, and an ester formulation of triclopyr. The triazine compound proved to be the most effective and longest lasting herbicide. However, most of the non-target vegetation in the area of application also was killed. The most effective (and labor intensive) control with limited non-target effects was achieved by using a low dose of triclopyr (basal application) (Ewel et al. 1982). A number of herbicides are currently recommended to control Brazilian peppertree in Florida (imazapyr, triclopyr ester and amine, dicamba, hexazinone, and tebuthiuron). These herbicides are applied specifically to the cut stump, basal bark, and foliage of the plant (Langeland 1990; Gioeli and Langeland 1997). Although herbicides are commonly used, they can remain in the soil and pose unwanted environmental effects. Cost also is an issue as are the unknown effects of abiotic factors such as rain, wind, temperature, soil chemistry, and water chemistry which can inhibit the efficacy of the herbicides (Langeland 1990).

Biological Control

A sustainable and environmentally friendly solution to control Brazilian peppertree is classical biological control. This process involves the introduction of natural enemies from the native range of the plant. Potential candidates must undergo a rigorous screening process to determine their host specificity. The goal is to permanently establish host-specific biological control agents that will reduce the competitiveness of the weed (Cuda et al. 1999; Scoles et al. 2005; Cuda et al. 2006). Results of exploratory surveys conducted in Brazil identified several
potential biological control agents of Brazilian peppertree. In the 1990s, three candidate biological control agents from Brazil were introduced under permit into an approved Florida containment facility (FBCL, Gainesville, FL). The three Brazilian peppertree natural enemies included a thrips *Pseudophilothrips ichini* Hood (Thysanoptera: Phlaeothripidae), a sawfly *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae), and a leafrolling moth *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae) (Cuda et al. 1999). To date, host-range testing has been completed for the thrips as well as the sawfly and is in the final stages for the leafroller.

*Episimus utilis* Zimmerman

**Taxonomy**

The larvae of the genus *Episimus* are commonly known as leafrollers or leafbud feeders. There are 32 New World species of *Episimus* found predominantly in tropical regions (Heppner 1994). Unfortunately, little is known about the biology of many of the species. There are nine species of *Episimus* in the United States (with all nine found also in Florida) ranging from northern Mexico to southern Canada. Many of the species are similar in appearance with minor differences in maculation and genitalia (Zimmerman 1978; Heppner 1994).

**Biology**

*Episimus utilis* was first described by Zimmerman (1978) who examined specimens collected in Hawaii where it was introduced in the 1950s (Krauss 1963) and in neotropical Brazil, which is part of its native range (Heppner 1994, Martin et al. 2004). Upon investigating the biology of *E. utilis* in a containment laboratory in Gainesville, FL, the entire life cycle (Fig 2-6) was found to be approximately 42 days, with multiple generations produced throughout the year (Martin et al. 2004). The duration of the larval stage also was found to be 24 days and included five instars (Martin et al. 2004). Four of the instars are pale green in color followed by a bright red 5th instar. Larvae range in size from 1.5 mm to 15 mm (Zimmerman 1978; Martin
et. al 2004). One of the diagnostic characteristics of the larval stage of *E. utilis* is a dark lateral bar on the head capsule (Zimmerman 1978). The larvae feed by scraping the surfaces of Brazilian peppertree leaflets (Fig 2-7). Early instars web together adjacent leaflets, while later instars usually roll a single leaflet to form a shelter in which to continue feeding (Yoshioka and Markin 1991). As the feeding process continues through larval development, the plant can be completely defoliated, resulting in injury or death of young plants and preventing reproduction of older plants (Cuda et al. 1999, 2006). Simulated herbivory studies have shown that growth and reproduction of Brazilian peppertree are inhibited when the plant is subjected to multiple defoliation events (Treadwell and Cuda 2007).

Pupae are brown in color and average 8 mm in length (Martin et al. 2004). During this 12-day stage, males and females can be separated by the number of abdominal segments. The female pupae have three movable segments with four fused segments in the terminal portion of the abdomen, whereas the male pupae have four movable segments and the apex with only three fused segments. The genital pore in female pupae also serves as a diagnostic characteristic for separating the sexes as it is “strongly drawn headward” (van der Geest 1991). Adults are small grayish brown moths with distinctive markings on the forewings (Zimmerman 1978; Martin et al. 2004). The adults also exhibit sexual dimorphism; the pale area on the male forewing can be used to separate the male from the female (Zimmerman 1978). The adults live an average of 7 days with females laying an average of 34.0 ± 5.1 eggs. The incubation period for the egg stage averaged 6 days (Martin et al. 2004).

