Mexican Rice Borer (Eoreuma loftini) Pheromone Trap Efficacy and Role in Invasive Species Monitoring and Pest Management

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MEXICAN RICE BORER (*EOREUMA LOFTINI*) PHEROMONE TRAP EFFICACY AND ROLE IN INVASIVE SPECIES MONITORING AND PEST MANAGEMENT

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

The Department of Entomology

by

Blake E. Wilson
B.S., Louisiana State University, 2009
M.S., Louisiana State University, 2011
May 2016
ACKNOWLEDGEMENTS

I would like to thank my major professor and mentor, Dr. T. E. (Gene) Reagan, for providing me with the opportunity to pursue my Ph.D. and the means to support a family throughout the duration of my graduate tenure at LSU. Gene’s dedication to integrated pest management research and to the Louisiana sugarcane industry embodies what I believe is the duty of agricultural scientists serving land grant institutions. Gene goes above and beyond to take care of his graduate students and provides them ample opportunity for success. It has truly been a pleasure to learn from one of the pioneers of IPM.

I also would like to thank Dr. Julien Beuzelin, my co-major advisor, colleague, and good friend. This work would not have been possible without Julien’s guidance and technical input in all aspects of this research project. Julien is a great scientist and has been a role model for me in the field of entomology ever since I first joined the department as a student worker.

Appreciation is expressed to long time lab-mate and good friend, Dr. Matthew VanWeelden. Matt and I worked closely on numerous research projects and I always know I can count on his support in good times and bad. I would also like to thank the members of my graduate advisory committee: Dr. Michael Stout, Dr. Rodrigo Diaz, and Dr. Jong Hyun Ham for their guidance. I would like to thank Dr. Jeremy Allison whose confidence in me early in my career encouraged me to pursue this project.

Gratitude is conveyed to all those who provided technical assistance to these projects including Jimmy Meaux, Dr. Mo Way and his crew at Texas A&M Beaumont, Tyler Torina, and Narinder Heer. I would also like to thank the numerous rice and sugarcane farmers in Louisiana and Texas who generously provided me with access to their farms for this research.
I would like to express gratitude to Dr. Kenneth Gravois, Dr. Bill White, Randy Richard, LSU AgCenter Cooperative Extension, and the American Sugarcane League for giving me the opportunity to interact with sugar growers and making me feel like I was part of the sugarcane research team. These interactions gave me the purpose and direction needed to see this project through.

I would like to thank the LSU AgCenter and Louisiana State University for all of the wonderful experiences I have had over my years here. LSU has been my “home away from home” for more than 10 years, and the Purple and Gold that runs through my veins will never fade. No matter where my career may take me, I will never forget where it started.

The deepest appreciation is expressed to my loving and supportive family. My amazing wife, Dr. Caitlin King, was by my side every step of the way. Caitlin is a brilliant scientist, a loving wife, and a devoted mother. She provided me with critical insight which frequently allowed me to see things from a unique perspective. Caitlin always supported my research even when it meant she had to care for two young girls while I was away from home at research sites and conferences. Lastly, her extraordinary culinary skills provided me with the nourishment I needed to get the job done. I also thank my two beautiful daughters, Rumi June and Bundle Wilde. Their smiling faces were a constant source of inspiration and helped to motivated me to push through during times of struggle. I am grateful for my parents, Jeff and Beth Wilson, for all of their support through my college career, and for allowing my love of wildlife to blossom from an early age. I would also like to thank Monica and Colleen King for doing whatever was needed to help out our family during the busiest of times.
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ABSTRACT

The Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), is an invasive pest of sugarcane, *Saccharum* spp.; rice, *Oryza sativa*; and other graminaceous crops along the U.S. Gulf Coast. Identification of *E. loftini* sex pheromones led to the development of pheromone baited traps. Studies were conducted to determine strategies for the use of *E. loftini* pheromone traps in invasive species monitoring and pest management.

A two-year field study demonstrated that *E. loftini* pheromone traps attract males from distances of up to 100m. A behavioral assay observed that detection of the pheromone by *E. loftini* males occurs at ≈48m from the source.

A network of pheromone traps monitored *E. loftini* range expansion from 2009–2015. *Eoreuma loftini* is now present in nine Louisiana Parishes: Calcasieu, Cameron, Beauregard, Allen, Jefferson Davis, Acadia, Vermilion, Evangeline, and St. Landry. Crop surveys observed *E. loftini* infesting Louisiana rice and sugarcane. The *E. loftini* population is advancing eastward at 11 km/yr. The population is characterized by high density clusters and may be limited at higher latitudes.

*E. loftini* is causing substantial yield reductions in unprotected commercial rice fields in southwestern Louisiana. Rice which received the Dermacor X-100® (chlorantraniliprole) seed treatment sustained reduced injury. Pheromone trap captures are correlated to larval infestations in adjacent unprotected rice fields.

Infestations of *E. loftini* in Louisiana sugarcane have not reached damaging levels. Sugarcane infested with *E. loftini* is being transported to sugar mills east of the pest’s known range, however, it has not established in these regions.
Studies indicated automated *E. loftini* pheromone trapping systems have potential to further reduce scouting efforts. This represents the first use of automated pheromone-based monitoring systems for Lepidopterous insect pests in field crops. Field studies indicate new diamide chemistries may improve chemical control of *E. loftini* in sugarcane.

This research expands the use of *E. loftini* pheromone traps in invasive species monitoring and pest management. Continued monitoring of *E. loftini* range expansion and the use of pheromone trap-based scouting techniques should be further pursued to mitigate the impact of this pest along the U.S. Gulf Coast.
CHAPTER 1: GENERAL INTRODUCTION

Management of invasive species is a growing concern in the U.S. with approximately 50,000 invasive species which are responsible for $137 billion in damages and control costs annually (Pimentel et al. 2005). Of those species, approximately 1,000 are crop pests which account for $14.4 billion annually in damages (Pimentel et al. 2005). One invasive insect that has become established as a major pest of sugarcane, *Saccharum* spp. L.; and rice, *Oryza sativa* L., in Texas is the Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae).

This species was first reported as a pest of sugarcane in the U.S. in the Rio Grande Valley of Texas in 1980 (Johnson and van Leerdam 1981), where it now accounts for >95% of the sugarcane stem borer population (Legaspi et al. 1997a). It has since spread northeast through the Texas rice belt along the U.S. Gulf Coast (Reay-Jones et al. 2007c). Despite a quarantine designed to prevent movement of Texas sugarcane into Louisiana, *E. loftini* was discovered in Louisiana in December 2008 (Hummel et al. 2010). Based on its current rate of expansion, *E. loftini* is predicted to infest the entire state by 2035, and it is projected to cause as much as $260 million in annual revenue loss to Louisiana agriculture (Reay-Jones et al. 2008).

Early research on *E. loftini* biology and management focused primarily on mitigating the pest’s impact to sugarcane in the Rio Grande Valley of Texas. While effective control strategies were to remain elusive for many years (Legaspi et al. 1997a), advancements were made nonetheless. One such advancement was the isolation and characterization of the female sex pheromone blend of *E. loftini* from ovipositor extracts (Brown et al. 1988). The pheromone blend was subsequently developed for use in population monitoring by determination of optimal blend concentrations and trap types (Shaver et al. 1990, 1991).
Traps baited with insect sex pheromones are frequently used in detection and population monitoring of pests. Pheromone traps are useful in monitoring population fluctuations and may provide improved early warning signs of pest outbreaks (Robacker and Landholt 2002). Additionally, pheromone baited traps are often able to detect the presence of an insect even at low population densities making them ideal for use in invasive species monitoring (Witzgall et al. 2010). Accordingly, pheromone trap monitoring is a major component of monitoring programs for many high profile invasive insect pests. Pheromone baited traps are also used in pest management programs (Witzgall et al. 2010). Effective means to monitor pest populations to more effectively time control measures is a cornerstone of integrated pest management (IPM) programs (Rabb and Guthrie 1970). Pest scouting often requires labor intensive and time-consuming sampling and quantification methods throughout the growing season to obtain accurate estimations of infestation levels (Pedigo and Buntin 1993). Pheromone traps can be used in IPM programs to improve scouting efficiency and focus sampling efforts when insect population densities are known to be high.

Bucket traps baited with the synthetic female sex pheromones provided a means to monitor *E. loftini* range expansion across Texas (Reay-Jones et al. 2007c) and detected the first *E. loftini* occurrence in Louisiana (Hummel et al. 2010). Pheromone traps have also been used to assist in scouting and timing of insecticide applications for control of *E. loftini* in sugarcane in Texas (Wilson et al. 2012b). However, pheromone trap monitoring for *E. loftini* appears vastly underutilized in comparison to the immense trapping programs used to combat other invasive insects such as the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) (Smith 1998), and the Gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae) (Sharov et al. 2002).
While more than 20 years have passed since the development of E. loftini pheromone traps, effective trapping protocols for use in invasive species monitoring and pest management have not yet been developed. Further, much about the functionality of these traps remains largely unknown. Development of refined pheromone trapping strategies will help to expand the use of pheromone traps in an effort to mitigate the impacts of E. loftini to agricultural production along the U.S. Gulf Coast.

This research aimed to determine the active space, or attractive distance, of E. loftini pheromone traps using a comprehensive approach involving field experiments and behavioral assays (Chapter 3). An extensive pheromone trap network program is monitored to provide early detection of the invasive pest in new areas and document occurrence of E. loftini in Louisiana field crops (Chapter 4). Pheromone traps and surveys of larval infestations assess the current pest status of E. loftini in Louisiana’s rice (Chapter 4) and sugarcane (Chapter 5) industries. Pheromone trap-assisted scouting strategies for use in IPM programs are evaluated in rice and sugarcane (Chapters 4, 5, and 6). These studies include examination of potential to use emerging pest detection technology developed by Spensa Technologies for use in E. loftini management (Chapter 5). Spensa Technologies’ electronic “Z-trap” systems use advanced computer algorithms to identify the target pests and record trap capture data which is automatically uploaded to an online database. Insecticidal management of E. loftini in rice (Chapter 4) and sugarcane (Chapter 6) are also investigated. Chemical control research documents the effect of widely used chlorantraniliprole seed treatments on E. loftini in commercial rice and assesses efficacy of recently labeled diamide insecticides for control of E. loftini in Texas sugarcane. Lastly, the potential to greatly improve the use of pheromone trapping in invasive species monitoring of E. loftini is demonstrated through the application of Geographical Information
Systems (GIS) to three years of pheromone trap data from 13 Louisiana Parishes. Collectively, this project greatly improves our understanding of *E. loftini* pheromone traps and highlights the potential to expand their use in invasive species monitoring and IPM.
CHAPTER 2: LITERATURE REVIEW

2.1. Distribution and Pest Status of E. loftini

The Mexican rice borer, Eoreuma loftini (Dyar), was first found infesting commercial sugarcane on the west coast of Mexico (Morill 1925, Van Zwaluwenburg 1926) and the range later expanded to include eastern and southeastern Mexico (Klots 1970, Rodriguez-del-Bosque and Smith 1991). The invasive pest was initially discovered in the U.S. in Arizona (Dyar 1917, Van Zwaluwenburg, 1926) and in the Imperial Valley in California (Osborn and Phillips 1946) in the early 1900s. Expansion into sugarcane production areas of north eastern Mexico had occurred by the mid-1970s and numerous interceptions of E. loftini infested sugarcane were made at the Texas-Mexico border in the 1950s and 60s (Johnson 1984). E. loftini was first reported as a pest of sugarcane in the Rio Grande Valley of Texas in 1980 (Johnson and van Leerdam 1981), and caused yield losses of 50–65% in some sugarcane fields within the first two years of its detection (Johnson 1984). The species soon became the dominant pest of sugarcane in the Rio Grande Valley (Legaspi et al. 1997a), and by the end of the 1980s its range had expanded northward well into the rice production area of Texas (Browning et al. 1989).

Pheromone trap monitoring was used to track the movement of E. loftini through the Texas rice belt. The species’ range included Calhoun, Jackson, and Matagorda counties by 1999; Wharton, Colorado, Fort Bend, Waller, and Brazoria counties by 2000; Austin and Harris counties by 2001; Galveston County by 2002; Liberty and Chambers counties by 2004; and Jefferson County by 2005 (Reay-Jones et al. 2007c). The range of E. loftini expanded northeastward at a rate of approximately 23 km/yr from 1980 to 2005 (Reay-Jones et al. 2007c). In December of 2008 E. loftini was detected in Louisiana in two pheromone traps near Vinton (Calcasieu Parish) (Hummel et al. 2010). While human aided movement is not thought to have played a major role
in *E. loftini* expansion across Texas and into Louisiana, it is suspected that human activities facilitated the recent transportation of *E. loftini* into Florida where it has become established in non-crop habitats of the south-central region of the state (Hayden 2012).


Once established in the Rio Grande Valley, *E. loftini* quickly surpassed the sugarcane borer, *Diatraea saccharalis* (F.), in economic importance, and now accounts for 95% of the sugarcane stem borer population in the region causing an estimated annual loss of $20 million to the Texas sugarcane industry (Legaspi et al. 1997a). The insect has more recently become an economic pest of rice in southeast Texas (Reay-Jones et al. 2007a, 2008), and poses an imminent threat to the Louisiana sugarcane and rice industries. The species is predicted to infest the entire state of Louisiana by 2035 and its establishment is expected to cause annual revenue losses as high as
$220 and $45 million for sugarcane and rice, respectively (Reay-Jones et al. 2008). The economic impact of *E. loftini* establishment on corn has not been examined, although research by Showler et al. (2012, 2013) suggests that susceptible cultivars of corn may be more preferred hosts than sugarcane. Although *E. loftini* exhibits only a weak diapause (van Leerdam 1986), it is more cold tolerant and has a higher overwintering survival rate than *D. saccharalis* (Rodriguez-del-Bosque et al. 1995) and can likely survive colder winters allowing the pest to infest corn central and northern Louisiana.

### 2.2. *Eoreuma loftini* Biology and Life cycle

Extensive studies of *E. loftini* biology and temperature-dependent development were conducted by van Leerdam (1986). *Eoreuma loftini* lays globular cream-colored eggs in clusters ≤100 typically laid on dry leaves of the lower portion of plants, between 0 and 80 cm above the soil surface (van Leerdam et al. 1984, van Leerdam 1986). In sugarcane, rice, and non-crop grasses, eggs are laid in cryptic sites including folds of dry leaves and leaf sheaths stems (Reay-Jones et al. 2007b, Showler and Castro 2010b, Beuzelin et al. 2013), limiting exposure to predators and parasitoids. The duration of the egg stage is inversely related to temperature lasting 14 days at 20°C and 5 days at 32°C (van Leerdam 1986). Upon hatching, larvae migrate to green parts of the plant and start to feed on leaf blades and sheaths. On sugarcane, larval exposure on plant surfaces is less than one week and some larvae have been documented entering into mid-rib tissue becoming protected within one day after eclosion (Wilson et al. 2012b). After stalk entry, larvae remain protected within frass-packed tunnels throughout their development until adult emergence. When reared in the laboratory, larvae undergo four to six molts, with five typical in males and six in females (van Leerdam 1986). The number of stadia and duration of larval development (21–78 days) are inversely related to temperature. The cream-colored larvae have
an orange-brown head capsule and dark-colored parallel broken stripes along their dorsal side and measure 19–25 mm before pupation (Osborn and Phillips 1946, Browning et al. 1989). Larval feeding in sugarcane stalks differs from that of *D. saccharalis* because *E. loftini* larvae tunnel horizontally as well as vertically (van Leerdom 1986). Additionally, *E. loftini* tunnels are tightly packed with frass making larvae and pupae less accessible to natural enemies in comparison to *D. saccharalis*, which pupates in a hollow cavity (Browning et al. 1989, Legaspi et al. 1997a, Showler and Reagan 2012).

The duration of the pupal stage is between 7–21 days depending on the temperature (van Leerdom 1986). The adult is a solid light-tan moth with a tiny (< 1 mm) dark spot in the center of each forewing. Adult *E. loftini* can be distinguished from similar looking Lepidopterans by the triangular gena and conical frons. However, definitive species level identification requires examination of the male genitalia (Reiss 1981, Agnew et al. 1988). The adult stage lasts about 7 days. Most adults of both sexes mate on the night after eclosion and likelihood of mating decreases with time after eclosion (Shaver et al. 1994). While both males and females are capable of mating with more than one partner, this behavior is only common to males as most females mate only once (Shaver et al. 1994). For both sexes, peak activity occurs between 7–9 hours after sundown, although females begin emitting pheromone at 5 hours after sundown (Shaver et al. 1994).

Fecundity ranges from 200–400 eggs/female and varies dependent on temperature (van Leerdom et al. 1986). Oviposition rates range from 29 eggs per day at 20°C to 64 eggs at 32°C, and the oviposition peak occurs during the first day of oviposition, usually 2 days after adult eclosion (van Leerdom et al. 1986). A linear relationship between fecundity and pupal weight exists (Spurgeon et al. 1995).
Browning et al. (1989) reported a general 45–50-day length for the duration of a generation under summer conditions in the Rio Grande Valley. Four to six overlapping generations occur annually in the region (Legaspi et al. 1997b), and all stages of *E. loftini* can be found in the field at any time of the year (Johnson 1985, Meagher et al. 1994, 1996b, Beuzelin et al. 2011b). Larvae may enter a facultative diapause during fall and winter months; however, adult moths are active throughout the year (Reay-Jones et al. 2007c, Hummel et al. 2010, Beuzelin et al. 2011b).

### 2.3. *Eoreuma loftini* IPM in Sugarcane

*Eoreuma loftini* damage in sugarcane results from internal tunneling which can impair growth, cause stalks to break and lodge, and reduce juice quality (van Leerdam 1986, Browning et al. 1989, Legaspi et al. 1999a). The level of sugarcane stem borer injury is most commonly assessed by determining the percentage bored internodes which provides a season long record of injury and is inversely related to sugar yield. Increased percentage bored internodes has been shown to be associated with reductions in yield parameters including juice purity, tonnage of sugarcane, sugar per ton of cane, and sugar per hectare (Long and Hensley 1972, Legaspi et al. 1999a, Reay-Jones et al. 2005b, White et al. 2008). Control of *E. loftini* in sugarcane can be achieved by a combination of chemical control, cultivar resistance, and production practices which reduce plant stress (Legaspi et al. 1997a, Reay-Jones et al. 2005b, Wilson et al. 2012b, 2015).

Chemical control of *E. loftini* in sugarcane has often not proven to be economical, and the approach has largely been abandoned by Rio Grande Valley sugarcane producers (Johnson 1985, Meagher et al. 1994, Legaspi et al. 1997a, Reay-Jones et al. 2005b). Overlapping generations and the cryptic nature of *E. loftini* larvae make timing of insecticide applications difficult (Meagher et al. 1994, Reay-Jones et al. 2005b, Wilson et al. 2012b). However, pheromone trap assisted
scouting can reduce scouting effort and improve application timing (Wilson et al. 2012b). New more selective insecticide chemistry and better timing of insecticide applications may improve the economics of *E. loftini* chemical control (Wilson et al. 2012b). Flubendiamide and chlorantraniliprole are two diamide insecticides recently labeled for sugarcane which provided good control of *D. saccharalis* (Wilson et al. 2012a, Beuzelin et al. 2014). However, insecticides alone are not expected be effective in managing *E. loftini* when it becomes established in Louisiana sugarcane (Reay-Jones et al. 2005b).

The use of stem borer resistant cultivars in combination with insecticides and irrigation has been shown to reduce *E. loftini* injury and improve sugar yield (Reay-Jones et al. 2005b). Additionally, resistant cultivars may prolong duration of larval feeding on plant surfaces and increase exposure to insecticide applications and natural enemies (Wilson et al. 2012b). Host plant resistance has long been cited as an effective management tactic for control of *E. loftini* (Pfannenstiel and Meagher 1991, Meagher et al. 1996a, Reay-Jones et al. 2003, 2005b; Wilson et al. 2012b, 2015). Host plant resistance as a component of an IPM program has potential to reduce pest injury and input cost associated with control (Smith 1989). However, the use of resistant cultivars in stem borer management is often neglected as growers opt for higher yielding cultivars, and in turn, resistant but low yielding cultivars are not available (Milligan et al. 1994, Legendre and Gravois 2006, Wilson et al. 2015). Expansive acreage of susceptible cultivars with elevated moth production increases endemic *E. loftini* populations and imposes additional pressure on the remaining acreage. Currently, the majority of sugarcane acreage in Louisiana and the Rio Grande Valley is planted with *E. loftini* susceptible cultivars (Reay-Jones et al. 2003, Wilson et al. 2015). The ability of cultivar resistance to reduce area wide pest populations led to the development of a moth production index based on adult emergence
(Bessin and Reagan 1990, Bessin et al. 1991, Reay-Jones et al. 2003). A relative resistance ratio was subsequently developed to incorporate both percentage bored internodes and survival to adulthood into a single index (Wilson et al. 2015). While host plant resistance is promising for *E. loftini* control, the mechanisms of resistance are not fully understood.

Reduced oviposition preference may play a role in resistance (Reay-Jones et al. 2007b, Showler and Castro 2010a,b, Showler and Regan 2012, Showler and Moran 2014). Oviposition preference may be linked the detection of primary or secondary compounds to assist females in accepting or rejecting a host plant (Ramaswamy 1988). Selected nutrients, particularly essential amino acids, have a well-documented influence on *E. loftini* oviposition preference (Reay-Jones et al. 2007b, Showler and Moran 2014). Cultivar resistance may also result from impediment of establishment of early instars (Coburn and Hensley 1972, Wilson et al. 2012b). Factors which hinder larval establishment of *D. saccharalis* are physical characteristics such as rind hardness and leaf sheath appression, and may also affect *E. loftini* larvae (Coburn and Hensley 1972, Martin et al. 1975). In addition to physical factors, concentrations of certain primary and secondary metabolites affect larval development. Differences in *E. loftini* larval weight and time to pupation may be linked to varying levels of allelochemicals among sugarcane cultivars (Meagher et al. 1996a).

