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# Improving the Deployment of Insecticidal Seed Treatments in Louisiana Rice in Accordance with Integrated Pest Management

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# **IMPROVING THE DEPLOYMENT OF INSECTICIDAL SEED TREATMENTS IN LOUISIANA RICE IN ACCORDANCE WITH INTEGRATED 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  
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December 2021

*This dissertation is dedicated to my wonderful grandmothers, Linda Griffiths and Christiane Mulcahy, who are sorely missed. Their strength, kind souls, adventurous spirits and inquiring natures taught me so much and I am forever grateful for their love and guidance. Their enthusiastic approach to life continues to inspire me.*

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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	i
TERMS, ABBREVIATIONS AND ACRONYMS .....	vi
ABSTRACT.....	viii
CHAPTER 1. GENERAL INTRODUCTION .....	1
1.1. Justification .....	1
1.2. Research objectives .....	2
CHAPTER 2. LITERATURE REVIEW .....	4
2.1. Rice production in Louisiana .....	4
2.2. Introduction to IPM.....	7
2.3. Insect pests of Louisiana rice .....	11
2.4. Pest control practices in Louisiana rice .....	16
2.5. Insecticidal seed treatments in Louisiana rice.....	19
2.6. Finding solutions to improve the use of insecticidal seed treatments .....	25
2.7. Goals.....	29
CHAPTER 3. SPATIAL DISTRIBUTION OF <i>LISSORHOPTRUS ORYZOPHILUS</i> (COLEOPTERA: CURCULIONIDAE) IN LOUISIANA RICE.....	31
3.1. Introduction .....	31
3.2. Methods.....	33
3.3. Results.....	38
3.4. Discussion .....	46
CHAPTER 4. SPATIAL DISTRIBUTION OF LEPIDOPTERAN STEM BORERS IN LOUISIANA RICE FIELDS .....	50
4.1. Introduction .....	50
4.2. Methods.....	52
4.3. Results:.....	55
4.4. Discussion .....	64
CHAPTER 5. INTEGRATION OF CHEMICAL AND CULTURAL CONTROL METHODS FOR THE CONTROL OF <i>LISSORHOPTRUS ORYZOPHILUS</i> (COLEOPTERA: CURCULIONIDAE) AND LEPIDOPTERAN STEM BORERS IN LOUISIANA RICE.....	68
5.1. Introduction .....	68
5.2. Methods.....	69
5.3. Results .....	72
5.4. Discussion .....	78
CHAPTER 6. ASSESSING THE FEASIBILITY OF USING REMOTE SENSING TO DETERMINE <i>LISSORHOPTRUS ORYZOPHILUS</i> INFESTATION LEVELS IN RICE .....	85
6.1. Introduction .....	85
6.2. Methods.....	87
6.3. Results .....	92

6.4. Discussion .....	101
CHAPTER 7. GENERAL CONCLUSIONS.....	107
7.1. Integrated Pest Management and Insecticidal Seed Treatments .....	107
7.2. Summary of Results and Recommendations.....	108
7.3. Opportunities for Future Research .....	111
REFERENCES CITED.....	114
VITA.....	138



## TERMS, ABBREVIATIONS AND ACRONYMS

Term	Description
CruiseMaxx®	An insecticidal and fungicidal seed treatment developed by Syngenta. The main active ingredient is thiamethoxam, a neonicotinoid.
Cruiser 5FS®	An insecticidal seed treatment developed by Syngenta. The main active ingredient is thiamethoxam, a neonicotinoid.
Dermacor® X-100	An insecticidal Seed treatment developed by Corteva Agriscience. The main active ingredient is chlorantraniliprole, an anthranilic diamide.
EPA	Environmental Protection Agency
Fortenza®	An insecticidal seed treatment developed by Syngenta. The main active ingredient is cyantraniliprole, an anthranilic diamide.
GIS	Graphic information system
Gi-statistic	Getis-Ord statistic. A statistic used to identify statistically significant spatial clusters of high values (hot spots) and low values (cold spots).
Global Moran's I	Global Moran's index. A statistic that measures spatial autocorrelation using feature locations and values. It determines whether the pattern variation of a given variable is spatially clustered, dispersed, or random.
GNDVI	Green normalized difference vegetation index
GPS	Global positioning system
GSAK	Geocaching Swiss army knife. A software that allows you to store and manage an offline database of geolocated waypoints.
IDW	Inverse distance weighting. A method of interpolation that estimates cell value using sample data points in the neighborhood of each processing cell.
IPM	Integrated pest management
IR	Imidazoline-resistant
MCARI	Modified chlorophyll absorption in reflectance index
MLR	Multiple linear regression
NDRE	Normalized difference RedEdge
NDVI	Normalized difference vegetation index
NipsIt Inside®	An insecticidal and fungicidal seed treatment developed by Valent. The main active ingredient is clothianidin, a neonicotinoid.

<b>Term</b>	<b>Description</b>
NIR	Near infra-red
RedEdge	A waveband of light ranging from 680-700 nm. It represents a region of rapid change in vegetation reflectance.
RWW	Rice water weevil
SAVI	Soil-adjusted vegetation index
UAV	Unmanned aerial vehicle
USDA	United States Department of Agriculture
VARI	Visual atmospheric resistance index
VI	Vegetation index
VRT	Variable rate technology
Whitehead	Panicle of unfilled grain that is used as an indicator of stem borer infestation and injury in rice plants.

## ABSTRACT

*Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), is the most destructive insect pest of rice in the United States. *Lissorhoptrus oryzophilus* larvae feed on rice roots, reducing yields by up to 25 %. Lepidopteran stem borers, in the family Crambidae, are also economically important pests of rice in Louisiana.

Currently, insecticidal seed treatments are used throughout the U.S. Mid-south rice industry to prophylactically control *L. oryzophilus*. Chlorantraniliprole seed treatments are also effective against stem borers. Thus, insecticidal seed treatments are used on >80 % of Louisiana rice acreage. Although seed treatments reduce damage inflicted by these pests, they are applied preventatively and do not adhere to the fundamentals of integrated pest management (IPM). Additionally, over reliance on a single insecticide promotes pesticide resistance through increased selective pressure. This dissertation aimed to improve the efficacy and sustainability of insecticidal seed treatments in accordance with IPM principles.

Two years of data were collected in Acadia, Jefferson Davis and Vermillion Parishes to understand the spatial distribution of *L. oryzophilus* and stem borers in Louisiana rice. Pest populations had an edge-biased distribution. *Lissorhoptrus oryzophilus* was also influenced by distance from overwintering/alternative non-crop habitat, while stem borer populations were influenced by region.

A three-year small-plot experiment investigated the integration of chemical and cultural control practices in rice. Results demonstrated that early-planting and delayed-flooding work synergistically to reduce *L. oryzophilus* and stem borer pest incidence and injury. Chlorantraniliprole was the most effective tool for managing both pests. However, the neonicotinoid, thiamethoxam, can be used in conjunction with cultural controls as an alternative to chlorantraniliprole, especially in areas with low stem borer populations.

Remote sensing, using unmanned aerial vehicles equipped with multi-spectral cameras, produced vegetation indices that were significantly correlated with *L. oryzaephilus* densities. Four indices can be used to predict yields and *L. oryzaephilus* induced crop stress. This could enhance monitoring of *L. oryzaephilus* on large commercial farms.

This research can be used to improve deployment of seed treatments and IPM implementation in Louisiana rice. It elucidates potential tools for moderating the risk of pesticide resistance, supports decision making processes and explores avenues for reducing input costs.

# CHAPTER 1. GENERAL INTRODUCTION

## 1.1. Justification

Rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), is the most destructive insect pest of rice, *Oryza sativa* L. (Poales: Poaceae), in the United States. (Way 2003). After the permanent flood has been established, *L. oryzophilus* larvae feed on rice roots, reducing plant growth and yields. Severe infestations of *L. oryzophilus* can cause up to 25% yield loss (Zou et al. 2004a). Lepidopteran stem borers, in the family Crambidae, are also among the most economically important pests of rice in Louisiana and Texas (Sidhu et al. 2014, Wilson et al. 2015, 2017). The stem borer complex found attacking rice in this region includes *Chilo plejadellus* Zink (rice stalk borer), *Diatraea saccharalis* F. (sugarcane borer), and the invasive pest, *Eoreuma loftini* Dyar (Mexican rice borer) (Beuzelin et al. 2016). Larvae of these pest species feed internally in rice stems. This hinders plant growth and development (Way 2003). The injury that stem borers cause typically results in dead hearts (dead tillers) and whiteheads (panicles with unfilled grains) (Way 2003).

Currently, insecticidal seed treatments are used throughout the U.S. Mid-south rice industry as a pre-emptive means of controlling *L. oryzophilus* (Lorenz and Hardke 2014). Additionally, the insecticidal seed treatment, chlorantraniliprole (Dermacor X-100) has been successfully used against both *L. oryzophilus* and stem borers (Sidhu et al. 2014). Therefore, seed treatments are used on >80 % of rice acreage in Louisiana (Bateman et al. 2020).

While seed treatments reduce the damage inflicted by these pests (Lanka and Stout 2015), they are typically applied as a preventative measure only, and therefore do not consider pest densities, thresholds, non-target effects, and cost-benefit ratios (Douglas and Tooker 2015). Thus, insecticidal seed treatments do not always adhere to the principles of integrated pest management (IPM), leading to questions about whether they can be used effectively in IPM programs (Tooker et al. 2017, Peterson et al. 2018, Mourtzinis et al. 2019,

Vojvodić and Bažok 2021). According to the United States Department of Agriculture (USDA) and the Environmental Protection Agency (EPA), all agricultural enterprises should strive to employ IPM as a “sustainable means of pest control” (USDA 2013). The broad-scale use of insecticides without considering pest pressure or economic benefits, defies the recommendations set forth in the National IPM Roadmap (USDA 2013). Furthermore, over reliance on any one insecticide, or insecticidal chemistry, promotes the evolution of pesticide resistance in pest populations through an increase in selective pressure (van den Bosch 1989).

The aim of this dissertation was to explore avenues for improving the efficacy and sustainability of insecticidal seed treatments in rice. The research and recommendations outlined herein can be used to help stakeholders make IPM-based decisions when applying insecticidal seed treatments. Additional goals included enhanced monitoring of insect pests in rice, the introduction of precision agriculture for rice pest management, and the development of potential strategies for reducing selective pressure in *L. oryzaephilus* and lepidopteran stem borer populations. These goals prioritize insecticide resistance mitigation and seek to preserve the longevity of insecticidal seed treatments. The project simultaneously addresses important IPM knowledge gaps, investigates the use of novel pest management techniques in rice and provides a base for future studies on targeted applications of insecticidal seed treatments. Overall, the project was conceived to support Louisiana rice farmers in their decision-making processes and to help them reduce their risks and input costs.

## **1.2. Research objectives**

Objective 1: To gain a thorough understanding of the spatial distribution of *L. oryzaephilus* in untreated rice fields in Louisiana (Chapter 3).

This study was conducted to improve *L. oryzaephilus* monitoring and sampling techniques, and to provide insights about the potential of using precision targeted applications of insecticidal seed treatments for *L. oryzaephilus* management.

Objective 2: To gain a thorough understanding of the spatial distribution of lepidopteran stem borers in untreated rice fields in Louisiana (Chapter 4).

Stem borers are also considered an important pest of rice in Louisiana. Therefore, information on the distribution of lepidopteran stem borers was needed to ensure *L. oryzaephilus* and stem borer management recommendations were compatible.

Objective 3: To assess the feasibility of integrating different insecticidal seed treatments with other pest control practices in rice (Chapter 5).

The goal of this objective was to determine whether cultural control strategies could be integrated with widely used chemical controls. This can help diversify pest management strategies, reduce farmer input costs, and decrease the risk of insecticide resistance.

Objective 4: To determine a model for the rapid estimation of *L. oryzaephilus* population densities using remote sensing technology (Chapter 6).

Unmanned aerial vehicles equipped with multi-spectral cameras are novel tools that can enhance monitoring of crop stress and inform management interventions. This study was conducted to ascertain whether remote sensing tools could be used to detect *L. oryzaephilus* injury in rice. The goals of this chapter were to assess the relationship between *L. oryzaephilus* density and NDVI values, and to determine which vegetation index is the most accurate for predicting *L. oryzaephilus* infestations and related yield loss. This can help increase sampling efficiency and improve our understanding of *L. oryzaephilus* yield impacts.

## CHAPTER 2. LITERATURE REVIEW

### 2.1. Rice production in Louisiana

Rice, *Oryza sativa* L., is the main staple food of over three billion people worldwide. Although the United States of America (U.S.) only produces about 2% of the world's rice, it is among the major exporters of rice, accounting for over 10% of annual global exports of the crop (Childs 2016). The local and international rice trade generates up to two billion dollars in annual revenue in the U.S. (Childs 2016).

Rice production is concentrated in six states, with Arkansas being the largest producer of rice in the country (USDA 2017). With approximately 470,000 acres (190,200 ha) of land dedicated to the production of long, and medium grain rice, Louisiana is considered the third most important rice producing state in the U.S. (Harrel 2020). As such, rice is not only an important contributor to the rural economy of Louisiana, but also to the livelihoods of the people who live in the state.

In Louisiana rice is grown on level land that is made up of silt loam or clay soils with an impermeable subsoil layer that minimizes the absorption of water. This allows farmers to decrease the number water retaining barriers or levees required, while also ensuring that their fields stay flooded during the growing season. Optimum seeding dates differ by location and by year according to environmental conditions, however plant survival improves greatly when the average daily temperature is above approximately 18 °C. Based on this information farmers in Southwest Louisiana are advised to plant their seeds between the 10<sup>th</sup> March and 15<sup>th</sup> April, while those in North Louisiana should begin seeding from the 1<sup>st</sup> April to the 5<sup>th</sup> May.



Rice in Louisiana is planted using three basic methods (Harrell and Saichuk 2014):

1. Water seeding — dry or pre-sprouted seed is dropped into an already flooded field.
2. Drill seeding — rice seeds are planted in moist soil using a drill, with a drill spacing of approximately 17–26cm
3. Dry broadcasting — dry seed is applied to a drained or dry field by ground equipment or airplane.

Both dry- and water-seeded planting practices are employed in Louisiana rice production. In water-seeded production, three different flood systems are commonly utilized. These include the delayed flood, the pinpoint flood, and the continuous flood. In a delayed flooding system, fields are drained following seeding for a period of 3–4 weeks before the permanent flood is established. For pinpoint flooding systems, farmers drain their fields 3–5 days after planting to allow the seedlings to establish before the rice is permanently flooded. In the continuous flooding system, flood water remains on the field from the time of seeding until draining for harvest. Although the numbers differ from year-year, approximately 35% of the total area planted with rice in Louisiana is water-seeded (Harrell and Saichuk 2014).

Water-seeding was once the most widely utilized method of planting rice, especially in Southwest Louisiana (Harrell and Saichuk 2014). Water-seeding, using a pinpoint flooding system, is an efficient mean of reducing weed infestations in cultivated rice (Dunand et al. 1985, Levy et al. 2006). This method of planting is particularly effective at decreasing the germination and subsequent infestation of red rice, a variety of *O. sativa* that acts as a weed and competes with cultivated rice, reducing grain yields and quality (Levy et al. 2006).

The discovery and development of imidazolinone-resistant (IR) rice in 1993 provided a new means of economical chemical weed management for red rice in cultivated IR rice varieties (Croughan 1994, Webster and Masson 2001, Carlson et al. 2012, Webster 2014). Farmers planting IR rice, also known as Clearfield<sup>®</sup> rice, are able to use imidazolinone

herbicides, such as imazethapyr and imazomox, against red-rice infestations (Webster 2014). These herbicides inhibit acetolactate synthase (ALS) in plants, thereby offering farmers a more effective means of controlling red rice and various other grass and broadleaf weed species (Webster and Masson 2001). The adoption of IR rice technology has therefore provided more flexibility when it comes to tillage, planting and flooding practices. This has allowed the majority of Louisiana farmers to switch to drill-seeded production systems, which are less knowledge and labor intensive (Harrell and Saichuk 2014).

When using a drill-seeded production system, the seed bed should be well-prepared and weed-free to allow for adequate seeding depths (these vary according to variety) (Harrell et al. 2021). This helps to ensure the establishment of a uniform rice field. The seed bed should also be moist when the rice is drill-seeded. Farmers can therefore plant seeds after a rainfall event, or they can flush their fields with water within four days of planting to improve germination. As an alternative to drill-seeding, rice can also be broadcasted onto a dry seedbed using ground or aerial equipment. However, broadcast-seeding reduces rice stand uniformity as compared to drill-seeding. Drill seeding requires additional seeds and the seeds need be covered with soil after planting. As such, this method is not as preferable as drill-seeding. Another benefit of the dry seeding methods is that it is easier to manage fertilizer and flooding regimes, which are similar for both drill-seeded and dry broadcast-seeded rice. Potassium (K) and phosphorous (P) can be incorporated into the seed bed prior to planting and Nitrogen (N) can be applied to the soil surface approximately three days before the permanent flood. In dry-seeded systems, the permanent flood should be established at the 4-5 leaf stage, however, full submergence of the plants should be avoided (Harrell et al. 2021).

Rice fields in Louisiana are often grown in rotation with red swamp crawfish, *Procambarus clarkii* Girard (Decapoda: Cambaridae), which are considered a major aquaculture commodity in the region (Lanka and Stout 2015). Although drill-seeding is the

preferred planting method in Louisiana, water-seeding still plays an important role in these crawfish/rice rotations. Farmers can also revert to water-seeding their rice in years when long-periods of rainfall affect the timing of dry planting systems (Harrell and Saichuk 2014).

Provisia rice varieties, that are resistant to the acetyl-coenzyme A carboxylase (ACCase) inhibitor herbicides, have been commercialized as an alternative herbicide-resistant rice technology for those seeking to manage ALS resistant weeds (Webster 2014). In addition to this, high-yielding hybrid varieties are continuously being developed and adopted in the U.S., since these varieties typically yield up to 20% more than conventional cultivars on similar land (Nalley et al. 2017). Thus, Louisiana rice farmers have more options available to them, in terms of varieties and management practices, than ever before.

Despite this, several factors act as constraints to rice production in Louisiana, and other rice producing regions in the United States. Low commodity prices, global competition, high input costs and an increase in the frequency of flooding events have affected farmer profits in recent years (Childs 2016, Schultz 2021). Currently, insect pests also act as a major constraint to rice production in Louisiana (Harrell 2021). Such pests account for millions of dollars in lost revenue due to reductions in yield, and the cost of chemical controls (Blackman et al. 2014, Diliberto et al. 2016). Therefore, one means of improving rice production in Louisiana is through the effective control and management of insect pest populations using integrated pest management (IPM).

## **2.2. Introduction to IPM**

Integrated pest management has been the main paradigm that has guided the research and implementation of insect pest management for over 50 years (Furlong and Zalucki 2010). However, the basic strategies of IPM were being employed long before this term was formally recognized (Smith et al. 1976). In the late nineteenth and early twentieth centuries farmers and agricultural specialists lacked efficient pesticides to manage pest populations.

Successful crop protection was dependent on an understanding of pest biology, agroecology, and cultural practices to produce pest control strategies that were multitactical, and which worked alongside natural biological control systems within the environment (Gaines 1957, Peshin et al. 2009). These tactics could be considered the precursors of the IPM programs used today (Peshin et al. 2009). During this time, researchers focused primarily on economically important pests. According to Kogan (1998), “*Pest control was understood as the set of actions taken to avoid, attenuate, or delay the impact of pests on crops, as such goals and procedures of pest control were clearly understood*”. This perspective changed with the discovery of the insecticidal properties of the chlorinated hydrocarbon DDT (dichlorodiphenyltrichlorethane) in 1939.

The period from the 1940s to the mid-1960s have been referred to by some as the dark ages of pest control (Kogan 1998). The “miracle” pesticide, DDT, was effective against a wide range of insect pests. It was also cheap, easy to use and long lasting (Flint and van den Bosch 1981). The increased use of chlorinated hydrocarbons to control insects, coupled with the discovery of the herbicide 2,4-D (2,4-Dichlorophenoxyacetic acid), sparked broadscale application and over-reliance on organo-synthetic pesticides in both agricultural and urban areas (Flint and van den Bosch 1981). Additionally, researchers tended to emphasize the development and testing of new chemical pesticides, neglecting previous holistic approaches to pest control. There was a distinct lack of concern regarding the economic importance of pests, and focus shifted towards the eradication of all unwanted insects, irrespective of the impact on beneficial organisms and non-target species (Peshin et al. 2009).

Unanticipated problems, following the widespread use of insecticides, soon emerged. Insect populations were being subjected to repetitive applications of a single class of insecticide, leading to extreme selective pressure. This resulted in the evolution of resistance to pesticides in several pest species. Resistance to DDT was first detected in houseflies and

mosquitoes as early as 1947 (Brown 1958). Furthermore, the destruction of beneficial natural enemies caused infestations of secondary pests, that were previously deemed unimportant (Flint and van den Bosch 1981, Kogan 1998, Peshin et al. 2009). To combat these problems farmers increased the frequency and dosage of insecticide applications. Eventually, insecticides would fail to provide meaningful crop protection. Thus, new and more potent insecticides were constantly being developed (e.g., organophosphates, carbamates and pyrethroids), leading to the selection of ever more resistant strains of the targeted pests (Kogan and Bajwa 1999). The continuous cycle of pesticide over-use and development in response to resistance has been referred to as the “pesticide treadmill” (van den Bosch 1978, Knight and Norton 1989). In the late 1950s it also became increasingly apparent that the massive, global use of pesticides was having severe ecological impacts (Kogan 1998, Kogan and Bajwa 1999). These impacts were expounded upon and sensationalized in books written by Rachel Carlson (1962) and Robert van den Bosch (1978). The concept of IPM was therefore developed in response to a mounting record of insecticide failures and to mitigate environmental disasters (Kogan and Baajwa 1999).

Integrated pest management itself can be loosely defined as a decision-making process, which is based on a thorough knowledge of the pest, and its tri-trophic and environmental interactions (Kogan 1998, Ehler 2006). It is concerned with using multiple pest management tactics in conjunction with threshold models, for the control of insect pests or diseases in agricultural crops (Kogan 1998, Ehler 2006). The main purpose of IPM is to prevent economically damaging out-breaks of pests and decrease pesticide resistance, whilst also reducing the risks to human health and the environment through the reduction of chemical insecticides, herbicides and fungicides on crops (Prokopy 2003).

The National IPM RoadMap is a federal program that was set in place to advocate for the development and adoption of IPM practices in U.S. agriculture. According to the United

States Department of Agriculture (USDA) and the Environmental Protection Agency (EPA), all agricultural enterprises should strive to employ IPM as a “sustainable means of pest control” (USDA 2013). This can be achieved by monitoring pests, applying chemicals only when needed (according to cost-benefit ratios), and above all, minimizing any harmful effects to the public and the environment (USDA 2013).

Although, federal programs advocate for the use of IPM programs, recent trends in agriculture undermine important principles of the IPM movement (Allen 2014, Peterson et al. 2018). Advances in genetic engineering have provided farmers with new herbicide-resistant varieties, and insect resistant crops that have been altered to express one or more proteins from the soil bacterium *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (Altieri 2004). In the past 20 years, the U.S. has seen the overwhelmingly successful adoption of prophylactic pest control tactics in the form of these transgenic crops and insecticidal seed treatments (Peterson et al. 2018). This has challenged the continuing development of IPM (Sappington 2014, Deguine et al. 2021).

Such tactics are used preventatively and on a broadscale (Sluijs et al. 2015). Genetically modified and chemically treated seeds are planted without first establishing whether economically damaging levels of pests exist within the crop environment (Sappington 2014, Douglas and Tooker 2015). Therefore, prophylactic technologies are typically intolerant to any injury done by the target pests, which is contrary to a key concept of IPM (Allen 2014, Deguine et al. 2021). This is especially true in major food and fiber crops, where scale and convenience drive the adoption of prophylactic pest control as a means of insurance (Sappington 2014, Peterson et al. 2018). Farmers using preventative measures, such as insecticidal seed treatments, rely less and less on the integration of other pest management tactics and the scouting services provided by crop specialists and extension personnel (Deguine et al. 2021, Vojvodić and Bažok 2021). Thus, a reduction in IPM infrastructure can

be seen in various regions of the Southern U.S (Allen 2014). Although many prophylactic pest control options are environmentally safer than the pesticides used in previous decades, they are also more expensive and subject to over-use (Sappington 2014, Sluijs et al. 2015). Many agricultural commodities (e.g., soybean, corn and cotton), are becoming increasingly reliant on limited insecticidal seed chemistries and Bt transgenic varieties (Sappington 2014, Mourtzinis et al. 2019, Bueno et al. 2021).

The broad-scale use of an insecticide, without taking into account pest pressure or economic benefits, defies the recommendations as set forth in the National IPM Roadmap (USDA 2013). Furthermore, an over reliance on any one insecticide or insecticidal chemistry promotes the buildup of pesticide resistance in pest populations through an increase in selective pressure (Sappington 2014). This necessitates a return to the principles of IPM in American agriculture (Peterson et al. 2018), as well as in Louisiana rice, which has seen a marked increase in the use of insecticidal seed treatments against economically important pests (Wilson and Stout 2017, Wilson et al. 2019).

### **2.3. Insect pests of Louisiana rice**

In Louisiana, rice is attacked by a multiplicity of aquatic, semi aquatic, and terrestrial insect pests (Harrell 2021). Some of the most damaging pests of rice are the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), and the complex of Lepidopteran stem borers. The following paragraphs discuss the biology, ecology and management of these pests in Louisiana rice:

#### *2.3.1. Biology and management of rice water weevil*

*Lissorhoptrus oryzophilus* Kuschel, commonly referred to as the rice water weevil (*L. oryzophilus*), is one of the most destructive insect pests of rice in the U.S. (Way 2003). This small, light brown, semi aquatic beetle is native to North American marshlands, where it originally attacked aquatic grasses and sedges in the plant families Poaceae and Cyperaceae

prior to the expansion of rice production in the early 1900's (Kuschel 1951, Tindall and Stout 2003, Aghaee and Godfrey 2014). The weevil began infesting rice in Louisiana as the crop's acreage was expanding in the region, with rice fields replacing marshland habitats (Aghaee and Godfrey 2014). *L. oryzophilus* is not only considered a pest in its native range, in the South-eastern rice growing regions of the U.S.A., (including Arkansas, Texas and Mississippi), but it has also spread to California as well as parts of Asia and Europe where it is likewise a pest of early season rice (Aghaee and Godfrey 2014, CABI 2017).

In California, and in other regions of the world where *L. oryzophilus* is invasive, only parthenogenetic females of the species are present in rice (Way 2003). However, in Louisiana, and other southern rice producing states, both females and males of *L. oryzophilus* are present (Way et al. 2003). In these areas, *L. oryzophilus* adults overwinter in bunch grass, leaf litter and forest habitats adjacent to rice fields (Way et al. 2003).

In early spring (late April–early May), both mated and un-mated adults emerge from diapause and start feeding on the leaves of rice and other non-crop host plants (Way 2003, Zou et al. 2004c). Adult *L. oryzophilus* are elongate-oval beetles that are approximately 5 mm in length (rostrum included). They are olive-grey to tan in color, with a dark V-shaped area on the elytra (Heinrichs 1994). The females of the species have larger abdomens and more distinct coloring (Saito et al. 2005). The weevils also have blade-like mid-tibiae, with dense elongate swimming hairs on inner and outer margins (Caldara et al. 2004). The weevils fly into paddy fields at night and swim beneath the water surface from plant to plant (Heinrichs 1994). Adults feed and rest on rice leaves and stems, leaving elongate feeding-scars on plant tissue (Saito et al. 2005). At the onset of permanent flooding, adult females begin to oviposit on rice leaf sheaths. The oblong eggs are about 0.8 mm in length and are placed longitudinally in the leaf sheath. The duration of the egg stage is between 4-9 days depending on temperature (Raksarart and Tugwell 1975). Once the eggs hatch, *L. oryzophilus* larvae



move to the base of the rice plant (Heinrichs 1994). The larvae are small, white, and legless. They have a brown head capsule and possess modified spiracles, which allow them to live and move in the soil and helps them acquire oxygen from the root aerenchyma (Everett and Trahan 1967). There are four larval instars (Kayumi and Sakashita, 1985) and the larvae feed on the roots of rice crops for approximately 21 days, growing up to 8 mm in length before pupating (Heinrichs 1994). Pupation takes place in the soil, in oval mud cells that are attached to the roots. Pupation lasts for 5-7 days, with the entire life cycle taking about 30 days to complete. In southern Louisiana, under optimal conditions, up to four generations of *L. oryzae* can occur in one year.

While all stages of *L. oryzae* infest rice, only the larvae are regarded as economically damaging. Pruning of rice roots by *L. oryzae* larvae leads to reductions in tillering, vegetative growth, panicle density and grain weight. Severe infestations of *L. oryzae* can cause over 25% yield loss (Zou et al. 2004a,b).

### 2.3.2. Stem borer complex

Lepidopteran stem borers are also considered important pests of rice in Louisiana (Sidhu et al. 2014, Wilson et al. 2015, 2017). The stem borer complex that can be found attacking rice in this region include rice stalk borer (*Chilo plejadellus* Zink), sugarcane borer (*Diatraea saccharalis* F.), and the invasive pest, Mexican rice borer (*Eoreuma loftini*) Dyar (Harrell et al. 2021). All of these Lepidopteran stem borers belong to the family Crambidae which are known to attack numerous crop and non-crop grasses (Showler and Reagan 2012).

In comparison to *D. saccharalis* and *E. loftini*, *Chilo plejadellus* is considered a sporadic pest of rice (Bowling 1975, Hummel et al. 2009). Therefore, the following paragraphs will focus on the biology and ecology of the other more economically damaging pest species.

