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Ecology and chemical ecology of plant-insect interactions in rice: implications for pest management

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ECOLOGY AND CHEMICAL ECOLOGY OF PLANT-INSECT INTERACTIONS IN RICE:
IMPLICATIONS FOR PEST MANAGEMENT

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
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in

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by
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ABSTRACT

Induced resistance to the rice water weevil, *Lissorhoptrus oryzophilus*, was assessed in greenhouse and field experiments. The fall armyworm,*Spodoptera frugiperda*, and an elicitor, jasmonic acid, were used to induce resistance. The effect of these treatments on rice resistance to oviposition varied between cultivar used, but significantly fewer larvae were found on plants exposed to *S. frugiperda* and jasmonic acid on both cultivars tested. Application of jasmonic acid significantly reduced the number of *L. oryzophilus* larvae per plant, and represents the first example of elicitor-induced resistance in rice in field experiments.

Oviposition by the sugarcane borer, *Diatraea saccharalis*, induced resistance to further oviposition by *D. saccharalis* in several cultivars. Plants with egg masses present received 33-50% fewer egg masses when exposed to gravid *D. saccharalis*. However, *D. saccharalis* oviposition on cultivar M202 rendered plants more susceptible to subsequent oviposition. M202 plants with egg masses present received 2-3 fold more egg masses when subsequently exposed to *D. saccharalis*.

The rice stink bug, *Oebalus pugnax*, was reared on rice (*Oryza sativa*), barnyardgrass (*Echinochloa crus-galli*) and amazon sprangletop (*Leptochloa panicoides*) and the metathoracic gland (MTG) contents were analyzed using GC/MS. Quantities of three compounds ((E)-2-decenal, (E)-2-hexenyl acetate and *n*-dodecane) are significantly influenced by host-plant. Crude metathoracic gland extracts attracted *O. pugnax* at low concentrations, and attraction decreased as the concentration increased, suggesting a bifunctional role of metathoracic gland compounds. Field experiments using a synthetic mixture of the four most abundant MTG chemicals significantly reduced *O. pugnax* in plots sprayed with this mixture. In addition, the host-plant on which *O. pugnax* was reared was found to significantly alter the ratio of four MTG chemicals, as

well as influence development time and adult weights.

The biological activity of four common phenolic compounds in rice (ferulic, *p*-coumaric, cinnamic and caffeic acids) were evaluated for their effects on the growth rate of *D. saccharalis* and *S. frugiperda* larvae. Levels of these compounds were quantified and then incorporated into diet bioassays. Despite minor structural differences, these compounds were found to have widely divergent effects on the larval weights of *D. saccharalis* and *S. frugiperda.*

INTRODUCTION

Worldwide, rice is planted on more than 150 million hectares (USDA FAS, 2005). Rice is a staple for more people than foods obtained from any other plant species, and a large majority of the world's population derives at least half of their caloric intake from rice (IRRI, 2000). Moreover, rice farms cover 11% of the world's arable land (IRRI, 2000). As a result, rice research and its applications have the potential to affect the well being of a large part of the world's population and will also have a substantial effect on the environment.

Globally, rice is cultivated in more than 50 countries across Asia, North and South America, Europe, Africa and Australia (USDA FAS, 2005). While accounting for only1.5-2% of global production, the United States is one of the largest exporters of rice, representing 14% of global rice exports (USDA FAS, 2005). In 2010, the value of the United States rice harvest was approximately $3.07 billion (USDA NASS, 2010), and in Louisiana, it was worth over $411 million (LA AgSummary, 2010).

The semi-aquatic environment in which rice is cultivated requires environmentally sound management strategies in order to reduce the adverse effects of insecticides on the unique fauna found in Louisiana rice paddies. In addition, the increasing co-production of crawfish and rice makes it absolutely necessary to investigate alternatives to the conventional management strategies historically used to control insect pests – insecticides. The research described in this dissertation was undertaken to understand aspects of the ecology and chemical ecology of rice and insect pests, with the broader goal of applying knowledge of plant-insect interactions to the development of novel management strategies.

In the southeastern United States, the rice water weevil, *Lissorhoptrus oryzophilus,* is an early season pest and also the most destructive insect pest of rice. Feeding by adults generally does not result in economic injury, but root pruning by larvae can severely reduce both the growth and yield of rice (Smith, 1983; Zou et al., 2004). In Louisiana, yield losses typically exceed 5 to 10% and can approach 25% or more (Stout et al., 2000). Induced resistance using a known elicitor of plant resistance, jasmonic acid, and another herbivore, the fall armyworm (*Spodoptera frugiperda*) was investigated in greenhouse and small-plot field experiments. Both treatments induced resistance in rice, and represent the first successful field-based application of jasmonic acid to reduce populations of an insect pest of *O. sativa*.

The rice stink bug, *Oebalus pugnax*, is the most important late season insect pest of rice in Louisiana. Broad-spectrum pyrethroid and organophosphate insecticides are commonly used for control of the rice stinkbug, although more selective neonicotinoid insecticides are beginning to be registered. Despite its significance as a pest, no non-chemical control methods have been implemented in production farms, and as a result, insecticides are the only control method farmers utilize.

Stink bugs are characterized by the production of large quantities of strong smelling and irritating defensive chemicals which are released from metathoracic glands (MTG) when the bugs are disturbed (Aldrich, 1988). Numerous studies have attested to their efficacy as a defensive response towards predators (Aldrich, 1988; Krall, et al. 1999; Staddon, 1979). They may also serve as alarm pheromones (Kou et al., 1989). Furthermore, studies have shown that male-produced pheromones are exploited by natural enemies (Aldrich et al., 1984; Aldrich, 1985; Aldrich et al., 1986; Hokkanen and Pimentel, 1989) and may help protect against entomopathogens (Sosa-Gomez et al., 1997; Milks and Hamm, unpublished). The multi-

functional role of MTG chemicals was investigated and shown to have concentration-dependent activity, with lower concentrations significantly attracting *O. pugnax* and higher concentrations repelling *O. pugnax*. This insight led to the application of a synthetic blend of *O. pugnax* MTG chemicals in field experiments, where significantly fewer *O. pugnax* were found in plots sprayed with this mixture. In addition, the host-plant on which *O. pugnax* was reared was found to significantly alter the ratio of four MTG chemicals, as well as influence development time and adult weights.

The use of host-plant resistance is crucial to development of sustainable integrated pest management programs. Induced resistance in rice to insect pests has been previously confirmed (Hamm et al., 2010; Karban and Chen, 2007; Stout et al., 2009). However, direct induced resistance resulting from oviposition by Lepidoptera has not been demonstrated in rice. Understanding cultivar-specific responses to oviposition represents an important first step in the development of cultivars with improved levels of resistance. In the cultivar M202 (a popular medium-grain developed in California), oviposition by the sugarcane borer, *Diatraea saccharalis,* was found to induce increased plant susceptibility to subsequent oviposition by the same species. Other cultivars (Cocodrie, Reiho, Rosemont) exposed to ovipositing *D. saccharalis* were found to be less preferred for oviposition following the initial oviposition event.

Phenolic compounds are a diverse group of secondary metabolites that are widespread in the plant Kingdom (Waterman and Mole, 1994). Studies on the activity and role of phenolic compounds have shown a wide range of effects on insects. The biological activity of four commonly found phenolic compounds in rice were evaluated for their effects on the growth rate of *D. saccharalis* and *S. frugiperda* larvae. Results from these diet incorporation experiments

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indicate different structure-activity relationships for each insect, and may provide some insight into the mode of action of phenolic compounds.

This work has provided novel insights into several aspects of the chemical ecology of rice insect pests and the interactions of these pests with rice. A greater understanding of the chemical ecology underlying insect-plant interactions will facilitate the development of novel management strategies in the near future. For example, the successful use of a plant-based elicitor to induce plant resistance in small-plot field experiments can provide a valuable framework for larger-scale studies now that the methodology has been established. In addition, *O. pugnax* produced volatiles have the potential to be used in a variety of methods, from use in monitoring traps to potentially attracting natural enemies and parasitoids and to even be used as a spray to repel *O. pugnax* from entering fields. The combined use of multiple control tactics in an integrated manner will aid in the development of a more sustainable approach to pest management for insect pests of rice in Louisiana.

