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Role of Varietal Resistance in Managing the Rice Water Weevil, a Major Insect Pest of Louisiana Rice

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ROLE OF VARIETAL RESISTANCE IN MANAGING
THE RICE WATER WEEVIL, A MAJOR INSECT PEST
OF LOUISIANA RICE

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To

my parents
Hjh. Razimah Kechik and Hj. Mohamad Saad Hj. Osman

my parents in law
Prof. Dato' Dr. Hj. Sufean Hussin and Datin Hjh. Noraihan Hj. Abd Rashid

To

my husband
Sharil Nizam Sufean

and our three lovely children,
Muhammad Rayyan Rifqi
Muhammad Dzukran Hadif
Muhammad Haris Zafran

For their endless love, believing in me, and vision.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Resistance of several commercial rice varieties widely grown in Louisiana was assessed against the rice water weevil (*Lissorhoptrus oryzophilus* Kuschel), the major insect pest of rice in the United States. A five-year field study was conducted to identify resistant genotypes among conventional inbred, herbicide tolerant, conventional hybrid, and herbicide tolerant hybrid varieties. Resistance was evaluated in two different locations to enable selection of rice varieties with wide adaptability over diverse environments. There were no large differences in the resistance found amongst commercial rice varieties over the five-year field study. Jefferson was frequently found to support lower larval densities than other varieties, while Jupiter often supported higher larval densities. Another assessment involved greenhouse experiments that evaluated adult preference for oviposition and survivorship of larvae on different varieties. Numbers of weevil eggs per plant differed significantly among varieties in choice tests but not in no-choice tests, while 1st instar densities in both choice and no-choice tests showed no significant differences between varieties. This suggests that inconsistency of oviposition preferences exist among the varieties. Nevertheless, analysis of mature instar data from the no-choice test showed that numbers of mature instars were significantly different among the varieties "Cheniere", "CL111", "CL151", "Cocodrie", "Jefferson", and "Jupiter". Percentage of larval survival showed no statistical difference between the four varieties tested. Our results from the field and the greenhouse suggest that none of the commercial varieties tested showed high levels of resistance to rice water weevil infestation, although Jupiter appears to be more susceptible than other varieties and Jefferson appears to be somewhat more resistant.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Rice, *Oryza sativa*, is the most widely consumed staple food for a large part of the world's human population, particularly in Asia. According to the World Rice Statistics, rice has the third-highest worldwide production and was planted on more than 160 million hectares in 2016. Apart from Asia, rice consumption continues to grow steadily in both the United States and the European Union as consumers diversify towards more fiber based diets and as numbers of Asian and Hispanic immigrants increase (Vargas and Jurado 2015).

The United States is among the largest exporters of rice globally with 10% of total global exports, trailing only Thailand (30% of total exports), Vietnam (20%), and India (11%) (ricepedia.org/rice-as-a-crop/rice-productivity). Rice is commercially produced in six states including California and the southern states near the Mississippi River (Arkansas, Louisiana, Missouri, Mississippi, and Texas), and rice is an important commodity in these regions. Rice production in Louisiana started in the early 1700s. It increased rapidly after the Civil War and today, Louisiana consistently ranks as the third leading rice producing state in the United States (USDA, 2017). In 2016, the Louisiana rice crop was valued at $298 million (USDA, 2017). Rice production in Louisiana is concentrated in the southwestern and northeastern parts of the state. As one of the state's top agricultural exports, Louisiana rice production accounts for thousands of jobs. Rice in Louisiana is grown as an irrigated crop on natural flatlands, which allows for mechanization and efficient crop management. However, this fragile ecosystem

usually focused on a single crop, creates a situation in which an entire crop can be wiped out by a single pest species. The rice agroecosystem in Louisiana and other southern states is exposed to biotic and abiotic stresses that may potentially reduce the yield and value of the rice grain. To ensure the stability of rice production or increase rice production in the state, it is vital to control the various stresses that are involved in rice production in Louisiana.

Among the biotic stresses, rice crop suffers from attacked by a number of insect herbivores throughout the growing season, some of which can cause serious economic losses. In Louisiana and nearby rice producing states, important rice insect pests include the rice stink bug (*Oebalus pugnax* F.), the fall armyworm (*Spodoptera frugiperda* J. E. Smith), stem borers (*Diatrea saccharalis* F., *Chilo plejedellus* Zink, and *Eoreuma loftini* Dyar), chinch bug (*Blissus leucopterus* Say), rice seed midge (*Chironomus* spp.), rice leaf miner (*Hydrellia griseola*), South American rice miner (*Hydrellia wirthi* Korytkowski), panicle rice mite (*Steneotarsonemus spinki* Smiley), colaspis (*Colaspis brunnea* and *Colaspis louisianae*), and black rice bug (*Amaurochrous dubius*) (Anonymous 2014). However, the major insect pest in Louisiana and the rest of the United States is the rice water weevil (*Lissorhoptrus oryzophilus* Kushel) (Coleoptera: Curculionidae) which injuries both foliage and roots and causes significant reductions in rice yield (Stout et al. 2000).

The rice water weevil is known as the key pest of rice in the United States (Smith and Robinson 1982, Way 1990, Aghaee and Godfrey 2014). This species is native to the New World, but has become a significant invasive pest in other rice growing regions of Asia and Europe (Smith and Robinson 1982, Saito et al. 2005, Stout 2014). Rice

water weevil adults feed on the young leaves, creating longitudinal scars, but it is root pruning by larvae that cause extensive damage to the root systems, promoting tiller abortion, and resulting in yield losses (Way 1990). Larval infestation reduce yields up to 25% in untreated plots, and losses can be higher under heavy pressure (Stout et al. 2000, Zou et al. 2004b).

Given the importance of rice water weevil in Louisiana rice ecosystems, this pest has received significant attention for the development of management programs. There are several management strategies available for control of the rice water weevil populations in the field. Chemical application has always been preferred tactic by growers for managing rice water weevil infestations and yield losses in Louisiana (Stout et al. 2000, Flint et al. 2013, Aghaee and Godfrey 2014). However, concern about the consequences of heavy use of insecticides to non-target organism and the possibility at the development of insecticide resistance have led to investigations of alternative approaches that allow for more sustainable rice production. Integrated pest management (IPM) for this insect pest has been developed as a way to prevent populations from reaching economically damaging levels without relying solely on chemical insecticides. Host-plant resistance is one of the tactics that can be incorporated into the IPM program.

1.2 Host-Plant Resistance

Rice host-plant resistance is an attractive alternative approach for sustainable rice production. Several prior studies suggest that varietal resistance has the potential to complement IPM programs currently being used to manage the rice water weevil (Stout et al. 2001, Zou et al. 2004). Growing resistant rice varieties is an effective tactic

3

because it offers a built-in, economical, and ecologically friendly tactic for protecting rice plants from the rice water weevil. Identification of rice varieties resistant to the rice water weevil is very useful for the immediate use or for the improvement of rice varieties via breeding. Unfortunately, no commercial rice varieties have been identified as highly resistant to the rice water weevil from previous studies. This remains an important deficiency in the literature concerning the source of resistance of Louisiana rice to the important root feeders on rice.

To evaluate the resistance to rice water weevil on several commercially grown rice varieties, we have conducted a five year field study in Louisiana, where rice water weevil is a well-established pest, the greenhouse experiments were also conducted as part of the study. This study will help shed light on the current status of rice resistance to rice water weevil.

1.3 Objectives of the Study

The study was designed to investigate the present status and provide more comprehensive information on the resistance of commercial rice varieties currently grown in Louisiana to the rice water weevil. The specific objectives of the study were:

1) To evaluate resistance of commercial varieties to the rice water weevil under field conditions over two locations

2) To evaluate whether adults or feeding scars are correlated with larval populations of rice water weevils in field plots

3) To evaluate the preference of female rice water weevils for oviposition on selected rice varieties under greenhouse conditions

4) To evaluate the survivorship of rice water weevil larvae on selected rice varieties under greenhouse conditions

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CHAPTER 2

REVIEW OF LITERATURE

This review of literature provides an overview of the origin, distribution, host plants, and biology of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel. This chapter will also provide information on the Integrated Pest Management (IPM) program used to manage this pest by rice growers in Louisiana. In addition, an in-depth description of studies on varietal resistance to the rice water weevil is provided.

2.1 Origin, Distribution, and Host Plants

The rice water weevil belongs to the order Coleoptera, family Curculionidae, subfamily Erirhininae, tribe Stenopelmini, and genus *Lissorhoptrus*. The species, which is native to the New World, was identified as *Lissorhoptrus oryzophilus* Kuschel (1952)*,* and was previously referred to as *Lissorhoptrus simplex* Say (Webb 1914). Several researchers have investigated the origin of this species and found that it is from the marshlands of the Mississippi basin (Newell 1913, Isely and Schwardt 1932). According to Newell (1913), the first report of the rice water weevil as an insect pest damaging rice fields was from Savannah, Georgia, by Riley and Howard in the report of the United States Commissioner of Agriculture for 1881 and 1882. Two decades after that, a great abundance of adult weevils were found in a rice field near Lake Arthur, Louisiana in 1909 after Mr. W. D. Pierce made mention of injury to rice by this species at Beaumont, Texas in 1904 in the Annual Report of the Nebraska State Board of Agriculture for 1906-07' (Newell 1913).

The rice water weevil is distributed throughout the rice growing regions in the U.S., but remained exclusively in the southern rice belt until the weevil was first

7

detected in the western rice belt (California) in 1958 (Lange and Grigarick 1959). The

rice water weevil is also known as a highly invasive species since it was unintentionally

introduced into Japan, presumably on infested hay from California, in 1976 (Iwata

1976). This invader succeeded in dispersing all over Japanese rice fields (Nagata 1990)

and also dispersed to Korea, Taiwan, and in 1988 to east China (Shih and Cheng 1993,

Jiang and Cheng 2003, Saito et al. 2005). In 2004, the first report of rice water weevil in

Europe was in Italy (Caldara et al. 2004) and more recently it has spread to the region

of Central Macedonia, Greece (Giantsis et al. 2017).

The rice water weevil feeds on various aquatic and semiaquatic grasses. Adult

weevils oviposit on many monocotyledonous plants in the families Poaceae and

Cyperaceae with banyardgrass, *Echinochloa crus-galli* Beauv., the most preferred for

feeding and oviposition as reported by Tindall and Stout (2003), Chen et al. (2005), and

Lupi et al. (2009). Moreover, adults may feed or larvae may survive on plants in the

dicotyledonous families Onagraceae, Amaranthaceae, Asteraceae, Convolvulaceae,

Euphorbiaceae, and Fabaceae (Tindall and Stout 2003, Lupi et al. 2009). Identification

of host range is important since host plants provide food and shelter to the rice water

weevil, especially near overwintering sites.

