INITIAL STEPS FOR DEVELOPING A RESISTANCE MANAGEMENT PROGRAM FOR THE SOUTHERN CHINCH BUG, *Blissus insularis* BARBER

By

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I dedicate this dissertation to my loving husband, Ricardo José Vázquez, for his unending love and support.

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Blissus insularis Barber, is a serious pest of St. Augustinegrass and has a history of resistance to insecticides in Florida. A resistance management program is needed for this pest but initial steps are required. The goals of this study were to 1) sample select *B. insularis* populations in Florida to describe their susceptibility to bifenthrin, document new locations of bifenthrin resistance, and evaluate another pyrethroid, permethrin, 2) develop a synchronous rearing method for *B. insularis*, and 3) develop an improved bioassay that could be used for detecting insecticide susceptibility differences between male and female *B. insularis*, evaluate and validate both the sprig-dip and the new bioassay under standardized conditions, and determine optimal exposure times and sample sizes to be used for each bioassay for selected insecticides.

The results of objective 1 suggest bifenthrin resistance continues to be problematic, is becoming more widespread, and there is a positive relationship between insecticide application and the development of bifenthrin resistance. This study documents the first case of insecticide resistance in the Florida Panhandle and first report of *B. insularis* resistance to permethrin.

Five different rearing methods were attempted for *B. insularis*. The use of glass jars and a combined diet of fresh corn cob and St. Augustinegrass proved to be the best synchronous

rearing method for producing *B. insularis* of known age and generation. No reduction in body size was observed after nine generations of rearing. In addition, the high number of brachypterus *B. insularis* produced indicates that populations were not stressed.

An airbrush bioassay for testing contact and systemic insecticides was developed, and evaluations were made of both the airbrush and sprig-dip bioassays under standardized conditions to determine sample size and duration of tests. The sprig-dip bioassay was more sensitive in detecting lower LC values than the airbrush bioassay when testing *B. insularis* against bifenthrin. The airbrush and sprig-dip bioassays will be useful tools for detecting and monitoring of insecticide resistance in *B. insularis*. The airbrush bioassay would be beneficial for use in studies concerning cross resistance, mechanisms, mode-of-inheritance, and stability of pyrethroid resistance because of the ability to easily detect differences between male and female *B. insularis* and reduced variability.

CHAPTER 1
LITERATURE REVIEW

Turfgrasses

Turfgrass is a vegetative ground cover used in landscapes and is the most widely used ornamental crop in the United States (Emmons 1995). Humans have used turfgrasses for more than 10 centuries as a means to enhance their environment and quality of life (Beard 1973, Beard and Green 1994). There are several functional, recreational, and aesthetic contributions of turfgrasses.

Functional Benefits

Turfgrasses are maintained in a long-term stable state and thus greatly aid in protecting nonrenewable soil resources from water and wind erosion (Kageyama 1982, Potter and Braman 1991, Beard and Green 1994). Once a vigorous and dense turf develops in the landscape, it also plays a significant role in reducing water runoff in urban and suburban areas, especially those near paved surfaces (Kageyama 1982, Potter and Braman 1991, Florida Department of Environmental Protection 2002, Bell and Moss 2008). In addition, the development of a healthy root zone allows greater infiltration of rain or irrigation by improving soil structure and reducing soil compaction (Florida Department of Environmental Protection 2002). The root zone also aids in facilitating biodegradation of organic pollutants, air contaminants, and pesticides used in lawns, as well as encouraging soil-building processes through the decomposition of organic matter and formation of humus. Healthy turfgrass also muffles noise, reduces glare, and modifies temperatures (Kageyama 1982, Potter and Braman 1991, Beard and Green 1994, Florida Department of Environmental Protection 2002). Also, a 15 m × 15 m turf area absorbs carbon dioxide, ozone, hydrogen fluoride, and perosyacetyle nitrate and can release enough oxygen to meet the needs of a family of four (Emmons 1995).

Recreational and Aesthetic Benefits

Healthy turfgrass provides a safe recreational surface with a cushioning effect that reduces injuries to humans compared to walking or running on poorly- or non-turfed soils (Beard and Green 1994). Also, the beauty of a well maintained lawn and landscape can have a positive impact on mental health by providing green space in urban areas, as well as increase property values by as much as 15% (Kageyama 1982, Potter and Braman 1991, Emmons 1995).

Turfgrass Industry in Florida

Many lawns in Florida are established through sodding. Sod is dense turf that is cut in pieces or strips from the soil and sold as ground cover for use in lawns (Emmons 1995, Christians 2004). In a national study, Florida was ranked first in terms of economic impact of sod production (Haydu et al. 2006). In 2003, the total sod production in Florida was estimated to be 93,000 ha, with 64% being St. Augustinegrass (Haydu et al. 2005). Only 3% of harvested sod is sold outside of Florida. With so much demand for sod in Florida, there is also a high demand for maintaining it. Florida is second only to California in terms of employment impacts of the turfgrass industry, providing 83,944 jobs in 2002 (Haydu et al. 2006).

St. Augustinegrass

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, is a warm-season, coarse-textured, aggressive, and stoloniferous grass (Turgeon 1996) that is believed to be native to the coastal regions of both the Gulf of Mexico and the Mediterranean (Trenholm and Unruh 2005). Carter and Duble (1976) estimated that St. Augustinegrass comprised as much as 96% of lawns in the Gulf Coast area. In Florida, the first known record of planting St. Augustinegrass was from a diary by A. M. Reed, where he wrote on November 11, 1880, "George planting St. Augustine grass in avenue in afternoon." It was planted as a turf alongside an avenue at A. M. Reed's Mulberry Grove plantation, at Yukon, near Orange Park, FL (Works Progress

Administration 1939, White and Busey 1987, Busey 1995). Today, it is the primary turfgrass in residential lawns and comprises ≈ 70% or 1.2 million ha in Florida (Hodges et al. 1994, Busey 2003). Most St. Augustinegrass cultivars have good salt (Dudeck et al. 1993) and shade tolerance (White and Busey 1987) and are usually established by plugs or sod (Christians and Engelke 1994, Christians 2004). St. Augustinegrass also grows well in most soils and climatic regions in Florida (Trenholm and Unruh 2005). Its aggressive growth habit gives it good recuperative capability, but it is prone to thatch buildup (Potter 1998).

Blissus insularis

Host Plants and Distribution

The southern chinch bug, *Blissus insularis* Barber (Barber 1918), is considered the most damaging insect pest of St. Augustinegrass (Reinert and Portier 1983, Busey and Coy 1988, Crocker 1993). *Blissus insularis* was at first believed to be a variety of *B. leucopterus* and a member of the *leucopterus* complex (Leonard 1966). However, Leonard showed *B. insularis* was genetically isolated from the other taxa of the *B. leucopterus* complex and gave it species rank. Originally known as the lawn chinch bug, the southern chinch bug was given its current name when it was designated as a distinct species (Stringfellow 1969, Sweet 2000). It was first documented as a pest of St. Augustinegrass in 1922 (Newell and Berger 1922). *Blissus insularis* also attacks other lawn grasses including bahiagrass (*Paspalum notatum* Fluegg), bermudagrass [*Cynodon dactylon* (L.) Pers.], centipedegrass [*Eremochloa ophiuroides* (Munto)], and zoysiagrass (*Zoysia* spp.), but most of the injury to these has occurred near heavily infested St. Augustinegrass (Kerr 1966). *Blissus insularis* has also been found in lawns that contained a mix of St. Augustinegrass and centipedegrass where the St. Augustinegrass was killed and the centipedegrass was left unharmed (Kerr 1966). Buss (E.A.B., unpublished data) observed *B. insularis* feeding and damage to a St. Augustinegrass lawn stopped abruptly where the

neighboring bahiagrass lawn started (Figure 1-1). Other hosts include crabgrass (*Digitaria floridana* Hitchc.), torpedograss (*Panicum repens* L.), and Pangolagrass (*Digitaria decumbens* Stent) (Slater and Baranowski 1990, Brandenburg and Villani 1995). *Blissus insularis* occurs in the southern U. S. coastal states, Hawaii, and Mexico (Henry and Froeschner 1988, Vittum et al. 1999, Sweet 2000).

Biology and Life History

Adult *B. insularis* are small insects with the adult body measuring between 2-4 mm long (Cherry and Wilson 2003) and 1 mm wide (Leonard 1968). Females are usually larger than males (Figure 1-2 A and B). The sclerites at the ventral tip of the abdomen are rounded in males and triangular in females (Figure 1-2 C and D). Wings are white with a distinctive triangular-shaped black marking in the middle of the outer edge of each wing and are folded flat over the back causing the tips to overlap. Populations may consist mostly of short-winged forms (brachypterous), long-winged forms (macropterous), or both [Figure 1-2 A and B] (Wilson 1929, Komblas 1962, Leonard 1966, Reinert and Kerr 1973). In Florida, macroptery is greatest during the summer and fall although reasons for this are unknown (Cherry 2001a). However, studies have shown that macroptery in the oriental chinch bug, *Cavelerius saccharivorus* Okajima is density dependent, and is strongly enhanced by seasonal factors (long day length, high temperature) (Fujisaki 2000).

The biology of *B. insularis* is well documented. When courting, males and females approach each other, make first contact with their antennae, then pair facing opposite directions (Vittum et al. 1999). Copulation may last as long as 2 h and during this time female *B. insularis* are more active than males and may walk about and/or feed (Leonard 1966, Vittum et al. 1999). Eggs are laid singly or a few at a time in sheaths, near the grass nodes, in soft soil, or in other protected areas (Beyer 1924a, Kuitert and Nutter 1952, Reinert and Kerr 1973). The eggs are

white when first laid (Eden and Self 1960), turning beige (Figure 1-3 A) then bright orange (Figure 1-3 B) just before hatching. Young nymphs are as small as 1.0 mm, are reddish-orange with a white band across the dorsal side of the abdomen, and become black in color as they mature (Figure 1-4 A-E). Many nymphs crawl between the folds of the sheath located at the lower portion of the grass leaf (Christians 2004), and may remain hidden for up to 10 d (Kerr 1966). Development from egg to adult depends on location and temperature. In Florida, Kerr (1966) reported *B. insularis* can complete development from egg to adult in 34.7 d at 28.3°C and in 93.4 d at 21.1°C. All life stages are present throughout the year in most of the state with three to four generations occurring in northern Florida and seven to ten in southern Florida each year (Kerr 1966, Reinert and Kerr 1973).

Feeding Habits and Damage

Although capable of flight, adult *B. insularis* move between lawns mainly by walking and many have been observed crawling across paved areas bordering heavily infested lawns (Kerr 1966). All life stages are distributed vertically through the turf thatch and into the upper organic layer of the soil, with densities of up to 2,000 *B. insularis*/0.1 m^2 being reported (Reinert and Kerr 1973). Light to moderate infestations are aggregated in small areas in the lawn, but *B. insularis* can occur throughout the entire lawn in heavily infested areas (Cherry 2001b). *Blissus* spp. are sap feeders (Slater 1976) and feed on the phloem and xylem in meristematic regions of the grass (Painter 1928) causing wilting, chlorosis, stunting, and eventually death (Painter 1928, Negron and Riley 1990, Spike et al. 1991). As the grass dies, the insects continue to move outward to feed on more-succulent grass, enlarging the damaged area (Figure 1-5), and may easily encroach onto neighboring St. Augustinegrass lawns (Figure 1-6). St. Augustinegrass cultivated on high, dry, sandy, or shell soil is especially vulnerable to *B. insularis* damage (Wilson 1929, Woods 2007).

Blissus insularis prefer open sunny areas of St. Augustinegrass, especially areas with abundant thatch (Reinert and Kerr 1973). Thatch is the layer of accumulated decomposing leaf blades, stems, and roots on top of the soil surface (Figure. 1-7) (Emmons 1995, Trenholm and Unruh 2005). Where temperatures are warmer, particularly in South Florida, the grass may grow continuously and create a thick, spongy thatch (Vittum et al. 1999). Thatch that is 10 – 15 cm thick is common and can be up to 30 cm deep (Vittum et al. 1999), providing *B. insularis* with shelter and possibly protecting them from predation and environmental stress (Reinert and Kerr 1973). The abundance of *B. leucopterus hirtus* Montandon was also closely linked to thatch thickness in lawns (Davis and Smitley 1990).

The effect of moisture on *B. insularis* populations and their feeding injury to turf is equivocal. *Blissus insularis* may thrive when the grass is most tender and succulent, and its feeding may prevent normal growth and cause a dwarfed condition to the grass (Beyer 1924a, Vázquez and Buss 2006). Warm and fairly dry weather is most favorable for hatching of *B. insularis* eggs (Beyer 1924a). *Blissus insularis* injury may be more evident during dry weather because dryness reduces turf vigor and favors the rapid increase in *B. insularis* populations (Wilson 1929). Kerr (1966) suggested that moisture had a marked but paradoxical effect on *B. insularis* populations. Heavy irrigation or rainfall may make the grass more succulent and able to tolerate some feeding damage, while at the same time making the grass more attractive to *B. insularis*. However, destructive outbreaks of *B. insularis* are sometimes prevented by heavy rainfall (Beyer 1924a) by killing the young nymphs, and this is true for other *Blissus* spp. as well (Webster 1907). Long-term *B. insularis* feeding damage may look like drought stress, but not be a result thereof. Also, *B. insularis* could already be present and feeding in a lawn, but a secondary stress, like drought, may intensify the damage (Vázquez and Buss 2006).

Several authors have attempted to rear *Blissus* spp. under laboratory and greenhouse conditions to better understand its biology, life history, and feeding habits. The following provides a brief review of previously reported rearing procedures for Blissidae.

Rearing of *Blissus* spp.

Yamada et al. (1984) reared the oriental chinch bug, *Cavelerius saccharivorus* Okajima, on maize, Kentucky bluegrass, sorghum, and sugarcane. Sugarcane leaves were the best diet on which to rear more than two generations of *C. sacchorivorus*. However, Yamada et al. (1984) reported that only 40% of the second generation successfully survived to the adult stage.

Dahms (1947) and Todd (1966) reared the common chinch bug, *Blissus leucopterus leucopterus* (Say), on plants maintained in a specially prepared nutrient solution. However, the insects were only maintained on a limited basis. Later, Parker and Randolph (1972) reared *B. l. leucopterus*, in the laboratory on alternating stacked layers of maize and sorghum stalk sections. Each stalk section end was dipped in melted paraffin wax and allowed to dry before placement in heat-sterilized 3.78-L cardboard cartons. Cartons were maintained in growth chambers at 32 ± 2°C with a 14L:10D photoperiod. Pathogens were controlled by washing the stalk sections with warm soapy water and rinsing in a 1.0% solution of benzalkonium chloride before placement in cardboard cartons. The carton tops were covered with heat-sterilized Purelin™ singlefold no. 515 towels. *Blissus l. leucopterus* eggs, nymphs, and adults were easily removed from the top stalks and used to start new colonies. Each 3.78-L cardboard carton could produce 800-1000 chinch bugs (Parker and Randolph 1972).

Wilde et al. (1987) also reared *B. l. leucopterus,* but used small grains, maize, sorghum, and millet. Ten to fifteen maize, sorghum, or millet plants were germinated in 15-cm pots. Two to 3 wk after planting, 25 unsexed adults were placed in each pot and confined with 15 × 45 cm plastic cages with ventilation holes on the side. Sand was used at the base with Teflon®

(DuPont, Wilmington, DE) sprayed on the upper inside surfaces of cages to prevent insect escape. Adults were transferred to new plants every 2 wk. Cages were maintained in the greenhouse with a 16L:8D photoperiod and 25-30°C. Between 300 to 400 chinch bugs developed on each plant. Meehan and Wilde (1989) also successfully reared *B. l. leucopterus* on pearl millet in the greenhouse (21 – 32°C) and in growth chambers (24 – 30°C) with a 16L:8D photoperiod.

Baker et al. (1981) attempted to rear the hairy chinch bug, *B. l. hirtus*, using Parker and Randolph's (1972) technique, but early-instar mortality was high, which appeared to be associated with fungal growth on the corn sections. When sections of young maize plants were treated with 2% sodium hypochlorite (instead of 1.0% benzalkonium chloride) and placed in 236.6-ml cardboard cartons in growth chambers [16L:8D photoperiod, at 26°C, and 40-75% RH], *B. l. hirtus* was reared year round (Baker et al. 1981). *Blissus l. hirtus* survival from egg to adult increased to 80%.

Busey and Zaenker (1992) maintained populations of the southern chinch bug on 10-20 stolon cuttings (~100 mm long with three to four nodes) of susceptible 'Florida Common' St. Augustinegrass for host-plant resistance studies. Insects were confined in plastic bins (14.5 × 18.0 × 9.0 cm deep) covered with a double sheet of cellulose tissue (Kimwipes, Kimberly-Clark, Roswell, GA) glued to the tops of the bins. Stolon cuttings were placed in water-filled glass vials that were sealed with parafilm and were replaced at least once a week (Busey and Zaenker 1992). Percentage survival, the number of generations produced, and the existence of overlapping generations were not reported. It is possible that the insects were only maintained long enough to complete the study.

Anderson (2004) reared *B. insularis* on 15-cm pots of 'Raleigh' St. Augustinegrass in a potting mixture of sand-soil-peat-perlite in a 2:1:3:3 ratio. Plants were covered with ventilated tubular 15 × 45 cm plastic cages that were embedded 2-3 cm into the soil. The cages were sealed with organdy fabric and sand was placed around the bottom of the cages to prevent insect escape. Infested plants were kept in a growth chamber at 28 ± 2°C with a 24L:0D photoperiod and 40 – 75% RH. As the plants began to die, insects were sifted through a 2-mm mesh screen, aspirated, and placed on new plant material. *Blissus insularis* was reared for five generations but the population peaked at a total of 500 insects and rapidly declined (Anderson 2004). Spider predation in the cages, limited air movement and fungal development due to caging negatively affected the population, and constant light may not have been suitable for *B. insularis* development (Anderson 2004).

Anderson (2004) also reared *B. l. leucopterus* and *B. l. hirtus* with the procedures described by Wilde et al. (1987). This method allowed the use of whole plants instead of stalk sections and did not require treating plants (Parker and Randolph 1972, Baker et al. 1981). One pot could support ~400 chinch bugs for about 3 wk. However, greenbug [*Schizaphis graminum* (Rondani)] populations would rapidly build, crowding out preferred chinch bug feeding sites and excreting copious amounts of honeydew, resulting in sooty mold (Anderson 2004).

Several authors successfully produced > 1 generation of *Blissus* spp. under greenhouse and growth chamber conditions (Wilde et al. 1987, Meehan and Wilde 1989, Anderson 2004). However mass-rearing of *B. insularis* in our greenhouse has not been feasible. Daily ambient summer temperatures in three of the greenhouses we used have exceeded 37.8 °C, which is lethal for *B. insularis* (personal observation), and St. Augustinegrass pots have become infested with aphids, thrips, scales, mites, other *B. insularis* populations, and natural enemies.

Management Practices

Biological Control

Reinert (1978) observed spiders (*Lycosa* sp.) and predatory insects such as *Pagasa pallipes* Stal (Hemiptera: Nabidae), *Xylocoris vicarius* (Reuter) (Hemiptera: Anthocoridae), *Lasiochilus pallidulus* Reuter (Hemiptera: Anthocoridae), *Sinea* spp. (Hemiptera: Reduviidae), *Labidura riparia* Pallas (Dermaptera: Labiduridae), and *Solenopsis geminata* (F.) (Hymenoptera: Formicidae), feeding on *B. insularis* but none were able to suppress *B. insularis* populations below damaging levels. Another pest in lawns, the red imported fire ant, *Solenopsis invicta* Buren, is also ineffective at controlling *B. insularis* populations (Cherry 2001c). *Beauveria bassiana* (Balsamo) Vuillemin was pathogenic to all life stages of *B. insularis*; however, was only present when moisture and humidity levels were high (Reinert 1978). Woods (2001) tested the virulence of *B. bassiana* on healthy *B. insularis* via direct contact and was only able to obtain a 6.1% rate of transmission.

Geocoris bullatus (Say) and *G. uliginosus* (Say) (Hemiptera: Geocoridae) have been observed preying on *B. insularis* (Reinert 1978). *Geocoris punctipes* (Say), also found in Florida turfgrass (Mead 2004), can prey on up to 19 first- to third-instar *B. insularis* nymphs in 24 h under laboratory conditions (Congdon 2004). However, young *B. insularis* nymphs can hide within the sheaths of St. Augustinegrass, thus avoiding predation by *Geocoris* spp. When *G. punctipes* were given a choice between pea aphids *Acyrthosiphum pisum* (Hemiptera: Aphididae) and nutritionally superior eggs of the corn earworm *Helicoverpa zea* (Lepidoptera: Noctuidae), *G. punctipes* consistently attacked *A. pisum* (Eubanks and Denno 2000). The authors reported that prey mobility, not prey nutritional quality, appeared to be the most important criterion used by *G. punctipes* in choosing their prey. Geocoridae are generalist predators and are cannibalistic (Chiravathanapong and Pitre 1980, James 2004, Mead 2004). Also, Geocoridae in turfgrass can

be mistaken for *B. insularis* and unnecessary insecticide applications can ensue (Caplan 1968, Mead 2004).

Perhaps the natural enemy that holds the most promise for controlling *B. insularis* can be found in the family Scelionidae. *Eumicrosoma* spp. (Hymenoptera: Scelionidae) are parasitic wasps (Figure 1-8 A-B) that attack the eggs of several *Blissus* spp. (McColloch and Yuasa 1914, 1915; Dicke 1937; Reinert 1972; Wright and Danielson 1992; Sadoyama 1998). *Eumicrosoma benefica* Gahan attacks the eggs of *B. insularis* year-round in Florida; Reinert (1972) found an average abundance of 35 wasps/0.1 m^2 in lawns containing *B. insularis* populations of 90.0/0.1 m^2 (Reinert 1972). However, research needs to be done to determine host-specificity and suitability for use in the biological control of *B. insularis*.

Host Plant Resistance

Tolerance of *B. insularis* is a major consideration in selection of St. Augustinegrass cultivars, as the success of 'Floratam' demonstrates. Floratam is an improved cultivar of St. Augustinegrass that was released as a chinch bug-resistant cultivar in 1973 by the University of Florida and Texas A&M (Anonymous 1973, Busey 1979, Horn et al. 1973, White and Busey 1987, Trenholm and Unruh 2005). After its release, Floratam was confirmed resistant to *B. insularis* (Reinert et al. 1980, Crocker et al. 1982, Reinert et al. 1986) and it quickly became the most widely used cultivar in South Florida (Busey 1986, Busey and Center 1987). Today, Floratam is the most widely produced and used cultivar of St. Augustinegrass and accounts for 80% of sod production in Florida (Haydu et al. 2005). It has a very coarse leaf texture, and poor cold and shade tolerance (Trenholm and Nagata 2005), but is resistant to the St. Augustine decline virus, and successfully minimized *B. insularis* problems for years (Busey 1979, Busey and Center 1987). However, *B. insularis* has overcome resistance to Floratam in Florida and Texas (Busey and Center 1987; Crocker et al. 1989; Busey 1990a, 1990b). Plant breeders

developed 'FX-10' St. Augustinegrass that was registered in 1993 and was resistant against *B. insularis* (Busey 1993). However, it was never extensively grown because of its very coarse texture, toughness, and poor ground coverage (Busey 1993, Nagata and Cherry 2003). Two additional cultivars, NUF-76 and NUF-216, were developed and found to be highly resistant to *B. insularis* (Nagata and Cherry 2003, Rangasamy et al. 2006). NUF-76, now known as Captiva™, has a lush, dark green color and can be mowed less frequently. It is now in production for use in lawns, but its long term effectiveness is yet to be determined.

Cultural Control

Proper cultural practices such as fertilization can help to promote healthy grass, which may be able to better tolerate chinch bug damage. Current nitrogen recommendations suggest a range from 100 to 300 kg N ha^{-1} yr^{-1} to maintain St. Augustinegrass lawns in Florida, depending on soil type, geographical location in the state, time of year, amount of shade in the lawn, and the preference for a high-or low-input lawn (Trenholm and Unruh 2007). Applications must be made carefully as heavy fertilization may cause excessive turfgrass growth (Christians 2004), thatch development, reduced tolerance to environmental stresses (Emmons 1995), and nutrient leaching (Kelling and Peterson 1975, Weaver et al. 1988, Petrovic 1990, Coale et al. 1994, Correll 1998, Sims et al. 1998, Wulff et al. 1998, Florida Department of Environmental Protection 2002, Sinaj et al. 2002, Park et al. 2008). Phytophagous insects such as *B. insularis* meet nutritional requirements by feeding on healthy host plants (Heinrichs 1988). Nitrogen appears to be a crucial, limiting factor in insect growth and survival (Mattson 1980, Scriber and Slansky 1981, Heinrichs 1988). With this said, high fertility with readily available N applied to St. Augustinegrass results in higher *B. insularis* populations than grass treated with lower N amounts (Horn and Pritchett 1963, Busey and Snyder 1993). Busey and Snyder (1993) noted that early population regulation effects, such as the number of eggs per female per week and rate

of development of early instars, may explain the observed response of *B. insularis* to high fertilization with quick-release fertilizers.

Irrigation and mowing are also important components in maintaining healthy St. Augustinegrass. Irrigation should occur on an as-needed basis (Emmons 1995, Trenholm and Unruh 2005), with St. Augustinegrass being watered at the first sign of rolling leaf blades, wilting, and/or footprints that remain on the lawn. At these signs of water deficit, applying 1.3–1.9 cm of irrigation to the entire lawn should supply water to a depth of ~15-23 cm for most Florida soils. Watering in this manner will help to encourage deep root growth (Emmons 1995). Excess irrigation may lead to problems such as a shallow root system, increased pest problems, and increased thatch (Emmons 1995, Trenholm and Unruh 2005). Mowing too infrequently can also cause a thatch buildup and excessive thatch (exceeding 2.5 cm) may need to be professionally removed by mechanical thatch removal (verticutting or aerification) (Christians and Engelke 1994, Christians 2004). Also, mower height should be properly set to avoid possible scalping. Most St. Augustinegrass cultivars should be mowed to a height of 8-10 cm. Newer semi-dwarf varieties can be mowed to a height of 3.8–6.3 cm (Trenholm and Unruh 2005).

Chemical Control

Control of damaging *B. insularis* populations is mainly achieved through insecticide use. Currently, 20-25 *B. insularis* per 1 ft2 warrant control (Short et al. 1982). Lawn care companies have at times made up to six to twelve insecticide applications a year to control this pest in Florida (Reinert 1978, Reinert and Niemczyk 1982). Insecticides historically used against *B. insularis* include tobacco dust, calcium cyanide, nicotine sulfate, DDT, parathion, dieldrin, aldrin, chlordane, chlorpyrifos, propoxur, diazinon, and bifenthrin (Beyer 1924b; Watson and Bratley 1929a, 1929b; Kelsheimer 1952; Wolfenbarger 1953; Kerr 1956; Brogdon and Kerr

1961; Reinert 1982a, 1982b; Reinert and Portier 1983; Cherry and Nagata 2005). Currently, organophosphates, carbamates, pyrethroids, neonicotinoids, and combination products are used for *B. insularis* control. The following provides a brief description of each insecticide class and their mode of action.

Organophosphates

The synthesis of organophosphates (OPs) began in the 1800's; however their potential toxicity went unrecognized until the 1930's (Chambers et al. 2001). By 1940, work conducted in England and Germany had produced several highly toxic compounds for possible use as chemical warfare agents. The most notable work was done in Germany by Gerhard Schrader with the development of nerve gases during World War II. It was not until the capture of Schrader's research records that interest in OP insecticides grew (Chambers et al. 2001). OPs are derivatives of phosphoric acid and are highly toxic (Yu 2008). There are several subclasses of OPs but, in general, they are considered to be biodegradable and nonpersistent.

OPs exert their toxic action by inhibiting acetylcholinesterase, an enzyme that occurs in the central nervous system (Scharf 2003, Yu 2008). Under normal circumstances, acetylcholinesterase removes acetylcholine from its postsynaptic receptor, resulting in hydrolysis of acetylcholine into acetate and choline. This initiates at precise intervals electrical impulses (action potentials) that travel along neurons and provide the basis of nervous system function (Scharf 2003). OPs attach to acetylcholinesterase resulting in prolonged binding of acetylcholine to its postsynaptic receptor, leading to death in the organism from prolonged neuroexcitation (Scharf 2003). The reaction with OPs and acetylcholinesterase is very slow, typically taking days or even weeks (Yu 2008). This is because OPs must be enzymatically activated by cytochrome P450s before they can effectively inhibit acetylcholinesterase and also because the nature of the OP-acetylcholinesterase interaction (Scharf 2003). As a result, OPs can become

irreversible inhibitors of acetylcholinesterase. The only OP currently registered for *B. insularis* control is trichlorfon (Dylox®, Bayer Environmental Science, Research Triangle Park, NC).

Carbamates

Carbamates are esters of carbamic acids (Plimmer 2001). Their herbicidal and fungicidal activities were demonstrated in the early 1930s, but interest in insecticidal activity did not begin until the mid-1950s (Ecobichon 2001). Carbamates are slightly to moderately soluble in water, moderately volatile, and readily biodegradable (Yu 2008). They are used to control a wide range of chewing and sucking insects. Like the OPs, carbamates are acetylcholinesterase inhibitors. However, carbamates are faster inhibitors than OPs, and are generally more hazardous although their effects are readily reversible (Scharf 2003). Carbaryl (Sevin™, Bayer Environmental Science, Research Triangle Park, NC) was the first carbamate to be commercially developed (Wickham 1995) and is the sole chemical in this class that is available for control of *B. insularis*.

Pyrethroids

Pyrethroids are synthetic insecticide derivatives of pyrethrum. Pyrethrum extracts are obtained from chrysanthemum flowers (*Chrysanthemum cinerariaefolium*). Originally grown in the former Yugoslavia, the ground, dried flower heads were later known as 'Dalmation Insect Powder' and the product was used to control body lice during the Napoleonic Wars (van Emden and Service 2004). Over the years, research was done to find synthetic replacements for agricultural use that had greater photochemical stability and longer field life (Plimmer 2001). The first pyrethroid, allethrin, was developed in 1949 (Ware and Whitacre 2004). Pyrethroids are divided into two groups, type I and type II; the difference between the two being that type II pyrethroids have an α-cyano group (Yu 2008). Type I pyrethroids permethrin and bifenthrin are used for control of *B. insularis*. Type II pyrethroids used to control *B. insularis* include beta-cyfluthrin, cypermethrin, deltamethrin, and λ-cyhalothrin. Pyrethroids are slightly soluble in

water and have minimal volatility. They have exceptional photostability and generally provide good residual control (Yu 2008). Pyrethroids are also lipophilic and adhere strongly to organic matter (Elliott et al. 1978, Laskowski 2002). Because of these characteristics, pyrethroids are widely used to control agricultural pests.