**History of Introduction of *E. utilis***

Measures to control Brazilian peppertree biologically in Hawaii were undertaken by the release of three different biological control agents in 1954 (Krauss 1963; Bennett et al. 1990; Habeck et al. 1994). However, only two of the three agents released actually established, one of
these was the moth *E. utilis*. Despite becoming widely distributed on the islands of Kauai, Oahu, Molokai, Maui, and Hawaii, *E. utilis* was not found to be sufficiently abundant to destroy a significant amount of foliage (Zimmerman 1978; Yoshioka and Markin 1991; Julien and Griffiths 1998). One of the reasons why *E. utilis* was unable to attain high population densities was due to biotic interference from non-specific parasitoids released in the early 1900s to control sugarcane pests, specifically leafrollers of the genus *Hedylepta (=Omodes) Meyrick* (Swezey 1907; Krauss 1963; Yoshioka and Markin 1991; Martin et. al 2004). However, *E. utilis* is still widely distributed on Brazilian peppertree throughout the islands.
Figure 2-1. Dense stands of *Schinus terebinthifolius* in Florida (Photo by Vic Ramey, University of Florida/IFAS Center for Aquatic and Invasive Plants. Used with Permission).
Figure 2-2. Morphology of *Schinus terebinthifolius* (Photo by Ann Murray, University of Florida/IFAS Center for Aquatic and Invasive Plants. Used with Permission).
Figure 2-3. Native distribution of Brazilian peppertree in South America by country (Cuda et al. 2006).
Figure 2-4. Worldwide distribution of Brazilian peppertree (Cuda et al. 2006).
Figure 2-5. Distribution of Brazilian peppertree in Florida (Wunderlin and Hansen 2004).
Figure 2-7. Effect of *E. utilis* on Brazilian peppertree, A) Larvae feeding by scraping the surface of a BP leaflet, B) Larval damage of a BP plant (right) followed by complete defoliation (left) (Photo credit: Veronica Manrique and J. P. Cuda).
CHAPTER 3
USE OF THE F₁ STERILE INSECT TECHNIQUE (F₁-SIT) AS A TOOL FOR FIELD HOST RANGE TESTING OF *Epismus utilis* ZIMMERMAN (LEPIDOPTERA: TORRICIDAE), A CANDIDATE BIOLOGICAL CONTROL AGENT OF BRAZILIAN PEPPERTREE

Introduction

Throughout history, humans have sought ways to better manage pests affecting our plants, animals, and health. In the early 20th century, three scientists (A.S. Serebrovskii, F.L. Vanderplank, and E.F. Knipling) independently established the basis of what would become known as the sterile insect technique (SIT) (Klassen and Curtis 2005). The idea was to release irradiated and, hence, sterile male insects to mate with wild females, which would lead to a reduction in pest populations and their damaging effects on crops and livestock (Klassen and Curtis 2005). Although a high dose of radiation is needed to provide complete sterility, the F₁ sterile insect technique (F₁SIT) uses a lower dose of radiation, providing partial sterility and a reduced number of progeny. Lepidoptera are very radioresistant and require a large dose of radiation to ensure sterility (LaChance 1985). With the use of F₁SIT, however, a lower dose could be applied resulting in a more competitive insect (North 1975). Various studies have used this technique to demonstrate control of populations of pest Lepidoptera, including the codling moth, *Cydia pomonella* (L.) (Bloem et al. 1999a,b), false codling moth *Thaumatotibia leucotreta* (Meyrick) (Bloem et al. 2003), and cactus moth *Cactoblastis cactorum* (Berg) (Carpenter et al. 2001a,b). Because application of F₁SIT has been widely studied in pest management programs, this well known approach has potential for evaluating the risks of releasing exotic lepidopteran candidates for weed biological control (Dunn 1978; Cullen 1990; Greany and Carpenter 2000).

Before a candidate weed biological control agent can be released into the environment, the safety of the organism must be demonstrated. Host range testing is a process of screening potential biological control agents to minimize the risk of damage to non-target plant species.
The tests involve several different plant species closely and distantly related to the family of the targeted host plant (Wapshere 1974). Related plant species that are economically important, endangered, or native are of high priority and also tested (McEvoy 1996; Schaffner 2001). Two phases in host-range testing, no-choice and choice tests, are performed in the laboratory (Marohasy 1998; Withers et al. 1999). No-choice tests involve larval development and oviposition tests on a single non-target species. Choice tests, however, involve exposing the insect to two or more plants and typically include the host plant (McEvoy 1996; Schaffner 2001). Although these tests are designed to provide a realistic estimate of field host range, caged laboratory tests often overestimate host range because of unnatural behavior exhibited by some natural enemies as a result of being in a caged environment. This type of behavior may produce “false positives,” or acceptance of plants as hosts that would not normally be accepted by the potential biological control agent in nature (Withers et al. 1999).