2.2 Biology of Rice Water Weevil

Rice water weevil adults are relatively small to medium in size. They are about

0.32 cm long by 0.16 cm wide, including the rostrum (snout). The adult body ranges in

color from dark-brown to grayish-black with an elongated dark-brown V-shaped mark on

the center of their elytra. In semi-aquatic environments such as rice fields, adult rice

water weevils not only fly but they are also strong swimmers as their mesothoracic legs

propel them, with their legs moving synchronously during protraction and retraction (Hix et al. 2000). According to Hix et al. (2000), weevils also can stay alive while immersed for more than 5 days. In rice fields, the adults are commonly found resting on leaf blades, "playing possum" when disturbed, dropping to the water and immediately escaping.

Adult females lay whitish, elongate-shaped eggs singly. Usually, the egg is laid inside the submerged part of the leaf sheath (Grigarick and Beards 1965) in a longitudinal pattern. Eggs hatch and first instar larvae feed on leaf sheath tissue for a short time before moving down to the mud, settling themselves, and continue feed on the roots. The white, c-shaped legless larvae colloquially known as 'root maggots' move through the mud from one root to other roots via modified spiracles that hook into the rice plant's tissue (Zhang et al. 2006). In addition, the modified spiracles also help in obtaining oxygen in flooded soils for breathing. The milk-white larvae pass through four instars before pupation occurs in mud cocoons also associated with the roots of rice plants. The pupal cell is smooth, oval in shape, and attached to the roots (Ingram 1927, Cave and Smith 1983, Zhang et al. 2006). The pupal period takes one to two weeks (Lange and Grigarick 1959). Adult weevils emerge from pupal cells, crawl up to the closest root and escape to the open air. According to Gifford (1973) and Zhang et al. (2006), rice water weevils spend their immature stages entirely under water. The life cycle from egg to adult can vary from 35 to 45 days depending on temperature (Lorenz and Hardke 2013, Zou et al. 2004b).

The rice water weevil generally reproduces sexually in its native range, although parthenogenic females were found in California when it was first detected in 1958

9

(Lange and Grigarick 1959), and Japan two decades later (Hirao 1978). In Louisiana rice growing areas, rice water weevils are multivoltine due to the longer growing season and ratoon/second stubble crop production (Ingram and Douglas 1930, Smith 1983). Understanding the biology of the rice water weevil is imperative as it reveals the potential distribution of this species. Chen et al. (2005) concluded that rice water weevil is spread by several ways; parthenogenesis biotype (no mating required), rice hay, flying, swimming, and by human transportation.

2.3 Damage

The rice water weevil is responsible for serious yield losses throughout its geographic range. In Louisiana, an average of 5-30% of the rice harvest is lost in untreated fields (Stout et al. 2013). During early spring, when influenced by the right temperatures (Zou et al. 2004a), adult rice water weevils begin to feed on numerous grasses to build up their flight wing muscles, then take off from overwintering sites to the rice field (Tindall and Stout 2003, Shang et al. 2004). Rice plants can be attacked by rice water weevil at any stage but are at greatest risk during early vegetative stages (Stout et al. 2002a). Adults feed directly on the leaves of young rice plants, creating translucent narrow scars that parallel the midrib of the leaf blade. This visible injury on the upper surface of the leaf does not typically cause economic losses to growers (Way and Wallace 1993). Typically, one month after sowing (for dry-seeded rice); growers establish a permanent flood. This event triggers the adult weevils to mate, and the females crawl down the rice stem and lay eggs in the rice leaf sheath, under the water surface.

After eclosion, the aquatic larvae feed on the roots of flooded rice plants for 3 - 4 weeks. Larval densities usually peak at 4 weeks after flooding (Shang et al. 2004). Root feeding by third and fourth instars larvae is a prolonged process and more importantly, severe injuries apparently caused extensive damage to the root systems. Another study by Zou et al. (2004c) indicated that larval feeding effects various vegetative and reproductive characters in rice. They found that both root and shoot biomasses were reduced significantly, together with lower number of tillers in weevil-infested plots. Not only that, root pruning by larvae affected number of grains per panicle and grain weight. These effects on yield components differ among different varieties. In some cases, the injury by larvae will be more severe in water-seeded rice field or for those growers who practice early flooding.

2.4 Control Strategies/Integrated Pest Management (IPM)

Being fully aware of the potential for devastating crop losses to occur, there are several control strategies that may help to reduce losses due to rice water weevil infestation. The appropriate control strategy to use in managing the rice water weevil can vary depending on the severity of the infestation in the fields. Currently, the management of rice water weevil is possible through the use of a combination of control options (cultural, chemical, and host-plant resistance) which encompasses the concept of Integrated Pest Management (IPM).

2.4.1 Cultural control

There are several cultural practices associated with water management that may help to reduce losses due to rice water weevil infestation. The earliest use of water management tactics to control rice water weevil was draining of rice fields infested with

11

rice water weevil larvae. The first use of this tactic was in 1881 by Charles Valentine Riley. Riley's idea then was supported by studies of Isely and Schwardt (1932), Douglas and Ingram (1942), and Thompson et al. (1994b) that found that timely drainage of rice fields lessens the root injury of rice plants by rice water weevil larvae. Draining fields, however, needs appropriate planning to minimize conflict with disease, fertilizer application, and weed infestation. Another practice that contributes to lower larval densities is delayed permanent flood as described by Rice et al. (1999). The research described by Rice et al. (1999) included measuring the efficacy of delayed flood and the use of herbicide resistant rice lines for rice water weevil and weed management, respectively. In a study by Stout et al. (2002b), the oviposition and behavior of rice water weevils were manipulated by flood depth under controlled conditions. Fewer larvae were found at 5 cm flood depth compared with 10 cm flood depth indicating that flooding may influence adult rice water weevil behavior both directly and indirectly. As stated in the same paper, the oviposition preference by adult females is strongly associated with presence of standing water and certain flooding depth. Early planting has long been recommended by numerous authors as another cultural practice to avoid damaging infestations of the rice water weevil (Isely and Schwardt 1932, Thompson et al. 1994a, Shang et al. 2004, Stout et al. 2011). This practice allows rice plants to grow past their most vulnerable growth stages before migration of weevils from overwintering sites. Thus, adoption of these approaches may benefit the growers without additional cost to their rice production. Practices other than manipulation of irrigation such as drill seeding (most common planting method in southern United States including Louisiana)

or removing weedy vegetation on the levees may also help growers in delaying or eliminating exposure of rice plants to weevil infestations (Palrang et al. 1994).

2.4.2 Insecticidal control

Insecticides remains the primary means of controlling the rice water weevil in the field (Lanka et al. 2015). In the southeastern United States region, rice growers mostly focus on two major approaches, namely prophylactic seed treatment and foliar applications. Currently, an anthranilic diamide insecticide (chlorantraniliprole) and two neonicotinoid insecticides, clothianidin and thiamethoxam are extensively used as prophylactic seed treatments (Lanka et al. 2014a, 2014b), especially in drill-seeded rice. Insecticidal seed treatments provide satisfactory control and superior larval suppression for this primary early season insect pest (Hamm et al. 2014). But, due to high price in the market, with the possibility of additional cost to their productions, some rice growers are reluctant to take this preventive treatment. They prefer to use applications of foliar insecticides to kill adults before they lay eggs at or near time of flooding. Applications are made on the basis of levels of weevil infestation: in IPM program, seed treatments may not always be needed in areas with lower insect pressure.

Pyrethroids are the most widely used group of foliar insecticides in controlling rice water weevil at the adult stage (Lanka et al. 2014a). However, timing of application is critical for pyrethroid-based insecticides and mistimed applications may result in insufficient control (Stout et al. 2000, Hummel et al. 2014). Early scouting for adult arrivals in the field is needed for a better decision making. Scouting for adults not only facilitates the timing of foliar application, but may help in prediction of larval abundance in the field. It was believed that the action thresholds and selection of a suitable

management approach can be developed for the rice water weevil based on adult densities and adult feeding scars in the field (Lorenz and Hardke 2013). However, none of any previous study ascertained a strong correlation either between adult densities or adult feeding scars with immature weevil densities in the field. Such methodology could be a valuable component of forecasting in IPM program.

2.4.3 Biological control

Biological control is another potential strategy for controlling rice water weevil. Some possible biological control agents include parasitic nematodes such as *Steinernema carpocapsae* and *Heterorhabditis* spp. (Bunyarat et al. 1977), and the entomopathogenic fungi *Beauveria bassiana* (Vuillemin) and *Metarhizium anisopliae* (Metschnikoff) (Huang 2017). Recently, entomophatogenic bacteria subspecies *Bacillus thuringiensis* spp. *galleriae* were considered to be promising biological control agents by Aghaee and Godfrey (2015) who published the first report on the effectiveness of this subspecies towards rice water weevil. Based on the author's observation, they found that granular formulations of toxins from these soil bacterium subspecies may have similar efficacy as the synthetic pyrethroid ʎ-cyhalothrin.

2.4.4 Nutrient amendment

Preliminary studies on Silicon (Si) soil amendment into rice field were conducted in several years of field trials in Louisiana. However, only a weak effect on weevil larval densities was observed in plots with addition of Si. (Villegas and Stout, personal communication).

2.4.5 Host Plant Resistance/ Varietal Resistance

Host plant resistance is always a component of an IPM program in rice. The use of resistant varieties in rice water weevil management offers a potential control measure that can result in an effective and less expensive control program. Information on the susceptibility of a variety to rice water weevil is critical to design of a management program against this pest. Resistant varieties can be harmoniously integrated with several control strategies to reduce amount of injury from rice water weevil and consequently lower yield losses. The following sections describe more details regarding research on varietal resistance to the rice water weevil.

2.5 Sustainable Rice Production of Louisiana

Sustainable rice production is an approach to growing rice consistent with ecological principles and in an ethically responsible manner. It is a response to the challenges of sustaining rice production with minimal effects on the biodiversity of the region where it is grown. In the context of controlling or managing the biotic constraints on rice production in Louisiana, for example the rice water weevil, our major concern is providing a healthy environment for the co-production of red swamp crayfish, *Procambarus clarkia,* in the rice ecosystem following rice harvesting season. In addition, conserving beneficial organisms is undoubtedly important. While several control strategies have been used to manage rice water weevil populations in the field, none have been fully effective when used as single approach. In spite of the robust application of chemical insecticides by the growers, the rice water weevil continues to be an important pest in the Louisiana rice ecosystem. Still, severe economic yield losses in rice will likely occur if growers continue to rely solely on insecticides. In

addition, there is strong evidence that resistance to insecticides, particularly aldrin, occurred in this pest in Arkansas, Louisiana, and Texas in the 1960s (Rolston et al. 1965, Graves et al. 1967, Bowling 1968). Therefore, implementation of IPM strategies in managing rice water weevil is the key to achieving sustainable rice production. An IPM approach creates a proper management program to keep the target pest density under economic threshold levels, meanwhile minimizing risks to human health and environment. Integrated Pest Management encourages increased use of alternatives to insecticide applications.