Currently, there are no commercially available sugarcane clones with genetically modified traits. However, genetically engineered clones expressing insecticidal snowdrop lectin proteins (*Galanthus nivalis* agglutinin) have been evaluated. A decrease in *E. loftini* larval survival, percentage of adult emergence, and fecundity was reported when fed with transgenic sugarcane (Sétamou et al. 2002a,b). In addition to cultivar resistance, cultural practices which reduce the attractiveness or suitability of hosts may mitigate *E. loftini* infestations.
The most common cultural practice used to manage *E. loftini* in sugarcane is irrigation. In the Rio Grande Valley, irrigation is a key practice in managing *E. loftini* infestations in sugarcane and reduced injury by as much as 2.5-fold in a 2-yr field experiment (Reay-Jones et al. 2005b). Infestations of *E. loftini* are enhanced by drought stress which leads to an increase in free amino acid concentrations (Reay-Jones et al. 2005b, Showler and Castro 2010a,b). Additionally, the greater number of dry senescing leaves likely make drought stressed sugarcane more attractive for *E. loftini* oviposition (Reay-Jones et al. 2007b, Showler and Castro 2010a). Because the use of chemical control against *E. loftini* in the Rio Grande Valley has not been economical (Legaspi et al. 1997a), most growers in the region use irrigation as the primary means of control.

Cultural control of stem borers in sugarcane has been more thoroughly examined with *D. saccharalis* than with *E. loftini*. Tactics including plowing stubble in fallow fields as early as possible and planting stem borer-free sugarcane seed pieces are currently recommended for *D. saccharalis* (LSU AgCenter 2010b). Planting and harvesting dates affect sugarcane phenology which can influence stem borer population dynamics. Although early planting usually provides higher yields, fields planted in August show increased *D. saccharalis* infestations (Charpentier and Mathes 1969, Beuzelin et al. 2011a). The effects of weed management in sugarcane field margins have on *E. loftini* infestations have not been examined; however, non-crop weed hosts have been shown to harbor substantial *E. loftini* populations (Beuzelin et al. 2011b). Because corn and sorghum potentially enhance stem borer populations when grown in sugarcane areas (Reagan and Flynn 1986, Showler et al. 2012), farmers are recommended to grow these two crops as far as possible from sugarcane fields (LSU AgCenter 2010b). This influence may be even more severe when considering *E. loftini* infestations, as corn has been shown to be preferred over sugarcane (Showler et al. 2012, Showler et al. 2013).
2.4. *Eoreuma loftini* Management in Rice

Similar to other crop hosts, *E. loftini* damage in rice results from internal larval tunneling in stems which cause deadhearts, broken culms, or incomplete panicles. Although injured culms usually remain green before heading, injury to the vascular tissue can kill the panicle and the developing grain, resulting in “whiteheads”. Whiteheads, or blanked panicles, are often used to assess stem borer damage in rice (Pathak 1968, Way 2003, Reay-Jones et al. 2007a). When injury occurs during ripening, the maturation of panicles suffers from a lack of uniformity in grain development and increased grain mortality. Mature panicles may also be lost because larval injury to the topmost node can cause the culm to break (Bowling 1975, Browning et al. 1989, Way 2003, Lv et al. 2008, 2010).

The reduced biomass of rice relative to sugarcane likely increases exposure of *E. loftini* larvae and improves the efficacy of control tactics. Currently, only two pyrethroid insecticides are labeled for stem borer control in rice in the U.S. Applications of pyrethroids are effective in reducing the number of whiteheads and improving rice yield (Reay-Jones et al. 2007a). Biorational insecticides including novaluron, diflubenzuron, and tebufenozide were not as effective as pyrethroids in controlling *E. loftini* (Reay-Jones et al. 2007a), possibly due to reduced contact toxicity relative to pyrethroids. The efficacy of insecticides for control of stem borers in rice is strongly influenced by application timing (Bandong and Litsinger 2005, Reay-Jones et al. 2007a), and insecticidal protection of rice may only be needed during the reproductive stages (Rubia et al. 1996). Applications made during the boot stage have been shown to be more effective than those made earlier in the growing season (Reay-Jones et al. 2007a). An economic threshold of 5% of stems infested has been used for control of the rice stem borer, *Chilo suppressalis* Walker, in Japan (Koyama 1975). Currently, no economic
thresholds are recommended for control of stem borers in rice in the U.S. and insecticide applications are often made along with fungicide applications regardless of infestation levels. Roughly 40% of growers in Texas apply at least one treatment with pyrethroids for *E. loftini* control (M.O. Way, personal communication). Insecticidal seed treatments are frequently applied to rice for control of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), and may provide some level of stem borer control (LSU AgCenter 2010a). Chlorantraniliprole seed treatments (Dermacor X-100) have activity against lepidopterans and may contribute to *E. loftini* control (Way et al. 2013, Sidhu et al. 2014). Widespread use of seed treatments has potential to reduce area wide pest populations. In addition to insecticidal control, cultivar resistance may also be incorporated into *E. loftini* management programs in rice.

Numerous studies have been conducted on stem borer resistance in rice in Asia, where the production of rice relies less on insecticides than in the U.S. (Chaudhary et al. 1984). Cultivar characteristics which have been reported to affect levels of resistance to stem borers include morphological characters such as plant height, culm diameter, and length and width of the flag leaf which have all been positively correlated with the percentage of infested tillers (Patanakamjorn and Pathak 1967). Increased levels of resistance have been associated with tight internode-wrapping leaf sheaths (Patanakamjorn and Pathak 1967) and thick layers of lignified tissues (Chaudhary et al. 1984). However, research on rice cultivar resistance to *E. loftini* is scarcer. Way et al. (2006) conducted a 4-yr study in Texas on rice yield loss as affected by genotype, and *D. saccharalis* and *E. loftini* injury level (measured as the number of whiteheads per m²). The most susceptible cultivar, Priscilla, demonstrated the highest injury levels in the main crop and the greatest yield losses. Despite varying levels of relative susceptibility among the years, Cocodrie was considered moderately susceptible in comparison to hybrid lines (e.g.,
XL8), which showed injury levels and yield losses lower than other cultivars. XL8, however, is more attractive for *E. loftini* oviposition than Cocodrie (Reay-Jones et al. 2007b). Way et al. (2006) suggested that cultivars such as XL8 could act as sinks for *E. loftini* infestations and decrease area wide stem borer populations. In addition to reduced injury, compensatory response to injury may also influence susceptibility to stem borers (Lv et al. 2010).

Stem borer infestations in rice are typically greatest in late planted main crop or early planted ratoon crops, and management of rice stubble may reduce stem borer populations (Beuzelin et al. 2012). Because substantial *E. loftini* infestations survive the harvest, rice stubble provides an important host during the fall and winter months (Beuzelin et al. 2012). Reducing the harvest cutting height from 40 to 20 cm has been shown to reduce *E. loftini* infestations in the ratoon crops. Additionally, mowing rice or removing stubble may enhance overwintering mortality and reduce area wide *E. loftini* populations (Beuzelin et al. 2012). However, lowering cutting height and mowing rice stubble requires increased input costs and affects ratoon crop maturity so growers must consider relative need and economic viability before changing agronomic practices.

Extensive studies by Beuzelin et al. (2011b, 2013) demonstrated numerous non-crop grasses play a major role in *E. loftini* dynamics. Weed management has been shown to affect stem borer populations in rice (Tindall 2004). While weed management efforts in rice fields are generally effective (Kendig et al. 2003), non-crop hosts in field margins support *E. loftini* infestations throughout the year (Beuzelin et al. 2011b). Non-crop hosts are of particular importance in the winter and early spring when rice crops are too young to support *E. loftini* infestations (Beuzelin et al. 2011b). Mowing, spraying herbicides, or manipulation of species composition in weedy
field margins may reduce *E. loftini* infestations (Beuzelin et al. 2011b); however, the effect of non-crop habitats on natural enemy populations must also be considered.

### 2.5. Lepidopteran Sex Pheromones

Attractant pheromones help one sex find or recruit the other. Attraction can be defined as a net displacement of one individual towards a chemical source (Bell and Cardé 1984). The use of chemical attractants in sexual recruitment is common to many groups of insects including Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, and Orthoptera (Bell and Cardé 1984). Lepidopteran sex pheromones represent one of the most impressive examples of long distance chemical communication in the animal kingdom (Greenfield 1981). Mate finding and mate selection are critical events in the survival and reproduction of insect species. In Lepidoptera these events are often facilitated by the use of pheromones. The use of olfaction to mediate sex attraction in Lepidoptera was first demonstrated by Mayer (1900), but the first pheromones were not chemically isolated until Butenandt et al. (1961) identified the female sex pheromone of the silk worm moth, *Bombyx mori* L. (Lepidoptera: Bombycidae). Since then, chemical ecology of lepidopteran sex pheromones has become widely studied with over 1600 moth species investigated (Arn et al. 1992, El-Sayed 2008).

The vast majority of lepidopteran species have females which emit pheromones from their abdomens to attract males of the same species. This “female signaler, male searcher” may have evolved because females are often required to expend additional energy locating suitable host plants and oviposition sites (Greenfield 1981). Lepidopteran sex pheromones are generally 10–18 carbon chain acetates, alcohols, or aldehydes (Cardé and Baker 1984). These pheromones differ between species by variations in the length of the carbon chain, functional groups, and positions/orientations of double bonds and asymmetric carbons (Tamaki 1977). This general
chemical structure allows compounds to possess both the necessary volatility as well as potential for uniqueness (Bossert and Wilson 1963). Different species use distinct pheromones to provide isolation from and prevent hybridization with other closely related species (Roelofs and Cardé 1974). However, in areas where a number of competitive species occur additional attractant specificity is required to reduce overlap. One way this is achieved is through the use of a blend of different proportions of chemicals in unique ratios. These blends are highly specific and small changes in the ratio of major to minor components can have significant effects on attractive properties (Roelofs and Cardé 1974, Cardé and Charlton 1984). For instance, an alteration of as little as 5% in the optimum ratio of the female sex pheromone blend of the red-banded leaf roller, *Argyrotaenia velutinana* (Walker) (Lepidoptera: Tortricidae), substantially reduces male capture (Klun et al. 1973). Similarly, Baker and Cardé (1979) showed minor variations in the female sex pheromone blend of the Oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), significantly reduced male attraction. However, highly specific pheromone blends may not be all that is required to reduce competition between species (Cardé and Baker 1984). Variations in diel and seasonal mating patterns can further reduce overlap, although most species use just one mechanism (Cardé and Baker 1984).

The attractive ability of female sex pheromones depends on a number of factors including physical properties of compounds as well as the males’ ability to detect pheromones in the environment. The behavior of pheromones in their environment, the response elicited in males, and the active space have become widely studied. However, interspecies variation and the influence of numerous environmental are still not fully understood.
2.6. Pheromone Dispersion and Active Space

Chemical signals are communicated from an emitting organism to a receiving organism by either direct contact or by dispersion through a medium such as air (Elkinton and Cardé 1984). Dispersion of chemicals in moving air is of particular interest, because lepidopteran sex pheromones are most often dispersed in wind. The active space is defined as the distance from a pheromone source inside which the odor concentration is above the level sufficient to produce a response in the receiving organism (Elkinton and Cardé 1984). The active space is dynamic and the factors which influence it are not easily understood. Active spaces are determined by the emission rate of the compound produced by the signaling organism and the behavioral threshold of the receiving organism. The ratio of pheromone emission rate to the behavioral threshold (Q/K) is a fundamental characteristic necessary for understanding chemical communication systems (Bossert and Wilson 1963). Unfortunately, these characteristics are difficult to measure in lepidopteran sex pheromones (Elkinton and Cardé 1984). The emission rates of most Lepidoptera are near 1 ng/hr and are difficult to measure even with gas chromatographs (Birch 1974). Additionally, behavioral thresholds are not easily determined. Some pheromones can elicit different behaviors at different concentrations (Baker and Cardé 1979) or if the receiving organisms detect brief versus sustained exposure (Cardé and Hagaman 1979). Environmental factors also heavily influence pheromone dispersion and active spaces. Numerous models have been developed to predict the active space of pheromones. The most commonly cited model which describes dispersion from a single, continuously emitting pheromone source was developed by Sutton (1953) and later adapted by Bossert and Wilson (1963) to estimate the maximum distance of communication. This equation was expanded to adjust for unstable conditions and led to the development of Gaussian plume equations (Gifford 1968, Fares et al.)
1980) which have dynamic dispersion coefficients. While these models were accurate to a degree under controlled wind conditions, none were able to predict active spaces which were consistent with observed field results (Elkinton et al. 1984). The Sutton equation and its derivatives have been widely studied and expanded in various models to account for the influence of other environmental factors. One such model was developed which adjusts for surface adsorption or ‘deposition’ (Nakamura 1976, Nakamura and Kawasaki 1977). Conversely, research by Mankin et al. (1980) suggests pheromone laden air will not sink to the ground because concentrations are too low to increase air density. Another model examined the effect of sudden changes in wind velocity or ‘gustiness’ (Hirooka and Suwani 1976), and suggested wind conditions have a greater effect on the width of the odor plume than the maximum distance of communication. Wind velocity has been shown to be particularly influential in insect communication and behavior. The active space may decrease with increasing wind velocity because the increased volume of air initially diluting the plume (Aylor et al. 1976, Miksad and Kittredge 1979) as well as increasing turbulence at higher wind speeds (Elkinton and Cardé 1984). However, the plume is being transported downwind more rapidly at high wind velocity. The active spaces of pheromones may be greatest at intermediate wind speeds. Nakamura (1976) documented Oriental leafworm moth, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), response was greatest at intermediate wind velocities and declined at both the highest and lowest velocities. This is also supported by increased calling by cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), females at intermediate wind velocities (Kaæe and Shorey 1972) and decreased flight at high wind speeds (Sower et al. 1973). However, the release rate from constantly emitting synthetic lures may be greater at increasing wind velocities (Elkinton et al. 1984). Air temperature also likely influences active space. Baker and Roelofs (1981) document increased distance at which Oriental fruit
moth, *Grapholitha molesta* (Busck) (Lepidoptera: Tortricidae), males responded to synthetic female sex pheromone lures at increasing temperatures. However, this could be a result of increasing release rates of synthetic pheromones with increasing temperatures (Tobin et al. 2011). Due to reduced variation in release rates relative to traps baited with live females, synthetic pheromone blends are most often used in assessments of active space.

The active space of lepidopteran pheromone traps has been evaluated for several species. However, many of these studies are difficult to interpret due to inconsistent methodology or failure to assess adequately large distances. The active space of *S. litura* pheromone traps was estimated to be ≈80 m in a series mark-release-recapture experiments conducted by Nakamura and Kawasaki (1977). Linn et al. (1987) determined the active space of *G. molesta* ranged from 30–60 m depending on pheromone blend concentration and temperature. This estimate was derived through direct observation of male response to pheromone detection when approaching the pheromone source from downwind. This method was also employed by Elkinton et al. (1984) to determine that male Gypsy moths, *Lymantria dispar* (L.) (Lepidoptera: Erebidae), can detect sex pheromones from distances of more than 80 m. However, estimates of active space under controlled conditions may not be accurate when applied in the field where numerous environmental factors can affect pheromone dispersion and detection (Elkinton and Cardé 1984).

One way active distance of pheromone traps can be assessed in the field is through examination of the effects of intertrap distance. Elkinton and Cardé (1988) used hexagonal arrays with varying intertrap distance as well as a 6 x 6 trap grid to evaluate *L. dispar* pheromone trap interactions. They determined that trap capture was maximized at an intertrap distance of 80 m. However, larger intertrap distances were not evaluated in that study and the maximum active space may not have been identified. Similarly, examination of the interference between traps was
used to provide an estimate of the active space of the summerfruit tortrix moth, *Adoxophyes orana* (Fischer von Röslerstamm) (Lepidoptera: Tortricidae), pheromone traps of 15–40 m, but larger distances were not evaluated (Van der Kraan and Van Deventer 1982). Wall and Perry (1980), determined the active space of the pea moth, *Cydia nigricana* (F.) (Lepidoptera: Tortricidae), pheromone baited traps may be in excess of 400 m; however, they were unable to determine a maximum distance of attraction. Forty percent of lesser peachtree borer moths, *Synanthedon pictipes* (Grote and Robinson) (Lepidoptera: Sesiidae), were captured in traps baited with virgin females 800 m upwind of their release point (Karandinos 1974). However, the distance at which the pheromone was detected was not determined by this study.

Because of the numerous abiotic factors affecting active space and the apparent variation between different groups of Lepidoptera (Elkinton and Cardé 1984), universal conclusions regarding active space of insect pheromones are rare. Continued examination of pheromone interactions among new model systems will broaden our knowledge of insect attraction.

### 2.7. Invasive Species Monitoring

The most commonly used and successful application of insect sex pheromones is in detection and population monitoring. Pheromone traps are useful in monitoring population fluctuations and may provide improved early warning signs of pest outbreaks (Robacker and Landholt 2002). Pheromone baited traps are particularly effective at detecting low level populations and are useful in early detection of invasive species entering new regions (Witzgall et al. 2010). Pheromone traps are widely used in monitoring and eradication of invasive insect pests. Some high-profile invasive insects for which pheromone trap monitoring programs are currently in place include the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) (Smith 1998); the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae); the pink bollworm,
*Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (Baker et al. 1990), and *Lymantria dispar* (L.) (Lepidoptera: Erebidae) (Sharov et al. 2002). Of these, the USDA APHIS sponsored programs, the Boll Weevil Eradication Program and the Slow the Spread program for *L. dispar* control, are two wide spread and successful examples of pheromone based monitoring of invasive insects. In order for *A. grandis* eradication to be successful, Knipling (1968) identified the need for a way to detect low level populations. Unlike pheromone trap monitoring of lepidopteran insects which primarily use female sex pheromones, the “grandlure” was developed from the aggregation pheromone produced by *A. grandis* males which attracts both males and females (Hardee et al. 1969). Since 1978, grandlure baited traps have been used successfully to detect low level populations and measure success of *A. grandis* eradication efforts throughout the southeast U.S. (USDA APHIS 2013). With the help of pheromone baited traps, the Boll Weevil Eradication Program has successfully eradicated the invasive pest from 98% of the U.S. cotton acreage (USDA APHIS 2013). Due to its success in the U.S., *A. grandis* pheromone trapping is now used on >250,000 ha in South America (Smith 1998). The Slow the Spread program is the largest invasive species monitoring program in existence which utilizes >80,000 pheromone traps throughout nine states (Sharov et al. 2002). The program uses a grid of pheromone baited traps spaced at 2-km intervals along the advancing edge of the range of *L. dispar* to detect isolated populations. These isolated populations primarily result from accidental transportation of egg masses because of the inability of females to fly and limited movement of larvae (Liebhold et al. 1992). Once detected, isolated populations are eliminated with insecticide applications. This program is estimated to reduce the annual expansion rate of *L. dispar* by ≈50% (Sharov et al. 2002).
In addition to uses in eradication and mitigation programs, pheromone trapping is used to
generate data to assess population dynamics such as rates of range expansion and landscape
effects of invasive species. The availability of Geographical Information Systems (GIS)
technology has allowed for landscape level applications of pheromone trapping data to document
geographic variation, predict pest outbreaks, and delimit invasive species (Witzgall et al. 2010).
The ability to predict spatial and temporal variability of invasive insect populations can aide in
development of effective strategies and policy. Tobin et al. (2007) utilized data from more than
100,000 uniformly spaced pheromone traps monitored from 2002-2006 to estimate the rate of
spread of *L. dispar* populations in five different regions of the U.S. Their results were
encouraging for other invasive species monitoring programs in that the predicted rates of spread
based measurements of boundary displacements from fixed points did not differ greatly from
estimates derived from more crude presence/absence county level data (Tobin et al. 2007).

*Lymantria dispar* range expansion varies from 5.8–18.0 km/yr across different regions of the
U.S. (Tobin et al. 2007). Augustin et al. (2004) determined pheromone traps were as effective as
more labor intensive damage assessments for monitoring of the invasive horse-chestnut leaf,
*Cameraria ohridella* (Lepidoptera: Gracillariidae), in France. In these studies, a combination of
leaf damage scores and pheromone trap captures was used to determine the range of *C. ohridella*
was expanding at ≈10 km/generation (40 km/yr).

### 2.8. *Eoreuma loftini* Pheromone Trapping

The presence of a female sex pheromone was first detected by Brown et al. (1988), and later
isolated from female ovipositor extracts (Shaver et al. 1988). The pheromone blend was
determined to be (Z)-13-octadecenyl acetate (Z-13-ODAc), (Z)-11-hexadecenyl acetate (Z-11-
HDAc), and (Z)-13-octadecenal (Z-13-ODAl) in a ratio of 8:1:1.3, respectively (Shaver et al.
Both field and laboratory assessments indicated all three components must be present to elicit full male response. A 2-component blend of Z-13-ODAc and Z-13-ODAl did have some activity, but no response to individual components alone was detected (Shaver et al. 1988). In initial evaluations of the blend in a ratio of 8:1:1.3 of Z-13-ODAc, Z-11-HDAc, and Z-13-ODAl, respectively, dosages of 200–600 μg caught comparable numbers of male *E. loftini* to traps baited with virgin females (Shaver et al. 1988). Increasing the concentration of the major component (Z-13-ODAc) relative to the minor ones did not improve trap performance (Shaver et al. 1990). The blend can be impregnated on rubber septa or other medium by diluting the blend in hexane to a concentration of 20 mg/ml, pouring it into the septa and allowing hexane to evaporate (Shaver et al. 1990). Rubber septa impregnated with 5.0 mg of pheromone blend caught significantly more moths than PVC rods, plastic vials, or multi-layered polymeric dispensers with the same concentrations of pheromone (Shaver et al. 1990). The attractive activity of rubber septa baited with the pheromone blend showed no reduction in attractive ability after three weeks of field use (Shaver et al. 1990). Rubber septa impregnated with 5.0 mg of the *E. loftini* female sex pheromone blend are now commercially available for purchase (Lure sept; Hercon Environmental, Emigsville, PA). Once the most attractive pheromone blend had been determined, researchers focused on development of trapping strategies for use in the field.

Field evaluations showed Universal Moth Traps (Great Lakes IPM, Vestaburg, MI) with green tops, yellow funnels, and white buckets (GYW Unitraps) caught significantly more moths than all other trap types tested (Shaver et al. 1991). Additionally, traps at heights of roughly 1 m above the ground placed within sugarcane fields outperformed other scenarios tested. Based on the research of Shaver et al. (1988, 1990, 1991) GYW Unitraps baited with rubber septa
impregnated pheromone blend hung at a height of roughly 1.0 m have been adopted as the standard for *E. loftini* pheromone traps. The recommended trapping protocol requires replacing pheromone lures every two weeks and insecticidal vapor strips (Vaportape II; Hercon Environmental, Emigsville, PA) every four weeks.