Pyrethroids interfere with voltage-gated sodium channels of both the peripheral and central nervous system. Voltage-gated sodium channels are responsible for the initiation and perpetuation of action and receptor potentials in neurons (Scharf 2003). Pyrethroids affect sodium channels by causing activation at lower thresholds or inactivation later than would occur under normal circumstances, resulting in prolonged flow of sodium currents into neurons and excessive neuroexcitation (Scharf 2003).

Neonicotinoids

Three generations of chemicals are involved in the history of nicotinoids (Yamamoto and Casida 1999), the first of which was nicotine. Nicotine was extracted from tobacco in 1828 by Posselt and Reimann and was named after Jean Nicot, who introduced tobacco to the French court around 1560 (Posselt and Reimann 1828, Ujváry 1999). Due to its high toxicity to mammals, research continued in search of more selective compounds. The second generation of chemicals emerged in 1970 when the Shell Development Company was investigating heterocyclic nitromethylenes as potential insecticides (Sheets 2001, Tomizawa and Casida 2005). Nitromethylenes had potency, selectivity, and systemic properties but were not photostable (Yamamoto and Casida 1999). Imidacloprid is the first commercial product of the third generation of chemicals, the neonicotinoids. Imidacloprid was developed by Bayer in 1984 and has greater insecticidal activity and lower mammalian toxicity than its predecessors (Sheets 2001). Neonicotinoids generally have low toxicity to mammals (Thyssen and Machemer 1999, Yamamoto 1999, Anatra-Cordone and Durkin 2005, Tomizawa and Casida 2005, birds (Anatra-

Cordone and Durkin 2005, Tomizawa and Casida 2005), and fish (TDC Environmental 2003, Tomizawa and Casida 2005, Jemec et al. 2007). Since the introduction of imidacloprid, neonicotinoids have become the fastest-growing class of insecticides introduced to the market since the commercialization of pyrethroids (Nauen and Bretschneider 2002, Jeschke and Nauen 2005). Neonicotinoids are broad-spectrum insecticides that possess contact, stomach, and systemic activity (Jeschke and Nauen 2005). Neonicotinoids labeled for control of *B. insularis* include imidacloprid, clothianidin, dinotefuran, and thiamethoxam.

Neonicotinoids act on the insect central nervous system as agonists of the postsynaptic nicotinic acetylcholine receptors (nAChRs) (Jeschke and Nauen 2005). nAChRs and muscarinic acetylcholine receptors are considered the two major acetylcholine receptors based on their sensitivity to agonists (Nauen et al. 2001, Scharf 2003). nAChRs are agonized by nicotine and muscarinic acetylcholine receptors are agonized by the mushroom toxin muscarine. nAChR is a highly insect-specific target site. Compared to the ~250:1 ratio of muscarinic to nicotinic receptors in the mammalian central nervous system, insects have more than 10 times as many nicotinic as muscarinic receptors (Scharf 2003). Neonicotinoids mimic acetylcholine by acting as agonists to activate the nAChR, causing an influx of sodium ions and the generation of action potentials (Yu 2008). Under normal conditions, the synaptic action of acetylcholine is terminated by acetylcholinesterase which hydrolyzes the neurotransmitter. However, neonicotinoids are not hydrolyzed by acetylcholinesterase and the persistent activation leads to hyperexcitation, convulsion, paralysis, and death of the insect (Yu 2008).

Insecticide resistance in *B. insularis*

Limited research on the effects of natural enemies on *B. insularis*, lack of host-plant resistance options for the last two decades, and minimal and/or improper use of cultural methods have helped generate near-constant reliance on chemical control of *B. insularis* populations. As

a result, *B. insularis* has developed resistance to organochlorines, organophosphates, carbamates, neonicotinoids, and pyrethroids (Wolfenbarger 1953; Kerr 1958, 1961; Reinert 1982a, 1982b; Reinert and Niemczyk 1982; Reinert and Portier 1983; Cherry and Nagata 2005, 2007).

Insecticide Resistance

Resistance is defined as a heritable physiological and/or behavioral (Sparks et al. 1989) adaptation that confers a selective advantage in the presence of a pesticide, and that leads to control failures (Sawicki 1987, ffrench-Constant and Roush 1990). In 2006, there were 550 arthropod species resistant to one or more pesticides worldwide (Onstad 2008, Whalen et al. 2008). Pesticide resistance has been estimated to have a global annual economic impact of over $4 billion dollars (Pimentel et al. 1991, Mota-Sanchez et al. 2002). Since the first report of pesticide resistance in 1914 (Melander 1914), significant progress has been made in 1) the development of methods for detection and documentation of resistance, 2) research on resistance mechanisms, 3) the identification of biotic, genetic, and operational factors influencing the evolution of resistance, and 4) pest management practices that incorporate resistance-delaying measures (Georghiou and Saito 1983).

Detection and Documentation

Early detection and documentation of insecticide resistance is an important step in resistance management because if resistance can be detected before control failures occur, then preventive measures possibly can be implemented (ffrench-Constant and Roush 1990). However, it is first important to have a standardized method for evaluating toxicity to the insect in question. The toxicity of an insecticide is typically determined using concentration- or dose-response bioassays with response expressed in terms of lethal concentration, dose, knockdown, or time (Yu 2008). Evaluation of toxicity to *B. insularis* is typically expressed as lethal concentration (LC_{50} and/or LC_{90}) with the response being death (concentration-mortality). For

analysis, the raw data are transformed to a log scale, forming a sigmoid or S-shaped curve (Yu 2008). The percentage mortality is then converted to probits using probit (for normally distributed data) or logit analysis to create a log concentration-probit (or logit) line from which the LC_{50} is estimated (Yu 2008). Ideally, five concentrations that cause mortality ranging from 5-95% with at least 100 insects per concentration should be used for estimation of LC_{50} (Robertson and Preisler 1992, Robertson et al. 2007). When conducting concentration-mortality tests it is important to choose an appropriate bioassay for measuring response. It is also important to standardize insecticide bioassays to reduce variability. Attributes to standardize bioassays inlcude temperature, daylength, relative humidity, sex of the insect, age of the insect, previous exposure(s) to other chemicals, and substrate. This will ensure that the observations made are due to the effect of the insecticide and not to some other variable.

Choice of Bioassay

Most insecticide bioassays can be classified based on the manner in which the pesticide is applied (i.e., dipping, topical, feeding, or residual). The dipping method involves dipping insects in solutions of known concentration (Yu 2008). This method is often regarded as unrealistic or imprecise because the results cannot be expressed in terms of toxicant per gram of body weight (ffrench-Constant and Roush 1990). While dipping tests ensure uniform contact, it would be extremely time consuming when working with small insects such as *B. insularis*. Topical application involves applying a known dose of insecticide directly to individual insects via microsyringe (Yu 2008). However, as with the dipping method, topical application is time consuming when dealing with small insects and the exact amount of pesticide penetrating the insect is not known. Feeding assays entail providing immature insects with insecticide-treated diets (Yu 2008). The residual or contact method consists of exposing insects to a dry residue of pesticide on a natural (e.g., leaf) or artificial (e.g., glass, filter paper) substrate (ffrench-Constant

and Roush 1990). Some researchers have reported that tests such as leaf-residue assays can improve the accuracy of resistance detection as well as help to establish the relationship between laboratory bioassays and field-control failures (ffrench-Constant and Roush 1990). However, others have expressed concern that insects may be repelled by treated leaves and never come in contact with the insecticide, and that the exact dose accumulated by the insect is unknown (Brown and Brogdon 1987, ffrench-Constant and Roush 1990). Nonetheless, the residual method has been used in many hemipteran studies (Studebaker and Kring 2003, Snodgrass et al. 2005, Fleury et al. 2007), including *B. insularis* (Reinert and Portier 1983; Congdon and Buss 2004, 2006; Cherry and Nagata 2005, 2007).

The most commonly used residual bioassay for evaluating insecticide efficacy in *B. insularis* is the sprig dip (Reinert and Portier 1983; Congdon and Buss 2004, 2006; Cherry and Nagata 2005, 2007). This method involves cutting sections of St. Augustinegrass stolons, dipping them into insecticide solutions, allowing them to dry, and placing them into petri dishes containing ten adult *B. insularis*. The set up for the sprig-dip bioassay can be conducted quickly and it is inexpensive. However, a large degree of variability in response occurs. Tests are usually conducted in different laboratories under varying environmental conditions, or with field-collected *B. insularis* of unknown age and/or from different locations (Reinert and Portier 1983; Cherry and Nagata 2005, 2007; Congdon and Buss 2006; Chapter 2). It would be beneficial to evaluate the sprig-dip bioassay under more standardized conditions to validate the use of the assay.

In addition to variability in the sprig-dip bioassay, scoring multiple individuals in the same dish can be cumbersome when they are not all moribund. A more standardized bioassay that could detect differences between male and female *B. insularis* would greatly aid in

understanding how insecticide resistance develops in this pest (i.e., mode of inheritance, stability of resistance).

Source of variability in insecticide bioassays

Replication and reliability of insecticide bioassays can depend on the stability of the environmental conditions (e.g., light, temperature, and humidity) (Sun 1960, Rozman et al. 2001) and the insects used for testing (Sun 1960). Any variability in the biological and/or environmental factors in a bioassay may change the insects' susceptibility to an insecticide. These factors can be divided into two categories, intrinsic and extrinsic (Busvine 1980).

Intrinsic factors

The insect species, stage, and strain used may generate variability in insecticide bioassays (Sun 1960). Data from one species cannot be used to detect resistance in another as differences in susceptibility may occur. However, related species tested using the same technique may result in similar susceptibility levels (Busvine 1980). Choosing the appropriate life stage is also important. Sun (1960) noted that inactive stages (eggs and pupae) are usually more tolerant of an insecticide than active stages (immatures and adults). Also, using homogeneous susceptible or resistant laboratory colonies will help to reduce variability in bioassays.

Other intrinsic factors that may introduce variability into insecticide bioassays include age, size, sex, and nutrition (Rozman et al. 2001). Adult insects are often more susceptible just after molting, followed by a period of greater tolerance, and then increasing susceptibility with advancing age (Busvine 1980). In immature insects, tolerance to insecticides increases as the insect gains weight (ie., first instar vs. fifth instar). In addition, male insects tend to be more susceptible to insecticides than females (Sun 1960). Insects in a single field population or laboratory colony have also shown changes in response to insecticides depending on the time of

day and/or season (Sun 1960). Also, well-nourished insects will generally be more tolerant to insecticides than malnourished ones.

Extrinsic factors

Temperature, humidity, and light can possibly alter tests by changing the effectiveness of the insecticide (Busvine 1980, Rozman et al. 2001) and/or insect activity (Busvine 1980). The relationship between temperature and insecticide toxicity in insects is well documented (Scott 1995, Valles et al. 1998), particularly for DDT and pyrethroids. DDT (Vinson and Kearns 1952) and type I pyrethroids may show a negative temperature coefficient, meaning the insecticide becomes more toxic to insects with decreasing temperature (Wadleigh et al. 1991, Valles et al. 1998, Musser and Shelton 2005). Type II pyrethroids may show a positive, negative (Scott and Matsumura 1983, Sparks et al. 1983), or no temperature coefficient (Toth and Sparks 1990). Most carbamates and organophosphates have a positive temperature coefficient (Norment and Chambers 1970, Chalfant 1973, Grafius 1986, Li et al. 2006b), as well as the neonicotinoid imidacloprid (Elbert et al. 1991, Richman et al. 1999). Low humidity is another extrinsic factor that can alter the outcome of insecticide bioassays because it can cause desiccation of sensitive insects (but should also be evident in the controls). Increased or constant light conditions may lead to greater insect activity and as a result, the insects would come into contact with the insecticide faster in residual tests (Busvine 1980).

While insecticide resistance has been documented in *B. insularis* populations in Florida, these conclusions were made based on tests either conducted in the field under varying environmental conditions (Kerr 1958, 1961) or under laboratory conditions using field-collected insects of unknown age and sex (Reinert 1982a; Reinert and Niemczyk 1982; Reinert and Portier 1983; Cherry and Nagata 2005). A more standardized method of testing conducted under

laboratory conditions is needed to better understand the development of insecticide resistance in *B. insularis*.

Resistance Mechanisms

There are several ways that insects can develop resistance to insecticides and these can be classified into two main categories: behavioral and physiological. Behavioral resistance is defined as the ability to avoid a dose or concentration that would prove lethal (Yu 2008). It is thought that insects with behavioral resistance contain receptors which can better detect low concentrations of insecticides than normal insects (Yu 2008). Physiological resistance has been shown to involve three factors: reduced penetration, target site insensitivity, and metabolism (increased detoxification) (Yu 2008). Penetration resistance occurs when resistant insects absorb toxins through the cuticle more slowly than susceptible insects. Reduced penetration by itself results in only slight resistance (Soderlund and Bloomquist 1990, Yu 2008). However, in the presence of other mechanisms, reduced penetration confers considerable resistance to some insecticides (Yu 2008).

Target site insensitivity usually involves point mutations (the replacement of one nucleotide by another [Hoy 2003]). There are three types of target site insensitivity involved in insecticide resistance in insects: nerve insensitivity, altered acetylcholinesterase, and reduction in midgut target site binding (Yu 2008). Nerve insensitivity is involved in organochlorine, pyrethroid, neonicotinoid, and phenylpyrazole insecticide resistance in many insects. For example, resistance to cyclodienes in *Drosophila melanogaster* (ffrench-Constant et al. 1993) and fipronil in diamondback moths (Li et al. 2006a) was due to a point mutation (substitution of alanine to serine) of the GABA receptor protein, causing receptor insensitivity. Also, knockdown resistance (kdr) to pyrethroids in *D. melanogaster* was due to several point mutations in the sodium channel gene (Yu 2008). Altered acetylcholinesterase is associated with resistance

to organophosphate and carbamate insecticides. This type of resistance occurs in several insect and acarine species, including cattle ticks, *Drosophila*, fall armyworms, houseflies, green rice leafhoppers, mosquitoes, tobacco budworms, and two-spotted spider mites (Smissaert 1964; Fournier and Mutero 1994; Gunning and Moores 2001; Yu 2006, 2008). Examples of reduction in midgut target site binding include insects resistant to *Bacillus thuringiensis* (Bt). Ferre and Van Rie (2002) reported that reduced binding of toxin is a primary mechanism of insect resistance to the Cry proteins of Bt, but some insects are able to alter the sugar structure of the glycolipid (receptors for Bt toxin) molecule so the Bt toxin cannot attach itself, and as a result, become resistant (Griffitts et al. 2005).

Metabolic resistance results when an insect detoxifies and excretes the toxin faster than a susceptible insect, enabling the resistant insect to quickly rid its body of the insecticide. Three detoxification enzymes associated with resistance in insects are cytochrome P450 monooxygenases, hydrolases, and glutathione S-transferases (GSTs) (Yu 2008). Resistance to insecticides can be due to enhanced oxidative metabolism caused by cytochrome P450 monooxygenases. This important enzyme is non-specific to organic compounds and can result in cross-resistance to other insecticides (Yu 2008). Carboxylesterases (hydrolases) are involved in resistance to ester-containing insecticides such as organophosphate, carbamate, and pyrethroid insecticides (Yu 2008). GST is a phase II enzyme associated with resistance to nearly all pesticide classes.

Cross resistance refers to a situation in which an insect population becomes resistant to two or more insecticides (with different active ingredients) as a result of selection by a single insecticide (Winteringham and Hewlett 1964). Multiple resistance occurs after simultaneous or

successive exposure to two or more insecticides. Resistance mechanisms are not known for *B. insularis*.

Biotic, Genetic, and Operational Factors

The development of resistance is determined by a variety of genetic, biological or ecological, and operational factors (Georghiou and Taylor 1986). Genetic factors would include the number, frequency, and/or dominance of resistant alleles; past selection with other chemicals; and the extent of integration of the resistance genes with fitness factors. Important biological factors include time per generation, offspring per generation, monogamy or polygamy, mobility, diet, and refugia (Georghiou and Taylor 1977a). *Blissus insularis* can be difficult to control in Florida because it produces multiple generations per year, has a high number of offspring per generation, is highly mobile and encroaches onto neighboring lawns, is able to survive on other grass sources until new St. Augustinegrass is located, and is able to avoid insecticides.

Operational factors that lead to resistance are those related to the application of pesticides and include the dosage used, treatment history, treatment schedule (rotation or no application), treatment thresholds, life stage selected, and method of application (Georghiou and Taylor 1977b). Operational factors are considered under human control and their manipulation may help to delay the onset of insecticide resistance. Multiple insecticide applications are made each year to control damaging *B. insularis* populations; however, it has been unclear whether treatment history plays a role in development of insecticide resistance in this pest. Also, with respect to treatment history, Streu (1973) suggested that excessive pesticide usage may cause stress in turfgrass that contributes to accumulation of thatch, possibly providing insect pests shelter from insecticides.

Resistance Management

Roush (1989) has suggested that if created at the earliest opportunity, a properly-structured resistance management (RM) program can be developed without having made a serious error in recommendations. Even if based on limited information of the insecticides used and the population dynamics of the pest involved, it may be possible to improve the design of the RM program as new information (ie., mechanisms, cross-resistance, mode of inheritance, and stability of resistance) is obtained (Roush 1989). However, the research involved in acquiring this information and the time needed to implement it into a RM program can take several years, and relies on employing the correct genetic model (Hoy 1995). Several models have been developed that evaluate options for RM and try to predict how quickly a pest will develop resistance if certain conditions are met.

Resistance Management Models

There are four RM management modeling approaches: analytical, simulation, optimization, and empirical (Tabashnik 1990). Analytical models (Tabashnik 1990, Hoy 1999) use simple mathematical descriptions and attempt to analyze general trends to define fundamental principles. These models do not provide realistic details and are relatively simple. Analytical models assume that insect population dynamics are simple with discrete generations and no age structure. Also, population growth is usually determined by some form of the logistic equation (Tabashnik 1990). However, few arthropods have discrete generations and may be prone to developing resistance (Hoy 1999). Also, many insects, such as *B. insularis*, are multivoltine and have overlapping generations.

Simulation models are more complex and realistic as they attempt to assess the influence of a large number of factors (e.g., biology, behavior, and ecology of the population) (Tabashnik 1990, Hoy 1999). These models may contain complex population dynamics, including age

structure, overlapping generations, and temporal and spatial variation in pesticide dose. Simulation models can be used to evaluate different options for delaying resistance by including empirical data in the parameters included in the model. Parameters can be varied in a systematic way to determine how important each is. However, the details of the population biology, ecology, and structure may influence the rate of resistance development. These models may become extremely complex or difficult to simulate field conditions.

Optimization models focus on economic analysis and evaluate which management strategy will maximize profit when pest susceptibility to a pesticide is considered a non-renewable natural resource. This approach aims to balance the future cost of reduction in pest susceptibility with the present losses in crop yield due to the effects of the target pest. However, information on the target pest is simplified (biology, ecology, behavior) and is often viewed as a constraint (Tabashnik 1990). As a result, optimization models may not properly predict the longevity of a product and lead to inaccurate predictions of the costs of losing a specific product (Hoy 1999).

Empirical models are based on actual observations among variables and no assumptions are made about causal mechanisms (Tabashnik 1990). These models are derived from data and may only be appropriate for the specific conditions of the observed populations (Tabashnik 1990, Hoy 1999). Empirical models may not be useful for developing a strategy for delaying resistance in an unknown situation if it is assumed that the important variables (mode of inheritance, cross resistance, fitness costs, allele frequency, and selection intensity) can vary between populations (Hoy 1999).

Mitigation models involve the use of mixtures, mosaics, rotations, natural enemies, and/or high-dose strategies (Tabashnik 1990, Hoy 1999). For mixtures to be appropriately applied, resistance to each product should be monogenic. No cross resistance can occur between

products in the mixture and they must have equal persistence. Also, some of the population must remain untreated (refuge). Mitigation models also assume that resistant individuals are rare in the population and that resistance is functionally recessive. While mixtures exist for control of *B. insularis*, it would be difficult to provide untreated refuges for this pest due to the amount of damage their feeding can cause. Also, as with the previous models, the genetic basis of insecticide resistance in *B. insularis* is not known. With a mosaic strategy, susceptible individuals are maintained and able to move into surrounding areas; this model may require negative cross-resistance or fitness costs associated with resistance (Tabashnik 1990, Hoy 1999).

Rotation strategies assume the frequency of individuals resistant to one product will decline after the application of an alternative product, which is true if there is negative cross-resistance, a fitness cost associated with the resistance, and/or immigration of susceptible individuals occurs (Hoy 1999). Natural-enemy strategies may be used if food limitations are sufficient to constrain the ability of natural enemies to develop resistance in the field. The high-dose strategy assumes complete coverage, effective kill of all individuals, and ignores negative effects on secondary pests, natural enemies, or the environment (Hoy 1999).

Hoy (1995) suggested that the development of resistance is likely inevitable and at best we can only delay the onset of resistance in order to preserve existing products. Long-term resistance management must be a broad-based multitactic endeavor, in which resistance management is combined with integrated pest management (IPM) and involves altering pesticide use patterns (Hoy 1995). IPM was first developed by Stern et al. (1959) for control of spotted alfalfa aphid, *Therioaphis maculata* (Buckton) (Homoptera: Aphididae), in alfalfa in California. The authors noted that IPM included a variety of tactics, involving monitoring, assessing economic injury levels, use of selective pesticides, and integrating chemical and biological

control (Stern et al. 1959). While some aspects of IPM exist for *B. insularis* (biological, cultural, and chemical control), research on some of these aspects has been limited. Historically, once *B. insularis* develops resistance to an insecticide (bifenthrin being the most current), that insecticide is replaced by another without an understanding of mechanisms, cross-resistance patterns, mode of inheritance, or stability of resistance. The distribution of bifenthrin resistance in Florida is not known. Several other conventional and newer insecticides are currently available for *B. insularis* control; however, baseline susceptibilities to them are also not known. In addition, it is unclear how effectivene the sprig-dip assay is for systemic insecticides and variability in this bioassay needs to be reduced. A resistance management program needs to be developed for this pest. However, it is important to obtain initial information upon which to build a foundation.

Research Objectives

With the above mentioned rationale in mind, the objectives of this research were to:

(1) sample select *B. insularis* populations in 2006 and 2008 in northern and central Florida to describe their susceptibility to bifenthrin, document new locations of bifenthrin resistance to bifenthrin, and evaluate another pyrethroid, permethrin (Chapter 2),

(2) develop a synchronous rearing method for *B. insularis* that produces insects of known age and generation (Chapter 3) , and

(3) develop an improved bioassay that could be used for detecting insecticide susceptibility differences between male and female *B. insularis*, evaluate and validate both the sprig-dip and the new bioassay under standardized conditions, and determine optimal exposure times and sample sizes to be used for each bioassay for selected insecticides (Chapter 4).

Figure 1-1. Severe damage from *B. insularis* feeding (right) that stops at the neighboring bahiagrass lawn (left) (Photo credit: E. A. Buss).

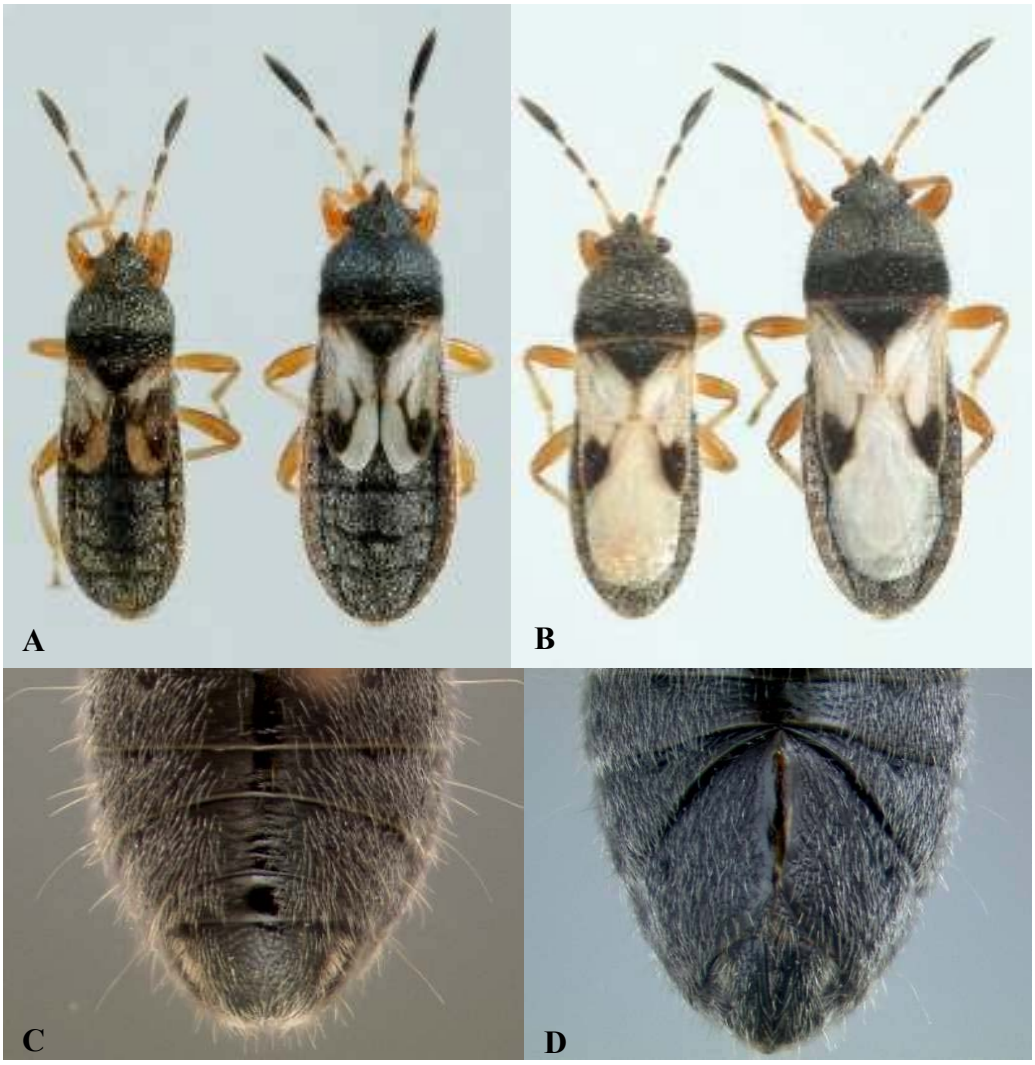

Figure 1-2. Images showing A) brachypterus and B) macropterus male (left) and female (right) *B. insularis*, respectively. The ventral tip of the abdomen of C) male and D) female *B. insularis* (Photo credit: L. Buss).

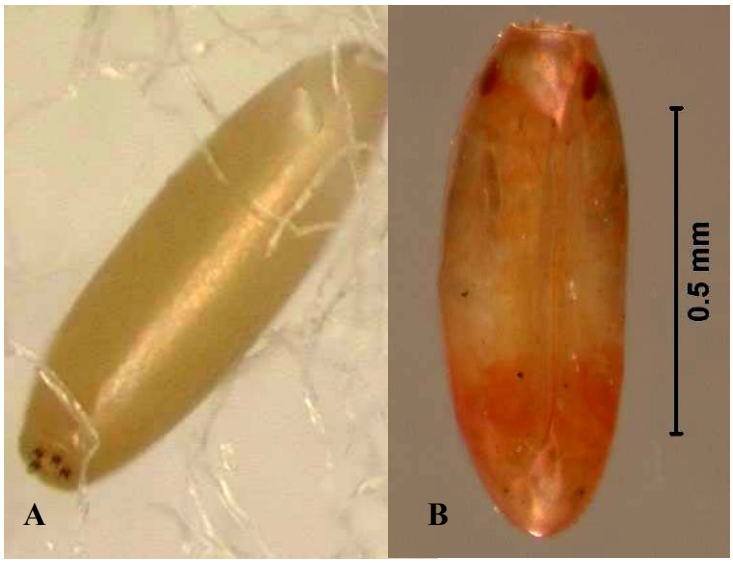

Figure 1-3. Photograph of A) healthy *B. insularis* egg in early development, and B) healthy *B. insularis* egg in late development (Photo credit: L. Buss).