*Eoreuma loftini* is a native pest of Mexico and is considered an invasive pest in Louisiana (Reay-Jones et al. 2008, Hummel et al. 2010, Wilson et al. 2015). The pest was first introduced to the Rio Grande Valley of Texas via sugarcane residues in 1980 (Johnson 1984). Since then, *E. loftini* has expanded its range towards the northeast and was first discovered in Louisiana in 2008 (Hummel et al. 2009). This species, also referred to as the Mexican rice borer (*E. loftini*), has become established in the southeastern regions of Louisiana and is continuing its expansion eastward (Wilson et al. 2015, 2017). Adult moths of *E. loftini* are usually 1.2 cm long and are solidly beige with almost no body patterning. The forewings have a small black central dot with two faint black streaks along the anterior margin of the wing. Mexican rice borer can be distinguished by its slightly conical frons and triangular shaped gena (Klotz 1970, Beuzelin et al. 2016). However, similarities among other related taxa, means that positive identification of *E. loftini* may require dissection of the genitalia (Reiss 1981, Agnew et al. 1988). Unlike some borers, *E. loftini* typically lays eggs in cryptic sites on rice, sugarcane and other host grasses. Oviposition sites can be found in folds and crevices on dead or dry leaves and in green leaf sheaths. This behavior reduces the exposure of eggs and young larvae to predators, parasitoids and foliar pesticides (Reay-Jones et al. 2007, Showler and Castro 2010a, Beuzelin et al. 2013). The yellowish, sub-globular eggs are laid in batches of 5 to 100 and hatch in 5-14 days depending on the temperature (van Leerdam et al. 1984, 1986). Once hatched, the pale cream-colored larvae move onto green tissue to feed, entering the stalk within a week (Wilson et al. 2012). Male larvae usually undergo 5 molts, while females undergo 6 molts (van Leerdam 1986). The larvae feed both horizontally and vertically within the stalk and reach a length of 25 mm when fully-grown. They have a light brown head capsule and two pairs of dark longitudinal stripes extending along the dorsal side of the abdomen (Browning et al. 1989). Larval development is inversely proportional to temperature, and they can take 21-78 days to reach pupation (van Leerdam

1986). The pupae of *E. loftini* are brown, nondescript, and can be found in thin cocoons near the emergence holes of feeding tunnels that have been tightly packed with frass (van Leerdam 1986). Adults emerge after 7-21 days, with the entire life cycle taking approximately 45 days during optimal summer conditions (Browning et al. 1989). Up to 6 generations can occur annually in this region (Legaspi et al. 1997), and while some larvae enter facultative diapause during winter, moths can be caught throughout the year (Reay-Jones et al. 2007, Hummel et al. 2010, Wilson et al. 2015, Beuzelin et al. 2016).

Like *E. loftini*, *D. saccharalis* is considered an invasive pest of rice and sugarcane in Louisiana (Mulcahy and Reagan 2019). However, it was introduced at a much earlier date (1850s) in sugarcane that was imported from the West Indies (Holloway et al. 1928). The adults of the sugarcane borer are similarly drab beige in color, although they have an inverted v-pattern of dots on their wings and have a wingspan of up to 2.8 cm in males, and 3.9 cm in females (Dyar and Heinrichs 1927). In rice agroecosystems *D. saccharalis* adults breed on various host plants until rice culms reach a large enough size for larval feeding (Bowling 1975, Ring et al. 1998). Female moths prefer to oviposit on rice that is at the boot and panicle differentiation stage and will usually oviposit on the upper reaches of the rice plants. Moths lay 2-100 eggs, which are deposited in overlapping clusters on either side of rice leaves. The eggs are yellowish, flat and oval in shape. Upon hatching, larvae migrate toward the space between leaf sheaths and stems. Larvae feed within leaf sheaths, until the third or fourth instar, when they tunnel into the stems. Larvae of *D. saccharalis* typically have five stadia, but some complete a fifth and even a sixth molt (Roe et al. 1982). Larvae are pale yellow with a head capsule that is a darker brown than those of *E. loftini* larvae. During the summer months, *D. saccharalis* larvae bear dark brown spots on each body segment, whereas the winter form lacks spots. The larval stage can take 18-34 days depending on temperature, with larvae reaching a size of up to 30 mm in length. Prior to pupation, larvae clean and expand

their feeding tunnels and pupate near the exit of the tunnel or between the leaves and stem (King et al. 1975). The pupae are brown in color and slender (16-20 mm in length), with the pupal stage lasting about 8-13 days (King et al. 1975). *Diatrea saccharalis* can complete four to five generations annually in Louisiana, with two to three generations occurring annually in rice fields (Hensley 1971). Oviposition can begin on rice as early as May, but economically damaging infestations generally do not occur until August or September (Bowling 1975, Ring et al. 1998). The late instar larvae of *D. saccharalis* also enter a facultative diapause, usually between October and December in Louisiana (Katiyar and Long 1961). Fluctuations in photoperiod and temperature during this time are the main factors influencing the initiation or termination of diapause in this species (Fuchs et al. 1979). Sugarcane trash left in the field is an important source of *D. saccharalis* re-infestations and the main overwintering habitats for larva are underground portions of stubble and newly planted stalks (Ingram et al. 1951).

In rice, Lepidopteran stem borers are typically a mid-season pest, inflicting damage on both the vegetative and early reproductive stages of the plant. The larvae of all these pest species bore into the culms of rice plants to complete their development and the damage that they cause typically results in “dead hearts” (dead leaves and tillers), and “whiteheads” (panicles with unfilled grains) (Way 2003, Beuzelin et al. 2016). There has been an increase in the incidence of stem borer infestations with the expansion *E. loftini* into the western rice growing regions of Louisiana (Wilson et al. 2017). Mild winters, dry conditions and reduced tillage in rice fields have also been linked to increases in stem borer populations in rice in different seasons (Castro et al. 2004, Showler and Castro 2010b, Sidhu et al. 2014).

#### **2.4. Pest control practices in Louisiana rice**

It is estimated that, in Louisiana, over \$13 million is spent each year in an effort to control the insect pests of rice (Blackman et al. 2014, Diliberto et al. 2016). Such input costs are necessary for the management of pest populations in Louisiana rice. If populations are left

unchecked, the insect pests mentioned above could cause damages that far exceed the cost of control. For instance, it is estimated that in Louisiana rice, *E. loftini* can cause up to 45 million dollars in lost revenue each year if it is not managed and is allowed to become fully established in the state (Beuzelin et al. 2016). If uncontrolled, *L. oryzaephilus* infestations could cause yield losses in excess of 25 % (Stout et al. 2000, Aghaee and Godfrey 2014). Such impacts would severely impact the economic viability of the rice industry in Louisiana. As such, the continued use of effective pest management practices is crucial for the sustainability of rice production in this state.

Several control practices have been shown to reduce the amount of damage done to rice by certain insect pests in Louisiana. Early planting, delayed flooding, field draining, and water depth management are effective at managing the build-up of *L. oryzaephilus* populations in Louisiana rice (Thompson et al. 1994, Rice et al. 1999, Stout et al. 2002a, Villegas et al. 2021a). Whereas, cultural control methods, such as good field hygiene practices (the removal of dried trash and litter, and overwintering residues), and the use of resistant cultivars, can be used to manage stem borer infestations (Way 1990, Way et al. 2006a, Sidhu et al. 2013a, Wilson et al. 2020). Sidhu et al (2013a) demonstrated that *D. saccharalis* relative growth rate and boring success was lower on Clearfield (CL151) and hybrid cultivars (XL723) than on some high-yielding long grain cultivars such as Cocodrie and Priscilla. The addition of silicon to the soil of rice fields has also shown potential as an effective mechanism for reducing the damage inflicted by Lepidopteran pests in rice (Sidhu et al. 2013b, Villegas et al. 2017).

The goal of cultural control practices is to reduce crop pest infestations through the manipulation of abiotic and biotic components within the agroecosystem (Bajwa and Kogan 2004). Abiotic factors subject to cultural control include site selection, planting date, harvest time, soil practices (tillage, irrigation, and fertilization), and the use of mulches and row

covers. Cultural controls can also be used to manipulate the biotic environment through crop rotation, intercropping, trap cropping, companion planting, and through the use of semiochemicals in antifeedants and "push-pull" strategies. However, crops are only a small part of a network of environmental interactions, therefore individual cultural pest control strategies need to be compatible with other IPM methods (biological, chemical, physical) in order to be successful (Gabrys and Kordan 2013). While cultural control practices are useful as a pest management strategy for rice farmers, there are some problems concerning their compatibility with rice farming practices in Louisiana. For example, delayed flooding and water depth management can result in an increase in weed abundance, this is particularly true of red rice, an undesirable, phenotypically similar variety of rice, which is considered the number one economically damaging weed in Louisiana (Rao et al. 2007, Webster 2014).

Although certain cultivars have shown resistance to stem borers, and even some mild resistance and tolerance to *L. oryophilus* damage (Saad et al. 2016, Villegas et al. 2021b), it can be difficult to understand the economic benefits of host plant resistance in rice. This is because resistance to one pest does not necessarily confer resistance to another pest, and because there is variability in the overall yield potentials of different resistant and susceptible cultivars (Stout et al. 2001, Beuzelin et al. 2016). Furthermore, there are many factors which influence variety choice, with pest resistance being only a small component of the management concerns faced by farmers (Harrell et al. 2021). Farmers can prioritize increased yield or disease resistance when making choices, and problems with identifying and transferring insect resistance to usable varieties can act as barriers to employing variety choice as an effective pest management tool (Gracen and Guthrie 2008). There is also a current lack of research and effort regarding the use and efficacy of employing biological control for the management of economically damaging pests of rice in Louisiana (Way 2003). As such, the rice industry in Louisiana, as well as those in other rice producing states, relies

heavily on the application of chemical insecticides to control insect pests (Aghaee and Godfrey 2014, Beuzelin et al. 2016, Bateman et al. 2020).

After the soil insecticide Carbofuran was banned in the 1990's, applications of foliar insecticides were primarily used to control *L. oryzaephilus* populations in rice, as well as outbreaks of other major insect pests (Stout et al. 2000). The main foliar insecticides used were lambda-cyhalothrin (a pyrethroid) and the growth regulator diflubenzuron (Stout et al. 2000). Fipronil, which belongs to the phenylpyrazole chemical family, was also used as an insecticidal seed treatment to control *L. oryzaephilus* (Hummel 2014). Despite the effectiveness of these insecticides for controlling the insect pests of rice, they were eventually deemed unsuitable for widescale use in Louisiana rice. Unfortunately, both fipronil and lambda-cyhalothrin have significant non-target effects on other aquatic arthropod species in the rice agroecosystem (Lanka and Stout 2015). This is particularly problematic because these insecticides can negatively affect the rice/crawfish crop rotations that are common to Louisiana rice production systems (Lanka and Stout 2015). As an insect growth regulator, diflubenzuron is far less toxic to non-insect organisms, and would therefore be safe to use on rice. However, this insecticide is highly susceptible to photodegradation, and therefore may not provide adequate long-term protection against insect pests (Mabury and Crosby 1996). Although foliar applications of pyrethroids and neonicotinoids are still used against outbreaks of rice stink bug (Bhavanam et al. 2021), vegetative rice is now primarily protected against insect pests through the use of a new class of insecticidal seed treatments.

## **2.5. Insecticidal seed treatments in Louisiana rice**

Preplant treatment of seeds with systemic insecticides has been widely adopted as a prophylactic pest control measure in row crops (Bradshaw et al. 2008, Gontijo et al. 2014a). Systemic insecticides are characterized by low lipophilicity, which helps to facilitate their translocation in plant tissues (Cloyd and Bethke 2011, Gontijo et al. 2014b). Systemic

insecticides can therefore be used to coat seeds, because the residues of such insecticides can be absorbed by the plant and distributed throughout its various tissues, including stems, leaves, roots, fruits, and flowers (Dively and Kamel 2012, Goulson 2013). Their use has, to some extent, reduced the need for broadcast applications of insecticides as well as decreased the amount of active ingredient being used. Thus, insecticidal seed treatments have the potential to reduce environmental contamination (and applicator exposure) and have generally lowered pesticide impacts on non-target organisms through ecological selectivity (Hull and Beers 1985, Albajes et al. 2003, Cloyd and Bethke 2011). Prophylactic treatments using systemic insecticides have proven to be successful in preventing arthropod damage to seeds and seedlings in many crops such as corn, soybean, sorghum and sunflower, among others.

Currently, insecticidal seed treatments are used throughout the rice industry as a pre-emptive means of controlling *L. oryzaephilus* and other pests (Lorenz and Hardke 2012). Five insecticidal seed treatments are presently being recommended for use against *L. oryzaephilus* (Everett et al. 2015). These are chlorantraniliprole (Dermacor X-100<sup>®</sup>), thiamethoxam (Cruiser Maxx<sup>®</sup> and Cruiser 5FS<sup>®</sup>), clothianidin (NipsIt Inside<sup>®</sup>), and cyantraniliprole (Fortenza<sup>®</sup>), which was recently approved for use in rice (Everett et al. 2015, Wilson et al. 2021a).

The active ingredient in Dermacor X-100, chlorantraniliprole, is an anthranilic diamide (Cordova et al. 2006, Cordova et al. 2007). This is a novel class of insecticide that targets the ryanodine receptors in insect muscles cells, causing a depletion of Ca ions that halts feeding, causes muscle spasm and eventually leads to death in targeted insects (Cordova 2006, Cordova et al. 2007). Thiamethoxam and clothianidin on the other hand both belong to the neonicotinoid group of insecticides, which selectively target nicotinic acetylcholine receptors in insects (Simon-Delso et al. 2015). Neonicotinoids cause overstimulation at low



concentrations and at higher concentrations they block receptors, leading to paralysis and ultimately death (Tomizawa and Casida 2005).

Hummel et al. (2014), showed that the use of insecticidal seed treatments provided significant control of *L. oryzaophilus* over four growing seasons. Chlorantraniliprole seed treatments are generally more effective against *L. oryzaophilus* than neonicotinoid seed treatments (Stout et al. 2009, Stout et al. 2011) and reduce larval densities by up to 94 % relative to controls (Hummel et al. 2014). The effectiveness of chlorantraniliprole is maintained even at application rates that are 5-fold lower than the recommended label rate (Lanka et al. 2013a, Villegas et al. 2019). In contrast to chlorantraniliprole, thiamethoxam and clothianidin treatments reduced *L. oryzaophilus* population by 39 to 50 % (Hummel et al. 2014). However, both neonicotinoid and diamide seed treatments perform better than single, pre-flood applications of foliar pyrethroids (Stout et al. 2011). Additionally, the seed treatments discussed here (Dermacor<sup>®</sup> X-100, CruiserMaxx<sup>®</sup>, Cruiser 5FS<sup>®</sup> and NipsIt Inside<sup>®</sup>) are not only less toxic to crawfish than fipronil and foliar pyrethroids (Barbee et al. 2010), but they also persist for a longer period of time in the environment, and therefore provide early, long lasting pest control (Lanka and Stout 2015).

While rice insecticidal seed treatments are effective against *L. oryzaophilus*, they can also be used to control other pests of rice in Louisiana. Neonicotinoid insecticides have little effect on lepidopteran pests, but they can be used to control early season insect pests. *Colaspis* spp. (Coleoptera: Chrysomelidae), the larvae of which generally feed on the roots of rice that grow in rice-soybean rotations (Heinrichs et al. 2017), can be managed using neonicotinoid seed treatments (Wilson et al. 2019). This can help to reduce seedling death and reduced stand count (Heinrichs et al. 2017). Chinch bugs, *Blissus leucopterus* Say (Hemiptera: Blissidae), and different species of Thrips can also be managed using neonicotinoids, however these pests are considered sporadic and do not usually cause

economic damage in Louisiana (Harrell et al. 2021). Chlorantraniliprole has also been shown to work effectively against lepidopteran pests such as the foliar feeder, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), and the stem borers, *C. plejadellus* D. *saccharalis* and *E. loftini*. Therefore, chlorantraniliprole is recommended as a useful tool for the management of stem borer populations in rice (Sidhu et al. 2014, Wilson et al. 2015, 2017). The effectiveness of chlorantraniliprole against such damaging pests means that Dermacor X-100 is now being used on >80% of Louisiana commercial rice farms (Bateman et al. 2020).

Despite the advantages of using prophylactic chemicals, research has revealed that insecticidal seed treatments do have a number of lethal and sublethal effects on non-target organisms (Gontijo et al. 2014a). There is a growing body of evidence, that demonstrates the negative impacts of neonicotinoid insecticides, such as thiamethoxam and clothianidin seed treatments, on native pollinators and honeybees, *Apis mellifera* L. (Hymenoptera: Apidae) (Krupke et al. 2012, Goulson 2013, van der Sluijs et al. 2013). Fear surrounding pollinator non-target effects, as a result of neonicotinoid use, could lead to further utilization of the anthranilic diamides, such as Dermacor X100, which are a more selective class of insecticides (Lanka and Stout 2015). Fortunately, this is not a concern for rice, which is a self-pollinating plant that is not frequented by pollinators (unlike other row crops such as corn, cotton, and soybean) (Stewart et al. 2014).

Systemic pesticide concerns extend beyond the impacts that they have on bees and other pollinators (Gross 2014). The high solubility and widespread use of systemic neonicotinoid insecticides in agriculture results in the contamination of soil and aquatic environments (Sanchez-Bayo et al. 2016). Such contamination of subsurface soil and water was found in cultivated potato systems that rely on neonicotinoid seed treatments (Huseth and Groves 2014). Anthranilic diamides can also build up in high levels in these

environments (USEPA 2008, EFSA 2013), and therefore have the propensity to contaminate waterways and crops through runoff and affect other communities of invertebrates in ways that are currently unknown (Rodrigues et al. 2015, Rodrigues et al. 2016). This is especially relevant to rice agroecosystems since flooding practices can readily result in the leaching of seed treatments into nearby water sources (Gupta et al. 2008). The levels of neonicotinoids found in some agricultural soils, waterways, field margins and floral resources are at concentrations that can sufficiently control crop pests, and often exceed the LC50 (concentration which kills 50% of individuals) for beneficial organisms (Goulson 2013). While the high toxicity of these insecticides to aquatic insects and other arthropods has been recognized, there is little understanding about the impacts that these chemicals can have on aquatic environments and agroecosystems (Sanchez-Bayo et al. 2016). Insecticidal seed treatments can have lethal and sublethal impacts on beneficial natural enemies too. Predatory insects, such as Coccinellid larvae, predacious Hemipterans, *Chrysoperla* spp. (Neuroptera: Chrysopidae) and Syrphid larvae, are negatively affected by exposure to both chlorantraniliprole and neonicotinoid seed treatments. Exposure can occur through zoophytophagy (omnivorous feeding behavior) and through the consumption of targeted prey (Moser and Obrycki 2009, Goulson 2013, Gontijo et al. 2014a, Gontijo et al. 2014b, van der Sluijs et al. 2015). To quote from van der Sluijs et al. (2015), “*The combination of prophylactic use, persistence, mobility, systemic properties and chronic toxicity is predicted to result in substantial impacts on biodiversity and ecosystem functioning*”. Thus, we can conclude that, while seed treatments are considered safer than broadcast insecticides, non-target effects are still possible. We should therefore be cautious before recommending the indiscriminate use of insecticidal seed treatments in Louisiana rice.

Additionally, seed treatments are typically applied as a preventative measure only (Hummel et al. 2014, Douglas and Tooker 2015). Therefore, their application does not

consider pest densities, economic thresholds, non-target effects, selection pressure, and cost-benefit ratios (Douglas and Tooker 2015). As such, insecticidal seed treatments are considered by some to be incompatible with traditional IPM concepts, which prioritize these decision-making strategies (Hummel et al. 2014, Douglas and Tooker 2015, Peterson et al. 2018, Vojvodić and Bažok 2021).

The integration of alternative management tools is needed to enhance the sustainability of these current control practices. This is particularly true in Louisiana, where the widespread use of chlorantraniliprole may contribute to resistance development (Denholm and Rowland 1992). This has occurred in the past with other insecticide chemistries in rice. For example, the broad-scale use of Aldrin seed treatments led to the development of insecticide resistance in *L. oryzae* populations within just eight years (Bowling 1968). As such, some level of insecticide resistance management (IRM) is needed to ensure chlorantraniliprole remains effective in future years. Furthermore, at approximately \$50 per hectare, chlorantraniliprole is far more expensive than alternate neonicotinoid options (Wilson and Stout 2017). This is compounded by the fact the recommended rate of Dermacor X100 varies according to farmers' seeding rate in order to maintain an even amount of insecticide per hectare (Wilson and Stout 2017). Conversely, neonicotinoids are applied according to the weight of seed being planted and are therefore more economical in situations where a lower seeding rate is required (Wilson and Stout 2017). With decreasing rice prices, and increasing input costs, farmers are seeking options for reducing the expenses of rice production. While farmers do have the option of using multiple different insecticides to improve pest control, research analyzing the cost effectiveness of such practices is lacking (Wilson and Stout 2017).

## 2.6. Finding solutions to improve the use of insecticidal seed treatments

### 2.6.1. Characterizing the spatial distribution of insect rice pests

IPM programs are typically “knowledge intensive” and require an in-depth knowledge of pest biology, ecology, and behavior within an agroecosystem (Kogan 1998). Therefore, it is vital that we gain an in depth understanding of plant-pest interactions in the rice agroecosystem, and how these interactions relate to the adequate deployment of insecticidal seed treatments. While the biology and ecology of *L. oryzaophilus* and Lepidopteran stem borers have been well researched, surprisingly little is known about the spatial distribution of these pests within commercial rice fields. A study done by Espino (2012) in California, showed that *L. oryzaophilus* populations tend to condense along the outer margins of rice fields, with relatively fewer weevils being found in the center of rice fields. However, as stated previously, the population of *L. oryzaophilus* in California is solely parthenogenetic, unlike the weevils found in Louisiana. Populations of the *L. oryzaophilus* in California are also lower, with only one generation per year, when compared to the multi-voltine populations of this pest in Louisiana, and in other regions where it is native (Heinrichs et al. 2017). This may mean that other factors are playing a role in the distribution and spread of the weevil in California. Research looking at the spatial distribution of the stem borer, *D. saccharalis*, in sugarcane, found that variable random and aggregated dispersal patterns can be observed depending on the density of the pest and the developmental stage of the larvae being sampled (Schexnayder et al. 2001). The same was found to be true of *E. loftini* (Meagher 1996).

More research needs to be conducted in Louisiana regarding the colonization of rice by these pests and the factors that may be influencing their distribution in the crop throughout the growing season. Understanding the spatial and temporal distributions of insect pests can lead to better implementation of IPM tactics by allowing for improved sampling techniques, favorable manipulations of the agroecosystem and targeted applications of chemical controls

(Nguyen and Nansen 2018). Such information could lead to better site specific, or precision targeted, management options.

### 2.6.2. Precision Agriculture

Site-specific management uses variable applications of an input or management operation in a localized/pin-pointed manner (Cassman 1999, Zhang et al. 2002). Using remote sensing as well as spatial and temporal data, seed, nutrients, water, and pest control measures can be deployed to meet the need-specific requirements of different locations within a field (Zhang et al. 2002). This is referred to as precision agriculture (Zhang et al. 2002). According to Nguyen and Nansen (2018), “*compared to conventional agriculture, precision agriculture focuses more on timely and targeted application of treatments to control insect pest infestations rather than field-wide and calendar-based spraying of chemicals*”. More efficiently targeted seed treatments have the potential to not only improve pest management in rice, but also help to reduce farmer input costs and decrease the build-up of pest resistance by reducing selective pressure (Nguyen and Nansen 2018).

From a modern perspective, precision agriculture can be loosely defined as the use of emerging technologies, including global position systems (GPS), geographical information systems (GIS), yield monitors, variable-rate technology (VRT), and remote sensing with unmanned aerial vehicles (UAVs) to achieve site-specific crop management (McBratney et al. 2005). Precision agriculture is characterized as a conceptualized systems approach, which seeks to restructure the traditional system of crop production towards a more low-input, high-efficiency, sustainable agricultural framework (Zhang et al. 2002). It is considered by some to be the next major step in the green revolution, because it has the potential to improve crop production, whilst also reducing overall inputs (Lambert and Lowenberg-DeBoer 2000, Tilman et al. 2002).

One of the most promising tools in precision agriculture, is in the use of remote sensing technologies, in conjunction with UAVs, to quickly and efficiently collect data across a vast acreage of crop production (Zhang and Kovacs 2012). Digital aerial imagery is an example of a remote sensing tool that can be used in this way. Cameras equipped with near-infrared spectrum capabilities can be used to photograph large agricultural fields from low-flying UAVs. These images can be used to determine the level of crop stress within a given area by measuring the amount of chlorophyll that is being produced by each plant in the field. A healthy plant produces more chlorophyll and therefore reflects green light. An unhealthy, stressed or damaged plant produces less chlorophyll and thus absorbs green light. The images, captured using the remote sensing cameras, can pick up minute changes in the amount of chlorophyll produced by individual plants over a large surface area. These images, along with the geographical data that they carry, can be used to create specialized maps displaying the normalized difference vegetation index (NDVI). NDVI maps highlight trouble spots and can ultimately pinpoint those areas that are of most and least concern, especially with regard to the nutrient status of the crop. Using this information farmers can use targeted inputs in those areas that are doing poorly, and thus save time and money when it comes to management decisions. The technology is still emerging and is not yet widely used, but with a high-level overall accuracy, aerial imagery is considered a quick, effective, and precise tool to improve crop scouting techniques.

Although remote sensing and precision agriculture have the potential to help target applications of insecticides and thus reduce the amount of the chemicals being used in rice production, it is also important that the compatibility of seed treatments with other control methods be assessed.

### *2.6.3. Integration of chemical and cultural controls*

Cultural controls, that are well suited to a cropping system, can form the basis of an effective IPM program by enhancing ecological processes that mitigate pest population expansion in agricultural habitats (Bajwa and Kogan 2004). Successful cultural controls are able to prevent or reduce pest outbreaks, and decrease pest survival, population dispersal and crop damage (Bajwa and Kogan 2004). As such, cultural controls form an important component of the IPM toolbox.

Some studies have shown that insecticidal seed treatments are congruent with cultural control practices and host plant resistance (Adams et al. 2015, Lanka et al. 2015). In fact, integration with such techniques has indicated that reduced rates of insecticidal seed treatments may be used with very little reduction in efficacy (Lanka et al. 2015, Villegas et al. 2019). Reductions in the amount of insecticide needed to control pests has the potential to reduce farmer costs, whilst concurrently decreasing possible harmful effects to the environment (Lanka et al. 2015). There is a debate as to whether reducing insecticide output is useful for insecticide resistance management (IRM), however, Gressler (2011), reports that using low rates of persistent insecticides (such as anthranilic diamides) may prevent the development of target site resistance in pest populations.

Although researchers generally acknowledge the important role that cultural control tactics play in IPM programs, these methods do not always suit current farming practices. Many of the cultural control practices recommended for rice insect pests are contrary with the wants and needs of rice farmers (Espino and Way 2014). Therefore, it is important that we discuss research outcomes and recommended agricultural practices with farmers before attempting to implement them.



#### 2.6.4. *A farmer first approach*

Unfortunately, it has been well documented that integrated pest management programs do not often consider the decision-making process of farmers and their specific reasons for choosing and employing pest management strategies (Hashemi and Damalas 2011). As such, practical recommendations for pest management often do not address farmer's needs or fail to be adopted (Hashemi and Damalas 2011). To improve the usefulness of IPM interventions, one must first gain a better understanding of farming systems, and farmers knowledge or perceptions of pest management strategies. It is also necessary to gain an appreciation of the current pest control methods used by farmers. This can help to direct research and develop extension programs to design more effective IPM implementation agendas and thereby improve the adoption of pest management strategies, whilst also ensuring that such strategies are well suited to the needs of farmers. By surveying Louisiana rice farmers, we can learn more about their preferred pest management practices and decision-making processes, as well as discover which regions need to be targeted for specific IRM outreach.

### 2.7. Goals

Using a combination of spatial ecology, precision agriculture, improved IPM practices and a better understanding of farmers pest management priorities, this study aimed to develop a strategy for enhancing the use of insecticidal seed treatments in Louisiana rice in an integrated and sustainable manner.

This was achieved in the following chapters by gaining a thorough understanding of the spatial distribution of *L. oryophilus* and Lepidopteran stem borers in Louisiana rice fields, so that precision targeting can be used to deploy insecticides in areas that require pest control. Remote sensing technologies were also evaluated as a tool to rapidly detect pest populations for management purposes. The feasibility of integrating current pest management

practices with alternative insecticides and control strategies was assessed under field conditions to further prevent the development of insecticide resistance in pest populations. Finally, farmers, extension personnel and consultants were surveyed to determine rice pest management practices throughout Louisiana. This helped to identify areas that are at risk of insecticide resistance and aid researchers in developing recommendations that can be tailored to suit the farming practices of rice farmers in different areas of the state.

## **CHAPTER 3. SPATIAL DISTRIBUTION OF *LISSORHOPTRUS ORYZOPHILUS* (COLEOPTERA: CURCULIONIDAE) IN LOUISIANA RICE.**

### **3.1. Introduction**

*Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), commonly referred to as the rice water weevil, is the most destructive insect pest of rice in the United States Mid-south, where it is a native marshland species (Way 2003). Rice water weevil is also considered an invasive pest of rice in California as well as in parts of Europe and Asia where it has been introduced (Aghaee and Godfrey 2014).

In spring, both mated and un-mated adults emerge from diapause in overwintering habitats (tree lines and bunch grasses on infield levees and field borders) and start feeding on the leaves of rice and other non-crop host plants (Way 2003, Zou et al. 2004c). Adults feed on rice leaves and stems, leaving elongate feeding-scars on the plants (Saito et al. 2005). At the onset of permanent flooding, adult females, begin to oviposit on rice leaf sheaths. Once the eggs hatch, *L. oryzophilus* larvae move to the base of the rice plant, where they feed on rice roots (Heinrichs 1994). While all stages of *L. oryzophilus* infest rice, only the larvae are regarded as economically damaging. Pruning of rice roots by *L. oryzophilus* larvae leads to reductions in tillering, vegetative growth, panicle density and grain weight. Severe infestations of *L. oryzophilus* can cause over 25 % yield loss (Zou et al. 2004a,b).