Since their development, *E. loftini* pheromone traps have been used to monitored the pest’s expansion across Texas and into Louisiana (Reay-Jones et al. 2007c, Hummel et al. 2010) as well as used to assist in pest scouting in sugarcane (Wilson et al. 2012b). However, much about trap efficacy remains unknown. All research on *E. loftini* pheromone traps was conducted in sugarcane agroecosystems in the Rio Grande Valley, and may not be applicable to other environments. Additionally, trap performance may also depend on environmental conditions such as wind speed (Sutton 1953, Bossert and Wilson 1963, Aylor et al. 1976, Elkinton and Cardé 1984), temperature (Bossert and Wilson 1963, Elkinton and Cardé 1984), and structural interference (Nakamura 1976, Nakamura and Kawasaki 1977, Elkinton and Cardé 1984). Pheromone dispersion and active space has been studied in a number of insects, but no research in these areas with *E. loftini* has been conducted.
CHAPTER 3: THE ACTIVE SPACE OF MEXICAN RICE BORER (LEPIDOPTERA: CRAMBIDAE) PHEROMONE TRAPS

3.1. Introduction

The Mexican rice borer, *Eoreuma loftini* (Dyar), is an invasive insect which originated in Mexico and has become established along the U.S. Gulf Coast. This invasive species is now present in Texas, Louisiana, and Florida where it is a pest of sugarcane, *Saccharum* spp., rice, *Oryza sativa* L., and other graminaceous crops (Legaspi et al. 1997a, Hayden 2012, Chapter 4). The range expansion of *E. loftini* has been monitored with traps baited with a female sex pheromone blend (Reay-Jones et al. 2007c, Hayden 2012, Chapters 4, 7). Pheromone traps are also used to assist in scouting for *E. loftini* in sugarcane and rice (Wilson et al. 2012b, Chapters 4–6). Initial studies by Shaver et al. (1990, 1991) determined the synthetic pheromone blend is as effective at attracting *E. loftini* males as traps baited with virgin females. These studies determined optimal concentrations of pheromone blends and trap designs to increase moth capture. Field studies which determined trap designs and trap deployment methods were conducted at intertrap distances of 50 m (Shaver et al. 1990, 1991). However, if mutual interactions between traps at this distance occur, experimental results from these studies may be invalid (Cardé and Baker 1984). Determination of the active space, or radius of attraction, of *E. loftini* pheromone traps will provide a critical piece of information to improve pheromone trapping strategies for this pest.

The active space is defined as the distance from a pheromone source inside which the odor concentration is above the level sufficient to produce a response in the receiving organism (Elkinton and Cardé 1984). The active space of female sex pheromones varies widely between Lepidopteran species, and the factors which influence it are not easily understood. Numerous methods have been employed to estimate the active space of Lepidopteran pheromone traps.
including mark-release-recapture experiments, wind tunnel assays, and field experiments which assess the effects of intertrap distance. Many of these experiments were conducted under controlled conditions or over small temporal periods and are unlikely to represent the wide range of environmental variables which are encountered in the field.

The active space of pheromone traps for *Cydia nigricana* (F.) (Lepidoptera: Tortricidae) was estimated to be greater than 100 m through a series of mark-release-recapture studies (Wall and Perry 1978). The active space of traps baited with virgin females and synthetic pheromone blends for *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) was estimated to be approximately 80 m. The active space of pheromone detection for males of the lesser peach tree borer, *Synanthedon pictipes* (Grote and Robinson) (Lepidoptera: Sesiidae) is among the greatest distances documented for Lepidoptera. In mark-release-recapture studies, 40% male *S. pictipes* were recovered at a pheromone source 800 m up wind of the release point (Karandinos 1974).

Although female sex pheromones have been identified for numerous Crambid species, the active space has not yet been evaluated for any member of this pestiferous Lepidopteran family.

Field studies which assess the effects on intertrap distance on trap interactions have been used to estimate the active space for *Lymantria dispar* L. (Lepidoptera: Erebidae) (Elkinton and Cardé 1988) as well as *Adoxophyes orana* (Lepidoptera: Tortricidae) (Van der Krann and Van Deventer 1982). A behavioral assay was used to assess the distance from a pheromone source at which male *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) detect on respond to female sex pheromones (Linn et al. 1987). A comprehensive approach involving a two-year field study and a behavioral assay based on these methods was used to estimate the active space of *E. loftini* traps. This information can be used to improve pheromone trapping strategies for use in pest management.
management and invasive species monitoring. This work provides the first estimation of the active space for a Crambid pest species.

3.2. Materials and Methods

3.2.1. Trap Interference Experiment

A two-year field study utilized hexagonal arrays of *E. loftini* pheromone traps (Figure 3.1) to determine the effect of intertrap distance on trap performance. Intertrap distance was equal between each perimeter trap and its two closest neighboring perimeter traps. The central trap is equidistant from all perimeter traps. When the intertrap distance is less than the active space of each trap, the trap capture in all traps will be reduced due to trap interference. This interference will also result in a reduced proportion of the total array trap capture which is caught by the central trap (Elkinton and Cardé, 1988).

The female sex pheromone blend consists of (Z)-13-octadecenyl acetate (Z-13-ODAc), (Z)-11-hexadecenyl acetate (Z-11-HDAc), and (Z)-13-octadecenal (Z-13-ODAl) in a ratio of 8:1:1.3, respectively (Shaver et al. 1988). Universal Moth Traps (Great Lakes IPM, Vestaburg, MI) with green tops, yellow funnels, and white buckets (GYW Unitraps) baited with a rubber septa impregnated with 5.0 mg of the *E. loftini* female sex pheromone blend (Luresept; Hercon Environmental, Emigsville, PA) placed at heights of approximately 1 m above the ground were used for all assays in accordance with recommendations from Shaver et al. (1991). Each trap contained an insecticidal strip (Vaportape II; Hercon Environmental, Emigsville, PA). Pheromone lures were replaced every two weeks, and insecticidal strips were replaced every four weeks according to label instructions. These traps are widely used in pest scouting and invasive species monitoring for *E. loftini* (Reay-Jones et al. 2007c, Wilson et al. 2012b, Chapters 4–7).
Arrays were deployed in homogenous rice stubble habitat at commercial farms in Chambers and Jefferson Counties, Texas (2011) and in Calcasieu Parish, Louisiana (2013). In each year, 5 arrays were deployed in each of two farms. Traps were checked and arrays re-randomized within each farm for each of five weeks for a total of 10 replications (farm × sampling period) each year. In 2011, intertrap distances of 5, 25, 50, 100, and 250 m were selected. The minimum distance between traps from different arrays was 280 m with a mean distance of 337 m. Trap arrays were initially deployed on 19 Oct 2011 and were checked and re-randomized to new locations within each farm on 25 Oct, 3 Nov, 10 Nov, 17 Nov, and 29 Nov 2011. Sampling periods in 2011 ranged from 6–12 days with an average of 7.3 days. In 2013, intertrap distances of 50, 100, 150, 225, and 300 m were evaluated. The minimum distance between arrays was 357
m with a mean distance of 2,028 m. Traps at the Lake Charles, LA farm were initially deployed 20 Sept 2013 and were checked and re-randomized on 25 Sept, 9 Oct, 17 Oct, 23 Oct, and 29 Oct 2013. Traps at the Vinton, LA farm were initially deployed 25 Sept 2013 and were checked and re-randomized on 3 Oct, 9 Oct, 17 Oct, 23 Oct, and 29 Oct 2013. Sampling periods in 2013 ranged from 5–14 days, with an average of 7.3 days. Data was not collected from the array at the Vinton farm with an intertrap distance of 50 m during the final sampling period in 2013 because flooded conditions hindered access to traps.

For all arrays in both years, each trap was given a designated “position” (either center or perimeter). Each perimeter trap was assigned two cardinal directions (Direction1 and Direction2) relative to the central trap to account for the effect of wind direction. Perimeter traps were either North(N) x North(N), South(S) x South(S), N x East(E), N x West(W), S x E, or S x W. Central traps were assigned a C x C designation. No prevailing wind directions are known to exist at any of the experimental farms, and wind speed and direction changed naturally throughout sampling periods (Texas Commission of Environmental Quality 2002, Windfinder 2016).

All trap capture data were converted to daily trap captures prior to statistical analysis. For each sampling period, the trap captures for each array were totaled and the proportion of the total array capture for each trap was calculated. Data from each year were analyzed separately due to differences in the intertrap distances evaluated and locations. Daily trap capture data were analyzed with ANOVAs with Gaussian distributions and intertrap distance as a fixed effect and farm, sampling period, farm × sampling period, and intertrap distance × farm × sampling period as random effects (Proc GLIMMIX; SAS Institute 2008). Daily trap capture data were also subjected to a two-way ANOVA with Direction1, Direction2, and the interaction as fixed effects to test for an influence of wind direction. The proportion of the total array capture data were
analyzed with two-way ANOVAs with Gaussian distributions and position, intertrap distance, and the interaction as fixed effects. Additionally, a linear regression (Proc REG; SAS Institute 2008) was conducted to examine the effect of intertrap distance on the proportion of the total array capture caught by center traps. This regression included data from the center trap of all arrays across years, farms, and sampling periods. The proportion of total array capture caught in the center trap provides a metric to compare the degree of trap interference across intertrap distances independent of environmental factors.

3.2.2. Behavioral Assay

An assay was conducted which observed the behavior of *E. loftini* males while approaching from downwind of the pheromone source according to the methods of Linn et al. (1987). The assay was conducted in an unoccupied livestock arena on the Campus of Louisiana State University, Baton Rouge, LA. An “airstream” was established using a series of floor fans. One fan was placed approximately 1 m behind the pheromone source (Universal bucket trap containing one pheromone lure). Pairs of fans angled inward were placed at 10, 20, 30, 40 and 60 m downwind of the pheromone source. The flow of air through the airstream was monitored using a stream of bubbles produced from a small bubble machine (Gazillion Bubble Hurricane; Funrise Toy Corporation, Van Nuys, CA) containing a standard glycerin-based bubble mixture. Fans were positioned to create a constant air stream approximately 2 m wide. A handheld digital anemometer (Model GM816; Benetech, Aurora, IL) was used to measure air velocity and temperature. The ambient temperature in the arena was 22.8 °C. Air velocity within the airstream was measured at 0, 5, 25, 35, 45, 55, and 65 m from the pheromone source and averaged 0.92 ± 0.05 [SE] m/s. Air velocity outside of the airstream was not able to be detected with the anemometer, indicating potential sources of interference were negligible.
Figure 3.2. Screen mesh cages holding *E. loftini* males during the behavioral assay. Cages were suspended from small twine and held at the same height as the pheromone source.

*Eoreuma loftini* males were obtained from a laboratory colony reared on artificial diet (Martinez et al. 1988) at 25°C, 65% RH, and a photoperiod of 14:10 (L:D). Two weeks prior to adult emergence, *E. loftini* larvae were placed under offset photoperiod so assays conducted between 10:00 AM and 2:00 PM would correspond to hours of scotophase when peak mating activity occurs under natural conditions (Brown et al. 1988, Shaver et al. 1994). Adult *E. loftini* males were placed in cages immediately after emergence from pupal casings approximately 24–48 hours prior to conducting the assays.

Males were placed in cylindrical cages with aluminum window screening (18 x 16 mesh) on all sides (Figure 3.2). An observer held the caged male at height even with the pheromone source (≈1 m above the ground) and walked upwind towards the pheromone source from an initial distance of 100 m. When males exhibited excited behavior (walking, wing fanning, taking flight, etc.), a marker flag was placed on the ground (Figure 3.4). Flags and cages were marked with numbers to designate males. This was repeated for 30 *E. loftini* males. After each initial exposure, males were placed in a dark box outside of the arena for a minimum of two hours and
the assay was repeated. The distance of initial response to detection for each male was recorded
for the first and second exposure. An ANOVA compared the distance of initial response between
first and second exposure to the pheromone blend. Individual males (n = 30) served as
replications.

### 3.3. Results

#### 3.3.1. Trap Interference Experiment

A total of 4,691 adult *E. loftini* males were captured in all traps over the 5 wk experiment in
2011. Differences were detected between intertrap distances in daily trap captures and the
proportion of the total array capture caught in center traps (Table 3.1). Daily trap capture ranged
from 0.51 to 4.22 *E. loftini*/trap/day and increased with increasing intertrap distance. Differences
in the proportion of the total array capture were detected between positions (*F* = 21.55, *df* = 1,
293; *P* < 0.001) and the position × distance interaction, but not between distances. The
proportion of the total array capture in the central trap was different (*P* < 0.05) from the
proportion caught in perimeter traps in arrays with intertrap distances of 5, 25, and 50 m. An
effect of direction relative to the central trap was not detected (*P* > 0.05) for Direction1,
Direction2, or the Direction1 × Direction2 interaction.

<table>
<thead>
<tr>
<th>Intertrap distance (m)</th>
<th><em>E. loftini</em>/trap/day (LS means ± 1.08 [SE])</th>
<th>Proportion of total array capture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Central Trap</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(LS means ± 0.029 [SE])</td>
</tr>
<tr>
<td>5</td>
<td>0.51a</td>
<td>0.056*</td>
</tr>
<tr>
<td>25</td>
<td>0.90a</td>
<td>0.044*</td>
</tr>
<tr>
<td>50</td>
<td>1.34a</td>
<td>0.069*</td>
</tr>
<tr>
<td>100</td>
<td>2.90b</td>
<td>0.102</td>
</tr>
<tr>
<td>250</td>
<td>4.22c</td>
<td>0.163</td>
</tr>
</tbody>
</table>

Table 3.1. The effect of intertrap distance on daily *E. loftini* trap capture and the proportion of
array total captured by center traps, Jefferson and Chambers Counties, Texas, 2011

Means which share a letter are not significantly different (Tukey’s HSD, *α* = 0.05)
*Indicates central trap is significantly less than perimeter traps (Tukey’s HSD, *α* = 0.05)
During the 2013 experiment a total of 9,544 E. loftini males were captured in all traps throughout the experiment. Differences were detected between intertrap distances in daily trap captures (Table 3.2). An effect of the position × distance interaction on the proportion of total array capture was detected (Table 3.2), but no effect of distance or position was observed ($P > 0.05$). The proportion of the total array capture by the center trap was less than the mean proportion caught by perimeter traps at an intertrap of 50 m, but not at other distances. Results from the 50 and 100 m intertrap distances were consistent with results at the same distances evaluated in 2011. An effect of direction relative to the central trap was not detected between Direction1 ($F$ = 2.40, df = 1, 286; $P = 0.12$), Direction2 ($F$ = 0.96, df = 3, 286; $P = 0.41$) or the Direction1 × Direction2 interaction ($F$ = 3.47, df = 1, 286; $P = 0.06$).

A significant relationship occurred ($F$ = 64.21, df = 1, 98; $P < 0.001$, $R^2 = 0.39$, Root MSE = 0.057) between the proportion of the total array capture caught by the central trap and the intertrap distance (Figure 3.3).

Table 3.2. The effect of intertrap distance on daily E. loftini trap capture and the proportion of array total captured by center traps, Calcasieu Parish, Louisiana, 2013

<table>
<thead>
<tr>
<th>Intertrap distance (m)</th>
<th>E. loftini/trap/day (LS means ± 0.76 [SE])</th>
<th>Proportion of total array capture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Central Trap (LS means ± 0.029 [SE])</td>
</tr>
<tr>
<td>50</td>
<td>1.45a</td>
<td>0.083*</td>
</tr>
<tr>
<td>100</td>
<td>2.66b</td>
<td>0.107</td>
</tr>
<tr>
<td>150</td>
<td>4.36c</td>
<td>0.146</td>
</tr>
<tr>
<td>225</td>
<td>5.04c</td>
<td>0.156</td>
</tr>
<tr>
<td>300</td>
<td>6.54d</td>
<td>0.189</td>
</tr>
</tbody>
</table>

$F = 16.94$  \hspace{1cm} 3.78

df = 4, 35.0  \hspace{1cm} 4, 287.0

$P = <0.001$  \hspace{1cm} 0.005

1Means which share a letter are not significantly different (Tukey’s HSD, $\alpha = 0.05$)

*Indicates central trap is significantly less than perimeter traps (Tukey’s HSD, $\alpha = 0.05$)
3.3.2. Behavioral Assay

All *E. loftini* males responded to detection of the pheromone within the airstream. Prior to detection of the pheromone blend all males remained motionless in the cages. When the males detected the pheromone they became excited, walking rapidly in small circles and exhibiting wing fanning. This behavior persisted for approximately 10–15 seconds in all males before they returned to a non-excited state. Once wing fanning and walking behaviors were observed, the observer opened the cage to allow the males to take flight. None of the males took flight or exhibited any responses to the pheromone other than the wing fanning and walking behavior.
observed at the initial detection. Responses to the second exposure to the pheromone blend did not differ from that of the first exposure. The distance from the pheromone source at which males response was observed ranged from 24.5–60.6 m (Figure 3.4) and had a mean of 47.6 m. Differences were not detected ($F = 1.85$, df = 1, 53.0; $P = 0.179$) between the distance from the pheromone source at first (46.5 m) and second (48.8 m) exposure to the pheromone blend.

3.4. Discussion

The hexagonal array experimental design originally adapted from Elkinton and Cardé (1988) provided a thorough assessment of the effects of intertrap distance on *E. loftini* trap performance under field conditions. By utilizing week-long sampling periods, multiple locations, and multiple years, this experimental design measured the active space under variable wind speeds, wind directions, temperatures, and other environmental conditions. Trap captures at commercial farms in Texas (2011) and in Louisiana (2013) were sufficiently high to detect a strong effect of trap interference. The daily trap captures observed in arrays which minimized trap interference were greater than those reported in other sugarcane and rice habitats (Wilson et al. 2012b, Chapters 4–6) indicating the stubble rice habitat where hexagonal arrays were deployed harbor high populations of *E. loftini* during the fall. The two-year study provides strong evidence that significant interference occurs between traps in rice habitat when the intertrap distance is less than 100 m. Results from previous studies which determined optimal trapping strategies in sugarcane (Shaver et al. 1991) were conducted with an intertrap distance of 50 m were likely affected by interference between traps. However, the active space of *E. loftini* pheromone traps in sugarcane habitat may differ where tall crop canopies likely influence pheromone dispersion. Deposition or surface adsorption has potential to influence pheromone dispersion (Nakamura 1976, Nakamura and Kawasaki 1977). The effects of pheromone deposition in our field assays
were likely minimized because the 1 m trap height was well above the stubble rice present in the fields.

Our results were consistent with previous studies which utilized hexagonal arrays to assess trap interference which reported decreased trap captures in central traps (Van der Krann and Van Deventer 1982, Elkinton and Cardé 1988). The proportion of the total trap capture caught in the central trap continually increased with increasing intertrap distance in our study. If trap interference was negligible at the greatest intertrap distance, the proportion caught in the central traps would be equal to the mean from perimeter traps. The numerically greater proportion caught by central traps in arrays with an intertrap distance of > 200 m observed in our study may have resulted from an edge effect which reduced trap captures in perimeter traps near field margins. The consistency in results from distances evaluated in both 2011 and 2013 indicates the experimental design effectively assessed interference between traps while minimizing the impact of other factors.

Figure 3.4. Response distance of *E. loftini* males to detection of the pheromone blend. Orange flags indicate the sites of individual male responses. Distances indicated by white lines are approximations
Results from the behavioral assay under controlled conditions confirmed that the active space of pheromone traps is approximately 50 m. The unfailing ability of males to detect and respond to the pheromone blend in our assay demonstrates that this methodology can be used to assess active space of Lepidopteran sex pheromones under controlled conditions. Wing-fanning and walking responses observed in our study were consistent with those reported in a similar assay assessing males of *G. molesta* (Linn et al. 1987). By designing a controlled airstream, our assay allowed for male response to be evaluated under stable conditions. Linn et al. (1987) noted logistical difficulties including excessive wind speeds while conducting similar assays under field conditions. By varying conditions within the air stream, the effect wind speed, wind direction, temperature, or other factors on active space could be evaluated with this approach.

The male response observed in our study was largely consistent with that reported from studies of *E. loftini* mating behavior (Brown et al. 1988); however, no males took flight in our study. Brown et al. (1988) reported that upon detection of the pheromone, *E. loftini* males immediately began to flutter wings and either walked or flew to the source. The cages prevented the males from attempting to walk towards the source and may have discouraged taking flight. Notably, Brown et al. (1988) reported that crawling toward the source was more prevalent than flying during the first 4 hours of scotophase while flying was more frequent during the latter hours of scotophase. The males used in our assays were held under a controlled photoperiod so that assays were conducted at times which would correspond to approximately 4–8 hours into scotophase. Further, males were kept in a dark enclosure at all times when not subjected to the assay, but a low level of artificial light which was required for observation may have influenced male behavior. The failure to detect a difference of male response between first and second exposures to the pheromone blend was also consistent with Brown et al. (1988) who did not
observe differences between first, second, third, or fourth exposures occurring on each subsequent night. Our results suggest the removing *E. loftini* males from the presence of the pheromone source for a period of two hours was sufficient for the males to return to a resting, non-excited state. Results from this observational assay encourage further evaluation of *E. loftini* active space under variable environmental conditions.

Based on results from our two-year field study in addition to the behavioral assay, we assert that the active space of *E. loftini* pheromone traps is between 50–100 m. Traps used for *E. loftini* invasive species monitoring, pest scouting, or in subsequent experiments should not be deployed with an intertrap distance < 100 m. Assuming that the active space functions as a radius of attraction around each trap, a single pheromone trap can monitor an estimated 1–3 ha. This information will improve deployment strategies for *E. loftini* pheromone traps used in invasive species monitoring (Reay-Jones et al. 2007c, Chapters 4,7) and in pest scouting in sugarcane and rice (Wilson et al. 2012b, Chapters 4–6).