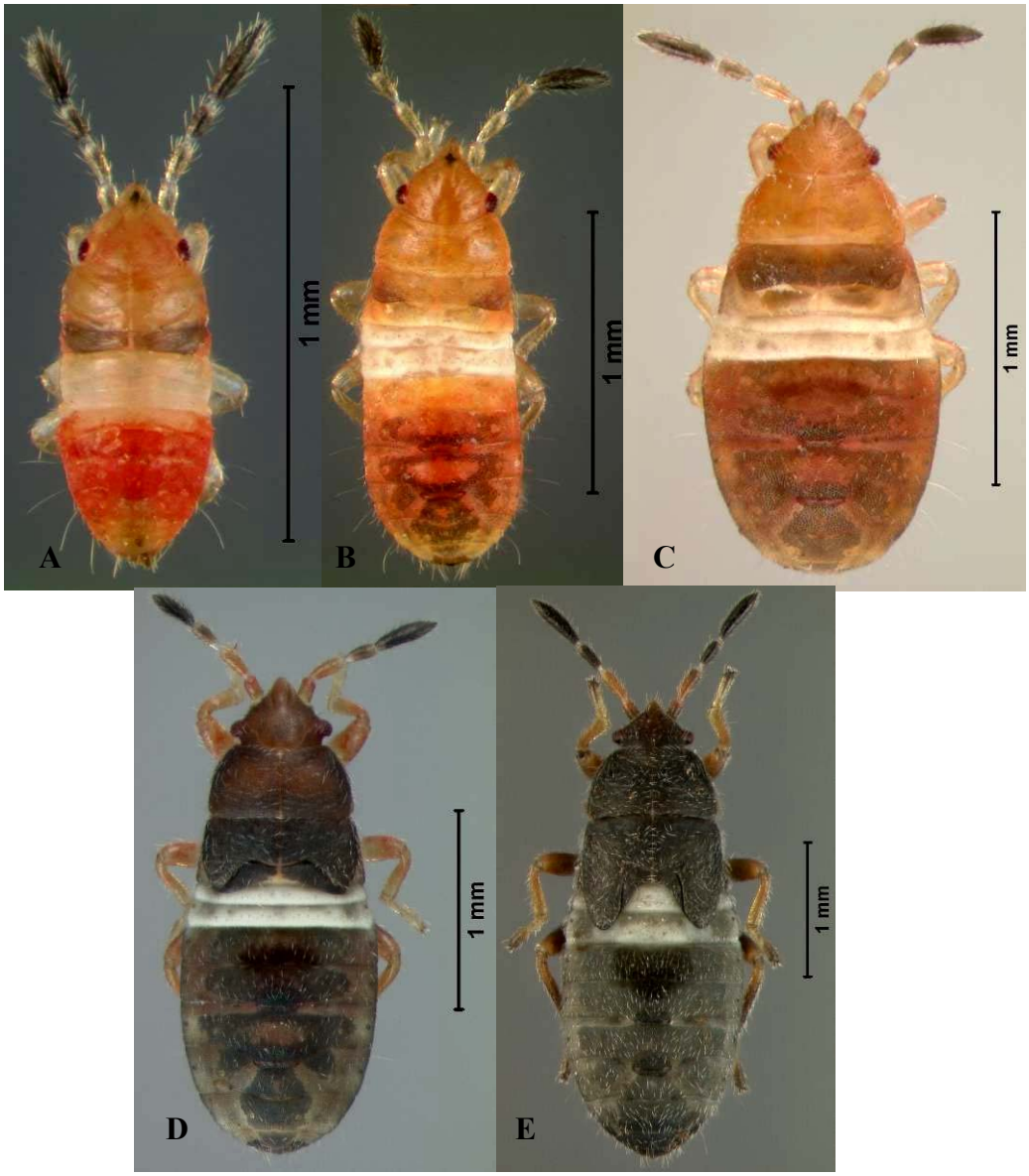

Figure 1-4. *Blissus insularis* A) first, B) second, C) third, D) fourth, and E) fifth instars (Photo credit: L. Buss).

Figure 1-5. Lawns damaged by *B. insularis* [Photo credit: A) Rick Lewis, B) and D) J. C. Vázquez, and C) R. Levin].

Figure 1-6. St. Augustinegrass lawns with *B. insularis* populations encroaching on neighboring lawns (Photo credit: R. Clemenzi).

Figure 1-7. St. Augustinegrass with excessive thatch (Photo credit: R. Clemenzi).

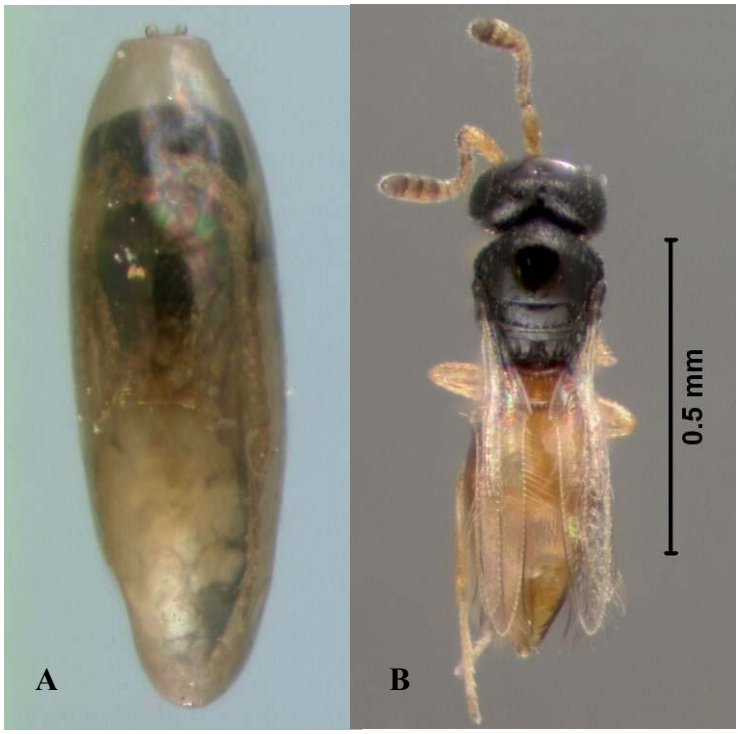

Figure 1-8. Photographs of A) a *B. insularis* egg parasitized by *E. benefica*, and an B) adult *E. benefica* (Photo credit: L. Buss).

CHAPTER 2
SUSCEPTIBILITY OF B. *insularis* POPULATIONS IN FLORIDA TO BIFENTHRIN AND PERMETHRIN

Introduction

St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze) is the most widely used lawn grass in tropical and subtropical climatic regions (Sauer 1972). It is the primary turfgrass in residential lawns and comprises ~70% or 1.2 million ha in Florida (Hodges et al. 1994, Busey 2003). The southern chinch bug, *Blissus insularis* Barber, is considered the most damaging insect pest of this grass (Kerr 1966, Reinert and Kerr 1973, Reinert and Portier 1983, Crocker 1993). Kerr (1966) speculated that *B. insularis* was one of the most economically important plant feeding arthropods in Florida, being second only to the citrus rust mite in amount of money spent for control. By 1983, the combined annual losses and cost in Florida to manage this pest was estimated at $5 million (Hamer 1985). Given that the number of housing units in Florida increased from ~3.9 million in 1980 to 8.5 million in 2006 (an increase of 118%), the potential for damage and increased cost for management is likely higher now. With over 18 million people and an annual growth rate of 1.8% (U.S. Census Bureau 2006), the demand for quality turf and maintenance in Florida continues to increase (Haydu et al. 2005). Florida is second only to California in terms of employment impacts of the turfgrass industry, providing 83,944 jobs in 2002 (Haydu et al. 2006).

Similar to other *Blissus* feeding habits, nymph and adult *B. insularis* damage St. Augustinegrass by feeding in the phloem sieve elements of the grass (Rangasamy et al. 2009) causing wilting, chlorosis, stunting, and eventually death (Painter 1928, Negron and Riley 1990, Spike et al. 1991). As the grass dies, the insects continue to move outward to feed on more-succulent grass, thus enlarging the damaged area.

Although capable of flight, adult *B. insularis* move between lawns mainly by walking and many have been observed crawling across paved areas bordering heavily infested lawns (Kerr 1966). All life stages are distributed vertically through the turf thatch and into the upper organic layer of the soil, with densities of up to 2,000 *B. insularis*/0.1 m^2 being reported (Reinert and Kerr 1973). Light to moderate infestations are aggregated in small areas in the lawn, but *B. insularis* can occur throughout the entire lawn in heavily infested areas (Cherry 2001b).

Blissus insularis can be difficult to control because it has overcome host-plant resistance (Busey and Center 1987, Cherry and Nagata 1997), it produces multiple generations per year, has a high number of offspring per generation, is highly mobile and disperses to neighboring lawns (i.e., encroachment), is able to survive on other grass sources until new St. Augustinegrass is located (Kerr 1966, Reinert and Kerr 1973), and is able to avoid insecticides. Currently, 20-25 *B. insularis* per 0.09 m^2 warrant control (Short et al. 1982). Insecticides are currently the only economical management option for lawn-care companies in Florida, with some making as many as twelve insecticide applications per year to control this pest (Reinert 1978, Reinert and Niemczyk 1982). With near-constant reliance on chemical control, this insect has developed resistance to organochlorines, organophosphates, and carbamates (Wolfenbarger 1953; Kerr and Robinson 1958; Kerr 1958, 1961; Reinert 1982a, 1982b; Reinert and Niemczyk 1982; Reinert and Portier 1983).

In a 2003 University of Florida survey, the pyrethroid bifenthrin was the insecticide used most by lawn and ornamental professionals in Florida (Buss and Hodges 2006). Cherry and Nagata (2005) reported resistance to bifenthrin in 14 *B. insularis* populations in central and south Florida. In 2006, our lab received multiple complaints of field failures with bifenthrin and other pyrethroids as far north as Pensacola, FL. Additionally, pyrethroids are widely available to

homeowners and professionals and their overuse may make pyrethroid resistance more widespread. In an effort to develop a resistance management program, it is important to determine where bifenthrin-resistant populations occur in the state and the severity of the problem. Thus, I tested 16 *B. insularis* populations in 2006 and 6 populations in 2008 in northern and central Florida to describe their susceptibility to bifenthrin, document new locations of resistance to bifenthrin, and evaluate another pyrethroid, permethrin.

Materials and Methods

St. Augustinegrass Maintenance

Commercially-obtained plugs of 'Palmetto' St. Augustinegrass were planted in 15.2-cm plastic pots filled with Farfard #2 potting soil (Conrad Farfard Inc., Agawam, MA). Plants were maintained in a University of Florida greenhouse in Gainesville, FL and held under a 14L:10D photoperiod with day and night temperatures of 27 and 24°C, respectively. Plants were fertilized weekly with a 20-20-20 water-soluble complete nitrogen source (NH_4NO_3) at 0.11 kg N/0.09 m^2, watered as needed, and cut to a height of ~7.6 cm.

2006 Collection Sites

Blissus insularis populations were collected between May and August 2006. Two populations were collected from areas where insecticides had not been used, three were randomly collected (treatment history unknown), and 11 were from lawns where control failures with bifenthrin had been reported (Table 2-1). The number of times lawns were treated prior to collection and the active ingredients used during 2006 were documented for each site, where possible, and GPS coordinates were recorded. Several populations were collected from the same neighborhood or street, but were considered distinct because their treatment history varied. Populations were named based on location within a neighborhood.

2008 Collection Sites

Blissus insularis populations were collected in July 2008. Six populations were from lawns where control failures with bifenthrin had been reported (Table 2-2). The active ingredients used during 2008 were documented for each site, however, I was unable to obtain the number of times lawns were treated. GPS coordinates were recorded. Populations were named based on location within a neighborhood.

Insects

Insects were collected using a modified Weed Eater Barracuda blower/vacuum (Electrolux Home Products, Augusta, GA) (Crocker 1993, Nagata and Cherry 1999, Congdon 2004), transported to the laboratory, sifted from debris, and fifth instars and adults were placed into colony as outlined in Chapter3.

2006 Tests

Bifenthrin

Tests were conducted using a sprig-dip bioassay similar to that of Reinert and Portier (1983) and Cherry and Nagata (2005). Bioassays were run for 72 h because mortality results after 24 and 48 h for some of the populations did not fit a probit or logit model. This could have been due to a delay in response or because some insects initially avoided the plant material. Serial dilutions were made with formulated bifenthrin (TalstarOne®, FMC Corporation, Philadelphia, PA) and prepared fresh on each test date. Eight concentrations were tested and mortality ranged from 5 to 95% with the exception of three outliers, populations DAR, HF and GE18 (Table 2-1). Fresh 'Palmetto' St. Augustinegrass stolon sections (5.0 – 6.4 cm long, with three leaflets and one node) were dipped in one solution and air dried on wax paper (~2 h). Ten unsexed adult *B. insularis* of unknown age were placed into plastic petri dishes (100 × 15 mm) containing one treated stolon and one 70-mm Whatman filter paper moistened with 0.5-ml of

distilled water to prevent desiccation. All tests were conducted between 1330 – 1500 h at room temperature (25 ± 2°C) and a 14L:10D photoperiod. The number of dead *B. insularis* was assessed at 24, 48, and 72 h using a dissecting microscope. Insects were scored as dead if they were on their backs or unable to walk.

Permethrin

One *B. insularis* population (JC) had control failures with both TalstarOne® and Permethrin-G Pro (permethrin, Gro-Pro™ LLC, Inverness, FL), so both products were tested. Permethrin-G Pro solutions and testing were conducted as described with TalstarOne®. Population HF was used as the susceptible standard.

2008 Bifenthrin Test

Tests were conducted using an airbrush bioassay as described in Chapter 4. A bifenthrin-susceptible laboratory population, LO (Chapter 4), was used as a standard in this test. Serial dilutions were made with formulated bifenthrin (TalstarOne®, FMC Corporation, Philadelphia, PA) and prepared fresh on each test date. Eight or nine concentrations were tested for each population and mortality ranged from 5-95%. Tests were held for 24 h and insects were scored as previously described.

Statistical Analysis

The LC_{50} and LC_{90} values, 95% confidence limits (CL), slopes of the regression lines, and the likelihood ratio test to test the hypothesis of parallelism and equality of the regression lines were estimated by logit analysis using Polo Plus (LeOra Software 2002). Differences in susceptibility between populations were tested by the 95% confidence limits (CL) of lethal concentration ratios (LCRs) at the LC_{50} and LC_{90} (Robertson and Priesler 1992, Robertson et al. 2007). Populations were individually compared to the most susceptible population (GE18) and LCR confidence intervals (95%) that did not include 1.0 were considered significant ($P < 0.05$)

(Robertson and Priesler 1992, Robertson et al. 2007). Conventionally, if the 95% confidence limits of the lethal concentrations overlapped, then the lethal concentrations were not considered significantly different. However, the ratio test has greater statistical power and lower Type I error rates, so this statistical test was used in this study (Wheeler et al. 2006, Robertson et al. 2007). The relationship between the number of insecticide applications made in 2006 and respective LCRs (at LC_{50}) was analyzed using regression analysis (Systat Software 2006).

Results and Discussion

2006 Tests

Bifenthrin

LC_{50} values for bifenthrin from the 16 *B. insularis* populations (Table 2-3) were highest in populations that received two or more insecticide applications. Populations P, BH, and JC received the most insecticide applications (8 – 11) and had the highest LC_{50} values for bifenthrin (3,835, 3,748 and 2,737 µg/ml, respectively). Populations that received two to five insecticide applications (V, GE12, LF4, FS, BP, and CT) had LC_{50} values for bifenthrin ranging from 93 – 1,127 µg/ml. Populations with one or no applications (DAL, DAR, HF, and GE18) had the lowest LC_{50} values, ranging from 0.9 – 42 µg/ml. LCR_{50} values for all populations (with the exception of DAR and HF) were significantly different from the most susceptible population, GE18, and increased with increasing insecticide applications (Figure 2-1).

LCR_{90} values for all populations treated with bifenthrin (with the exception of DAL and DAR) were significantly different from the most susceptible population, GE18 (Figure 2-2). The highest LCR_{90} values for bifenthrin were recorded from populations BH, JC, GE12, LF4, L, FS, and BP. LCR_{90} values for these populations indicated they were 1,077 – 13,000 µg/ml more resistant to bifenthrin than the most susceptible population, GE18 (Figure 2-2). LC_{90} values for these same populations ranged from 53,120 to 642,527 µg/ml. The lowest LC_{90} values were

from *B. insularis* populations GE18, DAR, and DAL (Table 2-3). Of the 11 populations collected, nine were actual control failures (highest label rate of TalstarOne® = 209 µg/ml). Populations DAL and DAR demonstrated LC_{90} values that were below the recommended label rate, but control failure in these two sites may have been due to application error. Alternately, it is possible that different resistance mechanisms are present in the DAL and DAR populations and the bioassay was unable to detect them.

These data describe new locations of bifenthrin-resistant *B. insularis* populations, as well as in counties similarly reported by Cherry and Nagata (2005) (Figure 2-3). In 2003, Cherry and Nagata (2005) reported 8 cases of *B. insularis* resistance to bifenthrin in Flagler, Hernando, Lake, Manatee, Monroe, Sarasota, and Volusia counties, showing a 4.6 – 736 – fold reduced susceptibility to bifenthrin. The data I collected in 2006 show a 45– to 4,099 – fold reduced susceptibility to bifenthrin in Citrus, Escambia, Flagler, Hillsborough, Orange, Osceola, and Volusia counties. These data are the first to report bifenthrin resistance in Citrus, Hillsborough, Orange, and Osceola counties. In addition, population P from Escambia County is the first known in the Florida Panhandle to be resistant to insecticides of any kind in *B. insularis* (Figure 2-3).

The results of the hypothesis tests of parallelism and equality show that the regression lines of 13 of the *B. insularis* populations collected in central and northern Florida in 2006 were parallel but not equal to the most susceptible population, GE18 (Table 2-4). Even though their intercepts differ significantly, their slopes are not significantly different. This could mean that the field-collected populations are heterogeneous and represent a range of susceptible and resistant individuals (as can be seen in population SCL with an LC_{50} of 47 and an LC_{90} of 4,039 µg/ml). Alternately, the hypothesis test results may indicate that the different *B. insularis*

populations have qualitatively identical but quantitatively different levels of detoxification enzymes (Robertson et al. 2007). Population DAL, with a steep slope of 4.3, had significantly different intercepts and slopes from the GE18 population. This may indicate that DAL was more uniform in its response to bifenthrin, their detoxification enzymes differ qualitatively, or that this population has entirely different detoxification enzymes (Robertson et al. 2007). Intercepts and slopes for populations DAR and GE18 were similar, demonstrating a similar response to bifenthrin.

It is interesting to note that the data obtained from the 2006 bifenthrin test indicate that individual lawns may represent a single *B. insularis* population. In Palm Coast, sites GE12 and GE18 were located a few houses from each other, on the same side of the street, and were maintained by the same company at the time of this study. GE12 had received four insecticide applications between January and July 2006 and the *B. insularis* collected from this lawn demonstrated an LC_{50} of 1,048 µg/ml and an LC_{90} of 186,000 µg/ml for bifenthrin. Meanwhile, lawn GE18 showed the presence of *B. insularis* damage for the first time in 2006 and thus had not been treated at the time of collection. The *B. insularis* collected from this lawn demonstrated an LC_{50} of 0.9 µg/ml and an LC_{90} of 49 µg/ml for bifenthrin. Also, population V was located in the same neighborhood, just one street away from GE12 and GE18. Although, the V population was under the same insecticide schedule as GE12, the *B. insularis* collected from this lawn demonstrated an LC_{50} of 1,127 µg/ml and an LC_{90} of 28,641 µg/ml for bifenthrin. Populations FS and L, also located in Palm Coast but in a different neighborhood, were located directly across the street from each other, and were not maintained by the same lawn care company. The FS population received three insecticide applications between January and July 2006 and the *B. insularis* collected from this lawn demonstrated an LC_{50} of 652 µg/ml and an LC_{90} of 53,120

µg/ml for bifenthrin, while the L population, with unknown treatment history had an LC_{50} of 521 µg/ml and an LC_{90} of 62,612 µg/ml for bifenthrin. Although, it is possible the *B. insularis* sampled from these lawns did not fully represent the population as a whole, treatment effects on individual lawns, effects of encroachment, and population dynamics of *B. insularis* within neighborhoods warrants further study.

Based on the known treatment history for the populations where control failures with bifenthrin were reported in 2006, the number of applications made with bifenthrin, carbaryl, clothianidin, cypermethrin, imidacloprid, permethrin, and/or trichlorfon was positively correlated to their respective bifenthrin lethal concentration ratio (at LC_{50}) values (Figure 2-4). While there are several documented cases showing a positive relationship between insecticide application frequency and selection for resistance (Georghiou 1986, Rosenheim and Hoy 1986, Croft et al. 1989, He et al. 2007, Magana et al. 2007), these studies were based on knowledge of treatment history over a period of several years. Because I was only able to obtain the treatment history for 2006, it is uncertain whether application frequency caused, or merely resulted from the development of resistance to bifenthrin in *B. insularis* in this study. However, it is well documented in other organisms that resistance to pyrethroids often evolves quickly on the foundation of DDT resistance (Chadwick et al. 1977, Prasittisuk and Busvine 1977, McDonald and Wood 1979, Omer et al. 1980, Priester and Georghiou 1980, Malcolm 1983, Miller et al. 1983, Georghiou 1986, Cochran 1995). Cases of DDT resistance in *B. insularis* were documented in Sarasota (Kerr and Robinson 1958) and Miami (Kerr 1958), but, it is unclear how widespread the problem was and if cross resistance to pyrethroids is currently occurring as a result. Due to the number of different insecticides used in 2006 to treat the populations I

collected, cross resistance and/or multiple resistance may have occurred, but I did not have enough insects to test this.

Permethrin

Population JC was 212.4-fold more tolerant of permethrin than the susceptible population, HF (Table 2-5). The hypothesis test for equality was rejected ($\chi^2 = 141$; df = 2; $P < 0.05$) and the hypothesis test for parallelism was not rejected ($\chi^2 = 0.53$; df = 1; $P = 0.47$) showing that intercepts differed significantly, while slopes did not. Population JC from Orange County represents the first report of permethrin resistance for the state. By 2007, Cherry and Nagata (2007) documented resistance to the pyrethroids deltamethrin and lambda-cyhalothrin, clearly indicating the occurrence of cross resistance in Florida. In addition, Cherry and Nagata (2007) documented the first case of resistance to a neonicotinoid, imidacloprid as well as finding six additional locations of bifenthrin resistance.

2008 Tests

LC_{50} values for bifenthrin from the 6 *B. insularis* populations collected in central Florida in 2008 ranged from 99 - 366 µg/ml compared to the LC_{50} of 3.0 µg/ml from the susceptible laboratory population, LO (Table 2-6). All 6 field-collected populations were actual control failures (highest label rate of TalstarOne® = 209 µg/ml), with LC_{90} values ranging from 293 – 1,439 µg/ml (Table 2-6). Slopes of the regression lines from the populations tested were steep, indicating a uniform response to bifenthrin, with the exception of population PA (Georghiou and Metcalf 1961; ffrench-Constant and Roush 1990; Prabhaker et al. 1996, 2006).

The regression lines of the 6 populations had significantly different intercepts from that of the most susceptible population LO (Table 2-7). The hypothesis test for parallelism was not rejected for populations JP, JH, and TG ($\chi^2 = 0.5$; df = 1; $P = 0.46$, $\chi^2 = 1.5$; df = 1; $P = 0.21$, and $\chi^2 = 0.2$; df = 1; $P = 0.66$, respectively). For these populations, the slopes were similar to that of

population LO. Populations LU, PA, and OR had significantly different intercepts and slopes from the LO population (Table 2-7). LCR_{50} values for all populations, ranging from 33 – 121 µg/ml, were significantly different from the most susceptible population, LO (Figure 2-5). LCR_{90} values for these populations indicated they were 19 – 98 µg/ml more resistant to bifenthrin than population, LO (Figure 2-6).

The results of this chapter show that bifenthrin resistance continues to spread and is particularly problematic in central Florida (Figure 2-3). Although, it is possible that pyrethroid resistance may be more widespread. In addition, these data along with reports by Cherry and Nagata (2007) show that cross resistance to other pyrethroids is occurring. Currently, pyrethroids, carbamates, neonicotinoids, and organophosphates are used for *B. insularis* control in Florida. Carbamate (propoxur) and organophosphate (chlorpyrifos) resistance was reported in the 1970s and 80s (Reinert and Niemczyk 1982, Reinert and Portier 1983). Cross-resistance patterns and the stability of propoxur and chlorpyrifos resistance in *B. insularis* are not known, making it unclear as to their effects on the current use of the carbamate, carbaryl, and the organophosphate, trichlorfon.

In addition, the impact of insecticide use on St. Augustinegrass grown in sod farms remains unknown. In a national study, Florida was ranked first in terms of economic impact of sod production (Haydu et al. 2006). In 2003, the total sod production in Florida was estimated to be 93,000 ha, with 64% being St. Augustinegrass (Haydu et al. 2005). Only 3% of harvested sod is sold outside of Florida. A summary of agricultural pesticide use in Florida in 1995-1998 and 1999-2002 noted that chlorpyrifos was the sole insecticide used in sod farms (Shahane 1999, 2003). It is likely that St. Augustinegrass sod has already received several insecticide applications before it is even planted in residential neighborhoods. I have observed *B. insularis*

already present in St. Augustinegrass sod before it had been planted in a residential lawn. A better understanding of insecticide use on sod farms would be greatly beneficial in understanding their role (if any) in selection for resistance to pesticides in *B. insularis* populations in Florida.

It is clear that further information is needed in order to solve the resistance problem in Florida. Once this is done, a resistance management strategy can be made. An effective resistance management strategy should be multi-tactic (Hoy 1999) and include not only traditional integrated pest management (IPM) strategies (monitoring pests, use of cultural controls, preservation of natural enemies, and host plant resistance) but also include the possible use of synergists, effective educational programs, and monitoring of progress to ensure tactics are properly put into place. This would not only require cooperation by professionals in academia and pest management, but should include sod growers, homeowner associations, and homeowners as well. In addition, solving the resistance problem in Florida will require much more research and cooperation and/or coordination with insecticide manufacturers. It is important to delay the development of resistance to chemical classes available for *B. insularis* control. Although doing so will be challenging because all registered products (carbamates, neonicotinoids, organophosphates, and pyrethroids) are currently used multiple times per year in attempts to control and prevent damage from *B. insularis* in Florida lawns.

Table 2-1. Collection sites and the number of insecticide applications made to the *B. insularis* populations in Florida in 2006 that were tested for susceptibility to bifenthrin.

Population	County	City	GPS coordinates	Month collected	No. insecticide applications in 2006	Active ingredients used[c]
P	Escambia	Pensacola	N30°28.70676, W87°11.7228	August	11	Bifenthrin Trichlorfon
BH	Citrus	Beverly Hills	N28°52.9644, W82°24.9684	August	11	Bifenthrin Carbaryl Imidacloprid Trichlorfon
JC	Orange	Windermere	N28°29.33244, W81°34.15464	June	8	Bifenthrin Permethrin Carbaryl Trichlorfon Acephate
V[a]	Flagler	Palm Coast	N29°34.81518, W81°10.87286	July	4	Bifenthrin Cypermethrin
GE12[a]	Flagler	Palm Coast	N29°34.78872, W81°10.93536	July	4	Bifenthrin Cypermethrin
LF4	Flagler	Palm Coast	N29°33.69246, W81°11.93052	July	3	Bifenthrin Cypermethrin
FS[b]	Flagler	Palm Coast	N29°32.994833, W81°10.11883	July	3	Bifenthrin Cypermethrin
L[b]	Flagler	Palm Coast	N29°32.98482, W81°10.161	July	unknown	
BP	Hillsborough	Sun City	N27°42.516, W82°21.618	May	2	Bifenthrin
CT	Hillsborough	Sun City	N27°44.416167, W82°20.86733	June	5	Bifenthrin Carbaryl Imidacloprid
PC	Flagler	Palm Coast	N29°32.2641, W81°9.55944	May	unknown	

Table 2-1. Continued

SCL	Osceola	St. Cloud	N28°15.20868, W81°19.0191	July	unknown	
DAL	Volusia	Port Orange	N29°6.101333, W81°8.952833	July	1	Bifenthrin[d] Imidacloprid[d]
DAR	Volusia	Port Orange	N29°6.3879, W81°3.33222	July	1	Clothianidin[e]
HF	Alachua	Gainesville	N29°35.83908, W82°26.0241	June–August	0	-------
GE18[a]	Flagler	Palm Coast	N29°34.78644, W81°10.93704	July	0	-------

[a] Denotes populations in the same neighborhood.

[b] Denotes populations located across the street from each other.

[c] Products are listed in descending order of application frequency.

[d] A single application of Allectus® SC was used at this site, which contains both bifenthrin and imidacloprid.

[e] Control failure with bifenthrin was reported in 2005 but, at the time of collection, only clothianidin had been used in 2006.

Table 2-2. Collection sites and the number of insecticide applications made to the *B. insularis* populations in Florida in 2008 that were tested for susceptibility to bifenthrin.

Population	County	City	GPS coordinates	Month collected	No. insecticide applications in 2008[*]	Active ingredients used[c]
LU	Lake	Clermont	N28°36.8664, W81°4.9164	July	N/A	Bifenthrin Trichlorfon
JP	Orange	Winter Garden	N28°32.6611, W81°38.9364	July	N/A	Bifenthrin Carbaryl Imidacloprid
JH	Orange	Winter Garden	N28°32.65, W81°38.5522	July	N/A	Bifenthrin Carbaryl Imidacloprid Fipronil
PA	Orange	Windermere	N28°30.0283, W81°33.7480	July	N/A	Bifenthrin Carbaryl Imidacloprid
TG	Orange	Windermere	N28°29.2447, W81°34.6830	July	N/A	Bifenthrin Carbaryl Imidacloprid
OR	Orange	Orlando	N28°27.07361, W81°30.31778	July	N/A	Bifenthrin Carbaryl Imidacloprid

[*] I was unable to obtain the number of insecticide applications that were made to these sites.

Table 2-3. Response of Florida *B. insularis* populations collected in 2006 to bifenthrin after 72 h using a sprig-dip bioassay at 25.5°C, 14L:10D photoperiod.

Population	*n*	Slope ± SE[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	χ^2 (df)[c]
P	240	2.0 ± 0.3	3,835 (1,619–8,923)	44,798 (17,078–273,547)	5.1(5)[d]
BH	80	1.0 ± 0.2	3,748 (678–18,707)	642,527 (89,477–47,530,686)	4.0(5)[d]
JC	240	1.1 ± 0.1	2,737 (1,058–6,557)	260,786 (81,799–1,538,682)	3.2(5)[d]
V	240	1.6 ± 0.2	1,127 (490–2,358)	28,641 (11,496–120,575)	2.0(5)[d]
GE12	240	1.0 ± 0.1	1,048 (48–10,027)	186,000 (16,864–285,753,603)	14.1(5)
LF4	240	1.1 ± 0.2	817 (18–10,187)	71,506 (6,573–785,990,382)	18.2(5)
FS	240	1.1 ± 0.2	652 (32–3,649)	53,120 (8,599–4,135,424)	8.0(5)[d]
L	240	1.1 ± 0.2	521 (41–2,347)	62,612 (12,217–1,833,448)	5.9(5)[d]
BP	240	0.9 ± 0.1	459 (31–3,122)	143,891 (14,638–67,945,632)	11.0(5)
CT	240	1.8 ± 0.3	93 (10–501)	1,447 (310–801,491)	14.0(5)
PC	240	2.0 ± 0.4	62 (24–128)	785 (345–3,435)	0.6(5)[d]
SCL	240	1.1 ± 0.2	47 (1–259)	4,039 (661–537,205)	8.2(5)[d]
DALL	240	4.3 ± 1.1	42 (18–69)	137 (84–349)	0.1(5)[d]
DAR	240	2.7 ± 0.7	10 (3–17)	62 (32–365)	0.2(5)[d]
HF	1,200	1.2 ± 0.1	8 (2–18)	652 (349–1,436)	4.0(4)[d]
GE18	240	1.3 ± 0.4	0.9 (0–5)	49 (11–467)	0.5(5)[d]

[a] Slope of the logit mortality line.