Currently, insecticidal seed treatments are used throughout the rice industry in the U.S. Mid-south as a pre-emptive means of controlling *L. oryzophilus* and Lepidopteran pests (Sidhu et al. 2014, Wilson et al. 2019). Four insecticidal seed treatment chemistries are presently being recommended for use against *L. oryzophilus*, but regional dependence on a single product is high (Wilson et al. 2021). While seed treatments have been shown to reduce the damage inflicted by *L. oryzophilus* and other pests, they are typically applied as a

preventative measure only (Douglas and Tooker 2015, Villegas et al. 2019). Therefore, their application does not consider pest densities, economic thresholds, non-target effects, selection pressure, and cost-benefit ratios, which are vital components of successful integrated pest management (IPM) programs (Douglas and Tooker 2015). However, state-wide surveys have shown that their use is justified in the majority of commercial fields in Louisiana because of the considerable yield loss potential from *L. oryzaephilus* (Hummel et al. 2014). Current usage rates exceed 80% throughout most of the Mid-South (Bateman et al. 2020). Unfortunately, the widespread reliance on insecticidal seed treatments has raised concerns about insecticide resistance development. Furthermore, the application of seed treatments over a vast acreage can be a costly endeavor for farmers (Lanka and Stout 2015). With decreasing rice prices, and increasing input costs (Childs 2016, Saichuk 2016), it is important to research strategies for improving the cost-effectiveness of rice production in this region. One way to improve efficiency of seed treatment deployment could be to target areas where *L. oryzaephilus* infestations are spatially clustered.

Improved understanding of the spatial distribution of pest infestations has enhanced efficiency of insecticide use in other agricultural systems (Park et al. 2007), but this approach has scarcely been investigated in rice. Several studies have reported the existence of edge-biased distributions of insects in open-field agricultural systems (Nguyen and Nansen 2018), including canola (Severtson et al. 2015), cotton (Toews and Shurley 2009, Reeves et al. 2010), wheat (Nansen 2005), potatoes (Parsa et al. 2012), soybean and corn (Venugopal et al. 2014). In these examples, pest population densities were negatively correlated with distance from field edges. Targeted or site-specific applications of insecticides along field edges have also been successfully employed to manage a wide range of pests in soybeans (Rice et al. 2014), cotton (Campanella 2000), wheat (Dammer and Adamek 2012), potatoes (Carroll et al. 2009), blueberries (Collins and Drummond 2004), peaches (Blaauw et al. 2015), blackberries

(Iglesias and Liburd 2017), and grapes (Sciarretta et al. 2014). The potential to utilize this approach for management of rice insects requires improved understanding of spatial distribution of pest populations.

Espino (2012) reported that *L. oryzaophilus* populations in California rice had an edge-biased population distribution and suggested that border insecticide applications could be effective. However, the population of *L. oryzaophilus* in California is solely parthenogenetic and univoltine, unlike the weevils found in the Mid-South (Heinrichs et al. 2017). Rice production in California also relies almost exclusively on water-seeded rice, a practice known to greatly influence *L. oryzaophilus* infestations (Everett and Trahan 1967, Stout et al. 2002). These differences in pest biology and production practices between California and the Mid-South may alter pest distribution. The objective of this study is to determine spatial distribution patterns of *L. oryzaophilus* in commercial fields of drill-seeded rice in Louisiana. Potential applications to integrated pest management are discussed.

## **3.2. Methods**

### *3.2.1. Site selection*

Data relating to the spatial distribution of *L. oryzaophilus* was collected over two years (2017 and 2018) in the southwestern Louisiana parishes of Vermillion Parish, Jefferson Davis Parish and Acadia Parish. These represent the areas of Louisiana with the highest acreage of commercial rice production (Harrell 2017). Farmers in the region typically grow drill-seeded rice, where rice seeds are planted in moist soil using a drill, with a drill spacing of approximately 17–26 cm (Saichuk 2016). All sites sampled in this study were located in commercial rice fields which did not receive insecticidal seed treatments. This ensured that *L. oryzaophilus* populations were indicative of the natural infestation levels experienced by rice farmers in the region. The fields were selected with the help of LSU AgCenter extension

personnel and the rice on all farms was planted in mid-March to early-April as per the recommendations set forth in the LSU AgCenter rice production guideline (Saichuk 2016).

In the 2017 season, eight fields were surveyed: four in Vermillion Parish and four in Jefferson Davis Parish. An additional five sites were surveyed in Acadia Parish during the 2018 field season. All field sites were surveyed prior to the commencement of the study and were mapped using Google Earth and GSAK (Garmin GPS software). The surveyed fields ranged in size from 18.2 ha to 65.7 ha (45–162 acres), with the overall average size being 38 ha (approximately 95 acres). Field boundaries were headlands, tree lines, and farm roads and sampling occurred across in-field levees.

### *3.2.2. Experimental design*

In 2017, the center of each field site was located using Google Earth, and four diagonal transects were used to connect the edges/margin of the field to the central point. Four equidistant sampling points were identified along each transect, starting at the edge and ending at the midpoint (Fig. 3.1.). Sample points marked with an A were near the edge of the field, and sampling points marked D were near the center of the field. Therefore, samples were labelled A, B, C, and D, with each letter corresponding to the distance of the sample from the edge of the field. The average distance between sample points was 128.5 m and the four sampling points located at D were spaced 1 m apart in the direction of each diagonal transect. In 2017 each field had 16 sampling points, with four replications at each distance away from the edge. Four sample points were discarded from an individual field ( $n = 124$  total samples collected in 2017).

In 2018, a different experimental design was chosen to increase sample number and improve sample distribution (Fig. 3.2.). Instead of transects, the sites selected in 2018 were sampled using perimeters. In the map, sample points marked A (near the edge of the field) were located along an outer perimeter that incorporated 90 % of the field. The perimeter used

to sample points marked B incorporated 60 % of the field. The inner perimeters used to sample points marked C and D (near the center of the field) incorporated 30 % and 10 % of the field, respectively. The average distance between perimeters was 96.5 m. There were 12 equidistant sample points for each of the four perimeters per field site, meaning 48 samples were collected at each site ( $n = 240$  total samples collected in 2018).

In both years, each sample point was marked as a way point in Google Earth and uploaded onto a hand-held Garmin GPS. The GPS was used in the field to locate the correct sampling areas for each site, so that every *L. oryzophilus* sample had a geo-location that could be used to measure the distance from the field edges and non-crop or overwintering habitat (infield levees, headlands, and forest margins).

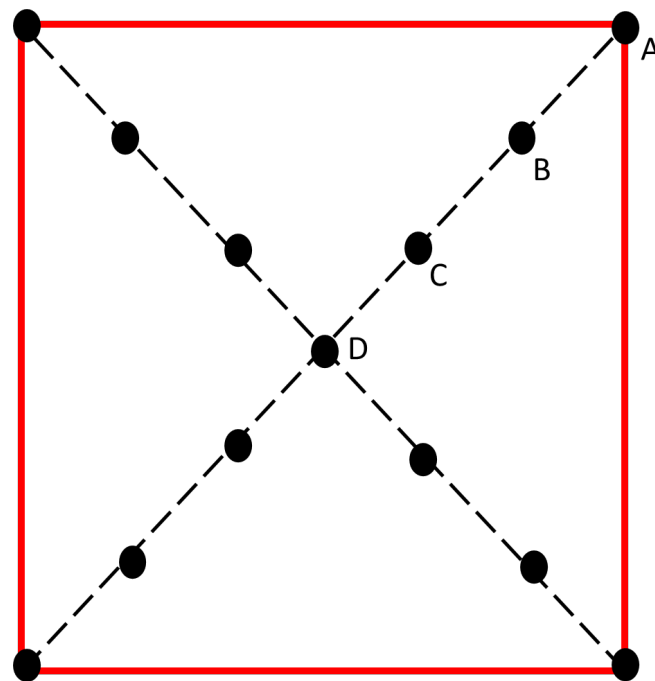


Figure 3.1. Map showing an example of the sampling design for farms sampled in 2017. Red lines depict the field boundary, black broken lines show the transects, black markers depict the sampling points, and the text labels indicate the name of each sampling point in relation to distance from the edge of the field.

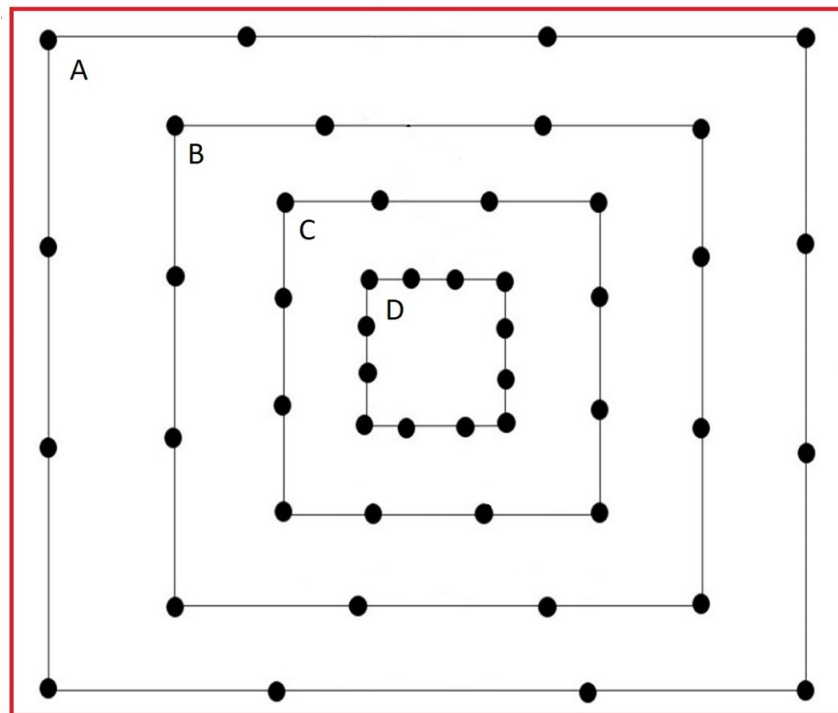


Figure 3.2. Map showing an example of the sampling design for farms sampled in 2018. Red lines depict the field boundary, black lines show the transects, black markers depict the sampling points, and the text labels indicate the name of each sampling point in relation to distance from the edge of the field.

### 3.2.3. Sample collection

Each site was sampled in late May through early June approximately four weeks after the permanent flood was established, corresponding to peak infestation periods (Zou et al. 2004a). A soil core (with a diameter and depth of 10 cm and 8 cm, respectively) was taken at each sampling point to determine the density of *L. oryzaophilus*. Soil cores samples were collected and washed according to methods used for over 50 years to accurately quantify populations of *L. oryzaophilus* larvae (Bowling 1968, Morgan et al. 1989, Hummel et al. 2014). At each core site, a PVC quadrat (measuring 1x1 m) was also used to assess rice stand counts. This was done to ensure that the density of rice was not a confounding factor contributing to fluctuations in *L. oryzaophilus* population distribution.



#### 3.2.4. Data analysis

Rice stand and *L. oryzaophilus* larval density data were analyzed using an ANOVA (PROC MIXED, SAS Institute 2011) with sample points on the transects/perimeters included as fixed effects and field as the random effect. Kenward-Rogers was used for all calculation of error degrees of freedom and means were separated using Tukey's HSD ( $\alpha=0.05$ ). Weevil larvae data from each field were spatially analyzed using GIS software (ArcGIS, Environmental Systems Research Institute 2011). Tests for degree of spatial autocorrelation among points were conducted using the Global Moran's I statistic (Moran 1950, Cocu et al. 2005, Wilson et al. 2017). Hot-Spot analysis (ESRI 2011) was used to calculate the Gi statistic, which tests for statistically significant clusters of high-densities (hot-spots) and low-densities (cold-spots) (Getis and Ord 1992). Distribution maps were generated for *L. oryzaophilus* larval density using spatial interpolation with inverse distance weighting (IDW) (Weisz et al. 1995). The IDW spatial interpolation uses the known values at sampled sites to predict the values of unknown sites based on the assumption that spatial points which are near to one another are more similar than those further apart (Wang 2015). Thus, nearby points are given high weights in comparison to points located at a greater distance. Weights are inversely proportional to a power of distance. The power selected for this IDW analyses was 2.00, which is commonly used by applied researchers (Pimentel et al. 2017). A spatial resolution of 1 m<sup>2</sup> pixels was also used.

The data was further analyzed using a multiple linear regression (MLR) model to assess the density of *L. oryzaophilus* populations in relation to three independent variables across both 2017 and 2018 samples. First the data was transformed due to the residuals being non-normal. The log of *L. oryzaophilus* larval density was used. PROC REG was used to construct an MLR model in SAS, and the ANOVA table, t-values, Type I and Type II sum of square errors and multicollinearity diagnostics were assessed. A Full Model was created

using the following three variables; Distance of the sampling point to the edge of the field, distance from overwintering habitat, and rice stand count at each point. Stepwise variable selection was used to determine the best variables for the model. A reduced model was then run excluding stand counts, which were not significantly related to *L. oryzaophilus* density.

### 3.3. Results

Rice stand counts did not differ significantly between samples points in either 2017 or 2018. Rice stand count was not significantly correlated with *L. oryzaophilus* and was excluded from the MLR models using the stepwise method (with alpha set at 0.1). Thus, stand count results are not presented herein.

The ANOVA results show differences in *L. oryzaophilus* larval density among transect points and perimeters located in different areas of fields sampled in both 2017 ( $F = 12.02$ ;  $DF = 3, 120$ ;  $P < 0.001$ ) (Fig. 3.3.) and 2018 ( $F = 9.25$ ;  $DF = 3, 236$ ;  $p\text{-value} < 0.001$ ) (Fig. 3.4.). In 2017 larval densities at point A (the edge of the field) were 1.8, 2.8, and 3.3-fold higher than the densities in the B, C, or D (center) regions of field sites, respectively. In 2018, *L. oryzaophilus* density did not differ between A and B regions. Points located on perimeter A were 1.5 and 2.0-fold greater than C and D regions, respectively.

The Moran's I correlation coefficient was above 0.9 ( $p\text{-values} < 0.001$ ) for all the sample sites indicating that the *L. oryzaophilus* distribution is clustered and non-random. Results of the IDW spatial interpolation, depicting the estimated distribution of *L. oryzaophilus* within the sampled sites, show that the larvae are generally concentrated along the margins of the commercial rice fields sampled in 2017 (Fig. 3.5.). Similar edge-biased larval distributions were observed in 2018 (Fig. 3.6.). Although, in two of the fields, densities were greater at only one edge of the field. In both years, larval density was relatively low in the central region of each field (between 0–10 *L. oryzaophilus* larvae). Thus, according to the

spatial interpolation maps and the Moran's I value, *L. oryzaophilus* distributions exhibit a clustering pattern within the commercial rice fields sampled in this study.

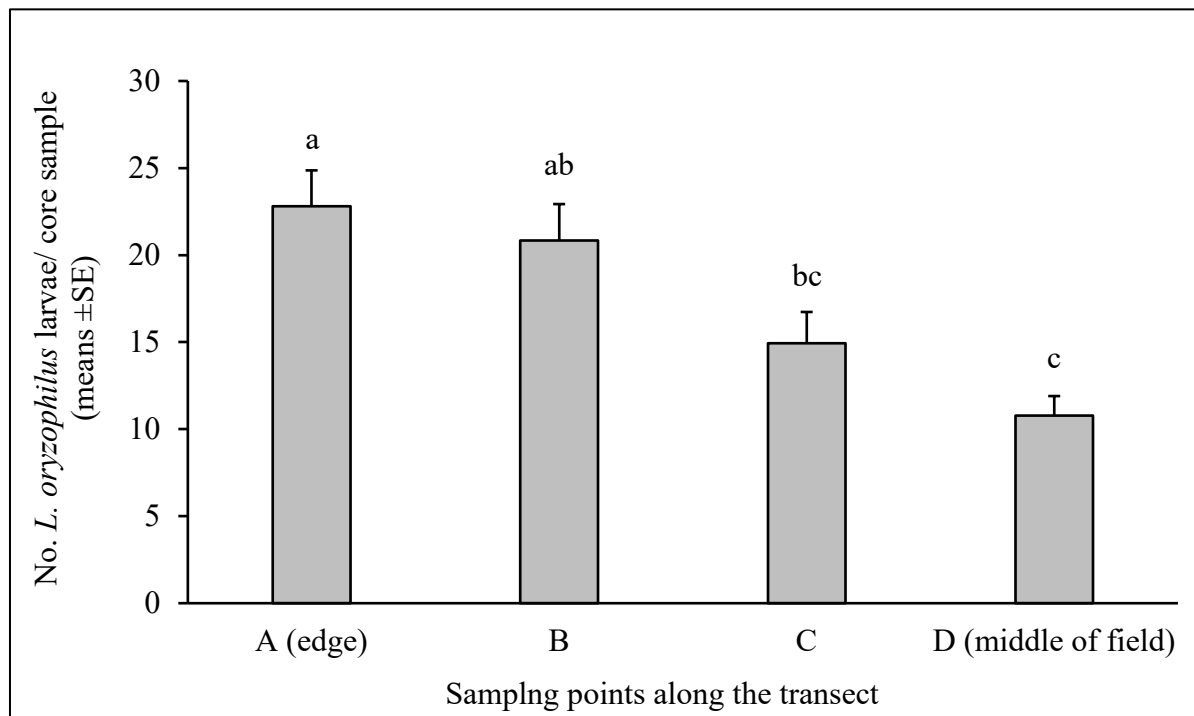


Figure 3.3. The distribution of *L. oryzaophilus* larvae along various sampling points of a transect progressing from the edge (A) to the center (D) of commercial rice fields in 2017. Letters indicate significance at  $p < 0.05$ .

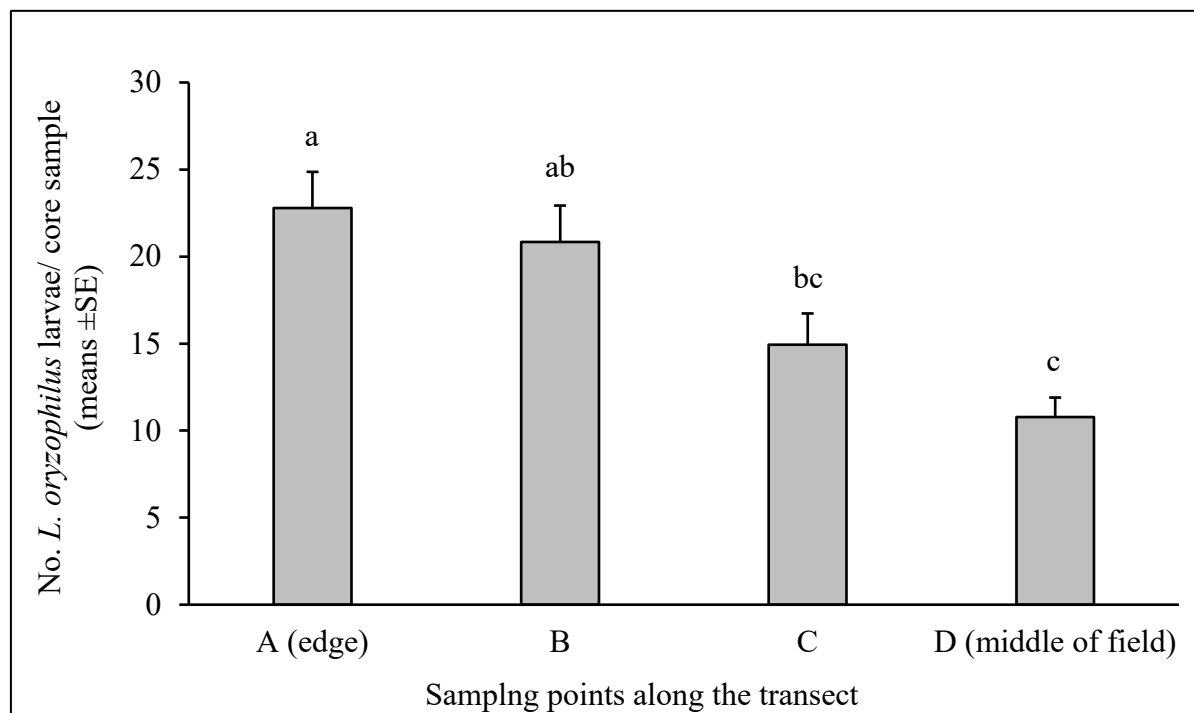


Figure 3.4. The distribution of *L. oryzaophilus* larvae along various sampling points of perimeters progressing from the edge (A) to the center (D) of commercial rice fields in 2018. Letters indicate significance at  $p < 0.05$ .

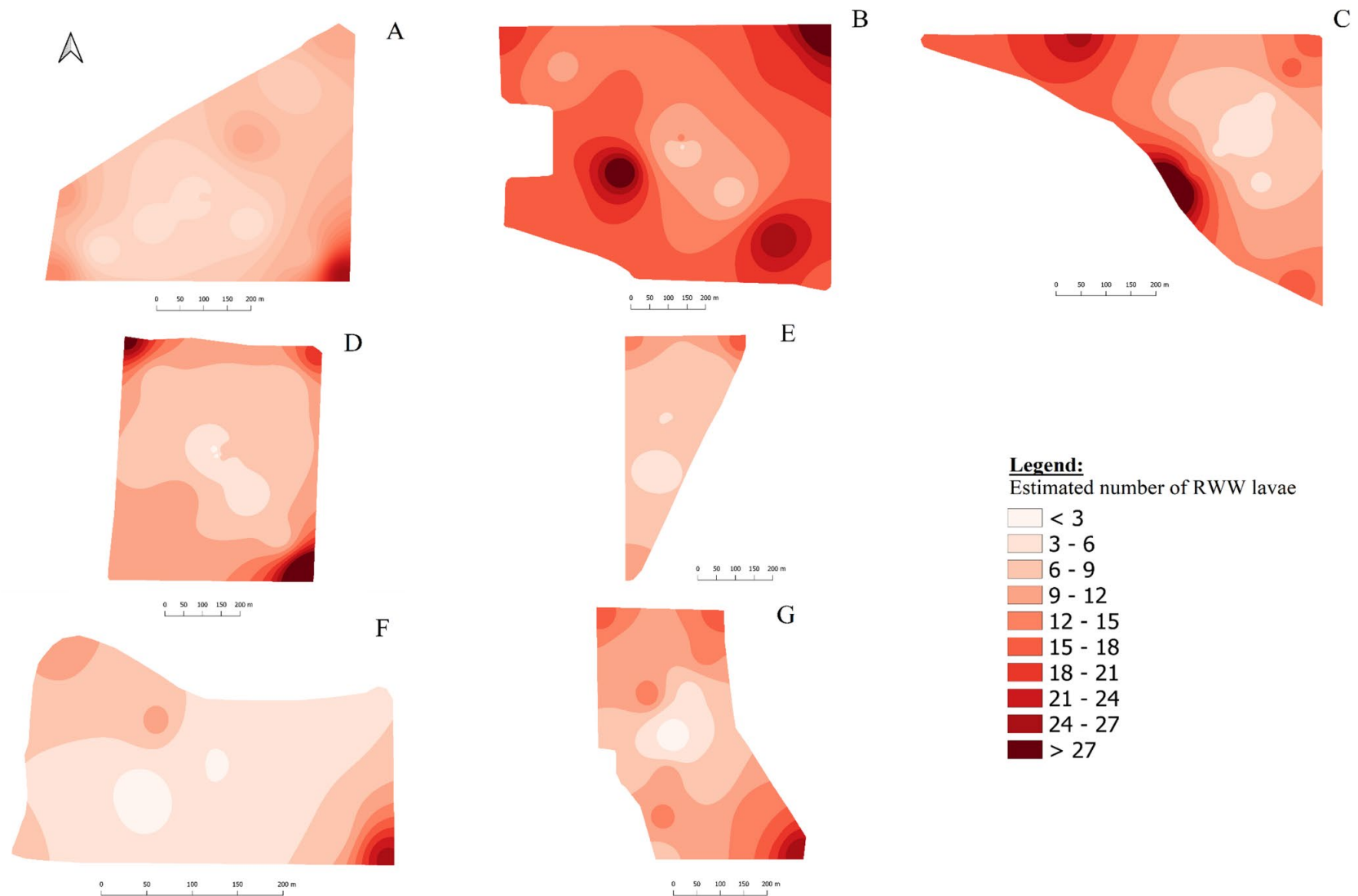


Figure 3.5. ARC GIS spatial interpolation showing the spatial distribution of *L. oryzae* larvae in untreated commercial rice fields during the 2017 growing season in Louisiana.

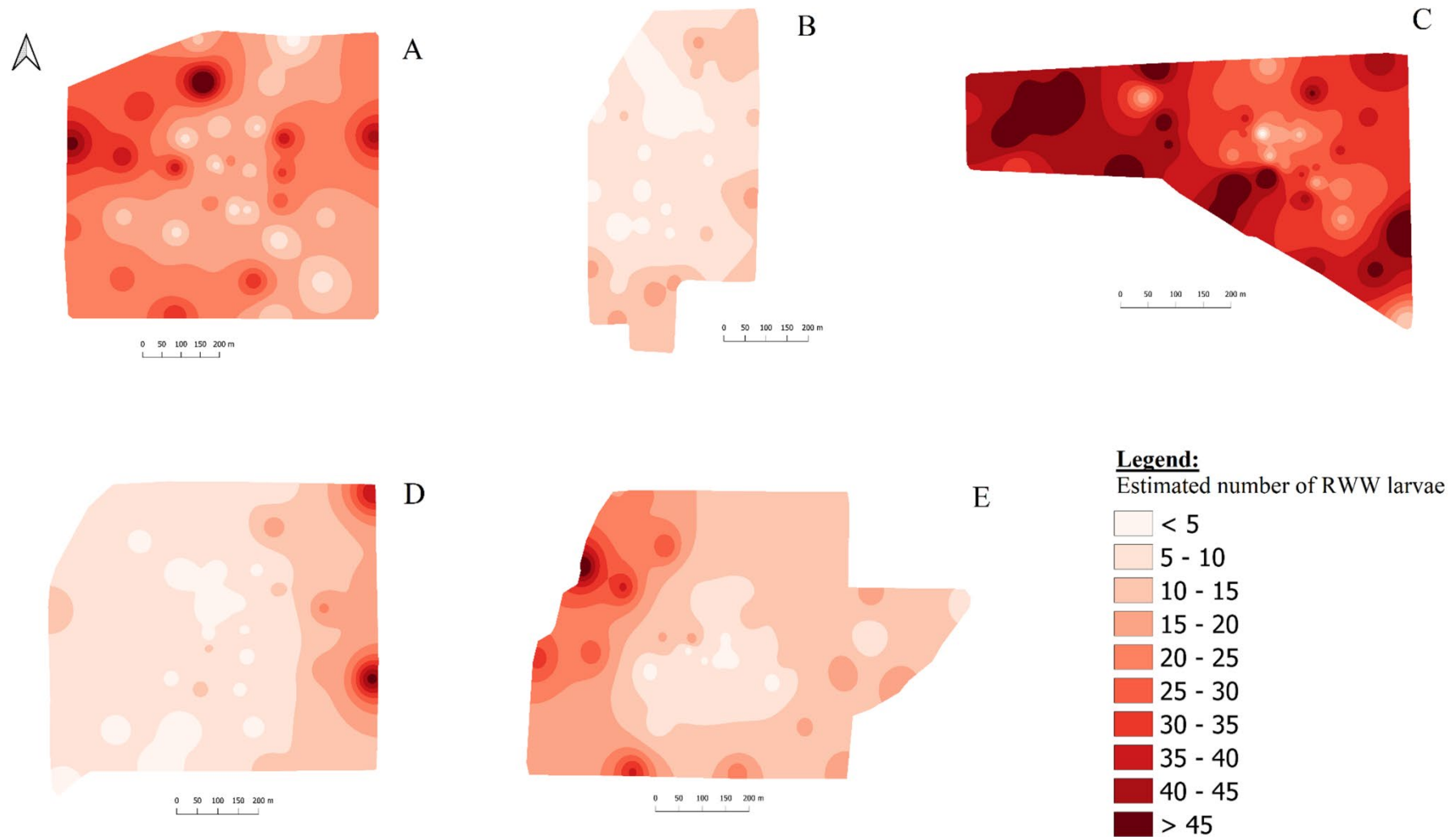


Figure 3.6. ARC GIS spatial interpolation showing the spatial distribution of *L. oryzophilus* larvae in untreated commercial rice fields during the 2018 growing season in Louisiana.

The 2017 field sites were excluded from hotspot analyses because the low number of sample points per site did not produce reliable results. The hotspot analysis of 2018 data revealed the Getis-Ord Gi-statistic for each point in the dataset and produced z-scores and p-values, which identify sites with significantly high (positive z-score; “hot spots”) or low (negative z-score; “cold spots”) *L. oryzophilus* density. A total of 24 significant ( $P < 0.05$ ) hot spots and 88 cold spots were detected in all fields in 2018 (Table 3.1.). High density hot spots were confined to points located in perimeter A and B. Most low-density cold spots were found on perimeters C and D (59.1% and 31.8% respectively), with only few cold spots found at A and B (1.14% and 7.95% respectively). Non-significant values were not depicted.

Table 3.1. Z-scores for sample points identified as significant Hotspots (positive score) or Cold-spots (negative score), according to the Getis-Ord spatial clustering analysis.

Field (2018)	Transect	Sample Point A	Sample Point B	Sample Point C	Sample Point D
<b>A</b>	1	-	-	-	-
	2	-	-	-	-
	3	2.23	2.86	-1.91	-1.85
	4	3.03	2.78	-2.32	-2.30
	5	-	-	-2.13	-2.92
	6	-	-	-3.12	-3.00
	7	-	-2.52	-2.24	-2.90
	8	-1.87	-	-	-2.44
	9	-	-	-	-2.90
	10	2.42	-	-2.58	-2.80
	11	-	-1.96	-	-2.25
	12	-	-	-	-
<b>B</b>	1	-	-1.94	-	-1.74
	2	-	-	-	-2.08
	3	-	-	-	-
	4	-	-	-	-1.79
	5	-	-	-	-2.08
	6	2.61	2.41	-	-1.97
	7	-	-	-	-1.94
	8	1.90	-	-	-
	9	-	-	-	-
	10	-	-	-	-
	11	-	2.23	-	-
	12	-	-	-	-1.74

(Table cont'd.)