CHAPTER 1: LITERATURE REVIEW

1.1. Rice Insect Pests in the Southeastern United States

A major limiting factor worldwide for rice production is damage by insect pests. In Louisiana, the main pests are the rice water weevil, *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae), the rice stink bug, *Oebalus pugnax* (Hemiptera: Pentatomidae) and a group of sporadic pests, the sugarcane borer, *Diatraea saccharalis* and the fall armyworm, *Spodoptera frugiperda*.

1.1.1. Rice Water Weevil

The rice water weevil, *Lissorhoptrus oryzophilus*, is considered to be the most important insect pest in Louisiana as well as in other Southern rice producing states (Smith, 1983; Way, 1990). It also has the potential to be an international pest due to its introduction into Japan in 1978 (Smith, 1983), Korea, Taiwan and mainland China in the 1990's (Heinrichs and Quisenberry, 1999). Adults feed on leaves, leaving longitudinal scars which are not considered economically important. Typically, oviposition does not begin until after the fields are flooded (Everett and Trahan, 1967; Muda et al., 1981; Smith, 1983; Stout et al., 2002b). Females oviposit in the leaf sheaths just below the water surface (Everett and Trahan, 1967; Raksarart and Tugwell, 1975; Smith, 1983; Way, 1990). Larvae eclose within four to nine days after oviposition and migrate to the roots (Everett and Trahan, 1967; Raksarart and Tugwell, 1975). Larval damage causes an average of a 10% loss in yield (Smith, 1983) and can result in losses up to $50 million annually (Spradley and Widham, 1995).

Non-chemical methods for rice water weevil control have been investigated, but have shown little success (Puissegur, 1976; Bunyarat et al., 1977; Smith, 1983; Way, 1990; Thompson et al., 1994; N'Guessan and Quisenberry. 1994; N'Guessan et al., 1994; Rice, 1996; Heinrichs

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and Quisenberry, 1999; Stout et al., 2001; Stout and Riggio, 2002). Draining and drying fields until the soil cracks during heavy infestations of larvae may be effective in some cases, but is not recommended due to frequent rain and the costs associated with reapplying herbicide and fertilizer (Way, 1990; Thompson et al., 1994) and pumping of water to reflood.

Thousands of rice lines have been screened for resistance to the rice water weevil, but only a few have shown low levels of resistance (N'Guessan and Quisenberry, 1994; N'Guessan et al., 1994; Heinrichs and Quisenberry, 1999; Stout et al., 2001; Stout and Riggio, 2002); nevertheless, even the most resistant lines do not avoid weevil damage and require additional control methods—usually insecticides.

As a result, chemical control of rice water weevil has been heavily relied upon. Carbofuran was found to provide effective control in the mid 1960's and was the primary means of control until the late 1990's. In 1998, carbofuran lost its registration for rice, and new insecticides were introduced in 1997. Icon was used heavily after the loss of carbofuran; however, it was removed after the 2005 growing season due to voluntary withdrawal by its manufacturers.

Early flooding applied at the two to three leaf stage is commonly practiced in southwestern Louisiana. This is especially important where red rice is a severe pest because it assists in red rice management. In areas where red rice is a less of a problem, floods are delayed until the four to five leaf stage. Nonetheless, the initiation of flooding induces rice water weevil oviposition, with more eggs oviposited in leaf sheaths of flooded rice plants than non-flooded plants. The depth of flood also influences oviposition; floods of 10.2 cm were the most preferred when rice water weevils were provided a choice between multiple flood depths (Stout et al., 2002b). Research has also shown that younger plants are more susceptible to rice water weevil

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injury (Stout et al., 2002a). The time at which permanent floods are applied also affects rice

water weevil injury to rice (Rice et al., 1999; Zou et al., 2004). When floods are delayed by two

weeks, numbers of rice water weevil larvae on roots were reduced by as much nine times that on

roots of early flooded rice (Rice et al., 1999; Zou et al., 2004). However, delaying floods has not

been readily adopted because it compromises red rice control (Dunand, 1988).

1.1.2. Rice Stink Bug

Rice stink bugs are attracted to rice during the reproductive stage of growth, especially

during the grain filling period. The grain filling period is separated into three stages, based on

liquid content within each grain -- the milk, soft and hard dough stages. Previously, Rolston et

al. (1966) observed that rice stink bugs demonstrated a preference for grain in the earlier stages

of development, showing that the injury caused by feeding could cause measurable damage by

arresting the development of older grains and/or completely damaging younger grains.

Depending on which stage is attacked, feeding results in yield loss and/or reduced quality

(Chambliss, 1920; Drees, 1983; Elliot et al., 1994; Gifford et al., 1968; Harper et al., 1983; Patel

et al., 2006a; Smith et al., 1986; Swanson, 1960; Swanson and Newsom, 1962). When kernels

are attacked during the milk stage, the contents are sucked out, rendering the grains empty - a

condition known as ‒false grains‖ (Bowling, 1956; Douglas and Ingram, 1942; Drees, 1983;

Genung et al., 1979; Hamer and Jarratt, 1983).

When grains are damaged during the soft and hard dough stages, only portions of the

seed contents are removed. The result is a chalky, discolored area around the feeding site

(Johnson et al., 1987; McPherson and McPherson, 2000; Ogdlen and Warren, 1962). The

resulting grain is of inferior quality, often known as pecky rice. Pecky rice is a general term for

discolored rice kernels resulting from both feeding and pathogen damage. Moreover, feeding

sites can serve as entry points for pathogens (Lee et al., 1993). Pecky rice is of reduced quality (McPherson and McPherson, 2000; Way, 1990) because it often breaks during milling due to the weakening at the feeding site (Johnson et al., 1987; Way, 1990). It is challenging to estimate losses from rice stinkbugs because feeding damage resulting in pecky rice is not apparent until the milling process, and pecky rice is not always attributable to injury by the rice stink bug.

Currently, two types of insecticides are used for *O. pugnax* control -- organophosphates (methyl parathion), pyrethroids (gamma-cyhalothrin, lambda-cyhaolthrin and zeta-cypermethrin) and neonicotinoids (clothianidin). Despite its significance as a pest, no other control methods have been implemented in production farms, and as a result, insecticides are the only control method farmers utilize.

In the near future, the insecticides used in the control of the rice stink bug may be removed due to environmental and human safety concerns in addition to the increasing cost of registration (McPherson and McPherson, 2000). These mounting concerns require the investigation of non-chemical control strategies for the rice stink bug. One major limiting factor in the development of ecologically conscious management tools is the brief time period rice is vulnerable to the rice stink bug. Way (1990) indicated that rice is vulnerable for approximately 30 days, but in a recent study by Patel et al. (2006a), it was shown that rice is most vulnerable to rice stink bug damage during the first two weeks following anthesis. The short window of vulnerability makes timing of control strategies critical, and in turn, necessitates the development of accurate and effective management strategies.

1.1.3. Sugarcane Borer

The sugarcane borer, *Diatraea saccharalis*, is a major pest of sugarcane in the western hemisphere, and in Louisiana, is responsible for up to 90% or more of the insect damage to

sugarcane (Reagan et al., 1972). Although sugarcane is the principal host, at least twenty other plants have been reported as hosts (Holloway et al., 1928). Of these, the most economically important hosts in Louisiana include sweet sorghum (*Sorghum bicolor* (L.) Moench), corn (*Zea mays* L.) and rice (*Oryza sativa* L.) (Roe et al., 1981).

Historically speaking, the sugarcane borer has been considered an infrequent pest of corn and rice in Louisiana. However, it has steadily dispersed into central and north-eastern Louisiana; in 2002, approximately 3,000 acres of rice in Concordia Parish were infested with sugarcane borers that destroyed from 70 to 95 percent of the rice crop on some farms (Castro et al., 2004). In the future, outbreaks of this magnitude will become more commonplace unless a multi-crop management program for the sugarcane borer is developed.