[b] LC_{50}, LC_{90}, and 95% confidence limits (CL) are expressed in µg/ml.

[c] Pearson chi-square statistic (degrees of freedom).

[d] Good fit of the data to the logit model ($P > 0.05$).

Table 2-4. Hypothesis tests comparing the slopes and intercepts of logit regression lines for 15 *B. insularis* populations in comparison to the most susceptible population, GE18, after exposure to bifenthrin for 72 h using a sprig-dip bioassay at 25.5°C, 14L:10D photoperiod.

Population	Hypothesis test for equality	Hypothesis test for parallelism
P	reject; $\chi^2 = 168$; df = 2; $P < 0.05$	accept; $\chi^2 = 1.7$; df = 1; $P = 0.19$
BH	reject; $\chi^2 = 74$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.4$; df = 1; $P = 0.56$
JC	reject; $\chi^2 = 126$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.1$; df = 1; $P = 0.71$
V	reject; $\chi^2 = 110$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.3$; df = 1; $P = 0.58$
GE12	reject; $\chi^2 = 97$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.47$; df = 1; $P = 0.49$
LF4	reject; $\chi^2 = 92$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.10$; df = 1; $P = 0.75$
FS	reject; $\chi^2 = 80$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.07$; df = 1; $P = 0.79$
L	reject; $\chi^2 = 78$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.23$; df = 1; $P = 0.63$
BP	reject; $\chi^2 = 78$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.88$; df = 1; $P = 0.35$
CT	reject; $\chi^2 = 44$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.97$; df = 1; $P = 0.32$
PC	reject; $\chi^2 = 33$; df = 2; $P < 0.05$	accept; $\chi^2 = 1.4$; df = 1; $P = 0.24$
SCL	reject; $\chi^2 = 29$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.08$; df = 1; $P = 0.78$
DALL	reject; $\chi^2 = 35$; df = 2; $P < 0.05$	reject; $\chi^2 = 10$; df = 1; $P = 0.001$
DAR	accept; $\chi^2 = 6$; df = 2; $P = 0.06$	accept; $\chi^2 = 3$; df = 1; $P = 0.08$
HF	reject; $\chi^2 = 14$; df = 2; $P = 0.001$	accept; $\chi^2 = 0.04$; df = 1; $P = 0.85$

Table 2-5. Response to permethrin after 72 h of two *B. insularis* populations collected in 2006 using a sprig-dip bioassay at 25.5°C, 14L:10D photoperiod.

Population	*n*	Slope ± SE[a]	LC_{50} (95% CL)[b]	LCR_{50} (95% CL)[c]	LC_{90} (95% CL)[b]	LCR_{90} (95% CL)[c]	χ^2 (df)[d]
JC	240	3.5 ± 0.7	341 (130 – 750)	212 (104 – 434)*	1,431 (668 – 9,885)	157 (53.7 – 457)*	6.0 (5)[f]
HF[e]	240	2.9 ± 0.5	1.6 (1.0 – 2.7)	1	9.1 (4.9 – 28)	1	4.4 (5)[f]

[a] Slope of the logit mortality line.

[b] LC_{50}, LC_{90}, and 95% confidence limits (CL) are expressed in mg/ml.

[c] Lethal concentration ratios with 95% confidence limits indicating the fold-difference for each population in comparison to the most susceptible population at LC_{50} and LC_{90}. Confidence limits that include 1.0 indicate no significant difference from the susceptible (HF) population. * Shows ratios that are significant ($P \leq 0.05$, Robertson and Preisler 1992; Robertson et al. 2007).

[d] Pearson chi-square statistic (degrees of freedom).

[e] Susceptible population.

[f] Good fit of the data to the logit model ($P > 0.05$).

Table 2-6. Response of Florida *B. insularis* populations collected in 2008 to bifenthrin after 24 h using an airbrush bioassay at 25.5°C, 14L:10D photoperiod.

Population	*n*	Slope ± SE[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	χ^2 (df)[c]
LU	270	5.0 ± 0.8	366 (291–463)	1014 (736–1770)	3.2(6)[d]
JP	54	4.1 ± 1.3	333 (172–1192)	1140 488–22,203)	3.6(5)[d]
JH	288	4.0 ± 0.5	129 (87–202)	457 (270–1417)	10.4(6)[d]
PA	288	2.1 ± 0.3	124 (54–578)	1439 (382–279,909)	21.2(6)[d]
TG	288	3.5 ± 0.6	116 (68–186)	499 (277–2,370)	9.6(6)
OR	288	4.7 ± 0.6	99 (49–208)	293 (156–3,084)	25.1(6)
LO	256	3.2± 0.4	3.0 (1–5)	15 (8–83)	12.3(5)[d]

[a] Slope of the logit mortality line.
[b] LC_{50}, $LC_{90,}$ and 95% confidence limits (CL) are expressed in µg/ml.
[c] Pearson chi-square statistic (degrees of freedom).
[d] Good fit of the data to the logit model ($P > 0.05$).

Table 2-7. Hypothesis tests comparing the slopes and intercepts of logit regression lines for 6 *B. insularis* populations in comparison to a susceptible laboratory colony, LO, after exposure to bifenthrin for 72 h using an airbrush bioassay at 25.5°C, 14L:10D photoperiod.

Population	Hypothesis test for equality	Hypothesis test for parallelism
LU	reject; $\chi^2 = 290$; df = 2; $P < 0.05$	reject; $\chi^2 = 4.5$; df = 1; $P = 0.03$
JP	reject; $\chi^2 = 142$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.5$; df = 1; $P = 0.46$
JH	reject; $\chi^2 = 260$; df = 2; $P < 0.05$	accept; $\chi^2 = 1.54$; df = 1; $P = 0.21$
PA	reject; $\chi^2 = 190$; df = 2; $P < 0.05$	reject; $\chi^2 = 5.25$; df = 1; $P = 0.02$
TG	reject; $\chi^2 = 228$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.20$; df = 1; $P = 0.66$
OR	reject; $\chi^2 = 257$; df = 2; $P = 0.001$	reject; $\chi^2 = 5.0$; df = 1; $P = 0.03$

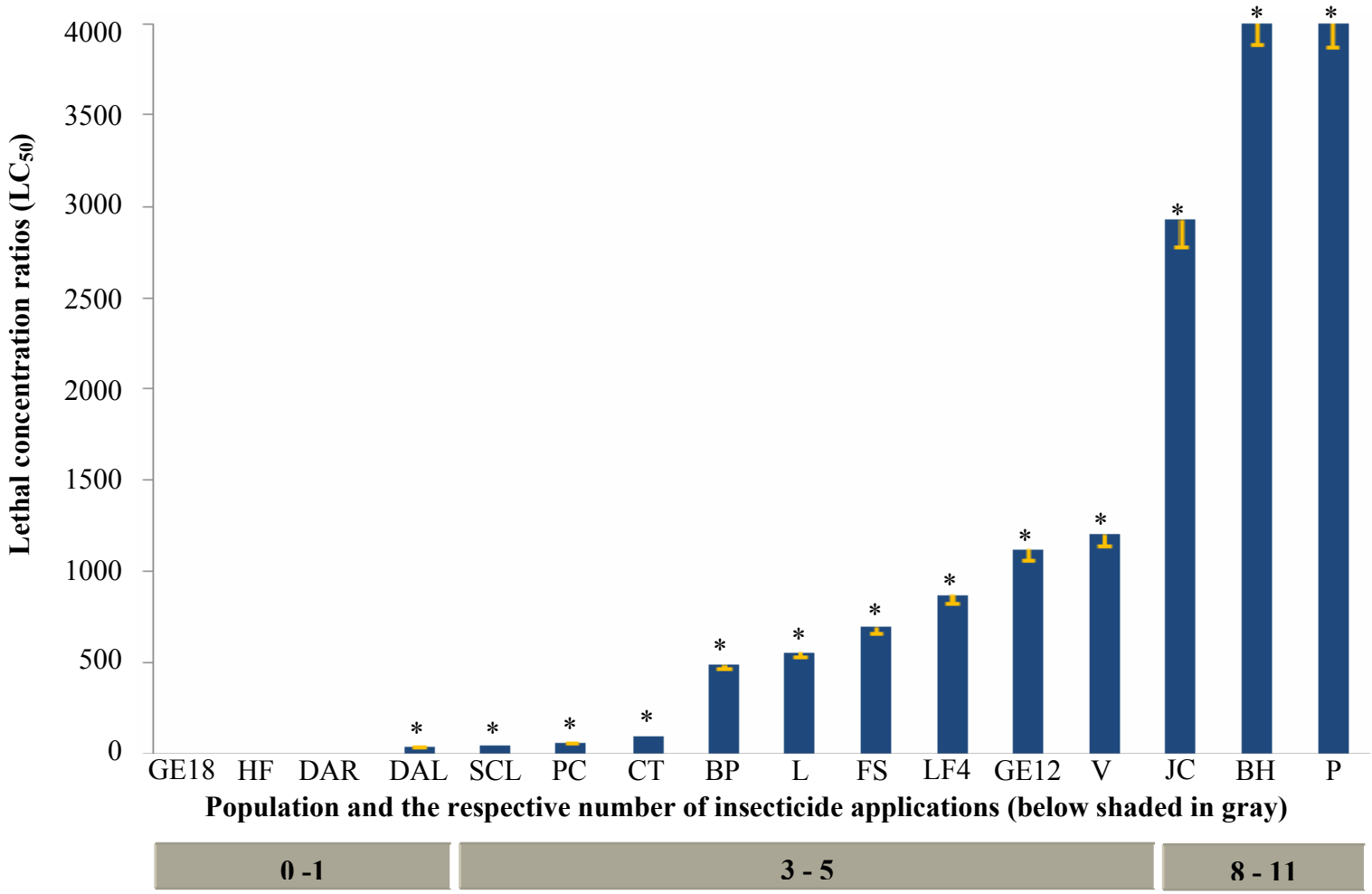

Figure 2-1. LC_{50} Lethal concentration ratios with lower 95% confidence limits indicating the fold difference for each population of *B. insularis* in comparison to the most susceptible population (GE18) when tested with bifenthrin. Lower confidence limits that do not include 1.0 are significantly different from the GE18 population. * Shows ratios that are significant ($P \leq 0.05$, Robertson and Preisler 1992, Robertson et al. 2007).

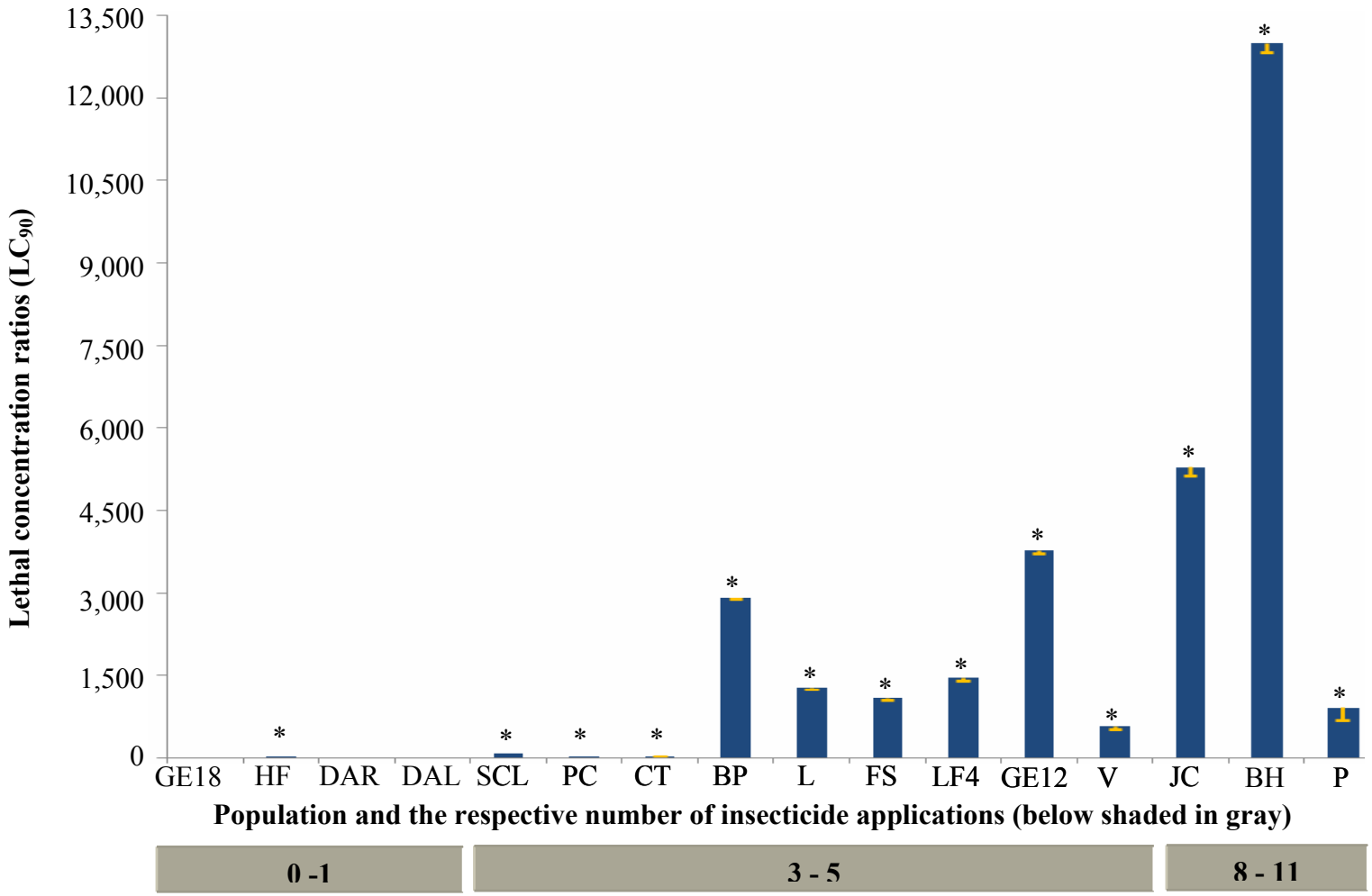

Figure 2-2. LC_{90} Lethal concentration ratios with lower 95% confidence limits indicating the fold difference for each population of *B. insularis* in comparison to the most susceptible population (GE18) when tested with bifenthrin. Lower confidence limits that do not include 1.0 are significantly different from the GE18 population. * Shows ratios that are significant ($P \leq 0.05$, Robertson and Preisler 1992, Robertson et al. 2007).

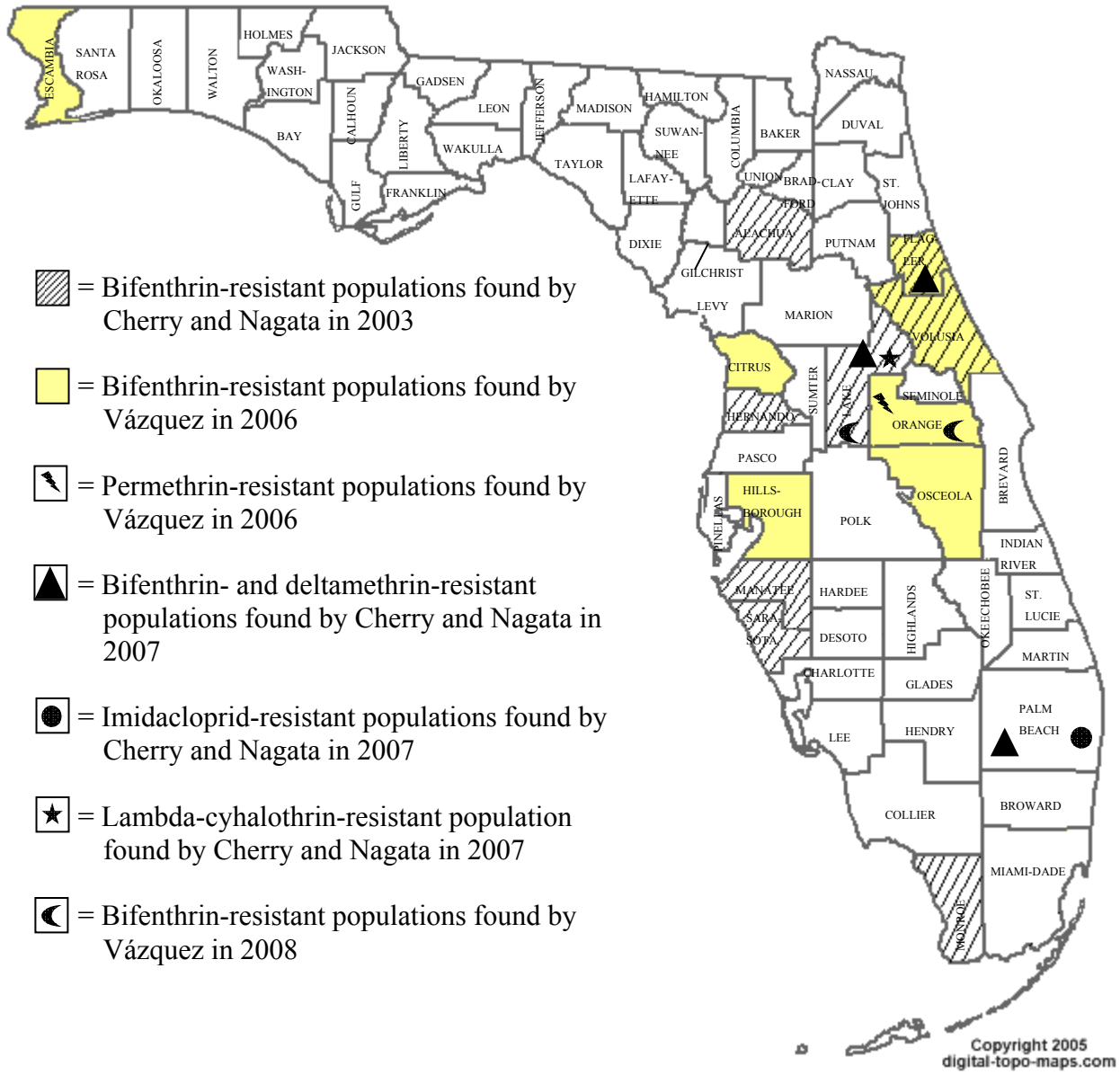

Figure 2-3. Map showing the distribution of insecticide-resistant *B. insularis* populations in Florida between 2003-2008. The legend identifies counties where populations have been found and identifying authors.

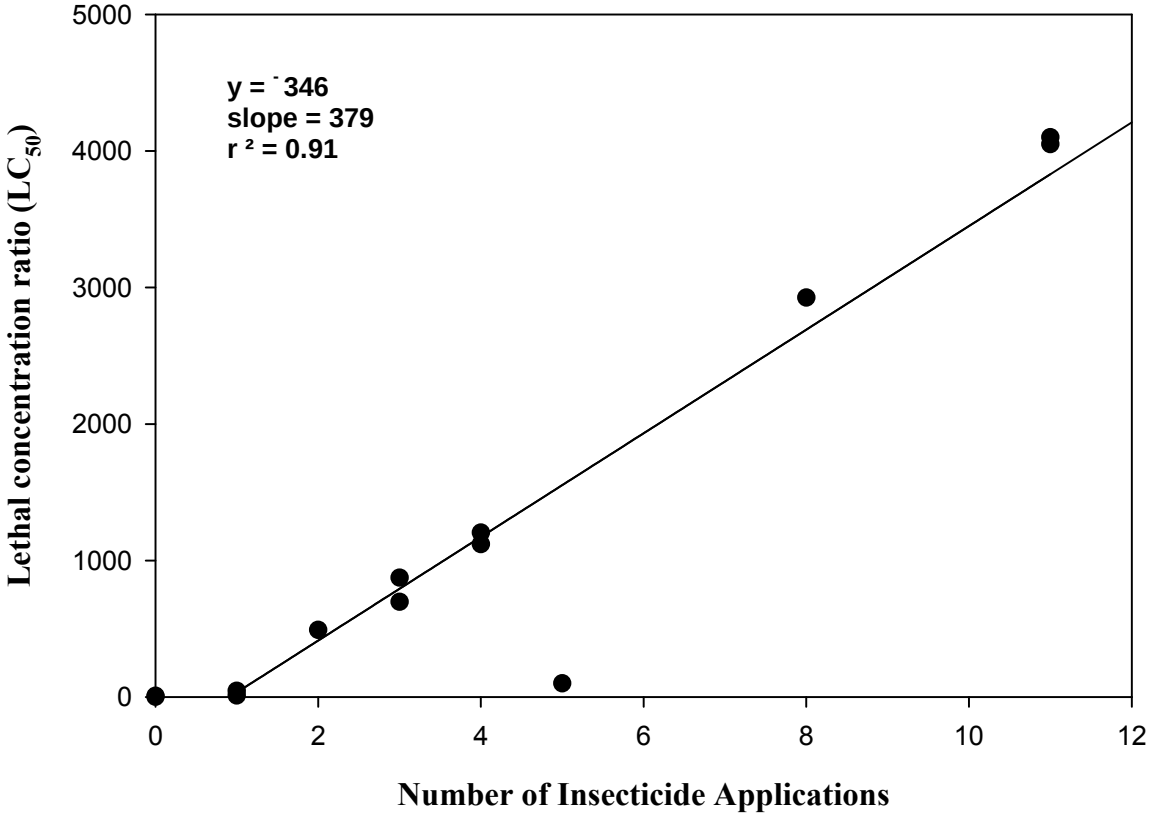

Figure 2-4. Bifenthrin resistance in *B. insularis* populations from central and northern Florida in 2006: relationship between the number of insecticide applications made (regardless of active ingredient used) and respective lethal concentration ratios (at LC_{50}) See Table 2-1 for locations sampled.

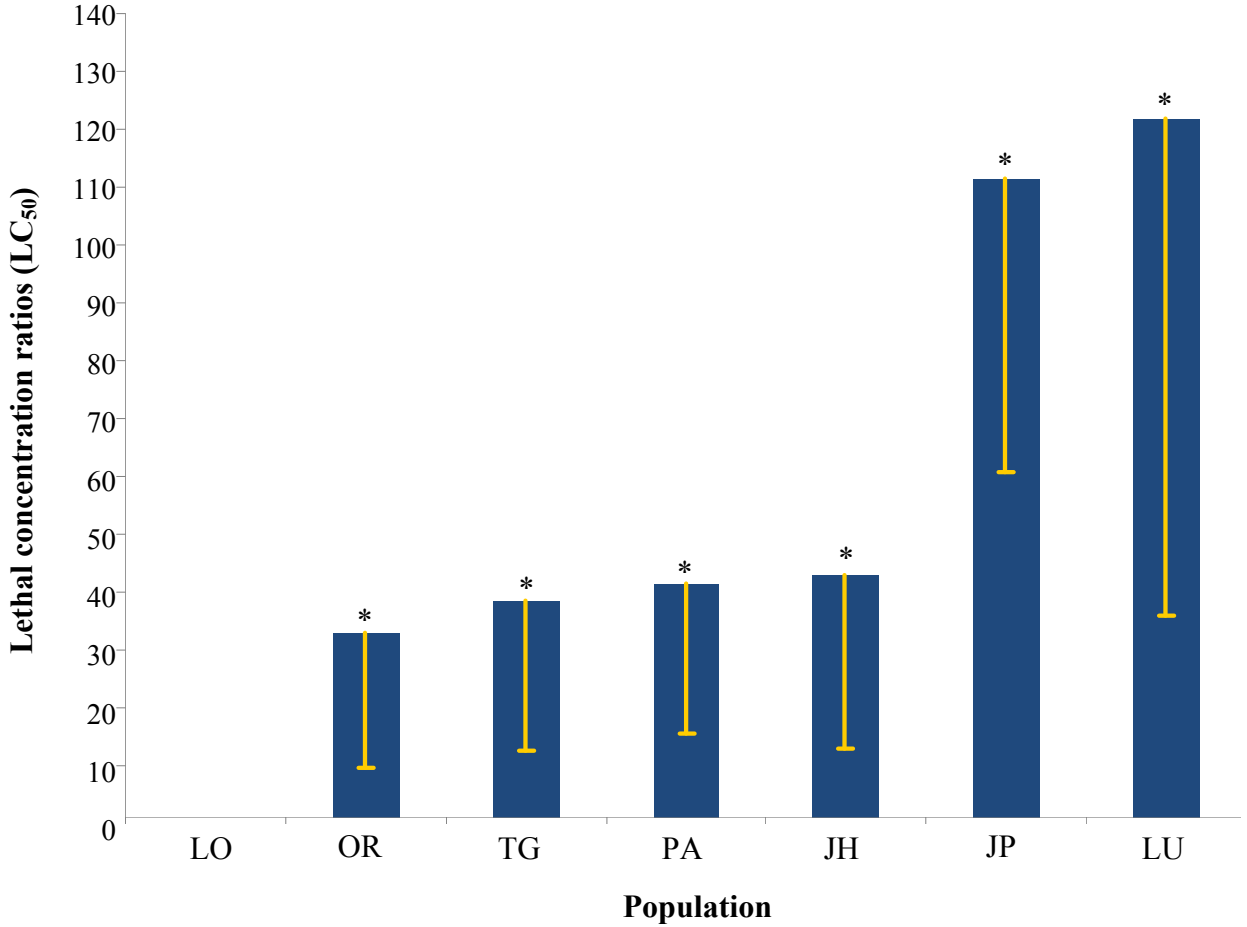

Figure 2-5. LC_{50} Lethal concentration ratios with lower 95% confidence limits indicating the fold difference for each population of *B. insularis* in comparison to the most susceptible population (LO) when tested with bifenthrin. Lower confidence limits that do not include 1.0 are significantly different from the LO population. * Shows ratios that are significant ($P \leq 0.05$, Robertson and Preisler 1992, Robertson et al. 2007).

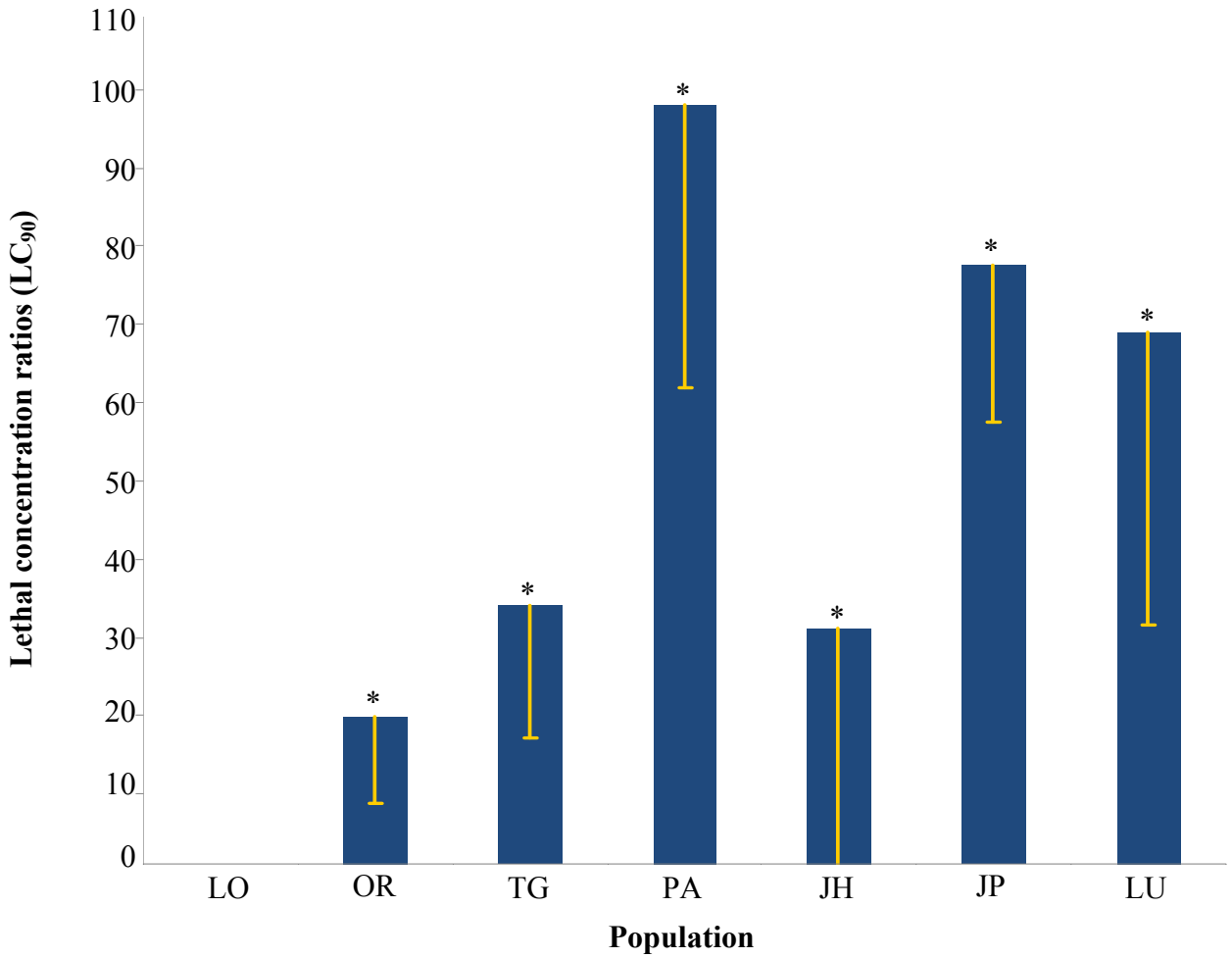

Figure 2-6. LC_{90} Lethal concentration ratios with lower 95% confidence limits indicating the fold difference for each population of *B. insularis* in comparison to the most susceptible population (LO) when tested with bifenthrin. Lower confidence limits that do not include 1.0 are significantly different from the LO population. * Shows ratios that are significant ($P \leq 0.05$, Robertson and Preisler 1992, Robertson et al. 2007).