This research builds on a growing body of literature investigating the active space of Lepidopteran pheromones. Although pheromone trapping strategies are being developed for several Crambid species (Witzgall et al. 2010), this work provides the first examination of active space of sex pheromones in this pestiferous family of Lepidoptera. Additionally, the comprehensive approach involving both field experiments and behavioral assays may be adapted to evaluate the active space of other Lepidopterans.
CHAPTER 4: EXPANSION OF THE MEXICAN RICE BORER (LEPIDOPTERA: CRAMBIDAE) INTO RICE AND SUGARCANE IN LOUISIANA

4.1. Introduction

The Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), is an invasive insect originating from Mexico which has become established as a major pest of graminaceous crops in Texas. *Eoreuma loftini* attacks sugarcane, *Saccharum* spp., rice, *Oryza sativa* L., corn, *Zea mays* L., sorghum, *Sorghum bicolor* L., and many non-crop grass species (Showler et al. 2011, 2012; Beuzelin et al. 2011b, 2013). *Eoreuma loftini* was first reported as a pest in the U.S. in 1980 on sugarcane in the Lower Rio Grande Valley of Texas (Johnson and van Leerdam 1981), where it now comprises >95% of the sugarcane stem borer population (Legaspi et al. 1997a). *Eoreuma loftini* has since spread northeast through the rice production area along the Texas Gulf Coast (Reay-Jones et al. 2007c) and was discovered in Louisiana in December 2008 (Hummel et al. 2010). A quarantine was implemented during the 2005 crop production season to prevent movement of infested Texas sugarcane into Louisiana (Reagan et al. 2005), which is estimated to have saved the Louisiana sugarcane industry $1.1–3.2 billion (Reay-Jones et al. 2008). Establishment of *E. loftini* throughout Louisiana is expected to cause annual revenue losses as great as $220 and $40 million to the sugarcane and rice industries, respectively (Reay-Jones et al. 2008). Because *E. loftini* uses a broad range of host plants, eradication of this pest is not feasible (Johnson and van Leerdam 1981, Showler et al. 2011, 2012; Beuzelin et al. 2011b, 2013). Development of population management strategies is the only viable approach to mitigating the pest’s impact (Showler and Reagan 2012).

The *E. loftini* female sex pheromone was isolated (Brown et al. 1988) and developed for use in pheromone traps (Shaver et al. 1988, 1990, 1991), which are deployed to monitor *E. loftini*.
range expansion (Reagan et al. 2005, Reay-Jones et al. 2007c, Hummel et al. 2010) and for pest scouting in individual sugarcane fields (Wilson et al. 2012b). The recent discovery of *E. loftini* in Florida (Hayden 2012) highlights the potential for rapid range expansion. Objectives of this work were to (1) monitor *E. loftini* distribution and expansion in southwestern Louisiana, (2) determine the severity of *E. loftini* infestations in rice and sugarcane, and (3) evaluate the potential for use of pheromone traps to improve scouting for *E. loftini* in rice.

**4.2. Materials and Methods**

4.2.1. Pheromone Trap Monitoring

Adult *E. loftini* populations were monitored using pheromone traps in southwestern Louisiana from 2009 through 2013. Standard green, yellow, and white bucket traps (Unitrap; Great Lakes IPM, Vestaburg, MI) were baited with synthetic *E. loftini* sex pheromone lures (Luresept; Hercon Environmental, Emigsville, PA). Each trap contained an insecticidal strip (Vaportape II; Hercon Environmental, Emigsville, PA). Traps were attached to metal poles 1 m above the soil surface to maximize trap performance (Shaver et al. 1991). Pheromone lures and insecticidal strips were replaced every 4 wk according to label instructions.

The number and locations of traps in each parish varied by year. Trap locations were recorded using a handheld GPS unit. The distance of each trap to its nearest neighboring trap was determined using GPS coordinates, and mean distance between traps was calculated for each year. Mean distances between traps during each year were 2.7, 3.1, 5.6, 6.1, and 9.0 km in 2009, 2010, 2011, 2012 and 2013, respectively. Prior to 2013, trap locations were 5–15 km east of the eastern edge of the known *E. loftini* range as determined by monitoring in the prior years. Trapping was expanded in 2013 to include areas where the pest was already known to occur. Hence, monitoring in 2013 involved Calcasieu, Cameron, Jefferson Davis, Beauregard, Allen,
and Acadia parishes, >15,000 km². Traps in southwestern Louisiana (Calcasieu, Cameron, Jefferson Davis, Beauregard, Allen, Acadia, and Vermilion parishes) were placed adjacent to rice fields, sugarcane fields, or pastures with wild hosts, and traps in St. Mary, St. Martin and Iberia parishes were located near sugar mills. Specimens which represented the possible initial detection of *E. loftini* in a parish were transported to the Louisiana State Arthropod Museum (Louisiana State University, Baton Rouge, LA) for identification (Reiss 1981, Agnew et al. 1988).

4.2.2. *Eoreuma loftini* Infesting Sugarcane and Rice

Monitoring of *E. loftini* larval infestations in sugarcane was conducted from May–October, 2013 in two fields in Calcasieu Parish (≈120 ha total; var. HoCP 04-226) and three fields in Jefferson Davis Parish (≈220 ha total; var. L 99-226 and HoCP 96-540). A pheromone trap was placed adjacent to each field within 1 m of field edges to monitor adult populations. Larval infestations were assessed by monthly examination (5 sampling events per field) of 100 randomly selected stalks for the presence of *E. loftini* larvae. Stalks were considered infested if at least one *E. loftini* larva was observed feeding within the stalk or on plant surfaces.

Surveys involved monitoring of adult population densities and larval infestations in rice fields treated with chlorantraniliprole (Dermacor X-100, E.I. du Pont de Nemours and Company, Wilmington, DE) applied to seed at 80 g a.i./ha and fields without insecticidal seed treatments. No other insecticides were used on experimental fields during the growing season. Eleven fields were surveyed in 2012 (four treated and seven nontreated) and 12 (six treated and six nontreated) in 2013. A replication consisted of a pair of one treated and one nontreated field, each 30–165 ha, within 3.6 km of each other. Rice cultivars reflected those most commonly grown in Louisiana and included CL111, CL151, XL745, XL729, Cheniere, Cocodrie, and Mermentau.
Due to lack of replication of cultivars in the experimental design, rice cultivar was not included as an effect in the statistical analysis. All fields surveyed were located in areas of Calcasieu Parish where development of substantial *E. loftini* populations was anticipated based on pheromone trap captures during the previous spring. A single pheromone trap was placed directly adjacent to each field and monitored throughout the growing season. These traps were monitored for the duration of the rice-growing season (May through August) and augmented the traps used in the pheromone trap monitoring described in the previous section. Fields were sampled for larval infestations six times in 2012 (30 May, 14 June, 7 July, 28 July, 12 August, 26 August) and eight times in 2013 (21 June, 27 June, 3 July, 10 July, 18 July, 29 July, 6 August, and 15 August). On each sampling date, 25 rice tillers were randomly selected within a 50-m radius of the pheromone trap, observed for stem borer injury, and dissected. No other species of stem borer was recovered during the surveys in either year, and all injury by stem borer larval feeding was assumed to have been caused by *E. loftini*. Once rice fields neared maturity (hard-dough stage, 29 July–26 August), they were sampled by counting the total number of rice tillers and the number of tillers with “whiteheads” [incompletely emerged panicles or panicles which do not produce grain resulting from insect injury to vascular tissue during plant growth] (Pathak 1968) within a randomly positioned 1-m² quadrat. Whiteheads present in each sample were dissected to verify injury was caused by stem borer feeding and all recovered larvae were verified as *E. loftini*. Whitehead data were not collected in one treated and two nontreated fields in 2012 because the fields were harvested before sampling.

Trap capture converted to daily estimates and percentage of injured tillers for each growing season were analyzed using a three-way ANOVA (PROC MIXED, SAS Institute 2008) with year, treatment, sampling date, year × treatment, sampling date × year, sampling date ×
treatment, and sampling date × year × treatment as fixed effects. ANOVA models comparing trap captures and percentage injured tillers included replication(year) and treatment × replication(year) as random effects to account for the effects of the design of the study and repeated measures (variance component covariance structure). The number of whiteheads per m² and the percentage of tillers with whiteheads were compared using a two-way ANOVA (PROC MIXED, SAS Institute 2008) with year, treatment, and year × treatment as fixed effects and replication(year) as a random effect. Tukey’s honestly significant difference test (α=0.05) was used for mean separations and Kenward-Roger method was used for calculation of error degrees of freedom (PROC MIXED, SAS Institute 2008). A multiple linear regression was conducted with capture per trap per day as the dependent variable and percentage injured tillers as the independent variable (PROC REG, SAS Institute 2008). A qualitative dummy variable, $z_1$, was used to differentiate between years (if year = 2012 then $z_1=0$, if year = 2013 then $z_1=1$) because trap captures were significantly higher ($P<0.05$) in 2013 than in 2012.

4.3. Results

4.3.1. Pheromone Trap Monitoring

*Eoreuma loftini* pheromone trap monitoring efforts from 2009–2013 captured a total of 19,496 moths at >100 trap locations throughout seven parishes in southwest Louisiana (Table 4.1). Although moths were not detected in 2009, two specimens were trapped in November–December 2010 in non-crop habitat south of Vinton (N 30° 5' 44.9010", W 93° 31' 5.9010") 22 km to the south and 7 km to the east of the 2008 detection (N 30° 17' 54.3480", W 93° 35' 21.3300"; Hummel et al. 2010). In 2011, *E. loftini* males were captured at 42 new locations in three additional parishes (Cameron, Jefferson Davis, and Beauregard; Figure 4.1). The easternmost *E. loftini* detection in 2011 was near a sugarcane field ≈26 km south of Welsh Table

<table>
<thead>
<tr>
<th>Parish</th>
<th>Year</th>
<th>No. trap sites</th>
<th>Date deployed</th>
<th>Date retrieved</th>
<th>No. times traps were sampled</th>
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<th>Total no. <em>E. loftini</em> captured</th>
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<td>12</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
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<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>18</td>
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<td>NA</td>
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<tr>
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<td>1/8/2014</td>
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<td>1/3/2011</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td></td>
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<td>8/27/2012</td>
<td>1/16/2013</td>
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<td>4</td>
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<tr>
<td>St. Mary</td>
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<td>3</td>
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</tr>
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<tr>
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<td>9/19/2013</td>
<td>2/16/2013</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NA = Not Applicable
(Cameron Parish; N 30° 11' 10.7154", W 92° 51' 19.6914") and ≈64 km east of the 2010 detection site. Deployment of traps in 2012 revealed little eastward expansion with a single specimen captured in northwestern Cameron Parish (N 29° 59' 57.7314", W 92° 47' 29.436") ≈4 km east of the 2011 easternmost edge. Expanded pheromone trap monitoring in 2013 resulted in the capture of >19,000 *E. loftini* adults. Adults were detected at 65 trapping sites, 30 of which were located in areas where *E. loftini* had not previously been found (e.g. Allen, Acadia, and Vermilion parishes, Figure 4.1). The range of *E. loftini* in 2013 extended eastward to traps positioned south of Estherwood (Acadia Parish; N 30° 7' 28.3794", W 92° 27' 20.4408"), ≈32 km further east than in 2012. Detection of *E. loftini* in Beauregard and Allen parishes as well as southern Cameron Parish revealed substantial north-south range expansion since 2009.

Figure 4.1. *Eoreuma loftini* range expansion in Louisiana, 2008–2013
The southernmost detection site (N 29° 46' 29.553", W 93° 27' 48.0522") was <1 km from the coast of the Gulf of Mexico, while the northernmost detection site (N 30° 51' 40.2438", W 93° 19' 27.6924") was 120 km north of the coast in northern Beauregard Parish. Between 2009 and 2013 the eastern edge of the range of *E. loftini* moved a total of 111 km east from the 2008 detection site (22.2 km per yr). The range of *E. loftini* encompassed all of Calcasieu, Beauregard, Cameron, and Jefferson Davis parishes and regions of Allen, Vermilion, and Acadia parishes. No *E. loftini* adults have been captured near sugarcane mills in Iberia, St. Mary, or St. Martin Parish as of December 2013.

### 4.3.2. *Eoreuma loftini* Infesting Sugarcane and Rice

The first larva found to be infesting sugarcane was collected in a field of first season sugarcane (var. L 99-226) near Iowa (Calcasieu Parish; N 30° 14' 49.1784", W 93° 3’ 13.7946") on 29 March 2013. Season long scouting for *E. loftini* larvae in sugarcane revealed infestations in ≈120 ha of sugarcane in Calcasieu Parish and 220 ha in Jefferson Davis Parish. The mean percentage of stalks with *E. loftini* larvae feeding on plant surfaces or inside stalks peaked in August with 11% and 8% of stalks infested in Calcasieu and Jefferson Davis parishes, respectively (Table 4.2).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcasieu</td>
<td>12</td>
<td>31</td>
<td>56</td>
<td>156</td>
<td>65</td>
<td>16</td>
</tr>
<tr>
<td>Mean % infested stalks</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Jefferson Davis</td>
<td>4</td>
<td>40</td>
<td>28</td>
<td>39</td>
<td>68</td>
<td>51</td>
</tr>
<tr>
<td>Mean % infested stalks</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Adult trap captures near rice fields varied throughout the growing season in both years (Figure 4.2), although differences between sampling dates were not detected (Table 4.3). Daily
trap captures were more than 3.5 fold greater in 2013 than in 2012 (Table 4.3, Figure 4.2). However, differences were not detected among the traps near chlorantraniliprole-treated and nontreated fields, nor were interactions detected (Table 4.3). Differences in percentage of tillers with larval injury were detected among treatments, sampling dates, and for the sampling date × treatment interaction, but not for other interactions (Table 4.3). The percentage of injured tillers increased throughout most of the growing season, peaking in late July and early August in both years (Figure 4.3). Late season injury in nontreated rice fields in 2013 rose to 21.2% ± 2.7[SEM] of tillers with *E. loftini* injury, while levels in 2012 peaked at 9.7% ± 2.1[SEM]. The percentage of tillers with *E. loftini* injury in chlorantraniliprole treated fields remained below 5% throughout the growing season in both years. A linear relationship occurred ($F = 8.79, \text{df} = 2.66, P < 0.001, R^2 = 0.2103, \text{Root MSE} = 6.601$) between the number of *E. loftini* per trap per day and the percentage of tillers with stem borer injury in nontreated rice fields. The dummy variable, z1, improved the regression model ($t = 3.08, P = 0.003$) by increasing the coefficient of the explanatory variable by 4.9288 for 2013 data (slope = 0.2817; intercepts = 2012: 2.160 and 2013: 7.085, Figure 4.4).

Table 4.3. Statistical comparisons of *E. loftini* daily trap captures and percentage of injured tillers from chlorantraniliprole treated and nontreated rice fields in Calcasieu Parish, Louisiana, 2012–2013

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>Daily trap capture F</th>
<th>df</th>
<th>P &gt; F</th>
<th>Percentage injured tillers F</th>
<th>df</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling date</td>
<td>1.22</td>
<td>7, 94.9</td>
<td>0.298</td>
<td>4.99</td>
<td>7, 84.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.02</td>
<td>1, 16.5</td>
<td>0.880</td>
<td>20.93</td>
<td>1, 16.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Year</td>
<td>17.41</td>
<td>7, 12.0</td>
<td>0.001</td>
<td>0.01</td>
<td>1, 12.6</td>
<td>0.906</td>
</tr>
<tr>
<td>Year × treatment</td>
<td>2.68</td>
<td>1, 10.8</td>
<td>0.130</td>
<td>0.0</td>
<td>1, 12.7</td>
<td>0.950</td>
</tr>
<tr>
<td>Treatment × sampling date</td>
<td>1.03</td>
<td>7, 94.7</td>
<td>0.415</td>
<td>3.45</td>
<td>7, 84.9</td>
<td>0.003</td>
</tr>
<tr>
<td>Year × sampling date</td>
<td>1.23</td>
<td>5, 93.1</td>
<td>0.300</td>
<td>0.58</td>
<td>4, 83.6</td>
<td>0.681</td>
</tr>
<tr>
<td>Year × treatment × sampling date</td>
<td>1.35</td>
<td>5, 93.2</td>
<td>0.181</td>
<td>0.81</td>
<td>4, 83.6</td>
<td>0.524</td>
</tr>
</tbody>
</table>
Differences were detected in the number of whiteheads per m\(^2\) and the percentage of whiteheads between years, treatments, and a year × treatment interaction occurred (Table 4.4). The mean number of whiteheads per m\(^2\) ranged from 1.00 (chlorantraniliprole treated) to 12.67 (nontreated).

Table 4.4. *Eoreuma loftini* infestations (LS means ± SEM) as affected by insecticidal seed treatments in rice in Calcasieu Parish, Louisiana, 2012–2013

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>Whiteheads/m(^2)</th>
<th>Percentage Whiteheads</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>2.90 ± 0.98a</td>
<td>0.90 ± 0.25a</td>
</tr>
<tr>
<td>2013</td>
<td>5.67 ± 0.81b</td>
<td>2.13 ± 0.20b</td>
</tr>
<tr>
<td><em>F</em></td>
<td>4.72</td>
<td>14.66</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.046</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>1.00 ± 0.98a</td>
<td>0.34 ± 0.25a</td>
</tr>
<tr>
<td>Nontreated</td>
<td>7.57 ± 0.81b</td>
<td>2.69 ± 0.20b</td>
</tr>
<tr>
<td><em>F</em></td>
<td>26.59</td>
<td>53.75</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Year × Treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012 Chlorantraniliprole</td>
<td>1.00 ± 1.55a</td>
<td>0.27 ± 0.39a</td>
</tr>
<tr>
<td>Nontreated</td>
<td>4.80 ± 1.20a</td>
<td>1.53 ± 0.30b</td>
</tr>
<tr>
<td><em>F</em></td>
<td>4.72</td>
<td>11.77</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.046</td>
<td>0.004</td>
</tr>
<tr>
<td>2013 Chlorantraniliprole</td>
<td>3.56 ± 1.20a</td>
<td>1.05 ± 0.30b</td>
</tr>
<tr>
<td>Nontreated</td>
<td>12.67 ± 1.10b</td>
<td>3.85 ± 0.27c</td>
</tr>
<tr>
<td><em>F</em></td>
<td>4.72</td>
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</tr>
<tr>
<td><em>P</em></td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>

Means in the same column for each fixed effect which share a lowercase letter are not significantly different (*P* > 0.05; Tukey’s HSD test). For all tests, df = 1, 15

4.4. Discussion

This study documents the occurrence of *E. loftini* in seven Louisiana parishes, and provides the first record of infestations in Louisiana field crops. The estimated rate of *E. loftini* range expansion during the 5-yr monitoring period (22 km per yr) is consistent with previous estimates (23 km per year from 1980–2005; Reay-Jones et al. 2007c); however, eastward movement was sporadic and varied greatly between years. Changing weather conditions and host availability probably influenced *E. loftini* range expansion, and widespread removal of rice straw after the
2011 growing season (Schultz 2011, Beuzelin et al. 2012) and above average rainfall (U.S. Dept. of Commerce National Oceanic and Atmospheric Administration 2014) might have reduced *E. loftini* populations and slowed range expansion in 2012. Variation in population distribution between years has been reported for other lepidopteran species and periods of reduced population growth of invasive species (Byers et al. 2002, Augustine et al. 2004) caused by biotic and abiotic factors likely have roles in range expansion.

A quarantine was initiated in 2005 by the Louisiana Department of Agriculture and Forestry and the Texas Department of Agriculture to prevent the transport of sugarcane from Texas into Louisiana for processing with the aim of reducing human-aided movement of *E. loftini* into Louisiana (Reagan et al. 2005). However, at present no regulations are in place preventing movement of *E. loftini* infested sugarcane from western Louisiana parishes to sugarcane mills further east. Based on its current distribution as estimated by pheromone trapping, it is likely that *E. loftini* infestations are present throughout the ≈600 ha of sugarcane in Calcasieu and Jefferson Davis parishes. Although the pest’s range expansion through Texas and into Louisiana is not known to have been a result of human-aided movement, movement of infested sugarcane could introduce *E. loftini* into the 45,000 ha of sugarcane in Iberia, St. Mary, and St. Martin parishes.

With expanded pheromone trap monitoring in 2013 substantial increases in the northern range limit of *E. loftini* were observed. Males were captured in the northernmost trap (≈120 km north of the Gulf Coast), indicating that the species may occur further north. Overwintering survival of *E. loftini* is greater than that of the sugarcane borer, *Diatraea saccharalis* (F.), (Rodriguez-del-Bosque et al. 1995), which occurs as far north as southern Arkansas (Lorenz and Hardke 2014). Hence, expanding pheromone trap monitoring might reveal a larger range for *E. loftini* than is currently known (Showler et al. 2012, Showler and Reagan 2012).
Figure 4.2. *Eoreuma loftini* daily pheromone trap captures (LS means ± SEM) in chlorantraniliprole treated and nontreated rice in (A) 2012 and (B) 2013. Differences were detected ($P < 0.05$) between years, but not among treatments, sampling dates, or the interactions.
Figure 4.3. Percentages of tillers with *E. loftini* injury (LS means ± SEM) in chlorantraniliprole treated and nontreated rice in (A) 2012 and (B) 2013. Differences were detected ($P < 0.05$) among sampling dates, treatments, and the sampling date × treatment interaction, but not among years or other interactions.
The increase of infestations from 2012 to 2013 in Calcasieu Parish suggests that the invasive pest has become established there. Abundances of whiteheads indicate substantial yield losses attributable to *E. loftini* in fields that were not protected with insecticides during 2013. Yield losses of 1.0–4.2% have been reported for every percentage increase in whiteheads caused by a complex of stem borers in Asia (Pathak 1968, Muralidharan and Pasalu 2006). Reay-Jones et al. (2007a) estimated a 2.28% decrease in yield results from every whitehead per m² caused by mixed infestations of *D. saccharalis* and *E. loftini*. Hence, we estimate yield losses of 4% to 28% in unprotected rice fields in 2013. The injury levels in Louisiana were comparable to those reported in Texas (1–20 whiteheads/ per m²) where *E. loftini* has been present in rice for more than 10 yr (Way et al. 2006, Reay-Jones et al. 2007a).

![Figure 4.4](image)

**Figure 4.4.** Relationship between daily adult trap captures and larval injury in nontreated rice fields. Multiple linear regression (*F* = 8.79; df = 2, 66; *P* < 0.001; *R*² = 0.2103)
Chlorantraniliprole seed treatments provided season-long protection from *E. loftini* injury. The similar numbers of adult *E. loftini* near chlorantraniliprole treated rice fields and nontreated fields suggests that non-crop hosts harbor substantial populations. On an area wide scale pheromone traps are useful (Reay-Jones et al. 2007c, Wilson et al. 2012b), and the correlation between trap captures and larval infestations in nontreated rice suggests the traps have potential to alert growers of increasing populations and the need to monitor larval infestations. Currently, insecticidal seed treatments including chlorantraniliprole and neonicotinoids are applied to most rice planted in Louisiana for control of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Stout and Gore 2014). Neonicotinoid seed treatments are relatively ineffective against stem borers (Way et al. 2011). Chlorantraniliprole seed treatments, in contrast, are effective against *D. saccharalis* (Sidhu et al. 2014) and can be used to control a complex of stem borers. Additionally, widespread adoption of chlorantraniliprole seed treatments in rice may contribute to reduced area wide pest populations. Rice cultivars have varying levels of susceptibility to stem borers (Way et al. 2006), and the effect of cultivar used in this study is unknown.