High levels of field stubble in rice, corn, sorghum and other crops, often a result of no-till or conservation tillage practices, can increase the number of diapausing larvae, and in turn, facilitate the buildup of early season sugarcane borer populations. Often, corn, sorghum and rice fields are planted adjacent to each other, and if one factors in native gramineous hosts, this creates a wide range of suitable host crops available for the development and buildup of sugarcane borer populations across large areas throughout the growing season. In turn, the early buildup of large populations can increase the costs associated with chemical control. Not surprisingly, timely insecticide applications should be an integral component of sugarcane borer management programs.

The first parasitoid introduced in the United States on sugarcane was the Cuban fly, *Lixophaga diatraeae* (Townsend) into Louisiana. This tachinid fly, introduced from Cuba, was released during different intervals from 1915 to the early 1970's throughout much of the southeastern United States (Rodriguez-del-Bosque and Smith, Jr., 1996).

Another parasitoid, *Agathis stigmatera* (Hymenoptera: Braconidae) was introduced from Peru into Florida in the early 1930's and into Louisiana in the late 1940's and early 1950's. In the Caribbean islands and much of South America, it had been known to be parasitizing *Diatraea* spp. since the 1920's (Smith et al., 1993). Other parasitoid species released in Louisiana after the original introductions included tachinids, braconids, and scelionids. Some of the above species have become established in Louisiana and Florida, but none have provided consistent stem borer population suppression (White and Reagan, 1999).

1.1.4. The Fall Armyworm

The fall armyworm, *Spodoptera frugiperda*, is a polyphagous insect which is an important pest on several crops (Luginbill, 1928). It was originally reported as a pest of rice in Georgia in 1881 (Riley, 1881). Although its host range is wide, cereals and grasses are the most preferred among it host plants (Crumb, 1927). When infesting rice, *S. frugiperda* rapidly defoliates seedlings. Larvae typically feed and develop and become fully grown in two to three weeks. Most larvae that develop on flooded rice never pupate, as larvae normally pupate in the soil, and because of this, are considered a sporadic pest of rice in the southern United States (Bowling, 1978; Smith et al., 1986). In other countries, however, *S. frugiperda* has been reported to cause severe damage to rice at the seedling stage (Chandler et al., 1977; Machado, 1978; Navas, 1976). Despite its ability to rapidly defoliate stands of rice, Lye and Smith (1988) found that maximum larval weight of *S. frugiperda* was higher when fed three-leaf stage *O. sativa* foliage, but weights were lowest when larvae were fed material from older plants. Similarly, Hardy et al. (1986) reported that both neonate and fourth instar *S. frugiperda* larvae feed more on new growth than on older growth of tall fescue grass.

1.1.5. Conclusions

For all of the above described pests of rice, there are significant gaps in the research into novel, non-insecticide based management strategies. The adoption of neonicotinoid seed treatments in conjunction with Clearfield seed technology and delayed flooding has significantly enhanced *L. oryzophilus* management. Despite this, insecticides still remain the sole option to treat for L. *oryzophius*. The goal of my research is to develop a methodology on the application of an elicitor, jasmonic acid, which can be used in a field experiment in order to reduce the number of *L. oryzophilus*.

The lack of an integrated pest management plan for *D. saccharalis* – and stem borers in general – will become a major problem if stem borers reach economically damaging levels throughout the state. The arrival of the Mexican rice borer, *Eoreuma loftini*, makes the development even more critical. Host-plant resistance is an important aspect of any management program, and my research will investigate induced responses in different cultivars of *O. sativa*, with the broader goal of understanding how *O. sativa* responds to oviposition by *D. saccharalis*.

The management of *O. pugnax* is solely reliant on insecticides and this represents a major gap in an integrated pest management program. In addition to concerns relating to the development of resistance towards organophosphate and pyrethroid insecticides, the lack of any premature monitoring of insects prior to infesting fields represents an area that could benefit from a better understanding of the chemical ecology of *O. pugnax*. Research into the multi-functional role of *O. pugnax* defensive chemicals can contribute to a diversification of management programs by deterring *O. pugnax* from entering fields, and may be able to be used in early monitoring programs.

While *S. frugiperda* is a sporadic pest of *O. sativa*, outbreaks happen rapidly, with large areas of unflooded fields being defoliated in a short time period. To protect itself from herbivory, *O. sativa* produces many secondary compounds, many of them phenolic acids. Understanding how these compounds and *S. frugiperda* interact and how *S. frugiperda* is able to effectively metabolize these compounds may enhance our understanding of the mode of action of these compounds. My research into effects of phenolic acids on the growth of *D. saccharalis* and *S. frugiperda* can lead to a greater understanding of the structure-activity relationship of these compounds.

CHAPTER 2. HERBIVORE AND ELICITOR-INDUCED RESISTANCE IN RICE (*ORYZA SATIVA*) TO THE RICE WATER WEEVIL (*LISSORHOPTRUS ORYZOPHILUS* KUSCHEL) IN THE LABORATORY AND FIELD[*]

2.1. Introduction

Feeding by arthropod herbivores often causes changes in the expression of plant resistance-related genes and traits, and these changes often result in plants becoming less suitable for subsequent herbivores. This phenomenon has been termed induced resistance (Karban and Baldwin, 1997). Induced resistance to herbivory can be broadly classified as direct or indirect. Direct induced resistance refers to changes that negatively affect herbivore behavior, growth or physiology and can be manifested in a variety of ways, such as reduced feeding, oviposition, fecundity and survival of herbivores on previously damaged plants (Walling, 2000). Indirect induced resistance refers to changes that attract or retain natural enemies of herbivorous arthropods (Dicke et al., 2003). Direct and indirect induced resistance have been reported in a wide variety of plants (see Constabel et al., 2000; Howe et al., 1996; Johnson et al., 1989; Pechan et al., 2002). However, such responses are less studied in rice and other economically important monocots (Karban and Chen, 2007; Kogel and Langen, 2005).

Jasmonic acid (JA) is a plant hormone that serves as an important signal molecule to mediate the expression of both direct and indirect defenses against herbivory (Browse and Howe, 2008; Thaler et al., 2002). JA accumulates rapidly in plant tissues near the site of herbivore attack (Korth and Thompson, 2006), and increases in endogenous JA lead, through a series of intermediary steps, to changes in the expression of resistance-related genes and metabolites and to enhanced resistance to herbivory (Bruinsma and Dicke, 2008; Korth and Thompson, 2006). Consistent with its role as an endogenous signal, treating plants with exogenous JA often

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simulates the changes induced by natural herbivory (Browse and Howe, 2008; Farmer and Ryan, 1990; Kessler and Baldwin, 2002).

The broad spectrum nature of induced resistance and the ability to stimulate induced resistance by applying exogenous elicitors like JA has raised the possibility of using induced resistance in agriculture. One way induced resistance could be used in agriculture is to use JA or other elicitors to stimulate resistance at appropriate times in the life cycles of the pest and crop (Stout et al., 2002a). Most tests of this idea have been conducted in greenhouses, and few studies have shown significant reductions in the preference, feeding and survival of pests in field settings (Black et al., 2003; Thaler et al., 1996).

Rice, which serves as a staple food for a large portion of the world's population, has been relatively neglected as a model for the study of induced resistance to herbivorous arthropods (Karban and Chen, 2007). What research that has been done has primarily focused on indirect induced resistance and/or induced responses following feeding by piercing/sucking insects (Bentur and Kalode, 1996; Kanno et al., 2005; Lou et al., 2005; Matsumura and Suzuki, 2003; Satoh et al., 2005; Seino et al., 1996; Xu et al., 2002, 2003). Recently, however, Stout et al. (2009) demonstrated direct induced resistance in rice to a chewing insect, the fall armyworm (*Spodoptera frugiperda* J.E. Smith), following earlier feeding by the same insect. The fall armyworm feeds on a wide variety of plants and is considered a minor pest of rice. The experiments described herein build upon this observation by studying the effects of prior armyworm herbivory and exogenous JA on resistance of rice to its major early-season insect pest in the United States, the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel.

Adult rice water weevils move from overwintering sites to rice fields in early spring and feed on rice leaves, resulting in longitudinal feeding scars that run parallel to leaf veins.