CHAPTER 3
SYNCHRONOUS METHOD FOR REARING *B. insularis* ON CORN AND ST. AUGUSTINEGRASS

Introduction

The southern chinch bug, *Blissus insularis* Barber, is the most destructive insect pest of St. Augustinegrass (Reinert and Kerr 1973, Bruton et al. 1983). Similar to other *Blissus* feeding habits, nymphal and adult *B. insularis* damage St. Augustinegrass by feeding in the phloem sieve elements of the grass (Rangasamy et al. 2009) causing wilting, chlorosis, stunting, and eventually death (Painter 1928, Negron and Riley 1990, Spike et al. 1991). Populations may consist mostly of long-winged forms (macropterous), short-winged forms (brachypterous), or both (Wilson 1929, Komblas 1962, Leonard 1966, Reinert and Kerr 1973). In Florida, macroptery is greatest during the summer and fall when populations are high (Cherry 2001a). Eggs are laid singly or a few at a time in leaf sheaths, soft soil, or in other protected areas. Eden and Self (1960) reported that *B. insularis* eggs hatch in 14 d in Mobile, AL, while Kelsheimer and Kerr (1957) state eggs can hatch in 7-10 d during the summer in Florida. Young nymphs are as small as 0.87 mm (Leonard 1968), are reddish-orange with a white band across the dorsal side of the abdomen, and become black in color as they mature. Development from egg to adult can vary depending on location and temperature (Sweet 2000): 35 d in Florida (Kelsheimer and Kerr 1957), 49-56 d in Alabama (Eden and Self 1960), and 30-45 d in Mississippi (Burton and Hutchins 1958). Kerr (1966) reported that *B.insularis* development from egg to adult is completed in 93 d at 21°C and in 35 d at 28°C. In addition, female and male longevity was 70.4 and 42.1 d, respectively, with females laying an average of 4.5 eggs per day under laboratory conditions (Kerr 1966).

Control for *B. insularis* is mainly achieved through insecticide use. Because *B. insularis* is multivoltine and has overlapping generations (Kerr 1966, Reinert and Kerr 1973), damaging

populations have received as many as 6 to 12 insecticide applications a year in Florida (Reinert 1978, Reinert and Niemczyk 1982). With near-constant insecticide exposure, *B. insularis* has developed resistance to organochlorines, organophosphates, carbamates, neonicotinoids, and pyrethroids (Kerr 1958, 1961; Reinert 1982a, 1982b; Reinert and Niemczyk 1982; Reinert and Portier 1983; Cherry and Nagata 2005, 2007; Chapter 2). To conduct insecticide-resistance studies, it is important to conduct tests with quality insects of known age and generation (ffrench-Constant and Roush 1990). Therefore synchronized rearing methods are needed for *B. insularis*.

Several attempts have been made to rear *Blissus* spp. under laboratory or greenhouse conditions (Parker and Randolph 1972, Baker et al. 1981, Yamada et al. 1984, Wilde et al. 1987, Meehan and Wilde 1989, Anderson 2004). However, laboratory-reared *Blissus* often incurred high mortality, overlapping generations were produced so that insect age could not be determined prior to bioassays, or percentage success in development from egg to adult was not reported. Several authors successfully produced > 1 generation of *Blissus* spp. under greenhouse and growth-chamber conditions (Wilde et al. 1987, Meehan and Wilde 1989, Anderson 2004). However, mass rearing *B. insularis* in the greenhouse at the Entomology and Nematology Department at the University of Florida has not been feasible because daily ambient summer temperatures in the greenhouses can exceed 37.8 °C, which is lethal for *B. insularis.* Also, potted St. Augustinegrass can become infested with aphids, thrips, scales, mites, other *B. insularis* populations, and natural enemies.

In 2004 and 2005, preliminary tests were conducted to evaluate appropriate rearing containers, food sources, and oviposition substrates to minimize handling while maximizing *B. insularis* growth and speed of development. Different food sources tried included ‘Palmetto’ St.

Augustinegrass (both stolon sections and ground plant material mixed with plain gelatin), green beans (*Phaseolus vulgaris, L.*); ryegrass (*Lolium spp.*); frozen peas (*Pisum sativum, L.*); and baby, canned, fresh, frozen, and organic seedling corn (*Zea mays, L.*). *Blissus insularis* attempted to feed on the green beans but were unable to insert their stylets into intact beans. Of all the food choices, *B. insularis* performed best on fresh St. Augustinegrass and corn on the cob. For the remaining food choices, either *B. insularis* did not feed or mold developed on food in ≤ 24 h.

Ovipositional substrates tried included 70-mm Whatman filter paper, colored felt cloth (black, white, and green) (Michaels Stores, Inc., Irving, TX), colored cardstock (black, white, and green) (Michaels Stores, Inc., Irving, TX), colored foam sheets (black, white, green) (Michaels Stores, Inc., Irving, TX), paper towels (brown and white), cheesecloth, moistened cottonballs, cotton diaper towel (Clean-Rite Products, Lincolnshire, IL), and moistened Publix brand cosmetic squares (Publix Super Markets, Inc., Lakeland, FL). *Blissus insularis* either didn't lay eggs on the substrates (felt, cardstock, foam, paper towels, cheesecloth) or nymphs became trapped and were unable to free themselves (moistened cottonballs and cosmetic squares). However, *B. insularis* nymphs were able to emerge without becoming trapped in the diaper towel.

To control moisture in the rearing chambers, Feline-Pine® cat litter (Nature's Earth Products, Inc., West Palm Beach, FL), compressed sponges (The Color Wheel Company™, Philomath, OR), Plaster of Paris (Lowes Companies, Inc., Mooresville, NC), and dental castone (Henry Schein Inc., Indianapolis, IN) were tried. Various sized plastic containers, petri dishes, cardboard containers, glass jars, and 15.2-cm potted 'Palmetto' St. Augustinegrass with 15.2-cm stolon sections enclosed in fiber floral sleeves (Temkin International, Inc., Miami, FL) were

evaluated. The Feline-Pine® cat litter and compressed sponges worked well at controlling excess moisture, but were suitable for shelter of *B. insularis* and as an oviposition substrate. This made it difficult to remove insects and to count eggs to determine percentage survival. The plaster of Paris was difficult to work with because it dried before being placed into the rearing containers. However, the dental castone stayed in a semi-liquid form long enough to work with. Plastic containers and glass jars proved to be the best for housing *B. insularis* because appropriate relative humidity could be maintained. Other materials allowed too much air flow so that eggs desiccated, resulted in excess moisture, or were not big enough to contain large numbers of insects. Thus, fresh corn cobs and St. Augustinegrass, cotton diaper towel, dental castone, plastic containers, and glass jars were chosen as candidate materials for rearing *B. insularis*. Several tests were then conducted between 2005 and 2008 to develop a synchronized rearing method to produce pure *B. insularis* populations of known age and generation for use in insecticide-resistance studies.

Materials and Methods

Test 1. Small-Scale Rearing of Adults on Corn and Nymphs on Grass

St. Augustinegrass maintenance

Commercially-obtained plugs of 'Palmetto' St. Augustinegrass were planted in 15.2-cm plastic pots filled with Farfard #2 potting soil (Conrad Farfard Inc., Agawam, MA). Plants were maintained in a greenhouse near the University of Florida Entomology and Nematology Department in Gainesville, FL, and held under a 14L:10D photoperiod with day and night temperatures of 27 and 24°C, respectively. Heating mats were used to keep plants from going dormant during the winter months. Because of the slightly acidic (pH=5.5) greenhouse water, all plants were provided with 32 ml of a hydrated lime solution on a weekly basis (Oldcastle Stone Products, Thomasville, PA) (10.6 g/3785 ml) to enhance nutrient uptake. Plants were fertilized

weekly with 20-20-20 (N-P-K) water-soluble fertilizer (United Industries, St. Louis, MO) at a rate of 0.11 kg N/0.09 m^2, watered as needed, and cut to a height of ~ 7.6 cm.

Corn preparation

Commercially obtained bushels of yellow corn cob were shucked, soaked in a 3% bleach solution (600 mL of 6% sodium hypochlorite in 1.94 L tap water) for 10 min, and rinsed. Corn cobs were then dried and stored in 7.6-L Ziplock® clear plastic bags (S. C. Johnson and Son, Inc., Racine, WI) containing four sheets of Bounty® paper towels (Proctor and Gamble Co., Cincinnati, OH) to absorb excess moisture, and refrigerated.

Insect collection

Blissus insularis were collected from St. Augustinegrass lawns using a modified Weed Eater Barracuda blower/vacuum (Electrolux Home Products, Augusta, GA) (Crocker 1993, Nagata and Cherry 1999, Congdon 2004) and transported in a mesh-covered bucket to the laboratory. Adults were aspirated from debris and placed into oviposition containers, as outlined below.

Oviposition and nymph container construction

Plastic containers (15.2-cm diameter, 6.4-cm deep) were soaked in a solution containing 1 L of 6% bottled bleach and 19 L tap water for 20 min, rinsed, and allowed to dry. Fluon® (Ag Fluoropolymers, Chadds Ford, PA) was applied to the top 3 cm of the container to prevent insect escape. Castone™ dental stone Type III (DentSply Inc., York, PA) was then poured to a 0.5-cm depth in each container and allowed to dry for 24 h. Four holes (2-cm diameter) were cut into the plastic lids and chiffon mesh was glued over the holes to allow airflow. For nymph containers, one hole (1.6-cm diameter) was cut 1.3 cm from the bottom of the container for placement of a 7.6-cm long water tube. A small hole (0.28-cm diameter) was drilled into the water tube so water could be added without disturbing the insects.

Egg harvest method

Cotton diaper towels were used to create 'egg rolls' for collecting *B. insularis* eggs. Egg rolls were created by cutting diaper towels into 7.6-cm × 7.6-cm sections and rolled to a 1.7-cm diameter by wrapping around a plastic 10-mL graduated cylinder.

Thirty unsexed *B. insularis* were placed into each of three oviposition containers and provided with one corn section (5 × 2.5 cm) and two egg rolls as an oviposition substrate (Figure 3-1 A). The egg rolls were collected daily for 4 d, eggs were counted, and placed into nymph containers containing one 15.2-cm long stolon of Palmetto St. Augustinegrass with the excised end inserted into a floral tube filled with water (Figure 3-1 B). There were 12 replicates. All oviposition and nymph containers were maintained in the laboratory under a 14L:10D photoperiod, 26-31°C and 60-70% RH. Stolons were changed weekly and tubes filled with water as needed. Dental castone was moistened with 2.5 ml of deionized water 5 days a week to maintain relative humidity. The number and percentage of adult *B. insularis* that successfully developed, sex ratio, and the ratio of wing type were determined for each replicate.

Test 2. Assessment of Time of Day for Oviposition

A subsequent test was designed to determine if the previous method would be appropriate for larger-scale rearing and to determine the time of day *B. insularis* oviposition was highest. Oviposition and nymph containers were set up and maintained as previously described.

The egg rolls were collected three times a day (0700, 1500, and 2300 h) from two oviposition containers (Figure 3-1A), eggs were counted, and transferred into nymph containers. There were 20 replicates for each 8-h interval. Nymph containers (Figure 3-1B) were checked daily until the first sign of adult emergence, then were checked twice a day (0800 and 1600 h), and newly emerged adults were counted and stored in vials of 95% EtOH. The number of eggs collected at each time interval, number of days for chinch bug development from egg to adult,

ratio of males to females, ratio of brachypterus and macropterus adults, and percentage survival were determined. An analysis of variance (ANOVA) was used to determine if the mean number of eggs collected differed among time intervals. Treatment means were analyzed using the Tukey's Studentized Range (HSD) test (SAS Institute, Inc. 2001).

Test 3. Rearing Nymphs on Planted Grass in Builder's Sand and Glass Jars

The purpose of this test was to reduce competition for food, which was observed in Test 2. To accomplish this, 7.6-L glass jars (Heritage Hill Collection, Anchor Hocking, Lancaster, OH) were used in place of plastic containers to house more *B. insularis* and food. The jars were washed, dried, and a 5-cm band of Fluon® was applied with a wash bottle to the inside top of the jars, and dried using a hairdryer. This created a barrier to prevent insects from escaping. At the bottom of each oviposition jar, dental castone was poured to a 1.3-cm depth and allowed to dry for 24 h.

Corn preparation

The corn was prepared as previously described. For placement into colony jars, the bottoms of 59-ml plastic soufflé solo cups (Gainesville Paper Supply, Gainesville, FL) were removed, the cups cut in half, and then inserted into the bottom of each cob to keep it from rolling. Excess moisture from around the cup 'stands' was blotted with paper towels to reduce the development of mold.

Insect collection and colony maintenance

Blissus insularis were collected as previously described. Adults were aspirated from debris and placed into jars containing two full-sized, surface-sterilized, fresh yellow corn cobs and 12 egg rolls provided as an oviposition substrate (Figure 3-2A and B).

Corn cobs and stands were replaced twice a week in jars containing adult *B. insularis*. An air stream (~ 1.4 m/s) was gently applied to the corn cobs to cause the insects to stop feeding and

fall to the bottom of the jars unharmed. Dead insects or eggs lying at the bottom of the jars were vacuum aspirated before adding new corn cobs and stands. Excrement that was deposited on the inner sides of the jars was wiped clean using Clorox® disinfecting wipes.

For nymph development, one Palmetto St. Augustinegrass (15.2-cm diameter) plant was planted in jars containing 1500 g of sterilized builder's sand (Figure 3-3) the week of egg introduction. Plants were watered as needed. Oviposition and nymph jars were enclosed with chiffon mesh after insects were introduced. All jars were maintained in the laboratory under a 14L:10D photoperiod, 26-31°C, and 70-85% RH. The egg rolls were collected once a week for 3.5 wk from six oviposition jars, eggs counted, and transferred into nymph jars. There were 21 replicates. The number and percentage of adult *B. insularis* that successfully emerged were determined for each replicate.

Test 4. Corn Only Rearing Method

This experiment was designed to determine if *B. insularis* could be solely reared on fresh corn on the cob. The oviposition jars (Figure 3-2 A) and castone for the nymph jars were constructed as described for the oviposition jars in Test 3.

The egg rolls were collected weekly for 7 wk from one oviposition jar and eggs present were counted (total: 7,092 eggs). Twelve egg rolls (Figure 3-2 B) with eggs were transferred into each nymph jar containing one fresh surface-sterilized corn cob placed on two plastic stands. One fresh corn cob was added every 3 - 4 d and stacked alternately over the old corn cobs. Old corn cobs were not removed because nymphs used them as shelter and were difficult to remove. To maintain high humidity, 50 ml of water was added to the dental castone each week. All oviposition and nymph jars were maintained in the laboratory under a 14L:10D photoperiod, 26-

31°C, and 70-85% RH. The number and percentage survival of *B. insularis* was determined after 8 wk.

Test 5. Improved Method Using Corn and Grass

An improved method of rearing *B. insularis* adults on corn and nymphs on grass in 7.6-L glass jars was created based on the results of Tests 1-4. The following methods were used to determine the success of the rearing method.

Colony jar construction

Clean jars had fluon applied as previously described. Jars were set over wax paper and a marker was used to trace around the bottom of the jar to create wax paper circles. The paper circles were then cut in half, taped to a 23 cm × 2 cm cardboard strip, and placed at the bottom of the jars (Figure 3-4A). Castone (453 g mixed in 200 mL water) was poured over the wax paper/cardboard assemblage, carefully making sure to cover any holes in the cardboard (Figure 3-4B). The castone was allowed to dry for 24 h.

Egg harvest method and nymph maintenance

The egg rolls were created as described in Test 1 and were replaced in oviposition jars every 2 d. Any adults on the egg rolls were carefully removed and egg rolls were placed into a separate jar containing castone. One air-dried, soil-free, 15.2-cm St. Augustinegrass plant was placed next to or on top of the egg rolls and jars were covered with chiffon nylon mesh held in place with a rubber band (Figure 3-5). A clear plastic shower cap was added to maintain RH>70%. One end of a pair of scissors was used to pierce the shower cap, creating five holes in a star design to allow ventilation. One fresh St. Augustinegrass plant was added twice per week to each jar. The older plant material was left in the jars so *B. insularis* nymphs could use it for shelter and molting. Nymphs were often distributed or concentrated at the bottom of the jar,

perhaps because of the higher RH near the castone bottom. As a result, new plant material was placed underneath or directly next to older plant material.

Determining quality and success of rearing method 5

To determine the quality of the insects and success rate of this rearing method, a *B. insularis* colony was reared for eight generations (Figure 3-2 A, 3-4, 3-5) before testing. Egg rolls containing ninth-generation eggs were removed as described and 400 eggs were counted, placed into a castoned jar, and held in a Percival growth chamber (model I36VLC8) under a constant temperature of 30°C and 14L:10D photoperiod. There were eight replicates for a total of eight jars and 3,200 *B. insularis* eggs.

Nymphs that emerged were maintained as previously described. Three days after the first sign of adult emergence, all live insects were removed, counted, and placed into vials containing 95% EtOH. All *B. insularis* were then sexed and wing-typed using a dissecting microscope. Thirty brachypterus females were randomly selected from each jar, mounted onto cardstock, and body length measured. The percentage survival, wing length, and body length (of brachypterus females) were used to determine environmental stresses (crowding) and food quality. Body length comparisons were made of offspring, parent, and field-collected (merged from four populations in central FL) brachypterus females to determine the relative fitness of the *B. insularis* colony after being reared for eight generations. Analyses of variance (ANOVA) were used to determine mean differences in body length of emerged brachypterus females by replicate and between offspring, parent, and field-collected brachypterus female *B. insularis*. Treatment means were analyzed using the Tukey's Studentized Range (HSD) test (SAS Institute, Inc. 2001).

Results and Discussion

Test 1. Small-Scale Rearing of Adults on Corn and Nymphs on Grass

The percentage survival of *B. insularis* that successfully developed from egg to adult in each replicate ranged from 61.1-100% (Table 3-1). The total average percentage survival from all replicates was 85%. The sex ratio was 1:1 and all *B. insularis* that emerged were brachypterus. However, because few eggs (1-22) were placed into each container, a larger-scale study was needed to determine if this method could be used for mass rearing of *B. insularis* and to determine the best time of day to collect eggs.

Test 2. Assessment of Time of Day for Oviposition

The greatest number of eggs was collected at 2300 h and the temperature was highest between 1500 and 2300 h (Table 3-2). This suggests that the best time to collect eggs from containers would be in the morning to minimize interfering with *B. insularis* oviposition. Females took slightly longer to develop than males, 42.5 ± 0.5 and 40.2 ± 0.5 d, respectively. The ratio of males to females was 1:1 and the ratio of brachypterus and macropterus adults that emerged was 7:1. Out of the 976 eggs collected, only 381 (39%) survived to the adult stage. It is possible that survival decreased with increasing egg density. In the containers with egg densities of 41 and higher, we had problems with chinch bug nymphs squeezing through the cap into the water tube and drowning, suggesting there was competition for food. Also, it is possible that nymphs may have been harmed or accidentally discarded when grass material was changed. We speculated that larger rearing containers, additional food, and leaving the grass material inside the chambers until the test is completed may increase the percentage survival.

Test 3. Rearing Nymphs on Planted Grass in Builder's Sand and Glass Jars

The 7.6-L glass oviposition jars worked well for obtaining large numbers of *B. insularis* eggs and were more suitable for housing more insects than the containers used in Tests 1 and 2.

However, of the 30,534 eggs collected and placed into nymph jars, only 1,011 (3.3%) survived to the adult stage (Table 3-3). Plants used in this test may not have been washed thoroughly, so predators remained hidden in the plants. *Blissus insularis* nymphs were not apparent after the first week and upon greater inspection, spiders were seen toward the top of some nymphal jars, in the egg rolls, or fell out as the plants were pulled apart. Spiders were present in all of the nymphal chambers and in one case each, small earwigs, centipedes, and one *Geocoris uliginosus* Say was found. Anderson (2004) also had problems with predators invading greenhouse populations of *Blissus* spp., resulting in difficulty in rearing multiple generations.

Grass in the jars containing >1000 *B. insularis* nymphs (Table 3-3) quickly wilted and died within the first 2 wk of the test, indicating that the 15.2-cm grass plant was not enough food to sustain the high number of *B. insularis* and allow them to complete development to the adult stage. While the nymphal chambers in this test were inadequate for use in mass rearing of *B. insularis*, the ovipositional chambers were superior to those used in tests one and two. The ovipositional chambers in this study allowed for more *B. insularis* to be housed and thus obtain more eggs.

Test 4. Corn Only Rearing Method

Blissus insularis nymphs could not complete development to the adult stage when reared solely on fresh corn cob. Out of 7,092 eggs, only 12 (0.17%) survived to the second instar after 2 months of being maintained on corn (Table 3-4). In addition, castone was difficult to remove in some of the jars because it would stick to the bottom. There are several factors that may influence an organism's chance to survive and multiply: 1) food, 2) weather, 3) other organisms, and 4) a place in which to live (Andrewartha 1965). The host plant used in rearing may affect one or more of these categories, directly or indirectly (Berlinger 1992). It is possible that the fresh corn cob did not meet the nutritional needs for *B. insularis* for growth and development.

The absence or imbalance of certain nutritional requirements may have prevented growth (Chapman 1969) or impaired the ability of *B. insularis* to molt.

In addition, host plant morphology may also affect the insect's microclimate (Berlinger 1992). Nymphs of *B. insularis* tend to settle or hide in narrow places such as inside the sheaths of St. Augustinegrass. This allows them to be in maximum contact with their surroundings (thigmotropism). It is possible that it allows them to be in close contact with food and/or provides protection from environmental factors or predators. In order to rear thigmotrophic insects (such as *B. insularis*) effectively, it is important to fulfill this requirement (Berlinger 1992). By eliminating the St. Augustinegrass from the *B. insularis* nymphal diet and replacing it with fresh corn, this may have eliminated their 'place to live'. This may have exposed them to the direct light in the rearing room or stressed them, causing death or slowed growth.

Test 5. Improved Method Using Corn and Grass

All adult generation-nine *B. insularis* emerged after 5.5 wk when reared at a constant temperature of 30 ± 0.1 °C and 14L:10D photoperiod. Percentage survival of successfully emerged *B. insularis* in each jar ranged from 56 – 79% (Table 3-5). The sex ratio in each jar was 1:1 with a 5:1 ratio of brachypterus to macropterus individuals. The body length of brachypterus females from each jar ranged from 3.67 ± 0.1 to 3.88 ± 0.2 mm and significant differences were observed (Table 3-1). Particularly, jars 5, 7, and 8 had brachypterus females with significantly longer body lengths than females from other jars. Jar 1 produced brachypterus females with the shortest body length.

Mean body length for brachypterus female offspring was similar to that of the 30 randomly chosen brachypterus female *B. insularis* collected from the parent colony (3.77 ± 0.01 and 3.83 ± 0.03 mm, respectively, for mean body length; $F = 3.47$; df = 1, 268; $P = 0.06$).

However, both parent and offspring of laboratory-reared *B. insularis* were significantly larger than field-collected *B. insularis* (3.32 ± 0.02 mm for mean body length for field-collected *B. insularis*; $F = 187$; df = 2, 323; $P < 0.0001$).

The size of brachypterus females that emerged in Test 5 indicates that a combination of fresh corn cob and St. Augustinegrass provides a suitable diet for rearing *B. insularis*. The St. Augustinegrass used in our study received a weekly nitrogen (N) supply at the highest recommended rate for Florida lawns (0.11 kg N/0.09 m^2 per wk). Busey and Snyder (1993) suggested that greater host plant quality is associated with faster development, greater survival, and higher fecundity in *B. insularis*. Likewise, an increase in N fertilization is usually followed by an increase in populations in other phytophagous arthropods (Harrewijn 1970, Wermelinger et al. 1985, Berlinger 1992). Realized fecundity is positively related to female body size in some insect species (Speight 1994, Preziosi et al. 1996, Tammaru et al. 1996, Sopow and Quiring 1998). In addition, Dahms (1947) conducted studies with sorghum and found that higher rates of nitrogen (N) fertilization were associated with increased *B. l. leucopterus* oviposition rates. It is possible that the laboratory-reared *B. insularis* in this study were provided with higher quality turfgrass than is found in residential lawns in Florida. This would explain why the *B. insularis* offspring from Test 5 were larger than the insects that were collected from residential lawns in central Florida. Although larger than the field-collected specimens, brachypterus female offspring in Test 5 had body lengths comparable to those found by Cherry and Wilson (2003) in Florida, which had body lengths ranging from 3.2-4.0 mm.

Time for development (~35 d) was similar to that of field populations of *B. insularis* in Florida (Kelsheimer and Kerr 1957) at 28°C. This would suggest that by using Test 5 methods, laboratory-reared *B.insularis* can be produced that are of high quality and comparable in

development times to field-collected *B. insularis* in Florida. This may be important if laboratory-reared colonies are used for developing baseline insecticide studies in place of field-collected insects of unknown age and quality.

The large number of male and female brachypterus *B. insularis* that emerged in Test 5 showed that environmental stress (e.g., climate, crowding, competition for food) was minimal after nine generations. Cherry and Wilson (2003) reported brachypterus female *B. insularis* contain more eggs, as determined by dissection, and laid significantly more eggs per female than macropterus females. Fujisaki (1985) noted more macropterus *Cavelerius saccharivorus* Okajima (Heteroptera: Blissidae) individuals emerged when placed under extremely crowded conditions. The number of macropterus *B. insularis* that emerge in colonies could be used as an index for quality control in *B. insularis* laboratory rearing.

This work presents the first successful synchronized rearing method for *B. insularis*. Other *Blissus* spp. have been reared on grass under laboratory conditions, but with minimal success. Yamada et al. (1984) reared the oriental chinch bug, *Cavelerius saccharivorus* Okajima, on maize, Kentucky bluegrass, sorghum, and sugarcane. However, only 40% of insects in the second generation successfully survived to the adult stage. Parker and Randolph (1972) reared the common chinch bug, *B. leucopterus leucopterus* (Say), in 3.78-L cardboard cartons on alternating stacked layers of maize and sorghum stalk sections. Each carton could produce 800-1000 chinch bugs (Parker and Randolph 1972). However, this method produced overlapping generations in each container and the authors did not report how many generations were reared.

Baker et al. (1981) attempted to rear the hairy chinch bug, *B. leucopterus hirtus* Montandon, using Parker and Randolph's (1972) technique, but early-instar mortality was high, which appeared to be associated with fungal growth on the corn sections. The authors increased

B. l. hirtus survival by surface-sterilizing corn sections with 2% sodium hypochlorite and frequently changing corn sections in containers. However, the authors still had an average of 17% mortality in the egg, 48.3% during the first and second instars, and 15.7% during the third through fifth instar stages. Only 80% of third-fifth instar *B. l. hirtus* successfully developed to the adult stage (Baker et al. 1981). The total percentage survival from egg to adult in their study was 20%. In this study, a combined total average of 67% of *B. insularis* successfully emerged from the rearing containers. However, four replicates had percentage survival rates ranging from 71-79%. Grass plants in nymphal jars sitting on the top shelf of the growth chamber dried faster than in jars located on lower shelves, possibly because the fans were directly above the top shelf and increased air flow. Excess air flow may have dried out eggs or reduced the food supply to early-instar *B. insularis*. Using shower caps without ventilation or placing the upper shelf farther from the fans appeared to increase survival. However, regardless of reduced survival in half of the jars, the total percentage survival in *B. insularis* in this study is the highest reported in any laboratory-rearing procedure published for *Blissus* spp.

Problems I encountered while rearing previous generations included contamination with predators, egg parasitoids, mold/fungal growth, and safety issues regarding removal of dental castone (ie., shards) from colony jars. Contamination with predators and egg parasitoids can be greatly reduced by carefully vacuuming *B. insularis* from field samples (not introducing other debris into colony jars) and thoroughly washing plant material before placing it into nymph jars. Mold was greatly reduced on corn cobs after surface sterilization and removal of excess moisture before placing the corn into jars. Another means of reducing mold was by eliminating the addition of water to the dental castone. The addition of water is needed when using the containers employed in Tests 1 and 2. The depth of the castone used in these containers was

small and so water readily evaporated from it. However, the thicker dental castone in the 7.6-L glass jars didn't require the additional water and helped maintain high humidity. Also, the thicker dental castone in the glass jars absorbed excess moisture from grass in nymphal jars, which greatly reduced problems with mold. Overall, sanitation was found to be crucial to the success of rearing *B. insularis* in the laboratory.

Safety also was of concern because of the difficulty in removing dental castone from jars. Dental castone can be difficult to remove without the use of the wax paper/cardboard assembly, but with this in place, jars can be set upside down and the dental castone will drop down and then can be folded in half and safely removed. If the dental castone doesn't fall, the cardboard strip in the center can be pulled out to remove the dental castone so jars can be cleaned and reused.

In terms of labor for Test 5 methods, it takes ~5.5 h per week to set up one oviposition jar and produce one jar of offspring (Figure 3-6). Not including supplies, the labor cost ($10 per hour) would be $55 so, if one jar of offspring produces 224-317 *B. insularis*, then the cost per insect is $0.17-0.24.

For the first time, we can produce large numbers of *B. insularis* of known age and generation for use in bioassays that are of high quality in comparison to the field-collected *B. insularis,* which could be stressed from previous treatments with pesticides or because of a poor-quality diet. Using reared insects will help to reduce variability in insecticide bioassays and can be used to develop insecticide-susceptible colonies for use as a baseline in bioassays. This work also provides a key step in building a resistance-management program for *B. insularis*. *Blissus insularis* colonies can now be selected for bifenthrin resistance (or any other insecticide) in the laboratory. Pure insecticide-susceptible and -resistant colonies can then be used in tests to

determine mechanisms, cross-resistance, mode of inheritance, and stability of resistance, providing key information regarding the genetics of resistance in *B. insularis*.

Table 3-1. The total number eggs in each replicate at the start of Test 1* and the number of female and male *B. insularis* that successfully emerged after 5.5 wk that were reared at 26-31°C, 60-70% RH, and a 14L:10D photoperiod.

Replicate	Total number eggs	Number adults emerged (♂:♀)	Percentage survival from egg to adult
1	1	1 (1:0)	100.0
2	13	12 (7:5)	92.3
3	6	5 (2:3)	83.3
4	3	3 (2:1)	100.0
5	10	7 (3:4)	70.0
6	9	8 (4:5)	88.9
7	6	6 (3:3)	100.0
8	18	11 (6:5)	61.1
9	6	6 (3:3)	100.0
10	11	8 (3:5)	72.7
11	22	22 (10:12)	100.0
12	21	18 (8:9)	85.7

* Small-scale rearing of adults on corn and nymphs on grass.