<b>Field (2018)</b>	<b>Transect</b>	<b>Sample Point A</b>	<b>Sample Point B</b>	<b>Sample Point C</b>	<b>Sample Point D</b>
<b>C</b>	1	-	-	-	-2.23
	2	-	-	-	-2.27
	3	-	-	-	-2.17
	4	-	-	-	-1.98
	5	-	2.10	-	-1.99
	6	1.71	1.98	-	-1.65
	7	-	-	-	-1.77
	8	-	-	-3.38	-2.58
	9	-	-1.86	-3.14	-2.36
	10	-	-2.51	-	-2.56
	11	-	-	-2.28	-1.80
	12	-	-	-2.38	-3.12
<b>Field (2018)</b>	<b>Transect</b>	<b>Sample Point A</b>	<b>Sample Point B</b>	<b>Sample Point C</b>	<b>Sample Point D</b>
<b>D</b>	1	-	2.55	-	-1.93
	2	-	-	-1.78	-2.10
	3	-	-	-1.84	-2.10
	4	-	-	-	-2.10
	5	-	-	-1.97	-2.49
	6	-	-	-2.18	-2.59
	7	-	-	-2.26	-2.31
	8	-	-	-1.80	-2.37
	9	-	-	-	-2.37
	10	2.78	2.49	-	-2.13
	11	1.66	-	-	-1.80
	12	3.64	2.36	-	-2.19
<b>E</b>	1	-	-	-3.26	-4.17
	2	-	-3.09	-4.07	-4.34
	3	-	-	-4.31	-4.31
	4	-	1.73	-3.35	-4.31
	5	2.15	3.01	-2.44	-3.60
	6	4.44	2.11	-2.31	-3.25
	7	4.18	1.92	-2.57	-2.99
	8	-	-	-2.57	-2.99
	9	-	-	-3.15	-3.71
	10	-	-2.49	-3.60	-4.15
	11	-	-	-3.71	-4.20
	12	-	-	-3.22	-4.17

A significant relationship ( $F = 67.89$ ;  $df = 2, 361$ ;  $P < 0.001$ ;  $R^2 = 0.27$ ) was found between the log of *L. oryophilus* density and distance in meters from the edge of the field, and from alternative habitat/feeding sites (tree margins, levees, and other in-field features) (Table 3.2.). The relationship is explained by the following equation:

$$\log(\text{RWW}) = 1.32047 - 0.00078D_E - 0.00853D_A$$

Where:

RWW = Rice water weevil larvae per core

$D_E$  = distance (m) from field edge

$D_A$  = distance (m) from alternative habitat

According to the estimated equation, *L. oryophilus* densities decrease when moving away from field edges and sites with alternative habitat (Table 3.3.). The reduced models were assessed to assure that the appropriate MLR assumptions were met. The residuals of the model were homogenous and the Shapiro-Wilks test for normality of the residuals yielded a non-significant p-value ( $p = 0.7843$ ). Therefore, the normality assumption was met. VIF values and the condition index for the model also indicated that multi-collinearity was not a problem. Thus, we can be confident in the predictions made using this model. According to the parameter estimates, the predicted geometric mean of rice water weevil larvae found per core is 20.91. For every unit of change in distance from field edges (m), weevil larvae per core is predicted to decrease by 0.18 % (1 larva/core for every 26.6 m). Similarly, one unit of change in distance from alternative habitat (m) is expected to decrease weevil populations by 1.98 % (1 larva/core for every 2.42 m).



Table 3.2. ANOVA source table for the reduced multiple linear regression model for number of *L. oryzaophilus* vs distance of samples from the edge of rice fields and from overwintering habitats

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
<b>Model</b>	2	16.3499	8.1749	67.89	<.0001
<b>Error</b>	361	43.4686	0.1204	-	-
<b>Corrected Total</b>	363	59.8185	-	-	-

Table 3.3. Parameter estimates for the reduced multiple linear regression model for number of *L. oryzaophilus* vs distance of samples from the edge of rice fields and from alternative habitats/feeding sites.

Variable	DF	Parameter Estimate	Stand. Error	t Value	Pr >  t	Type I SS	Type II SS	Sq. Semi-partial Correlation Type I	Sq. Semi-partial Correlation Type II	Variance Inflation
<b>Intercept</b>	1	1.32047	0.02996	44.07	<.0001	371.68	192.32	.	.	0
<b>Dist. from edge</b>	1	-0.00078	0.00019	-4.17	<.0001	3.08	1.99	0.097	0.033	1.074
<b>Dist. from overwintering site</b>	1	-0.00853	0.00097	-8.78	<.0001	2.95	10.56	0.176	0.195	1.074

### 3.4. Discussion

This study is the first to investigate spatial distribution of *L. oryzaophilus* in Louisiana rice. It is the most robust examination of *L. oryzaophilus* density with a total of 364 sampling sites in 13 commercial fields over two years, and the first to utilize comprehensive spatial analysis. Our results are in agreement with research in California that showed infestations of *L. oryzaophilus* larvae were concentrated along field edges (Espino 2012) suggesting the distribution is consistent across varied environments. Populations in California are solely parthenogenetic and water seeding is the predominant planting method there. Similarly, higher populations were typically found near edges in other invaded regions including Italy (Lupi et al. 2010) and China (Chen et al. 2005). Other studies in Southern states, such as Texas and Florida, demonstrated that weevil populations and injury are distributed more uniformly within fields (Way and Wallace 1984, Cherry et al. 2013). These studies focused on adult feeding scars and had low numbers of weevils in comparison to the sites sampled here. Our results have confirmed that the distribution of *L. oryzaophilus* in untreated Louisiana rice fields is neither random, nor uniform. Populations of the pest decrease towards the center of rice fields and high densities of *L. oryzaophilus* tend to cluster at or near field margins. Our findings together with the other studies (Chen et al. 2005, Lupi et al. 2010, Espino 2012) suggest rice water weevil distribution in Texas and Florida as well as in the Mississippi river delta rice production region warrants further examination.

The spatial interpolation maps in 2018 show that this edge-bias is not always even across all field margins, and that high densities of *L. oryzaophilus* can occur on only one or two sides of the field. This is confirmed through the hotspot analysis, which shows statistically significant hotspot clusters along just a few field margins. The distribution patterns observed here provide evidence that field composition in relation to field margins may be driving the spatial distributions of this pest in addition to distance from field edges.

This is supported by results from our MLR which included the distance of sampling points to alternative habitats (infield levees and tree lines). The regression confirmed that *L. oryzophilus* densities decline with increasing distance from both field edges and nearby overwintering/non-crop habitats. Thus, *L. oryzophilus* spatial distribution is simultaneously influenced by the availability of overwintering habitat, alternative non-crop host plants, short-distance dispersal patterns, and ovipositional preference in females (Hortal et al. 2010). Research on the spread of *L. oryzophilus* in Italy (Lupi et al. 2010) and China (Chen et al. 2005) discussed the distribution of *L. oryzophilus* in relation to field structure and field adjacent habitat. The composition and spatial arrangement of landscape elements were key determinants of *L. oryzophilus* population dynamics and spread (Wang et al. 2011). These studies also reported the presence of levees in addition to field margins as important influences on larval densities. Adult *L. oryzophilus* were found to prefer treelines, meadows and river bunds adjacent to paddies. Weedy habitats near or within rice fields allowed populations to persist when rice was not available and facilitated colonization of fields, especially in early planted rice (Chen et al. 2005). In China, treatment of non-rice habitat on field edges has been proposed as an effective tool for curbing infestations in rice fields. This is likely also a key factor in Louisiana where *L. oryzophilus* adults overwinter in both woody habitats in field margins and in bunch grasses within fields or on levees (Shang et al. 2004).

Alternative host plants such as barnyard grass, johnsongrass (*Sorghum halepense* L.), yellow nutsedge (*Cyperus esculentus* L.) and red rice typically grow on levees, or headlands, within and around rice fields in Louisiana (Tindall and Stout 2003, Showler et al. 2011). These plants are likely influencing *L. oryzophilus* populations, with vegetated levees being associated with high *L. oryzophilus* numbers in California rice fields (Palrang et al. 1994). As such, other biological and ecological aspects need to be studied and considered when predicting infestations of this pest in Louisiana rice, including preferred alternative host

plants, colonization in relation to overwintering sites, suitability and location of habitat patches, and adult flight/dispersal, densities and in-field diffusion patterns. Our results suggest movement of *L. oryzophilus* adults away from overwintering sites may be limited, but the degree of dispersal during colonization of rice fields needs further examination.

*Lissorhoptrus oryzophilus* adults are thought to fly only short distances (tens of meters), arresting when they encounter water (Palrang and Gigarik 1993). Further dispersal occurs from plant to plant, or via swimming (Aghaee and Godfrey 2014). These behaviors could explain the concentration of larval distribution near overwintering sites. Better understanding of colonization of rice fields could be exploited for novel management tactics, such as the manipulation of visual cues or deployment of physical barriers.

Edge-biased distribution could allow for targeted chemical controls. Insecticidal seed treatments could potentially be applied selectively within rice fields. By precisely targeting areas, which are at a higher risk of infestation, we can improve the application efficiency of insecticidal seed treatments (Douglas and Tooker 2015). Selective applications of insecticides are commonly practiced in California rice to manage weevil infestations (Espino et al. 2015). However, the rice industry in California relies on foliar insecticides to control *L. oryzophilus* instead of insecticidal seed treatments (California Rice Production Workshop 2018). The utility of this approach in Louisiana may be limited, as however, densities at field centers in most fields exceeded the economic threshold of three larvae per core (Hummel et al. 2014). Potential to deploy targeted insecticidal seed treatments according to pest density and population dynamics on large-scale commercial rice fields in the Mid-South should be further investigated. This would not only reduce selective pressure on *L. oryzophilus* populations, and thus mitigate the evolution of resistance to seed treatments, but it can also improve cost-benefit ratios for farmers applying these insecticides. At approximately \$50 per hectare, the chlorantraniliprole seed treatment is a costly management tool. Even small reductions in the

amount of this insecticide being used, through border applications, could translate to a meaningful financial gain for farmers. While further research is needed in this area, our results provide farmers with a rough guide to predict which areas may be most susceptible to *L. oryzaophilus*. These areas can be monitored to assess the on-going efficacy of seed treatments.

Collectively, our results provide further support for edge-biased distribution of *L. oryzaophilus* larval infestations and shed new light on the role of overwintering habitats on larval density. Further examination of field colonization and potential for targeted chemical controls is needed for improvement of *L. oryzaophilus* IPM. Additionally, farmers need to be consulted to determine whether targeted applications are well suited to their management practices and whether it is in fact economically viable.

## CHAPTER 4. SPATIAL DISTRIBUTION OF LEPIDOPTERAN STEM BORERS IN LOUISIANA RICE FIELDS

### 4.1. Introduction

Lepidopteran stem borers (Crambidae) are considered among the most important pests of rice in Louisiana (Sidhu et al. 2014, Wilson et al. 2015, 2021a, b). The stem borer complex attacking rice in this region includes rice stalk borer (*Chilo plejadellus*), sugarcane borer (*Diatraea saccharalis*), and Mexican rice borer (*Eoreuma loftini*) (Saichuk 2016). In rice, Lepidopteran stem borers are mid-season pests, inflicting injury on both the vegetative and early reproductive stages of the plant. Larvae of these pest species bore into the culms of rice plants causing “dead hearts” (dead leaves and tillers) and “whiteheads” (panicles with unfilled grains) (Way 2003, Beuzelin et al. 2016). Although previously considered only sporadic pests, the expansion and proliferation of *E. loftini* in Texas and southwest Louisiana has led to stem borers being a consistent and economic pest of rice (Wilson et al. 2017, 2021b). Annual economic losses in excess of \$40 million are expected to result from expansion of *E. loftini* across the Louisiana rice industry, without effective controls (Reay-Jones et al. 2008). Therefore, development of successful management strategies for this pest is a rice research priority.

Cultural control methods, such as field hygiene practices, the use of resistant cultivars, and the manipulation of soil fertility have all shown potential as tools for reducing the injury inflicted by lepidopteran pests in rice (Way 1990, Way et al. 2006, Sidhu et al. 2013, Villegas et al. 2017). Foliar applications of pyrethroids, insect growth regulators, and diamides have also been used to manage stem borer infestations in the past (Beuzelin et al. 2016, Villegas et al. 2018, Villegas et al. 2021a). However, insecticidal seed treatments are the preferred method of stem borer management for Louisiana rice growers.

Insecticidal seed treatments are used throughout Louisiana as a preemptive means of controlling rice water weevil, *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae), which is considered the most damaging pest of rice in the United States (Lorenz and Hardke 2018, Wilson et al. 2019). Chlorantraniliprole, cyantraniliprole, thiamethoxam, and clothianidin are insecticidal seed treatments that are currently being recommended for use against *L. oryzophilus* (Wilson et al. 2019, 2021a). The diamide insecticide, chlorantraniliprole, is also an effective management tool against lepidopteran pests including foliar feeders, such as *Spodoptera frugiperda* (Lepidoptera: Noctuidae), and the stem borers, *D. saccharalis* and *E. loftini* (Sidhu et al. 2014, Wilson et al. 2015, 2021b). The efficacy of chlorantraniliprole against multiple damaging pests means that it is now being used on >80 % of rice acres in Louisiana and Texas (Bateman et al. 2020). Although chlorantraniliprole is highly effective, its widespread use as a preventative pest management tool has potential insecticide resistance implications.

Understanding the distribution of pest populations in agricultural ecosystems can help mitigate resistance to widely used chemistries by improving scouting and treatment regimens (Nguyen and Nansen 2008). Recent research has focussed on characterizing the spatial distribution of *L. oryzophilus* in Louisiana rice to determine whether insecticidal seed treatments can be used selectively to target the pest (Mulcahy et al. 2021). *Lissorhoptrus oryzophilus* populations have an edge-biased distribution and infestations levels of the pest are negatively correlated with increasing distance from overwintering sites and levees that contain alternative host plants or bunch grasses (Palrang et al. 1994, Espino 2012, Mulcahy et al. 2021). This information can be used to improve the deployment of seed treatments, which in turn can lead to cost saving strategies, increased efficiency, and enhanced scouting techniques (Douglas and Tooker 2015).

Although *L. oryzaephilus* is considered the most important pest of rice, it is important to develop integrated pest management (IPM) strategies that consider multiple pests within the agroecosystem (USDA 2013). Since chlorantraniliprole is used throughout the Mid-South to control both *L. oryzaephilus* and lepidopteran stem borers, an IPM strategy involving insecticidal seed treatments should not be based solely on the distribution of a single species. Thus, the objective of this study is to determine spatial distribution patterns of stem borers in commercial fields of drill-seeded rice in Louisiana, serving as a companion study to Chapter 3. Potential applications to IPM are discussed.

## **4.2. Methods**

### *4.2.1. Site selection*

Stem borer spatial distribution data were collected in 2017 and 2018 in the southwestern Louisiana parishes of Vermillion, Jefferson Davis, and Acadia Parishes. These areas have the highest acreage of commercial rice production in Louisiana and are known to harbor high populations of stem borers (Harrell 2017, Wilson et al. 2017). All sites sampled in this study were located in commercial rice fields which did not receive insecticidal seed treatments. Rice was planted in mid-March to early-April in accordance with LSU AgCenter rice recommendations (Saichuk 2016).

In the 2017 season, eight fields were surveyed: four in Vermillion Parish and four in Jefferson Davis Parish. An additional five sites were surveyed in Acadia Parish during the 2018 field season. Field sites were mapped using Google Earth and GSAK (Garmin GPS software). The fields ranged in size from 18.2 ha to 65.7 ha (45–162 acres), with the overall average size being 38 ha (approximately 95 acres). Field boundaries were headlands, farm roads, or tree lines directly adjacent to fields. Fields were bisected by infield levees.



#### *4.2.2. Experimental design*

In 2017, the center of each field site was located using Google Earth. Four diagonal transects were used to connect the edges/margin of the field to the central point. Four equidistant sampling points were identified along each transect, starting at the edge and ending at the midpoint. Sample points were labeled from A–D, with A being near the edge of the field and D being at the center of the field. The average distance between sample points was 128.5 m. The four sampling points located at D were spaced 1 m apart in the direction of the diagonal transects. In 2017 each field had 16 sampling points, with four replications at each distance away from the edge ( $n = 128$  total samples collected in 2017).

In 2018, a different experimental design was chosen to increase sample number and improve sample distribution. Instead of transects, 2018 sites were sampled using perimeters. Sample points marked A (near the edge of the field) were located along an outer perimeter that incorporated 90% of the field. Sample points marked B incorporated 60% of the field. The inner perimeters used to sample points marked C and D (at the center of the field) incorporated 30% and 10% of the field, respectively. On average the perimeters were spaced 96.5 m apart, and each of the four perimeters per field site had 12 equidistant sample points. Thus, 48 samples were collected at each site ( $n = 240$  total samples collected in 2018).

In both years, sample points were marked as way points in Google Earth and uploaded onto a Garmin GPS. The GPS was used in-field to locate the correct sampling areas. Therefore, each sample had a geo-location that could be used to measure distance from field edges and other non-crop or overwintering habitat (infield levees, headlands, and forest margins). See Chapter 3 (Fig. 3.1. & 3.2.) for maps of the experimental design.

#### 4.2.3. Sample collection

Field sites were sampled for stem borer presence in August, when rice in each field was in the soft dough stage. Stem borer counts were taken at each sample point using a 1 m<sup>2</sup> PVC sampling quadrat. Within these quadrats, stem borer infestations were quantified by counting the number of rice stems with characteristic “whiteheads” that result from internal borer feeding during panicle development (Reay-Jones et al 2008, Wilson et al. 2015). The rice stems were collected and dissected, and any stem borer larva found were identified as either *C. plejadellus*, *D. saccharalis* or *E. loftini*, according to distinguishing characteristics (Beuzelin et al. 2016). Prior to the removal of whiteheads, stand counts were assessed in each 1 m<sup>2</sup> quadrat. This was done to ensure rice density was not a confounding factor affecting the distribution of stem borer populations.

#### 4.2.4. Data analysis

Rice stands and stem borer larval density data were analyzed using an ANOVA (PROC MIXED, SAS Institute 2011) with sample points on the transects and perimeters, for 2017 and 2018 respectively, included as fixed effects and field as the random effect. Kenward-Rogers was used for all calculation of error degrees of freedom and means were separated using Tukey’s HSD ( $\alpha=0.05$ ). Stem borer larval data from each field were spatially analyzed using GIS software (ArcGIS, Environmental Systems Research Institute 2011). Tests for spatial associations and clustering among points were conducted using Moran’s I statistic (Moran 1950, Wilson et al. 2017). Hot-Spot analysis (ESRI 2011) was used to calculate the Gi statistic, which tests for statistically significant clusters of high-densities (hot-spots) and low-densities (cold-spots) (Getis and Ord 1992). Distribution maps were generated for stem borer larval density using spatial interpolation with inverse distance weighting (IDW) (Weisz et al. 1995). The IDW spatial interpolation uses known values at sampled sites to predict the values of unknown sites based on the assumption that spatial

points which are near to one another are more similar than those further apart (Wang 2015). Thus, nearby points are given high weights in comparison to points located at greater distances. Weights are inversely proportional to a power of distance. The power selected for this IDW analyses was 2.00, which is commonly used by applied researchers (Pimentel et al. 2017). A spatial resolution of 1 m<sup>2</sup> pixels was also used.

The data was further analyzed using a negative binomial regression model to assess the density of stem borer populations in relation to different independent variables across both 2017 and 2018 samples. PROC GENMOD was used to construct the negative binomial model in SAS, and the maximum likelihood parameter estimates, Chi-square (X<sup>2</sup>) values, standard errors and Akaike information Criterion (AIC) diagnostics were assessed (SAS 2010). A Full Model was created using the following variables: Distance of the sampling point from the edge of the field, distance from alternative non-crop habitat, sample point (A, B, C & D), parish location of sampling site, year, and rice stand count at each sample point. A reduced model was selected by assessing AIC scores and X<sup>2</sup> values (Cameron and Trivedi 1998). The model with the lowest AIC score was considered the most appropriate for describing the distribution of stem borer populations, as indicated by whitehead counts per m<sup>2</sup>. The reduced model excluding stand counts, distance from alternative non-crop habitat, sample point (A, B, C & D), year, and rice stand count at each sample site which were not significantly related to stem borer density.

#### **4.3. Results:**

Of the 322 recovered stem borers, 89.7 % were identified as *E. loftini*, 7.8 % were *C. plejadellus* and 2.5 % were *D. saccharalis*. Rice stand counts did not differ significantly between samples points in either 2017 or 2018. Rice stand count was not significantly correlated with number of whiteheads found per m<sup>2</sup> and was therefore excluded from the negative binomial model. Thus, stand count results are not presented herein.

The ANOVA results show that stem borer damage differs significantly between transect points and perimeters located in different regions of fields sampled in both 2017 ( $F = 14.16$ ;  $df = 3, 120$ ;  $P < 0.001$ ) (Fig. 4.1.) and 2018 ( $F = 9.61$ ;  $df = 3, 235$ ;  $P < 0.001$ ) (Fig. 4.2.). In 2017, stem borer damage at point A (the edge of the field) was 1.7, 4.8, and 4.2-fold higher than the damage recorded at points B, C, or D (center), respectively. In 2018, stem borer damage did not differ between field regions marked A and B. However, points located on perimeter A had 3.4 and 3.9-fold more whiteheads per m<sup>2</sup> than points on perimeters C and D, respectively.

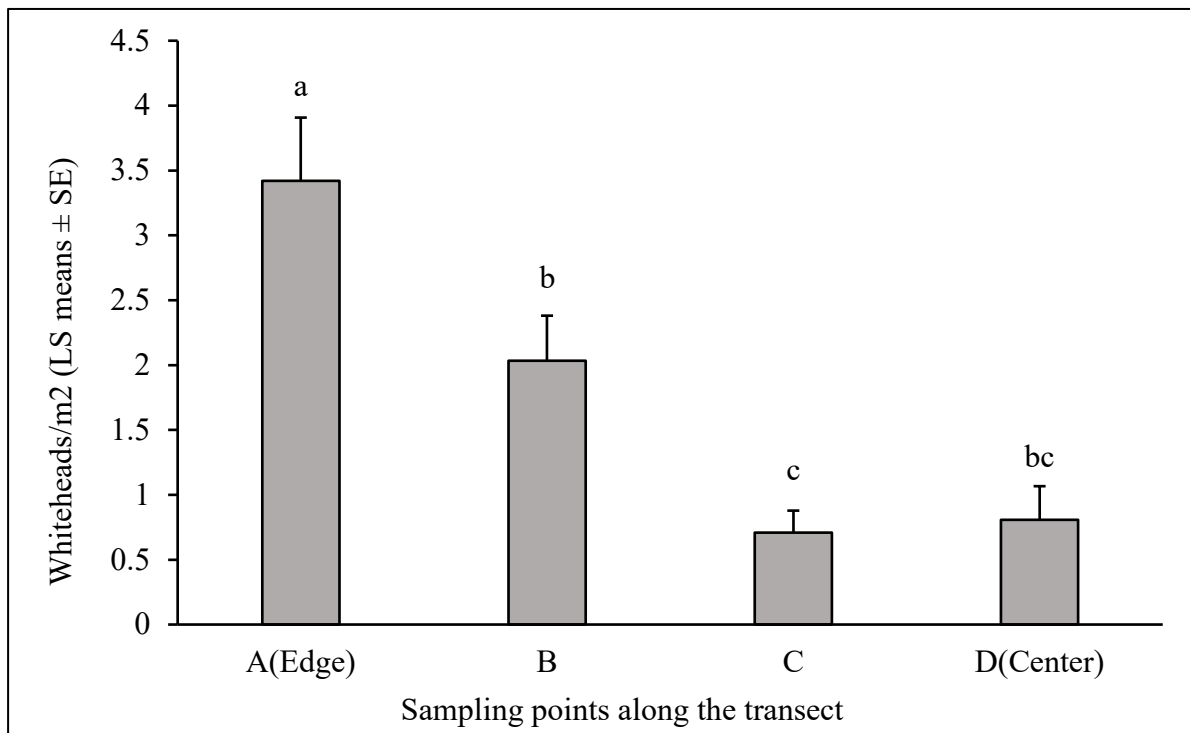


Figure 4.1. The distribution of stem borer damage (whiteheads per m<sup>2</sup>) along various sampling points of a transect progressing from the edge (A) to the center (D) of commercial rice fields in 2017. Letters indicate significance at  $p < 0.05$ .

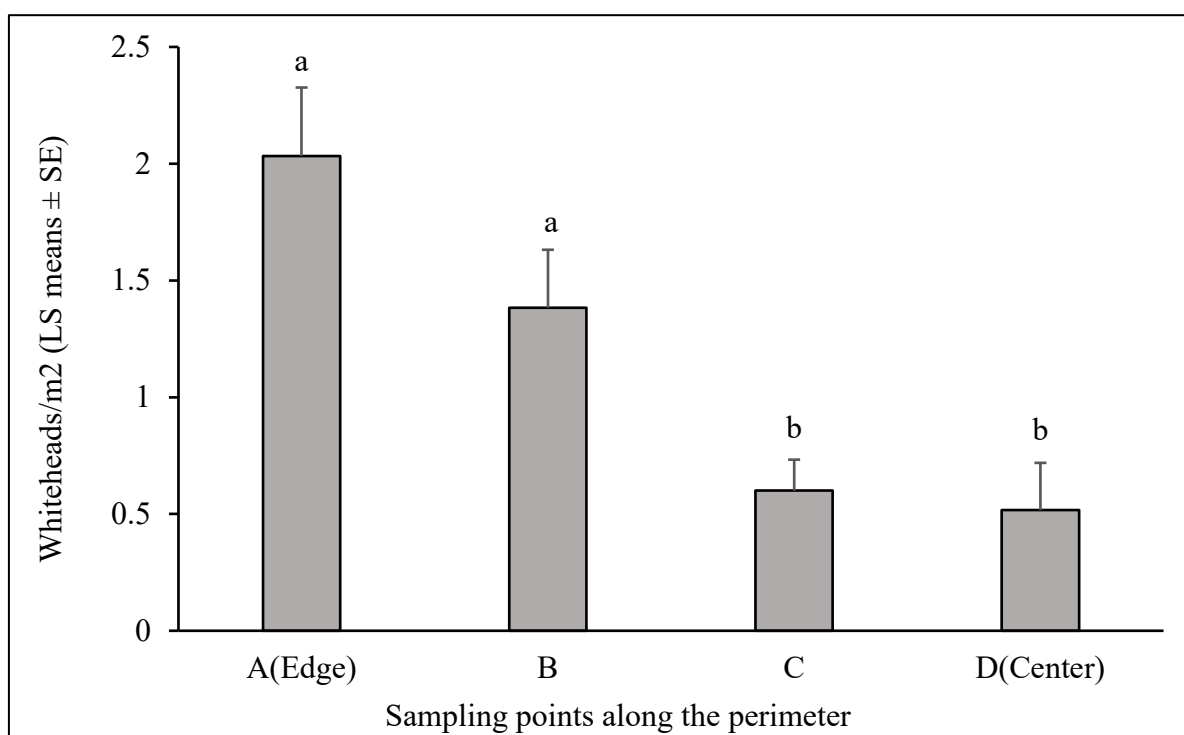


Figure 4.2. The distribution of stem borer damage (whiteheads per m<sup>2</sup>) along various sampling points of perimeters progressing from the edge (A) to the center (D) of commercial rice fields in 2018. Letters indicate significance at  $p < 0.05$ .

Results of the IDW spatial interpolation, depicting the predicted distribution of stem borers within the sampled sites, show that higher numbers of stem borer damaged plants were found along field margins in the commercial rice fields sampled in 2017 (Fig. 4.3.). Although populations of stem borers were concentrated near field edges in 2018, the distribution is more clustered (Fig. 4.4.). High stem borer densities occur in isolated patches along certain edges and areas of the sampled fields. In both years, stem borer damage was relatively low in the central region of each field (between 0–1 whiteheads per m<sup>2</sup>). However, some fields (Fig. 4.3.C and 4.4.C & D) did have densities of up to 3 whiteheads per m<sup>2</sup> at central points. The Moran's I correlation coefficient was significant ( $P < 0.05$ ) for all the sample sites except fields C and D in 2018 (Fig. 4.4.). In 2018, the Moran's I analysis indicated that stem borers in Field C had a random distribution (MI = 0.037,  $P = 0.206$ ), while those in Field D were only loosely clustered (MI = 0.077,  $p\text{-value} = 0.0561$ ).