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Oviposition commences when rice fields are flooded (Everett and Trahan, 1967). Upon hatching, neonate larvae migrate down the plant to roots, where they feed and pass through four larvae instars in 21-27days (Zou et al., 2004). Feeding by adults generally does not result in economic injury, but root pruning by larvae can severely reduce both the growth and yield of rice (Smith, 1983; Zou et al., 2004). In Louisiana, yield losses typically exceed 5 to 10% and can approach 25% or more (Stout et al., 2000). The objectives of this study were to determine if defoliation by *S. frugiperda* herbivory and exogenous JA applications induce resistance to *L. oryzophilus*.

2.2. Materials and Methods

2.2.1. Greenhouse Experiments

Plant and Insect Culture. A total of four experiments were conducted in a greenhouse on the campus of Louisiana State University, Baton Rouge. For each experiment, seeds were planted in a sterilized soil mix (2:1:1, soil: peat moss: sand) in 11.4cm^2 square pots and plants were maintained in greenhouse conditions under ambient lighting at approximately 29°C- 33°C. At the time of planting, approximately 1.2g of 19:6:12 controlled release fertilizer (Osmocote, Scotts Miracle-Gro, Marysville, OH) was added to soil. Plants were grown and experiments were conducted in large wooden basins lined with heavy black plastic that allowed plants to be flooded. Plants were thinned to a density of three plants per pot five to seven days after planting.

Adult rice water weevils used in these experiments were collected from rice fields at the LSU AgCenter's Rice Research Station in Crowley, Acadia Parish, Louisiana, one day prior to use in experiments. Weevils were maintained until use in large plastic containers with water and rice leaves. In order to ensure an equal ratio of males and females, weevils were captured in-copula and placed in small plastic cups just prior to use in experiments.

Fall armyworm larvae used to damage plants and to evaluate resistance were obtained

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from a colony maintained year-round on artificial diet in the laboratory. The colony originated from larvae collected in bermudagrass pastures near Baton Rouge in 1997. Insects collected from pastures or rice fields are added annually to the colony to maintain genetic variability and vigor.

Characterization of Induced Resistance Following Fall Armyworm Herbivory. Two separate experiments were conducted to assess whether resistance to *L. oryzophilus* was induced by prior *S. frugiperda* herbivory. In the first experiment, the rice variety 'Rosemont' was used, and in the second, 'Jackson' was used. These varieties were used because prior studies had shown that they are very responsive to fall armyworm feeding and JA treatment (Stout et al., 2009). Rice seedlings were grown to the early three-leaf stage as described above. Pots were then randomly assigned to two treatment groups, 'control' and 'damaged.' Plants were damaged by confining one fourth to fifth instar *S. frugiperda* larvae per plant using cages. Cages were constructed of clear plastic cylinders (8.5cm diameter, 23cm height) with one end inserted into the soil and the top end covered with a mesh-screen lid. The cylinders had two mesh-lined holes to allow for air circulation. Larvae were allowed to feed for four to six hours, and on average, consumed
between 20% and 30% of total leaf area, typically damaging portions of every leaf. Cages with no larvae were placed over plants assigned to the control group. Cages and larvae were removed from plants after four to six hours of feeding and plants were maintained in the greenhouse for later evaluation of resistance to *L. oryzophilus*.

Evaluations of resistance to *L. oryzophilus* were conducted approximately 13 to 15 days after injury by *S. frugiperda*. By this time a new leaf or, in some cases, two new leaves had emerged on both damaged and control plants. Four pots of each treatment were placed into infestation cages, which were constructed of cylindrical wire frames (46 cm diameter, 61 cm tall)

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covered with a mesh fabric screening. A total of five cages were used in each experiment.

Weevils were then placed in cages at a density of one male:female pair per plant (48 weevils per

cage). Basins were flooded to a depth of ≈24cm, and weevils were allowed to feed, mate and

oviposit on plants in cages for four days. Plants were then removed from cages and any weevils

found on plants were removed.

Densities of eggs and first instars on or associated with plants were used to estimate

levels of weevil infestation on plants. Procedures for estimating egg and larval densities were

adapted from Heinrichs et al. (1985) and Stout and Riggio (2002). Estimating egg densities

provides information regarding oviposition preference, and estimating larval densities provides

further information on oviposition preference and possibly on survival of eggs and early instars.

Densities of eggs were determined by removing one plant from each pot. Soil was carefully

removed from the roots, and plants were then labeled and placed in 75% ethanol until bleached.

The numbers of eggs on plants were determined by carefully examining plants under a dissecting

microscope (Meiji Techno Co. Ltd, Tokyo, Japan).

The densities of first instar larvae emerging rom plants were determined by removing two

plants from each pot, carefully washing soil from the roots and suspending individual plants in

test tubes containing distilled water. Test tubes were labeled, arranged in a rack, and placed in a

growth chamber (28°C,14:10 L:D). Weevils infesting plants treated in this manner hatch from

eggs, emerge from leaf sheaths, and settle on the bottom of test tubes (Heinrichs et al., 1985).

First instars were removed by shaking roots free of larvae and then pouring water from test tubes

into a Petri dish for counting. Plants were placed back into their respective test tubes

immediately after counting and replenished with distilled water. Larvae were counted daily until

no larvae were found for three consecutive days.

Characterization of Induced Resistance Following Jasmonic Acid Application.

Jasmonic acid induced resistance was assessed in two experiments, each of which used the two rice varieties, 'Jasmine' and 'Rosemont.' Elicitor treatments were prepared by dissolving 21mg (1mM) or 105mg (5mM) jasmonic acid (Sigma-Aldrich, St. Louis, MO) in 1mL of 95% ethanol and adding the ethanol solution to 100mL distilled water. Three weeks after planting, six pots of each variety were assigned to treatment groups and were sprayed with 100mL of a 1mM or 5mM JA solution using a hand held aerosol sprayer until run-off, and control plants were sprayed with 1mL ethanol dissolved into 100mL water. Each plant received approximately 5.5mL of elicitor or control solutions. The following day, one pot of each treatment and variety combination was placed in each of six infestation cages, resulting in six pots per cage, and plants were infested with weevils as described earlier. Estimation of larvae and egg densities were carried out as previously described (egg densities were not determined in the second experiment).

Analysis of Data. Data for each of the four experiments described above were analyzed separately. For each experiment, counts of eggs and first instar larvae were taken using separate plants and provided independent measures of plant resistance. Data from *S. frugiperda* experiments were analyzed as a completely randomized block design using a mixed-model analysis of variance (ANOVA) (PROC MIXED) in SAS (SAS Institute, 2007), with damage treatment (damaged or control) as a fixed effect and cage as a random effect. Data from JA experiments were analyzed as a 2x3 factorial using PROC MIXED, with infestation cage as a random effect and variety and JA concentration (0, 1 and 5 mM) as fixed effects. Means were separated using least significant differences (LSD) test.

2.2.2 Field Experiments

Plants. Two experiments were conducted during the 2008 growing season at the Louisiana State University Agricultural Center Rice Research Station, Crowley, Acadia Parish,

Louisiana. In both experiments, rice was hand planted in plots using a plywood template. This template measured 1.5m x 1.5m, with 3cm diameter holes spaced 7.5cm apart and arranged in five rows and five columns. In each 3cm hole, two seeds were inserted into soil at a depth of approximately 2cm. The template and plants within were considered a plot, and plots were spaced 1m apart in a completely randomized design. In the first experiment, rice variety 'Jackson' was hand planted on 8 April with six plots (replicates) of each of three treatments. Treatments consisted of exogenous applications of 1mM and 5mM solutions of JA and an untreated control; JA and control solutions were prepared as described in greenhouse experiments. On 29 April, 21 days after planting, plots were fertilized at a rate of 68kg per acre of nitrogen as urea and plants were subsequently sprayed with JA until runoff. A permanent flood was established one week later.