Table 3-2. Mean number (± SEM) of *B. insularis* eggs collected at each 8-h interval in Test 2† and respective average temperature (C°) and % RH (± SEM).

Collection Time (h)	Mean number eggs ± SEM	Average air temp ± SEM (C°)	Average % RH ± SEM
0700	3.0 ± 0.7	26.8 ± 0.3	65.0 ± 2.2
1500	16.5 ± 3.5	31.2 ± 0.5*	60.5 ± 2.2
2300	29.2 ± 5.1*	29.9 ± 0.5*	65.6 ± 2.9

* Mean ± SEM within a column followed by * are significantly different ($P < 0.05$) by Tukey-Kramer HSD test ($F = 13.2$; df = 2, 57; $P < 0.0001$ for mean number of eggs; $F = 24.2$; df = 2, 57; $P < 0.0001$ for temperature; and $F = 1.32$; df = 2, 57; $P = 0.27$ for % RH).

† Assessment of time of day for oviposition.

Table 3-3. The total number eggs in each replicate at the start of Test 3* and the number of *B. insularis* adults that successfully emerged after 6 wk that were reared at 26°C, 60-70% RH, and a 14L:10D photoperiod.

Replicate	Total no. eggs in jar	Number adults emerged	Percentage survival from egg to adult
1	620	33	5.3
2	1113	48	4.3
3	2484	119	4.8
4	1448	137	9.5
5	532	15	2.8
6	759	17	2.2
7	1897	101	5.3
8	2977	110	3.7
9	581	56	9.6
10	909	0	0
11	637	0	0
12	930	0	0
13	605	20	3.3
14	572	27	4.7
15	1842	91	4.9
16	1336	129	9.6
17	1312	73	5.6
18	2732	30	1.1
19	459	5	1.1
20	395	0	0
21	6394	0	0

* Rearing nymphs on planted grass in builder's sand and glass jars.

Table 3-4. The total number eggs in each replicate at the start of test four* and the number and stage of *B. insularis* found after 8 wks that were reared at 26-31°C, 70-85% RH, and a 14L:10D photoperiod.

Replicate	Total number of eggs in jar	Number found alive	Stage of development
1	1497	0	
2	1215	0	
3	953	8	2nd instar
4	1383	4	2nd instar
5	996	0	
6	530	0	
7	518	0	

* Corn only rearing method.

Table 3-5. The number of emerged generation-nine *B. insularis* adults, percentage survival, wing type, and comparison of mean (± SEM) body length (mm) of brachypterus females by replicate for test five[†] that were reared at a constant 30°C, 75 ± 5% RH, and a 14L:10D photoperiod, using fresh corn cob, St. Augustinegrass, and glass jars.

Jar	Number adults emerged ♂:♀	Percentage survival	Number of brachypterus:macropterus	Body length in mm (± SEM)*
1	258 (141:117)	64.0	218: 40	3.67 ± 0.02^{ad}
2	236 (121:115)	59.0	201: 35	3.76 ± 0.03^{ac}
3	296 (146:150)	74.0	249: 47	3.75 ± 0.02^{ac}
4	288 (150:138)	72.0	245: 43	3.74 ± 0.02^{ac}
5	317 (174:143)	79.2	253: 64	3.88 ± 0.03^{bc}
6	283 (147:136)	70.7	247: 36	3.75 ± 0.02^{ac}
7	224 (118:106)	56.0	198: 26	3.79 ± 0.02^{bc}
8	237 (136:101)	59.2	204: 33	3.84 ± 0.02^{bc}

* Mean ± SEM within a column followed by the same letter are not significantly different ($P < 0.05$) by Tukey-Kramer HSD test ($F = 187$; df = 2, 323; $P < 0.0001$).

† Improved method using corn and grass.

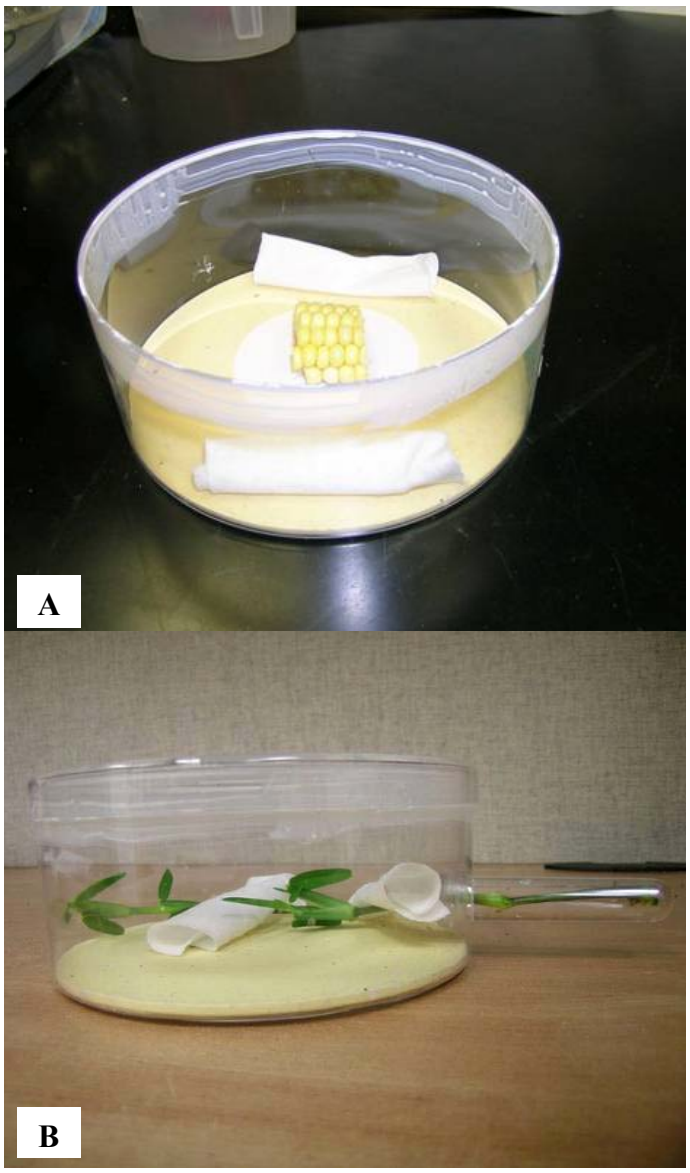

Figure 3-1. Experimental design of Tests 1 and 2: A) oviposition container used to maintain adults and collect eggs, and B) container used for *B. insularis* nymph development. These containers were limited by the amount of food and number of *B. insularis* that could be housed.

Figure 3-2. A 7.6-L oviposition jar (A) used for maintaining *B. insularis* adults and collecting eggs, and (B) a partially unrolled egg roll used in Tests 3, 4, and 5 displaying *B. insularis eggs*. This method worked best for housing adult *B. insularis* adults and oviposition.

Figure 3-3. A 7.6-L glass jar with grass planted in sterilized builder's sand for nymph development used in Test 3. This method failed because of contamination with predators and possibly limited food source.

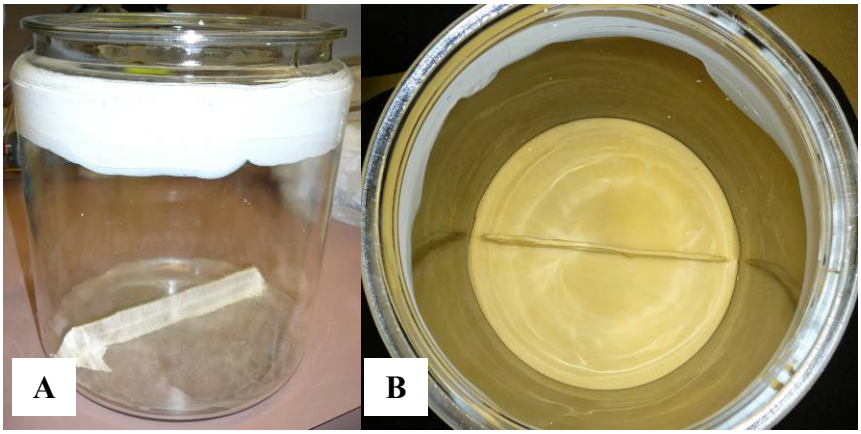

Figure 3-4. A 7.6-L glass jar with A) wax paper and cardboard assemblage at the bottom, and B) completely constructed jar with dental castone used in Test 5, which allowed dental castone to be removed easily and safely so jars could be cleaned and reused.

Figure 3-5. 7.6-L glass jar containing St. Augustinegrass for development of *B. insularis* nymphs used in Test 5. Fresh grass is added twice per week and placed near the bottom of the jar. This method worked best for producing the highest number of *B. insularis* adults.

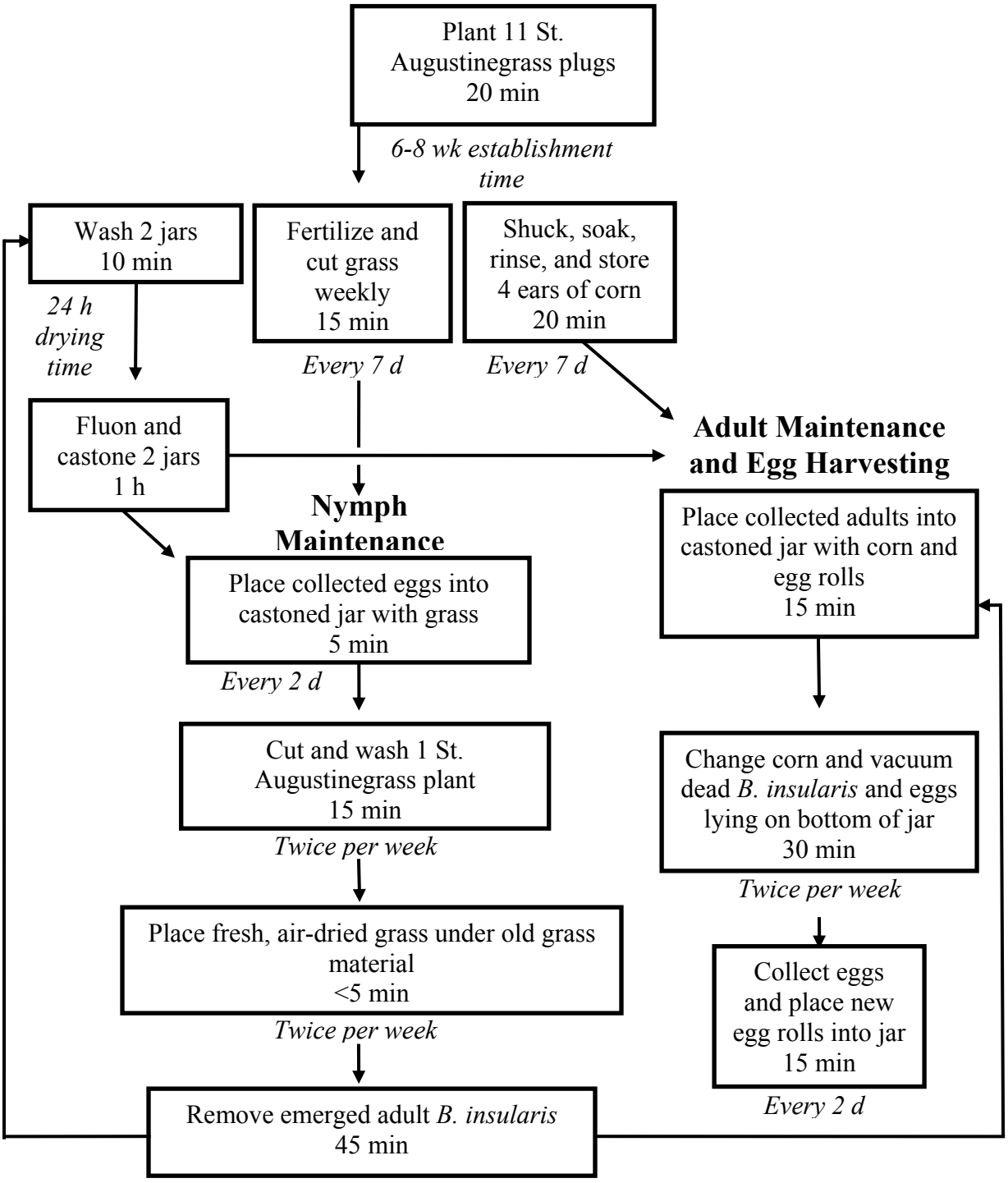

Figure 3-6 Flow chart of steps and approximate time and labor required to rear one jar of *B. insularis* in a synchronous laboratory system (test five) at a constant 30°C and 14L:10D photoperiod. Each jar could produce 224 to 317 adults if initiated with 400 eggs.

CHAPTER 4
CONCENTRATION-MORTALITY RESPONSES TO FIVE INSECTICIDES BY A SUSCEPTIBLE COLONY OF *B. insularis* USING AN AIRBRUSH BIOASSAY

Introduction

The southern chinch bug, *Blissus insularis* Barber, is the most damaging insect pest of St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, in Florida causing stress and death of turfgrass (Reinert and Kerr 1973, Reinert and Niemczyk 1982, Bruton et al. 1983). All life stages are present throughout the growing season in densities of up to 2,000 chinch bugs/0.1 m^2 (Reinert and Kerr 1973). In northern Florida, three to four generations of *B. insularis* may occur from March to October, but seven to ten generations per year may occur in southern Florida (Kerr 1966, Reinert and Kerr 1973).

Control of *B. insularis* is mainly achieved through insecticide use and, because it is multivoltine, insecticides may be applied up to 12 times a year in Florida (Reinert 1978, Reinert and Niemczyk 1982). As a result of consistent insecticide selection, *B. insularis* has developed resistance to organochlorines, organophosphates, carbamates, neonicotinoids, and pyrethroids (Kerr 1958, 1961; Reinert 1982a, 1982b; Reinert and Niemczyk 1982; Reinert and Portier 1983; Cherry and Nagata 2005, 2007; Chapter 2). Given the history of *B. insularis* in developing insecticide resistance, it is important to implement resistance management strategies that can prolong the effectiveness of existing or new insecticides for this pest.

As with any resistance management program, it is important to obtain information on current insecticide susceptibility levels in *B. insularis* populations so that baselines can be established and changes in susceptibility over time and in different locations can be detected. The most commonly used laboratory bioassay for evaluating insecticide efficacy against *B. insularis* is the sprig-dip test (Figure 4-1) (Reinert and Portier 1983; Cherry and Nagata 2005, 2007; Congdon and Buss 2006; Chapter 2). This method involves cutting sections of St.

Augustinegrass stolons, dipping them into insecticide solutions, allowing them to dry, and placing them into petri dishes containing ten adult *B. insularis*. The set up for the sprig-dip bioassay can be conducted quickly and it is inexpensive. However, a large degree of variability in response occurs. Tests are usually conducted in different laboratories under varying environmental conditions, or with field-collected *B. insularis* of unknown age and/or from different locations (Reinert and Portier 1983; Cherry and Nagata 2005, 2007; Congdon and Buss 2006; Chapter 2). It would be beneficial to evaluate the sprig-dip bioassay under standardized conditions to validate the use of the assay.

In addition to variability in the sprig-dip bioassay, scoring multiple individuals in the same dish can be cumbersome when they are not all moribund. A standardized bioassay that could detect differences between male and female *B. insularis* would also greatly aid in understanding how insecticide resistance develops in this pest (i.e., mode of inheritance, stability of resistance). Thus, bioassays that made scoring easier would be an important asset for *B. insularis* resistance monitoring.

The goal of this study was to 1) evaluate the sprig-dip bioassay under standardized conditions, 2) develop a bioassay that could be used for detecting insecticide susceptibility differences between male and female *B. insularis*, and 3) validate both bioassays and determine optimal exposure times and sample sizes to be used for each bioassay for selected insecticides.

Materials and Methods

St. Augustinegrass Maintenance

Commercially-obtained plugs of 'Palmetto' St. Augustinegrass were cut in half, planted in 8.9-cm plastic cups filled with Farfard #2 potting soil (Conrad Farfard Inc., Agawam, MA), and roots were allowed to establish for 3-4 wk before use in experiments. Plants were maintained in a University of Florida greenhouse in Gainesville, FL, and held under a 14L:10D photoperiod

and day and night temperatures of 27 and 24°C, respectively. Plants were fertilized weekly with 20-20-20 water-soluble complete N source (NH_4NO_3) at 0.11 kg N/0.09 m^2, and watered and cut to a height of 7.6 cm as needed.

Insect Collection and Maintenance

Blissus insularis fifth instars and adults were collected from St. Augustinegrass on the University of Florida campus using a modified Weed Eater Barracuda blower/vacuum (Electrolux Home Products, Augusta, GA) (Crocker 1993, Nagata and Cherry 1999, Congdon 2004). This area was not treated with insecticides within 1 yr of this study and preliminary tests indicated that this population was the most susceptible compared to 16 populations tested against bifenthrin in Chapter 2. Insects were sorted from the debris and placed into 7.6-L glass colony jars. Each jar contained two full-sized, surface-sterilized, fresh yellow corn cobs and 12 pieces (7.6 × 7.6-cm) of cotton diaper towel (Tiger Accessory Group, LLC, Lincolnshire, IL) rolled to 1.7-cm diameter, then provided as an oviposition substrate. Cotton rolls were collected after 1 wk and placed into 7.6-L glass jars containing Palmetto St. Augustinegrass plants that were cut at the crown and washed. Two 15.2-cm plants were added weekly until adults emerged. Second- and third-generation males and females were used for this study.

Insecticides

Formulated products commonly used for *B. insularis* control in Florida were chosen to represent four classes of insecticides (Table 4-1). Five to eight concentrations plus a water control were tested for each insecticide to establish probit lines with mortality ranging from 5 to 95% for unsexed *B. insularis*. The range of concentrations tested for each insecticide is shown in Table 4-1. All bioassays were conducted between 13:30 and 15:30 h and held in growth chambers with a constant temperature of 26ºC and a 14L:10D photoperiod.

Spray Application Device

All treatments in the airbrush bioassays were applied using a single-action Paasche airbrush (Figure 4-2A) (Model: H-set, Paasche Airbrush Company, Harwood Heights, IL), using the nozzle provided to deliver a fine aerosol spray. Lesco® Tracker® spray indicator dye (Lesco, Inc., Strongsville, OH) was used in initial tests to ensure that all insecticides were sprayed to runoff. New attachments were used for each insecticide to eliminate cross-contamination. Half the solution was sprayed onto plant material, plants were rotated 90°, and the remainder of the solution was sprayed to provide uniform coverage. Airbrush parts were cleaned with acetone.

Determining Uptake for Systemic Insecticides—Using an Airbrush Bioassay

To determine uptake time for systemic insecticides, two Palmetto St. Augustinegrass plants planted in 8-cm plastic cups were placed into the center of a 929-cm^2 cardboard tray and sprayed with clothianidin (1, 3, or 7 d before bioassay) and allowed to dry at room temperature (25 ± 2°C) and a 14L:10D photoperiod. Sections 1 cm in length containing a single node were cut from treated plants and placed into each cell of a BioServe bioassay tray (Figure 4-2B) (BAW128, Bio-Serve, Frenchtown, NJ) that had been swabbed with unscented Bounce® fabric softener (Procter & Gamble, Cincinnati, OH) to reduce static electricity. Control cells were also swabbed to verify that the fabric softener did not affect the insects. One adult *B. insularis* (2–3 wk old) of unknown sex was introduced into each cell. Cells were sealed with BioServe perforated tray lids (BACV16) and all trays were placed into closed plastic containers (35.6 cm × 26.7 cm) lined with moistened paper towels to maintain humidity. The number of dead *B. insularis* was assessed after 4, 8, 24, 48, and 72 h. *Blissus insularis* were scored as dead if they were on their backs or unable to walk. A total of 144 *B. insularis* and nine concentrations were tested for each spray time.

Comparison of Airbrush and Sprig Dip Bioassays

A side-by-side comparison of the airbrush and sprig-dip bioassays was performed using 280 *B. insularis* for bifenthrin (TalstarOne®) and 720 *B. insularis* for imidacloprid (Merit® 2F). The airbrush bioassays were performed as previously described (except that insects were scored after 24, 48, and 72 h) and the sprig-dip bioassays were conducted as described in Chapter 2. Both bioassays were placed into closed plastic containers as previously described. Because response to insecticides in individual insects cannot be determined when using the sprig-dip bioassay, insects in comparison tests were unsexed.

Airbrush Bioassay

Plants were treated with contact insecticides 1 d before the bioassay, except trichlorfon. Because trichlorfon degrades rapidly, plants were only dried for 2 h before the bioassay. Systemic insecticides were applied 3 d before the bioassay to allow for root uptake. The bioassay was set up as previously described.

Each treatment was replicated six times for a total of 96 insects per concentration (n = 672 *B. insularis* for bifenthrin, carbaryl, and clothianidin; 864 for imidacloprid; and 576 for trichlorfon). The location of each insect (on or off the plant) and the number of dead *B. insularis* were assessed after 1, 4, 8, 24, 48, and 72 h. *Blissus insularis* mortality was scored as previously described. Insects were sexed at the end of the experiment using a dissecting microscope.

Statistical Analysis

The LC_{50} and LC_{90} values, 95% confidence limits (CL), slopes of the regression lines, and concentration-response relationships were estimated by probit analysis. In addition, likelihood ratio tests to examine the hypothesis of parallelism and equality of the regression lines among individual replicates were used to determine variability in the bioassays in the comparison test using PoloPlus (LeOra Software 2002).

To determine the appropriate exposure time for insecticides used in all tests, differences between LC_{50} and LC_{90} values for the different scoring intervals within each sex or bioassay were determined by the 95% CL of lethal concentration ratios (LCRs). LCR confidence limits (95%) that did not include 1.0 were considered significant ($P < 0.05$) (Robertson and Priesler 1992, Robertson et al. 2007). Conventionally, if the 95% confidence limits of the lethal concentrations overlapped, then the lethal concentrations were not considered significantly different. However, the ratio test has greater statistical power and lower Type I error rates, so this statistical test was used in this study (Wheeler et al. 2006, Robertson et al. 2007).

Subsamples of the comparison test data were taken in order to determine if smaller sampling sizes could be used for the sprig-dip and airbrush bioassays. This was done by taking the raw concentration-mortality data from the optimal exposure time (as described above) for both bioassays and for each insecticide tested (bifenthrin and imidacloprid), and entering into columns in Mircrosoft Excel. In empty columns next to each data set, random numbers were assigned to raw data using the formula =RAND() and typing Ctrl + Enter. The formula was then dragged down each column, assigning random numbers to all cells in the adjacent column. Columns were then sorted (ascending to descending) and subsamples were chosen starting with the first cell and subsequent cells until the desired subsample was taken (i.e., sample of 10, 20, 30 for each concentration). Data sets were re-sorted for each subsample taken. Subsamples were analyzed using PoloPlus and LCRs (Robertson and Priesler 1992, Robertson et al. 2007) were used to determine significant differences between LC_{50} and LC_{90} values compared to the original sample size used in the comparison test.

For the airbrush bioassay, the significance of differences between LC_{50} and LC_{90} values for male and female *B. insularis* recorded at 24, 48, and 72 h was determined by the 95% CL of

LCRs at the LC_{50} and LC_{90} (Robertson and Priesler 1992, Robertson et al. 2007). In addition, an analysis of variance (ANOVA) was conducted to determine differences between male and female *B. insularis* in their ability to locate plant material within the first hour of the airbrush bioassay. If significant, treatment means were analyzed using the Tukey-Kramer (HSD) test using Jmp® (SAS Institute Inc. 2001).

Results and Discussion

Determining Uptake for Systemic Insecticides

The concentration-mortality data for *B. insularis* exposed to St. Augustinegrass treated with clothianidin 1, 3, and 7 d before bioassay are shown in Table 4-2. LC_{90} values obtained at the 4- and 8-h scoring intervals for *B. insularis* exposed to plants treated 1 d prior to testing were significantly higher than LC_{90} values from respective scoring intervals for plants treated 3 and 7 d previously. However, LC values obtained from *B. insularis* exposed to 3- and 7-d-treated plants were not different. Because clothianidin and imidacloprid are similar in water solubility, a 3-d interval from spray to test was chosen for both products in the airbrush bioassay.

Comparison of Airbrush and Sprig-Dip Bioassays

Bifenthrin

The sprig-dip bioassay produced significantly lower LC_{50} values at all scoring intervals, as well as LC_{90} values at 48 and 72 h, compared to the airbrush bioassay for bifenthrin (Table 4-3). The slope values for the sprig-dip bioassay were also lower than those for the airbrush bioassay (1.2-1.4 and 2.1-2.3, respectively). Hypothesis tests for equality and parallelism of the regression lines for each replicate for the sprig-dip bioassay show that slopes were not significantly different, but the intercepts were (Figure 4-3 A and C). The intercept of a probit or logit regression should correspond with the response that occurs with no treatment (Robertson et al. 2007), but control mortality was not observed. Differences between intercepts could have

been due to physical processes (i.e., absorption through the cuticle or gut, target site sensitivity, or excretion) (Robertson and Preisler 1992, Robertson et al. 2007). However, if physical processes were the cause, it would likely have shown up in the airbrush bioassay, as well, because *B. insularis* from the same colony, generation, and age were used for both bioassays and all assays were conducted at the same time under the same conditions. It is possible that variability between replicates in the sprig-dip bioassay occurred due to the large degree of untreated surface area in petri dishes, resulting in differences in the ability of *B. insularis* to locate plant material. Regression lines for replicates in the airbrush bioassay were more similar (Figure 4-3 B and D) and results of the hypothesis tests for equality and parallelism of the regression lines for each replicate showed that slopes and intercepts were not significantly different (Figure 4-3 B and D). This suggests that there was a more uniform response among insects in the airbrush bioassay than in the sprig-dip bioassay.

Comparisons of LC_{50} and LC_{90} values within each bioassay to determine appropriate exposure time for bifenthrin show the response at 24 and 48 h was similar to 72-h values with use of the airbrush bioassay [LCR_{50} for 24 h: 0.8 (0.5-1.2), LCR_{50} for 48 h: 1.0 (0.7-1.4); LCR_{90} for 24 h: 0.9 (0.5-1.5), LCR_{90} for 48 h: 1.0 (0.6-1.7)]. For the sprig-dip bioassay, the LC_{50} values for the different scoring intervals were not different; however, the 24-h LC_{90} values were significantly higher than respective values for 48 and 72 h [LCR_{50} for 24 h: 0.5 (0.3-1.1), LCR_{50} for 48 h: 0.9 (0.4-1.9); LCR_{90} for 24 h: 0.3 (0.2-0.8), LCR_{90} for 48 h: 0.8 (0.4-1.6)]. This suggests that when using the airbrush method for testing bifenthrin, assays can be run for 24 h to estimate reliable LC_{50} and LC_{90} values. However, when using the sprig-dip bioassay, bifenthrin assays should be run for a minimum of 48 h to generate both LC_{50} and LC_{90} values. Considering the fast action of pyrethroids, the longer test time required for the sprig-dip bioassay may be due

to the larger untreated surface area in the petri dishes, resulting in differences in the time needed for *B. insularis* to locate plant material. This factor may also explain the high variability observed between replicates (Figure 4-3 A and C). Alternatively, pyrethroids often act as repellents and thus may have caused *B. insularis* to avoid the plant material in the petri dishes. However, in the airbrush bioassay, the close quarters in the trays greatly reduces this variable.

Imidacloprid

The sprig-dip bioassay produced significantly lower LC_{50} values at the 48- and 72-h scoring intervals and higher LC_{90} values at 24 h compared to the airbrush bioassay for imidacloprid (Table 4-3). However, *B. insularis* were more susceptible to imidacloprid after longer exposure (48 and 72 h) in both bioassays (Table 4-3). The LCR results comparing 72-h LC values to respective results obtained at 24 h within each bioassay show both bioassays had significantly higher LC_{50} and LC_{90} values at 24 h compared to respective 72-h values [sprig-dip: LCR_{50} for 24 h: 0.4 (0.3-0.6); LCR_{90} for 24 h: 0.2 (0.1-0.3)], [airbrush: LCR_{50} for 24 h: 0.5 (0.4-0.7); LCR_{90} for 24 h: 0.6 (0.4-0.9)]. However, 48-h LC values were similar to those for 72 h for both bioassays [sprig-dip: LCR_{50} for 48 h: 0.8 (0.6-1.0); LCR_{90} for 48 h: 0.8 (0.6-1.2)], [airbrush: LCR_{50} for 48 h: 0.8 (0.6-1.0); LCR_{90} for 48 h: 0.9 (0.6-1.3)]. This would suggest that assays should run for at least 48 h in both bioassays to account for increased susceptibility to imidacloprid and to obtain both LC_{50} and LC_{90} values. These data are similar to other reports of increased susceptibility to neonicotinoids after longer exposure times. Prabhaker et al. (2006) also reported increased susceptibility to the neonicotinoids acetamiprid and imidacloprid after 48 h compared to 24 h in populations of *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae).

Results for comparisons of replicates within each bioassay show wide variability at all scoring intervals for the sprig-dip bioassay for imidacloprid (Figure 4-4 A and C). Hypothesis tests of equality and parallelism of the different regression lines for each replicate show that

slopes and intercepts were significantly different at the 24-h scoring interval, but after 48 h slopes were similar while intercepts remained different (Figure 4-4 A and C). By contrast, the slopes and intercepts of regression lines for the different replicates were similar and more consistent for the airbrush bioassay (Figure 4-4 B and D), suggesting that insects were more uniform in response and in their ability to come into contact with plant material.

Subsampled comparison data--bifenthrin

The subsamples taken of the comparison study with bifenthrin using the airbrush bioassay produced similar LC_{50} values and narrow CLs compared to the original sample size of 280 (Table 4-4). Subsample sizes from 70-210 had slopes ranging from 1.7-2.7. Slopes and intercepts of regression lines for each subsample were similar to those of the original sample size of 280 (hypothesis test of equality = accept: $\chi^2 = 11.5$, df = 6, $P = 0.07$; parallelism = accept: $\chi^2 = 3.5$, df = 3, $P = 0.37$). These findings suggest that when using the airbrush bioassay to test bifenthrin, a sample size of 70 could be used to determine a reliable LC_{50} value. However, for estimation of reliable LC_{90} values, CL limits widen greatly as sample sizes are reduced from 210 to 70 (Table 4-4). Thus, to avoid excessively wide confidence limits at higher probit mortality levels, a sample size of 210 would be best.