Surveys of sugarcane in Calcasieu and Jefferson Davis parishes in 2012 and 2013 led to detection of the first *E. loftini* larval infestation in Louisiana sugarcane. While infestations did not reach economically damaging levels in 2013, the pest is capable of inflicting revenue losses of up to $220 million annually to the sugarcane industry (Reay-Jones et al. 2008). Infestations in sugarcane in the Lower Rio Grande Valley consistently cause $>20\%$ bored internodes when left unmanaged (Legaspi et al. 1997a, Reay-Jones et al. 2005b, Wilson et al. 2012b), and severe infestations have caused fields to be unharvestable (Reagan et al. 2005, Showler and Reagan 2012). Although judiciously timed insecticide applications might reduce sugarcane yield losses from *E. loftini* (Wilson et al. 2012b), adequate control in sugarcane is difficult to achieve because

Reductions in populations of the stem borers *D. saccharalis, Diatraea lineolata* (Walker), and *Diatraea magnifactella* Dyar have been observed following the establishment of *E. loftini* in northeastern Mexico and southern Texas (Legaspi 1997b, Rodriguez-del-Bosque et al. 2011, Rodriguez-del-Bosque and Reyes-Méndez 2013). Although changes in stem borer species composition may have been associated with competitive displacement by *E. loftini*, reductions in *D. saccharalis* populations in Mexico and Texas have been attributed to the establishment of the parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) (Fuchs 1979). In Louisiana, *D. saccharalis* shares many host plants with *E. loftini* (Beuzelin et al. 2011b), but releases of *C. flavipes* have not been successful (White et al. 2004) and host crops and weeds (Showler et al. 2011, Showler and Moran 2014) are abundant. In addition, the two stem borer species exhibit differences in larval tunneling behavior (Legaspi et al. 1997b, Beuzelin et al. 2012, Showler and Reagan 2012), oviposition substrate preference (Showler and Castro 2010a,b), and seasonal activity (Rodriguez-del-Bosque et al. 1995, Beuzelin et al. 2011b, Showler and Reagan 2012). Coexistence of both species in Louisiana is therefore anticipated.

Because eradication of this invasive pest is not thought to be feasible (Johnson and van Leerdam 1981, Showler et al. 2011, 2012; Beuzelin et al. 2011b, 2013), reducing human-aided movement and implementing effective area wide management tactics will be critical to mitigating the impact of *E. loftini* in Louisiana rice and sugarcane. As *E. loftini* expands its geographical range, susceptible cultivars of corn and sorghum grown in the central and northern regions of Louisiana will also be affected (Showler et al. 2012, 2013). The availability of corn and sorghum is also anticipated to influence area wide populations (Showler and Reagan 2012).
Widespread monitoring through pheromone trapping and larval surveys will help to understand *E. loftini* population dynamics and range expansion. Combining climate data and host plant distributions might facilitate a role for geographical information systems for projecting further range expansion, identifying high economic risk areas, and enhancing integrated pest management strategies.
CHAPTER 5: MONITORING MEXICAN RICE BORER (LEPIDOPTERA: CRAMBIDAE) POPULATIONS IN SUGARCANE AND RICE WITH CONVENTIONAL AND ELECTRONIC PHEROMONE TRAPS

5.1. Introduction

The Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), is a major pest of sugarcane, *Saccharum* spp., rice, *Oryza sativa* L., and other graminaceous crops in Texas and Louisiana (Reay-Jones et al. 2008, Showler et al. 2012, Chapter 4). Timing of insecticidal control of *E. loftini* is made difficult by overlapping generations and limited exposure of larvae (Meagher et al. 1994, Wilson et al. 2012b). The development of pheromone traps to monitor adult *E. loftini* populations (Shaver et al. 1990, 1991) has potential to improve chemical control strategies for this pest in sugarcane. Pheromone baited traps are utilized in pest management (Witzgall et al. 2010) because effective means to monitor pest populations to more efficiently time control measures is a cornerstone of integrated pest management (IPM) programs (Rabb and Guthrie 1970). Pheromone traps can be used to assist in scouting for *E. loftini* in sugarcane in the Rio Grande Valley of Texas as well as rice in southwest Louisiana (Wilson et al. 2012b, Chapter 4,6). Annual economic losses to sugarcane in Louisiana up to $220 million may be sustained once *E. loftini* is established throughout the state (Reay-Jones et al. 2008). However, all research on *E. loftini* management has been conducted in south Texas where environmental conditions are much different from those in Louisiana. Investigation of the current status of *E. loftini* in Louisiana sugarcane and potential pheromone trap-assisted monitoring strategies is needed.

Electronic traps baited with pheromone blends have been developed for automated monitoring of insect populations. These traps are capable of accurately measuring insect populations in the field while reducing labor costs associated with manually monitoring traps.
Monitoring with electronic traps provides growers with real-time data on Lepidopteran pest populations in apple orchards (Holguin et al. 2010), but this technology has not been investigated in row crops. The evaluation of electronic traps for monitoring of *E. loftini* in sugarcane and rice habitats presented here provides the first documented use of automated pheromone-based pest scouting for use in row crops IPM.

### 5.2. Materials and Methods

#### 5.2.1. Monitoring *E. loftini* in Commercial Sugarcane

Populations of *E. loftini* were monitored in commercial sugarcane fields in southwestern Louisiana throughout the 2014 and 2015 growing seasons. Three fields were located in Calcasieu Parish and five fields were located in Jefferson Davis Parish. Fields ranged in size from 20–60 ha and were of one of three cultivars (HoCP 96-540, L 99-226, L 99-233) of either plant cane, first ratoon, or second ratoon. Commercial growers confirmed that all fields monitored in the survey did not receive any insecticide applications during the 2014 or 2015 growing seasons. Standard green, yellow, and white bucket traps (Unitrap; Great Lakes IPM, Vestaburg, MI) were baited with synthetic *E. loftini* sex pheromone lures (Luresept; Hercon Environmental, Emigsville, PA). Each trap contained an insecticidal strip (Vaportape II; Hercon Environmental, Emigsville, PA). Traps were attached to metal poles 1 m above the soil surface and placed approximately 1 m from field margins to maximize trap performance (Shaver et al. 1991). Pheromone lures and insecticidal strips were replaced every 4 wk according to label instructions. In 2014, traps were checked 12 times during the growing season (29 Mar, 1 May, 20 May, 29 May, 25 June, 1 July, 15 July, 7 Aug, 28 Aug, 9 Sept, 2 Oct, and 30 Oct). Traps were checked 9 times during the 2015 growing season (4 April, 14 May, 4 June, 30 June, 16 July, 29 July, 18 Aug, 24 Sept, and 9 Oct). Monitoring of larval infestations was conducted in each field on the same dates by examining 50
randomly selected stalks from each field for signs of stem borer injury. A stalk was considered injured if the presence of leaf sheath feeding, a stalk entry hole, or an *E. loftini* larva was detected. The sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), was also present, but injury from this pest can be distinguished from that of *E. loftini* by differences in feeding and tunneling behavior (Legaspi et al. 1997b, Showler and Reagan 2012, Wilson et al. 2012b).

At the end of the growing season (4 Nov 2014 and 29 Oct 2015) two 25-stalk samples were collected from opposite sides of each field and the number of internodes, bored internodes, stem borer larvae, and adult emergence holes were recorded. Bored internodes were classified as either *E. loftini* or *D. saccharalis* based on characteristic feeding signs. Horizontal and diagonal tunneling and packed frass were the primary characteristics used to indicate *E. loftini* injury (Showler and Reagan 2012).

All trap capture data were converted to daily trap captures prior to data analysis. Daily trap captures and percentage injured stalks were analyzed with Generalized Linear Mixed Models (PROC GLIMMIX, SAS Institute 2008) with year, parish, sampling period, and their interactions as fixed effects and field(Parish) and field(Parish) × year as random effects. A simple linear regression was conducted to determine the relationship between daily trap capture and percentage of injured stalks (PROC REG, SAS Institute 2008). The data collected at time of harvest including percentage of *E. loftini* bored internodes, percentage of *D. saccharalis* bored internodes, and the number of live *E. loftini* larvae per sample were analyzed with PROC GLIMMIX (SAS Institute 2008) with parish, year, and the parish × year interaction as fixed effects and field(Parish) and field(Parish) × year as random effects. A binomial distribution was used for analysis of bored internode data; a Poisson distribution was used for larvae counts. The
number of live *E. loftini* larvae per ha was estimated by multiplying the number of larvae per stalk by 30,000 stalks/ha, a stand estimate based on sugarcane cultivar trials (Tew et al. 2005). A second two-way ANOVA examined the effects of sugarcane cultivar, crop year, and their interaction on the same three parameters. Tukey’s honestly significant difference test ($\alpha=0.05$) was used for all mean separations and the Kenward-Roger method was used for all calculations of error degrees of freedom.

5.2.2. Spensa Z-traps

Electronic automated pheromone traps henceforth, Spensa Z-traps, were evaluated for potential to monitor *E. loftini* populations in sugarcane and rice agroecosystems at the Texas A&M AgriLife research station in Jefferson County, Texas in 2013 and 2015. The Spensa Z-traps (Figure 5.1; Spensa Technologies, West Lafayette, Indiana, PCT International Patent 56555) were baited with the same pheromone lures as the conventional traps. Moths which enter the trap and contact an electric grid are electrocuted and fall into the trap bucket below. The electric grid contains a bio-impedance sensor programed with algorithms developed for the coddling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). When an insect contacts the grid, a discharge occurs and the microcontroller stores the time of the event. Data are then communicated wirelessly to a base station and subsequently uploaded to the online data management system, MyTraps.com. The prototypes used in 2013 had the electric rods encircling the pheromone lure within an orange delta-shaped cover. These communicated to transmitters which then sent the signal to a base station receiver which was linked to a laptop computer with a wired internet connection. The system used in 2015 had the electronic rods positioned in two parallel rows on either side of the pheromone lure. The 2015 system was connected to a Verizon
wireless internet signal. Data was collected by the base station and uploaded to the MyTraps once every 24 hours online database.

In 2013, two Spensa Z-traps and one manual trap were placed near each of two fields of sugarcane/bioenergy crops. Antennae were mounted on PVC poles approximately 3 m above the ground to improve communication with transmitters. The west site contained sugarcane,

![Image](image.jpg)

Figure 5.1. Spensa Z-traps used in automated pheromone-based monitoring of *E. loftini*. (A) Spensa Z-trap near a sugarcane field, Jefferson County, TX, 2015. (B) Schematic design of the electric grid based trap

energycane, sweet sorghum (*Sorghum bicolor* L. inbred), and high-biomass sorghum (*S. bicolor x drummondii* [Sudangrass]). The east site contained sugarcane, energycane and *Miscanthus* hybrids. Both sites were surrounded by experimental rice fields which dominate the acreage at the Texas A&M AgriLife research station at Beaumont. Traps were initially deployed September 11, 2013 and were checked on eight dates (18 Sept, 23 Sept, 2 Oct, 9 Oct, 15 Oct, 22 Oct, 5 Nov, and 15 Nov 2013). The number of *E. loftini* captured in each trap was recorded and pheromone
lures were replaced every 4 weeks. Insecticidal vapor strips were present in manual traps, but not in Spensa Z-traps. One of the Spensa Z-traps at the east site was not functional and was not included in the analysis. Daily trap capture was compared between Spensa Z-traps and manual traps with PROC GLIMMIX (SAS Institute 2008) with a Gaussian distribution which included trap type as a fixed effect and site and trap(site) as random effects. A simple linear regression (PROC REG, SAS Institute 2008) was conducted to determine the relationship between electronically reported trap captures and manually recorded captures in Spensa Z-traps.

In 2015, assays were conducted in rice and sugarcane habitats at the same Texas A&M AgriLife research station. Four sites were in rice habitat and four were in sugarcane habitat. Each site contained one manual pheromone trap and one Spensa Z-trap. Both trap types contained insecticidal vapor tapes. Traps were initially deployed on 3 Aug 2015 and were check on 9 Aug, 17 Aug, 25 Aug, 1 Sept, 9 Sept, 14 Sept, 30 Sept, 13 Oct, and 22 Oct 2015. Daily trap capture data were analyzed with PROC GLIMMIX (SAS Institute 2008) with a Gaussian distribution and trap type, sampling period, habitat, and their interactions as fixed effects. Site(habitat) and site(habitat) × trap type were included as random effects. A simple linear regression (PROC REG, SAS Institute 2008) determined the relationship between electronically reported trap captures and manually trap captures in Spensa Z-traps.

5.3. Results

5.3.1. Monitoring *E. loftini* in Commercial Sugarcane

Differences were detected in the daily *E. loftini* trap capture between years and sampling periods; all other effects were not significant (Table 5.1). Trap captures near sugarcane fields in 2014 peaked in late October at 0.99 *E. loftini*/trap/day (Figure 5.2, a.). Adult *E. loftini* population increases were also observed in April and June in 2014. Daily trap capture recorded between 21
Table 5.1. Statistical comparison of *E. loftini* daily trap captures and percentage of injured stalks from sugarcane fields in Calcasieu and Jefferson Davis Parish, Louisiana, 2014–2015

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>Daily trap capture</th>
<th>Percentage injured stalks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year</strong></td>
<td>F = 8.58</td>
<td>57.79</td>
</tr>
<tr>
<td></td>
<td>df = 1, 7.4</td>
<td>1, 7.1</td>
</tr>
<tr>
<td></td>
<td>P = 0.021</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Parish</strong></td>
<td>F = 3.67</td>
<td>3.42</td>
</tr>
<tr>
<td></td>
<td>df = 1, 6.3</td>
<td>1, 6.7</td>
</tr>
<tr>
<td></td>
<td>P = 0.102</td>
<td>0.109</td>
</tr>
<tr>
<td><strong>Sampling period</strong></td>
<td>F = 2.85</td>
<td>16.91</td>
</tr>
<tr>
<td></td>
<td>df = 11, 108.5</td>
<td>11, 108.3</td>
</tr>
<tr>
<td></td>
<td>P = 0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Year × Parish</strong></td>
<td>F = 0.14</td>
<td>7.40</td>
</tr>
<tr>
<td></td>
<td>df = 1, 7.43</td>
<td>1, 7.1</td>
</tr>
<tr>
<td></td>
<td>P = 0.721</td>
<td>0.030</td>
</tr>
<tr>
<td><strong>Year × Sampling period</strong></td>
<td>F = 1.00</td>
<td>16.07</td>
</tr>
<tr>
<td></td>
<td>df = 8, 108.3</td>
<td>8, 108.3</td>
</tr>
<tr>
<td></td>
<td>P = 0.442</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Parish × Sampling period</strong></td>
<td>F = 1.29</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>df = 11, 108.5</td>
<td>11, 108.3</td>
</tr>
<tr>
<td></td>
<td>P = 0.242</td>
<td>0.424</td>
</tr>
<tr>
<td><strong>Year × Parish × Sampling period</strong></td>
<td>F = 0.65</td>
<td>2.38</td>
</tr>
<tr>
<td></td>
<td>df = 8, 108.3</td>
<td>8, 108.3</td>
</tr>
<tr>
<td></td>
<td>P = 0.735</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Jul and 13 Aug 2015 reached >1.2 *E. loftini/trap/day*, the highest level recorded in our survey.

Increases in daily trap capture in 2015 occurred at different periods in the growing season than in 2014. Differences in the percentage of injured stalks were detected between years, sampling periods, year × parish, year × sampling period, and the year × parish × sampling period interaction (Table 5.1). The percentage of *E. loftini* injured stalks was >5-fold greater in 2015 than in 2014 (Table 5.1, Figure 5.2, b.). Stalk injury from *E. loftini* was not observed prior to mid-July in 2014, and remained below 4% of stalks throughout the growing season. Injury from
*E. loftini* in 2015 occurred as early as mid-May and continued to rise throughout the season. A linear relationship occurred ($F = 37.00, \text{df} = 1, 160, P < 0.001, R^2 = 0.183, \text{Root MSE} = 0.46$) between the percentage of injured stalks and daily trap capture (Figure 5.3). This relationship had a slope of 0.074 and an intercept of 0.479, therefore a 1% increase in percentage injured stalks would correspond to an increase in daily trap capture of 0.074.

The percentage of *E. loftini* bored internodes was >9-fold greater in 2015 compared to 2014 (Table 5.2). Differences in percentage of *E. loftini* bored internodes were not detected between parishes or the parish × year interaction. Similarly, the number of live *E. loftini* larvae recovered was 15-fold greater in 2015 than in 2014. A total of 2 and 30 *E. loftini* larvae were recovered from samples at time of harvest in 2014 and 2015, respectively. The percentage of *D. saccharalis* bored internodes did not differ between years, parishes, or their interaction. *Diatraea saccharalis* was responsible for the majority of stem borer injury in 2014, but caused less than half the injury as *E. loftini* in 2015. No *D. saccharalis* larvae were recorded at time of harvest in 2014, and only three larvae were recorded in 2015. Differences were not detected between sugarcane cultivars, crop years, or the interaction in either of the parameters examined, and data are not presented.

### 5.3.1. Spensa Z-traps

Daily trap capture in 2013 was not different between manual traps (1.45 *E. loftini*/trap/day) and Spensa Z-traps (1.12), and differences in sampling dates or the sampling date × trap type interaction were not detected. Batteries in one of the wireless transmitter nodes were exhausted between 2 Oct and 15 Oct 2013, resulting in no data being reported to the MyTraps system for two of the eight sampling periods. A linear relationship was not detected ($F = 0.28, \text{df} = 1, 16; P = 0.606, R^2 = 0.017$) between the manually recorded trap captures in Spensa Z-traps and those reported to the MyTraps system in 2013.
Table 5.2. Stem borer injury and infestation (LS means ± SEM) at time of harvest in commercial sugarcane fields

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>Percentage <em>E. loftini</em> bored internodes</th>
<th>Percentage <em>D. saccharalis</em> bored internodes</th>
<th>Live <em>E. loftini</em> larvae/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>0.20 ± 0.08a</td>
<td>1.65 ± 0.70</td>
<td>353 ± 255a</td>
</tr>
<tr>
<td>2015</td>
<td>1.89 ± 0.45b</td>
<td>0.82 ± 0.35</td>
<td>5194 ± 1251b</td>
</tr>
<tr>
<td>F =</td>
<td>52.90</td>
<td>1.31</td>
<td>13.51</td>
</tr>
<tr>
<td>P =</td>
<td>&lt;0.001</td>
<td>0.274</td>
<td>0.001</td>
</tr>
<tr>
<td>Parish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcasieu</td>
<td>1.02 ± 0.40</td>
<td>0.81 ± 0.39</td>
<td>1936 ± 1099</td>
</tr>
<tr>
<td>Jefferson Davis</td>
<td>0.38 ± 0.14</td>
<td>1.68 ± 0.62</td>
<td>948 ± 524</td>
</tr>
<tr>
<td>F =</td>
<td>3.30</td>
<td>1.43</td>
<td>0.81</td>
</tr>
<tr>
<td>P =</td>
<td>0.110</td>
<td>0.254</td>
<td>0.375</td>
</tr>
<tr>
<td>Year × Parish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>Calcasieu</td>
<td>0.42 ± 0.20</td>
<td>456 ± 278</td>
</tr>
<tr>
<td>Jefferson Davis</td>
<td>0.10 ± 0.06</td>
<td>3.10 ± 1.57</td>
<td>273 ± 189</td>
</tr>
<tr>
<td>2015</td>
<td>Calcasieu</td>
<td>2.47 ± 0.92</td>
<td>8216 ± 2769</td>
</tr>
<tr>
<td>Jefferson Davis</td>
<td>1.44 ± 0.44</td>
<td>0.90 ± 0.48</td>
<td>3283 ± 1131</td>
</tr>
<tr>
<td>F =</td>
<td>2.15</td>
<td>0.79</td>
<td>0.08</td>
</tr>
<tr>
<td>P =</td>
<td>0.153</td>
<td>0.392</td>
<td>0.784</td>
</tr>
</tbody>
</table>

Means followed by different letters are significantly different (*P* < 0.05; Tukey’s HSD test). For all tests, df = 1, 28

All of the Spensa Z-trap prototypes evaluated in 2015 functioned correctly throughout the duration of the assay. Differences were detected between trap types (*F* = 23.57, df = 1, 84.5; *P* < 0.001) with Spensa Z-traps capturing >3-fold more *E. loftini* adults than conventional manual traps (Figure 5.4). Daily trap capture was also affected by sampling period (*F* = 4.04, df = 10, 78.6; *P* < 0.001) and the crop × trap type interaction (*F* = 7.04, df = 10, 84.6; *P* = 0.010). Spensa Z-traps in the rice habitat caught the greatest numbers of *E. loftini* adults reaching a mean daily capture rate of 5.6 between 13 Oct and 22 Oct 2015. A linear relationship occurred (*F* = 113.2, df = 1, 84; *P* < 0.001, $R^2 = 0.57$, Root MSE = 7.26) between the manually recorded trap captures and those reported electronically to the My traps website (Figure 5.5).
Figure 5.2. *Eoreuma loftini* populations and injury in commercial sugarcane fields in Calcasieu and Jefferson Davis Parishes, Louisiana, 2014–2015, (A) Mean daily pheromone trap captures and (B) larval injury.
Figure 5.3. Relationship between daily *E. loftini* trap capture and the percentage of injured stalks in commercial sugarcane fields in Calcasieu and Jefferson Davis Parishes, Louisiana, 2014–2015. The linear regression has a slope = 0.074 and an intercept = 0.479

5.4. Discussion

After the initial detection of *E. loftini* in Louisiana sugarcane in 2012 (Chapter 4), infestations in commercial fields have remained relatively low. The highest level of *E. loftini* injury reported here (2.5% bored internodes in Calcasieu Parish in 2015) is below the level of injury which would justify insecticidal protection (Wilson et al. 2012b). The numbers of *E. loftini* captured in pheromone traps in Calcasieu and Jefferson Davis Parishes are comparable to trap captures in the Rio Grande Valley of Texas where *E. loftini* routinely causes significant economic losses in sugarcane (Legaspi et al. 1997a, 1999a; Wilson et al. 2012b). Infestations previously reported from commercial rice fields also indicate populations of *E. loftini* are high in southwestern Louisiana (Chapter 4, 7). One possible explanation for the lack of *E. loftini* pest pressure in Louisiana sugarcane could be increased rainfall in Louisiana which receives
Figure 5.4. Daily trap captures in conventional bucket traps and Spensa Z-traps from 3 Aug to 22 Oct 2015 as affected by crop habitat in Jefferson County, Texas.