A second experiment using rice variety 'Rosemont' was planted on 10 June using the same templates as above with 1.8m spacing between plots. The experimental design was a completely randomized design with two treatments – control and JA (5mM) – with 15 replicates for each treatment. On 10 July, 30 days after planting, plots were fertilized at a rate of 68kg per acre of nitrogen as urea and plants were subsequently sprayed with JA until runoff and permanent flood was established the following day.

Estimation of Egg and Larval Densities. In the first field experiment, plants were sampled for eggs six and 12 days after establishment of permanent flood (13 and 19 days after JA treatment). In the second experiment, plants were sampled for eggs two days after permanent flood was established (three days after JA treatment). For egg sampling, two plants from each plot were removed and soil was washed from roots. Plants were labeled and stored in 75% ethanol until bleached. The numbers of eggs on plants were determined by examining the leaf sheaths under a dissecting microscope (Meiji Techno Co. Ltd, Tokyo, Japan).

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Plot densities of *L. oryzophilus* larvae and pupae were determined using a soil-root core sampler with a diameter of 9.2cm and a depth of 7.6cm. Core samples were taken 19 and 27 days after permanent flood in the first experiment, and 14 and 19 days after permanent flood in the second experiment. For each sampling date, one to four core samples were taken from each plot. Core samples were processed by placing them in a sieve bucket (40-mesh screen) and washing soil from roots. Buckets were then placed into plastic basins containing salt water, which facilitated larval and pupal counts as they floated to the water surface (N'Guessan et al., 1994).

Data Analysis. Prior to analysis, the number of immature *L. oryzophilus* observed in each core sample was converted to number of larvae per plant by dividing the total number of immature larvae by the number of plants in each core sample. Generally, one or two plants were contained in each core sample. Data were analyzed as completely randomized design experiments by one- way ANOVA using PROC MIXED with treatment (JA or control) as a fixed effect. Means were separated using least significant difference (LSD) test.

2.3. Results

Characterization of Induced Resistance Following Fall Armyworm Herbivory. In the first experiment using variety 'Rosemont' significantly fewer larvae emerged from plants previously damaged by *S. frugiperda* larvae two weeks earlier than from undamaged plants (Figure 2.1; $F_{1,33}$=5.30, P=0.028). The number of eggs per plant did not significantly differ between control and damaged plants ($F_{1,33}$=1.66, P=0.21). In the second experiment using variety _Jackson,' we found that both eggs ($F_{1,33}$=8.78, P=0.0056) and larvae ($F_{1,33}$=15.02, P=0.0005) of *L. oryzophilus* were significantly reduced in plants that were previously fed upon by *S. frugiperda* than in undamaged plants (Figure 2.2).

Characterization of Induced Resistance Following JA Applications. In our initial JA

experiment, number of eggs were significantly lower in plants treated with 1mM and 5mM JA when compared to control plants in both varieties (Figure 2.3; $F_{2,50}$=12.39, P<0.0001). There were fewer eggs per plant on 'Rosemont' than on 'Jasmine' ($F_{1,50}$=5.89, P=0.02).
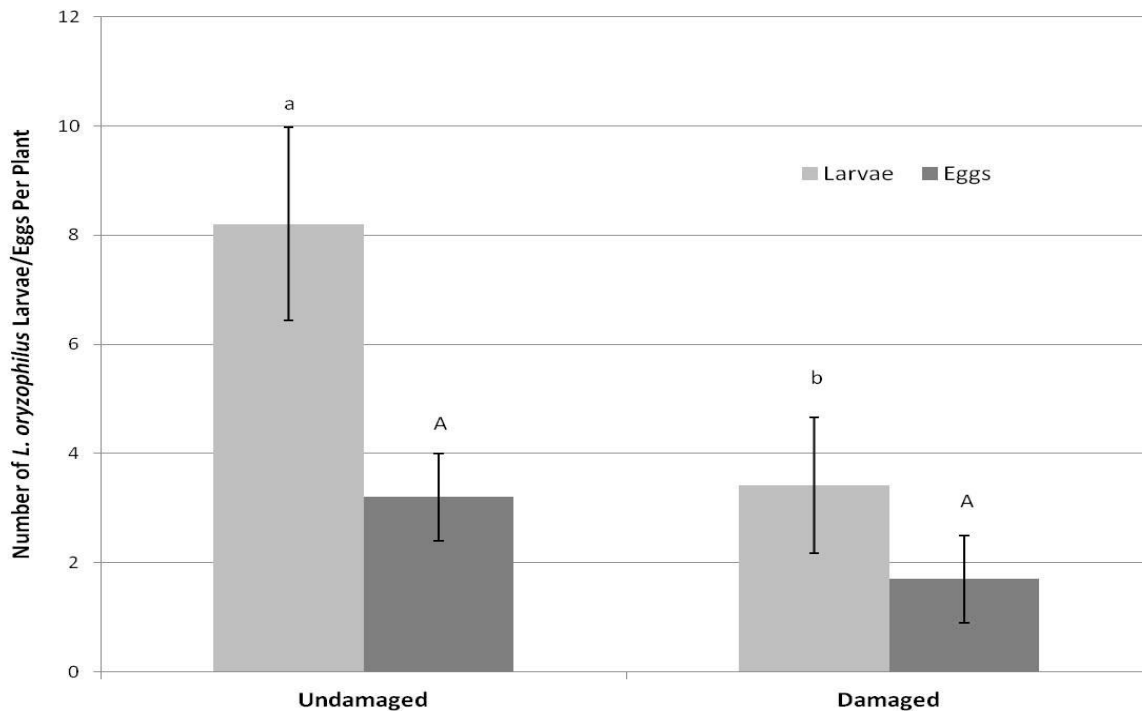
Figure 2.1 Mean number of *L. oryzophilus* larvae and eggs per plant (± se) in initial experiment using *S. frugiperda* damaged and undamaged plants of rice cultivar Rosemont. Plants were damaged by allowing one fourth-fifth instar *S. frugiperda* to feed on each plant for four to six hours. Means with different letters indicate a significant difference ($P \leq 0.05$).

The interaction between variety and treatment was not significant ($F_{2,50}$=0.39, P=0.68) indicating that the effect of variety was not as strong as the JA effect. Exogenous JA applications reduced the number of first instar larvae in the 5mM treatment compared to both 1mM and untreated plants (Figure 2.3; $F_{2,20}$=6.82, P=0.006). No varietal effect on first instars was observed ($F_{1,20}$=2.51, P=0.13) and the interaction between variety and treatment was not significant ($F_{2,20}$=0.21, P=0.81).

In the second greenhouse experiment, the numbers of larvae in both the 1mM JA and