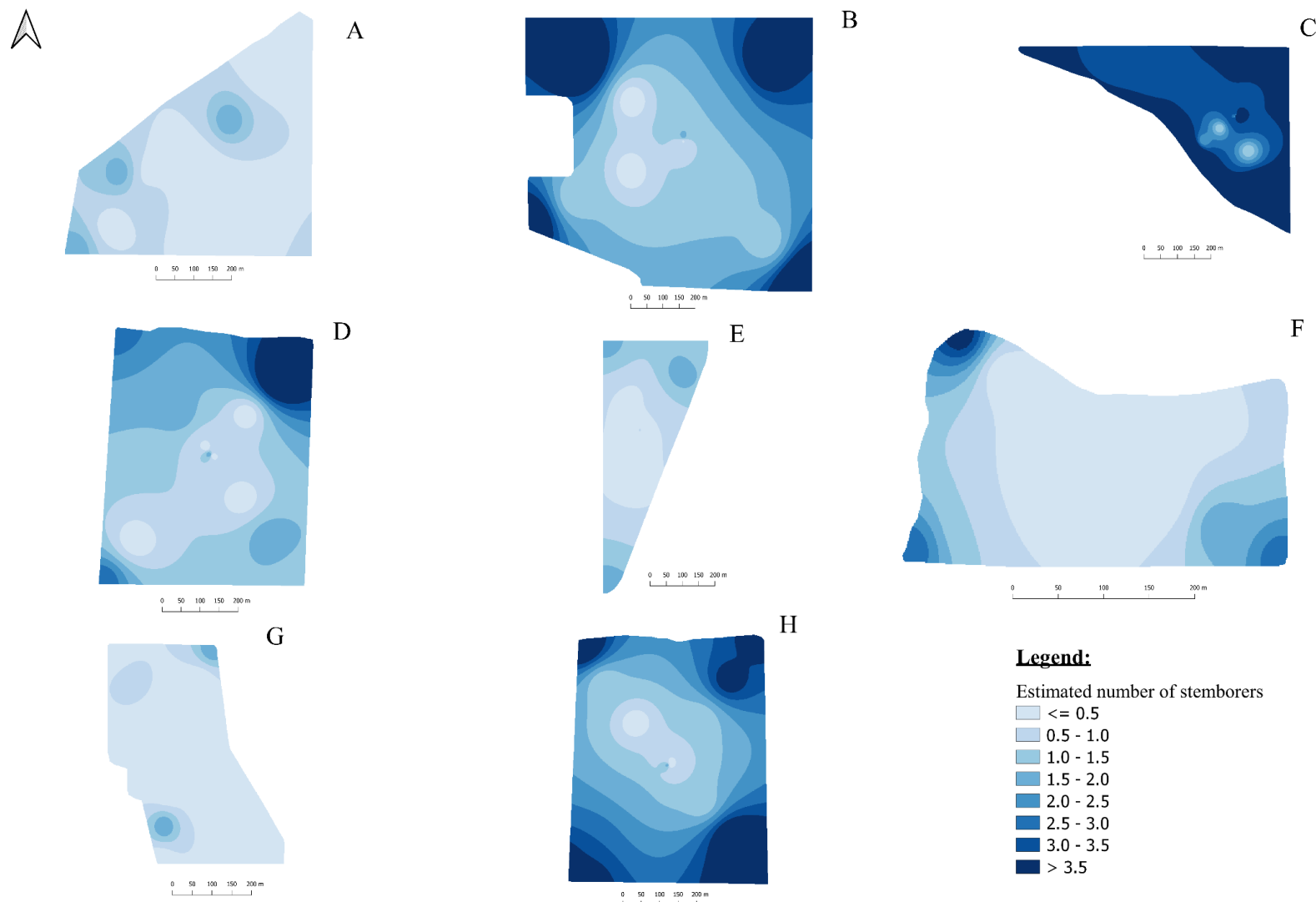


Figure 4.3. ARC GIS spatial interpolation showing the spatial distribution of stem borer damage (whiteheads per m<sup>2</sup>) in untreated commercial rice fields during the 2017 growing season in Louisiana.

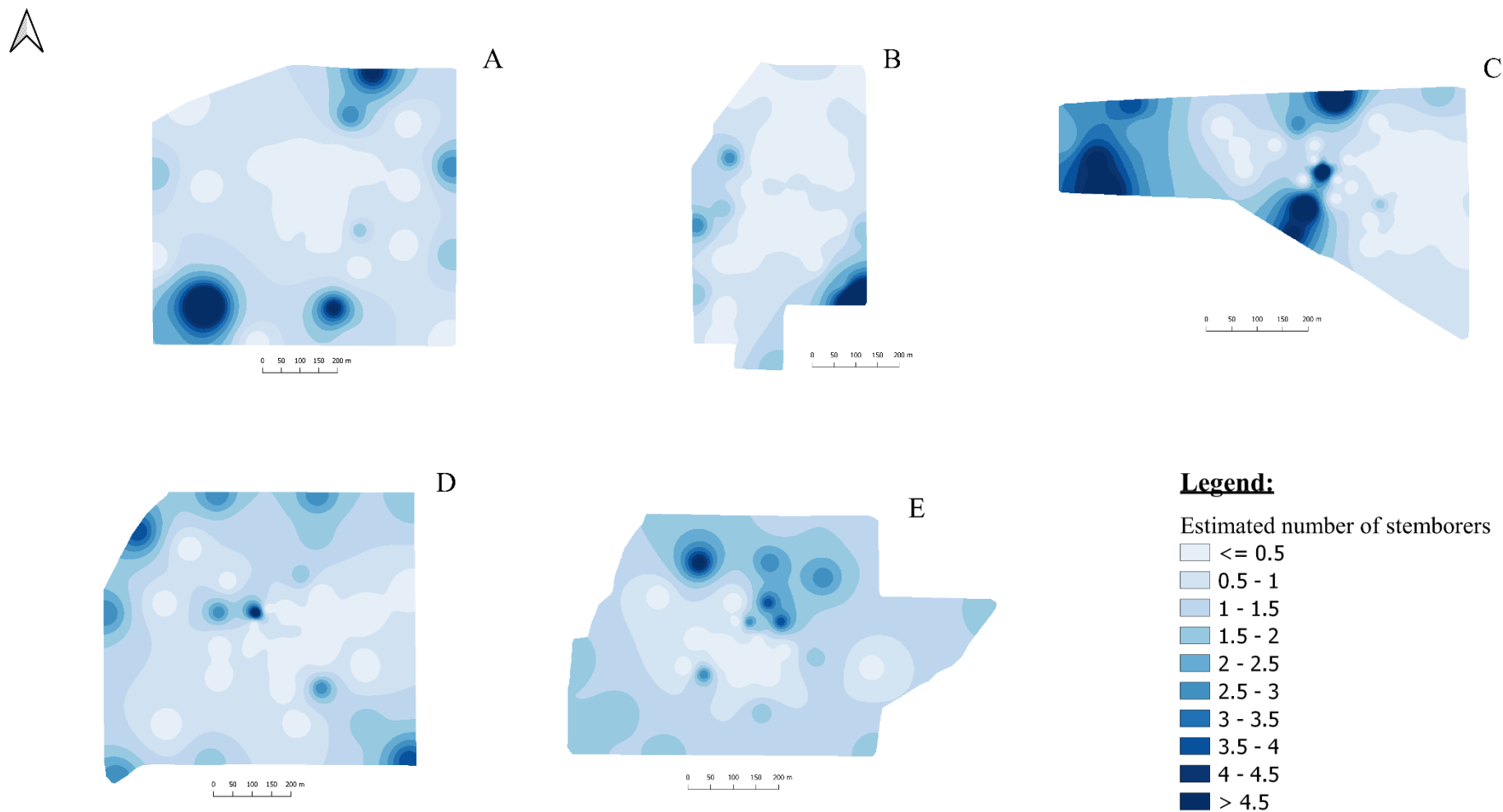


Figure 4.4. ARC GIS spatial interpolation showing the spatial distribution of stem borer damage (whiteheads per m<sup>2</sup>) in untreated commercial rice fields during the 2018 growing season in Louisiana.

The 2017 field sites were excluded from hotspot analyses because sample point allocation was too widely spread, producing unreliable results. A hotspot analysis of 2018 data revealed the Getis-Ord Gi-statistic for each point in the dataset. The corresponding z-scores and p-values identified sites with significantly high (positive z-score; “hot spots”) or low (negative z-score; “cold spots”) whitehead densities. A total of 13 significant hot spots and 48 cold spots were detected in all fields in 2018 (Table 4.1). High density hot spots were confined to points located on perimeter A and B. A majority of low-density cold spots were found on perimeters C and D (41.7% and 52.1% respectively), with only 6.2% of cold spots occurring on perimeter B and none on perimeter A. Non-significant values were not depicted.

Table 4.1. Z-scores for sample points identified as significant Hotspots (positive score) or Cold-spots (negative score), according to the Getis-Ord spatial clustering analysis.

Field (2018)	Transect	Sample Point A	Sample Point B	Sample Point C	Sample Point D
<b>A</b>	1	-	-	-	-1.98
	2	-	-	-	-1.94
	3	-	1.97	-	-1.78
	4	3.88	3.20	-	-1.98
	5	-	-	-	-2.13
	6	-	-	-	-2.38
	7	-	-	-	-2.49
	8	-	-	-1.70	-2.67
	9	2.59	-	-	-2.34
	10	-	-	-	-2.34
	11	-	-	-1.94	-2.16
	12	-	-	-	-1.98
<b>B</b>	1	1.93	-	-1.68	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	-	-	-
	7	-	-	-	-
	8	-	-	-	-
	9	-	-	-	-
	10	-	-	-	-
	11	-	-	-	-
	12	-	-	-	-

(Table Cont'd)



Field (2018)	Transect	Sample Point A	Sample Point B	Sample Point C	Sample Point D
<b>C</b>	1	-	-	-	-2.50
	2	-	-	-2.09	-
	3	1.67	-	-	-
	4	-	-	-	-
	5	2.3	-	-	-
	6	-	1.88	-	-
	7	-	-	-	-
	8	-	-	-	-
	9	-	1.71	-	-
	10	-	-	-1.94	-
	11	-	-	-2.38	-
	12	-	-	-1.95	-
<b>D</b>	1	1.93	-	-1.68	-2.51
	2	-	-	-2.30	-
	3	-	-	-2.10	-
	4	-	-	-2.10	-
	5	-	-	-1.67	-2.09
	6	-	-	-1.66	-2.11
	7	-	-	-	-
	8	-	-	-	-1.67
	9	-	-	-	-1.67
	10	-	-	-	-2.10
	11	-	-1.77	-1.67	-
	12	-	-	-3.13	-
<b>E</b>	1	-	-	-	-1.92
	2	-	-2.78	-2.10	-1.97
	3	-	-1.75	-1.92	-1.92
	4	-	-	-3.36	-1.92
	5	-	-	-3.18	-2.37
	6	-	-	-1.7	-
	7	1.8	-	-	-
	8	-	2.63	-	-
	9	-	2.75	-	-
	10	-	-	-	-
	11	-	-	-	-
	12	-	-	-	-1.92

A significant negative-binomial relationship was found between the whiteheads per m<sup>2</sup> and distance in meters from the edge of the field ( $X^2 = 55.52$ ,  $df = 1$ ,  $P < 0.001$ ), and the Parish location ( $X^2 = 39.37$ ,  $df = 2$ ,  $P < 0.001$ ) across the sampled fields (Table 4.2.). The relationship is explained by the following equation:

$$\log(\text{Whiteheads}) = 0.7457 + 0.6411P_J - 0.7975P_V - 0.0057D_E$$

Where:

Whiteheads = Whiteheads per m<sup>2</sup>

$P_J$  = Jefferson Davis Parish

$P_V$  = Vermilion Parish

$D_E$  = distance (m) from field edge

According to the estimated equation, stem borer densities decrease when moving away from field edges. The number of whiteheads per m<sup>2</sup> decreases by approximately 0.6 % for every unit of increase in distance from the edge of the field (approximately 1 whitehead per m<sup>2</sup> for every 30 m from the edge). An analysis of the contrast estimates shows that whitehead densities for Jefferson Davis Parish and Vermilion Parish are 1.899 and 0.45-fold the incident rate of the reference (Acadia Parish), respectively. The location of farms had a significant effect on the number of whiteheads recorded across sites, with predicted values of whiteheads per m<sup>2</sup> showing significant differences between parishes (Fig. 4.5.). The model was assessed for goodness of fit by comparing the model  $df$  to the deviance and Pearson  $X^2$  values (Table 2). The deviance indicated an appropriate negative binomial fit ( $\sigma = 1.03$ ), with the Pearson  $X^2$  indicating likewise ( $\sigma = 1.4$ ). According to the assertions of Payne et al. (2018) that  $\sigma \leq 1.5$  is an acceptable threshold for determining goodness of fit for a negative binomial model. To test whether the data was over dispersed due to zero inflation, the model parameters were used to simulate negative binomial data. The distribution of the simulated was similar to the original data set and the number of zeroes did not differ significantly ( $X^2 = 1.615$ ,  $DF = 1$ ,  $P = 0.204$ ). Thus, the model adequately fits the data and can be used to make inferences.

Table 4.2. Analysis of maximum likelihood parameter estimates for the negative binomial regression model for number of whiteheads vs distance of samples from the edge of rice fields in different parishes.

Variable	DF	Parameter Estimate	Std. Error	Wald 95 % Confidence Limits		Wald Chi-Square	Pr>ChiSq
<b>Intercept</b>	1	0.7457	0.1134	0.5234	0.9680	43.23	<0.0001
<b>Dist. from edge</b>	1	-0.0057	0.0008	0.0072	0.0042	56.27	<0.0001
<b>Parish (Jeff Davis)</b>	1	0.6411	0.1553	0.3367	0.9454	17.04	<0.0001
<b>Parish (Vermilion)</b>	1	-0.7975	0.2132	1.2155	0.3796	13.99	0.0002
<b>Dispersion</b>	1	0.6546	0.1275	0.4469	0.9590		

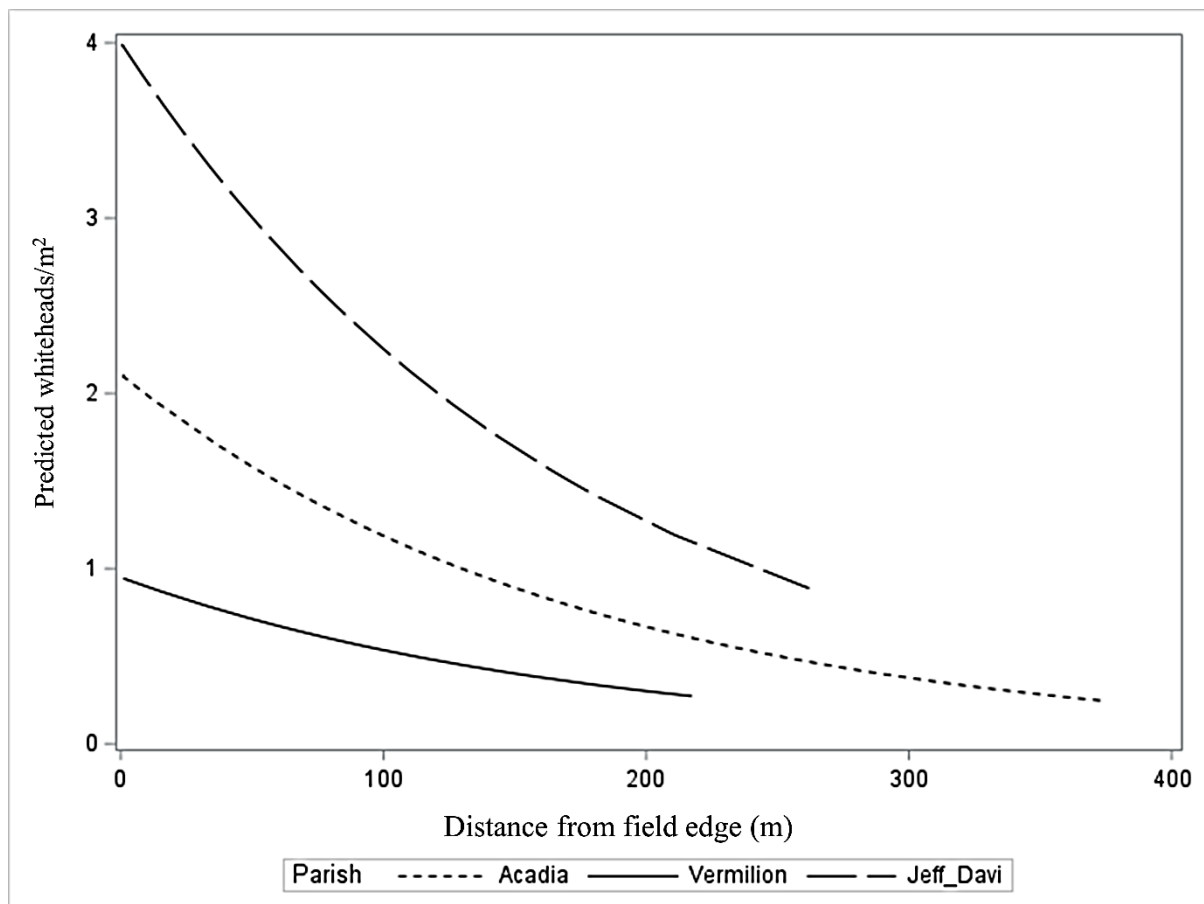


Figure 4.5. Negative binomial regression depicting change in stem borer damage (whiteheads per m<sup>2</sup>) according to distance from the edge of the field, with regional differences separated by the location (Parish) of each farm sampled.

#### 4.4. Discussion

This work is the first to describe the spatial distribution of lepidopteran stem borers in Louisiana rice fields. There is very little research detailing the in-field abundance of stem borer populations in any rice growing regions. Therefore, this study can help researchers better understand stem borer colonization and infestation of rice agroecosystems. The data presented here show that stem borers have an edge biased negative binomial, distribution. Further, this study demonstrates that stem borer infestations decrease as distance from the edge of rice fields increases. These findings are supported by January et al. (2018), who demonstrated that populations of stem borers and their parasitoids were concentrated along the edges of rice fields in Tanzania. Another survey of rice stem boring species, including *Chilo partellus*, *Semamia calamistis* (Lepidoptera: Noctuidae) and *Maliarpha separatella* (Lepidoptera: Pyralidae) in Tanzania, found that an increase of larval abundance was associated with proximity to field edges (Leonard and Rwegasira 2015). In Arkansas, stem borer damage (as indicated by whitehead abundance) in rice is reportedly more severe on field margins, beside levees and on plants near drainage ditches (Lorenz et al. 2018). Fields bordered by weedy ditches or wooded areas often have higher infestations (Lorenz et al. 2018). Edge biased distributions in lepidopteran pest populations are also reported for *Ostrinia nubilalis* (Lepidoptera: Crambidae) and *Busseola fusca* (Lepidoptera: Noctuidae) in corn (Olson and Andow 2008, Merrill et al. 2013), *Endopiza viteana* (Lepidoptera: Tortricidae) in grapes (Martinson et al. 1991) and *Cydia pomonella* (Lepidoptera: Tortricidae) in apple orchards (Gutt and Brunner 1998).

Studies suggests that the source of stem borer infestations in rice is related to gramineous alternative host habitat in adjacent areas (Gounou and Schultess, 2004, Wilson et al. 2017, January et al. 2018). Cereal stem borers are typically polyphagous and are known to attack several gramineous crops and other non-cultivated wild host plants (Showler 2019). Non-crop

grasses prevalent in rice agro-ecosystems which are frequently attacked by *E. loftini* include johnsongrass (*Sorghum halepense*) (L.), Vasey grass (*Paspalum urvillei*), and barnyardgrass (*Echinochloa crus-galli*), which provide suitable early season and overwintering habitat (Showler et al. 2011, Beuzelin et al. 2013). These plants can act as stem borer reservoirs, facilitating infestations in Louisiana agricultural systems (Beuzelin et al. 2011). Furthermore, Showler et al. (2011) reported an increase in abundance of *E. loftini* in sugarcane fields infested with grasses and broad leaf weeds. Unlike populations of *L. oryzophilus* (Mulcahy et al. 2021), stem borer distributions in Louisiana rice were not significantly influenced by the presence of levees other in-field sources of alternative host plants.

Maps depicting the IDW interpolation, in conjunction with the Moran's I and Gestis-Ord values, indicate that stem borer larval populations are not uniform and that they are typically aggregated along the edges of rice fields. Only 2 of 13 fields surveyed had stem borer damage patterns that were considered random. Thus, it is evident that whitehead distribution ordinarily occurs in clusters at or near field margins, but that non-random distribution is not universal. Aggregated spatial patterns in stem borer populations have been well-documented in numerous cropping systems using a multitude of different indices, namely Morister's index, Taylor's law, Green's index and Iwao's mean crowding regression (Setamou et al. 2000, Parian et al. 2012, Ndjomatchoua et al. 2016, Nikpay et al. 2019, Arbabtafti et al. 2021). Similar results are described for a wide range of stem borer species, including *S. calamistis*, *B. fusca* and *Eldana saccharina* (Lepidoptera: Pyralidae), in various host plants across West Africa (Schultess et al. 1997, Gounou and Schultess 2004).

Although many insect species show some degree of aggregation, species distribution is influenced by a wide range of factors (Taylor 1984). In-field stem borer distribution can be affected by population dynamics and ecological conditions, such as pest abundance, species interactions, adult dispersal, oviposition preference, developmental stage, landscape

composition, host plant availability and geographic region (Parian et al. 2012). For example, in Florida sugarcane the distribution of *Diatraea saccharalis* was only aggregated at high population densities (Hall 1986). Aggregation of this species also occurred in larger fields, with small fields having more regular distribution patterns (Hall 1986). Schexnayder et al. (2001) determined that the distribution pattern of *D. saccharalis* was random in Louisiana sugarcane, while Showler et al. (2012) discovered an edge bias in corn, but not in sugarcane or sorghum. In Texas sugarcane, the spatial distribution of small *E. loftini* larvae was aggregated, but medium and large larvae had a random distribution pattern (Meagher et al. 1996). Thus, it is evident that although stem borers have an aggregated distribution pattern in Louisiana rice, there are many factors influencing their distribution that can cause population densities to deviate from the norm.

The negative binomial regression found that location of sampled farms also influenced in-field stem borer distribution in Louisiana. Farms located in Jefferson Davis Parish had the highest levels of stem borer damage, followed by Acadia Parish, with Vermilion Parish having the fewest whiteheads per m<sup>2</sup> sampled. Regional differences in stem borer population abundances may be related to the increasing prevalence and range expansion of *E. loftini*. The majority of larvae recovered from collected whiteheads were identified as *E. loftini*. This supports claims that *E. loftini* is the most prevalent and problematic stem borer in Louisiana rice (Villegas et al. 2021a,b). *Eoreuma loftini* is native to Mexico and was first introduced to the Rio Grande Valley (Reay-Jones et al. 2008, Hummel et al. 2010, Wilson et al. 2015). It is evident that the invasive pest has become well-established in Louisiana and has become a consistent threat to rice production in the state (Wilson et al. 2015, 2021a,b, Villegas et al. 2021a,b). The continued eastward spread of this pest coincides with increases in the incidence of stem borer infestations in Louisiana rice, with regional differences in *E. loftini* populations being identified through hotspot analyses in Wilson et al. (2017).

In conclusion, this study provides scientists with a better understanding of stem borer distribution in rice agroecosystems. This can help improve scouting techniques and resistance management, by providing stakeholders with information on which areas are most susceptible to stem borer attack. The use of insecticidal seed treatments is the key method of stem borer control in Louisiana. Our results indicate that the greatest return on seed treatment investment would be along field edges, where the majority of stem borer damage occurs. Additionally, chlorantraniliprole seed treatments are used widely against stem borers and *L. oryzaephilus*. The edge biased distribution of both stem borers and *L. oryzaephilus* suggests that applications of chlorantraniliprole could be restricted to field borders without compromising management for either pest (Mulcahy et al. 2021). The differences in regional distribution of stem borer populations also indicates that farmers can consider different methods of pest management depending on their location and infestation levels (Park et al. 2007). This has important implications for resistance management. However, controlled experiments (testing the effectiveness of targeted seed treatments) need to be conducted before any recommendations are made. Targeted treatments may also be at odds with farmers preferred planting practices. Thus, more research should be done to assess the applicability and feasibility of using these precision agriculture techniques.

## **CHAPTER 5. INTEGRATION OF CHEMICAL AND CULTURAL CONTROL METHODS FOR THE CONTROL OF *LISSORHOPTRUS ORYZOPHILUS* (COLEOPTERA: CURCULIONIDAE) AND LEPIDOPTERAN STEM BORERS IN LOUISIANA RICE**

### **5.1. Introduction**

Over-reliance on chemical controls threatens the sustainability of *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae) and lepidopteran stem borer management in rice. Integration of cultural controls with insecticidal seed treatments is needed to allow for diversification of management tactics and to mitigate development of insecticide resistance. Alteration of water management in rice has shown potential as a cultural control for *L. oryzophilus* because of the pest's dependence on flooded conditions for oviposition and larval survival (Zou et al. 2004b, Adams et al. 2015, Lanka et al. 2015). Previous research has shown that delayed flooding significantly reduces rice water weevil larval populations within rice fields (Rice et al. 1999). Planting date can also be used as a management tool for the control of *L. oryzophilus*. Rice that was planted late (mid-April onwards) had reduced yields and higher levels of *L. oryzophilus* damage than rice planted at an earlier date. While early-planted rice did not escape damaging populations of *L. oryzophilus*, it was able to tolerate infestations without major yield loss (Thompson et al. 1994). The benefits of early planting in drill seeded rice are less pronounced (Wilson et al. 2021a). Although prior work has been done on the benefits of using cultural controls to manage *L. oryzophilus*, none of these studies have looked at the synergistic, or antagonistic, effects between different cultural and chemical controls in Louisiana rice (Stout et al. 2011, Lanka et al. 2015). Moreover, we do not understand how *L. oryzophilus* cultural controls impact infestations of Lepidopteran stem borers. Furthermore, neonicotinoid insecticides have little effect on lepidopteran pests. Therefore, it is important to determine how different insecticides and cultural control practices affect populations of stem borers to understand whether neonicotinoid seed



treatments can be used as alternative insecticides in rice IPM programs. If neonicotinoids can provide effective control when used along with cultural controls, they may be able to help mitigate selection pressure for chlorantraniliprole resistance.

The objective of this study is to assess the feasibility of integrating insecticidal seed treatments with other pest control practices in rice. Thus, we aim to identify cultural control strategies that can be integrated with widely used chemical controls in order to diversify pest management, reduce farmer input costs and mitigate risk of insecticide resistance. Although chlorantraniliprole is the preferred insecticide used by Louisiana rice farmers, this study also investigates neonicotinoid seed treatments. This was done to assess the feasibility of using neonicotinoids together with different cultural strategies as a potential tool for mitigating resistance build-up against chlorantraniliprole.

## **5.2. Methods**

A series of field experiments evaluated the potential of integrating cultural control tactics and insecticidal-seed treatments for the management of *L. oryzae* and lepidopteran stem borers. The 3-year study was conducted in 2018, 2019, and 2020 at the LSU AgCenter H. Rouse Caffey Rice Research Station in Crowley, Louisiana. For each year, early-planted (March) and late-planted (May) experiments were conducted with exact planting dates varying among years (Table 5.1.). Plots in each experiment were subjected to two different flooding regimes (split-plot) and three different insecticidal seed treatments (split-split-plot) using a randomized complete block design with four replicates (24 plots per planting date). Plots measured 11 m in length with 21 rows spaced approximately 18 cm apart. All plots were drill-planted with the cultivar CL153 at a seeding rate of 67 kg ha<sup>-1</sup>. Fertilizer was applied to plots 1–2 days before the permanent flood and weed management and other production practices followed LSU AgCenter recommendations (Saichuk 2014).

Table 5.1. Planting and harvesting dates for experimental fields.

Activity	2018		2019		2020	
	Early Planted	Late Planted	Early Planted	Late Planted	Early Planted	Late Planted
Planting	15 <sup>th</sup> March	17 <sup>th</sup> May	22 <sup>nd</sup> March	17 <sup>th</sup> May	16 <sup>th</sup> March	22 <sup>nd</sup> May
Flood (Normal)	25 <sup>th</sup> April	22 <sup>nd</sup> June	19 <sup>th</sup> May	3 <sup>rd</sup> July	29 <sup>th</sup> April	1 <sup>st</sup> July
Flood (Delayed)	15 <sup>th</sup> May	13 <sup>th</sup> July	5 <sup>th</sup> June	24 <sup>th</sup> July	13 <sup>th</sup> May	16 <sup>th</sup> July
Harvest	16 <sup>th</sup> August	25 <sup>th</sup> Sept.	22 <sup>nd</sup> August	23 <sup>rd</sup> Sept.	12 <sup>th</sup> August	6 <sup>th</sup> October

Plots were planted with seed treated with either chlorantraniliprole (Dermacor X-100, Corteva Agriscience, Wilmington, Delaware) at a rate of 78.5 g ai ha<sup>-1</sup> or thiamethoxam (Cruiser 5FS, Syngenta Group, Basel, Switzerland) at a rate of 186.8 g ai ha<sup>-1</sup> in addition to nontreated controls. Plots were then subjected to an early (approx. 21 days after planting) or delayed (approx. 42 days after planting) flooding regime.

Plots were surveyed twice for *L. oryzaephilus* larvae and pupae at 4–6 weeks after the permanent floods (early and late) were established, using common soil coring protocols (N’guessan and Quisenberry 1992, Stout et al. 2001). In 2018 and 2019 five core samples were taken from each plot per sample date (480 samples per year). In 2020 six core samples were taken for each plot per sample date (576 samples). Thus, 1,536 samples were collected in total. Each core sample contained at least one rice plant with intact roots and the surrounding soil. The soil was washed from the roots using a 40-mesh sieve bucket. The bucket was placed in a saltwater bath and *L. oryzaephilus* larvae were counted as they floated to the surface. The bucket was then removed from the bath and inspected for pupae. Mean number of larvae and pupae were calculated per plot for both sample dates prior to data analysis. Once rice reached the 100 % heading stage, stem borer density was estimated by collecting and processing injured plants or “whiteheads” (Way 2003). The dissected whiteheads were examined for frass and other signs of stem borer feeding and any larvae found were collected and identified.

Plots were harvested at grain maturity using a small plot combine (Wintersteiger Delta Plot Combine, Wintersteiger Inc., Salt Lake City, UT) and the yield per plot was recorded using rough grain weights. For yield data, rough grain weights were adjusted to 12 % moisture and converted to kg ha<sup>-1</sup>.

A generalized linear mixed model was used to analyze the effects of planting date, flooding regime, and insecticidal seed treatment on *L. oryzaephilus*, stem borer, and yield data

(PROC GLIMMIX, SAS v 9.4). Response variables were non-normal, therefore the distribution of each data set was assessed and modelled accordingly (PROC SEVERITY, SAS v 9.4). The *L. oryzaophilus* and yield models were analyzed using a gamma distribution and the stem borer models were analyzed using a log-normal distribution. For each response variable, data were analyzed with planting date, flooding regime, insecticidal seed treatment and their interactions included as fixed effects. Field rotation ensured that, for each year, experiments were located in different areas at the Crowley Rice Research Station. Therefore, in the models, year (blocking factor) was included as a random effect to account for variation in soil and pest pressure. Kenward-Rogers was used for the calculation of error degrees of freedom and a post-hoc Tukey HSD analysis was conducted to separate the means ( $\alpha = 0.05$ ).