For the sprig-dip bioassay, the subsamples taken of the comparison study with bifenthrin produced LC values that were not significantly different from the original sample size of 280 (Table 4-4). The slopes ranged from 1.3-1.9 and slopes and intercepts of regression lines for each subsample were similar to those of the original sample size of 280 (hypothesis test of equality = accept: $\chi^2 = 2.3$, df = 6, $P = 0.89$; parallelism = accept: $\chi^2 = 2.1$, df = 3, $P = 0.55$). However, the subsamples only produced an LC_{50} value for one of the subsamples (210) (Table 4-4). Concentration-mortality lines could not be produced for subsample sizes of 140 and 70

because variability was too high. Based on these results, a minimum of 210 *B. insularis* could be used when testing bifenthrin using the sprig-dip bioassay for estimating LC_{50} and LC_{90} values.

Subsampled comparison data--imidacloprid

A subsample size of 90 *B. insularis* was sufficient (Table 4-5) for estimation of LC_{50} and LC_{90} values for imidacloprid using the airbrush bioassay (Table 4-5). LC_{50} values obtained for all subsample sizes were similar to those of the original sample size (LC_{50} = 4.2 and LC_{90} = 20.9) and CL limits were relatively narrow (Table 4-5). The slopes ranged from 1.5-2.1 and slopes and intercepts of regression lines for each subsample were similar to those of the original sample of 720 (hypothesis test of equality = accept: $\chi^2 = 6.0$, df = 14, $P = 0.97$; parallelism = accept: $\chi^2 = 5.1$, df = 7, $P = 0.65$).

Concentration-mortality data for the sprig-dip bioassay also demonstrated similar LC_{50} and LC_{90} values compared to the data from the original sample size of 720. The slopes were more variable, ranging from 1.6-2.4 and slopes and intercepts of regression lines for each subsample were similar to those of the 720-sample size (hypothesis test of equality = accept: $\chi^2 = 6.9$, df = 14, $P = 0.94$; parallelism = accept: $\chi^2 = 4.5$, df = 7, $P = 0.94$). However, PoloPlus was unable to provide estimates for the 180-sample size. This may have been an artifact of the subsample obtained or it may indicate this is too small a sample for estimation of LC values for imidacloprid using the sprig-dip bioassay. If the latter condition is true, then a sample size of 270 should be used for estimation of LC_{50} as CL limits are still narrow at this sample size. A sample size of 360 *B. insularis* should be used for estimation of LC_{90} values for imidacloprid because CL limits widen considerably when smaller sample sizes were used (Table 4-5).

Robertson et al. (1984) determined that a sample size of 120 was the minimum necessary for calculating a reliable LC_{50} estimation, but for increased precision sample sizes of 240 or more are often necessary (Robertson et al. 1984, 2007; Robertson and Preisler 1992). However, the

authors noted that further investigation is needed to explore other combinations of sample size and dose placement in different bioassays (Robertson et al. 2007). Cherry and Nagata (2007) reported LC_{50} values for *B. insularis* exposed to imidacloprid for 24 h using the sprig-dip bioassay with sample sizes ranging from 120-360 *B. insularis*. In this study, LC values and sample sizes varied depending on the insecticide tested and bioassay used. These subsample data indicate that when using the sprig-dip bioassay, the optimal exposure time is 48 h using a minimum of 270 *B. insularis* when testing approximately 8 concentrations of imidacloprid for estimation of LC_{50} values and 360 *B. insularis* for estimation of the LC_{90}.

In terms of cost for supplies for each bioassay, the BioServe bioassay trays (BAW128) and perforated tray lids (BACV16) used in the airbrush bioassay cost 3.5¢ per insect. The cost of petri dishes (cat. number 08-757-12, Fisher Scientific, Pittsburgh, PA) and 70-mm Whatman filter paper (cat. number 1002 070, Whatman International Ltd, Maidstone, England) for use in the sprig-dip bioassay is 4.0¢ per insect. The time to set up each bioassay will vary depending on the number of concentrations tested and the number of replicates at each time interval. Not including the making of serial dilutions or treatment of plant material, one person can set up a single replicate of the airbrush bioassay with eight concentrations in 1-2 h. For the sprig-dip bioassay, one replicate with the same number of concentrations could be set up in 30 min to 1 h. The set up times for both bioassays may be greatly reduced if insects are anesthetized before introduction into tests; however, the effects of anesthetizing insects prior to insecticide exposure currently are not known. While the sprig-dip bioassay takes less time to set up per replicate, these data indicate that larger sample sizes and/or exposure times (for bifenthrin) are required for estimation of LC_{50} and LC_{90} values. One will need to take into consideration increased costs for

collecting or rearing larger numbers of insects in addition to increased costs for supplies and labor when using the spring-dip bioassay.

The BioServe bioassay trays were useful for testing insecticides against *B. insularis* and have been used successfully with Coleoptera (Lalitha et al. 2005), Lepidoptera (Bomford and Isman 1996, Simmonds and Stevenson 2001, Kokubun et al. 2003, Zoerb et al. 2003, Akhtar and Isman 2004, Oigiangbe et al. 2007), and Thysanoptera (Brown et al. 2003). The trays allowed easier observation of individual insects (including differences between males and females, behavior, recovery time) and reduced evaluation time compared to the sprig-dip bioassay. Prior bioassays involving sprig dips (Congdon and Buss 2004, 2006) were time consuming when scoring and observations of individual insects were difficult. The BioServe perforated lids helped keep St. Augustinegrass succulent for *B. insularis* feeding for the duration of each test while allowing adequate ventilation. The use of Bounce® fabric softener for static charge reduction did not appear to affect *B. insularis* as there was at most 2 – 3% mortality observed in controls after 72 h.

Airbrush Bioassay

Male and female *B. insularis* located plant material equally well in all tests within 1 h (Table 4-6). Due to variability in the time at which insects were in contact with and/or fed on plant material, concentration-mortality lines were only determined for the 24-, 48-, and 72-h intervals. LC_{50} and LC_{90} values were within the range of concentrations tested for each insecticide (Table 4-1) with the exception of bifenthrin. The LC_{90} values for females after 24, 48, and 72 h were greater (32.9, 31.9, and 30.5 µg/ml, respectively, Table 4-7) than the highest concentration of bifenthrin used in this study (26 µg/ml) indicating these are calculated estimates. Using a higher concentration may have provided true LC_{90} values for females exposed to bifenthrin.

Of the five insecticides tested using the airbrush bioassay, clothianidin and imidacloprid were most toxic to *B. insularis* (regardless of sex), particularly after 48 and 72 h, followed by bifenthrin (Table 4-7). Carbaryl and trichlorfon were the least toxic of the insecticides tested, which may in part be due to their short residual and/or volatility in turfgrass. Bifenthrin, carbaryl, and trichlorfon are often used for control of surface- and/or subsurface-feeding pests in turf (Clark and Kenna 2001). Neonicotinoids, such as clothianidin and imidacloprid, are known to be highly effective against piercing-sucking insects (Cahill et al. 1996; Elbert et al. 1996; Nauen et al. 1996, 1998; Tomizawa and Casida 2005; Magalhaes et al. 2008). The low affinity of neonicotinoids for vertebrate compared to insect nicotinic acetylcholine receptors (Tomizawa and Casida 2005), along with a long residual life, allows clothianidin and imidacloprid to be applied at lower rates compared to insecticides with shorter residual lives such as carbaryl and trichlorfon.

The slope values obtained after each scoring interval for the contact insecticides (bifenthrin, carbaryl, and trichlorfon) are steep, suggesting that *B. insularis* males and females responded uniformly to these products (Georghiou and Metcalf 1961; ffrench-Constant and Roush 1990; Prabhaker et al. 1996, 2006). However, the slope values obtained for the systemic insecticides (clothianidin and imidacloprid) were lower and more variable across scoring intervals for male and female *B. insularis*, particularly for imidacloprid. Alternatively, it is possible that the *B. insularis* population that was tested was more heterogeneous in its response to these insecticides as compared to the contact insecticides (Table 4-6).

LC_{50} values at all scoring intervals showed that male *B. insularis* were significantly more susceptible to bifenthrin, carbaryl, clothianidin, and imidacloprid than females (Table 4-7). However, the LC_{90} values for clothianidin are not different between male and female *B.*

insularis. Both sexes demonstrated an equal response to trichlorfon at all scoring intervals (Table 4-7).

The baseline data generated using the airbrush bioassay showing that female *B. insularis* are more tolerant to most of the insecticides tested is not unusual, given that females are significantly larger than male *B. insularis* (Cherry and Wilson 2003). However, size cannot always be used as an indicator for predicting sexual differences in susceptibility. For example, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) males were more tolerant to thiomethoxam despite being smaller than females (Nielson et al. 2008). Also, one sex may show more tolerance to one insecticide but not another (de Lane et al. 2001, Shearer and Usmani 2001). Sexual differences in insecticide susceptibility also occur in other insects, including *Cimex lectularius* L. (Hemiptera: Cimicidae) (Busvine and Lien 1961), *Diglyphus begini* (Ashmead) (Hymenoptera: Eulophidae) (Rathman et al. 1992), *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) (de Lame et al. 2001, Shearer and Usmani 2001), and *H. halys* (Nielson et al. 2008). This study is the first to document differences in insecticide susceptibility between male and female *B. insularis*.

Analysis of LC_{50} and LC_{90} values for the different scoring intervals within each sex show that the responses of male and female *B. insularis* after 24 and 48 h were similar to their responses after the 72-h interval for the contact insecticides bifenthrin, carbaryl and trichlorfon (Table 4- 8). This suggests that when using the airbrush method for testing these insecticides, tests can be scored after 24 h to obtain both LC_{50} and LC_{90} values for male and female *B.insularis*. However, when testing the systemic insecticides clothianidin and imidacloprid using the airbrush bioassay, tests should be run for at least 48 h before scoring results. The LCR_{50} for clothianidin showed LC_{50} values after 24 h for females were significantly higher than their

respective 48-h and 72-h values (Table 4-9). LC_{50} and LC_{90} values recorded for males and females after 24 h were significantly higher for imidacloprid (Table 4-9).

The results of this study indicate that when using the airbrush bioassay, contact insecticides should have an exposure time of 24 h while systemic ones should run for 48 h. These data are consistent with the results of the airbrush bioassay conducted in the comparison test, but it would be useful to compare the two bioassays using other insecticides and *B. insularis* populations because responses may differ (ffrench-Constant and Roush 1990, Scharf et al. 1995, Studebaker and Kring 2003).

As part of a resistance management program, it is recommended that the sprig-dip bioassay be used for detection of bifenthrin-resistant *B. insularis* populations because it was more sensitive in detecting lower LC values in this study. The airbrush bioassay would be beneficial for use in studies concerning cross resistance, resistance mechanisms, mode-of-inheritance, and stability of pyrethroid resistance in *B. insularis*. In addition, the airbrush bioassay could be used for determining a diagnostic dose for pyrethroid- and imidacloprid-resistant *B. insularis* populations because this bioassay results in less variance.

Table 4-1. Insecticides tested against a susceptible colony of *B. insularis*

Class	Compound	Registered name	Recommended label rate*	Range used	Company
Carbamate	Carbaryl	Sevin® SL	5.622 – 7496 µg/ml	7.3-468 µg/ml	Bayer Environmental Science, Research Triangle Park, NC
Neonicotinoid	Clothianidin	Arena™ 50 WDG	368 µg/ml	0.7-23 µg/ml	Arysta Life Science, San Francisco, CA
	Imidacloprid	Merit® 2F	375 µg/ml	0.7-93 µg/ml	Bayer Environmental Science, Research Triangle Park, NC
Organophosphate	Trichlorfon	Dylox® 80 T & O	976 – 1,464 µg/ml	23-366 µg/ml	Bayer Environmental Science, Research Triangle Park, NC
Pyrethroid	Bifenthrin	TalstarOne®	209 µg/ml	0.8-26 µg/ml	FMC Corporation, Philadelphia, PA

* Label rates calculated using a spray application volume of 11.3 L/92.9 m^2.

Table 4-2. Concentration-mortality data (at LC_{50} and LC_{90}) at different exposure times for a susceptible *B. insularis* laboratory colony exposed to St. Augustinegrass treated with clothianidin 1, 3, and 7 d before bioassay.

Treatment time (d)	*n*	Exposure time (h)	Slope ± SE	LC_{50} (95% CL)[a]	LCR_{50} (95% CL)[b]	LC_{90} (95% CL)[a]	LCR_{90} (95% CL)[b]	x^2 (df)[c]
7	144	4	1.0 ± 0.2	14.5 (3.5–34.6)	---	236 (80.3–6,284)	---	10.0 (6)[d]
		8	1.5 ± 0.3	8.5 (1.4–19.9)	---	58.6 (24.4–690)	---	12.1 (6)[d]
		24	1.3 ± 0.4	1.7 (0.1–3.8)	---	15.7 (8.3–51.0)	---	5.7 (6)[d]
		48	1.3 ± 0.4	1.3 (0.02–3.0)	---	11.4 (5.4–38.5)	---	3.4 (6)[d]
		72	1.5 ± 0.5	1.3 (0.04–3.1)	---	9.3 (4.8–30.4)	---	1.1 (6)[d]
3	144	4	1.4 ± 0.2	15.9 (5.1–36.1)	0.9 (0.4–1.8)	121(49.1–1,692)	1.9 (0.5–6.4)	13.3 (6)[d]
		8	1.2 ± 0.2	8.4 (0.5–22.9)	1.0 (0.4–2.5)	101 (35.3–3,773)	0.6 (0.1–1.7)	11.2 (6)[d]
		24	1.9 ± 0.6	2.0 (0.2–3.6)	0.8 (0.1–3.8)	9.3 (5.6–31.4)	1.7 (0.6–4.3)	3.1 (6)[d]
		48	1.9 ± 0.6	2.0 (0.2–3.6)	0.6 (0.1–3.4)	9.3 (5.6–31.4)	1.2 (0.4–3.1)	3.1 (6)[d]
		72	1.9 ± 0.7	1.8 (0.09–3.4)	0.8 (0.1–3.9)	8.4 (4.9–29.9)	1.1 (0.4–2.7)	1.7 (6)[d]
1	144	4	1.7 ± 0.3	7.9 (4.7–11.7)	1.8 (0.5–6.4)	45.3 (28.0–104)	5.2 (1.6–16.4)*	4.7 (6)[d]
		8	2.0 ± 0.4	4.9 (2.8–7.3)	1.7 (0.8–3.4)	21.9 (14.2–49.6)	2.7 (1.1–6.3)*	4.3 (6)[d]
		24	2.1 ± 0.7	2.0 (0.2–3.5)	0.8 (0.1–3.5)	8.4 (5.3–25.3	1.9 (0.7–4.6)	1.1 (5)[d]
		48	2.0 ± 0.7	1.9 (0.1–3.3)	0.6 (0.1–3.5)	7.6 (4.8–23.8)	1.5 (0.6–3.7)	0.5 (5)[d]
		72	2.1 ± 0.7	1.9 (0.1–3.3)	0.7 (0.1–3.4)	7.6 (4.8–23.8)	1.2 (0.5–2.9)	0.5 (5)[d]

[a] LC_{50} and LC_{90} values in µg/mL (95% confidence limits).

[b] Lethal concentration ratios with 95% confidence limits indicating the fold-difference for males for each insecticide in comparison to the respective female *B. insularis* scoring interval at LC_{50} and LC_{90}. Confidence limits that include 1.0 indicate no significant difference from female scoring interval ($P \leq 0.05$). ***** Shows ratios that are significant.

[c] Pearson chi-square statistic (degrees of freedom).

[d] Good fit of the data to the probit model ($P > 0.05$).

Table 4-3. Comparison of concentration-mortality data (at LC_{50} and LC_{90}) for a susceptible *B. insularis* laboratory colony to bifenthrin and imidacloprid at 24, 48, and 72 h using the airbrush and sprig-dip bioassays.

Bioassay used	Insecticide tested	Test time (h)	Slope ± SE	LC_{50} (95% CL)[b]	LCR_{50} (95% CL)[c]	LC_{90} (95% CL)[b]	LCR_{90} (95% CL)[c]	x^2 (df)[d]
Sprig-dip	Bifenthrin	24	1.2 ± 0.2	1.4 (0.3-2.8)	0.2 (0.1–0.4)*	17.1 (7.6-221)	0.8 (0.4–1.7)	7.4 (4)[e]
		48	1.3 ± 0.2	0.8 (0.1–1.6)	0.2 (0.1–0.3)*	7.6 (4.1-33.5)	0.4 (0.2–0.8)*	5.6 (4)[e]
		72	1.4 ± 0.2	0.8 (0.04–1.6)	0.2 (0.1–0.3)*	6.1 (3.0-68.4)	0.3 (0.2–0.6)*	9.1 (4)[e]
Airbrush		24	2.3 ± 0.2	5.6 (3.2-10.6)	---	20.2 (10.7-111)	---	12.6 (4)
		48	2.2 ± 0.2	4.8 (3.1-7.0)	---	17.6 (10.7-40.9)	---	6.7 (4)[e]
		72	2.1 ± 0.3	4.5 (1.7-7.4)	---	18.0 (10.6-78.8)	---	6.8 (4)[e]
Sprig-dip	Imidacloprid	24	1.1 ± 0.1	5.9 (3.9-8.8)	1.0 (0.7–1.2)	82.4 (44.3–223)	2.8 (1.6–4.5)*	12.0 (6)
		48	1.8 ± 0.1	3.1 (2.5-3.8)	0.7 (0.5–0.9)*	16.5 (13.1-22.1)	0.8 (0.5–1.1)	6.0 (6)[e]
		72	1.7 ± 0.1	2.5 (1.8-3.2)	0.7 (0.5–0.9)*	14.0 (10.3-21.1)	0.8 (0.5–1.1)	6.5 (6)[e]
Airbrush		24	1.9 ± 0.1	6.1 (5.2-7.1)	---	29.7 (23.4–38.9)	---	4.7 (6)[e]
		48	1.8 ± 0.1	4.2 (3.2-5.3)	---	21.1 (15.4-31.7)	---	7.8 (6)[e]
		72	1.7 ± 0.1	3.3 (2.6-4.1)	---	18.3 (13.7-27.0)	---	6.3 (6)[e]

[a] $n = 280$ *B. insularis* were tested for each bioassay for bifenthrin and 720 for each bioassay for imidacloprid.

[b] LC_{50} and LC_{90} values in µg/mL (95% confidence limits).

[c] Lethal concentration ratios with 95% confidence limits for the sprig dip bioassay indicating the fold-difference for each test time in comparison to respective airbrush test times at LC_{50} and LC_{90}. Confidence limits that include 1.0 indicate no significant difference from the respective airbrush test time ($P \leq 0.05$). ***** Indicates ratios that are significant.

[d] Pearson chi-square statistic (degrees of freedom).

[e] Good fit of the data to the probit model ($P > 0.05$).

Table 4-4. Comparison of subsampled[a] concentration-mortality data (at LC_{50} and LC_{90}) for a susceptible *B. insularis* laboratory colony exposed to bifenthrin using the airbrush and sprig-dip bioassays.

Bioassay used	Sample size	Slope ± SE	LC_{50} (95% CL)[b]	LCR_{50} (95% CL)[c]	LC_{90} (95% CL)[b]	LCR_{90} (95% CL)[c]	x^2 (df)[d]
Sprig-dip	280	1.3 ± 0.2	0.8 (0.1–1.6)	---	7.6 (4.1–33.5)	---	5.6 (4)[e]
	210	1.3 ± 0.2	0.9 (0.2–1.7)	0.9 (0.4–1.9)	8.7 (4.6–40.4)	0.9 (0.4–1.8)	4.2 (4)[e]
	140	N/A	N/A	N/A	N/A	N/A	N/A
	70	N/A	N/A	N/A	N/A	N/A	N/A
Airbrush	280	2.3 ± 0.2	5.6 (3.2–10.6)	---	20.2 (10.7–111)	---	12.6 (4)
	210	2.7 ± 0.3	5.9 (3.5–10.7)	0.9 (0.7–1.2)	17.6 (10.0–83.1)	1.1 (0.6–1.9)	10.0 (4)
	140	2.4 ± 0.4	7.5 (3.5–23.7)	0.7 (0.5–1.0)	25.0 (11.4–852)	0.8 (0.4–1.5)	11.3 (4)[e]
	70	1.7 ± 0.4	3.8 (2.2–6.6)	1.5 (0.8–2.5)	21.6 (11.0–108)	0.9 (0.3–2.5)	3.1 (4)[e]

[a] Subsamples were selected from the comparison test using data from the 48 h sprig-dip and 24 h airbrush bioassays for bifenthrin. Scoring intervals were chosen based on the appropriate amount of time needed to run each bioassay for bifenthrin.

[b] LC_{50} and LC_{90} values in µg/mL (95% confidence limits).

[c] Lethal concentration ratios with 95% confidence limits for each bioassay indicating the fold-difference for subsamples within each bioassay in comparison to respective original sample size at LC_{50} and LC_{90}. Confidence limits that include 1.0 indicate no significant difference from the respective original sample size ($P \leq 0.05$).

[d] Pearson chi-square statistic (degrees of freedom).

[e] Good fit of the data to the probit model ($P > 0.05$).

Table 4-5. Comparison of subsampled[a] comparison test concentration-mortality data (at LC_{50} and LC_{90}) for a susceptible *B. insularis* laboratory colony exposed to imidacloprid using the airbrush and sprig-dip bioassays.

Bioassay used	Sample size	Slope ± SE	LC_{50} (95% CL)[b]	LCR_{50} (95% CL)[c]	LC_{90} (95% CL)[b]	LCR_{90} (95% CL)[c]	x^2 (df)[d]
Sprig-dip	720	1.8 ± 0.1	3.1 (2.5–3.8)	---	16.5 (13.1–22.1)	---	6.0 (6)[e]
	630	1.8 ± 0.1	3.2 2.3–4.1)	1.0 (0.7–1.3)	15.8 (11.5–24.1)	1.0 (0.7–1.5)	6.8 (6)[e]
	540	1.6 ± 0.1	3.0 (2.1–4.2)	1.0 (0.7–1.4)	18.3 (12.5–31.4)	0.9 (0.5–1.3)	7.6 (6)[e]
	450	1.7 ± 0.1	3.6 (2.3–5.0)	0.9 (0.6–1.2)	20.6 (13.4–39.0)	0.8 (0.5–1.2)	8.5 (6)[e]
	360	1.7 ± 0.2	3.4 (2.0–5.0)	0.9 (0.6–1.3)	18.8 (11.9–38.4)	0.9 (0.5–1.3)	8.2 (6)[e]
	270	2.0 ± 0.3	3.2 (0.8–7.0)	1.0 (0.6–1.4)	14.0 (6.5–125)	1.2 (0.7–1.9)	24.8 (6)
	180	2.0 ± 0.3	N/A	N/A	N/A	N/A	N/A
	90	2.4 ± 0.6	4.3 (2.0–7.0)	0.7 (0.4–1.3)	14.9 (8.9–43.4)	1.1 (0.5–2.2)	3.7 (6)[e]
Airbrush	720	1.8 ± 0.1	4.2 (3.3–5.3)	---	20.9 (15.4–31.7)	---	7.8 (6)[e]
	630	1.8 ± 0.1	4.1 (3.1–5.4)	1.0 (0.8–1.3)	21.0 (14.7–35.0)	1.0 (0.6–1.4)	9.3 (6)[e]
	540	1.8 ± 0.1	4.0 (3.2–4.8)	1.0 (0.8–1.3)	21.1 (16.2–29.5)	1.0 (0.6–1.4)	5.7 (6)[e]
	450	1.8 ± 0.1	4.1 (2.9–5.7)	1.0 (0.7–1.3)	20.4 (13.2–40.3)	1.0 (0.6–1.5)	10.6 (6)[e]
	360	2.1 ± 0.2	4.0 (3.3–5.0)	1.0 (0.8–1.3)	16.6 (12.5–24.4)	1.3 (0.8–1.9)	5.7 (6)[e]
	270	1.8 ± 0.2	4.2 (2.8–6.0)	1.0 (0.7–1.3)	22.5 (14.0–48.0)	0.9 (0.5–1.5)	7.1 (6)[e]
	180	1.5 ± 0.2	4.5 (3.0–6.5)	0.9 (0.6–1.4)	33.8 (20.5–74.1)	0.6 (0.3–1.2)	4.4 (6)[e]
	90	1.8 ± 0.3	4.2 (2.5–6.7)	1.0 (0.6–1.6)	21.8 (12.3–63.5)	1.0 (0.4–2.1)	1.6 (6)[e]

[a] Subsamples were selected from the 48-h scoring intervals for both the sprig-dip and airbrush bioassays for imidacloprid. Scoring intervals were chosen based on the appropriate amount of time needed to run each bioassay for imidacloprid.

[b] LC_{50} and LC_{90} values in µg/mL (95% confidence limits).

[c] Lethal concentration ratios with 95% confidence limits for each bioassay indicating the fold-difference for subsamples within each bioassay in comparison to respective original sample size at LC_{50} and LC_{90}. Confidence limits that include 1.0 indicate no significant difference from the respective original sample size ($P \leq 0.05$). ***** Shows ratios that are significant.

[d] Pearson chi-square statistic (degrees of freedom).

[e] Good fit of the data to the probit model ($P > 0.05$).

Table 4-6. The mean number ± SEM of male and female B. insularis that located treated plant material within 1 h of introduction into the airbrush bioassay.

Insecticide	Mean no. ♀ ± SEM	Mean no. ♂ ± SEM	*F*-value	df	*P*-value
Bifenthrin	0.34 ± 0. 3	0.33 ± 0.02	0.27	2, 670	0.76
Carbaryl	0.47 ± 0.03	0.41 ± 0.03	2.20	2, 670	0.14
Clothianidin	0.48 ± 0.03	0.41 ± 0.03	3.48	2, 670	0.05
Imidacloprid	0.50 ± 0.02	0.46 ± 0.02	1.49	2, 862	0.22
Trichlorfon	0.64 ± 0.03	0.62 ± 0.03	0.14	2, 574	0.70

Table 4-7. Concentration-mortality data (at LC_{50} and LC_{90}) compared for males and females from a susceptible *B. insularis* laboratory colony treated with five insecticides after 24, 48, and 72 h using the airbrush bioassay.

Insecticide tested	n	sex	Test time (h)	Slope ± SE	LC_{50} (95% CL)[a]	LCR_{50} (95% CL)[b]	LC_{90} (95% CL)[a]	LCR_{90} (95% CL)[b]	x^2 (df)[c]
Bifenthrin	376	Males	24	2.5 ± 0.2	6.4 (5.0–8.4)	0.5 (0.4–0.8)*	21.4 (15.1–36.6)	0.6 (0.3–1.3)	4.1 (4)[d]
			48	2.4 ± 0.3	6.0 (4.5–8.1)	0.6 (0.4–0.8)*	20.6 (13.9–39.2)	0.6 (0.3–1.3)	5.4 (4)[d]
			72	2.4 ± 0.2	5.5 (4.3–7.2)	0.6 (0.4–0.7)*	18.6 (13.0–32.3)	0.6 (0.4–0.9)*	4.6 (4)[d]
	296	Females	24	2.8 ± 0.6	11.5 (9.2–16.6)	---	32.9 (21.1–90.6)	---	1.7 (3)[d]
			48	2.7 ± 0.6	10.7 (8.4–15.1)	---	31.9 (20.3–93.1)	---	2.2 (3)[d]
			72	2.6 ± 0.3	9.7 (7.9–11.9)	---	30.5 (22.8–47.6)	---	2.2 (4)[d]
Carbaryl	334	Males	24	2.9 ± 0.3	104 (88.0–123)	0.6 (0.5–0.8)*	287 (229–391)	0.5 (0.3–0.7)*	1.0 (4)[d]
			48	2.6 ± 0.3	90.1 (59.5–134)	0.6 (0.5–0.8)*	281 (178–694)	0.7 (0.4–1.0)	8.6 (4)
			72	2.6 ± 0.3	88.8 (59.7–130)	0.6 (0.5–0.9)*	276 (178–643)	0.7 (0.4–1.0)	8.0 (4)
	338	Females	24	2.4 ± 0.3	167 (121–241)	---	572 (361–1,410)	---	3.4 (3)[d]
			48	2.7 ± 0.3	142 (119–169)	---	424 (329–606)	---	2.1 (3)[d]
			72	2.7 ± 0.4	139 (111–169)	---	407 (315–603)	---	1.0 (3)[d]
Clothianidin	344	Males	24	1.9 ± 0.2	4.1 (3.3–5.0)	0.6 (0.4–0.9)*	18.8 (13.7–29.2)	0.6 (0.3–1.2)	3.4 (4)[d]
			48	2.0 ± 0.2	3.7 (3.0–4.5)	0.7 (0.5–0.9)*	16.7 (12.3–25.5)	0.7 (0.4–1.5)	2.0 (4)[d]
			72	2.1 ± 0.2	3.2 (2.2–4.3)	0.7 (0.5–0.9)*	12.1 (8.2–22.6)	0.6 (0.3–1.1)	4.5 (4)[d]
	328	Females	24	1.8 ± 0.2	6.4 (3.7–22.6)	---	32.6 (12.7–1,619)	---	6.3 (3)[d]
			48	2.1 ± 0.3	5.4 (3.6–9.7)	---	22.2 (11.6–112)	---	4.0 (3)[d]
			72	2.0 ± 0.2	4.5 (2.8–8.7)	---	19.1 (9.5–139)	---	5.4 (3)[d]
Imidacloprid	456	Males	24	2.5 ± 0.2	10.5 (8.9–12.4)	0.6 (0.5–0.8)*	34.1 (27.1–45.9)	0.4 (0.3–0.7)*	2.5 (6)[d]
			48	1.8 ± 0.2	3.1 (2.1–4.3)	0.7 (0.5–0.9)*	15.7 (10.3–30.9)	0.7 (0.4–1.1)	11.0 (6)
			72	1.5 ± 0.2	2.2 (1.3–3.2)	0.5 (0.4–0.8)*	14.8 (9.1–32.9)	0.6 (0.3–0.9)*	11.0 (6)
	408	Females	24	2.0 ± 0.2	17.4 (14.3–21.3)	---	74.7 (55.8–110)	---	5.3 (6)[d]

Table 4-7. Continued.