A more natural or realistic testing approach is the use of open field tests. These tests can be done only in the native range of the target weed. However, there are certain limitations including the need to import test plant species that may be subjected to regulatory restrictions, availability of the potential biological control agent, and possible mortality of the agent by specialized predators and parasitoids. Field testing in the area of introduction could be done in a safe, temporary manner for potential lepidopteran biological control agents by using the F1 sterile insect technique. Advantages of this approach include the exposure of the biological control agent to the actual environmental conditions it would experience if approved for release, prediction of true field host range, and ability to reverse releases of the biological control agent without permanent establishment if non-target damage is detected (Bax et al. 2001; Carpenter et al. 2001a).
An example of a situation where F1SIT could be used is in classical biological control of Brazilian peppertree (*Schinus terebinthifolius*). This invasive, exotic weed is native to Brazil, Paraguay, and Argentina and was introduced into Florida as an ornamental in 1898 (Austin 1978; Ewel et al. 1982). Brazilian peppertree is distributed widely throughout central and southern Florida and listed by the Florida Exotic Pest Plant Council (FLEPPC) as a “Category 1” invasive weed because it is drastically altering native plant communities. The invasive nature of Brazilian peppertree also has been documented in Hawaii (Hight et al. 2002), California (Randall 2000), and Texas (Gonzalez and Christoffersen 2006) as well as in the subtropical regions of at least 20 additional countries (Ewel et al. 1982).

As a result of field surveys conducted in Brazil, three potential biological control candidates were imported into a Florida containment laboratory in 1994. One of these was a leafroller *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae). The insect was released in 1954 in Hawaii, but despite becoming widely distributed throughout the islands, it was not found to be sufficiently abundant to severely damage populations of the plant (Bennett et al. 1990; Yoshioka and Markin 1991; Habeck et al. 1994). Later, it was discovered that biotic interference from generalist parasitoids and predators probably prevented the insect from reaching its full biotic potential (Krauss 1963; Martin et al. 2004). Although *E. utilis* was ineffective in providing successful control of Brazilian peppertree in Hawaii, it could provide effective control in Florida because Florida may provide more favorable ecological conditions and less biotic mortality from introduced and native parasitoids and predators (Martin et al. 2004). Larvae of *E. utilis* inflict damage by feeding on leaflets, which can eventually lead to the defoliation of the plant (Cuda et al. 1999). Recently, a simulated herbivory study was conducted that showed Brazilian peppertree is vulnerable to sustained defoliation (Treadwell and Cuda 2007).
biology of *E. utilis* and methodology for its laboratory rearing were investigated by (Martin et al. 2004).

Laboratory no-choice tests with *E. utilis* produced ambiguous results; non-target plant species including fragrant sumac (*Rhus aromatica* L.), winged sumac (*Rhus copallinum* L.), poison sumac (*Toxicodendron vernix* (L.) Kuntze), pistachio (*Pistacia vera* L.) and cashew (*Anacardium occidentale* L.) were unexpectedly accepted as developmental hosts (Fig 3-1) (J.P. Cuda unpubl. data). We believe that the broad physiological host range exhibited by *E. utilis* is not indicative of the true behavior of this insect in a natural environment (ecological host range). For these cases where the results of laboratory tests lead to false positives, F1SIT could be an additional tool to elucidate the actual or ecological host range of the candidate biological control agent. The objectives of the current study were to determine a dose of radiation that would sterilize the F1 generation of *E. utilis* and to verify the effects of radiation in *E. utilis*.

**Materials and Methods**

**Colony Rearing**

Pairs of adult *E. utilis* moths (24 - 48 hr old) were set up on individual Brazilian peppertree plants planted in 3.8 L (1-gallon) pots (20 cm height x 22.5 cm diam.). Each plant was enclosed in a clear acrylic cylinder (45 cm height x 15 cm diam.) with six evenly spaced ventilation holes (6.5 cm diam.). The top of the cylinder was covered with a sheer polyester fabric (Jo-Ann Fabrics® #449-1676 white casa organza) and all six circular ventilation holes each were covered with a mesh, screen size of 150µ x 150µ (Green.tek® Inc., Edgerton, WI). The sheer polyester fabric was fastened to the top of the cylinder by a metal ring clasp (14.3-21.6 cm) and further sealed with a rubber band to prevent small larvae from escaping (Fig 3-2). Two additional access holes in the cylinder (2.5 cm diam) were plugged with #5 rubber stoppers. Each cylinder also was provided with a Gatorade® feeder (15 ml glass vial with a 5 cm piece of dental wick
soaked in Gatorade®) as a nectar source for the adults. The use of Gatorade® was previously shown to increase lifespan of adults and fecundity (total number of eggs laid) in females (Martin et. al 2004). When approximately 90% larval damage of the plant was observed, bouquets of five stems of Brazilian peppertree leaves (1-5 days old) in plastic vials (40 ml) with water (field collected in Ft. Pierce, FL) were placed near the top of the plant in each cylinder as needed.