Zou et al. (2004b) provided evidence that the combination of delayed flooding with the use of the more tolerant variety "Cocodrie", resulted in less yield losses than use of the less tolerant variety "Bengal". They found that cultural practices such as delayed flooding create a more suitable environment for expression or optimization of plant resistance. These findings support a previous study conducted by Stout et al. (2001). They evaluated the compatibility of using varietal resistance, delayed flooding, and seed treated with fipronil (Icon). Another recent study of Lanka et al. (2015) on integrating shallow flooding and resistant varieties with chlorantraniliprole seed treatments also demonstrated positive results. Importantly, no antagonistic interactions have been found in any of these studies. Reductions in rice water weevil densities resulting from the use of resistant varieties may complement reductions from seed treatment and cultural practices.

2.6 Research on Varietal Resistance to Rice Water Weevil

Research on host-plant resistance to herbivore attack throughout the past few decades has highlighted the potential of varietal resistance in enhancing the profitability

of several agricultural crops including rice (Painter 1968, Luginbill 1969, Heinrichs et al. 1985, Teetes 1985). The development and implementation of varietal resistance in rice has been successful against several planthoppers such as brown planthopper (*Nilaparvata lugens* Stal.), whitebacked planthopper (*Sogatella furcifera* Horvath), green leafhopper (*Nephotettix virescens*), and against the rice gall midge (*Orseolia oryzae*) (Habibuddin et al. 2000, Vijaykumar et al. 2009, Liu et al. 2016). Contrary to the success in producing resistant varieties to planthoppers and rice gall midge, less effort has been made in breeding resistance against the rice water weevil (Way 1990). One rapid approach in identifying varietal resistance or new sources of rice resistance is comparative screening of available commercial varieties in the market.

Throughout the long history of research on the rice water weevil, Isely and Schwardt (1934) initially raised the possibility of using resistant varieties against the rice water weevil. Beginning in the early 1960s, Bowling (1963) conducted a series of variety trials to determine a varietal response to rice water weevil. Failure in obtaining differences in larval populations among eight varieties tested in a field evaluation did not stop the author. Laboratory screening procedures were later developed by Bowling (1973) with a focus on adult preference for oviposition and the survival of early instars. In Louisiana, more than 50 varieties have been released by the LSU AgCenter or Texas A&M AgriLife since 1917 to 2015 (Anonymous 2017). From the standpoint of pest management, the screening program focuses exclusively on rice diseases prior to release of a new variety. All recommended varieties for Louisiana rice growers are screened for resistance to sheath blight, which is the most important disease in Louisiana, while screening for resistance to insects is lacking.

A few rice lines from the World Collection have been reported to support lower

densities of rice water weevil larvae than the most susceptible standard (Smith and

Robinson 1984, N'Guessan and Quisenberry 1994, N'Guessan et al. 1994b, Heinrichs

and Quisenberry 1999). Several breeding lines possessing low to moderate levels of

tolerance to weevil injury have also been identified (Gifford and Trahan 1976,

N'Guessan et al. 1994a, 1994b, 1994d). Numerous other studies done by field

entomologists have identified commercial varieties with significant variation in

susceptibility and tolerance to the rice water weevil, either in the field (Smith and

Robinson 1982, Stout et al. 2001) or greenhouse (N'Guessan et al. 1994c, Stout and

Riggio 2003). Nonetheless, most of the varieties in these studies showed only low or

moderate levels of resistance and none of them are widely grown anymore. The

absence of a source of resistance is currently the main obstacle for successful breeding

and deployment of rice water weevil resistant rice variety.

Evaluation of resistance to rice water weevil is crucial and needs to be

considered for a dynamic rice breeding program. Field evaluation is needed especially

under natural infestations as this condition enables the realistic measurement of the

actual abundance of rice water weevil populations and local adaptations to the rice

genotype. Greenhouse evaluations are ideal for the characterization of resistance as

they are done under more controlled conditions (Stout and Riggio 2003).

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CHAPTER 3

SUSCEPTIBILITY OF COMMERCIAL VARIETIES OF RICE TO THE RICE WATER WEEVIL

3.1 Introduction

Rice, *Oryza sativa*, is an important commodity in the southern region of the United States, and Louisiana ranks as the third leading rice-producing state (USDA, 2017). Rice is a short season crop that is cultivated in a monocropping system in most areas during spring and summer months. This planting system increases the vulnerability of rice to various biotic and abiotic stresses. Through the decades, insect pests have been serious impediments to rice yields in Louisiana and nearby rice producing states. The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), is regarded as the major insect threat to rice in the United States (Smith and Robinson 1982, Way 1990, Aghaee and Godfrey 2014).

Lissorhoptrus oryzophilus is indigenous to the Unites States (Isely and Schwardt 1932) and also occurs in Central America, the Caribbean, and South America (CABI/EPPO 2011). The rice water weevil has become a significant invasive pest in several countries of Asia including China, the Korean Peninsula, Taiwan, and India (Nagata 1990, Shih and Cheng 1993, Jiang and Cheng 2003, Stout 2014) since it was inadvertently introduced into Japan on infested rice hay from the West Coast of the USA in 1976 (Iwata 1976, Saito et al. 2005). The first discovery of this weevil in Europe was in mainland Italy in 2004 (Caldara et al. 2004).

The status of the rice water weevil as a ubiquitous and major pest of rice in Louisiana is due to several factors. First, adult rice water weevils are specialists on

grasses and sedges, feeding on three plant families (Poaceae, Cyperaceae, and Onagraceae) with rice apparently the primary host (Isely and Schwardt 1932, Lange and Grigarick 1959, Tindall and Stout 2003). Adult weevils feed directly on young rice plants, scraping leaf tissues and producing longitudinal scars parallel to leaf veins, but this injury, in most cases, is not economically important. On other hand, feeding by the larval stage on the roots of flooded rice plants throughout the vegetative stages of crop development reduces root and shoot biomass, decreases plant vigor and growth, and results in lodging in extreme cases (Smith and Robinson 1982, Stout et al. 2000, Zou et al. 2004b).

Second, in the southern United States, the rice water weevil is multivoltine, capable of having up to 3-4 generations per year (Smith 1983). Shang et al. (2004) indicated that immature weevils furthermore were apparently capable of completing their development on ratoon-crop rice. Third, *L. oryzophilus* cryptically overwinter as adults on levees, in bunchgrasses and leaf litter in riparian areas, or under vegetative cover adjacent to rice fields (Shang et al. 2004). Weeds found in levee areas could serve as hosts for rice water weevil flight initiation once adults emerge from overwintering sites (Tindall and Stout 2003, Shang et al. 2004).

Larval infestations can reduce grain yields up to 25% and losses can be higher in areas with heavy weevil densities (Stout et al. 2000, Zou et al. 2004b). Yield reductions attributable to this insect have forced rice growers to depend heavily on prophylactic insecticidal seed treatments for control, which consequently, have contributed to increases in production costs (Hummel et al. 2014). Cost-effective insect management practices are imperative in maintaining the economic viability of rice production in

Louisiana. A key component of managing this early-season insect pest is the development of alternative management practices, including varietal resistance.

Varietal resistance is an important facet of host-plant resistance (HPR) and historically has played an important role in integrated pest management (IPM) in rice (Heinrichs 1994, Kogan 1994). Host-plant resistance can be defined as the heritable ability of a plant to resist or mitigate damaging attacks by insect herbivores (Smith and Clement 2012). Resistant rice varieties have been considered an economical, convenient, long-lasting, non-hazardous strategy (Pedigo and Rice 2014). Host-plant resistance is compatible with other IPM strategies, easy and inexpensive for rice producers to implement, and is cumulative in its impact on herbivore populations in the field. These characteristics, aside from reducing management costs for growers, are also beneficial in improving long-term sustainability and providing significant positive environmental impacts.

Adoption of varieties of rice with inherent resistance to the rice water weevil in Louisiana has the potential to reduce rice water weevil abundance and reduce dependence on chemical insecticides. Previous studies have documented significant variation in the susceptibilities of rice genotypes to the rice water weevil. Since the 1960s, a large number of germplasm accessions have been screened for resistance to the rice water weevil in a collaborative program conducted by USDA and Louisiana State University scientists under both field (Smith and Robinson 1982, 1984, N'Guessan and Quisenberry 1994, Rice et al. 1994, Heinrichs and Quisenberry 1999, Stout et al. 2001) and greenhouse conditions (N'Guessan et al, 1994, Stout and Riggio 2003). Nevertheless, none of the varieties screened previously are planted extensively at the

27

present except a few varieties tested in recently published article by Vyavhare et al. (2016). It is crucial to identify potentially resistant varieties so they can be incorporated into management programs.

In the present study, we investigated the susceptibility of widely grown commercial rice varieties to the rice water weevil. Data from field evaluations over several years at two locations allowed us to test the stability of expression of resistance. We were also interested in determining whether adult population densities or numbers of feeding scars could be correlated with larval populations so that improved recommendations for scouting could be made to growers. Finally, we compared the results of field evaluations with results of greenhouse evaluations of adult oviposition and larval survivorship of rice water weevil larvae on selected rice varieties. We also examined plant morphological structures potentially associated with weevil oviposition, including plant height and intraveinal distance.

3.2 Materials and Methods

3.2.1 Field evaluations (Cultivar Trials)

Seven field experiments were conducted over five consecutive growing seasons (2013-2017) to evaluate the resistance of varieties grown widely in Louisiana to the rice water weevil. Five experiments were conducted at the H. Rouse Caffey Rice Research Station located near Crowley, Acadia Parish, LA (30° 14' 34" N, 92° 21' 36" W). Two additional experiments were conducted in 2015 and 2016 at a site near Lake Arthur, Vermilion Parish, LA (30° 3' 34" N, 92° 38' 13" W) to evaluate resistance of the same varieties in a different environment.

Fields at the H. Rouse Caffey Rice Research Station have been in a rice-fallow rotation for over 20 years. The soil type at this location is a Crowley silt loam (fine, montmorillonitic, thermic Typic Albaqualf) (Stout et al. 2011). Experimental plots at this site historically suffer heavy infestations of rice water weevils. Fertilizer was applied at recommended rates based on the Louisiana Rice Production Handbook (Anonymous 2014) after soil analyses were obtained. The experimental design in all five years was a completely randomized block with each variety replicated four times. By using a grain drill mounted on a tractor, plots were drill seeded on March 18 (2013), April 1 (2014), March 24 (2015), April 23 (2016), and March 15 (2017). Each plot measured 5.4 m x 1.8 m with 7 rows of rice spaced 17.5 cm apart. Each plot was separated from neighboring plots by at least 1.2 m on all sides. Permanent flood was applied when rice plants possessed four to five fully expanded leaves approximately four weeks after planting and fields were kept flooded until they were drained for harvest.