Figure 5.5. Relationship between manually recorded *E. loftini* trap capture in Spensa Z-traps and trap capture recorded electronically by the MyTraps system. The linear regression has a slope = 0.468 and an intercept = 5.10.
approximately double the annual precipitation as the Rio Grande Valley (U.S. Weather Service 2016). Drought stressed sugarcane has been shown to be preferred by *E. loftini* for oviposition (Reay-Jones et al. 2007b, Showler and Castro 2010a,b), and irrigation of sugarcane fields reduces infestations (Reay-Jones et al. 2005b). However, recent studies conducted in sugarcane, energycane, and sorghum in Jefferson County, TX which has similar climactic conditions as southwestern Louisiana reported much greater levels of *E. loftini* pest pressure than was observed in this survey (VanWeelden et al. 2015, Wilson et al. 2015). Although, experimental fields in these studies were directly adjacent to rice fields which likely influenced *E. loftini* populations. This indicates factors other than climactic differences are influencing *E. loftini* pest pressure in Louisiana.

The prevalence of *E. loftini* susceptible cultivars in the Rio Grande Valley may also contribute to the higher infestations observed there (Wilson et al. 2015). However, HoCP 96-540 which accounted for 60% of the fields included in our survey was shown to be susceptible to *E. loftini* in cultivar resistance studies (Wilson et al. 2015). Competition with other stem boring species has also been proposed to influence *E. loftini* populations (Rodrigues-Del-Bosque et al. 2011, Rodriguez-Del-Bosque and Reyes-Méndez 2013). However, the low levels of *D. saccharalis* observed in our surveys were unlikely to have an adverse effect on *E. loftini* populations. Despite the low levels of *E. loftini* injury observed in commercial sugarcane in Louisiana in 2014 and 2015, potential for highly damaging infestations to develop remains high.

Based on estimates from our surveys, hundreds to thousands of immature *E. loftini* are present in each hectare of sugarcane in southwestern Louisiana at time of harvest each year. As increasing amounts of infested sugarcane are transported to sugar mills in the center of the sugarcane production region, the rate of *E. loftini* range expansion is anticipated to increase.
Once the entirety of sugarcane acreage in Louisiana is infested with *E. loftini*, tens of millions of dollars in annual revenue losses could occur even if pest pressure does not reach the levels observed in the Rio Grande Valley (Reay-Jones et al. 2008). Reay-Jones et al. (2008) estimated $220 million in annual revenue loss could result from infestation of *E. loftini* throughout Louisiana’s sugarcane based on an injury level which was recorded in Texas of 57% bored internodes. This level of injury is nearly 30-fold higher than the level observed in 2014–2015. Therefore, it is likely that annual losses would only approach $220 million under extreme circumstances.

The use of pheromone trap-assisted scouting has shown potential to improve insecticide application timing in the Rio Grande Valley where trap captures were correlated with infestations of treatable larvae (Wilson et al. 2012b, Chapter 6). While treatable infestations were not present during our surveys, the relationship between larval feeding signs and pheromone trap capture we observed suggests these traps will be useful scouting tools in Louisiana as well. Crop consultants in Louisiana actively monitor sugarcane fields for stem borer infestations throughout the growing season. Improved scouting efficiency through the use of pheromone traps could reduce the labor costs associated with pest scouting. This scouting method should continue to be investigated under varying degrees of *E. loftini* pest pressure in Louisiana once the species has become established throughout the sugarcane production area.

The use of Spensa Z-traps has potential to further reduce the labor required for scouting for *E. loftini*. Although the prototypes evaluated in 2013 had some functionality problems, the improved design in 2015 was demonstrated to be much more effective. The parallel rows of electric rods and placement of an insecticidal strip in the bucket drastically improved trap performance. The 3-fold increase in daily trap capture by Spensa Z-traps over conventional
pheromone traps may not be the result of solely the presence of electric rods. The orange delta-shaped top and opaque bucket of the Spensa Z-traps may also be more attractive to *E. loftini* males than the conventional green/yellow/white universal moth trap currently used. These results suggest that previously reported trap preferences (Shaver et al. 1991) may no longer be consistent with *E. loftini* behavior. More comprehensive studies are needed to reevaluate *E. loftini* trap preferences.

The Spensa MyTraps system which was used in 2015 accurately reported trap captures in real-time over the course of 10 weeks. This work represents the first evaluation of automated pheromone trap monitoring for pests of row crops. Based on these results, Spensa Z-traps should be evaluated for potential to improve monitoring of Lepidopteran pest species in a variety of systems. The technology which was developed for monitoring of *C. pomonella* in apple orchards could be adapted to improve monitoring of other high profile pests including the Gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae) and the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), which currently have active pheromone trapping programs in place (Witzgall et al. 2010). The addition of the Verizon® wireless communication capabilities will allow this system to be deployed under most field conditions.

While further research is needed into pheromone trap-assisted scouting with both manual traps as well as Spensa Z-traps before this system is widely adopted for *E. loftini* in sugarcane and rice, our concept shows the potential to improve current pest monitoring tactics. Reliable and efficient pest sampling is critical to the success of IPM programs. The use of manual and electronic pheromone based monitoring should be evaluated for use in a variety of crop-pest systems.
CHAPTER 6: THE ROLE OF PHEROMONE TRAPS IN INSECTICIDAL CONTROL OF THE MEXICAN RICE BORER (LEPIDOPTERA: CRAMBIDAE) IN SUGARCANE

6.1. Introduction

The Mexican rice borer, *Eoreuma loftini* (Dyar), is an invasive pest of graminaceous crops which was first detected in the Rio Grande Valley of Texas in 1980 (Johnson and van Leerdam 1981). The pest quickly became the primary pest of sugarcane in the area, and within one year of its detection yield losses attributable to *E. loftini* damage of 50–65% were recorded (Johnson 1984). In the years following *E. loftini* establishment, development of effective control strategies was critical to the Rio Grande Valley sugarcane industry. Numerous insecticide application and timing strategies were investigated with little success (Johnson 1985, Meagher et al. 1994). Reductions in *E. loftini* injury were achieved, but these were not sufficient to reduce yield losses to acceptable levels. Yield losses were suffered in fields which received as many as 19 insecticide applications during the growing season (Meagher et al. 1994). Insecticide timing strategies included larval infestation thresholds as well as timing applications based on crop phenology (Pfannenstiel et al. 1990, Ring et al. 1991, Meagher et al. 1996b); however, economic returns on insecticide applications were rarely achieved. Nearly 20 years after *E. loftini* became established in the Rio Grande Valley, insecticidal control had not improved and most growers had abandoned the practice altogether (Legaspi et al. 1997a).

Overlapping generations and reduced exposure of eggs and larvae are thought to be the primary reason for insecticidal control failures (Meagher et al. 1994). The cryptic nature of larvae and rapid entry of neonates into sugarcane tissues where they are protected combined with the high biomass of sugarcane limit the ability of foliar applied insecticides to contact larvae (Wilson et al. 2012b). Development of new reduced risk insecticides which were shown to be effective against the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae),
offered potential to improve *E. loftini* pest management (Legaspi et al. 1999b, Reagan and Posey 2001). Applications of the insect growth regulator, tebufenozide, were shown to reduce *E. loftini* injury, but sugar yields were not improved (Reay-Jones et al. 2005b). Similarly, the chitin synthesis inhibitor, novaluron, was shown to be effective against the *D. saccharalis* in sugarcane (Beuzelin et al. 2010). This chemical reduced *E. loftini* injury with only a single application when timed according to a pheromone trap-assisted threshold (Wilson et al. 2012b).

Wilson et al. (2012b) monitored *E. loftini* populations in the Rio Grande Valley with pheromone traps and developed a threshold of 20–25 *E. loftini*/trap/week (2.85–3.5 *E. loftini*/trap/day) which was used to indicate the need for larval scouting. Correlation of trap captures to larval infestations from five commercial sugarcane fields revealed this trap capture threshold corresponded to a larval infestation level of approximately 5% of stalks with treatable larvae on plant surfaces (Wilson et al. 2012b). There was considerable variation in trap captures and larval infestations between fields, and this threshold was based only on a single year of data (Wilson et al. 2012b). Additionally, fields in that study were monitored weekly throughout the growing season for larval infestations. Hence, Wilson et al. (2012b) suggested further evaluation of pheromone trap-assisted scouting methods should be conducted in order to validate the application of this strategy to individual sugarcane fields.

This study evaluates the potential to use pheromone traps to indicate the need for larval scouting and improve timing of insecticide applications against *E. loftini* in sugarcane. Additionally, efficacy of recently labeled diamide insecticides (flubendiamide and chlorantraniliprole) for *E. loftini* control is assessed.
6.2. Materials and Methods

Pheromone trap-assisted scouting and insecticidal control of *E. loftini* were evaluated in large plot field trials in 2012, 2014, and 2015 in the Rio Grande Valley. Adult populations were monitored with traps baited with the synthetic *E. loftini* female sex pheromone blend. In all experiments, Universal Moth Traps (Great Lakes IPM, Vestaburg, MI) with green tops, yellow funnels, and white buckets (GYW Unitraps) baited with a rubber septa impregnated with 5.0 mg of the *E. loftini* female sex pheromone blend (Luresept; Hercon Environmental, Emigsville, PA) placed at heights of approximately 1 m above the ground were used for all assays in accordance with recommendations from Shaver et al. (1991). Traps were placed at distances of >100 m between traps to reduce interference among traps (Chapter 3).

Pheromone traps were monitored weekly during the growing season of each year and lures and insecticidal strips were replaced every four weeks according to label instructions. Experimental fields and trap monitoring periods varied between years (Table 6.1). All experimental fields in each year were located in Hidalgo and Cameron Counties, Texas. Pheromone trap data were converted to mean daily trap captures prior to analysis. In 2012 and 2014, trap capture data were analyzed with a generalized linear model (PROC GLIMMIX; SAS Institute 2008) with sampling period as a fixed effect and field and trap(field) as random effects. The same analysis was conducted with data from 2015 with trap as the only random effect because all traps were located at a single experimental field. Each of five fields served as a block (replication) in the 2012 and 2014 experiments, while a single experimental field was divided into four sections which served as blocks (replications) in the 2015 experiment.

<table>
<thead>
<tr>
<th>Year</th>
<th>Experimental fields</th>
<th>Cultivar</th>
<th>Plot size</th>
<th>Traps</th>
<th>Traps Monitored</th>
<th>Percentage of stalks with live larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>5 (23–43 ha)</td>
<td>Ratoon CP 72-1210</td>
<td>3.3–4.1 ha</td>
<td>2/field</td>
<td>21 June 2012–21 Sept 2012</td>
<td>&gt;5%</td>
</tr>
<tr>
<td>2014</td>
<td>5 (23–43 ha)</td>
<td>Ratoon CP 72-1210</td>
<td>3.3–4.1 ha</td>
<td>2/field</td>
<td>5 June 2014–4 Sept 2014</td>
<td>1.8%</td>
</tr>
<tr>
<td>2015</td>
<td>1 (72 ha)</td>
<td>Ratoon CP 72-1210</td>
<td>2.9 ha</td>
<td>8</td>
<td>14 July 2015–30 Sept 2015</td>
<td>14.2%</td>
</tr>
</tbody>
</table>

Larval scouting was initiated after mean daily pheromone trap captures exceeded 3.0 *E. lofii* trap/day the first week of August in 2012. Larval scouting was conducted 21 Aug 2012 by examining 100 stalks on opposite sides of each experimental field. Larval scouting in 2015 was conducted on 11 Aug by examination of 100 stalks in each replication (n = 4). Insecticide applications were made and injury and yield data collected in the 2012 and 2015 tests.

Applications in both years were made with fixed wing aircraft with a licensed commercial aerial applicator (Table 6.2).

Table 6.2. Aerial insecticide application, Hidalgo Co., TX 2012 and 2015

<table>
<thead>
<tr>
<th>Aerial Application Methods</th>
<th>2012</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application Date</td>
<td>22 Aug 2012</td>
<td>11 Aug 2015</td>
</tr>
<tr>
<td>Flight speed</td>
<td>145 mph</td>
<td>145 mph</td>
</tr>
<tr>
<td>Swath width</td>
<td>60 ft</td>
<td>60 ft</td>
</tr>
<tr>
<td>Nozzles</td>
<td>CP-03</td>
<td>CP-03</td>
</tr>
<tr>
<td>Nozzle deflection</td>
<td>45°</td>
<td>45°</td>
</tr>
<tr>
<td>Application volume (L/ha)</td>
<td>90.85</td>
<td>45.4</td>
</tr>
</tbody>
</table>

Injury data were collected by destructive sampling and recording the number and position of bored internodes, the number of total internodes, and the number of emergence holes for each stalk. Injury data in the 2012 study were collected on 29 Oct 2012 by taking samples of 15 randomly selected stalks from opposite sides of each experimental plot (n = 50 samples). Injury data in 2015 were collected on 10–11 Nov 2015 from two 20-stalk samples per plot (n = 40).
samples). Data were analyzed using generalized linear mixed models (PROC GLIMMIX; SAS Institute 2008) with binomial distributions for percentage bored internodes and Poisson distributions for emergence holes. Analyses included replication and treatment × replication as random effects. Because extensive injury was present in lower internodes prior to the insecticide application in 2015, an additional analysis compared injury among treatments in internodes above the seventh internode from the base of the stalk.

Experimental fields from 2012 were harvested on 19 Dec 2012 (n = 2), 8 Feb 2013 (n = 1), and 17–20 March 2013 (n = 2). Yield data were for each plot collected by the Rio Grande Valley Sugar Growers laboratory using the core sampling method (Birkett 1975, 1979) including percentage brix and percentage sucrose determined through direct polarization. The ratio of sucrose to all other dissolved solids, or juice purity, is expressed as a percentage. Commercially recoverable sugar was recorded for each core sample and extrapolated to one ton of cane. Tons of sugar per acre was calculated by Eq. 6.1.

\[
TSA = \frac{\text{Mean CRS } \times \text{TCA}}{2000} \quad \text{(6.1)}
\]

where:

- TSA = Tons of sugar per acre
- CRS = Commercially recoverable sugar in pounds sugar per ton of cane
- TCA = Tons of cane per acre

Yield data were analyzed using generalized linear mixed models (Proc GLIMMIX, SAS Institute 2008) with Gaussian distributions which included replication and treatment × replication as random effects. Means were converted to metric units after analysis.
6.3. Results

A significant effect of sampling date on the mean daily trap capture was detected in 2012 ($F = 3.94; df = 10, 86.2; P < 0.001$), 2014 ($F = 2.30; df = 13, 117; P = 0.010$), and 2015 ($F = 8.23; df = 10, 77; P < 0.001$) (Figure 6.1). Daily *E. loftini* capture peaked in 2012 at 3.1 in early August. Daily *E. loftini* trap captures in 2014 were highest (0.62) during early June, and declined throughout the growing season. Low levels of *E. loftini* infestations in 2014 negated the need for an insecticide application. Daily *E. loftini* trap captures in 2015 peaked at 0.97 in early August.

Larval scouting on 11 Aug 2015 revealed heavy *E. loftini* infestations, and extensive damage had already occurred with larval entry holes present in most stalks. Active infestations exceeded the threshold of 5% stalks with larvae on plant surfaces (Table 6.1), and the application was made.

Differences were detected in the percentage of bored internodes in 2012 with chlorantraniliprole and flubendiamide achieving the highest level of control (Table 6.3). Differences were also detected in the number of adult emergence holes, with flubendiamide less than the nontreated control. Differences in cane yield and sugar yield were detected between treatments; however, none of the treatments was greater than the nontreated control (Table 6.3).

Tebufenozide-treated plots yielded less than flubendiamide-treated and nontreated plots.

**Table 6.3. Eoreuma loftini injury and sugarcane yield as affected by a single insecticide application, Rio Grande Valley, Texas, 2012**

<table>
<thead>
<tr>
<th>Insecticide treatment</th>
<th>Rate (g AI/ha)</th>
<th>% Bored internodes</th>
<th>Emergence stalk</th>
<th>Metric tons of cane/ha</th>
<th>Metric tons of sugar/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>NA</td>
<td>12.64a</td>
<td>0.46a</td>
<td>87.9ab</td>
<td>10.1a</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>136.1</td>
<td>7.82ab</td>
<td>0.32ab</td>
<td>73.1b</td>
<td>8.2b</td>
</tr>
<tr>
<td>Novaluron</td>
<td>84.7</td>
<td>5.62ab</td>
<td>0.21ab</td>
<td>85.1ab</td>
<td>9.9ab</td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>73.1</td>
<td>3.55b</td>
<td>0.13b</td>
<td>90.2a</td>
<td>9.9ab</td>
</tr>
<tr>
<td>Flubendiamide</td>
<td>136.1</td>
<td>3.36b</td>
<td>0.22ab</td>
<td>94.2a</td>
<td>10.4a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>df = 4, 18.75</th>
<th>4, 20.62</th>
<th>4, 16.00</th>
<th>4, 16.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F = 6.21$</td>
<td>2.98</td>
<td>4.48</td>
<td>4.23</td>
</tr>
<tr>
<td>$P= 0.0023$</td>
<td>0.0432</td>
<td>0.0128</td>
<td>0.0159</td>
</tr>
</tbody>
</table>

Means within a column which share a letter are not significantly different (Tukey’s HSD; $\alpha = 0.05$)
Figure 6.1. *Eoreuma loftini* pheromone trap captures in experimental fields in the Rio Grande Valley, Texas. (A) 2012, (B) 2014, and (C) 2015
Differences in the percentage of bored internodes or the number of emergence holes per stalk were not detected between treatments in 2015 (Table 6.4). All treatments sustained *E. loftini* injury of >10% bored internodes. Differences were detected in percentage of bored internodes when only internodes above the seventh internode were considered with chlorantraniliprole-treated plots having reduced injury relative to the nontreated plots. Tebufenozide- and novaluron-treated plots were not significantly different from nontreated control plots in any of the parameters measure in either 2012 or 2015.

Table 6.4. *Eoreuma loftini* injury as affected by a single insecticide application, Rio Grande Valley, Texas, 2015

<table>
<thead>
<tr>
<th>Insecticide treatment</th>
<th>Rate (g AI/ha)</th>
<th>% Bored internodes (whole stalk)</th>
<th>% Bored internodes (top only)</th>
<th>Emergence/stalk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>NA</td>
<td>15.6</td>
<td>12.9a</td>
<td>0.27</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>136.1</td>
<td>16.7</td>
<td>11.1a</td>
<td>0.29</td>
</tr>
<tr>
<td>Novaluron</td>
<td>84.7</td>
<td>13.7</td>
<td>9.2a</td>
<td>0.22</td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>73.1</td>
<td>10.1</td>
<td>3.7b</td>
<td>0.21</td>
</tr>
<tr>
<td>Flubendiamide</td>
<td>136.1</td>
<td>10.8</td>
<td>5.6a</td>
<td>0.20</td>
</tr>
</tbody>
</table>

\[
\begin{array}{cccc}
\text{df} & 4, 12.0 & 4, 12.0 & 4, 12 \\
\text{F} & 1.23 & 3.95 & 0.59 \\
\text{P} & 0.351 & 0.029 & 0.674 \\
\end{array}
\]

Means within the same column which share a letter are not significantly different (Tukey’s HSD; \( \alpha = 0.05 \))

### 6.4. Discussion

This research adds to a growing body of evidence documenting the difficulty in achieving effective chemical control of *E. loftini* in sugarcane in the Rio Grande Valley of Texas (Meagher et al. 1994, Legaspi et al. 1997a, Reay-Jones et al. 2005b). Reduced risk chemistries applied with novel timing strategies showed improved efficiency over previous studies which required multiple applications to achieve control (Meagher et al. 1994, Legaspi et al. 1999b, Reay-Jones et al. 2005b). However, our research demonstrates that although successful and economical
control of *E. loftini* has been documented (Wilson et al. 2012b), the pest remains a challenge for Rio Grande Valley sugarcane growers to effectively manage.

Pheromone traps provide a means to effectively and efficiently monitor *E. loftini* populations in agricultural systems including rice (Chapter 4) and sugarcane (Wilson et al. 2012b, Chapter 5). However, the pheromone trapping data reported herein indicate that monitoring adult populations alone is not sufficient to make informed pest management decisions, and larval scouting should remain the primary means to quantify *E. loftini* infestations. Mean daily *E. loftini* pheromone trap captures in 2012 reached levels above the threshold suggested by Wilson et al. (2012b), and subsequently larval densities were present in treatable levels. These data were means of trap captures and larval infestations levels from five experimental fields, and the applicability of the monitoring strategy to individual fields is still questionable. Potential for failures of this monitoring strategy at the individual field level was demonstrated by data from the 2015 experiment. Mean daily pheromone trap captures from eight traps in the experimental field never exceeded 1 despite a high level of *E. loftini* infestation present in the field. The insecticide application in this experiment was made after significant *E. loftini* injury had been sustained, and insecticides failed to prevent insect injury. The failure of pheromone traps to detect rising populations in 2015 may have resulted from late deployment of traps. Traps were deployed in mid-July in 2015 rather than in early June as in the 2012 study and in the experiment reported by Wilson et al. (2012b). Pheromone trap captures in 2014 were successful indicators of low *E. loftini* populations. In this scenario, labor intensive larval scouting could have been minimized because trap captures documented unusually low *E. loftini* populations were occurring. Pheromone trap-assisted monitoring of *E. loftini* for use in pest management provides an expedient scouting tool which likely has a role in IPM programs. However, it may be more
suitable in area wide or farm-level monitoring programs, and should not be the primary means to monitor pest pressure in individual fields.

Insecticidal control of *E. loftini* in our tests was variable between years and chemical treatments. Sugar yield was not improved in 2012 despite a reduction in *E. loftini* injury, indicating the pest may have not been the primary yield limiting factor during that crop production cycle. This discrepancy may have been influenced by the amount of time (>3 months in two fields) between collection of injury data and harvest of experimental fields. The inability to detect improved yield resulting from reduced *E. loftini* injury is not uncommon (Meagher et al. 1994, Legaspi et al. 1997a, Reay-Jones et al. 2005b), despite a well-documented relationship between stem borer injury and yield loss in sugarcane (Hensley 1971, Legaspi et al. 1999a, White et al. 2008, VanWeelden et al. 2015). While detection of a reduction in injury among top internodes in the 2015 study provides information on the relative efficacy of insecticides, it is unlikely these treatments had an appreciable impact on sugar yields. Lower, more mature internodes are the greatest contributors to sugar yields and protection of newly formed internodes late in the season would be expected to provide little benefit (Ring et al. 1991, White and Hensley 1987, White et al. 2008).