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5mM JA treatments were significantly reduced compared to untreated control plants (Figure 2.4; $F_{2,20}=21.57$, $P<0.0001$). We also observed a varietal difference in larval numbers ($F_{1,20}=4.92$, $P=0.038$). The interaction between variety and treatment was not significant ($F_{2,20}=0.04$, $P=0.96$).
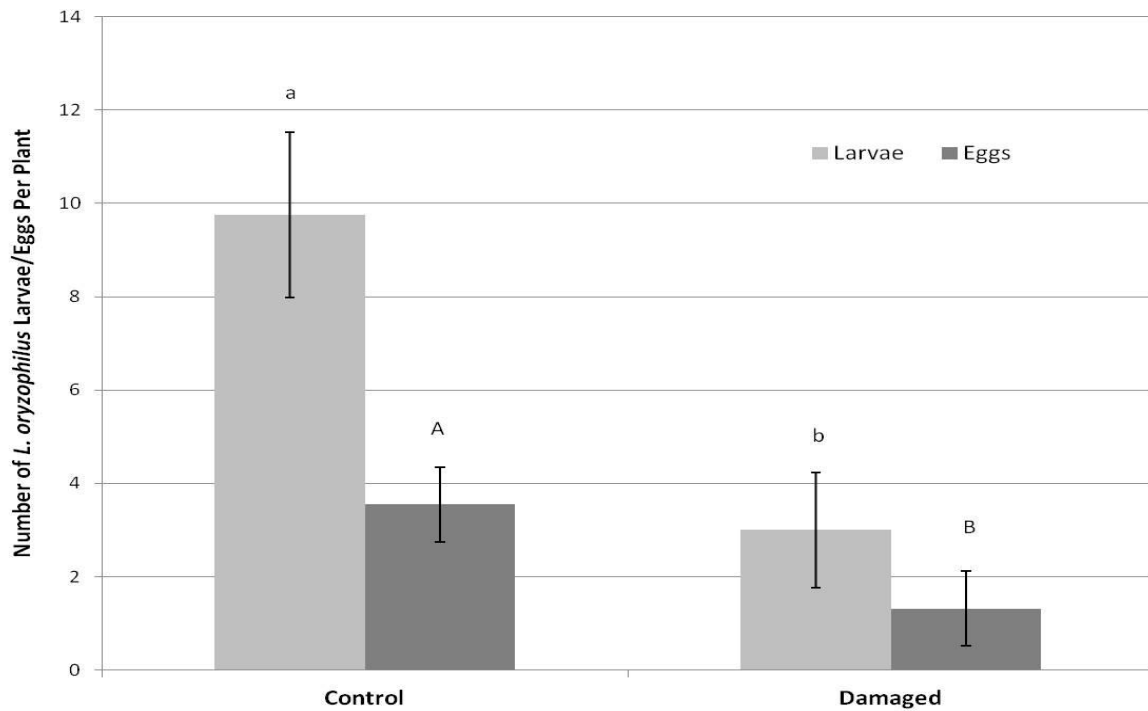
Figure 2.2. Mean number of *L. oryzophilus* larvae and eggs per plant ($\pm$ se) in second greenhouse experiment using *S. frugiperda* damaged and undamaged plants of rice cultivar Jackson. Plants were damaged by allowing one fourth-fifth instar *S. frugiperda* to feed on each plant for four to six hours. Means with different letters indicate a significant difference ($P \leq 0.05$).

Field Experiments. In the first field-planted experiment using variety 'Jackson,' the number of eggs per plant was not significantly different among treatments at six and 12 days after flood (Table 2.1; $F_{2,15}=0.53$, $P=0.60$ and $F_{2,15}=0.15$, $P=0.86$, respectively). The number of *L. oryzophilus* larvae per plant in the first core sampling was 52% and 62% lower in the 1mM and 5mM JA treated plants, respectively, compared to control (Table 2.1; $F_{2,15}=3.90$, $P=0.04$). In the second core samples, the number of larvae per plant was not significantly different among treatments ($F_{2,14}=0.71$, $P=0.51$). In the second field-planted experiment using

cultivar 'Rosemont' there was no significant difference in eggs per plant between treated and untreated plots. The numbers of *L. oryzophilus* larvae per plant in the first core sampling was significantly lower in the 5mM JA treated plots than in control plots (Table 2.2; $F_{1,20}=4.23$, $P=0.05$). The second core sampling showed no treatment effect on the number of larvae per plant ($F_{1,18}=0.36$, $P=0.55$).
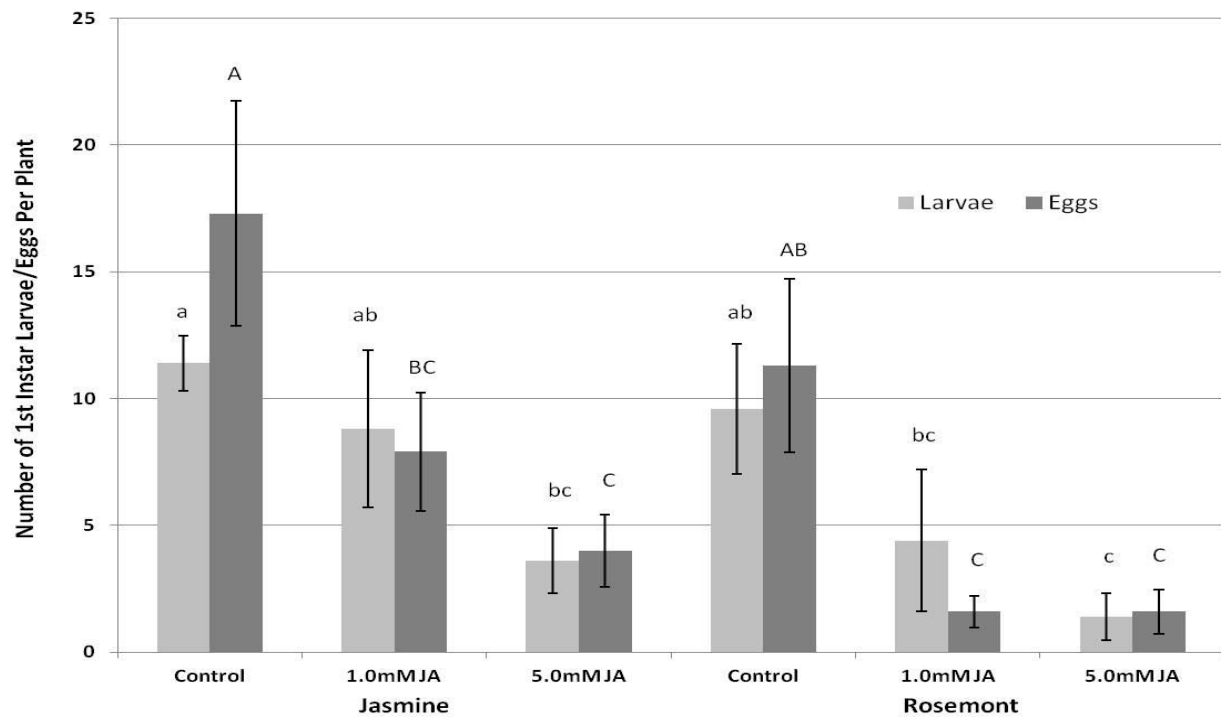
Figure 2.3 Mean number of larvae and eggs per plant (± se) in greenhouse two-way experiment using two different cultivars and three elicitor treatments. Means with different letters indicate a significant treatment effect ($P \leq 0.05$). Uppercase letters refer to egg data and lowercase letters refer to larval data.

2.4. Discussion

There is a growing body of literature pertaining to induced responses to insects in rice (Karban and Chen, 2007). However, most of this research has involved piercing-sucking insect pests of rice or induced volatile emissions (Lou et al., 2005; Matsumura and Suzuki, 2003; Xu et al., 2002; Zhou et al., 2003). Rice thus remains a relatively under-utilized model for the study of
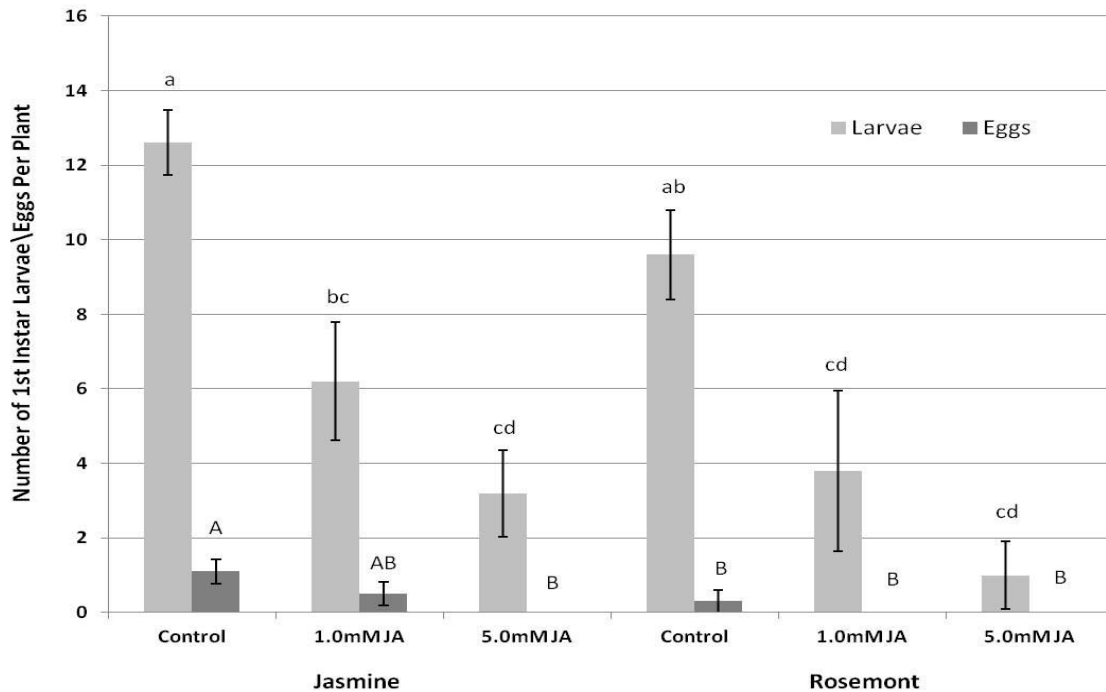
Figure 2.4. Second greenhouse experiment using two different cultivars and three elicitor treatments. Means with different letters indicate a significant treatment effect ($P \le 0.05$). The effect of cultivar on both eggs ($P=0.026$) and larvae ($P=0.038$) is also significant. Uppercase letters refer to egg data and lowercase letters refer to larval data.