### 5.3. Results

Flood timing, insecticidal seed treatment and their interaction affected the number of weevils found per core, but differences among planting dates or other interactions were not detected (Table 5.2.). The results of the main effects show that a delayed flood can reduce weevil pressure by approximately 46.4 % (Fig. 5.1.B). In terms of insecticidal seed treatments, chlorantraniliprole was the most effective management tool (Fig. 5.1.C). Weevil numbers in plots treated with chlorantraniliprole were 51.9 % and 72.8 % less than thiamethoxam-treated plots and untreated plots, respectively. Thiamethoxam decreased weevil numbers by 43.4 % relative to untreated plots. Thiamethoxam-treated plots did not differ from chlorantraniliprole-treated plots in early-planted  $\times$  delayed-flood rice (Fig. 5.2.). Delayed flood reduced weevil densities in thiamethoxam-treated and untreated plots regardless of planting date, as well as late-planted chlorantraniliprole treated plots.

Table 5.2. Results of the general linear mixed model (GLIMMIX) for mean rice water weevil density per core, stem borers per m<sup>2</sup> and plot yields in field trials at Crowley, Louisiana.

Fixed Effect	DF	DenDF	Weevils/core		Whiteheads/m <sup>2</sup>		Yield (kg/ha)	
			F Value	P Value	F Value	P Value	F Value	P Value
Date	1	2	1.09	0.2980	0.21	0.6442	202.85	<0.0001
Flood	1	4	45.84	<0.0001	30.83	<0.0001	21.87	<0.0001
Date*Flood	1	4	2.31	0.1308	3.20	0.0759	137.41	<0.0001
Treatment	2	124	103.32	<0.0001	71.15	<0.0001	95.33	<0.0001
Date*Treatment	2	124	0.34	0.7098	1.17	0.3127	0.15	0.8803
Flood*Treatment	2	124	4.49	0.0130	0.18	0.8325	0.46	0.6306
Date*Flood*Treatment	2	124	1.59	0.2087	1.16	0.3181	0.77	0.4640

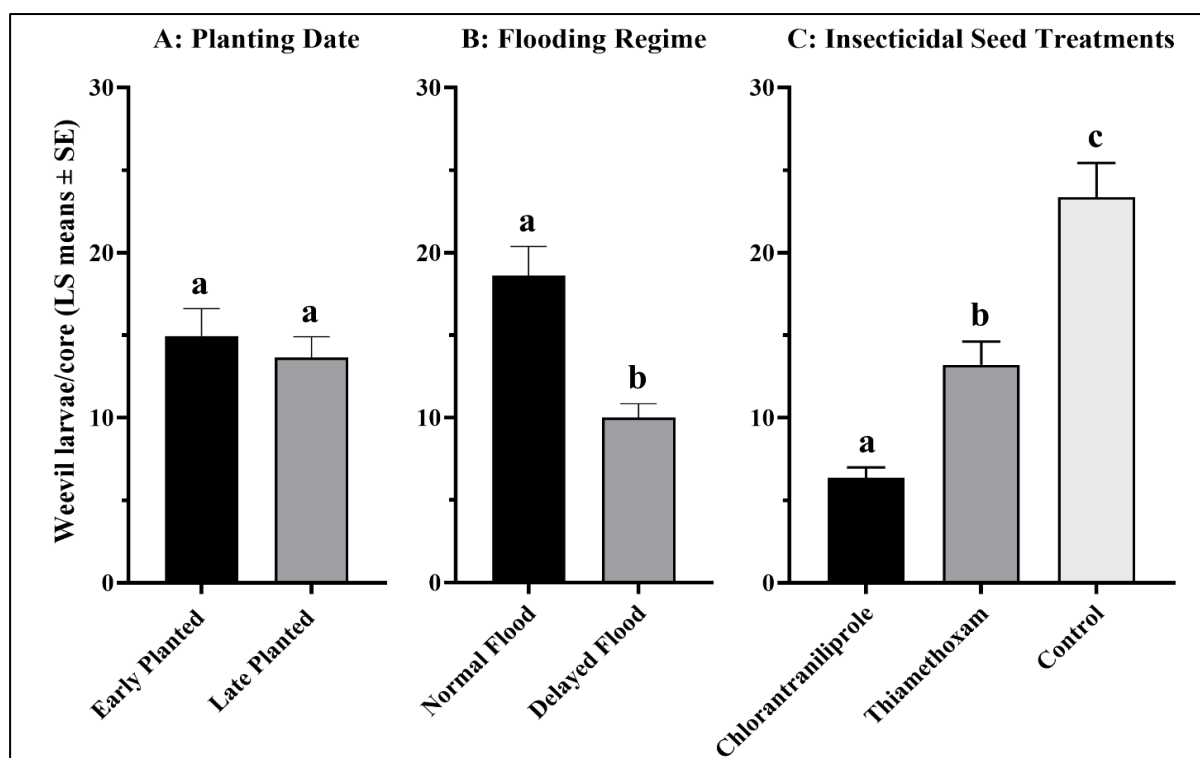


Figure 5.1. The main effects of planting date (A), flooding regime (B) and insecticidal seed treatment (C) on mean number of rice water weevil larvae found per soil-core in rice plots planted in Crowley, Louisiana across 3-years (2018, 2019 and 2020). For each main effect bars accompanied by the same letter do not differ significantly (Tukey's HSD:  $\alpha > 0.05$ ).

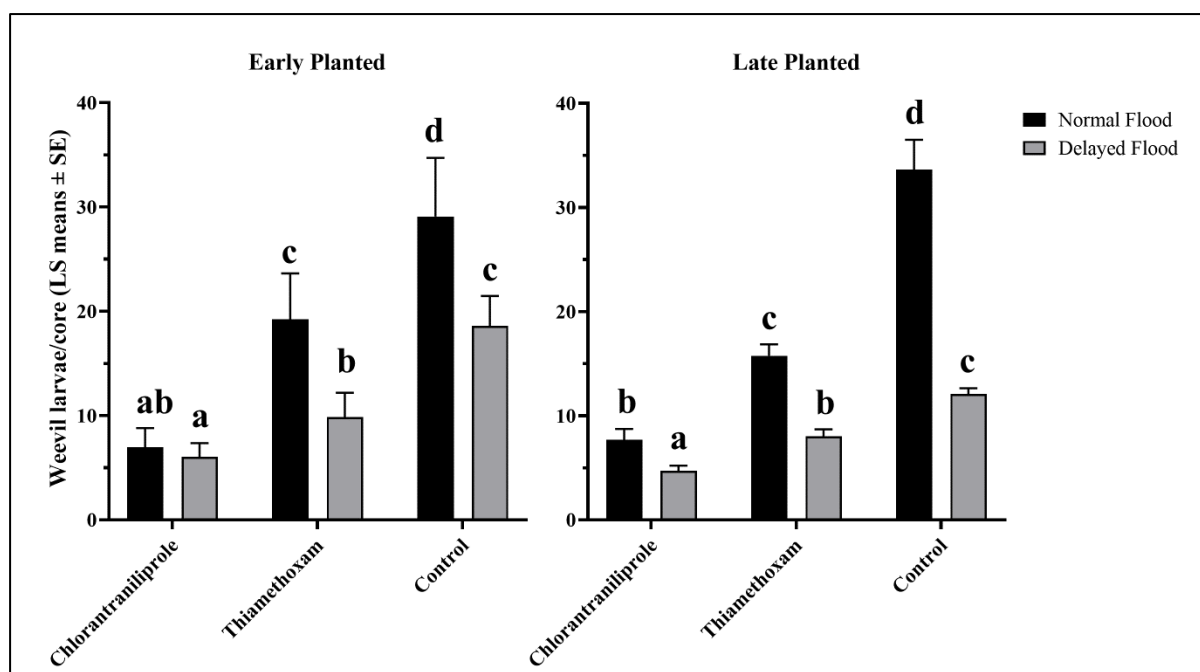


Figure 5.2. Mean number of rice water weevil larvae found per soil-core as affected by the flooding regime  $\times$  insecticidal seed interactions across 3-years (2018, 2019 and 2020), Crowley, Louisiana. Figures are separated by planting date and within each planting date bars accompanied by the same letter do not differ significantly (Tukey's HSD:  $\alpha > 0.05$ ).

A total of 1,693 stem borer larvae were collected from the 4,119 damaged rice plants (whiteheads) that were identified and collected during the study. Of those larvae, 91.8 % were identified as *E. loftini*, 5.1 % were *C. plejadellus*, and 0.9 % were *D. saccharalis*. A further 2.2 % of recovered larvae were damaged and therefore unidentifiable. The results of the model show that insecticidal seed treatment and flooding regime influenced whitehead density, but not planting date or any of the interactions (Table 5.2.). Delayed flooding reduced number of whiteheads collected per plot by 44.9 % (Fig. 5.3.B). Chlorantraniliprole decreased whitehead density by 77.6 % and 70.3 % relative to thiamethoxam treated plots and untreated plots, respectively (Fig. 5.3.C). Thiamethoxam was not efficient at managing stem borer populations and the overall number of whiteheads collected from thiamethoxam treated plots did not differ from those collected in untreated plots (Fig. 3C).

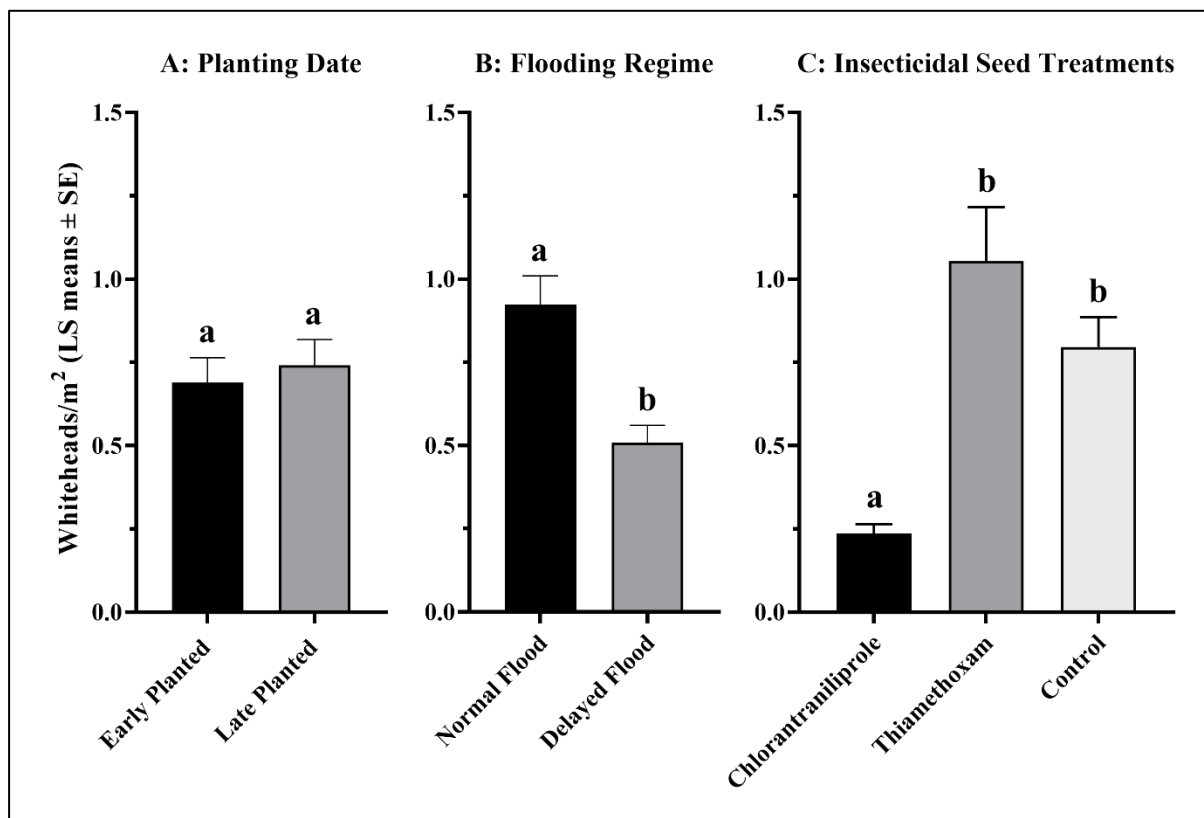


Figure 5.3. The main effects of planting date (A), flooding regime (B) and insecticidal seed treatment (C) on mean number of whiteheads found per m<sup>2</sup> in rice plots planted in Crowley, Louisiana across 3-years (2018, 2019 and 2020). For each main effect bars accompanied by the same letter do not differ significantly (Tukey's HSD:  $\alpha > 0.05$ ).

Both early-planted and late-planted thiamethoxam treated plots experienced the highest levels of injury from stem borers during the experiment, particularly in plots subjected to a normal flooding regime (Fig. 5.4.). Flooding significantly affected the number of whiteheads present in thiamethoxam treated plots and late-planted untreated plots. Flooding did not influence stem borer damage in plots treated with chlorantraniliprole (Fig. 5.4.).

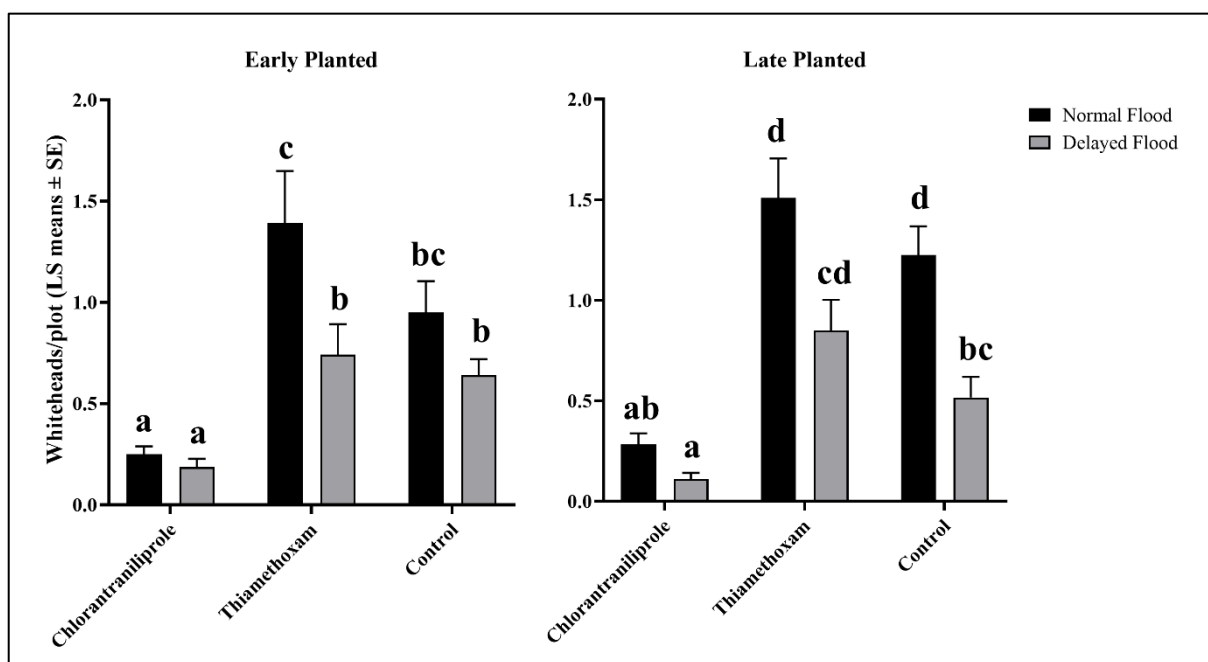


Figure 5.4. Mean number of whiteheads found per m<sup>2</sup> as affected by the flooding regime × insecticidal seed interactions across 3-years (2018, 2019 and 2020), Crowley, Louisiana. Figures are separated by planting date and within each planting date bars accompanied by the same letter do not differ significantly (Tukey's HSD:  $\alpha > 0.05$ ).

Yield was significantly influenced by planting date, flooding time, insecticidal seed treatment and by the date × flood interaction, but not by other interactions (Table 5.2.). Late-planted rice yielded >20 % (approximately 1940 kg. ha<sup>-1</sup>) less relative to early-planted rice across all years, flooding regimes, and insecticidal seed treatments (Fig. 5.A). The main effects for flooding regime show an approximate 6 % decrease in yield when a delayed flood is applied relative to normal flood timing (Fig. 5.5.B). Insecticidal seed treatment had a



significant influence on yield. Thiamethoxam and chlorantraniliprole treated plots yielded 1.2- and 1.3-fold greater than untreated plots, respectively (Fig. 5.5.C).

When the impact of planting date is considered together with the effects of flooding regime on yield, it is evident that early-planted rice benefited significantly from a delayed flood (Fig. 5.6.). In contrast to this, late-planted rice was negatively impacted by a delayed flood. This is particularly true of late-planted untreated rice, which had the lowest yields in the study with an average of 5334.7 kg. ha<sup>-1</sup> (Fig. 5.6.). The yield in early-planted/delayed-flood thiamethoxam treated plots did not differ significantly from early-planted plots treated with chlorantraniliprole (Fig. 5.6.).

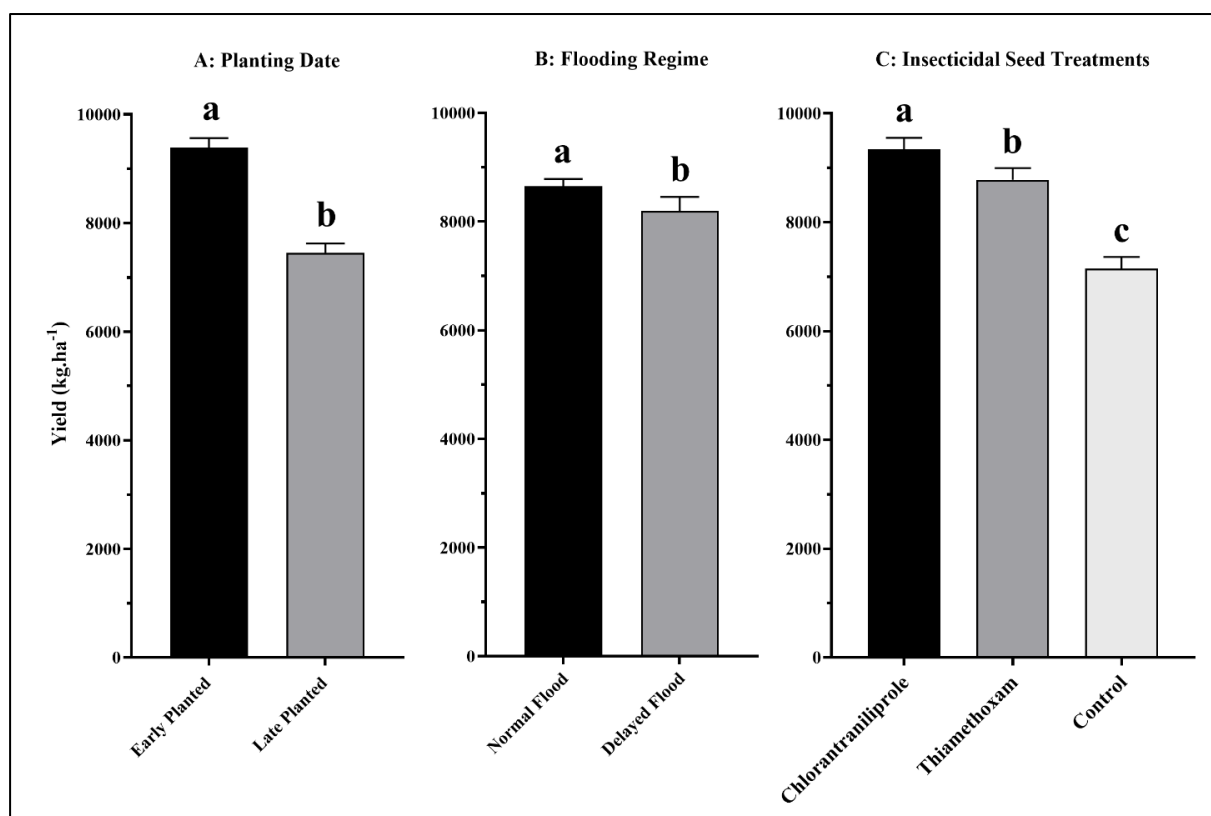


Figure 5.5. The main effects of planting date (A), flooding regime (B) and insecticidal seed treatment (C) on the average yield (kg/ha) recorded from rice plots planted in Crowley, Louisiana across 3-years (2018, 2019 and 2020). For each main effect bars accompanied by the same letter do not differ significantly (Tukey's HSD:  $\alpha > 0.05$ ).

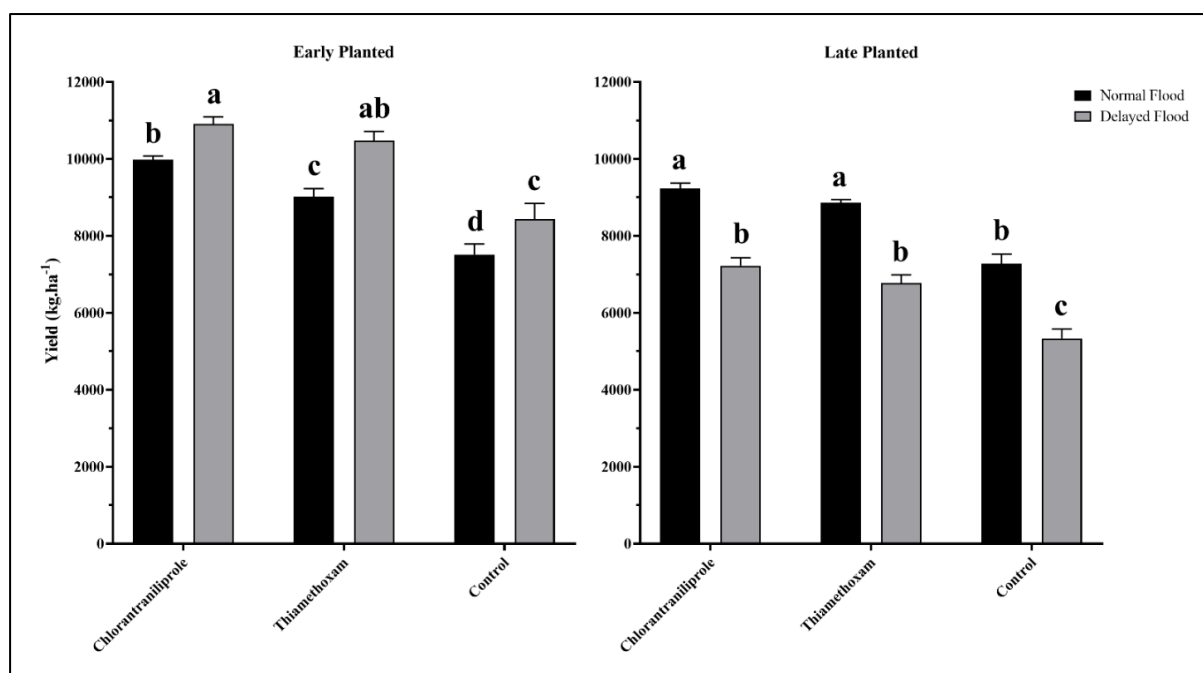


Figure 5.6. The average yield (kg/ha) recorded per rice plot as affected by the flooding regime  $\times$  insecticidal seed interactions across 3-years (2018, 2019 and 2020), Crowley, Louisiana. Figures are separated by planting date and within each planting date bars accompanied by the same letter do not differ significantly (Tukey's HSD:  $\alpha > 0.05$ ).

## 5.4. Discussion

This is the first study that has considered the effects of planting date and flooding time on different insecticidal seed treatments and their efficacy against multiple pest species. The results of this study show that planting date, flooding time and insecticidal seed treatment can be used in concert to improve *L. oryzaephilus* and stem borer pest management and increase rice yields. Thus, cultural, and chemical control tactics are compatible if used correctly in IPM programs designed for rice agriculture.

Our findings that planting date did not impact *L. oryzaephilus* infestation levels is in contrast to prior research that documented a decrease in *L. oryzaephilus* infestations in early-planted rice (Stout et al. 2001, Stout et al. 2011). However, a number of recent studies have found that there is a negligible effect of planting date on *L. oryzaephilus* populations in Louisiana rice (Wilson et al. 2021a, Villegas et al. 2021a). This may be due to the weevils emerging earlier in the rice growing season. The cessation of diapause and regeneration of

flight muscles in overwintering *L. oryzophilus* is highly dependent on temperature (Grigarik et al. 1991, Zou et al. 2004c, Agahee and Godfrey 2014), with date of emergence linked to springtime temperatures (Morgan et al. 1984). Climate data shows that increasing temperatures in the southeastern United States is hastening the onset of spring in the region (Climate Central 2020, NOAA 2021). Such changes are predicted to have widespread differential effects on the phenology of plants and animals (Pearson 2019), especially on the growth and management of crops and crop pests (Deutsch et al. 2020, Jabran et al. 2020). The seasonal dynamics of *L. oryzophilus* in Louisiana should be re-examined in future studies.

The absence of change in stem borer infestations between early and late-planted rice reported herein is also contrary to previous studies conducted in Louisiana, which found a four-fold increase in whitehead density in rice planted in May and June (Wilson et al. 2021a). This corresponds with data collected by Beuzelin et al. (2011), showing an accretion in *E. loftini* larvae in grassy habitats adjacent to rice fields from April-October. However, the effects of planting date on stem borer density and damage have not yet been thoroughly explored in Louisiana rice. Villegas et al. (2021a), also considered the influence of planting date on stem borer activity, however the results of that study were inconsistent between years. Furthermore, research in Texas found that early-planted rice had significantly higher stem borer injury than rice planted later in the season (Way 2006). The discrepancies could be related to adult flight and oviposition activity, specifically as it relates to *E. loftini*, the predominant species observed in this study. *Eoreuma loftini* moth peaks typically occur in March-April and again in September-November (Beuzelin et al. 2016, Wilson et al. 2017). Differences in infestation trends between years and studies may be due to shifts that can occur within moth peak intervals according to annual variations in thermal accumulation, or degree-days (Fand et al. 2021). It is evident that additional studies, involving multiple

planting dates, are needed to accurately determine how populations of *E. loftini* and other stem borers (and their subsequent injury) fluctuate throughout the rice growing season.

In comparison to planting date, flooding regime had a marked effect on the number of pests recorded in rice plots. A delayed flood was able to significantly decrease both *L. oryzophilus* and stem borer infestations. Manipulation of flooding regimes is a recognized method of cultural control for *L. oryzophilus* in Louisiana (Rice et al. 1999, Stout et al. 2000, 2001, 2002a, Zou et al. 2004b, Villegas et al. 2021a). Rice water weevil oviposition and feeding behavior is closely related to both the timing and depth of flooding in rice fields (Hesler et al. 1992, Tindall et al. 2013, Lanka et al. 2014a). In fact, oviposition is triggered by the permanent flood. The reduced number of larvae under delayed flooding conditions can be explained by a decrease in adult feeding and oviposition, and thus larval infestation, in the absence of flooding (Stout et al. 2002a). In contrast to the large body of work documenting the effects of flood management on *L. oryzophilus* populations, very little research has been conducted on how flood management influences stem borer injury and infestations. In Asian rice growing regions some flooding practices, such as alternate wet and dry irrigation schemes, are known to decrease infestations of stem borers and other insect pests (Chapagain et al. 2011, Arora et al. 2016, Hasan et al. 2016, Bigornia et al. 2016). Delayed flooding could have both direct and indirect effects on stem borers. Direct effects include changes to the micro-climate and the physical crop environment that could lead to increased mortality of eggs and larvae (Zhi 2001, Bolarinwa et al. 2021, Luo et al. 2021). Changes in water management can influence nutrient uptake and quality in rice plants (Beyrouy et al. 1994), which could indirectly affect the feeding and incidence of stem borers and other insect pests (Stout et al. 2002a). The indirect effects of water management and plant nutrition on *E. loftini* abundance have been observed in trials focusing on the use of irrigation in sugarcane (Reay-Jones et al. 2005). Although Villegas et al. (2021a) also found significant reductions in

whitehead densities in rice plots subjected to a delayed flood, more research is needed to understand why delayed flooding decreases stem borer injury, and whether it affects all stem boring species.

This study provides further evidence that both chlorantraniliprole and thiamethoxam insecticidal seed treatments provide significant control of *L. oryzaophilus*. The benefits of using insecticidal seed treatments have been consistently demonstrated in recent years (Hummel et al. 2014, Sidhu et al. 2014, Everett et al. 2015, Lanka and Stout 2015, Wilson et al. 2021b). Chlorantraniliprole was typically more effective against *L. oryzaophilus* than the neonicotinoid seed treatment, thiamethoxam, which is similarly consistent with the literature (Stout et al. 2009, Stout et al. 2011). Studies have shown that the effectiveness of chlorantraniliprole is so pronounced that significant reductions in weevil infestations are maintained (relative to untreated rice) even at application rates that are up to 75 % lower than the recommended label rate (Lanka et al. 2013a, Villegas et al. 2019). However, the efficacy of thiamethoxam seed treatments is greatly enhanced if the treatment is used together with a delayed flood. Our finding that thiamethoxam achieved the same level of *L. oryzaophilus* control as chlorantraniliprole when plots were planted early and subjected to a delayed flood suggests it could provide a suitable alternative when used along with cultural controls. Compared to chlorantraniliprole, which accumulates in rice roots and has a strong larvicidal effect (Lanka et al. 2014a), concentrations of thiamethoxam are highest in the leaves and stems of rice plants (Lanka et al. 2014a, Lanka et al. 2014b). Therefore, thiamethoxam has greater activity against adult *L. oryzaophilus*. Preventing weevil oviposition by delaying the permanent flood may simultaneously lead to greater adult mortality and reductions in the number of eggs laid due to potential sub-lethal effects, thereby decreasing larval abundance (Lanka et al. 2013b). Contrastingly, delaying the permanent flood did not significantly improve the performance of chlorantraniliprole. A delayed flood also reduced weevil

numbers in untreated plots, to such an extent that larval densities were the same as normal flooded plots treated with thiamethoxam.