Plants used for colony rearing were sprayed twice weekly with an organic insecticide consisting of 1 Tbsp (15 ml) each of isopropyl alcohol (70%), insecticidal oil, and Ivory® liquid soap mixed in 1 gallon (3.8 liters) of water to protect against soft-bodied pests. Average temperature and humidity of the rearing room were maintained at 25.8 ± 4.0°C and 40-70% RH as recorded by a Fisher Scientific® Thermo-Hygro® digital maximum-minimum temperature and relative humidity recording instrument. Temperature and relative humidity recorded within the cylinder were 24.9 ± 3.8°C and 60-80% RH, respectively. A photoperiod of 14:10 (L:D) was maintained by a programmable timer connected to sets of two 60 cm 20 watt fluorescent bulbs (one standard and one Gro-Lux®) per shelf of colony plants. Colony rearing and experiments with E. utilis were conducted at the University of Florida, Department of Entomology and Nematology Containment Facility (Gainesville, FL).

**Radiation Biology Study**

The procedures used for the radiation biology study were based on methodologies developed previously for the codling moth Cydia pomonella (L.) (Bloem et al. 1999a,b), false codling moth Thaumatotibia leucotreta (Meyrick) (Bloem et al. 2003), and cactus moth Cactoblastis cactorum (Berg) (Carpenter et al. 2001a,b). The E. utilis moths were collected from the colony at the 5th instar (red larval stage) or the pupal stage and placed individually into separate clear plastic 30 ml (1 oz) diet cups with a 3.5 cm piece of moistened filter paper added to each cup to maintain humidity. The cups were checked each morning at the same time and
adults were removed upon emergence. Male and female virgin *E. utilis* adults (<24hr old) were collected and individually exposed to gamma radiation in plastic snap-cap vials (12 ml) within an aluminum-lined cardboard canister (8.8 cm height x 7.5 diam.) (Fig 3-3). Doses of 0, 50, 100, 150, 200, 250, and 300 Gy were administered by using a Cesium-137 Gammacell® 1000 irradiator with a dose of 12-13 Gy/min (Fig 3-4) (F.A.S.T. Gainesville, FL). The dose rate was determined by a dosimetry study using Far West® dosimetry film. Two canisters each containing 10 of the plastic vials were placed on top of each other within the irradiator. Dosimetry film was placed in three different positions within four different vials (one from the top and bottom of each canister). Results indicated that to minimize the variance between the levels of irradiation, the canister containing the plastic vials should only be positioned at the bottom of the irradiator.

Five treated (T) male or female moths were placed inside a triangular waxed paper oviposition chamber (30 x 19 x 12cm) with an equal number of either treated (T) or nontreated (N) adult moths of the opposite gender (Fig 3-5). The oviposition chamber was then placed inside a 1-gallon plastic sealable freezer bag (Ziploc®) to maintain relative humidity and suspended on a string line to maximize the use of the limited amount of space in the containment laboratory. Each oviposition chamber included a 2 cm piece of cotton dental wick soaked in Gatorade® as a nectar source and a small leaf disc of *Pistacia vera* L. (2.4 cm x 2.4 cm). Due to inconsistent oviposition in preliminary experiments with nontreated moths, small leaf discs of Brazilian peppertree were substituted with *Pistacia vera* L. A phytochemical study of the leaves and bark of *Schinus terebinthifolius* had previously found that its compounds show a greater similarity to compounds isolated from *Pistacia* species than to those isolated from other species of *Schinus* (Campello and Marsaioli 1975). It was later determined that the leaf material was not a factor in the preliminary results, yet the *Pistacia vera* L. was used throughout the rest of the
experiments for consistency. The moths were allowed to mate and lay eggs for 2 intervals of 5 days to take into account the 7 day average lifespan for the adults (Martin et al. 2004). After the first 5-day period, they were transferred to a new oviposition chamber. At the end of the 10-day period, the females were collected, preserved in ethyl alcohol (80%), and subsequently dissected to determine their mating status (presence of spermatophores or inflated bursa copulatrix) (Ferro and Akre 1975). The egg sheets were then incubated for a period of 7 days at 24.6 ± 4.5°C, 50-80% RH, and a photoperiod of 14:10 L:D which corresponded to the developmental time of the egg stage (Martin et al. 2004). The total number of eggs laid (fecundity) and the number of eggs that hatched (fertility) were then counted for each egg sheet per radiation dose. Five replicates of 3 different crosses (treatments) (N♀ x T♂, T♀ x N♂, and T♀ x T♂) were completed for each dose of radiation. Temperature and relative humidity in the experiment room were 24.6 ± 4.5°C and 50-80% RH, respectively, with a photoperiod of 14:10 (L:D). Temperature and relative humidity within the oviposition chamber also were recorded as 24.1 ± 3.8°C and 60-80% RH, respectively. The newly emerged *E. utilis* moths were irradiated using a Cesium-137 Gammacell® 1000 irradiator (Fig 3-4) at the Florida Accelerator Services and Technology, Florida Department of Agriculture and Consumer Services, Division of Plant Industry (Gainesville, FL).