Although a single application of novaluron has reduced *E. loftini* injury in previous trials (Wilson et al. 2012b), it did not provide a good level of control in either the 2012 or 2015 experiment. Similarly, tebufenozide, a widely used chemistry for control of *D. saccharalis* in Louisiana sugarcane (Gravois 2014), was largely ineffective. Insecticide resistance to tebufenozide has been reported in *D. saccharalis* populations (Reay-Jones et al. 2005a, Akbar et al. 2008), however, this is not a likely explanation for control failures against *E. loftini* because the compound has scarcely been used on sugarcane in the Rio Grande Valley. Interestingly,
tebufenozide treated plots yielded markedly less sugar than nontreated controls despite having a numerically lower level of borer injury. This suggests the tebufenozide treatment may have negatively impact some aspect of sugarcane physiology. No effect of tebufenozide on plant growth in sugarcane has been documented, but the insecticide has been shown to influence gene expression in tobacco (Padidam 2003).

Chlorantraniliprole and flubendiamide provided the highest level of control in both experiments, and may offer a new class of reduced risk insecticides for stem borer management in sugarcane. Both diamide chemistries are effective against *D. saccharalis* in sugarcane and provide superior control to tebufenozide (Wilson et al. 2012a, Beuzelin et al. 2014). Chlorantraniliprole usage in Louisiana sugarcane is increasing (J. M. Beuzelin, personal comm.), but flubendiamide may no longer be available for commercial use. The U.S. Environmental Protection Agency is currently in legal proceedings which may result in the loss of registration of flubendiamide due to potential effects on nontarget invertebrates (Keller 2016).

Effective insecticidal management strategies are needed to mitigate the impact of *E. loftini* in sugarcane in Texas as well as in Louisiana where the pest is increasingly investing sugarcane (Chapter 5). Pheromone trap-assisted scouting and efficacious insecticides including chlorantraniliprole have potential to improve chemical control of *E. loftini* in sugarcane, but more research is needed to document consistent results. Despite >30 of years applied research in to *E. loftini* chemical control, reliable insecticidal management tactics are still not available to Rio Grande Valley sugarcane growers. Fortunately, alternative control strategies have potential to mitigate the pest’s impact. Biological control of *E. loftini* has been largely unsuccessful to date (Meagher et al. 1998), but the red imported fire ant, *Solenopsis invicta* Buren, can reduce *E. loftini* survival in sugarcane (VanWeelden 2015). Sugarcane cultivars with resistance to *E. loftini*
have been identified (Wilson et al. 2015) and have potential to improve chemical control by prolonging larval exposure on plant surfaces (Wilson et al. 2012b). Irrigation of sugarcane reduces attraction for *E. loftini* oviposition (Reay-Jones et al. 2007b, Showler and Castro 2010a,b) and is a viable management strategy for inclusion in integrated control programs (Reay-Jones et al. 2005b).
7.1. Introduction

Increasing globalization and frequency of natural disturbances have created ample opportunity for biological invaders to inhabit and exploit new habitats (Elton 1958). Invasive species are a leading cause of biodiversity loss and have cause enormous economic damage across the world (Pimentel et al. 2005). The high dispersal capacity and short generation time of insects has allowed them to become some of the most successful invaders (Lawton and Brown 1986). Globally, insects are among the most widespread and damaging groups of invaders (Pimentel et al. 2005).

Effective field-level control strategies will remain the first line of defense against the impacts of invasive insect pests of agriculture. However, improved understanding of population dynamics of nonindigenous species at a landscape level will allow for development of models to forecast range expansion and mitigate areawide impacts of invasive insects (Liebhold and Tobin 2008). Determination of the rates of spread and predicting species distributions are important to risk assessments and policy decisions regarding management of invasive species (Pyšek and Richardson 2010). The rate spread of nonindigenous insects varies greatly depending on the type dispersal and degree of human-aided movement among other variables (Liebhold and Tobin 2008).

The Mexican rice borer (Lepidoptera: Crambidae), *Eoreuma loftini* (Dyar), is an invasive insect which poses a significant threat to graminaceous crops along the U.S. Gulf Coast (Reay-Jones et al. 2008, Showler et al. 2012, Chapters 4, 5). The pest was first detected in Texas in 1980 in the Rio Grande Valley (Johnson and van Leerdam 1981) where it quickly became the dominant pest of sugarcane (Legaspi et al. 1997a). It has since spread through the Texas rice
production area along the Gulf Coast towards Louisiana. Reay-Jones et al. (2007c) used pheromone traps to track *E. loftini* expansion in Texas from 2000–2005 and determined the leading edge of the infestation spread at a rate of 16.5 km/yr during that time. As predicted by Reay-Jones et al. (2007c), *E. loftini* was first detected in Louisiana in 2008 (Hummel et al. 2010). From 2009–2013, *E. loftini* continued its expansion into Louisiana, and was detected in a total of seven Parishes (Chapter 4). The rate of spread during this period was estimated to be 22 km/yr based on the location of eastern-most trap captures. Relying solely on the leading edge of the invasion may not represent the nature of the invading population, however, because characteristics of range expansion vary between different species and environments (Liebhold and Tobin 2008). Additionally, these studies provided only county/parish-level estimates of *E. loftini* populations and much about the pest’s distribution remains unknown. Recent introduction of *E. loftini* into Florida (Hayden 2012) highlights the need for more extensive monitoring of the pest’s population distribution.

This research relies on an extensive pheromone trap network in 13 parishes in southwest Louisiana to document *E. loftini* expansion and distribution from 2013–2015. Geographical Information Systems (GIS) analysis is applied to pheromone trap captures to determine the rate of spread based on the weighted mean population center and identify the spatial cluster patterns of *E. loftini* population distribution.

### 7.2. Materials and Methods

#### 7.2.1. Pheromone Trap Monitoring

All pheromone trap monitoring was conducted using practices consistent with optimal trap placement and maintenance (Brown et al. 1989, Shaver et al. 1990, 1991; Chapter 3). Standard green, yellow, and white bucket traps (Unitrap; Great Lakes IPM, Vestaburg, MI) were baited
with synthetic *E. loftini* sex pheromone lures (Luresept; Hercon Environmental, Emigsville, PA). Each trap contained an insecticidal strip (Vaportape II; Hercon Environmental, Emigsville, PA). Traps were attached to metal poles 1 m above the soil surface and placed approximately 1 m from field margins to maximize trap performance (Shaver et al. 1991). Pheromone lures and insecticidal strips were replaced every 4 weeks according to label instructions.

A total of 77 pheromone traps in 13 southwest Louisiana parishes were monitored from March 2013 to Jan 2016 (Figure 7.1). Trap locations remained constant throughout the survey, while surrounding habitat varied with changes in land use between years (Table 7.1). The number of times each trap was checked varied between traps and years. Traps were checked approximately biweekly from April–October, and approximately monthly from November–March of each year. Traps were checked less frequently during winter months because lower numbers of *E. loftini* adults and non-target insects reduced the amount of material collected in bucket traps and allowed for accurate quantification of *E. loftini* males. Trap captures of >100 *E. loftini* males were placed in zip-top bags and quantified in the lab to ensure accurate counts.

![Figure 7.1. *Eoreuma loftini* pheromone trap locations in southwestern Louisiana. Seventy-seven traps were monitored from 2013–2015](image-url)
All trap capture data were converted to daily trap captures and monthly totals were calculated using Eq. 7.1, 7.2, and 7.3.

\[ DTC_{ijk} = \frac{C_i}{D_j - D_k} \]  
\[ (7.1) \]

\[ MTC_{im} = (M_{mjk} \times DTC_{ijk}) + (M_{kl} \times DTC_{ikl}) \]  
\[ (7.2) \]

\[ MDC_{im} = \frac{MTC_{im}}{D_m} \]  
\[ (7.3) \]

where:

- \( DTC_{ijk} \) = daily trap capture in trap \( i \) during sampling period \( jk \)
- \( C_i \) = the total number of \( E. loftini \) recorded in trap \( i \)
- \( D_j \) = date of previous sampling
- \( D_k \) = date of sampling
- \( MTC_{mi} \) = total capture in trap \( i \) for month \( m \)
- \( M_{mjk} \) = the number of days during sampling period \( jk \) falling in month \( m \)
- \( MDC_{jm} \) = mean daily trap capture in trap \( i \) for month \( m \)
- \( D_m \) = number of days in month \( m \)

Yearly mean daily trap captures for each trap were calculated by summing the \( MTC_{mi} \) for each month and dividing by 365 days. Yearly mean daily trap captures were analyzed separately for each year using a two-way ANOVA (PROC MIXED, SAS Institute 2008) with parish, habitat type, and parish \( \times \) habitat type as fixed effects and trap(Parish) as a random effect. Only data from trap \( n = 59 \) located in parishes in which \( E. loftini \) was detected were included in this analysis. Habitats directly adjacent to traps were classified as rice, sugarcane, wild hosts, or pasture. Seasonal population dynamics were assessed from trap captures from Calcasieu, Cameron, and Jefferson Davis Parishes where \( E. loftini \) populations are well established. Mean
daily trap captures for each month from these parishes were analyzed with an ANOVA with year, month, and year × month as fixed effects, and trap as a random effect.

7.2.2 Spatial Analysis

The spatial analysis was conducted using ArcGIS software, and ArcMap was used to generate all figures (ESRI 2011). Shapefiles containing parish boundaries were obtained from the Louisiana Department of Transportation and Development (LDOTD 2007). Yearly mean daily trap capture data for all traps were imported as a shapefile into ArcGIS and subjected to point-based spatial interpolation using inverse distance weighting (IDW) to create continuous surface of estimated trap capture values across the 13-parish study area. This interpolation method is appropriate for the trap capture data which has a low density of known data points (Childs 2004). The IDW interpolation method estimates unknown values as the weighted average of its surrounding points with known values, in which the weight is the inverse of the distance raised to a power. The IDW is expressed using Eq. 7.4 (Wang 2015).

\[ z_u = \frac{\left( \sum_{i=1}^{s} z_i d_{iu}^{-k} \right)}{\left( \sum_{i=1}^{s} d_{iu}^{-k} \right)} \]

(7.4)

where:

- \( z_u \) is the unknown value to be estimated at point \( u \)
- \( z_i \) is the attribute value at a known point \( i \)
- \( d_{iu} \) is the distance between point \( i \) and point \( u \)
- \( s \) is the number of known points used in the estimation
- \( k \) is the power

The higher the power, \( k \), the stronger the effect of distance decay is (i.e. nearby points are weighted higher than remote ones). Interpolation with IDW using power \( (k=2) \) and \( s = 8 \) with a
maximum search radius of 50 km was applied to daily mean trap captures for each year, and each quarter of each year (3 month period). This ensured data from traps in a variety of habitats were included in estimations of unknown points across the study area.

The IDW interpolation results in a raster (image) output from which estimated trap capture data was extracted using the Sample Extraction Tool (ESRI 2011). This resulted in mean daily trap capture estimates for a total of 10,803 uniform polygons (squares) each with an area of 2.47 km\(^2\) encompassing the entire study area. Layer files were created which had estimated mean daily trap captures for each of the three years as well as each yearly quarter. These data layers were then used to calculate weighted mean centers and subjected to cluster analysis.

The weighted mean center for each three month period was calculated by applying the Weighted Mean Center Tool (ESRI 2011) to each daily trap capture layer. The weighted mean center is the geographic center of a set of points adjusted for the influence of a value associated with each point. The weighted mean center is calculated with Eq. 7.5 (Burt and Barber 1996).

Weighted Mean Center \((\bar{X}, \bar{Y})\)

\[
\bar{X}_w = \frac{\sum_{i=1}^{n} w_i X_i}{\sum_{i=1}^{n} w_i} \quad \quad \quad \quad \quad \bar{Y}_w = \frac{\sum_{i=1}^{n} w_i Y_i}{\sum_{i=1}^{n} w_i}
\] (7.5)

Tests for spatial associations and global clustering among daily trap captures for each year were conducted using Moran’s I statistic and the general G statistic using the Spatial Autocorrelation tool and the High/Low Clustering Tool, respectively (ESRI 2011). Moran’s I statistic (Moran 1950) was used to test for the presence of spatial autocorrelation across the data set. Moran’s I is the correlation coefficient between a variable and its spatial lag which detects
whether nearby areas have similar or dissimilar attributes overall (Wang 2015). The general $G$ statistic (Getis and Ord 1992) indicates clustering of high and low values based on statistical significance (Wang 2015). Because tests for spatial association indicated data were spatially autocorrelated ($P < 0.05$), Hot-Spot Analysis (ESRI 2011) was conducted for daily trap captures for each year. This calculates the $G_i$ statistic (Gertis and Ord 1992) which tests for statistically significant clusters of high values (hot-spots) or low values (cold-spots).

### 7.3. Results

Over the entire study area a total of 45,845 *E. loftini* males were trapped between Mar 2013 and Jan 2015. Detections of *E. loftini* were made for the first time in Evangeline Parish in 2014. Additionally, two *E. loftini* males were captured in St. Landry Parish in November of 2015. Trap capture totals were 17,284, 11,103, and 17,458 in 2013, 2014, and 2015, respectively. No *E. loftini* adults were captured in Lafayette, Iberia, St. Mary, or St. Martin Parishes during any year of the survey. Differences in mean daily trap captures (Table 7.1) in 2013 were detected among parishes ($F = 9.49$, df = 7, 40; $P < 0.001$), habitat types ($F = 7.00$, df = 3, 40; $P < 0.001$), and their interaction ($F = 3.79$, df = 8, 40; $P = 0.002$). Mean daily trap captures in 2014 differed between parishes ($F = 3.86$, df = 7, 40; $P = 0.003$), but not between habitat types or their interaction. Similarly, mean daily trap captures in 2015 differed only between parishes ($F = 2.27$, df = 7, 40; $P = 0.048$). Populations peaked in March–April of each of the three years, while the lowest populations were detected in January and February (Figure 7.2). Lesser population increases were also observed in July and November. While populations in 2013 and 2014 were on the decline in August–September, a steep rise was observed during the same period in 2015.
Differences in mean daily trap captures from Calcasieu, Cameron, and Jefferson Davis Parishes were detected between years \((F = 3.83, \text{df} = 2, 891; P = 0.022)\), months \((F = 7.25, \text{df} = 11, 891; P < 0.001)\), and the year by month interaction \((F = 2.62, \text{df} = 20, 891; P < 0.001)\).

Mean daily trap captures were >3.5 in some areas in each of the three years (Figure 7.3). A high degree of spatial autocorrelation in mean daily trap captures was detected in each of the three years based on both the Moran’s I and general G statistics (Table 7.2). These trends were confirmed with the hot-spot (local \(G_i\)) analysis (Figure 7.4). Clusters of high trap captures (hot-spots) were present in most of Calcasieu and Cameron Parishes in 2013 and 2014. The primary hot-spot in 2015 occurred in Jefferson Davis Parish and eastern portions of Calcasieu and Cameron Parishes. Smaller hot-spots were also detected in western Calcasieu and central Acadia Parish in 2015. Clusters of low trap captures (cold-spots) were detected in large areas of

![Graph showing Eoreuma loftini seasonal population dynamics](image)

**Figure 7.2.** *Eoreuma loftini* seasonal population dynamics. Daily trap captures (LS means) for each month in Calcasieu, Cameron, and Jefferson Davis Parishes, 2013–2015
<table>
<thead>
<tr>
<th>Parish</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>No. of traps sites</td>
<td>E. loftini /trap/day</td>
<td>No. of traps sites</td>
<td>E. loftini /trap/day</td>
<td>No. of traps sites</td>
<td>E. loftini /trap/day</td>
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<td>10</td>
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</tr>
<tr>
<td></td>
<td></td>
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<td>4</td>
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<tr>
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<td>3</td>
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<td>0.01</td>
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<td></td>
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<td>Wild hosts</td>
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<td>1</td>
<td>0.06</td>
</tr>
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</table>
Figure 7.3. *Eoreuma loftini* population distribution in southwestern Louisiana based on mean daily pheromone trap captures, 2013–2015 based on spatial interpolation using inverse distance weighting.

Figure 7.4. Statistically significant (G_i statistic) high/low clusters of mean daily *E. loftini* trap captures, 2013–2015.
the more northern Beauregard, Allen, and Evangeline Parishes, as well as in eastern Parishes of St. Landry, Iberia, St. Mary, and St. Martin where *E. loftini* is not thought to be present.

Table 7.2. Statistical tests for spatial associations of mean daily *E. loftini* trap captures, 2013–2015

<table>
<thead>
<tr>
<th></th>
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<th>2015</th>
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<tr>
<td><strong>Spatial Autocorrelation</strong></td>
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<td><em>Moran’s I</em></td>
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<tr>
<td><em>Z-score</em></td>
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<td>147.88</td>
<td>242.71</td>
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<td><em>P value</em></td>
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<td>&lt;0.0001</td>
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<td><strong>High/Low Clustering</strong></td>
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<tr>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The weighted mean population center moved eastward in each quarter with the exception of the third quarter (July–Sept) of 2014 and the second quarter (April–June) of 2015 which were located slightly west of the weighted mean center in the previous quarter (Figure 7.5). The weighted mean center in the last quarter of 2015 was 34 km east of the second quarter in 2013 indicating the population is moving eastward at a rate of approximately 11 km/yr. The eastern most trap capture detected during this survey was located south of Rayne, LA (30.122850°, -92.266650°). Eastward expansion based on the eastern most trap capture rate indicated populations advanced 16.5 km between Jan–Dec 2014 and 6.7 km between Jan–Dec 2015. This averages to 11.6 km/yr during this period.

Figure 7.4. Weighted mean population centers for *E. loftini* in Louisiana, 2013–2015
7.4. Discussion

This work builds on more than a decade of pheromone trap based monitoring of *E. loftini* in Texas and Louisiana (Reay-Jones et al. 2007c, Hummel et al. 2010, Chapter 4). The high trap density in our survey provides the most extensive assessment of *E. loftini* distribution and range expansion to date. The rate of eastward expansion estimated from initial detection in Texas counties from 1980–2005 was 23 km/yr (Reay-Jones et al. 2007c). Reay-Jones et al. (2007c) estimated that the weighted mean population center moved eastward at 5.5 km/year while the estimated leading edge advanced 16.6 km/yr from 2000–2005. When the rate of eastward expansion was based solely on the location of easternmost trap captures, expansion occurred at a rate of 22 km/yr from 2008–2013 (Chapter 4). In both the Texas (Reay-Jones et al. 2007c) and Louisiana (Chapter 4) monitoring surveys, substantial variation in eastward expansion occurred between years. When based on eastern-most trap captures, expansion ranged from 4 km in 2012 to 64 km in 2011. Weighted mean centers reduced this variation between years and more consistently measured expansion rates. In this study, the rate of expansion estimated from the weighted mean center and that based on eastern most trap captures were similar, 11.3 and 11.6 km/yr, respectively. Reay-Jones et al. (2007c), however, observed significant differences in expansion rates between the two methods. This may be the result of differences in the study areas between the two surveys. The distance between our westernmost trap and the leading edge of the population in this survey was 126 km, less than half of the distance covered by Reay-Jones et al. (284 km). This may have resulted in an underestimation of the rate of expansion when measured based on weight population centers in the Reay-Jones (2007c) survey.

Many previous examinations of the rates of range expansion in insect invasions have focused on radial expansion from a known point of invasion (Liebhold and Tobin 2008). Because
monitoring of *E. loftini* range expansion has largely been focused on predicting movement towards the economically important Louisiana sugarcane industry, little is known about the current western and northern boundaries of the population in Texas. The result is estimations of directional range expansion rather than radial range expansions observed in other insect invasions. Similarly, the northern boundary of the range of *E. loftini* in Louisiana has not been determined as the northern-most traps in this survey detected the pest’s presence.

Our data adds to a mounting body of evidence suggesting that *E. loftini* range expansion is occurring at a relatively stable rate and appears to follow reaction-diffusion model which combines population growth with random dispersal (Liebhold and Tobin 2008). The reduced populations present near the leading edge of the invasion observed in this survey are further evidence supporting the spread of *E. loftini* is occurring through simple diffusion. It is more common for invasive insects’ range expansion to follow a pattern of stratified dispersal characterized by sporadic long distance movement followed by coalescence of isolated populations (Liebhold and Tobin 2008). This is often the result of rapid expansion from human aided transport of infested material such as the case for *Lymantria dispar* L. (Lepidoptera: Erebidae) (Liebhold et al. 1992), and the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) (Muirhead et al. 2006). No direct evidence of human aided expansion of *E. loftini* has been found in Texas or Louisiana. This is particularly astonishing considering the substantial amount of infested material currently being transported to sugar mills east of the known *E. loftini* distribution (Chapter 5). Detection of *E. loftini* in Florida in 2012 (Hayden 2012) further adds to the uncertainty of potential for rapid advancement into new areas. Although it is doubtful the direct cause of the Florida introduction will ever be elucidated, human-aided transport is a likely scenario. The Florida population appears to be the result of an isolated introduction occurring
near the Goeth State Forest in Levy County, and the pest is now known to occur in three additional counties (University of Florida 2015).

In addition to assessment of range expansion, our pheromone trapping survey allowed for determination of *E. loftini* population distribution throughout southwestern Louisiana. High *E. loftini* populations observed near the southern border of Jefferson Davis Parish in all three years of our survey indicate the pest has become firmly established and will pose a consistent threat to graminaceous crop production in that region. Although a northern boundary of *E. loftini* distribution was not determined, consistently low trap captures in more northern parishes suggest *E. loftini* populations will rarely be an economic pest above 31° N in latitude. However, cold tolerance data indicate *E. loftini* is able to survive winter temperatures at these latitudes (Rodriguez del Bosque et al. 1995). Therefore, it is likely that *E. loftini* will become established in many areas of central and northern Louisiana, but potential to become a damaging pest in those regions may be limited relative to more southern areas.