direct induced resistance to chewing insects. This is a critical lack of knowledge, as many of the most important pests of rice in the U.S. and globally are chewing insects. Recently, Stout et al. (2009) showed that feeding by *S. frugiperda* and exogenous JA induced a long lasting systemic resistance to subsequent feeding by *S. frugiperda*. The results of this prior study led us to hypothesize that *S. frugiperda* herbivory and JA would induce resistance to *L. oryzophilus*, the most important insect pest of rice in the United States (Smith, 1983; Way, 1990). The goal of this study was to provide further information on the nature and importance of direct induced resistance in rice and its possible use in pest management.

Our results demonstrate that feeding by *S. frugiperda* induces resistance to an unrelated insect species (*L. oryzophilus*), which is consistent with the broad spectrum nature of induced resistance in many systems (Stout and Bostock, 1999). Plants that were previously injured by *S.*

frugiperda received 37%-53% fewer eggs from *L. oryzophilus* females than did undamaged

plants. Additionally, 30%-40% fewer *L. oryzophilus* first instars were recovered from previously

damaged plants than from undamaged plants. Reduction in *L. oryzophilus* eggs and first instars

on armyworm-damaged plants is unlikely to be due to a reduction in oviposition sites for weevils

because *L. oryzophilus* females oviposit inside leaf sheaths and not in leaf blades (Stout and

Riggio, 2002), the primary tissue removed by armyworm feeding. Also, plants had put on

between 1-2 new leaves between the time of armyworm feeding and weevil infestations, and

weevils prefer the sheaths of younger leaves for oviposition (Stout et al., 2002a).

In addition, exogenous JA stimulated resistance to rice water weevils. Plants treated with

1mM or 5mM exogenous JA received 54% to 66% fewer eggs on varieties 'Jasmine' and

'Rosemont' respectively, than untreated plants. Moreover, exogenous JA reduced the number of

L. oryzophilus larvae per plant by 23% to 69% in 'Jasmine' and 54% to 85% in 'Rosemont'

(1mM and 5mM respectively). There was also a significant effect of variety in one of the JA

experiments (more eggs and larvae were found on 'Jasmine' than on 'Rosemont', but the effect

of JA on egg and larval mortality was stronger than the varietal effect. Our results are consistent

with research in many dicot species that has found the activation of the JA pathway can provide

generalized protection against a variety of herbivorous insects (Inbar et al., 1998; Omer et al.,

2000, 2001; Thaler 1999).

Our demonstration of direct induced resistance in rice contributes to a growing

literature documenting responses induced by chewing insects in rice. Recently, Yuan et al.

(2008) identified genes underlying enhanced volatile emission from rice plants damaged by *S.*

frugiperda herbivory. They also demonstrated that induced volatiles from *S. frugiperda*

herbivory were highly attractive to female *Cotesia marginiventris* (Cresson) parasitoids. Xu et

al. (2002) demonstrated increased volatile emission following *S. litura* herbivory compared

with *Nilaparvata lugens* (Stål) damaged, mechanically damaged and undamaged rice plants. Moreover, *S. litura* females avoided plants infested with *N. lugens* in a dual-choice flight tunnel bioassay. In our study utilizing *S. frugiperda* damaged plants, *L. oryzophilus* females avoided ovipositing on previously damaged plants when given a choice in greenhouse experiments.

Table 2.1. Mean number of *L. oryzophilus* eggs and larvae per plant (± SE) in field samples using rice variety 'Jackson.' Egg samples were taken 13 and 19 days after exogenous JA applications. Core samples were taken 19 and 27 days after permanent flood was established. Means within the same column followed by different letters indicate a significant difference ($P \leq 0.05$).

	Egg Samples		Core Samples	
	First	Second	First	Second
Treatment	Eggs/Plant	Eggs/Plant	Larvae/Plant	Larvae/Plant
Control	0.83 ± 0.74a	2.17 ± 1.48a	4.84 ± 1.32a	7.4 ± 1.7a
1 mM JA	0.17 ± 0.16a	1.82 ± 0.8a	2.31± 0.50b	4.76 ± 0.75a
5 mM JA	0.67 ± 0.33a	4.10 ± 1.8a	1.92 ± 0.50b	6.32 ± 1.1a

Table 2.2. Mean number of *L. oryzophilus* eggs and larvae per plant (± SE) in field samples using variety 'Rosemont.' Egg samples were taken 3 days after exogenous JA applications. Core samples were taken 14 and 19 days after permanent flood was established. Means within the same column followed by different letters indicate a significant difference ($P \leq 0.05$).

	Egg Sample	Core Samples	
	First	First Core Sample	Second Core Sample
Treatment	Eggs/Plant	Larvae/Plant	Larvae/Plant
Control	2.18 ± 0.7a	2.88 ± 0.68a	8.12 ± 2.56a
5 mM JA	0.88 ± 0.43a	1.46 ± 0.34b	6.54 ± 1.33a

The ability of JA to induce resistance to *L. oryzophilus* in greenhouse experiments led us to hypothesize that exogenous JA would induce resistance in field grown plants. Although adult rice water weevils can be found in rice fields both before flooding, oviposition and larval infestations largely commence after flooding (Everett and Trahan, 1967). Densities of eggs in

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rice fields in Louisiana are generally highest one to three weeks after flooding, while peak larval

densities usually two to three weeks later (Stout et al., 2000; Shang et al., 2004; Zou et al., 2004).

Because our greenhouse experiments had shown that JA-induced resistance reduces oviposition,

applications of JA were made 7 days or 1 day prior to flooding in the first and second field

experiments, respectively. Although densities of rice water weevil eggs were not significantly

reduced in JA treated plants compared with untreated plants, consistent trends in the data

suggest that JA-treatment made plants less attractive for oviposition (see Tables 1 and 2). Most

probably, sampling of eggs was not extensive enough (both in terms of number of plants sampled

and frequency of sampling) to detect transient JA-induced differences in egg densities. This

interpretation is particularly likely in light of the results of the core sampling , which in both

experiments were consistent with the hypothesis that JA treatment induces a transient increase in

rice resistance to rice water weevils. In both experiments, initial core samplings,which were

conducted 15 to 28 days after flooding, revealed significant and substantial (up to 60%)

reductions in densities of larvae and pupae in JA treated plants compared to untreated plants.

However, weevil densities in the second core sampling were not reduced in JA treated plants

compared to untreated plants in either experiment.

There are few studies that have examined the use of JA on economically important crops

in field settings (but see Black et al., 2003; and Thaler, 1999) and, to our knowledge, the results

reported here are the first to describe direct, JA-induced resistance in rice to herbivores in field-

based experiments. The use of chemical elicitors, such as JA, holds potential as a tool for use in

agriculture (Karban and Chen, 2007). Negative effects associated with a reliance on

conventional insecticides, such as the development of insecticide resistance, environmental

contamination and threats to human safety, can be mitigated by the use and exploitation of

elicitor-induced host plant resistance. Elicitors can stimulate broad-spectrum resistance to a

variety of pests; in rice, for example, this study and prior studies (Stout et al., 2009; Mei et al., 2006) have shown that exogenous JA induces resistance against a variety of important insect and disease pests. In addition, the use of elicitors allows producers to manage both the intensity and timing of induction. However, the relative high cost of elicitors compared to insecticides, coupled with the transient nature of elicitor-induced resistance, may serve as limiting factors in the widespread adoption and use of elicitors in agriculture.