The conventional inbred 'Jefferson', six additional conventional inbred varieties, four herbicide tolerant varieties, a hybrid variety, and a herbicide tolerant hybrid variety were included in this 5-year field study (Table 3.1). Jefferson is a long grain conventional variety developed in Texas. It is not widely grown in Louisiana but was used as resistant standard in experiments based on its superior resistance in several prior field and greenhouse evaluations (Stout et al. 2001, Stout and Riggio 2003). In 2013, nine varieties comprising six conventional inbred and three herbicide tolerant inbred were evaluated. In 2014, seven varieties from 2013 were maintained, with Mermentau added. Varieties selected for study in 2015, 2016, and 2017 differed

Table 3.1. Rice varieties evaluated for resistance to the rice water weevil in fields and greenhouse experiments, 2013-2017

Variety	Variety Type	Grain Type	Year Released	Acreage[a] (Hectare)	Year of evaluation				
					2013	2014	2015[b]	2016[b,c]	2017
Catahoula	Conventional Inbred	Long	2008	n/a	√				
Cheniere	Conventional Inbred	Long	2003	17,578.33	√		√	√	√
Cocodrie	Conventional Inbred	Long	1997	2,825.92	√	√	√	√	√
Jefferson[d]	Conventional Inbred	Long	1997	0.00	√	√	√	√	√
Jupiter	Conventional Inbred	Medium	2005	24,421.16	√	√	√	√	√
Mermentau	Conventional Inbred	Long	2012	6,552.27		√	√	√	√
LA110	Conventional Inbred	Medium	1979	0.00*	√	√	√	√	√
CL111	Herbicide Tolerant Inbred	Long	2010	58,114.88		√	√	√	√
CL151	Herbicide Tolerant Inbred	Long	2008	10,294.80			√	√	√
CL152	Herbicide Tolerant Inbred	Long	2011	6,741.66	√	√			
CL261	Herbicide Tolerant Inbred	Medium	2010	202.34	√	√			
XL753	Conventional Hybrid	Long	2011	10,030.13			√	√	
CLXL745	Herbicide Tolerant Hybrid	Long	2007	20,218.50					√

[a]Numbers of hectares planted with variety in 2014 in Louisiana
[b]Two locations: Rice Research Station (Crowley) and Lake Arthur
[c]Greenhouse experiments
[d]Resistant standard
*Not grown commercially in Louisiana

somewhat from varieties used in 2013-2014 and collectively represented more than 70% of the rice acreage in Louisiana (Saichuk, personal communication). CL111, Jupiter, LA110, Mermentau, Jefferson, and Cocodrie were used in all three years, with CL151, Cheniere, hybrid XL753, and the herbicide tolerant hybrid CLXL745 used in at least one of the years. The variety Cheniere was used for border rows in all experiments. Rice plots were not treated with insecticides throughout the entire planting season to allow for natural infestations of rice water weevils.

The experimental design at the Lake Arthur site was identical to that of the Crowley site in 2015 and 2016. The soil type at this location is a Kaplan silt loam. Plots were drill seeded on March 24 (2015) and March 21 (2016). The same nine rice varieties used at the Crowley site were planted each year at the Lake Arthur site (Table 3.1). Fertilizer applications and other agronomic practices followed recommendations of the LSU AgCenter for drill-seeded rice in southwestern Louisiana and were similar to practices used at the Crowley site.

For experiments at both locations, population densities of rice water weevil immature (larvae and pupae) in plots were determined using a root-soil core sampler with a diameter of 9.2 cm and a depth of 7.6 cm. Root-soil core samples were taken twice in each year of evaluation to estimate population densities of rice water weevils only at Crowley site. The first core (Core1) was sampled at approximately three weeks after permanent flooding (WAF), and the second core (Core2) was taken the following week in all year except in 2013. Core samples were collected from one sample date at Lake Arthur site at approximately 3 WAF. Two core samples were taken per plot in Lake Arthur site and three core samples were taken per subplot in Crowley site. Core

31

samples were taken haphazardly from the interior rows of plots and individually bagged in pre-labeled 6" x 3" x 15" plastic bags. Core samples containing plants and soil were processed at a washing station. The roots of core samples were washed vigorously under pressure in a sieve bucket composed of 40-mesh screening. Screen buckets were then placed into basins of saturated salt water. Larvae and pupae dislodged from roots during washing were counted as they floated to the surface of the salt solution (N'Guessan and Quisenberry 1994). In addition, in 2015, 2016, and 2017, feeding scars were estimated from an average of 10 plants in each plot two days before flooding. Adult densities were estimated from an average of four quadrats (0.1 square meters) in each plot a week after permanent flood was applied. Both feeding scars and adult densities were estimated by visual counting.

3.2.2 Greenhouse Studies

The varieties chosen for study in both choice and no-choice experiments in a greenhouse were the same varieties that were used in 2015 and 2016 field evaluations, namely CL111, CL151, Jupiter, Mermentau, Cheniere, Cocodrie, and XL753. In addition, the long-grain variety 'Jefferson', which had been previously shown to be resistant to infestation by the rice water weevil (Stout et al. 2001) was again used as a resistant standard. Soil used in all greenhouse experiments was obtained from fields at the H. Rouse Caffey Rice Research Station, Crowley.

Unless specifically mentioned otherwise, rice water weevil adults or larvae used in the greenhouse experiments were collected from untreated rice fields at the Rice Research Station, 1 or 2 days prior to conducting experiments. Adult weevils were kept in a glass jar provided with water and rice leaves as a food source. Mating pairs of rice

water weevils were selected from jars and used in both choice and no-choice experiments to ensure a sex ratio of 1:1. The rice water weevil larvae used in the larval survivorship experiment were washed and collected from roots of plants immediately prior to conducting experiment from rice plants taken from Rice Research Station as well.

3.2.2.1 Choice and no-choice experiments

Choice and no-choice experiments were conducted during the summer of 2016 to assess the preference of adult rice water weevils for oviposition. Experiments were conducted in a greenhouse on the campus of Louisiana State University (30° 24'16" N, 91° 10' 40" W). The experimental procedure was similar to that used in a previous study (Stout and Riggio 2003). Six rice seeds of a single variety were sown in 10-cm diameter round pots (500 ml), with eight or ten pots per variety for choice and no-choice experiments, respectively. Each pot represented a replication. Plants were maintained in large wooden basins lined with heavy black plastic liner that allowed plants to be flooded. Seedlings were thinned to three seedlings per pot one week after sowing. Experiments were conducted 30-35 days after planting, when plants of all varieties possessed four to five leaves. Plant height measurement was done prior to imposition of rice water weevil infestation.

In the choice experiment, one pot of each variety was enclosed in 61-cm in height by 46-cm diameter infestation cage covered with a fine brown mesh fabric. Eight infestation cages containing eight randomly distributed pots (one of each variety) were set up in a greenhouse bench. The bench was then flooded to a depth of 22 cm prior to weevil infestation. Each cage was infested using 48 adult weevils at a density of two

weevils per plant, and weevils were allowed free access to the plants for five days. After five days of exposure to adults, pots were then removed from cage and any weevils found on plants were killed. Two plants were promptly removed from each pot to count total eggs per plant and first instar larvae. The third plant in each pot was maintained in the basins under flooded conditions for another 20 days, and then evaluated to assess densities of late instars.

For the no-choice experiment, three mating pairs at a density of two weevils per plant were confined to a pot containing three plants of a single variety using transparent cylindrical cages (23-cm height by 8.5-cm diameter) with the top end covered with a mesh cloth. The cylindrical cages fitted snugly in pots, and each cage had two mesh-covered windows to let water move through them. The experiment was arranged in a randomized complete block design with 10 replications. The cages were left for five days to allow weevils to feed, mate, and oviposit. All steps after weevil infestation were similar as described in the choice experiment.

Egg and larval densities from both choice and no-choice experiments were quantified using procedures similar to those used by Stout and Riggio (2003). To determine egg densities, plants were removed from pots after cage removal, washed gently under running water, and labeled properly before placing in 95% ethanol until entirely bleached. Numbers of eggs per plant were estimated by examining rice plants under a dissecting microscope equipped with an eyepiece micrometer (MA524, 1 mm divided into 100 U for a minimum unit of 0.01 mm, Meiji Techno America, San Jose, CA). Additionally, distances between leaf veins were measured for each variety while

34

counting densities of eggs. Intraveinal distances were estimated from an average of four points in each plant.

The densities of first instars were determined from the second plant that was removed from each pot after the five days of adult infestation. Each rice plant was washed and transferred to a 20 x 150mm labeled test tube containing approximately 20ml water. Test tubes were organized in test tube racks and placed in a growth chamber at 28° - 30° C (14:10h L: D). First instar larvae enclosing from eggs were counted beginning the day after by shaking roots in the test tubes, and then pouring the water into a Petri dish. Water was replenished after the plants were placed back into the test tubes and emerging larvae were counted daily until no larvae were found for three consecutive days.

Densities of mature instars were determined from the third plant that was retained in pots and maintained for another 20 days after the termination of the adult infestation period. Late instars and pupae were counted in each pot, using procedures identical to those used in the field evaluation.

3.2.2.2 Larval survivorship experiment

Larval survival on four varieties (Jefferson, Jupiter, Cocodrie, and CL151) was assessed in a no-choice experiment. The methods for preparing the test plants in this experiment were similar to methods previously described for the choice and no-choice experiments, except seedlings were thinned to two seedlings per pot. Pots were organized in a randomized complete block design with 10 replications on a greenhouse bench. When plants had reached the 3-4 leaf stage, the bench was flooded to just below the rim of the pots. Each pot was infested with five 1st or 2nd instar rice water

weevils by carefully placing the larvae close to the base of a plant using a small paint brush. On the 3rd day after larval infestation, water was added to the bench to a depth of ≈22 cm above the soil line. Larvae of rice water weevil were given ample time to move to the root systems of the rice plants. Infested plants were retained under flooded conditions in the greenhouse for 20 days, after which they were washed and the surviving larvae and pupae were counted and recorded. From these data, the percentage of survival larvae was calculated as; *percentage of survival = (number of surviving larvae and pupae/ number of larvae used to infest plants) x 100*.

3.2.3 Data Analysis

For the field evaluations, effects of rice variety on numbers of immature weevils were analyzed separately for each core sampling using analysis of variance (ANOVA) in randomized complete block design (RCBD) in SAS 9.4 (PROC MIXED SAS Institute Inc 2013). The variety was considered a fixed effect, while block was considered a random effect. Immature weevil counts from core sample in each plot were averaged to obtain a mean value for each plot for each of the two core sampling dates, and these mean values were entered into the ANOVA analysis. Tukey's honest significant difference (HSD) test was used to assess differences among treatment means (α=0.05). Effects of variety on numbers of eggs, first instars, late instars, and percentage of larval survival were also analyzed separately. Possible correlations between adult densities or number of scars and immature weevil densities and between egg densities and morphological traits were examined using Pearson correlation coefficients in PROC CORR in SAS.

3.3 Results

3.3.1 Fields experiments

Significant differences in immature weevil infestations among commercial varieties in field evaluations at Crowley Rice Research Station were found in 2014, 2015, 2016, and 2017 but not in 2013 (Table 3.2 and Figure 3.1).

Immature weevil densities at Lake Arthur site showed patterns similar to those at the Rice Research Station. Differences among varieties were not significant in 2015 (Table 3.2), although numerically Jefferson suffered the lowest infestation. Differences among varieties were significant in 2016 (Table 3.2). Lower immature weevil densities were found in plots of CL111 and Jefferson, while highest densities were found on Mermentau plots (Figure 3.2: B).