The lack of stem borer control by thiamethoxam reported herein is consistent with previous studies demonstrating neonicotinoid seed treatments do not provide adequate control against these pests (Wilson et al. 2021a,b). The lack of efficacy of neonicotinoid seed treatments on stem borer populations is a major driver of regional differences in insecticide use in Louisiana rice (Wilson et al. 2019). Over 80 % of rice acreage in Louisiana is treated with chlorantraniliprole (Bateman et al. 2020) with most of it in the southern rice growing regions, where stem borers are more prevalent. Neonicotinoid seed treatments are almost exclusively used in northeast Louisiana, where it is used against *L. oryzaephilus* and grape colaspis, *Colaspis brunnea* F. (Coleoptera: Curculionidae) (Heinrichs et al. 2017, Wilson et al. 2019).

Regardless of the lack of effect of planting date on pest incidence, it is clear from the yield data that early-planted rice performs better than late-planted rice. The optimal dates for planting rice in southwest Louisiana are from the 10<sup>th</sup> of March to the 18<sup>th</sup> of April (Harrell et al. 2021). Stand establishment is negatively affected by planting later in the season, and high temperatures during the reproductive phase can decrease the yield potential and quality of rice grains (Cerioli et al. 2021). Flooding also had a significant effect on rice yield. When planted early, a delayed flood can significantly increase rice yields at least in part because of reduced pest infestations. Research on delayed floods indicates that this practice can make rice more tolerant of pest injury. A delayed flood allows rice to accumulate more growing degree days before the fields are permanently flooded. This means that, the plants are more mature when they are attacked by *L. oryzaephilus* larvae. The older plants are less preferable to *L. oryzaephilus* (Stout et al. 2013) and they are more tolerant to weevil injury (Wu and Wilson 1997, Stout et al. 2002a, Villegas et al. 2021a, b). Therefore, delayed flooding not

only reduces pest incidence, but also reduces the susceptibility of rice to insect pests. However, it is important to note that delayed flooding negatively affects the yields of late-planted rice. Delaying the permanent flood in late-planted fields likely exposes the rice to drought-stress, which can exacerbate the adverse effects of planting after recommended dates. Villegas et al. (2021a) reported a decrease in weevil and stem borer related yield loss in plots that had a delayed flood, especially in late-planted plots. Our study demonstrates that these reductions in yield loss do not account for the decrease in overall yield in late-planted/delayed flooded rice plots. A normal flood is therefore preferable for late-planted rice, regardless of pest pressure.

The use of insecticidal seed treatments has a clear significant effect on yield, with chlorantraniliprole treated plots having greater yields than both thiamethoxam treated plots and untreated plots. Nevertheless, it is interesting to note that when a delayed flood is applied, the early-planted thiamethoxam treated plots had yields comparable to those treated with chlorantraniliprole. This is despite a significant increase in the number of whiteheads in the late-planted/delayed flooded thiamethoxam treated plots. This indicates that *L. oryzae* infestations have a higher impact on rice yield than stem borer infestations. Wilson et al. (2021a) compared the relative yield loss between fully protected and borer-only infested plots. Differences in yield loss were only observed in one of four experiments, where *E. loftini* infestations levels were fourfold greater than in other trials. With a predicted 1.7 % increase in yield loss with every whitehead per m<sup>2</sup> it is possible that high infestations of stem borers (>8 whiteheads/m<sup>2</sup>), can lead to significant damage (Wilson et al. 2021a, Way et al. 2006). However, the low-mid level infestations reported in this study likely caused minimal yield loss in comparison to *L. oryzae*. Therefore, when used in conjunction with cultural control practices, thiamethoxam can act as an alternative to chlorantraniliprole, especially in areas where stem borer populations are low. This can help to curb the regional reliance on

chlorantraniliprole, which can in turn reduce the risk of resistance developing against this chemistry in rice pest populations.

The cultural control practices and insecticidal seed treatments used in this study are compatible and can be used successfully in an IPM program against multiple pests. Based on the results, we recommend that farmers plant their rice fields early and delay the permanent flood to reduce infestations of both *L. oryzaophilus* and stem borers. Yield data shows that use of insecticidal seed treatments is justified in areas where *L. oryzaophilus* numbers are problematic. Although chlorantraniliprole is the most effective insecticide against both pests, if used correctly on well managed fields, thiamethoxam can provide similar levels of weevil control. Thiamethoxam can also be safely used in areas with low stem borer populations, and it can therefore be used as an alternative to chlorantraniliprole. Current increases in stem borer populations in rice are related to the spread of *E. loftini* in the region. Therefore, continued monitoring of this pest in Louisiana rice is important to help farmers make informed decisions. Future research should focus on assessing the feasibility of using other pest management techniques in rice integrated pest management. Other insecticides, such as the clothianidin (a neonicotinoid) and cyantraniliprole (an anthranilic diamide) have known activity against *L. oryzaophilus* and could also be used as potential alternatives to chlorantraniliprole (Wilson et al. 2021b). Different cultivars have shown resistance to *E. loftini*, and host plant resistance could be incorporated into an IPM program to further reduce stem borer yield impacts so that *L. oryzaophilus* management can be further prioritized (Way et al. 2006, Villegas et al. 2021a, b, Wilson et al. 2021a). Further investigation is also needed to look at the compatibility of delayed flooding with weed management.



## **CHAPTER 6. ASSESSING THE FEASIBILITY OF USING REMOTE SENSING TO DETERMINE *LISSORHOPTRUS ORYZOPHILUS* INFESTATION LEVELS IN RICE**

### **6.1. Introduction**

Precision agriculture, variable rate technology and remote sensing practices are often used for monitoring and enhancing crop yields in relation to soil fertility and water management (Griffin et al. 2005, Schimmelpfennig and Ebel 2016). Recently there has been an increase in the use of these technologies for disease, weed and insect pest management (El-Ghany et al. 2020, Filho et al. 2020). Current advances in diagnostic tools, and in our ability to collect high resolution digital aerial images using unmanned aerial vehicles (UAVs) and satellites, have the potential to improve pest detection via remote sensing (Wójtowicz et al. 2016). However, the ease of access and the applicability of using aerial imagery for monitoring crop pests is still in the early stages of development, and the efficacy of such techniques for accurately predicting pest populations and damage is still being researched (Zhang et al. 2019). There is immense potential for remote sensing technologies to reduce input costs through precision targeting of insecticide applications, and thus it is worth exploring (Brenner et al. 1998).

The ability to understand, predict, and map the spatial distribution of insect populations is critical in the development of precision targeted Integrated Pest Management (IPM) strategies (Fleisher et al. 1999). Multi-spectral digital aerial images are used to calculate various vegetation indices that can help locate and quantify pest-induced crop stress and yield loss on commercial farms (Wójtowicz et al. 2016). The use of aerial imagery in conjunction with geostatistics is a pest sampling technique that has been shown to efficiently and accurately assess pest densities in multiple crops. For example, work done on boll injury in cotton demonstrated that normalized difference vegetation index (NDVI) can be used as a component in a site-specific management plan for stink bugs in the crop (Reisig et al. 2015,

Reay-Jones et al. 2016). Furthermore, NDVI has shown potential for detecting early outbreaks of Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), in potato (Hunt and Rondon 2017), as well as hessian fly, *Mayetiola destructor* Say (Diptera: Cecidomyiidae), infestations in wheat (Battarai et al. 2019). This indicates that insect feeding, which results in reduced canopy cover and crop growth, can be readily detected using multi-spectral images (Zhang and Kovacs 2012).

As a root feeding insect, the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), directly impacts the uptake of nutrients in rice (Zou et al. 2004a). This in turn effects the tillering and vegetative growth of the crop. Therefore, it is possible that remote sensing, using UAVs equipped with multi-spectral cameras, will be able to accurately detect *L. oryzophilus* infestations and related damage within rice fields.

The goal of this study was to assess the effectiveness of using UAVs and digital aerial imagery to predict the distribution of *L. oryzophilus* in controlled experiments at the LSU AgCenter rice research station in Crowley, LA. Various vegetation indices were compared to ground data to determine which index accurately predicted both weevil pressure and rice yields. Potential applications of remote sensing to rice insect management include the detection of damage from soil insects, such as *L. oryzophilus*, which are not easily sampled on large commercial fields. The ability to estimate *L. oryzophilus* densities with UAVs would allow for rapid determination of spatial distribution of infestations in commercial rice fields. This would greatly improve our understanding of the pests impacts on rice and potentially lead to precision targeted control tactics. Remote sensing of weevil populations could also improve our ability to monitor the effectiveness of widely applied prophylactic insecticidal seed treatments, so that appropriate measures can be taken if failures occur due to the development of insecticide resistance.

## 6.2. Methods

A two-year experiment (2019 and 2020) was conducted at the H. Rouse Caffey Rice Research Station in Crowley, LA to assess the feasibility of using remote sensing technology to predict *L. oryzaophilus* populations in rice (Table 6.1.). Different insecticidal seed treatments were applied to drill seeded rice plots (1.65 m × 6.0 m in size) using a completely randomized design. This ensured that *L. oryzaophilus* were excluded from some rice plots and allowed to feed on others. Research has shown that the seed treatments, chlorantraniliprole (Dermacor X100) and thiamethoxam (Cruiser 5SF), act on different life stages of *L. oryzaophilus* (Lanka et al. 2013b). Chlorantraniliprole accumulates in the roots of rice plants, providing adequate control of weevil larvae for up to 6 weeks after permanent flooding (Lanka et al. 2013a, Villegas et al. 2019). Chlorantraniliprole typically reduces *L. oryzaophilus* infestations by approximately 90 % relative to untreated plots (Hummel et al. 2014). In contrast to this, thiamethoxam moves systemically through rice plants, with higher concentrations of the insecticide being found in the leaves (Lanka et al. 2013a). Thiamethoxam is more effective at controlling adult weevils, which reduces the number of eggs and larvae of the pest (Lanka et al. 2012, Lanka et al. 2013b). After the 5–6 leaf stage, concentration of thiamethoxam in rice foliage decreases, resulting in reduced effectiveness against weevil larvae in the latter stages of rice growth (Lanka et al. 2012, Lanka et al. 2013a). Thiamethoxam typically reduces *L. oryzaophilus* populations by up to 50 % relative to untreated plots (Hummel et al. 2014). Using this information, we manipulated *L. oryzaophilus* populations using insecticidal seed treatments, thereby subjecting rice plants to variable levels of weevil pressure.

Table 6.1. Planting, harvesting and activity dates for remote sensing fields.

Activity	2019	2020
Planting	17 <sup>th</sup> April	2 <sup>nd</sup> April
Flood	5 <sup>th</sup> June	2 <sup>nd</sup> June
Flight 1	25 <sup>th</sup> June	19 <sup>th</sup> June
Flight 2	5 <sup>th</sup> July	26 <sup>th</sup> June
Flight 3	19 <sup>th</sup> July	2 <sup>nd</sup> July
Harvest	29 <sup>th</sup> August	28 <sup>th</sup> August

The upper label rate of Dermacor X-100 (80 g AI ha<sup>-1</sup>) was used to achieve a high level of weevil control. These were considered low-pressure plots. A high, (186.8 g AI ha<sup>-1</sup> – full recommended rate) medium (93.4 g AI ha<sup>-1</sup> – half the recommended rate) and low (46.7 g AI ha<sup>-1</sup> – one quarter of the recommended rate) rate of Cruiser 5SF was used to simulate different levels of *L. oryzaophilus* feeding pressure. Untreated control plots were used to generate high weevil pressure data. In 2019, each treatment was replicated 5 times, so that the experiment consisted of 25 plots in total. In 2020, an additional 5 untreated plots were planted so that the experiment had 30 plots in total. The weevil populations were augmented on these plots by adding 50 adult weevils per plot after the permanent flood was applied. Adult weevils were hand collected from untreated rice plants in buffer plots planted on the Crowley Rice Research Station.

Digital aerial images were captured using an UAV (DJI MatricePro<sup>TM</sup> drone, Da-Jiang Innovations, Shenzhen, China) equipped with a multi-spectral camera (MicaSense RedEdge-MX, AgEagle Inc., Kansas, USA). The camera captured wavelengths of light in the blue (475 nm), green (560 nm), red (668 nm), red-edge (717 nm) and near-infrared (840 nm) spectral range. The images were analyzed using various vegetation indices to see which one was most suitable for predicting *L. oryzaophilus* infestations and related yield loss. Normalized Difference Vegetation Index (NDVI), Normalized Difference Red Edge (NDRE), Modified

Chlorophyll Absorption in Reflectance Index (MCARI), Green Normalized Difference Vegetation Index (GNDVI), Soil-Adjusted Vegetation Index (SAVI) and the Visual Atmospheric Resistance Index (VARI) were used. These indices use different wavelengths of light to calculate plant cover and production, and each has its pros and cons (Zhang et al. 2019) (Table 6.2.). The most commonly used vegetation index is NDVI (Prabhakar et al. 2011, Zhang and Kovacs 2012). It determines the density of green vegetation in an area of land by measuring the amount of red and near-infrared (NIR) light being reflected by plants.

Higher NDVI values indicate healthy dense vegetation, while lower NDVI values indicate sparse vegetation (Prabhakar et al. 2011). Unfortunately, NDVI is sensitive to the amount of light reflected by background soil or water and the amount of light scattered due to atmospheric conditions (Al-Khindi et al. 2017, dela Torre et al. 2021). Therefore, other vegetation indices, that adjust for these issues, were used to test the accuracy of the NDVI results and to determine whether other measures can be used more reliably in rice systems.

Flight missions commenced 20 days after permanent flooding, and three missions were conducted during the experiment (approximately 10 days apart) (Table 1). Flights were conducted on clear, sunny, windless days to reduce the amount of reflectance variation caused by water movement and atmospheric back scattering. Solar zenith angle can significantly influence light reflectance and therefore the consistency of vegetation indices, especially in rice paddies (Ishihara et al. 2015). Changes in light intensity typically decrease with increasing zenith angle. Therefore, flights were conducted between 10:00 am and 11:00 am. These times were chosen to accommodate optimum zenith angle, whilst also reducing the effects of sun glint on flooded plots (Jiang et al. 2019). For each mission a black and white calibration plate was used to calibrate the images taken, according to baseline reflectance. A downwelling light sensor was used to correct for unexpected changes in light intensity during flight missions.

Table 6.2. Description of different vegetation indices and their calculation formulas (VI = Vegetation Index).

VI	Formula	Description	Reference
NDVI	$(\text{NIR} - \text{Red})/(\text{NIR} + \text{Red})$	Green biomass, canopy greenness, phenology	Rouse et al. 1973
GNDVI	$(\text{NIR} - \text{Green})/(\text{NIR} + \text{Green})$	Green biomass, canopy greenness, phenology (more sensitive to chlorophyll-concentration)	Gitelson et al. 1996
MCARI	$[(\text{RedE} - \text{Red}) - (\text{RedE} - \text{Green})] \times (\text{RedE}/\text{Red})$	Canopy chlorophyll content, canopy phenology, leaf area index	Maccioni et al. 2001
NDRE	$(\text{NIR} - \text{RedE})/(\text{NIR} + \text{RedE})$	Sensitive to changes in medium to high levels of chlorophyll content, within field variation	Daughtry et al. 2000
SAVI	$[(\text{NIR} - \text{Red}) / (\text{NIR} + \text{Red} + 0.5)] \times (1.5)$	Plant structure, reduced background noise	Huete et al. 1988
VARI	$\text{VARI} = (\text{Green} - \text{Red}) / (\text{Green} + \text{Red} - \text{Blue})$	Vegetation fraction, low sensitivity to atmospheric effects	Gitelson et al. 2002

The drone used in the experiment was flown at a height of 10m, 30m and 60m at speed of 5m/s for each mission. Approximately 750 images were taken for each of the 5 color bands (3750 images per mission date). Each raw image was saved as a .tiff and the optical, red edge and infrared imagery was orthorectified and stitched/mosaicked using Pix4Dmapper<sup>®</sup> software (Zhang et al. 2014). Thus, images were corrected according to geographical and topographical data and stitched to form a complete map of the surveyed plots (Brewster et al. 1999). A front overlap and side overlap of 85% and 65% was used, respectively. This level of overlap enhances the reliability and accuracy of the image stitching process and the resultant mosaic (Zhang et al. 2014). Pix4Dmapper<sup>®</sup> was also used to generate images depicting values from the various vegetation indices for each of the plots (Zhang et al. 2014). The plots were then isolated using a boundary tool and the image with related geospatial data was saved for further processing in QGIS. To ensure accuracy, images were further trimmed to fit the precise plot size (1.65 m × 6.0 m). The images and data were imported to QGIS as raster files and subsequently rendered using the single band pseudo-color setting. A red-green color ramp was used to depict the differences in values within each plot. The zonal and raster statistics plug-ins was then used to calculate average vegetation index value for each of the various indices per plot.

Three soil core samples were taken from each plot after every flight mission. The core samples were used to quantify mean *L. oryzaephilus* density per plot. This served as ground-truth data to determine the relationship between weevil density and average vegetation index values for each sampling date and flight height. At the end of the growing season plots were mechanically harvested and grain yield and moisture were recorded.

A One-Way Analysis of Variance (ANOVA) was conducted in Python, using the Pingouin and Statsmodels packages, to compare weevil data across each treatment type (McKinney 2010, Seabold et al. 2010, Vallat 2018). A Tukey HSD analyses was conducted

to separate means using the same Python packages. Linear regression models were used to compare values from different vegetation indices to *L. oryzaophilus* densities and yield data. The models were assessed for goodness of fit and accuracy to determine whether remote sensing can accurately estimate weevil density and yield, and to determine which vegetation index was the most reliable. Linear regressions and cross-validation processes were conducted in Python using the NumPy and Sci-kit learn packages (McKinney 2010, Pedregosa et al. 2011, Harris et al. 2020).

### 6.3. Results

One flight mission (Flight 2, 2019) was excluded from the analyses, due to errors related to unforeseen climatic factors during data collection. Data and results collected from flights conducted at 10 m are not included because the orthomosaic stitching process was not reliable at that height. Yield data collected in 2020 was severely affected by winds from Hurricane Laura (Schultz 2020). Therefore, yield results from the 2020 experiment are not reported herein.

The ANOVA results show differences in *L. oryzaophilus* larval density among treatments in plots sampled in both 2019 ( $F = 14.55$ ,  $DF = 4, 19$ ;  $P < 0.001$ ) (Fig. 6.1.A) and 2020 ( $F = 11.68$ ;  $DF = 5, 23$ ;  $P < 0.001$ ) (Fig. 6.1.B). The Shapiro-Wilks test indicated the residuals were normally distributed in both the 2019 ( $w = 0.97$ ,  $P = 0.63$ ) and 2020 ( $w = 0.96$ ,  $P = 0.53$ ) models. Additionally, the Bartlett's test was conducted to ensure homoscedasticity in both the 2019 ( $t = 2.96$ ,  $P = 0.57$ ) and 2020 ( $t = 8.73$ ,  $P = 0.12$ ) models.

In 2019 larval densities in chlorantraniliprole plots were 1.9, 2.1, 2.2 and 2.7-fold lower than the densities in thiamethoxam (high, medium, and low) treated plots and untreated plots, respectively. Furthermore, plots treated with a high rate of thiamethoxam had larval densities that were 1.5-fold lower than untreated plots.



Plots planted in 2020 experienced weevil pressure that was on average 1.5-fold greater than those planted in 2019. Larval densities in chlorantraniliprole treated plots did not differ significantly from plots treated with a high rate of thiamethoxam. However, relative to chlorantraniliprole treated plots, *L. oryzaephilus* densities increased significantly in mid-rate and low-rate thiamethoxam plots (1.7 and 2.03- fold increase, respectively), as well as in untreated (2.3-fold increase) and augmented plots (1.9-fold increase) (Fig. 6.1B). A high-rate of thiamethoxam significantly decreased weevil numbers when compared to low-rate thiamethoxam plots, untreated plots, and augmented plots. Thus, plots planted in both years experienced a range of weevil pressure.

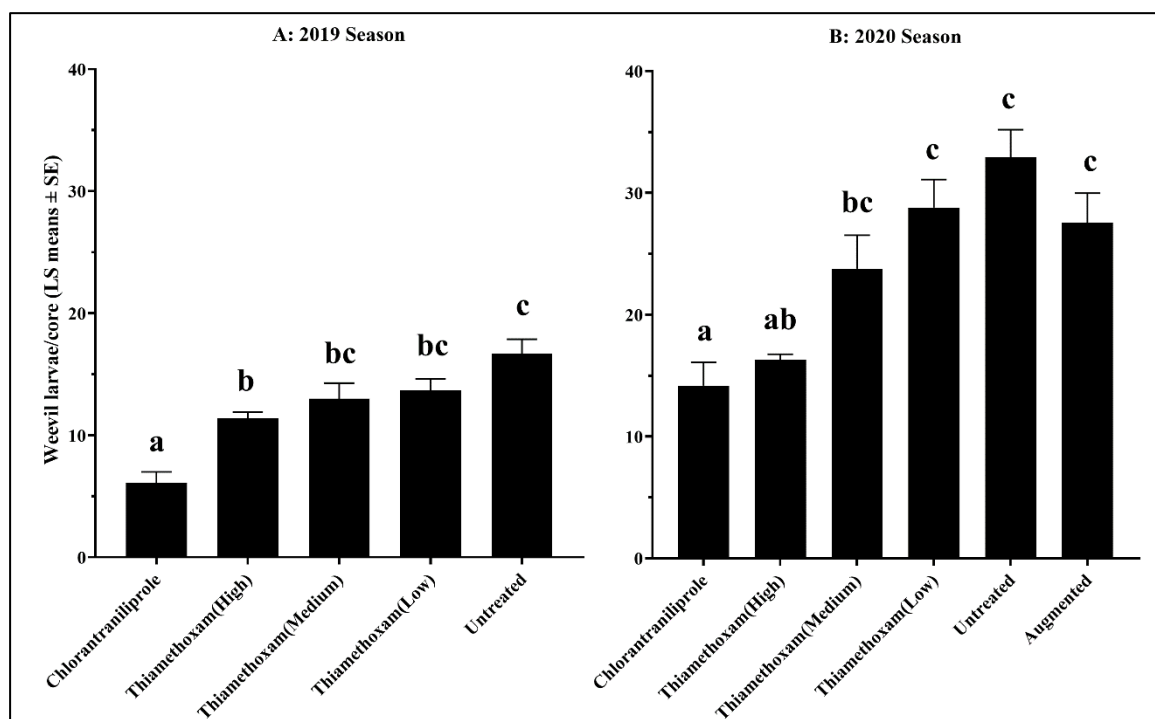


Figure 6.1. *Lissorhoptrus oryzaephilus* larval density per core in plots subjected to different insecticidal seed treatments in the 2019 (A) and 2020 (B) growing season in Crowley, Louisiana. Bars within a figure accompanied by the same letter do not differ significantly (Tukey's HSD:  $\alpha > 0.05$ ).

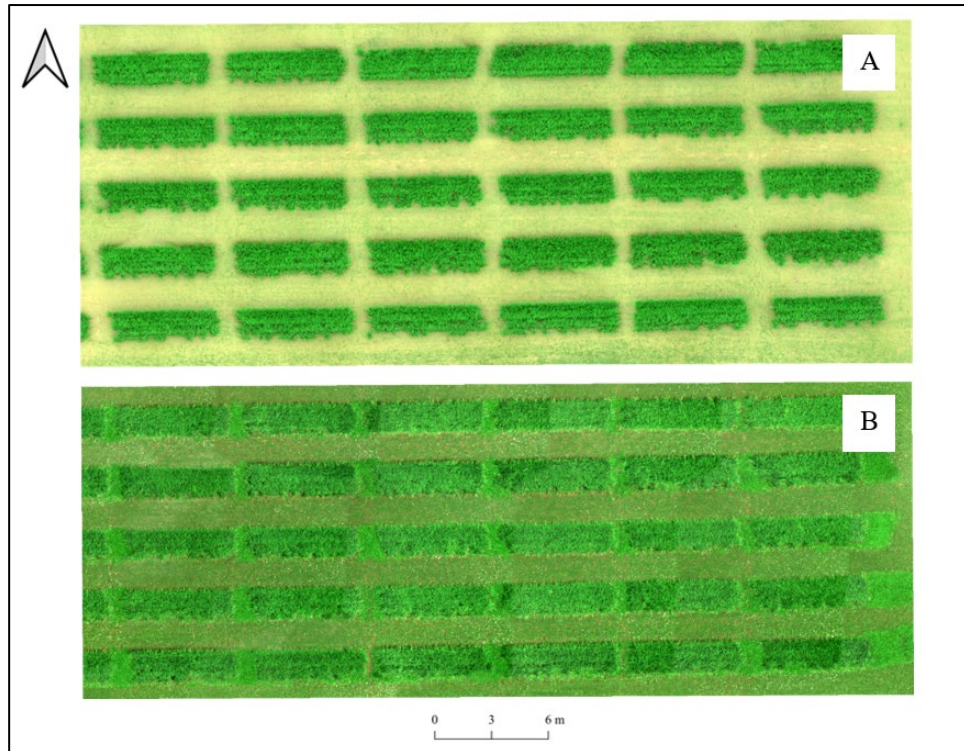


Figure 6.2. Orthomosaic showing differences between images collected at Flight 1 (A) and Flight 3 (B) from remote sensing plots planted in 2019 Crowley, Louisiana.

A comparison of linear regression models, showing the relationship between different vegetation indices and *L. oryzaephilus* densities in 2019, reveals that the strength and reliability of correlations vary according to the height and date of remote sensing flights (Table 6.3.). Flight 1 produced more significant relationships (with the exception of VARI data collected at 60 m) and better correlations relative to Flight 3. With the exception of GNDVI, all indices were more strongly correlated with weevil density when taken at 30 relative to 60 m. Spectral data from Flight 3 was influenced by an increased presence of duckweed, *Lemna* spp. (Alismatales: Araceae), which was in the latter part of the season (Fig. 6.2.). Although the MCARI vegetation index produced the best correlations, the log-likelihood and AIC values for this index were highly variable. This indicates that predictions based on the MCARI models were not reliable as predictions produced by other indices. The goodness of fit statistics suggests that the NDVI, GNDVI, NDRE and SAVI models produce more reliable relationships between vegetation index values and weevil density.

Table 6.3. Results of linear regressions assessing the relationship between various vegetation indices at 30 and 60 m flight heights and *L. oryzophilus* densities in plots planted in 2019 in Crowley, Louisiana (DF = 1, 23).

Mission	VI	Adj. R-Square		Log-Likelihood		AIC		F-Value		P-Value	
		30	60	30	60	30	60	30	60	30	60
<b>Flight 1</b>	NDVI	0.53	0.35	73.27	74.53	-142.5	-145.1	28.13	13.74	<0.001	0.001
	GNDVI	0.22	0.25	73.02	78.14	-142.0	-152.3	7.59	9.134	0.011	0.006
	MCARI	0.51	0.46	55.8	56.25	-107.6	-108.5	24.31	22.12	<0.001	<0.001
	NDRE	0.39	0.13	75.75	83.73	-147.5	-163.5	14.4	4.54	0.001	0.044
	SAVI	0.49	0.14	69.45	64.88	-134.9	-125.7	23.83	4.722	<0.001	0.041
	VARI	0.37	0.12	64.11	47.22	-124.2	-90.43	14.89	0.36	<0.001	0.851
<b>Flight 3</b>	NDVI	0.06	0.15	75.89	94.91	-147.8	-185.8	2.6	3.97	0.12	0.058
	GNDVI	0.05	0.04	70.08	88.27	-136.2	-172.1	1.28	0.85	0.269	0.367
	MCARI	0.46	0.07	86.76	41.41	-169.5	-78.82	21.6	1.574	<0.001	0.222
	NDRE	0.04	0.001	72.93	83.8	-141.9	-163.6	1.94	0.2	0.177	0.659
	SAVI	0.17	0.07	82.14	66.86	-160.3	-129.7	5.952	1.69	0.023	0.207
	VARI	0.22	0.35	58.54	77.41	-113.1	-151.5	7.49	13.72	0.012	0.001

In 2020, a comparison of linear regression models show that vegetation indices performed well across Flights 1, 2 and 3 (Table 6.4.). All relationships, with the exception of one (NDRE data collected at 30 m on Flight 3), were significant. The correlations between vegetation indices and weevil densities were usually stronger for Flights 1 and 2. However, the Flight 3 linear regressions modelling the relationship between weevil density and the MCARI, SAVI and VARI indices also had high  $R^2$  values. Index values from images taken at 60 m were better correlated with *L. oryophilus* numbers, except for the VARI models for Flight 3. Once again, the log-likelihood and AIC values for the MCARI models indicate that this vegetation index performs poorly relative to other indices. The goodness of fit statistics for 2020 suggest that the NDVI, GNDVI and NDRE models produce more reliable relationships between vegetation index values and weevil density.

Yield and vegetation indices are positively correlated. The correlations between yield and vegetation indices are particularly high across all indices for images taken at 30 m for Flight 1, with  $R^2$  values ranging between 0.51 for GNDVI, and 0.73 for NDVI (Table 6.5.). These models were also highly significant. The relationships were more variable among indices for other heights and dates. Again, spectral interference from large stands of duckweed may have influenced the reliability of vegetation indices measured during Flight 3. Log-likelihood and AIC values for models of Flight 1 at 30 m suggest that the MCARI vs weevil density relationship was the least reliable for yield. For this same date and height, NDRE was considered the best model, while NDVI was the index with the strongest relationship to yield ( $R^2 = 0.73$ ).

Table 6.4. Results of linear regressions assessing the relationship between various vegetation indices and *L. oryzophilus* densities in plots planted in 2020 in Crowley, Louisiana (DF = 1, 28).