**Statistical Analysis for Radiation Biology Study**

In order to determine the effect of radiation dose on fecundity, linear regressions using fecundity as the response variable (Y) and radiation dose as the treatment variable (X) were performed. A separate model was fitted for each of the treatments.

The effect of radiation dose on fertility was determined by performing simple linear regressions of radiation dose predicting fertility for each of the crosses. In some cases, a polynomial model was indicated by the scatter plots of the data. The alpha level for all of the
regressions was $p=0.05$. The sex ratio was recorded for F1 males and analyzed using a simple linear regression. All regression analyses were performed using S-plus® 7.0 for Windows® (Insightful Corp.).

**Inherited Sterility Methodology**

Based on the findings from the radiation biology study, five doses of radiation were chosen to be additionally evaluated for the cross $N♀ \times T♂$ (nontreated female x treated male) in order to achieve inherited sterility. These doses included 125, 150, 175, 200, 225 Gy and a control (0 Gy). The protocol was the same as previously described, with the exception that the F1 egg sheets were each placed on a Brazilian peppertree plant in a 3.8 L (1-gallon) pot enclosed by a clear acrylic cylinder (45 cm height x 15 cm diam.) in order to rear the F1 generation. Average temperature and relative humidity recorded within a cylinder were $25.2 \pm 3.6°C$ and 70-90% RH, respectively. When the larvae hatched, they were allowed to develop on the plant. At the 5th instar (red larval stage) or the pupal stage, the insects were collected and each individual was placed in a separate clear plastic diet cup 30 ml (1 oz) with a 3.5 cm piece of moistened filter paper to maintain humidity. Upon emergence, each F1 female or male was outcrossed with a nontreated adult moth of the opposite sex. These F1 crosses were done in single pairs (1 female x 1 male). The protocol for the single pair crosses was the same as previously described for the radiation biology study. Ten crosses of F1 females and males were attempted for each dose, but due to virgin females and/or limited emergence of adults, there was a range of 5-12 replications for each gender per dose. Temperature and relative humidity in the experiment room were $25.6 \pm 4.4°C$ and 40-70% RH, respectively, with a photoperiod of 14:10 (L:D). The temperature and relative humidity recorded inside the oviposition chamber were slightly higher, averaging $26.1 \pm 4.9°C$ and 50-60% RH, respectively.
Statistical Analysis for Inherited Sterility

To determine the effects of radiation on the reproductive biology of F1 offspring of irradiated males, linear regressions of radiation dose administered on fecundity and fertility of the offspring were performed, fitting polynomial functions where appropriate. The sex ratio was recorded for F1 males and analyzed using a simple regression. Alpha level for each of the factors was p=0.05. The regression analyses also were performed using S-plus® 7.0 for Windows® (Insightful Corp.).

Results

Radiation Biology Study

The effects of the radiation treatments on the adults of E. utilis were dependent upon the dose of radiation and gender irradiated. In irradiated males, no significant changes in fecundity of mated females were observed as radiation dose increased (N♀ x T♂; F = 3.673; df = 1, 31; P = 0.06456), whereas in irradiated females, significantly fewer eggs were laid as dose increased (T♀ x N♂; F = 11.62; df = 1.32; P < 0.05; T♀ x T♂; F = 16.85; df = 1, 31; P < 0.05) (Fig. 3-6). Fertility for treated females also decreased with increasing radiation dose (T♀ x N♂; F = 56.31; df = 2, 30; P < 0.05; T♀ x T♂; F = 53.74; df = 2, 30; P < 0.05) and the same effect was observed for treated males crossed with nontreated females (N♀ x T♂; F = 57.35; df = 1,31; P = < 0.05) (Fig. 3-7). Additionally, the dose of radiation at which treated females were found to be 100% sterile was 200 Gy, whereas males irradiated at 200 Gy still had a residual fertility of 18%. Mating was confirmed in all adult female moths used in the experiments as determined by the presence of spermatophores or inflated bursa copulatrix (Ferro and Akre 1975).