Table 3.2. Summary of ANOVA with respect to five-year field evaluations at Crowley Rice Research Station and Lake Arthur, Louisiana, in 2013-2017

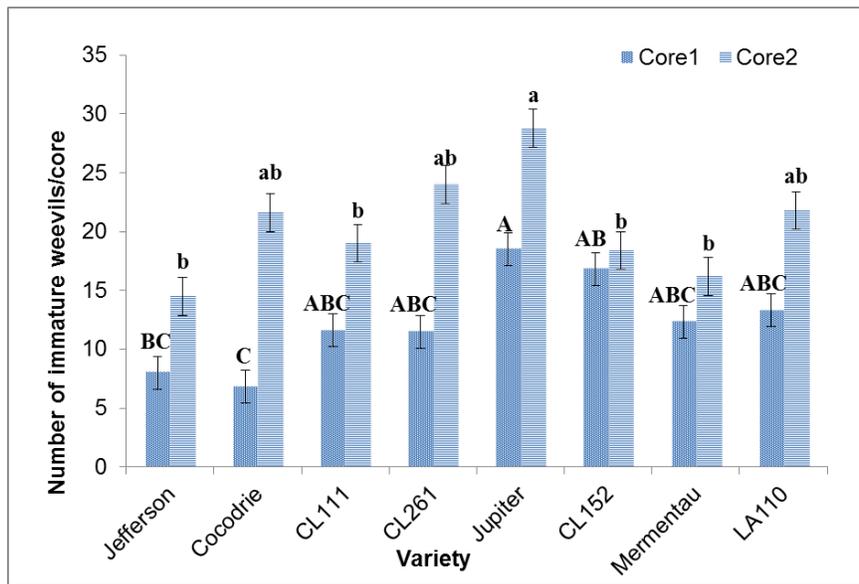| Experiment	ANOVA	
	Core1	Core2
Crowley		
2013	F=1.83; df=8,20; P=0.1309	n/a
2014	F=4.38; df=7,21; P=0.0039	F=5.07; df=7,21; P=0.0017
2015	F=1.12; df=8,24; P=0.3844	F=3.27; df=8,24; P=0.0115
2016	F=1.31; df=8,24; P=0.2854	F=4.37; df=8,24; P=0.0023
2017	F=1.40; df=8,24; P=0.2461	F=4.68; df=8,24; P=0.0015
Lake Arthur		
2015	F=1.23; df=8,24; P=0.3270	n/a
2016	F=3.21; df=8,24; P=0.0126	n/a

(A) Year 2013
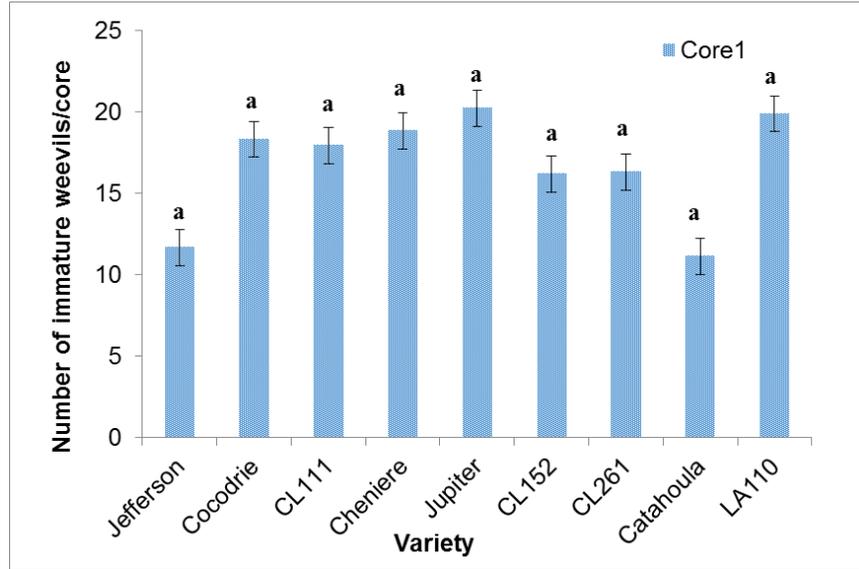
(B) Year 2014

Figure 3.1. Mean numbers of rice water weevil immature per core ± SE in field evaluations at Rice Research Station (Crowley) during 2013 (A) and 2014 (B). Uppercase and lowercase letters represent Core1 and Core2, respectively. Value across all varieties tested in each figure followed by a same letter are not significantly different (alpha=0.05).
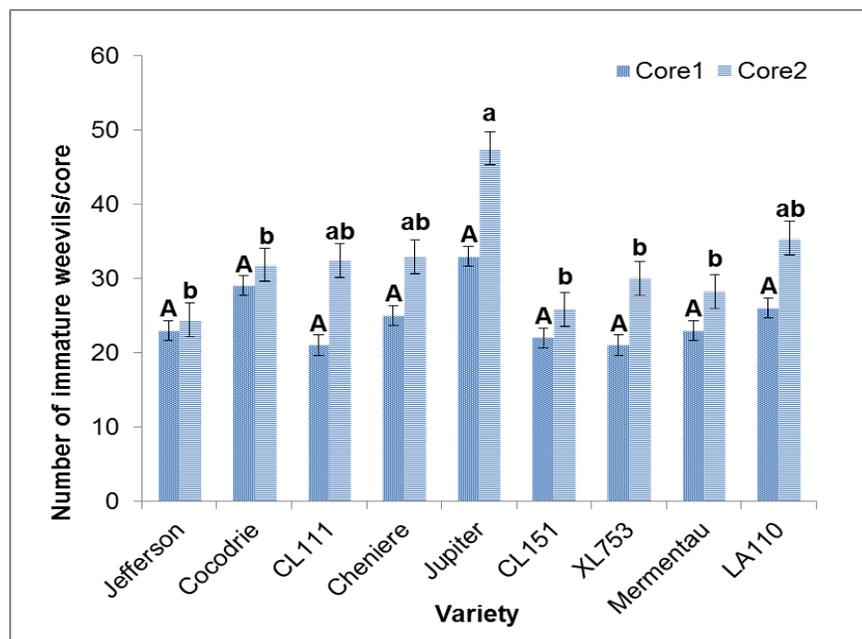
(Figure 3.1 continued)

(C) Year 2015
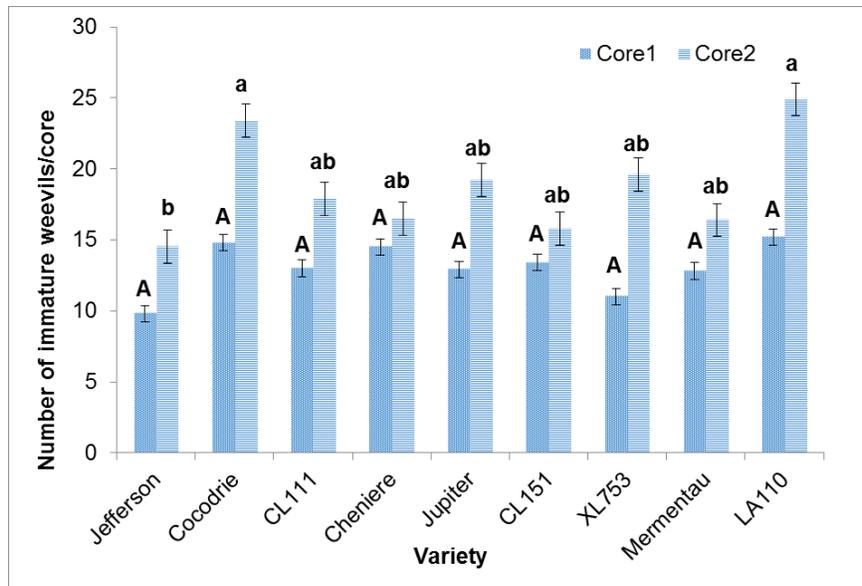
(D) Year 2016

Figure 3.1. (Cont.). Mean numbers of rice water weevil immature per core ± SE in field evaluations at Rice Research Station (Crowley) during 2015 (C) and 2016 (D). Uppercase and lowercase letters represent Core1 and Core2, respectively. Value across all varieties tested in each figure followed by a same letter are not significantly different (alpha=0.05).
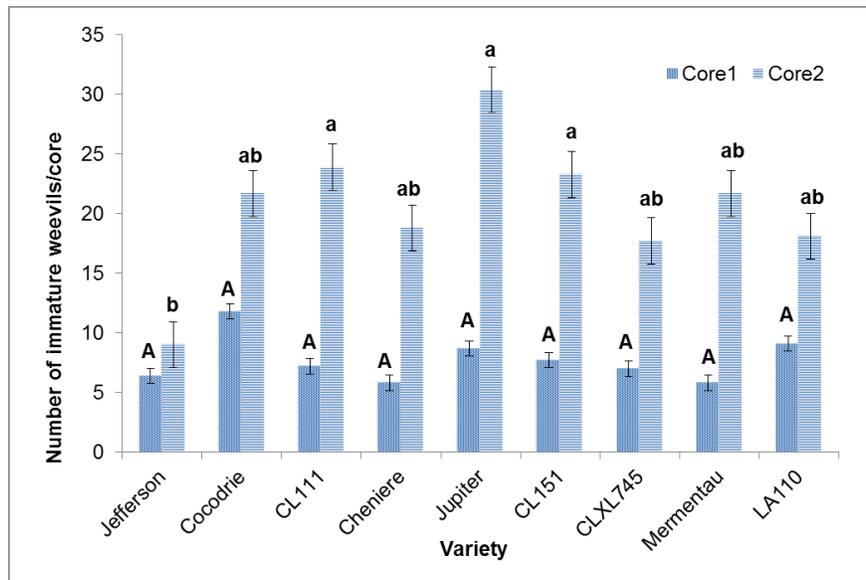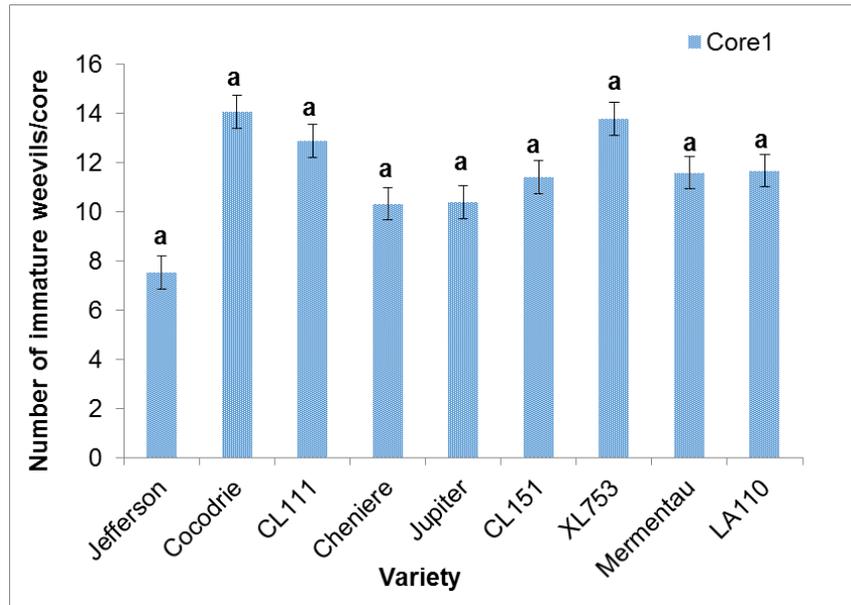
(Figure 3.1 continued)