The ability to identify hot-spots of high potential for damaging *E. loftini* infestations to develop will be critical to mitigating the impact of this invasive pest. This monitoring program will provide information which can be used to direct resources to areas where *E. loftini* is most likely to be an economic pest. This includes increasing education and outreach to farmers and other stakeholders in these regions providing them with the materials they need to make informed pest management decisions. The ease of pheromone trapping relative to more labor intensive insect sampling methods could allow the monitoring program to be maintained by county level extension agents. This would allow extension agents to provide their clientele with accurate and up to date information about the threat of *E. loftini* in their area. This program could be combined with online data base technology similar to Spensa Technologies’ MyTraps website.
(Chapter 5) to allow extension agents to monitor a small number of traps in their area, and combine data to maintain a statewide record of *E. loftini* populations.

Despite substantial variations in populations between geographic areas, differences in trap captures between habitat types were not as apparent in this survey. Although, *E. loftini* is known to infest numerous non-crop grasses (Beuzelin et al. 2011b), crop hosts including rice, sugarcane, and corn are thought to be preferred hosts (Showler et al. 2011, 2012; Beuzelin et al. 2013). Failure to detect differences in trap captures between habitat types in two of the three years in our survey suggests that factors other than availability of host plants may also be important influences of *E. loftini* distribution. Habitat heterogeneity, habitat fragmentation, disturbance frequency, and other environmental variables are known to be important factors affecting landscape level variation in insect population distributions (Turner 1989). Seasonal peaks observed in November of each year during our study were consistent with those observed in rice habitats in Texas (Beuzelin et al. 2011b). However, spring peaks during each year of our survey occurred earlier (March–April) than those observed by Beuzelin et al. (2011b) in Texas. This may have resulted from inclusion of traps in strictly non-crop habitats in addition to rice habitats. Adult emergence from these weedy hosts following overwintering may have contributed to the earlier population increases. Overall, trap captures in our study were substantially lower than those reported by Beuzelin et al. (2011b) which documented > 12.0 *E. loftini*/trap/day during population peaks in Texas.

Pheromone trap monitoring of *E. loftini* should be continued throughout Louisiana as the pest continues its range expansion into the state. The ability of *E. loftini* to utilize numerous crop and non-crop grasses (Beuzelin et al. 2011b, 2013; Showler and Reagan 2012) and the establishment in Florida indicate the pests is likely to invade most areas along the U.S. Gulf Coast.
Development and maintenance of the pheromone trap monitoring program in Louisiana could provide a framework for use of similar programs in other states. Development of effective monitoring and management strategies for *E. loftini* is critical to mitigation of the impact of this invasive pest to agricultural production in the southeastern U.S.
CHAPTER 8: SUMMARY

The Mexican rice borer, *Eoreuma loftini*, is a key pest of graminaceous crops including sugarcane, rice, and corn along the Gulf Coast. The invasive pest has recently become established in Louisiana where it threatens to cause substantial revenue losses to rice and sugarcane, two of the state’s most important commodities. Additionally, *E. loftini* has been introduced into Florida where it poses a risk to ≈500,000 acres of sugarcane and ≈20,000 acres of rice. Management of *E. loftini* in Texas, where the insect has been the key pest of sugarcane for more than 30 years, remains challenging and effective control tactics are badly needed. Traps baited with synthetic female sex pheromone are effective at monitoring *E. loftini* populations, but are currently underutilized in efforts to mitigate the impact of this pest. Thus, studies were conducted with aim of improving the understanding of *E. loftini* pheromone trap efficacy and expanding the role of these traps in invasive species monitoring and pest management.

The active space, or radius of attraction, for *E. loftini* pheromone traps was determined to be between 50 and 100 m through a two-year field study and a behavior assay. Based on results from these experiments, a single *E. loftini* pheromone trap can monitor an area of 1–3 ha. Experimental methods used in these studies can be adapted to other insect/cropping systems to study pheromone trap activity directly in the field environments where they will be deployed.

Monitoring an extensive network of pheromone traps throughout southwestern Louisiana documented *E. loftini* range expansion into nine new parishes. The pest is now known to be established in Calcasieu, Cameron, Beauregard, Allen, Jefferson Davis, Acadia, Evangeline, Vermilion, and St. Landry Parishes. In areas where it has been established for >2 years, *E. loftini* appears to be ubiquitous and can be detected in virtually all habitat types. Clusters of high density populations (hot-spots) were identified in southeastern Calcasieu and southern Jefferson
Davis Parish in each of three years from 2013–2015. Consistently low pheromone trap captures in the northern parishes of Beauregard, Allen, and Evangeline indicate *E. loftini* populations may be limited by colder winter temperatures at higher latitudes. Although it is likely that the pest will become established in regions of central and north Louisiana, it is not predicted to be an economic pest in these areas. The *E. loftini* population is advancing eastward into Louisiana at a rate of approximately 11 km/year. Although transportation of *E. loftini* infested sugarcane to sugar mills east of the pest’s current known range poses an immediate risk of introducing the species into the heart of the sugarcane production region, no specimens have been recorded from Iberia, St. Martin, or St. Mary Parishes to date.

Infestations of *E. loftini* in commercial rice reached damaging levels in fields which did not receive insecticidal seed treatments in Calcasieu Parish. It is likely that in regions where high populations of *E. loftini* are present, insecticidal protection of rice will be required to reduce revenue losses from this pest. Chlorantraniliprole (Dermacor X-100) seed treatments are effective at reducing *E. loftini* injury and can mitigate yield losses. Widespread application of these seed treatments which may be present in reduced concentrations late in the season or in ratoon rice has potential to select for insecticide resistance among stem borers and other rice pests. Alternative control methods for *E. loftini* management in rice should continue to be explored.

Contrary to expectations, *E. loftini* infestations in Louisiana sugarcane did not approach the levels frequently incurred in sugarcane production regions of Texas. There remains potential for highly damaging infestations to occur, particularly if drought conditions prevail which can exacerbate *E. loftini* populations. The sugarcane borer, *Diatraea saccharalis*, remains the key pest of Louisiana sugarcane at this time. Mixed infestations of *D. saccharalis* and *E. loftini* are
expected to become increasingly common as the latter continues its eastward range expansion further into the Louisiana sugarcane production region. Relative densities of each stem borer species will likely fluctuate in response to varying environmental conditions. Although *E. loftini* management is made difficult by reduced exposure of larvae relative to *D. saccharalis*, it is hopeful that sugarcane growers in Louisiana will be able to mitigate losses from *E. loftini* to levels lower than what has been historically suffered in Texas sugarcane.

Pheromone trap-assisted scouting has potential to be a valuable tool in integrated pest management (IPM) programs for *E. loftini* in rice and sugarcane. Trap captures are correlated to larval densities in both crops, and may be used to indicate potentially damaging populations are present in an area. Monitoring adult populations is drastically less labor intensive than sampling for larvae in the field, and pheromone-based techniques can substantially improve scouting efficiency. More comprehensive studies are needed before this strategy can be reliably used as a primary pest monitoring method in IPM programs. There remains a risk that damaging larval infestations can occur without detection of increasing adult densities, and pest management decisions should not be made solely on pheromone trap captures.

The development of automated pheromone trapping systems of electronic Z-traps which upload capture data to online data bases has potential to further reduce scouting effort. These systems allow for pest populations to be accurately monitored online or with a mobile phone application, removing the need to sample in the field. Automated pheromone-based monitoring systems are used to monitor for Lepidopteran pests in apple orchards and other fruit crops. Evaluations of Z-traps for *E. loftini* monitoring demonstrated the system can monitor pest populations in sugarcane and rice equal to or better than the currently used conventional traps. Pest management of *E. loftini* in sugarcane and rice may not provide the optimal systems for
widespread adoption of automated pheromone based monitoring, but this study demonstrated the potential for use of Z-traps to monitor Lepidopteran pests in field crops IPM.

The polyphagous nature of *E. loftini* and its widespread utilization of both crop and weedy host plants eliminate the potential for eradication of the pest in its introduced range. All evidence to date indicates the invasive pest will continue its incursion along the U.S. Gulf Coast. The newly introduced population in Florida suggests this insect has potential to be spread through human activities and will continue to threaten agriculture production in new areas. The pheromone trapping strategies demonstrated by this research project will provide a foundation of knowledge to improve monitoring and management of this invasive pest as it becomes established in new environments.
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APPENDIX A: LETTER OF PERMISSION FOR CHAPTER 4

From: Lisa Junker
To: E. Alan Cameron, Blake E Wilson
Subject: RE: Permission to include paper in dissertation
Date: Monday, March 07, 2016 8:26:48 AM

Dear Blake:

Thank you for contacting us about this! You do not need to request permission to republish your paper as part of your dissertation, as long as your dissertation is not going to be commercially published. All of our authors retain the right to use their papers in dissertations and master’s theses (see this page for more information, under “Rights retained by All Oxford authors”: http://www.oxfordjournals.org/en/access-purchase/rights-and-permissions/publication-rights.html)

If you are going to have your dissertation commercially published, let me know and I can connect you with our publisher’s permissions department.

Thank you again for your email, and good luck with your thesis.

Best regards-

Lisa Junker

Lisa Junker, CAE / Director of Publications & Communications
Entomological Society of America / 3 Park Place, Suite 307 / Annapolis, MD 21401-3722 / USA
Phone: +1 301-731-4535 ext. 3020 / Direct Dial: +1 240-696-3749 / Email: ljunker@entsoc.org

From: E. Alan Cameron [mailto:environmentalentomologyeditor@gmail.com]
Sent: Sunday, March 06, 2016 7:30 PM
To: Blake E Wilson <bwilson@lsu.edu>
Cc: Lisa Junker <ljunker@entsoc.org>
Subject: Re: Permission to include paper in dissertation

Blake;

By copy of thin note, I am passing your request on to Lisa Junker, Director of Communications for the Society. She is the one who handles this kind of request.

Thank you for choosing Environmental Entomology as the outlet for your research results.

Cheers!

Alan

On Sun, Mar 6, 2016 at 2:42 PM, Blake E Wilson <bwilson@lsu.edu> wrote:

| Dr. Cameron, |
APPENDIX B: SELECTED SAS PROGRAMS FOR CHAPTER 3

Hexagonal arrays 2011

dm='output;clear;log;clear';
Title1='Hex Arrays 2011';
data data1;
input per$ Days$ Farm$ Site$ Dist$ Trt$ Pos$ Dir1$ Dir2$ Total Catch CPD Prop;
cards;

; ODS HTML FILE='C:\Documents and Settings\tregexan\Desktop\Blake Wilson\Active Space MS\Hex Arrays 2011 Final.html' style = minimal ;
proc glimmix data=data1;
   Class Per Days Farm Site Dist Trt Pos Dir1 Dir2;
   Model CPD = Dist / htype=3 dist=Gaussian;
   Random Farm Per Per*Farm Dist*Per*Farm;
   lsmeans Dist / ilink diff cl;
   ods output diff=ppp lsmeans=mmm;
   ods listing exclude diffs lsmeans;
run;
%include 'C:\Documents and Settings\tregexan\Desktop\Blake Wilson\Stats\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.10,sort=yes);
run;
proc glimmix data=data1;
   Class Per Days Farm Site Dist Trt Pos Dir1 Dir2;
   Model Prop = Dist|Pos / htype=3 dist=Gaussian;
   Random Farm Per Per*Farm Dist*Per*Farm;
   lsmeans Dist|Pos / ilink diff cl;
   ods output diff=ppp lsmeans=mmm;
   ods listing exclude diffs lsmeans;
run;
%include 'C:\Documents and Settings\tregexan\Desktop\Blake Wilson\Stats\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.10,sort=yes);
run;
proc glimmix data=data1;
   Class Per Days Farm Site Dist Trt Pos Dir1 Dir2;
   Model CPD = Dir1|Dir2 / htype=3 dist=Gaussian;
   Random Farm Per Per*Farm Trt*Per*Farm;
   lsmeans Dir1|Dir2 / ilink diff cl;
   ods output diff=ppp lsmeans=mmm;
   ods listing exclude diffs lsmeans;
run;
%include 'C:\Documents and Settings\tregexan\Desktop\Blake Wilson\Stats\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.10,sort=yes);
run;

Hexagonal arrays, 2013 dm='output;clear;log;clear';
Title1='Hex Arrays 2013';
data data1;
Proportion in center trap regression

```
input per$ Days$ Farm$ Site$ Dist$ Trt$ Pos$ Dir1$ Dir2$ Catch CPD Total Prop;
cards;

;  
ODS HTML FILE='C:\Documents and Settings\treagan\Desktop\Blake Wilson\Active Space MS\Hex Arrays 2013 Final.html' style = minimal ;
proc glimmix data=datal ;
Class Per Days Farm Site Dist Trt Pos Dir1 Dir2 ;
Model CPD = Dist / htype=3 dist=Gaussian ;
Random Farm Per Per*Farm Dist*Per*Farm;
lsmeans Dist / ilink diff cl ;
ods output diffs=ppp lsmeans=mmm ;
ods listing exclude diffs lsmeans ;
run ;
%include 'C:\Documents and Settings\treagan\Desktop\Blake Wilson\Stats\pdmix800.sas' ;
%pdmix800 (ppp,mmm,alpha=.10,sort=yes) ;
run ;
proc glimmix data=datal ;
Class Per Days Farm Site Dist Trt Pos Dir1 Dir2 ;
Model Prop = Dist|Pos / htype=3 dist=Gaussian ;
Random Farm Per Per*Farm Dist*Per*Farm;
lsmeans Dist|Pos / ilink diff cl ;
ods output diffs=ppp lsmeans=mmm ;
ods listing exclude diffs lsmeans ;
run ;
%include 'C:\Documents and Settings\treagan\Desktop\Blake Wilson\Stats\pdmix800.sas' ;
%pdmix800 (ppp,mmm,alpha=.10,sort=yes) ;
run ;
proc glimmix data=datal ;
Class Per Days Farm Site Dist Trt Pos Dir1 Dir2 ;
Model CPD = Dir1|Dir2 / htype=3 dist=Gaussian ;
Random Farm Per Per*Farm Trt*Per*Farm;
lsmeans Dir1|Dir2 / ilink diff cl ;
ods output diffs=ppp lsmeans=mmm ;
ods listing exclude diffs lsmeans ;
run ;
%include 'C:\Documents and Settings\treagan\Desktop\Blake Wilson\Stats\pdmix800.sas' ;
%pdmix800 (ppp,mmm,alpha=.10,sort=yes) ;
run ;
```

```
Proportion in center trap regression

dm'output;clear;log;clear';
title1'Hex Arrays all PropC';
data data;  
input Year$ Farm$ Per$ Dist$ Distance PropC;  
cards;  
Proc Reg data=data;  
title2'PropC Reg';  
Model PropC = Distance;  
Run;
```
APPENDIX C: SELECTED SAS PROGRAMS FOR CHAPTER 4

Rice surveys, ANOVA

dm'output;clear;log;clear';
Title1'Paddy rice survey All Proc Mixed';
data data1;
input YEAR$ Trt$ Rep$ Per$ Days$ Month$ Stage$ Catch CPD Shoots FS PctI ;
cards;
ODS HTML FILE='C:\users\tregan\Desktop\Blake Wilson\Rice surveys Jun 26.html' style = minimal
;
%include 'C:\Users\tregan\Desktop\Blake Wilson\Stats\pdmix800.sas';
%pdmix800( ppp,mmm,alpha=.05,sort=yes);
run;

proc reg data=data1 ;
model cpd = PctI z1 / xpx influence ;
proc graph
run;

Rice Regression

dm'output;clear;log;clear';
Title1'PTAS Rice All Regression';
data data1;
input YEAR$ Trt$ Rep$ Per$ Days$ Month$ Stage$ Catch cpd Shoots FS PctI ;
if year = '2013' then z1=1 ; else z1=0;
cards;
ODS HTML FILE='C:\users\tregan\Desktop\Blake Wilson\PTAS Reg dummy variable outlier.html' style = minimal
;
proc reg data=data1 ;
model cpd = PctI z1 / xpx i influence ;
proc graph
run;
APPENDIX D: SELECTED SAS PROGRAMS FOR CHAPTER 5

Within season sampling: ANOVA and Regression

dm 'output;clear;log;clear';
Title1 'PTAS LA Sugarcane';
data data1;
input Year$ Field$ Trap$ Lat$ Long$ Par$ grower$ Var$ Crop$ Date$ Catch Days CPD PctI PctT ;
cards;

ODS HTML FILE='C:users\treagan\Desktop\Blake Wilson\PTAS LA Sugarcane all no verm.html' style = minimal;
proc glimmix data=data1;
class Year Field Trap Lat Long Par grower Var Crop Date;
model CPD = Year Par Year*Par / htype=3 ddfm=kr dist=Gaussian ;
random Field Field*year;
lsmeans Year Par Year*Par / ilink diff cl adjust=tukey;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'C:users\treagan\Desktop\Blake Wilson\Stats\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
proc glimmix data=data1;
class Year Field Trap Lat Long Par grower Var Crop Date;
model PctI = Year Par Year*Par / htype=3 ddfm=kr dist=Gaussian ;
random Field Field*year;
lsmeans Year Par Year*Par / ilink diff cl adjust=tukey;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'C:users\treagan\Desktop\Blake Wilson\Stats\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
proc glimmix data=data1;
class Year Field Trap Lat Long Par grower Var Crop Date;
model PctT = Year Par Year*Par / htype=3 ddfm=kr dist=Poisson ;
random Field Field*year;
lsmeans Year Par Year*Par / ilink diff cl adjust=tukey;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'C:users\treagan\Desktop\Blake Wilson\Stats\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
proc reg data=data1;
model cpd = PctI ;
proc graph
run;
proc reg data=data1;
model cpd = PctT ;
proc graph
run;
Z-traps 2015

*Z-traps 2015*

```
dm 'output; clear; log; clear';
title1 'Z-traps 2015';
data data;
input Date1$ Date2$ Days$ Wk$ Trap$ Crop$ Area$ Type$ Catch CPD MyTraps;
cards;

ODS HTML FILE='C:\Users\tregan\Desktop\Blake Wilson\Z-traps 2015 All.html'
style = minimal;
proc glimmix data=data;
title2 'Z vs M';
class Date1 Date2 days trap Crop Wk Area Type;
model CPD = crop|Type|Wk / htype=3 ddfm=kr dist=Gaussian;
random Area Area*Type;
lsmmeans Type|Wk / diff adjust=tukey;
ods output diffs=ppp lsmmeans=mmm;
ods listing exclude diffs lsmmeans;
run;
%include 'C:\Users\tregan\Desktop\Blake Wilson\Stats\pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
run;
Proc Reg data=data;
Model MyTraps = Catch;
Run;
```
APPENDIX E: SELECTED SAS PROGRAMS FOR CHAPTER 6

Pheromone Trap Capture, 2015

dm'output;clear;log;clear';
Title1'LRGV 2015 Trap Data';
data data1;
  input Days$ Per$ Trap$ Catch CPD ;
cards;
ODS HTML FILE='C:\Documents and Settings\taregan\Desktop\Blake Wilson\LRGV 2015 by Trap Data by field.html' style = minimal;
Proc glimmix data=data1 ;
class Days Per Trap;
model CPD = Per / htype=3 ddfm=kr dist=Gaussian ;
random Trap;
lsmeans Per / ilink diff cl adjust=tukey;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'C:\Users\taregan\Desktop\Blake Wilson\Stats\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;

Injury data ANOVA, 2015

dm'output;clear;log;clear';
title'LRGV MRB Injury 2015';
data data;
  input Rep$ Trt$ Sample$ TotalInt TotalBored BottomInt BottomBored TopInt TopBored;
pBored= TotalBored/TotalInt*100;
pBotBored= BottomBored/BottomInt*100;
pTopBored= TopBored/TopInt*100;
cards;
ODS HTML FILE='F:\Stats\Insecticides 2015\Output_MRB_LRGV_2015.html' style = minimal;
Proc sort;
  by Trt Sample;
run;
proc means;
  var pBored pBotBored pTopBored;
  by Trt ;
run;
proc glimmix data=data;
title2'GAUSSIAN Total Bored Internodes';
class Rep Trt ;
model pBored = Trt / htype=3;
random Rep Rep*Trt;
lsmeans Trt / diff adjust=tukey lines;
run;
proc glimmix data=data;
title2 'GAUSSIAN Top Bored Internodes';
class Rep Trt ;
model pTopBored = Trt / htype=3;
random Rep Rep*Trt;
lsmeans Trt / diff adjust=tukey lines;
run;

proc glimmix data=data;
title2 'BINOMIAL Total Bored Internodes';
class Rep Trt Sample ;
model TotalBored/TotalInt = Trt / htype=3 dist=binomial;
random Rep Rep*Trt Sample(Rep*Trt);
lsmeans Trt / ilink diff adjust=tukey lines;
run;

proc glimmix data=data;
title2 'BINOMIAL Top Bored Internodes';
class Rep Trt Sample ;
model TopBored/TopInt = Trt / htype=3 dist=binomial ;
random Rep Rep*Trt Sample(Rep*Trt);
lsmeans Trt / ilink diff adjust=tukey lines;
run;
quit;
VITA

Blake Emerson Wilson was born into a loving family in 1987 in Tulsa, Oklahoma. He spent the early years of his life in Austin, Texas, and Shreveport, Louisiana, before landing in Mandeville, Louisiana, at the age of 9. He graduated from Mandeville High School in 2005. He received his Bachelor of Science degree in biology from LSU in 2009. It was during his final semester as an undergraduate that he developed an interest in entomology. With help and encouragement from Dr. Gene Reagan, Blake enrolled in a master’s program in entomology at Louisiana State University. Blake received his M.S. in Entomology with a minor in Experimental Statistics in 2011. The title of his thesis was “Advanced Management of the Mexican Rice Borer (Eoreuma loftini) in Sugarcane”. This research was conducted in the Rio Grande Valley of Texas in conjunction with Dr. Allan Showler of the USDA-ARS.

Blake then joined the Reagan lab as a Research Associate in 2011. Blake’s work during 2011–2013 focused on management of the stem borers, Diatraea saccharalis and Eoreuma loftini in bioenergy cropping systems. Sugarcane varietal resistance to E. loftini was also studied.

Blake enrolled as a graduate student in 2013 to pursue his Ph.D. Blake’s PhD research focused development of pheromone traps for monitoring and management of E. loftini. Blake is currently completing requirements for the degree of Doctorate of Philosophy. Blake will remain in the Reagan lab as a postdoctoral researcher studying the sugarcane aphid, Melanaphis sacchari in sugarcane and sorghum. He plans to pursue a career in agricultural pest management research and extension.

Blake lives in a humble home under a large live oak tree in Baton Rouge, LA with his sweet and beautiful wife, Dr. Caitlin King, and his two daughters, Rumi and Bundle.