			48	1.9 ± 0.2	4.6 (2.8–6.9)	---	22.3 (13.4–54.4)	---	11.0 (5)
			72	1.6 ± 0.1	4.0 (3.0–5.2)	---	25.3 (18.4–38.5)	---	3.6 (6)[d]
Trichlorfon	277	Males	24	2.6 ± 0.3	79.2 (34.1–172)	1.0 (0.8–1.4)	244 (127–3,202)	0.9 (0.6–1.5)	12.6 (3)
			48	2.9 ± 0.3	69.6 (45.1–102)	1.1 (0.8–1.4)	194 (127-480)	0.9 (0.6–1.4)	4.9 (3)[d]
			72	3.1 ± 0.3	64.3 (37.8–102)	1.0 (0.8–1.4)	167 (105–541)	0.9 (0.6–1.3)	6.9 (3)
	299	Females	24	2.4 ± 0.4	74.7 (45.8–110)	---	254 (161–692)	---	4.5 (3)[d]
			48	2.5 ± 0.2	64.7 (43.7–88.2)	---	210 (144–423)	---	3.1 (3)[d]
			72	2.5 ± 0.3	61.0 (37.9–86.6)	---	193 (128–451)	---	4.1 (3)[d]

[a] LC_{50} and LC_{90} values in µg/mL (95% confidence limits).
[b] Lethal concentration ratios with 95% confidence limits indicating the fold-difference for males for each insecticide in comparison to the respective female *B. insularis* scoring interval at LC_{50} and LC_{90}. Confidence limits that include 1.0 indicate no significant difference from female scoring interval ($P \leq 0.05$). ***** Shows ratios that are significant.
[c] Pearson chi-square statistic (degrees of freedom).
[d] Good fit of the data to the probit model ($P > 0.05$).

Table 4-8. Analysis of LC_{50} values for 24, 48, and 72 h within each *B. insularis* sex to determine bioassay time for the contact insecticides bifenthrin, carbaryl, and trichlorfon.

Insecticide	Sex	Time (hours)	LCR_{50}[a]	95% CL	LCR_{90}[a]	95% CL
Bifenthrin	M	24	0.9	(0.7-1.1)	0.9	(0.6-1.3)
		48	0.9	(0.7-1.2)	0.9	(0.6-1.4)
	F	24	0.8	(0.6-1.1)	0.9	(0.5-1.5)
		48	0.9	(0.7-1.2)	0.9	(0.5-1.5)
Carbaryl	M	24	0.8	(0.7-1.1)	1.0	(0.6-1.4)
		48	1.0	(0.8-1.3)	1.0	(0.6-1.4)
	F	24	0.8	(0.6-1.0)	0.7	(0.4-1.1)
		48	1.0	(0.7-1.2)	1.0	(0.6-1.5)
Trichlorfon	M	24	0.8	(0.6-1.0)	0.7	(0.4-1.0)
		48	0.9	(0.7-1.2)	0.8	(0.5-1.2)
	F	24	0.8	(0.6-1.0)	0.8	(0.5-1.1)
		48	0.9	(0.7-1.2)	0.9	(0.6-1.4)

[a] Lethal concentration ratios with 95% confidence limits indicating the fold-difference in 48 h scoring times in comparison to the respective 24-h scoring interval within each sex for each insecticide at LC_{50} and LC_{90}. Confidence limits that include 1.0 indicate no significant difference from 24-h scoring interval ($P \leq 0.05$). ***** Shows ratios that are significant.

Table 4-9. Analysis of LC_{90} values for 24, 48, and 72 h within each *B. insularis* sex to determine bioassay time for the systemic insecticides clothianidin and imidacloprid.

Insecticide	Sex	Time	LCR_{50}	95% CL	LCR_{90}	95% CL
Clothianidin	M	24	0.8	(0.6-1.0)	0.6	(0.4-1.0)
		48	0.9	(0.6-1.1)	0.7	(0.4-1.2)
	F	24	0.7	(0.5-0.9)*	0.6	(0.3-1.0)
		48	0.8	(0.6-1.0)	0.7	(0.4-1.2)
Imidacloprid	M	24	0.2	(0.1-0.3)*	0.4	(0.2-0.6)*
		48	0.7	(0.5-1.0)	0.9	(0.5-1.4)
	F	24	0.2	(0.1-0.3)*	0.3	(0.2-0.5)*
		48	0.9	(0.6-1.2)	1.2	(0.7-1.9)

[a] Lethal concentration ratios with 95% confidence limits indicating the fold-difference in 48-h scoring times in comparison to the respective 24-h scoring interval within each sex for each insecticide at LC_{50} and LC_{90}. Confidence limits that include 1.0 indicate no significant difference from 24 h scoring interval ($P \leq 0.05$). * Shows ratios that are significant.

Figure 4-1. The sprig-dip bioassay conventionally used for testing insecticides against *B. insularis*.

Figure 4-2. The (A) Paasche airbrush and (B) BioServe bioassay tray and lid used in the airbrush bioassay. Photos by C. Vázquez.

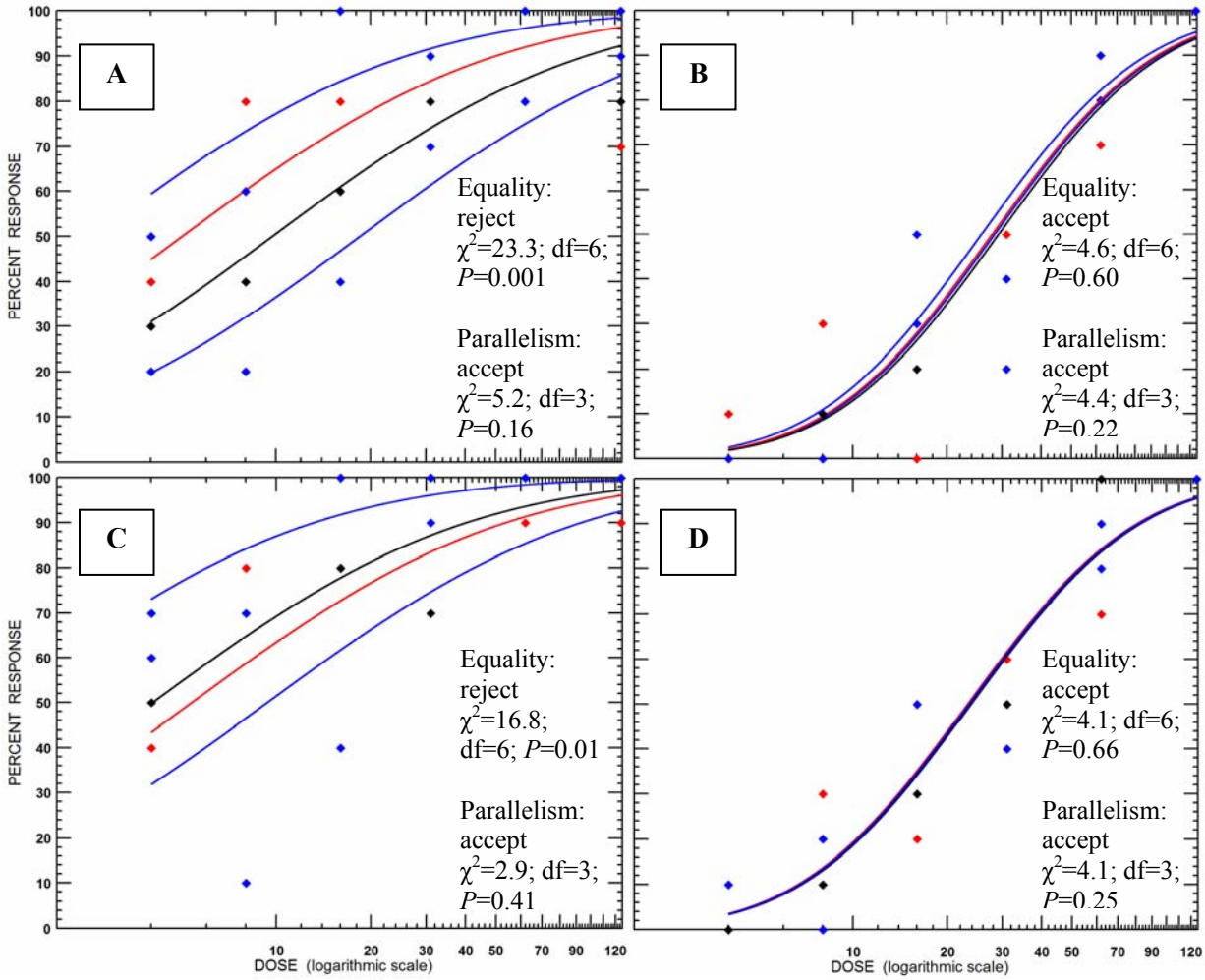

Figure. 4-3. The differences in variability between replicates of bifenthrin (n=280) for the (A) sprig-dip bioassay after 24 h, (B) airbrush bioassay after 24 h, (C) sprig-dip bioassay after 48 h, and (D) airbrush bioassay after 48 h. Each regression line within a graph represents one replicate. The results of hypothesis tests for equality and parallelism of the regression lines among individual replicates at each time interval are also shown.

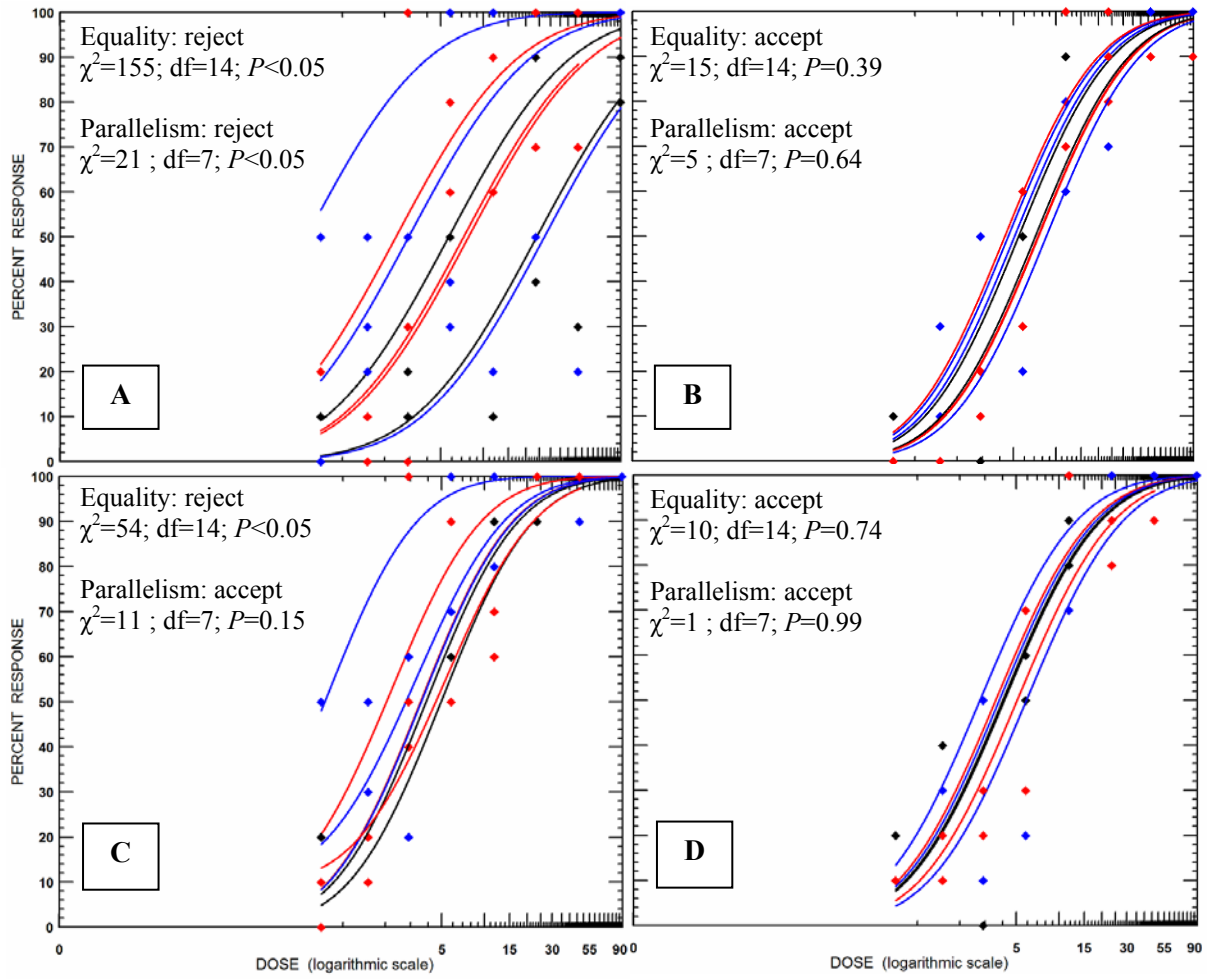

Figure. 4-4. The differences in variability between replicates of imidacloprid (n = 720) for the (A) sprig-dip bioassay after 24 h, (B) airbrush bioassay after 24 h, (C) sprig-dip bioassay after 48 h, and (D) airbrush bioassay after 48 h. Each regression line within a graph represents one replicate. The results of hypothesis tests for equality and parallelism of the regression lines among individual replicates at each time interval are also shown.

CHAPTER 5
CONCLUSIONS

The goals of this dissertation were to 1) sample select *B. insularis* populations in 2006 and 2008 in northern and central Florida to describe their susceptibility to bifenthrin, document new locations of bifenthrin resistance, and evaluate another pyrethroid, permethrin (Chapter 2), 2) develop a synchronous rearing method for *B. insularis* that produces insects of known age and generation (Chapter 3) , and 3) develop an improved bioassay that could be used for detecting insecticide susceptibility differences between male and female *B. insularis*, evaluate and validate both the sprig-dip and the new bioassay under standardized conditions, and determine optimal exposure times and sample sizes to be used for each bioassay for selected insecticides (Chapter 4).

The results of Chapter 2 show that bifenthrin resistance continues to be problematic, is becoming more widespread, and that there is a positive relationship between insecticide application and the development of bifenthrin resistance. Given the high number of insecticide applications observed in this study, resistance is likely to continue to spread into surrounding areas within the state unless management tactics are changed. In addition, the occurrence of cross resistance to other pyrethroids is evident from my data (population JC to permethrin) and that of Cherry and Nagata (2007). Florida's warm climate and high number of pests increases the need for lawn care professionals, and results in greater use of pesticides compared to other states (Short et al. 1982). Olkowski et al. (1978) reported pesticide use in the landscape is usually the result of response to aesthetic damage, rather than a reaction to medical problems or economic losses. While homeowners find St. Augustinegrass damage aesthetically displeasing, it can create economic losses when sod needs to be replaced or multiple insecticide applications are required to gain control of damaging *B. insularis* populations. One of the challenges that we

face in dealing with the resistance problem in Florida will be to change the mindset of lawn care professionals, homeowners, and homeowner associations.

Potter (1993) suggested that unnecessary or excessive use of pesticides can increase problems with thatch and pests by reducing beneficial organisms already present in the landscape, encouraging the development of resistance, or enhanced microbial degradation. Potter et al. (1990a, 1990b) demonstrated that Kentucky bluegrass plots treated with either chlordane or carbofuran greatly reduced earthworm numbers and resulted in increased thatch compared to untreated controls. Reinert (1978) observed *B. insularis* populations remained low in Florida St. Augustinegrass lawns that had an abundance of natural enemies and had not been treated with insecticides. Conversely, the author reported *B. insularis* populations reached outbreak densities on insecticide-treated lawns (Reinert 1978). It is clear from the many cases of resistance that have been reported over the last several decades (Wolfenbarger 1953; Kerr 1958, 1961; Reinert 1982a, 1982b; Reinert and Niemczyk 1982; Reinert and Portier 1983; Cherry and Nagata 2005, 2007) that many of Florida lawns can be considered high maintenance and receive considerable amounts of pesticides.

Florida is second only to California in terms of employment impacts of the turfgrass industry, providing 83,944 jobs in 2002 (Haydu et al. 2006). Considering the number of housing units in Florida increased from ~3.9 million in 1980 to 8.5 million in 2006 (an increase of 118%), the demand for quality turf and maintenance has likely increased (Haydu et al. 2005). In addition to meeting the demands of homeowners for high quality turf, lawn-care companies may also face high turnover of employees. New employees may not have developed the proper skills or been properly trained to monitor and manage *B. insularis* damage in lawns. Fothergill (1982) conducted a study in Massachusetts and found that the damage rate in lawns greatly increased as

the length of employee service decreased. Furthermore, the author noted that damage rate was more dependent on the experience and level of training employees received during and prior to their being allowed to monitor and treat lawns without direct supervision (Fothergill 1982).

Another issue that is likely adding to resistance problems in Florida lawns is the use of insecticides by sod growers, lawn-care companies, and homeowners for *B. insularis* control. Often the same active ingredients are available to all users at the same time year round (personal observation). While current pesticide use by homeowners is not known, Lipsey (1980) conducted a survey in Florida and found that homeowners used over 2,000,000 lbs of pesticides in a 12-month period during 1978-1979. Currently, pyrethroids, carbamates, neonicotinoids, and organophosphates are used for *B. insularis* control in Florida. Carbamate (propoxur) and organophosphate (chlorpyrifos) resistance was reported in the 1970s and 80s (Reinert and Niemczyk 1982, Reinert and Portier 1983). Cross-resistance patterns and the stability of propoxur and chlorpyrifos resistance in *B. insularis* are not known, making it unclear as to their effects on the current use of the carbamate, carbaryl, and the organophosphate, trichlorfon. Meanwhile, sod growers, lawn-care companies, and homeowners continue to use the same active ingredients, and quite possibly increase the rate of insecticide resistance development in *B. insularis*.

The problem of encroachment of *B. insularis* on to neighboring St. Augustinegrass lawns may also be an important factor in the development of resistance. Encroachment was observed in almost all of the lawns I collected from in 2006 and 2008 and the results of chapter 2 indicate that an individual lawn may represent a single *B. insularis* population. If the latter is true, theoretical studies suggest that the evolution of insecticide resistance may occur more rapidly in small, subdivided populations rather than large ones (Wright 1931, Crow and Kimura 1970,

Roush and Daly 1990). Also, effects of immigration on insecticide-resistant arthropods has been well discussed and show that immigration of susceptible individuals into treated areas can slow resistance development by increasing the frequency of susceptible alleles in a treated population (Comins 1977, Georghiou and Taylor 1977a, Curtis et al. 1978, Taylor and Georghiou 1979, Tabashnik and Croft 1982, Roush and Daly 1990, Tabashnik 1990). Alternately, emigration of resistant individuals from treated areas speeds the resistance development in the untreated area (Comins 1977). Sutherst and Comins (1979) indicated that acaricide-resistant cattle ticks, *Boophilus microplus* (Acari: Ixodidae), in Australia were spread primarily by emigration.

Immigration (or emigration) of *B. insularis* between lawns is poorly understood. In addition, flight patterns have not been evaluated in *B. insularis* and so it is unclear how far they can travel in order to find a new food source. Methods to measure immigration/emigration and wing polymorphism warrants further investigation to understand their impact on resistance development in *B. insularis*. Cherry (2001a) documented that macroptery is greatest in denser *B. insularis* populations. The use of aggregation or sex pheromone traps for determining increases in macropterus *B. insularis* for monitoring population increases in lawns would be useful. Traps and dye-marked *B. insularis* may also be of use to investigate movement patterns between lawns. Mark-release-recapture programs are frequently used for investigating animal populations (Southwood 1971) and dyes have been used for marking termites in studies involving foraging populations of termites (Lai 1977, Lai 1977 et al., Su and Scheffrahn 1988, Grace 1990, Jones 1990). Laboratory studies using test 5 rearing methods (chapter 3) could be used to facilitate studies in identifying *B. insularis* aggregation and/or sex pheromones. Aggregation pheromones have been identified for several heteroptera including *Eurydema rugosa* Motschulsky and *Euschistus* spp. (Hemiptera: Pentatomidae) (Ishiwatari 1976, Aldrich et al. 1991), *Oncopeltus*

fasciatus (Dallas) and *Lygaeus kalmii* Stål (Hemiptera: Lygaeidae) (Aller and Caldwell 2008), *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) (Leal et al. 1994), and *Riptortus clavatus* Thunberg and *Neomegalotomus parvus* (Westwood) (Hemiptera: Alydidae) (Numata et al. 1990, Ventura and Panizzi 2003). Also, sex pheromones have been found in heteroptera including *Maconellicoccus hirsutus* (Green) and *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) (Zada et al. 2004, Zhang et al. 2004, Zhang and Nie 2005, Zhang and Amalin 2005), and *Phorodon humuli* (Schrank) and *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) (Campbell et al. 1990; Dawson et al. 1987, 1988, 1989, 1990).

In addition to encroachment issues, Reinert (1982b) speculated that the tropical climate in south Florida, the high number of generations per year, all life stages being present each month of the year, and the monoculture of St. Augustinegrass in residential lawns along Florida's southeastern coast may influence the development of insecticide resistance in *B. insularis*. He also reported that it was not uncommon for lawns to be treated six to twelve times per year in some areas, with lawns possibly receiving less than recommended rates and exposing *B. insularis* to sublethal doses each year (Reinert 1982b). These observations were made during the 1980s when insecticide resistance was almost exclusively in the southeastern coast of Florida. The increase in housing development in Florida has helped increase the number of neighborhoods that are also a monoculture of St. Augustinegrass (personal observation). The results of chapter 2 and the data from Cherry and Nagata (2005, 2007) show that insecticide resistance is no longer restricted to south Florida and can be a problem in all areas of the state (south, central, north) although to varying degrees of severity.

Several factors can influence the selection of resistance to insecticides in field populations of insects, including genetic (frequency and number of R alleles, dominance of R alleles, past

selection by other chemicals), biological (biotic, behavioral/ecological), and operational factors (nature and persistence of insecticide, relationship to earlier used chemicals, number of applications, application methods) (Georghiou and Taylor 1986). While the genetic factors are currently unknown, the development of a synchronous rearing method (test 5, Chapter 3) and the development of the airbrush bioassay and improved sprig dip bioassay (Chapter 4) will be useful tools for insecticide resistance studies in *B. insularis*.

In chapter 3, test 5 proved to be the best method for synchronized rearing of *B. insularis*. For the first time, *B. insularis* colonies of known age and generation can now be selected in the laboratory for bifenthrin resistance over multiple generations (or any other insecticide one chooses). Pure insecticide-susceptible and -resistant colonies can then be used in mode-of-inheritance studies and characterization of mechanisms. Mode-of-inheritance tests with susceptible and resistant males and virgin females would provide key information regarding the genetics of resistance in *B. insularis*. First, one could determine if resistance is dominant or recessive. If resistance in *B. insularis* was shown to be dominant, a rotation strategy as part of a resistance management program would be ineffective. Second, one could determine if resistance is sex-linked or autosomal (Georghiou and Saito 1983). Comparison of XX and XY individuals of the F_1 progeny could determine whether or not a sex chromosomal resistance factor had been involved in the parental R strain. This information could help to determine the rate of evolution of resistance and which management strategies to use (Georghiou and Saito 1983).

The rearing methods outlined in test 5 (Chapter 3) will be useful when characterizing mechanisms of resistance in *B. insularis* because pure insecticide resistant and susceptible colonies can be developed. This is important because it may be difficult to distinguish if different responses (e.g., to a pesticide with or without a synergist) are due to a physiological

resistance mechanism or the differences that can occur between different populations (Scott 1990). In addition, the use of pure *B. insularis* colonies will allow for determining if a putative mechanism is really the one that determines resistance (e.g., one that has been identified by bioassay in a resistant vs. susceptible insect). The expression of the mechanism in reciprocal F_1 (resistant × susceptible) progeny should be correlated with the level of resistance seen in the bioassay (Scott 1990).

There are other ways that the test 5 rearing method can be used. First, studies determining the number of eggs laid per laboratory-reared female and mating habits would provide additional insight into the biology of *B. insularis* (ie., how long to hold each generation for testing). Second, the effect of density on *B. insularis* size and wing polymorphism in tests using laboratory colonies along with studies of field populations, would be of use in determining population dynamics in St. Augustinegrass lawns. Sasaki et al. (2002) found that environmental factors such as high temperature, long photoperiod, and crowding during nymphal development stimulated the production and increase in *Dimorphopterus japonicus* Hidaka (Hemiptera: Lygaeidae). Third, as previously mentioned, studies determining the presence of aggregation and/or sex pheromones would be useful for development of monitoring techniques. Fourth, rearing studies could be conducted with natural enemies of *B. insularis*. Quality *B. insularis* could be reared as a food or oviposition source and for use in studies with *Geocoris* spp. as well as *E. benefica*. Last, it would be of benefit to determine the presence of gut symbionts in *B. insularis*. Various true bugs in the order Hemiptera contain large masses of bacteria in the alimentary tract and are thought to have a symbiotic association with them (Brues 1946). Forbes (1892) found the presence of bacteria in the alimentary canal of the common chinch bug, *B. l. leucopterus,* and later found them in other Hemiptera. Glasgow (1914) discovered that specific

types of bacteria were characteristic of each species he examined. Later, Kuskop (1924) suggested that bacteria are passed from parent to offspring by entering the egg from a surface contamination at the time of oviposition, and reach the alimentary canal before hatching, accumulating in the caeca to which they are generally confined. If gut symbionts exist in *B. insularis*, there may be ways to alter the relationship and potentially lead to novel approaches to pest management. Several studies have demonstrated that when experimentally deprived of the symbiont, host stinkbugs suffer retarded growth and high mortality (Buchner 1965, Abe et al. 1995, Fukatsu and Hosokawa 2002, Hosokawa et al. 2006).

Monitoring for resistance is considered essential to insecticide and acaricide resistance management (Dennehy and Granett 1984, Staetz 1985, Roush and Miller 1986, Denholm 1990). In chapter 4, I developed an airbrush bioassay for testing contact and systemic insecticides and evaluated both the airbrush and the sprig-dip bioassay under more standardized conditions. These bioassays will be useful tools for detection and monitoring of insecticide resistance in *B. insularis*. Furthermore, I recommended that the sprig-dip bioassay be used for detection of bifenthrin-resistant *B. insularis* populations because it was more sensitive than the airbrush bioassay in detecting lower LC values. The airbrush bioassay would be better than the sprig-dip bioassay for use in studies concerning cross resistance, mechanisms, mode-of-inheritance, and stability of pyrethroid (and other chemical classes) resistance in *B. insularis*.

Future studies using the airbrush bioassay could include penetration studies, cross-resistance patterns, insecticide synergists, enzyme assays, metabolic detoxication, and target site sensitivity (Matsumura and Brown 1963; Plapp and Hoyer 1968; Scott and Georghiou 1986; Scott 1990; Bull and Patterson 1993; Scharf et al. 1998a, 1998b, 1999, 2000a, 2000b, 2001; Scharf and Siegfried 1999; Wu et al. 1998; Liu and Yue 2000; Miota et al. 2000; Valles et al.

2000; Ahmad et al. 2006). In addition, the airbrush bioassay could be used for determining a diagnostic dose for pyrethroid- and imidacloprid-resistant *B. insularis* populations because the results of chapter 4 demonstrate this bioassay results in less variance. Also, the airbrush bioassay has the added benefit of distinguishing differences between males and females (Chapter 4), and documenting behavioral differences could be of importance when monitoring and documenting resistance in *B. insularis* populations.

In addition, I also addressed questions about sample size and duration of the airbrush and sprig-dip bioassays for response of *B. insularis* to bifenthrin and imidacloprid (Chapter 4). Robertson et al. (1984) determined that a sample size of 120 was the minimum necessary for calculating a reliable LC_{50} estimation, but for increased precision sample sizes of 240 or more are often necessary (Robertson et al. 1984, 2007; Robertson and Preisler 1992). However, the authors noted that further investigation is needed to explore other combinations of sample size and dose placement in different bioassays (Robertson et al. 2007). Tabashnik et al. (1993) evaluated the duration of bioassays for *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) against the microbial insecticide, *Bacillus thuringiensis* Berliner. The authors demonstrated that bioassays for *P. xylostella* against *B. thuringiensis* could be run using shorter time intervals and a single concentration with little loss of information compared to the standard bioassays (Tabashnik et al. 1993). The results of comparison tests and subsampled data in chapter 4 indicated that smaller sample sizes could be used when testing bifenthrin for a shorter duration compared to the sprig-dip bioassay. While smaller sample sizes could be used for testing imidacloprid, the airbrush bioassay required a similar duration to that of the sprig-dip bioassay.

Future studies measuring wing polymorphism and the weight of the insects in each of the bioassays to determine differences in efficacy would be of value because size is not always an

indicator for greater response to an insecticide. In addition, studies should be conducted to determine if either bioassay closely mimics response of *B. insularis* to insecticides under field conditions. Nonetheless, the development of the airbrush bioassay and synchronous rearing method provide valuable tools that can be used to further investigate *B. insularis* biology, population dynamics, response to insecticides, and how insecticide resistance develops in this pest. A greater understanding of how insecticide resistance develops in *B. insularis* will provide researchers, chemical companies, and lawn-care companies the means to make responsible and sound decisions for resistance management of *B. insularis*.

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BIOGRAPHICAL SKETCH

Julie Cara Congdon Vázquez was born in 1970 in St. Petersburg, Florida. After spending most of her childhood in Gainesville, she moved to Elma, Washington, and attended Elma High School. After enjoying 10 years in Washington, she returned to Gainesville to pursue a college degree. She enrolled at Santa Fe Community College and developed an interest in entomology after taking several honors courses. In 1998, Cara entered the University of Florida as an undergraduate entomology major. While at the University of Florida, Cara gained practical experience in both pest control and research by working for the Florida Pest Control and Chemical Company, the University of Florida's Entomology and Nematology Department (urban entomology laboratory), and United States Department of Agriculture (USDA).

Cara received her bachelor's degree in entomology and nematology with a specialization in urban pest management in May 2001. Afterwards, she was hired by the FMC Corporation as a summer intern and provided technical and sales support to golf course superintendents, pest management professionals, and distributors. Cara started her graduate studies in August 2001 at the University of Florida under the guidance of Dr. Eileen A. Buss. During her master's research Cara developed a fondness for southern chinch bugs. She completed her Master of Science degree in May 2004 and immediately started work on her Ph.D. She is a member of the Entomological Society of America, Entomology and Nematology Student Organization (ENSO), Florida Entomological Society, Florida Turfgrass Association, Gamma Sigma Delta, and the Urban Entomological Society (UES). After completing her dissertation, Cara will work as a Research Scientist for Scynexis, Inc., in the Research Triangle Park, NC.