(E) Year 2017

Figure 3.1. (Cont.). Mean numbers of rice water weevil immature per core ± SE in field evaluations at Rice Research Station (Crowley) during 2017 (E). Uppercase and lowercase letters represent Core1 and Core2, respectively. Value across all varieties tested in each figure followed by a same letter are not significantly different (alpha=0.05).
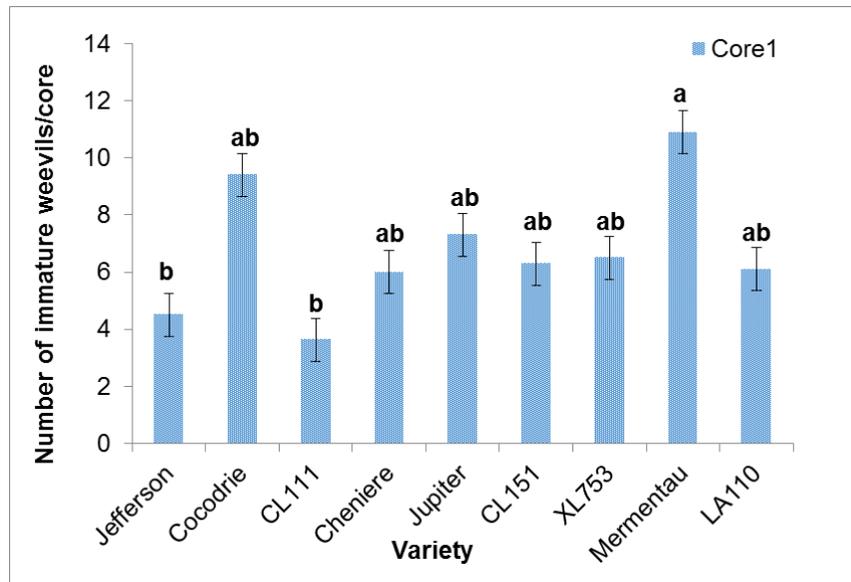
(A) Year 2015

(B) Year 2016

Figure 3.2. Mean numbers of rice water weevil immature per core ± SE in field evaluations at Lake Arthur during 2015 (A) and 2016 (B). Value across all varieties tested in each figure followed by a same letter are not significantly different (alpha=0.05).

3.3.2 Correlation among adult densities and feeding scars and immature weevil densities in the field

No significant correlations among densities of adult weevils or feeding scars and densities of immature weevils were detected in 2015, 2016, or 2017 (Figure 3.3 and Figure 3.4).
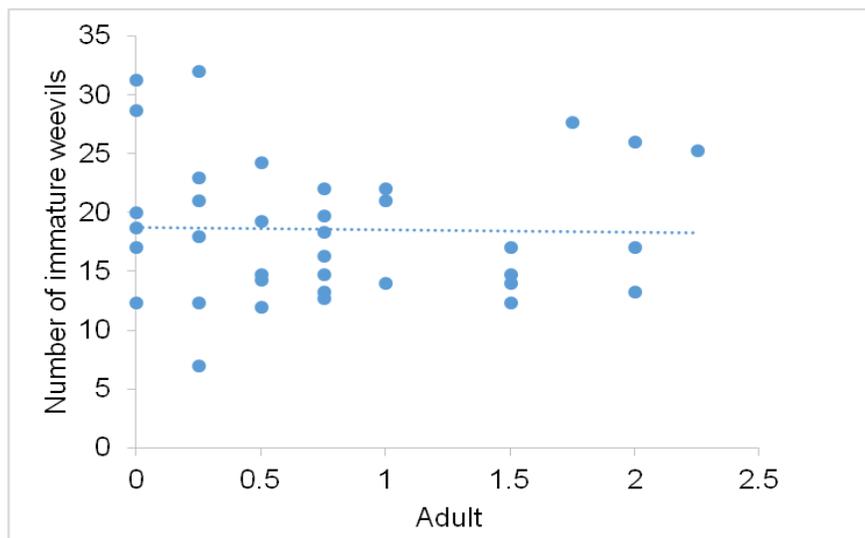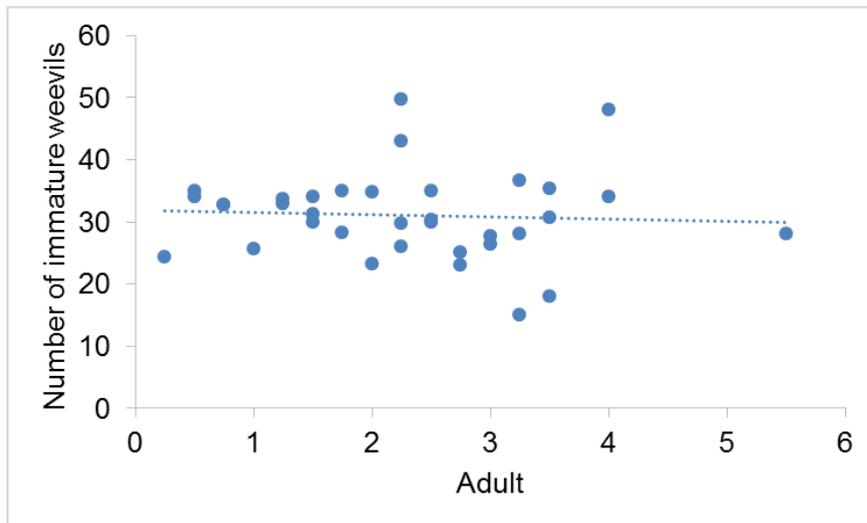
(A) Year 2015 (r = -0.02316, p = 0.8933)

Figure 3.3. Relationship between adult populations and immature weevil densities in field evaluations at Rice Research Station (Crowley) during 2015 (A). The immature weevil densities are from the second core samples (26 days after flooding (DAF) in 2015; 28 DAF in 2016, and 30 DAF in 2017).

(Figure 3.3 continued)

(B) Year 2016 (r = -0.0556, p = 0.7512)
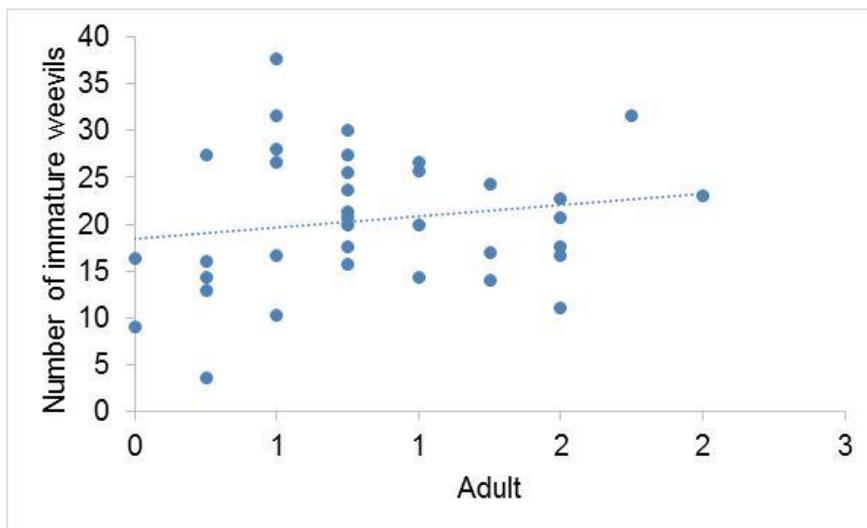

(C) Year 2017 (r = 0.1624, p = 0.3441)

Figure 3.3. (Cont.). Relationship between adult populations and immature weevil densities in field evaluations at Rice Research Station (Crowley) during 2016 (B) and 2017 (C). The immature weevil densities are from the second core samples (26 days after flooding (DAF) in 2015; 28 DAF in 2016, and 30 DAF in 2017).

(A) Year 2015 (r = 0.162, p = 0.3452)

(B) Year 2016 (r = 0.0922, p = 0.5985)

Figure 3.4. Relationship between feeding scars and immature weevil densities in field evaluations at Rice Research Station (Crowley) during 2015 (A) and 2016 (B). The immature weevil densities are from the second core samples (26 days after flooding (DAF) in 2015; 28 DAF in 2016, and 30 DAF in 2017).

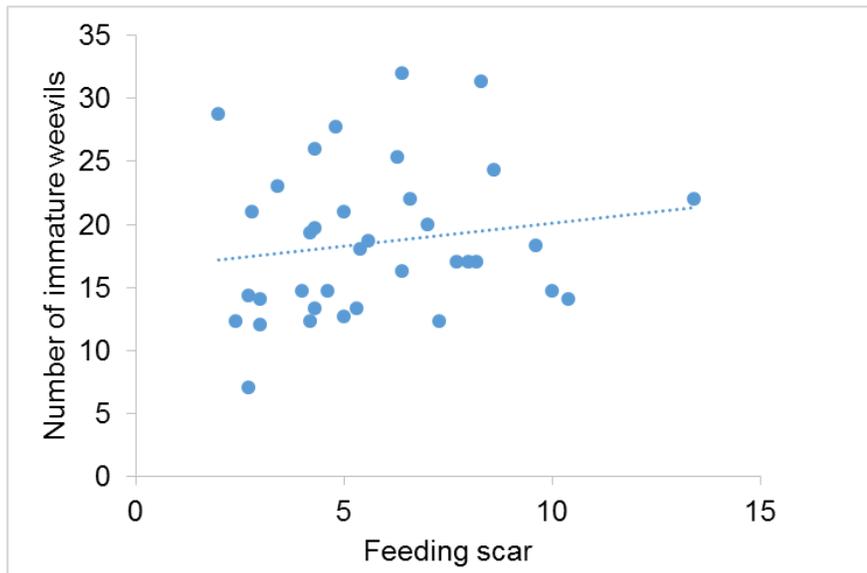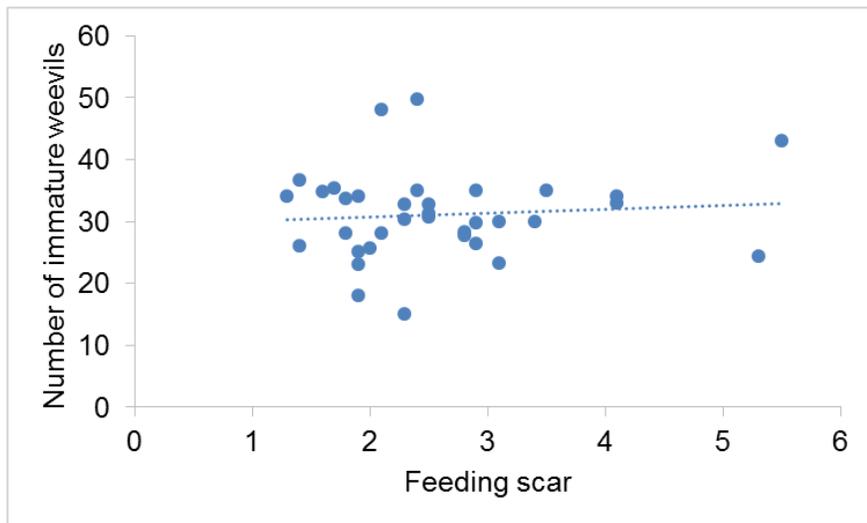(Figure 3.4 continued)