Inherited Sterility

With respect to the fecundity for F1 females, there was no significant relationship between the dose of radiation administered to the treated male in the parental cross and fecundity.
observed in the F1 generation (F1♀ x N♂; F = 0.06699; df = 1, 42; P = 0.797; N♀ x F1♂; F = 1.114; df = 2, 52; P = 0.336) (Fig. 3-8). Percent egg hatch for treatments with the F1 males and females both declined with an increased dose of radiation (N♀ x F1♂; F = 40.31; df = 2, 52; P < 0.05; F1♀ x N♂; F = 58.28; df = 1, 43; P < 0.05) (Fig. 3-9). For both the F1 female and male treatments, 100% sterility was found at 225 Gy, which was the minimum dose at which no viable offspring could survive. In addition, the ratio of F1 males to females was more or less correlated with an increase in radiation dose, although the p-value was borderline (F = 7.517; df = 1, 4; P = 0.05181) (Fig. 3-10).

Discussion

The F1 sterile insect technique (F1SIT) has been used in several studies to control various pest Lepidoptera. The technique provides a safe, environmentally friendly approach to pest management. Early studies by Proverbs (1962), who first documented partial sterility in the codling moth found that when males were partially sterilized and mated to wild females the number of progeny was reduced and they were mostly male and highly sterile. Subsequent studies by North (1975) and LaChance (1985) compared the use of partial sterility with complete sterility in Lepidoptera. They determined that a partially sterilizing dose of radiation would increase competitiveness, possibly cause a delay in development, and lower quality sperm in the F1 generation.

Recent laboratory and field studies have confirmed these effects in the codling moth, (Bloem et al. 1999a,b) and false codling moth (Bloem et al. 2003), both belonging to the Family Tortricidae. The results of this study were similar to the results found in the previous studies. Higher doses of radiation resulted in an increase in sterility, a higher ratio of F1 males to females, and a declining trend in fecundity for both treated female crosses. Irradiated females of E. utilis were found to be 100% sterile at 200 Gy, which is similar to the female false codling moth but
was more radioresistant than the female codling moth where complete sterility was observed at 100 Gy. When treated males of *E. utilis* were mated with nontreated females, the sterility of the F₁ generation was likewise similar to that reported for the other tortricids. In particular, the dose at which *E. utilis* was found to be 100% sterile was 225 Gy whereas the dose for the codling moth was 250 Gy (Bloem et al. 1999a,b), and the range of partial sterility for the false codling moth was 150-200 Gy (Bloem et al. 2003). Because our findings were similar to the other studies, we determined that a dose of radiation of 225 Gy will provide 100% sterility and ensure a reduced number of progeny which are more sterile than their parents and mostly male. The F₁ sterile insect technique could therefore be appropriately applied in other areas of pest management including the testing procedures of potential biological control agents involved in control of invasive, exotic weeds. For example, Brazilian peppertree is a highly invasive, exotic weed in Florida and *E. utilis* historically is an established biological control agent of Brazilian peppertree in Hawaii where no non-target impacts have been documented. Laboratory no-choice and multiple choice host range tests performed with *E. utilis* showed that the physiological host range of this insect is broader than expected (J.P. Cuda unpublish. data). The cage environment in laboratory screening tests probably inhibited the behavior of the insects and produced “false positive” results, where the insects accept plants that they would not normally accept in nature (Withers et al. 1999). Therefore, field testing in the proposed area of introduction would provide the most accurate results. In this study, we found that a dose of 225 Gy can be applied to *E. utilis* adult male moths and upon mating with nontreated female moths, complete sterility in the F₁ generation is assured.

Based on fecundity results of nontreated females mated with irradiated male parents, no significant relationship was found between treatment dose and fecundity of females, therefore
suggesting that the number of eggs laid would be similar to non-irradiated moths. Fertility recorded in the irradiated male moth treatments (N♀ x T♂), however, would be greatly reduced. Normal oviposition behavior as well as larval damage and feeding would occur under natural conditions except that the F₁ generation would be unable to reproduce. Performance of irradiated E. utilis males should be similar to nontreated males based on results of previous studies examining the effects of radiation on other tortricid moths (Bloem et al. 1999a,b, 2003) The safety of the technique can also be further insured by the fact that most of the F₁ progeny will be males, therefore limiting the number of matings.