Mission	VI	Adj. R-Square		Log-Likelihood		AIC		F-Value		P-Value	
		30	60	30	60	30	60	30	60	30	60
<b>Flight 1</b>	NDVI	0.38	0.45	73.31	79.18	-142.6	-154.4	17.39	24.69	<0.001	<0.001
	GNDVI	0.25	0.41	71.3	80.09	-138.6	-156.2	10.70	20.87	0.003	<0.001
	MCARI	0.46	0.52	44.11	51.04	-84.22	-98.08	25.62	31.81	<0.001	<0.001
	NDRE	0.31	0.33	73.76	81.20	-143.5	-158.4	14.18	14.97	0.001	0.001
	SAVI	0.43	0.53	65.80	72.39	-127.6	-140.8	22.96	33.90	<0.001	<0.001
	VARI	0.45	0.47	66.82	69.25	-129.6	-134.5	25.01	27.23	<0.001	<0.001
<b>Flight 2</b>	NDVI	0.37	0.38	88.14	95.03	-172.3	-186.1	18.42	18.76	<0.001	<0.001
	GNDVI	0.28	0.281	92.86	98.43	-181.7	-192.9	12.05	12.34	0.002	0.002
	MCARI	0.38	0.49	42.98	46.92	-82.0	-89.84	18.25	29.8	<0.001	<0.001
	NDRE	0.17	0.22	86.77	93.78	-169.5	-183.6	6.873	9.03	0.014	0.006
	SAVI	0.39	0.47	68.19	71.20	-132.4	-138.4	19.47	26.96	<0.001	<0.001
	VARI	0.33	0.43	62.18	72.99	-120.4	-142.0	15.42	22.39	0.001	<0.001
<b>Flight 3</b>	NDVI	0.28	0.33	102.84	97.69	-201.7	-191.4	12.32	15.56	0.002	<0.001
	GNDVI	0.11	0.21	101.64	91.96	-199.3	-179.9	4.61	8.77	0.041	0.006
	MCARI	0.37	0.56	41.96	54.26	-79.9	-104.5	18.49	38.38	<0.001	<0.001
	NDRE	0.062	0.14	94.34	88.96	-187.7	-173.9	2.92	5.52	0.098	0.026
	SAVI	0.38	0.54	69.73	80.61	-135.5	-157.2	18.97	36.04	<0.001	<0.001
	VARI	0.35	0.28	76.02	67.62	-148.0	-131.2	16.73	12.01	<0.001	0.002

Table 6.5. Results of linear regressions assessing the relationship between various vegetation indices and yield in plots planted in 2019 in Crowley, Louisiana (Df = 1, 23).

Mission	VI	Adj. R-Square		Log-Likelihood		AIC		F-Value		P-Value	
		30	60	30	60	30	60	30	60	30	60
<b>Flight 1</b>	NDVI	0.73	0.71	79.84	84.54	-155.7	-165.1	63.49	58.83	<0.001	<0.001
	GNDVI	0.51	0.66	78.68	87.781	-153.4	-171.6	25.13	46.47	<0.001	<0.001
	MCARI	0.71	0.78	62.65	67.36	-121.3	-130.7	58.8	86.75	<0.001	<0.001
	NDRE	0.60	0.05	81.58	82.12	-159.2	-160.2	36.66	1.205	<0.001	0.284
	SAVI	0.66	0.08	74.53	63.54	-142.6	-123.1	47.33	1.92	<0.001	0.180
	VARI	0.69	0.02	73.13	47.27	-142.3	-90.45	54.92	0.055	<0.001	0.816
<b>Flight 3</b>	NDVI	0.22	0.10	77.59	94.17	-155.2	-184.3	6.354	2.44	0.019	0.132
	GNDVI	0.16	0.02	72.09	88.01	-140.2	-172.0	5.517	0.35	0.028	0.561
	MCARI	0.59	0.45	90.47	48.68	-177.0	-93.37	37.01	20.98	<0.001	<0.001
	NDRE	0.23	0.02	75.76	83.90	-147.5	-161.4	8.270	0.41	0.009	0.531
	SAVI	0.10	0.39	81.11	72.68	-158.2	-141.4	3.652	16.33	0.069	0.001
	VARI	0.43	0.13	62.48	73.62	-118.5	-140.8	18.77	3.410	<0.001	0.078

Considering the results from the yield regressions, and the results of the regressions in 2019, all image data from Flights 1 (both 2019 and 2020,  $n = 50$ ) taken at a height of 30 m were selected for further analysis using a test/train split and cross validation procedure in Python. The test size was set to 0.2 (11 observations). This was done to determine whether the relationship between weevil density and different vegetation indices could be used to make reliable predictions.

All vegetation indices had a significant negative relationship with number of *L. oryzoophilus* larvae found per soil core (Table 6.6.). However, the correlations between weevil density and the MCARI ( $R^2 = 0.07$ ) and VARI ( $R^2 = 0.11$ ) vegetation indices were weak (Figure. 6.3. C & F). These two vegetation indices were also subject to the most error as revealed by our test data (test size = 0.2). The mean difference between actual and predicted values for MCARI and VARI was 7.24 % and 4.68 %, respectively (Table 6.6.). This represents a 3.2 to 5.2-fold decrease in predictive capability for MCARI, and a 2.1 to 3.3-fold decrease in predictive capability for VARI, relative to the other indices. This is reflected in the root mean squared error (RMSE) for each of the indices.

In contrast to this, mean NDVI, GNDVI, NDRE and SAVI values were strongly related to weevil densities (Fig. 6.3. A, B, D & E). The SAVI model had the highest  $R^2$  value, while the GNDVI model was subject to the least error. According to the values for the intercepts and regression coefficients, the NDRE model was the most sensitive to changes in weevil populations among plots. An increase in 7.9 weevils per core is predicted to decrease the mean NDRE value by 0.05 per plot. Increases of 10.8, 19.2 and 21.7 weevils per core are predicted to result in similar decreases in SAVI, GNDVI and NDVI values, respectively. All models satisfied the linear regression assumptions, except for the SAVI model, which had negatively skewed data (skewness = -1.02, kurtosis = 3.8).

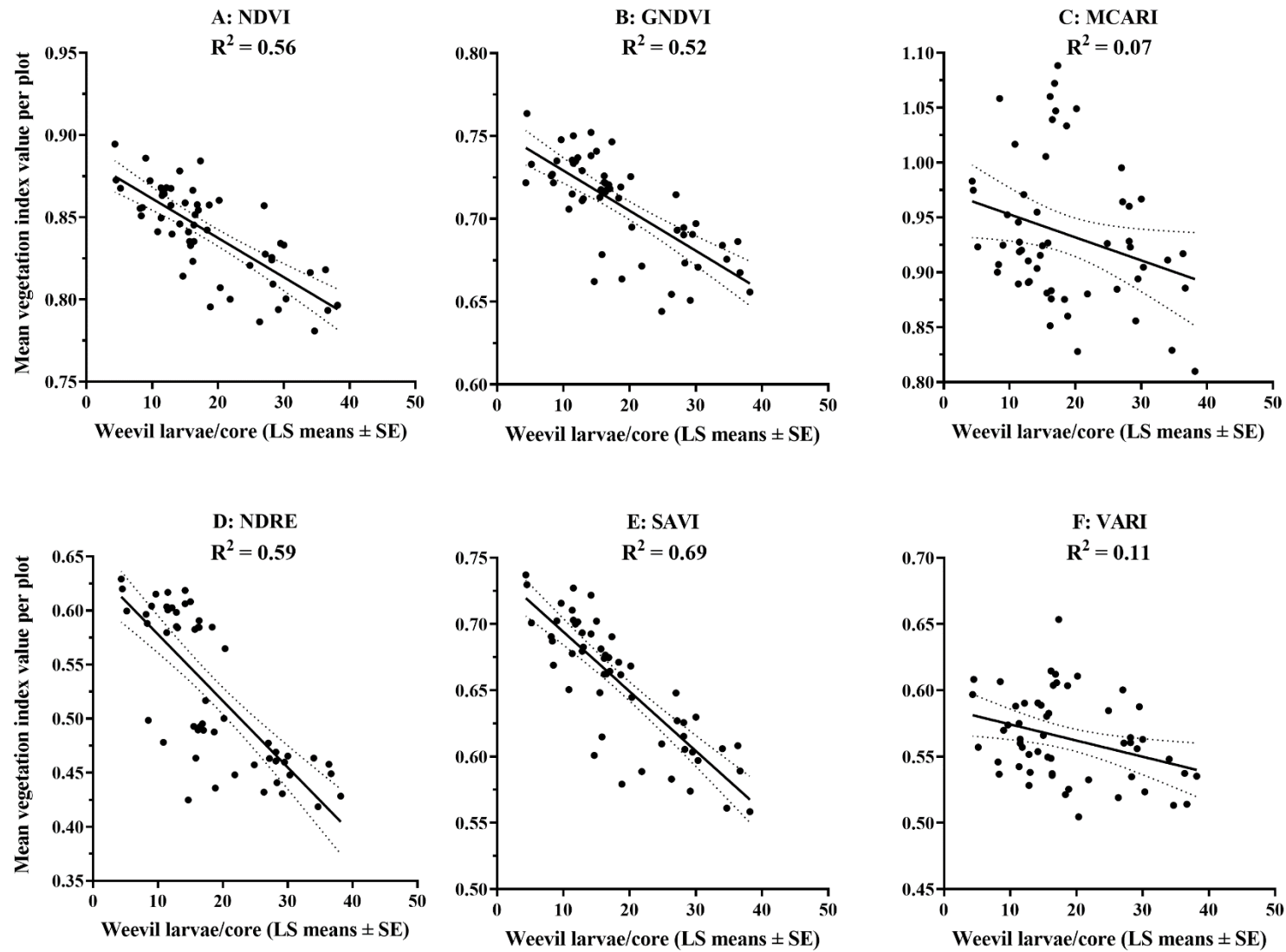


Figure 6.3. Linear regression depicting the relationship between *L. oryzaephilus* density and different vegetation indices in plots planted in 2019 and 2020, for Flight 1 at 30 m above ground level.



Table 6.6. Results of cross validated linear regressions assessing the relationship between various vegetation indices and *L. oryzaophilus* densities in plots planted in 2019 and 2020, for Flight 1 at 30 m above ground level. Test size = 11 data points. (Df = 1, 53).

VI	Intercept	Regression Coefficient	RMSE	Actual vs Predicted (% difference)	F-value	P-value
NDVI	0.88014	-0.00231	0.02382	2.06	70.1	<0.001
GNDVI	0.75394	-0.00256	0.01896	1.40	60.28	<0.001
MCARI	0.96133	-0.00221	0.09919	7.24	4.51	0.038
NDRE	0.64374	-0.00629	0.02920	1.78	77.5	<0.001
SAVI	0.73756	-0.00458	0.01981	2.24	123.3	<0.001
VARI	0.57838	-0.00108	0.04662	4.68	6.7	0.012

#### 6.4. Discussion

Manipulation of weevil populations was successfully achieved in plots subjected to different insecticidal seed treatments. The results show that different levels of *L. oryzaophilus* related stress and injury can be detected by reflectance data collected via airborne remote sensing devices. This the first study to record significant relationships between vegetation indices and *L. oryzaophilus* densities in rice agroecosystems. It provides further support for the use of UAVs as a promising and useful tool for insect population estimation and IPM, especially for cryptic pests or crops that are difficult sample (Nansen and Elliot 2015).

Canopy multispectral reflectance can provide information on the growth and health of rice plants, but paddy ecosystems are notoriously difficult to evaluate using digital aerial imagery and multispectral cameras. This is because, in addition to various factors, such as soil background and canopy structure, reflectance from the flooded surfaces of rice paddies can result in additional background noise, or back scatter, that can affect the performance of vegetation indices and therefore reduce accurate detection of crop stress (Liu et al. 2020).

Nevertheless, the formulas of different indices can account for variation in reflectance data as related to ground, atmospheric and climatic conditions (Al-Khindi 2017, Zhang et al. 2019).

It is evident from our data that vegetation indices are not equally useful for detecting *L. oryzaephilus* injury. Multiple studies have assessed the effectiveness of using NDVI to assess nitrogen uptake in rice, to predict rice yields at different growth stages and to monitor diseases such as sheath blight and blast (Tubana et al. 2012, Liu et al. 2015, Guan et al. 2019, Wang et al. 2019, Zhou et al. 2020). This commonly used vegetation index can likewise be employed to accurately assess *L. oryzaephilus* density. Research by Wang et al. (2018) and Ernawati et al. (2019) has shown that NDVI can also be used to predict brown plant hopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae), and rice leaf folder, *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Crambidae) incidence and injury in rice.

Although NDVI is undoubtedly a suitable index, our train/test split data show that GNDVI and NDRE can more accurately predict *L. oryzaephilus* populations. This is likely due to the decreased levels of red-band saturation associated with these indices (Thompson et al. 2015, Brinkhoff et al. 2019). Instead of Red light, GNDVI and NDRE calculate canopy chlorophyll content using Green and Red Edge wavelengths of light, respectively (Liu et al. 2021). These wavelengths of light can better penetrate the canopy of crops and can therefore provide a more accurate readings of photosynthetic rates and chlorophyll concentrations (Duan et al. 2019). NDRE is particularly sensitive to differences in more homogenous areas that are characterized by higher productivity or greater crop coverage (Brinkhoff et al. 2019). Thus, NDRE-based models often detect smaller changes in productivity and generate more accurate predictions in rice crops (Thompson et al. 2015, Brinkhoff et al. 2019, Duan et al. 2019, Zhang et al. 2019). The SAVI model had the highest  $R^2$  value, suggesting this index is closely correlated with *L. oryzaephilus* injury. The Huete et al. (1988) paper demonstrated the enhanced performance of SAVI in areas where vegetative cover was poor and in areas with

increased amounts of bare exposed soil, as is the case in small plot experiments. SAVI corrects for dynamic soil-vegetation spectral behavior and therefore accounts for greater background variation than other indices, such as NDVI. The SAVI model also provides an assessment of plant structure, which is likely influenced by *L. oryzaephilus* root feeding (Zou et al. 2004a).

The VARI and MCARI models did not adequately describe fluctuations in weevil density. These indices suffer from issues related to saturation and background noise (Stroppiana et al. 2009, Cui et al. 2019). They tend to perform more reliably if employed earlier in the rice growing season (Zhou et al. 2017). In fact, accurate yield predictions in rice are typically influenced by the growth stage of the crop (Harrell et al. 2011), with the predictive capability of many vegetation indices decreasing after the boot stage (Wang et al. 2019). Inaccurate predictions are particularly problematic after the ripening and heading stages (Mihn et al. 2013, Guan et al. 2019). This is likely due to increases in plant dry matter and panicle interference (Sakamoto et al. 2011, Zhang et al. 2019). The advantage of remote sensing in younger rice is supported by our finding that predictive ability was best at the earliest flights. Although, the accuracy of yield multi-spectral data in this study was affected by the presence of weeds at later sampling dates, it is important to note the best times and growth stages for predicting yields when planning future remote sensing experiments. For insect pest management, early season information is preferable because farmers need to make management decisions before insect populations become damaging (Mihn et al. 2013). Therefore, the reduced reliability of reflectance data in later season rice is not concerning from a pest management perspective.

Unfortunately, the impacts of Hurricane Laura on 2020 yield data mean our conclusions regarding yield predictions are based on only a single year of data. However, we are confident the ability to predict yield would likely have been validated in the second year.

This is because of the extremely well-documented relationship between weevil density and rice yield (Zou et al. 2000, Wilson et al. 2021 a,b). Our results from 2019, along with previous studies indicate that NDVI, GNDVI, NDRE and SAVI can also be used to predict both yields and weevil-related yield loss.

Our study also provides insights on the influence of UAV flight height on remote sensing ability. Unfortunately, flights at 10 m were impacted by distortion errors, indicating this height may not be suitable for remote sensing in rice. Reduction in drone speed may help to overcome these issues. Although vegetation indices performed well in assessing weevil density at a height of 60 m in 2020, the 2019 weevil models and yield models were more stable at a height of 30 m. Siefert et al. (2019) shows that UAV flights conducted at low altitudes (15–30 m above canopy), with a high forward overlap rate between images, result in improved reconstruction detail and accuracy when measuring vegetation. Analysis of individual, unstitched photos is possible. The high resolution provided by images taken at 10 m would be useful for such an analysis. However, careful pre-flight planning would be needed to ensure plots were fully captured in the individual images.

Many aspects of the cropping environment impact the measurement and accuracy of reflectance data and how they relate to pest populations and yields. Crop stress is influenced by cultivar, nutrient and water availability, and by numerous diseases, weeds, and insect pests. The combined effects of these factors feature in the reflectance patterns that are recorded via digital aerial images. This can reduce the reliability of results, especially when one is trying to identify or predict the source of crop stress. Although our study demonstrates the ability of multiple indices to accurately predict weevil infestations, the trials were conducted with a single cultivar and similar production practices across years. Thus, the predictive models presented herein are not likely applicable across the diverse rice production systems in the southern US. Future studies should aim to examine the relationship between

vegetation indices and weevil infestations under a variety of production conditions in order to develop predictive models that can be more widely utilized.

*Lissorhoptrus oryzophilus* management is currently prophylactic and therefore early detection is unlikely to influence management decisions, however the availability of quick, flexible and accurate sampling techniques can provide stakeholders with an efficient tool for scouting farms for pesticide resistance. Prior to the adoption of seed treatments, foliar insecticides targeted adult weevils and provided little relief of active larval infestations (Stout et al. 2011). The severity of larval infestations often went unnoticed until severe impacts to plant health, such as uprooted and floating rice were observed (Way 2016). If these infestations were detected early, corrective action such as draining and drying could be taken. Future availability of chemical controls targeting larvae could further increase the utility of this sampling approach.

Future research should aim to re-examine remote sensing for weevil sampling across a larger sampling area, with an increase in the number of flight missions conducted per season, to improve model parameters and predictions. Additional integrated vegetation indices and index transformations can be explored and used to determine how reflectance data is specifically influenced by *L. oryzophilus* feeding and resultant changes in crop physiology. Deep learning algorithms and improved image processing techniques, such as object-oriented segmentation (which allow for the isolation of plots), can also be used to improve predictions by reducing background noise (Ma et al. 2019, Qiu et al. 2020). Finally, other active and passive remote sensing platforms, such as satellite imagery and lidar (light detection and ranging) have shown potential for monitoring numerous stationary pests and flying insect species (Kirkeby et al. 2016, El-Ghany et al. 2020). These additional platforms could be further explored as potential avenues for improved pest sampling in rice agroecosystems.

In conclusion, our study provides the first demonstration that remote sensing can accurately estimate *L. oryzaophilus* infestations in rice. Further research is needed before the technology can be utilized in *L. oryzaophilus* management in a commercial setting. This is another example of a growing body of evidence demonstrating the potential for incorporation of UAV sampling strategies in row crop IPM.

## CHAPTER 7. GENERAL CONCLUSIONS

### 7.1. Integrated Pest Management and Insecticidal Seed Treatments

Integrated pest management is the prevailing paradigm for the sustainable reduction of damaging insect pest populations in applied agricultural research and crop production. It is a commonly recognized and used term among pest management stakeholders and there are numerous IPM practitioners and success stories. The foundations of IPM lie in the integrated use of multiple biological, cultural, physical, and chemical intervention measures for the control of pests within a crop. These seek to identify, manage, and reduce the risks associated with pest infestations in a way that decreases the economic, health, and environmental consequences of chemical control practices. Therefore, IPM is a vital approach to crop protection that helps to facilitate the success and sustainability of agriculture worldwide. However, multiple challenges exist that hinder the development and adoption of successful IPM programs, resulting in lack of adherence to its conceptual foundations.

The widespread adoption of prophylactic pest control tactics, such as transgenic crops and seed treatments, is one of the challenges facing IPM implementation. The ease of applicability of these control tactics serves as a convenient safety net for farmers, leading to underutilization of important IPM practices, including the use of thresholds and pest scouting procedures. As such, chemical controls increasingly form the basis of crop protection programs and preventative treatments have led to the extensive use of a limited number of products and chemistries.

In Chapter 2, challenges to the development of a successful IPM program in Louisiana rice were discussed. Rice production in the state is confronted by numerous constraints, including damaging infestations of insect pests. Infestations of rice water weevil, *L. oryophilus*, and lepidopteran stem borers, namely *E. loftini*, *D. saccharalis* and *C. plejadellus*, can cause substantial economic losses. These pests are typically managed using

insecticidal seed treatments, particularly chlorantraniliprole, which is applied as a precautionary insecticide on > 80 % of rice acreage in both Texas and Louisiana. Large scale applications of a single insecticidal chemistry, in the absence of diversified management tools, pest monitoring programs and integrated techniques, could have negative environmental impacts and result in pesticide resistance problems.

This work argues that a return to the fundamental principles of IPM, and a renewed focus on the ecological functioning of rice agroecosystems, could enhance rice pest management by improving the deployment of insecticidal seed treatments. The aim of the dissertation was to assess the feasibility of using different pest management tactics to reframe the way that we think about insecticidal seed treatments as a control tactic. Traditional and modern pest management practices were explored in small-plot experiments and research was conducted in an effort to gain a better understanding of pest spatial distributions in commercial rice fields. These applied and ecological studies can expand current pest management recommendations to include insecticidal seed treatments more effectively in rice IPM and resistance management programs. Efforts were made to ensure recommendations prioritized the management of both *L. oryzaophilus* and stem lepidopteran stem borers and that they were compatible with current agronomic practices.

## **7.2. Summary of Results and Recommendations**

Extensive surveys of *L. oryzaophilus* larvae and stem borer injury in untreated commercial fields located in Acadia, Jefferson Davis and Vermillion Parishes revealed that the pests had a significant edge-biased distribution in rice. Populations were neither uniformly nor randomly distributed. Pest incidences were typically clustered near the margins of field sites, with the majority of hotspots occurring at edge-based sampling points. Multiple linear regression showed that the number of *L. oryzaophilus* larvae found per core is predicted to decrease by 1 larva for every 26.6 m moved away from the field edge. Similarly, stem



borer damage is expected to decrease by 1 whitehead per m<sup>2</sup> for every 30 m from the edge. The surveys further demonstrated that *L. oryzaephilus* numbers are influenced by distance from overwintering/alternative non-crop habitat (such as tree lines and levees containing bunch grasses), while stem borer populations were heavily influenced by region, or parish location. These data have improved our understanding of the spatial distribution of *L. oryzaephilus* and stem borers in Louisiana rice. Results also indicate that targeted applications of insecticidal seed treatments are possible. Insecticidal seed treatments could theoretically be applied solely to field margins and areas adjacent to levees, without sacrificing pest management. The generated equations can be used in conjunction with economic thresholds to calculate what percentage of commercial fields require treatment. Since pest populations are similarly distributed, targeted applications of seed treatments for *L. oryzaephilus* are likely to be compatible with stem borer management goals.

A three-year small plot experiment assessing the feasibility of integrating different insecticidal seed treatments with cultural control practices was conducted at the H. Rouse Caffey Rice Research Station in Crowley, Louisiana. The results of the study show that seed treatments are indeed important for *L. oryzaephilus* control. Consistently high levels of *L. oryzaephilus* in experimental plots demonstrate that the prophylactic use of chlorantraniliprole is justified and consistent with the goals of IPM. This finding is supported by multiple sources in the literature and by further evidence in chapter 3, showing high levels of *L. oryzaephilus* in commercial fields. Although cultural controls alone did not sufficiently decrease pest populations, they can be used successfully to improve the efficacy of insecticidal seed treatments. Early-planting improves rice yields and response to pest injury and delayed-floods work reduce both *L. oryzaephilus* and stem borer pest incidence. Although chlorantraniliprole remains the most effective tool for managing *L. oryzaephilus*, the neonicotinoid thiamethoxam can be used as an alternative to chlorantraniliprole if it is

applied correctly in conjunction with recommended cultural control practices. This is particularly true in areas with low stem borer populations. Results from the commercial fields show that stem borer populations differ significantly between parishes, and small plot experiments indicate that low levels of injury ( $< 2$  whiteheads per  $m^2$ ) do not coincide with perceptible reductions in yield. Therefore, thiamethoxam is recommended for use against *L. oryzaophilus* in Louisiana, as an effective insecticide that can help farmers reduced reliance on chlorantraniliprole.

The viability of using remote sensing to quantify insect pest populations is currently being investigated, but research indicates that it is a promising scouting tool for pests that directly affect the uptake of nutrients in plants and reduce crop growth and foliage. The efficacy of using this novel pest management tool against *L. oryzaophilus* was investigated in experimental plots at the H. Rouse Caffey Rice Research Station in Crowley, Louisiana. The *L. oryzaophilus* numbers were successfully manipulated in plots using various application rates of insecticidal seed treatments. An unmanned aerial vehicle (UAV), equipped with a 5-band multi-spectral camera, produced vegetation indices that were significantly correlated with *L. oryzaophilus* densities. High resolution, early season flights worked best for measuring *L. oryzaophilus* populations. Four different indices (NDVI, GNDVI, NDRE and SAVI) can be used to reliably predict yields and *L. oryzaophilus* induced crop stress. These indices have formulas, which calculate reflectance using NIR light, in combination with various other wavebands (Green, Red, RedEdge), to determine crop stress. Although further research is needed to test the consistency of the results and confirm the findings, this is the first study showing that remote sensing tools can be used to identify *L. oryzaophilus* stressed plants. This represents a major contribution to the rice pest management literature and indicates that remote sensing can be used for precision agriculture or employed as a scouting method for *L. oryzaophilus*, which is a difficult pest to sample for, especially in large commercial fields.

Overall, this study has elucidated the distribution of pest populations in Louisiana rice, providing insights into the potential of deploying targeted applications of insecticidal seed treatments. It has also demonstrated that a neonicotinoid seed treatment can be used as an alternative to chlorantraniliprole in areas with low levels of stem borer infestation. At approximately \$50 per hectare, chlorantraniliprole is an expensive insecticide for farmers to apply. Reductions in the amount of chlorantraniliprole applied to fields (through targeted treatments or through rotations with thiamethoxam) can reduce input costs and improve the profit margins of rice production. This also has integrated resistance management (IRM) implications. The pervasive use of chlorantraniliprole in Louisiana rice, and the resultant selective pressure on pest populations, is a recipe for the development of insecticide resistance. The creation of refuges within commercial fields and the use of alternative chemistries, together with cultural control practices, are IRM strategies that can mitigate or delay resistance and improve the longevity of widely used insecticides. Improved knowledge of pest distribution provides stakeholders with information that can help them pinpoint those areas in a field that are susceptible to pest infestations. These areas can be monitored to check for insecticide failures so that measures can be taken to prevent the spread of resistance. Directed scouting techniques could be further enhanced with the help remote sensing models. Additionally, remote sensing tools can be used (with ground-truth data) to quickly assess fields that are utilizing IPM-recommended practices to ensure they are working effectively to manage *L. oryzaephilus* and stem borer pest populations.

### **7.3. Opportunities for Future Research**

A high proportion of recovered stem borers (up to 90 %) were identified as *E. loftini*. The increasing incidence of stem borer infestations in Louisiana rice coincides with the expansion of *E. loftini* into the western rice growing regions of the state. This research shows that the impacts of low-level stem borer injury on rice yields is negligible compared to *L.*

*oryzophilus*. However, efforts should be made to monitor the continued expansion of *E. loftini* to ensure the above recommendations are regionally appropriate. Annual counts of whiteheads in rice at the soft dough stage by farmers and extension specialists can help them track the incidence of these pests to determine whether pest densities are increasing. Pheromone trapping by researchers can also be used to track the numbers and spread of *E. loftini* in different parishes.

This dissertation reveals that there is potential for the use of targeted applications of insecticidal seed treatments, but this has not yet been tested in the field. Controlled small-plot and large-scale experiments need to be conducted to investigate the feasibility of effectiveness of applying targeted seed treatments against insect pests. These experiments have to be completed before any further recommendations can be made. Farmers should be consulted regarding the suitability of targeted treatments relative to preferred management practices and planting techniques.

The development of economic thresholds for *E. loftini* and other stem borers is also a research priority. Although prior studies have stated that the action threshold for *L. oryzophilus* is 5 larvae/core, this figure also needs to be updated. Information on economic thresholds is vital for the development of a targeted application plan for insecticidal seed treatments. Thresholds can also be used to monitor the ongoing efficacy and cost-benefit ratio of seed treatments.

The integration of insecticidal seed treatments and cultural control practices was successful, but further research using other chemistries is necessary. Clothianidin, cyantraniliprole and seed treatment combos are also used against *L. oryzophilus* populations. Together with thiamethoxam, these insecticidal seed treatments could be used as an additional tool to combat resistance to chlorantraniliprole, through insecticide rotation programs. However, the efficacy of using these seed treatments together with cultural

controls should also be researched to ensure the recommended practices are applicable and to ensure they don't exacerbate stem borer injury. Different cultivars and practices such as silicon amendment have been used successfully against stem borer populations. Thus, studies assessing the impact of host plant resistance on efficacy of different insecticides and cultural control practices should also be conducted.

Finally, remote sensing of *L. oryzaephilus* populations needs to be further explored. Supplementary ground samples and digital images should be collected to build a robust model for the prediction of *L. oryzaephilus* populations. These models should be tested in commercial field settings to assess how in-field variability affects the results. This is an exciting avenue of research, which may prove to be extremely useful and illuminating.

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## VITA

Megan Marie Mulcahy was born in Pretoria, South Africa to Henry and Geraldine Mulcahy. She matriculated in 2008 from Scottburgh High School and received a bursary to attend Rhodes University in Grahamstown, Eastern Cape Province. Before attending Rhodes University in 2010 to pursue a Bachelor of Science Degree, she lived and travelled abroad in Wales and Scotland. During her studies at Rhodes, Megan discovered her love of plant-insect interactions and conservation. She went on to graduate in 2013 with a double major in Entomology and Botany. During her bachelor's degree she completed two undergraduate theses. The first centered on the pollination biology of *Nymphaea nouchali* and the second investigated the thermal ecology of the diamondback moth on South African cabbage farms. Upon graduation she pursued a B.Sc. Honors degree in Entomology before starting her Master of Science at Rhodes University in 2014. Her M.S. thesis was completed in collaboration with the South African Sugarcane Research Institute and focused on the implementation of a push-pull program for the control of *Eldana saccharina* (Lepidoptera: Pyralidae) in Kwa-Zulu Natal sugarcane. Her M.S. experience solidified her passion for integrated pest management and extension.

In Spring 2017 Megan accepted a graduate research assistantship at Louisiana State University and travelled to the USA to start her Ph.D. in Entomology working in rice pest management. She is currently a doctoral candidate in the Department Entomology at Louisiana State University and is pursuing a minor in Experimental Statistics. Upon completion of her doctorate, she plans to return to South Africa to continue her work in plant-insect interactions, extension and sustainable agriculture.