(C) Year 2017 (r = 0.06629, p = 0.7009)
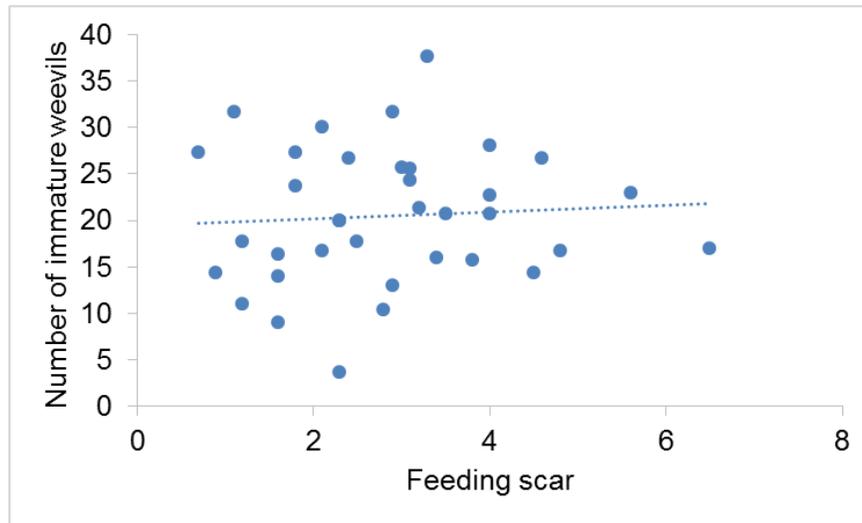
Figure 3.4. (Cont.). Relationship between feeding scars and immature weevil densities in field evaluations at Rice Research Station (Crowley) during 2017 (C). The immature weevil densities are from the second core samples (26 days after flooding (DAF) in 2015; 28 DAF in 2016, and 30 DAF in 2017).

3.3.3 Choice experiment

In the ovipositional preference (choice) experiment, gravid females laid significantly fewer eggs on the resistant standard Jefferson compared with the herbicide tolerant variety CL111 ($F = 2.73$; df = 7, 49; $P = 0.018$) (Table 3.3). No significant differences in densities of 1st instar were found among varieties. Numbers of late instars associated with Mermentau and CL151 were significantly lower than numbers of late instars associated with the hybrid variety XL753 ($F = 3.03$; df = 7, 49; $P = 0.008$) (Table 3.3).

3.3.4 No-choice experiment

In the no-choice (oviposition) experiment, no significant differences were observed in rice water weevil egg densities ($F = 1.67$; df = 7, 63; $P = 0.133$), and 1st

instar densities ($F = 0.90$; df = 7, 63; $P = 0.5124$) among varieties. Late instars

densities, however, did differ significantly among varieties (F = 4.14; df = 7, 63; P <

0.0001). Late instars densities were highest in variety Jupiter, intermediate in XL753,

and lower in the remainder of the varieties (Table 3.3).

Table 3.3. Mean (±SE) numbers of eggs, 1st instars, and late instars in choice and no-choice greenhouse experiments investigating ovipositional preference for eight varieties, Baton Rouge, Louisiana, 2016

Variety	(A) Choice			(B) No-choice		
	Eggs	1st instars	Late instars	Eggs	1st instars	Late instars
CL111	22.4 ± 5.0a	9.3 ± 1.6a	10.1 ± 0.9ab	13.4 ± 4.1a	14.3 ± 2.9a	9.2 ± 1.3b
CL151	9.4 ± 2.1ab	13.3 ± 3.3a	9.8 ± 2.3b	14.0 ± 2.3a	9.7 ± 2.9a	8.6 ± 1.7b
Cheniere	17.8 ± 6.0ab	17.1 ± 2.5a	10.5 ± 1.9ab	6.7 ± 2.5a	11.4 ± 2.2a	7.0 ± 1.4b
Cocodrie	12.5 ± 2.2ab	12.3 ± 2.6a	13.8 ± 1.7ab	10.0 ± 2.2a	12.9 ± 5.0a	9.0 ± 1.8b
Jefferson	5.1 ± 1.0b	13.5 ± 2.4a	10.9 ± 1.4ab	14.7 ± 3.7a	11.4 ± 3.2a	8.0 ± 1.4b
Jupiter	9.4 ± 1.4ab	14.4 ± 1.8a	14.1 ± 1.8ab	18.9 ± 3.7a	20.1 ± 5.0a	16.6 ± 2.2a
Mermentau	12.9 ± 3.8ab	7.1 ± 2.1a	7.9 ± 1.4b	16.1 ± 4.0a	17.1 ± 3.6a	8.1 ± 1.7b
XL753	16.1 ± 3.3ab	13.0 ± 2.6a	18.4 ± 2.8a	18.7 ± 3.7a	10.9 ± 3.2a	13.5 ± 1.5ab

Densities of eggs, 1st instars and late instars are means ± SE; (A) n=8, and (B) n=10. Egg, larval and pupa densities are expressed as number of egg or larvae plant^{-1}.
Means followed by the same letter are not significantly different ($P = 0.05$) according to Tukey's HSD.

3.3.5 Survival of rice water weevil larvae on four tested varieties

After 20 days of larval infestation, no significant differences were detected in the percentage of larval survival across the four varieties tested (F = 1.77; df = 3, 27; P = 0.1774). Nevertheless, visible differences on size categories of survival larvae across the variety are shown in Figure 3.5.
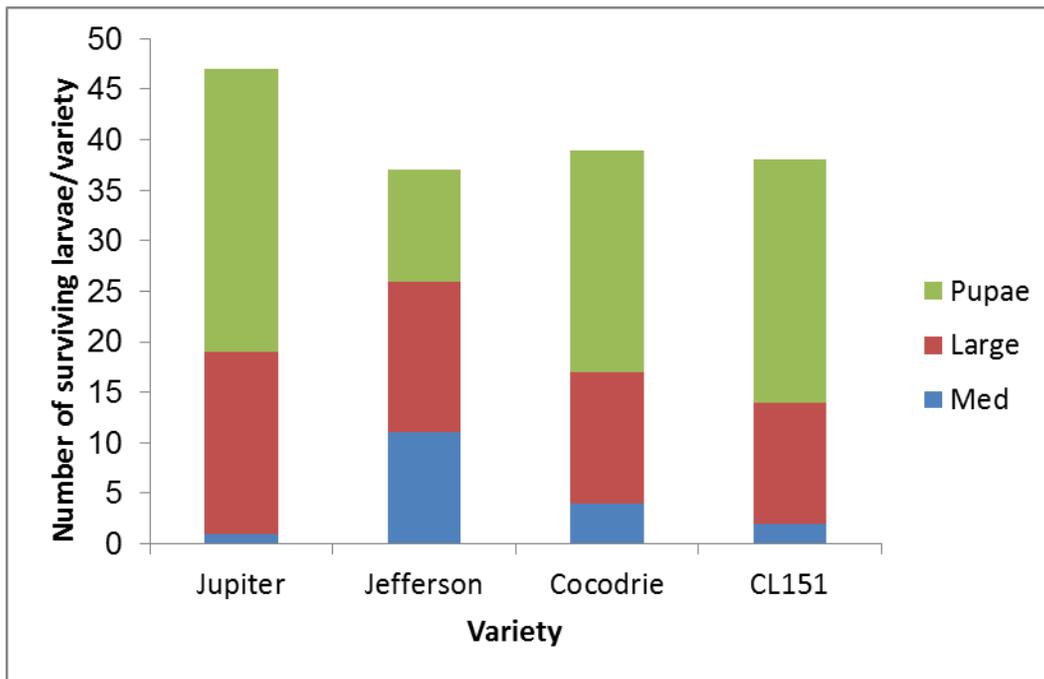
Figure 3.5. Survival larvae in different size categories in rice variety evaluated for larval survivorship, Baton Rouge, Louisiana, 2016.

3.3.6 Predictors of ovipositional preference

No statistically significant linear relationship was found between mean of number of eggs laid by female weevils and plant height (Figure 3.6: A). There is also no evidence of differences of an effect of intraveinal distance on female oviposition (Figure 3.6: B).

(A) Plant height (r =0.03342, p = 0.7932)
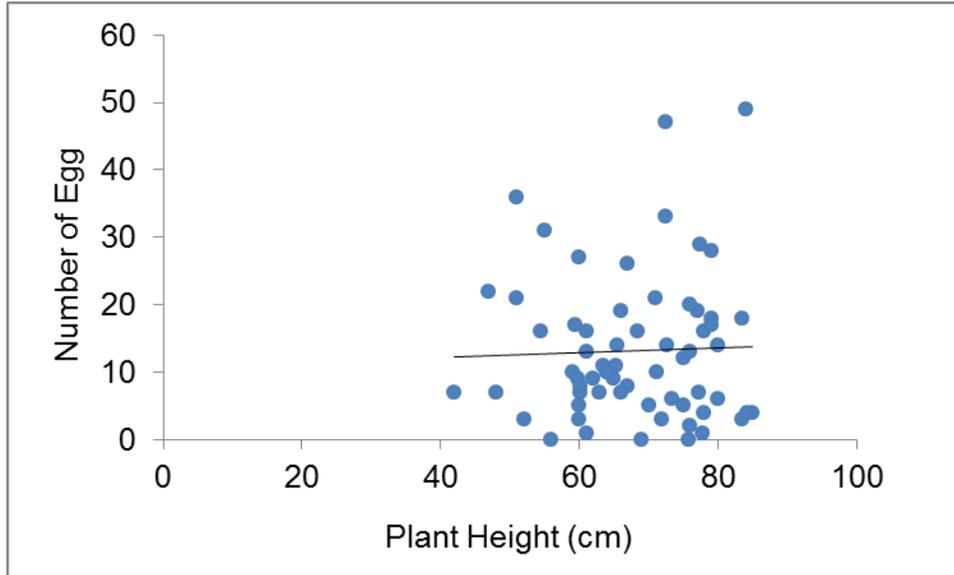
(B) Intraveinal distance (r = 0.00594, p = 0.9628)

Figure 3.6. Relationship between plant morphological traits; (A) plant height and (B) intraveinal distance with egg densities in choice experiment, Baton Rouge, Louisiana 2016. Plant heights were measured prior to imposition of adult weevil infestation. Distance between leaf veins were measured while counting densities of eggs (an average of four points in each plant).