The objectives of this study were to identify a dose of radiation that when administered to E. utilis, a candidate biological control agent for control of Brazilian peppertree, would result in complete sterility in the F₁ generation and confirm the effects of radiation in E. utilis. Using the F₁ sterile insect technique (F₁SIT) in addition to laboratory host range testing can provide a temporary and reversible way to test potential biological control agents in the proposed area of release as proposed by Bax et al. 2001. Further studies will be needed to address the performance of irradiated biological control agents including oviposition, larval feeding preferences and survival, and host-finding behavior.
Figure 3-1. Results of laboratory no-choice tests performed with *E. utilis* (ANOC - Cashew, COCO - European Smoke Tree, PICH - Chinese Pistachio, RHAR - Fragrant Sumac, RHCO - Winged Sumac, RHGL - Smooth Sumac, SCTE - Brazilian peppertree, TOVE - Poison Sumac).
Figure 3-2. Potted BP plant covered by a clear acrylic cylinder.
Figure 3-3. Materials used for irradiation of *E. utilis* moths.

Figure 3-4. Cesium-137 Gammacell® 1000 irradiator (F.A.S.T. Gainesville, FL).
Figure 3-5. Waxed paper oviposition chamber for mating and ovipositing by *E. utilis* moths.
Figure 3-6. Fecundity (mean number of eggs laid) per mated female of *E. utilis* adults for three crosses (N♀ x T♂, T♀ x N♂, and T♀ x T♂) treated with increasing doses of gamma radiation. Lines shown are least-squares regression lines, T♀ x T♂; y = 81.7731 – 0.2125x; R² = 0.352; T♀ x N♂; y = 71.6725 – 0.1511x; R² = 0.266; N♀ x T♂; y = 68.6534 – 0.0946x; R² = 0.106.
Figure 3-7. Fertility (mean percentage of eggs that hatched) of *E. utilis* adults for three crosses (N♀ x T♂, T♀ x N♂, and T♀ x T♂) treated with increasing dose of gamma radiation. N♀ x T♂; $y = 64.4286 - 0.1999x$; $R^2 = 0.649$; T♀ x N♂; $y = 60.0056 - 0.6843x + 0.0017x^2$; $R^2 = 0.790$; T♀ x T♂; $y = 63.6209 - 0.7619x + 0.0020x^2$; $R^2 = 0.782$. 
Figure 3-8. Fecundity (mean number of eggs laid) of F1 crosses (F1♀ x N♂, N♀ x F1♂) of *E. utilis* adults as a result of radiation administered to parental males. Lines shown are least squares regression lines, F1♀ x N♂: y = 148.0383 – 0.0536x; $R^2 = 0.002$; N♀ x F1♂: y = 99.3422 + 0.2139x – 0.0017x^2; $R^2 = 0.041$. 
Figure 3-9. Fertility (mean percentage of eggs that hatched) of F₁ crosses (F₁♀ x N♂, N♀ x F₁♂) of *E. utilis* adults as a result of radiation administered to parental males. F₁♀ x N♂; y = 63.8547 – 0.3176x; R² = 0.575; N♀ x F₁♂; y = 55.1634 – 0.6017x + 0.0016x²; R² = 0.608.
Figure 3-10. Percentage of $F_1$ *E. utilis* adult males as a result of radiation administered to parental males. Line shown is least-squares regression line, $y = 54.9846 + 0.1159x$, $R^2=0.653$. 

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Figure 3-10. Percentage of $F_1$ *E. utilis* adult males as a result of radiation administered to parental males. Line shown is least-squares regression line, $y = 54.9846 + 0.1159x$, $R^2=0.653$. 

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58
CHAPTER 4
CONCLUSIONS

Host range testing in weed biological control has long been a heavily debated issue. Typical protocols involve the exposure of one or more life stages of an insect to a variety of plants in no-choice and multiple choice conditions to determine if the potential biological control agent would damage or reproduce on non-target hosts: plant species thereby rendering it unsafe for release. These host range tests typically are conducted in cages in an artificial laboratory environment and may result in behavioral responses that would not be seen in nature. This type of testing procedure often can result in “false positives” where the insect may accept plants outside of their normal host range (Withers et al. 1999). Because of federal restrictions, actual field testing can only be done in the native range of the target weed. While this type of testing would provide the most realistic approximation of the true host range for the potential biological control agent several obstacles are involved. Critical test plant species of interest to the USA may not be available in the native range, and if so, there may be resistance by foreign governments to their importation. Additionally, it may be difficult to find qualified people with the facilities necessary to conduct the studies, and sending trained scientists and materials to the native range may be very costly.