3.4 Discussion

Varietal resistance potentially plays an important role in IPM programs in rice, but less attention has been given to insect resistance as opposed to disease resistant varieties (Heinr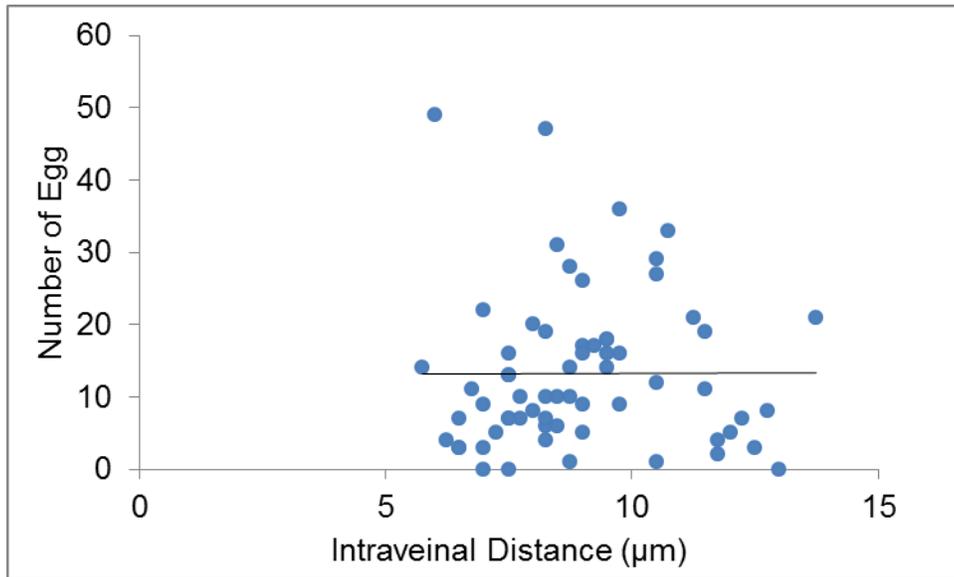ichs 1994).The main purpose of this study was to investigate the susceptibility of commercial rice varieties commonly grown in Louisiana to rice water weevil. Thirteen commercial varieties were evaluated for resistance by comparing densities of immature weevils. Because resistance to insects can be influenced by environmental factors (cultural practices, soil type, insect pest pressure, etc.), resulting in phenotypic variability (Gratani 2014), resistance was assessed over multiple years and locations and under field and greenhouse conditions. We found significant variation in susceptibility among the rice varieties tested.

The field study conducted over five consecutive years showed that Jefferson had numerically lower densities of immature weevils than the other commercial varieties in all seven field evaluations, five of them at Crowley and two at Lake Arthur (Figure 3.1 and Figure 3.2), and densities on Jefferson were significantly lower than at least other variety in five years. For example, in the last field evaluation in 2017, the extremely low numbers of immature weevils were recovered from the root wash of Jefferson plants, meanwhile immature weevil densities were 50% higher than Jefferson were observed in medium grain variety Jupiter (Figure 3.1 and Figure 3.2). Cocodrie and CL111 tended to increase in susceptibility to rice water weevil infestation over the seven-year study except in the second year of evaluation at the Lake Arthur site. Notably, Mermentau had lower immature weevil densities in its first and third year of evaluations at the Crowley site, but at the Lake Arthur site, Mermentau was among the varieties with the highest

number of immature weevil densities. The herbicide tolerant varieties CL151 and CL152, and the conventional hybrid XL753 only appear one time with lower weevil densities. The variety 'Cheniere' consistently showed an intermediate level of susceptibility compared to the resistant standard Jefferson. Field performance in this and other studies (Stout et al. 2001) has suggested that Jefferson has relatively greater durability of rice water weevil resistance than any other rice varieties tested. Jefferson retains a moderate level of resistance to rice water weevil in both high and low weevil pressure areas in Louisiana.

In a previous greenhouse experiment by Stout and Riggio (2003), resistance in Jefferson was expressed as antixenosis. Antixenosis or preference refers to an effect on insect behavior that reduces oviposition and/or feeding. In the choice experiment conducted under greenhouse conditions, egg densities were 4.4 times lower in Jefferson than egg densities found in the most widely grown variety CL111 (Table 3.3). This finding implies that Jefferson was less preferred for oviposition by female rice water weevils and thus possesses some antixenotic trait. However, antixenosis was not expressed consistently in the greenhouse as numbers of 1st instars found on Jefferson in the same experiment did not differ significantly from other varieties. In the larval survivorship experiment, the differences in percentage of larval survival on Jefferson and the other tested varieties were not significant ($F = 1.77$; df = 3, 27; $P = 0.1774$). It seems that antibiosis which is defined as an adverse effect of the host-plant on larval development, growth, or physiology is not responsible for resistance on Jefferson in this experiment, similar to earlier study by Zou et al. 2004a. However, numbers of late instars and pupae recovered from Jefferson plants were still the lowest among the four

varieties tested as shown in Figure 3.5. Differences in the size distribution of surviving

larvae in each category might be used to determine the antibiosis effect (Smith and

Robinson 1982, N'Guessan and Quisenberry 1994). The pattern of rice water weevil

resistance in Jefferson in the larval survival experiment, relative to that of the other

varieties, is similar and consistent with the results observed for the late instars densities

in the no-choice ovipositional experiment and also the performance of Jefferson under

field conditions. Our non-significant result was, therefore, probably due to lack of

replication and number of larvae used in this experiment. Furthermore, the number of 1st

or 2nd instars larvae rice water weevil/pot (n=5) in our study were low, which made it

difficult to determine the antibiosis.

Morphological traits often act as the first line of defense against herbivory attack

(Hanley et al 2007). There is a hypothesis that an element of physical obstruction may

influence host-plant selection (preference) for oviposition or feeding by insect herbivore.

According to Perrin (1977), plant height was considered to be a factor that influenced

insect pest movement within the cropping system for host-plant searching. The impact

of plant height in US rice was shown to affect infestation by skipper, *Ancyloxypha*

numitor (F) (Smith and Robinson 1983). To further investigate the factors that may

explain variation in ovipositional preference by female weevils, we measured variation

on plant height and intraveinal distance among varieties. None of the measured traits

were significantly related to ovipositional preference. The result with plant height was

similar to result from Finch and Kienegger (1997) findings. These authors found that

differences in plant height were not sufficient on their own to reduce the number of

insect pests eggs laid in brassica crop. Morphological traits may not adequately predict the degree of ovipositional preference by rice water weevils.

In an IPM approach, scouting is a forecasting tool that enables better decision-making in managing pests. It is important to do early scouting before any insecticide application. Timing of application is vital, and unnecessary insecticide applications increase costs of production to the growers. In this case, applications of adulticides for the control of eggs or larvae are ineffective and additional larvicidal insecticides are needed if adulticides applications are mistimed. In this study, we investigated if correlations existed between adult population densities or number of feeding scars and immature weevil densities, so that improved recommendations for scouting could be made to growers. Over three years, we found no significant correlations among adult weevil densities (determined 4-5 days after flooding) and immature weevil densities. Likewise, we found no significant correlations among scarring (determined two days before flooding) and immature weevil densities. These results indicate that these measurements cannot be used to determine a need for adulticides.

The rice water weevil continues to be one of the most important endemic pests of rice in Louisiana and neighboring states. Concomitantly, in accord with previous research studies by Stout and Riggio (2003), none of the currently grown varieties possess high levels of resistance to the rice water weevil. Therefore, using resistant varieties cannot be a primary strategy to control rice water weevil. Resistant varieties must be combined with other control measures to achieve adequate control. A recent study on host-plant resistance by Vyavhare et al. (2016) likewise showed small differences in varietal susceptibilities to the rice water weevil. However, the article urges

the idea that more research should be conducted to evaluate more rice germplasm. It should be noted that this study examined only the levels of resistance in several commercial rice varieties. We did not identify whether resistance mechanism in the various varieties were conferred by any specific resistance genes. Future studies are needed to identify the mode and inheritance of resistance in Jefferson. Because of it's resistance potential, considerable attention has been directed over the past few years to this particular variety. Knowledge of the inheritance of a trait is critical in designing appropriate breeding strategies for incorporating such a trait into economically useful populations.

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CHAPTER 4

SUMMARY AND CONCLUSIONS

Rice production in Louisiana could be increased by controlling the damage (yield losses) caused by biotic stresses, particularly the rice water weevil. Over the last decades, efforts to identify resistant rice varieties, *Oryza sativa* L., with acceptable levels of host-plant resistance (HPR) to the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel were facilitated by robust field and greenhouse screening.

This study elucidated the present status of resistant of commercial rice varieties currently grown in Louisiana to the rice water weevil. Choice field evaluations with high insect pressure allowed a large number of germplasm lines and varieties to be screened, and long-term evaluations allowed us to determine the stability of expression of resistance. Greenhouse evaluations (both choice and no-choice experiments), however, enabled confirmation of resistance in a controlled environment.

Findings of this study demonstrated that none of the varieties tested showed high levels of resistance to rice water weevil. In field evaluations over five years, Jefferson was frequently found to support lower larval densities than other variety, whereas Jupiter supported higher larval densities. In addition, adult population densities or feeding scars are not an indicator of immature weevil abundance in the field and thus both visible clues are not suitable to be used as indicator of action thresholds. Furthermore, no evidence was detected on how plant height or intraveinal distance might affect ovipositional preference by female weevils. Some of the main considerations regarding the limitations of this study are the lack of replication and lower

number of larvae used in the greenhouse experiments. Due to this conceivable

constraint, levels of resistance could not be expressed.

The findings from this study, as well as the studies cited in the literature review

can be used as a starting point for future research in evaluation for multiple pests and

evaluation for plant tolerance. Jefferson is a long-grain variety that possesses some

resistance to rice blast and sheath blight diseases. Jefferson has been used as a donor

in breeding programs to develop weevil resistant. In conclusion, the host-plant approach

to rice water weevil management has potential as this approach provides a framework

for researchers wishing to suppress rice water weevil in the field.

VITA

Maisarah Mohamad Saad was born and raised in Bentong town in the state of Pahang Darul Makmur, Malaysia. After she received her high school education in the same town, she obtained her Bachelor of Science degree in Bioindustry (Major: Pest Management) from University Putra Malaysia, Serdang Campus in 2005. In 2007, she worked as a Research Officer at Malaysian Agricultural Research and Development Institute (MARDI) at the Rice Research Station in Seberang Perai, Penang. With eight years of experience working with rice, she got an opportunity to pursue a master's degree in the United States with a scholarship given by MARDI. Maisarah enrolled into a Master program in August 2015 under the supervision of Dr. Michael J. Stout in the Department of Entomology at Louisiana State University and Agricultural and Mechanical College. Her thesis research involves investigation of varietal resistance of Louisiana rice against the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel in field and greenhouse studies. She is a member of the Entomology Society of America (ESA) under PI-E Section and also an active member of the LSU Entomology Club.