A possible solution to field testing in the area of introduction may lie in the use of insects that have been reproductively inactivated by the F1 sterile insect technique (F1SIT). Numerous studies involving important economic Lepidopteran pests have documented the effects of using radiation to achieve inherited sterility. Some of these effects include increased competitiveness compared with complete sterility, a decline in the number of progeny, a predominantly male biased sex ratio, and a highly sterile F1 generation that may experience delayed development along with producing lower quality sperm (Proverbs 1962, North 1975, LaChance 1985).
Results from studies by Bloem et al. (1999a,b, 2003) showed that the performance of irradiated adult moths were comparable to non-irradiated moths.

The purpose of this study was to determine the dose of radiation that would ensure full sterility in the F1 generation and to verify effects of radiation in *E. utilis*, a candidate biological control agent of Brazilian peppertree. The situation presents a prime example of an invasive, exotic weed that has a promising potential biological control agent, yet host specificity tests in the laboratory proved to be ambiguous. We found a broader host range than what was observed in Brazil, the insect’s native range or Hawaii, where the insect was released and attacks only Brazilian peppertree. Identifying the dose of radiation that produces a fully sterile F1 generation in *E. utilis* would allow the biological control agent to be field tested in Florida in a safe and non-permanent manner (Bax et al. 2001). This additional tool would provide a realistic indication of host range because *E. utilis* could be released in the area of potential introduction. The sterile F1 generation could then be allowed to develop normally on appropriate test plants.

Results for *E. utilis* were similar to other inherited sterility studies on tortricid moths including those reported for the codling moth (Bloem et al. 1999a,b) and false codling moth (Bloem et al. 2003). The fecundity of irradiated *E. utilis* females in the parental generation decreased significantly with increased dose, whereas fecundity for nontreated females outcrossed to irradiated males did not differ from the controls. However, the fertility of both irradiated females and males significantly declined with increased dose, especially for irradiated females due to a higher sensitivity to radiation. When increasing doses of radiation were applied to parental males outcrossed to nontreated females, there was no significant effect on the fecundity of the F1 females or males when outcrossed to nontreated moths of the opposite sex. Fertility of the F1 females and males declined significantly with increased dose, yet F1 males were found to
be more sterile than F₁ females. A significantly greater number of F₁ males were produced with higher doses of radiation applied to the parental male. For all three tortricids, effects of increased dose of radiation resulted in an overall decline in the fecundity and fertility of the parental and F₁ generations. Additionally, with increased dose of radiation, a higher ratio of F₁ males to females was found. The dose at which irradiated *E. utilis* females were 100% sterile was the same as for the false codling moth, yet *E. utilis* proved to be more resistant to radiation than irradiated codling moth females. In terms of the dose of radiation required for inherited sterility, *E. utilis* proved to be more radioresistant than the false codling moth, but was comparable to the codling moth.

The results of this study have shown that a dose of radiation can be applied to *E. utilis* adult males resulting in a reduced number of progeny that are sterile and largely male. The use of this technique for field host range testing can be done in a safe manner without posing any environmental risks because the F₁ generation will be sterile. This tool, used in addition to laboratory testing, would be useful for clarifying any unnatural behavior observed in the laboratory leading to overestimation of host range. Further study of the performance of irradiated insects compared to nontreated insects will need to be completed to ensure that host-finding behavior, oviposition, and larval survival along with feeding preferences can be compared in laboratory tests with field tests using reproductively inactivated biological control agents. If approved by state and federal regulatory officials, future studies will involve the use of partially sterilizing doses of radiation applied to males outcrossed to nontreated females released into field cages with appropriate test plants.
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BIOGRAPHICAL SKETCH

Onour received a BS in marine science and biology with a minor in chemistry at the University of Miami in 2002. Immediately following graduation, she received an internship at the Smithsonian Marine Station in Fort Pierce that involved working on the interaction of nutrients and salinity in the mangroves of the Indian River Lagoon. Following the internship, Onour held positions at the Indian River Research and Education Center working for Dr. William Overholt on the Biological Control of Weeds and at the USDA in Fort Pierce with Dr. Erin Rosskopf on Alternatives to Methyl Bromide-Weed and Disease Control. She quickly developed an interest in the biological control of weeds and working with insects which lead to her pursuit of a master’s degree in entomology and nematology at the University of Florida.