CHAPTER 3: INDUCED RESISTANCE IN RICE TO OVIPOSITION BY THE SUGARCANE BORER

3.1. Introduction

The interactions of herbivorous insects with their host plants can trigger a variety of responses in plants, and often these responses make the plants less suitable for subsequent herbivores. Induced responses in plants can have a direct negative effect on herbivores by reducing oviposition, feeding, survival and fecundity (Walling, 2000). In addition, induced responses can have an indirect effect on herbivores by attracting or retaining natural enemies of herbivorous arthropods (Dicke et al., 2003).

The focus of the majority of studies on induced resistance has been on responses induced by herbivore feeding (Dicke and van Loon, 2000; Hamm et al., 2010; Karban and Baldwin, 1997; Stout et al., 2009; Tumlinson et al., 1993; Turlings and Wäckers, 2004). However, most herbivorous insects start attacking a plant first through oviposition. Many herbivorous Lepidoptera and Hymenoptera do not feed upon leaves as adults, but will oviposit where newly hatched larvae will presumably find suitable food. For herbivores that share a feeding niche between immature and adults, such as many Coleoptera and Heteroptera, selection of an oviposition site is also crucial. Thus, it is not surprising that plants respond to oviposition as well as feeding (Hilker and Meiners, 2006).

Direct induced resistance to oviposition has been documented in several plants (Hilker and Meiners, 2006). For example, oviposition by the whitebacked planthopper, *Sogatella furcifera* Horváth, on rice has been shown to induce the formation of benzyl benzoate, an ovicidal compound that kills eggs (Seino et al., 1996; Suzuki et al., 1996; Yamasaki et al., 2003). Oviposition has also been shown to to mediate tritrophic interactions, typically attracting

parasitoids to the host plant (Colazza et al., 2004; Hilker et al., 2002, 2005; Meiners and Hilker, 1997, 2000). In some cases, responses to oviposition lead to both direct and indirect induced responses. Oviposition by the spotted stem borer, *Chilo partellus* (Swinhoe), on *Bracharia brizantha,* rendered the plants less preferred for subsequent oviposition. Moreover, oviposition suppressed emission of a green-leaf volatile ((Z)-3-hexenyl acetate), resulting in altered ratios of the green-leaf volatile to other volatile compounds. This change in volatile emission resulted in increased attractiveness of plants to a parasitoid wasp, *Cotesia sesamiae* (Bruce et al., 2010).

Worldwide, stem borers are the most important group of insect pests. In every rice producing region, stem-boring Lepidoptera are major pests (Kiritani, 1979; Pathak, 1968). Host-plant resistance in Asia has been a key component of IPM programs targeting rice stem borers for the past 50 years (Chaudhary et al., 1984). However, research on resistant rice cultivars to stem borers in the United States has been sparse, partly due to many years of reduced incidence of stem borers (Oliver et al., 1973).

Induced resistance in rice to insect pests has been previously confirmed (Hamm et al., 2010; Karban and Chen, 2007; Stout et al., 2009). However, direct induced resistance resulting from oviposition by Lepidoptera has not been demonstrated in rice. The objective of the experiments described herein was to test the hypothesis that oviposition by *D. saccharalis* induces resistance to further oviposition by the same species.

3.2. Materials and Methods

Experiments were conducted in a greenhouse on the campus of Louisiana State University, Baton Rouge, Louisiana in 2009 and 2010. Seeds were generously provided by personnel at the LSU AgCenter Rice Research Station and the National Plant Germplasm System, Genetic Stocks-Oryza (GSOR) Collection, United States Department of Agriculture

(Stuttgart, AR). Seeds were planted in a sterilized soil mix (2:1:1, soil: peat moss: sand) in 15cm diameter pots and plants were maintained in greenhouse conditions under ambient lighting at approximately 29°C- 33°C. At the time of planting, 1.2g of 19:5:8 controlled release fertilizer (Osmocote, Scotts Miracle-Gro, Marysville, OH) was added to soil. Plants were thinned to a density of one plant per pot five to seven days after planting. Experiments were conducted when plants reached the R2-R3 stage (Counce et al., 2000). Plants at this stage have formed a flag leaf collar and have a partly exerted panicle from the enlarged stem.

Adult *D. saccharalis* used in experiments were obtained from a colony maintained year-round on artificial diet in the laboratory. The colony originated from larvae collected in rice fields near Crowley, LA in 2005. Insects collected from rice fields are added annually to the colony to maintain genetic variability.

For all experiments, treatment groups were established by placing half of the plants of each cultivar inside pvc cages (122cm x 61cm x 61cm) covered with Econet B insect screening (AB Ludvig Svensson, Charlotte, NC) Newly eclosed *D. saccharalis* adults were added to cages at the rate of one pair per plant and allowed to oviposit over a period of four days. The remaining plants were designated controls and were placed in cages without insects for the same duration as the treatment group. Plants were then removed from cages and thoroughly inspected for egg masses. Adults that were alive were immediately discarded, as were carcasses of dead adults. Each egg mass was marked by placing a small black dot adjacent to it using a Sharpie[®] felt tip pen in order to distinguish it from any new egg masses deposited in the subsequent oviposition period. The mean number of egg masses per plant on the treated group was calculated and control plants received the same number of markings with a Sharpie[®] felt tip pen randomly distributed on the plant. Three to four days later, once egg masses began to darken,

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one plant from each treatment group was randomly placed inside cages and adult *D. saccharalis* were added to cages at the rate of one pair per plant. Care was taken to ensure that egg masses did not hatch either prior to or during the experimental period. Adults were allowed to oviposit inside cages for another four days, after which plants were removed and new egg masses counted.

In 2009, five experiments were conducted, three with 'M202' one with 'Rosemont' and another with 'Cocodrie.' Replication in these experiments ranged from five to six cages per experiment, with each cage containing one to two plants of each treatment group per cage. In 2010, three additional studies were conducted. Each of these studies consisted of two separate experiments, run in parallel, and utilized 'M202'and a second cultivar ('Cocodrie,' 'Reiho' or 'Rosemont'). The number of replications for each cultivar ranged from three to four cages per experiment, with each cage containing one plant of each treatment group. Previous experiments carried out by our laboratory have shown that'M202'and 'Rosemont' exhibit a relatively strong response when induced with jasmonic acid (Hamm et al., 2010) or with *Spodoptera frugiperda* J.E. Smith herbivory (Stout et al., 2009). Cultiva 'Reiho' has been shown to respond to oviposition by the white backed planthopper (*Sogatella furcifera*) by producing an ovicidal compound (Suzuki et al., 1996). 'Cocodrie' is a common long grain cultivar planted throughout Louisiana.

Numbers of egg masses per plant were analyzed as a completely randomized block design with cage as a random effect and treatment (control plants and plants previously exposed to ovipositing females) as a fixed effect using a mixed model analysis of variance (SAS, 2007). Means were separated using Tukey's adjustment.

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3.3. Results

Five experiments in 2009 used cultivars 'Cocodrie', 'M202'and 'Rosemont.' When presented with a choice of 'Rosemont' plants with or without eggs, female *D. saccharalis* chose to oviposit significantly more egg masses on plants without prior egg masses (3.6 ± 0.8) than on plants with egg masses present (0.5 ± 0.3) (Figure 3.1; $F_{1,12}=26.55$, *P*=0.0002). Females also chose to oviposit significantly more egg masses on 'Cocodrie' control plants (3.4 ± 0.6) than on plants with egg masses already present (1.0 ± 0.4) (Figure 3.1; $F_{1,4}=96.00$, *P*=0.0006). However, when presented with a choice of 'M202' plants with or without eggs, there was no significant difference in the number of egg masses on control plants (6 ± 1.8) and on plants with egg masses already present (11.6 ± 2.1) (Figure 3.1; $F_{1,14}=4.15$, *P*=0.06).
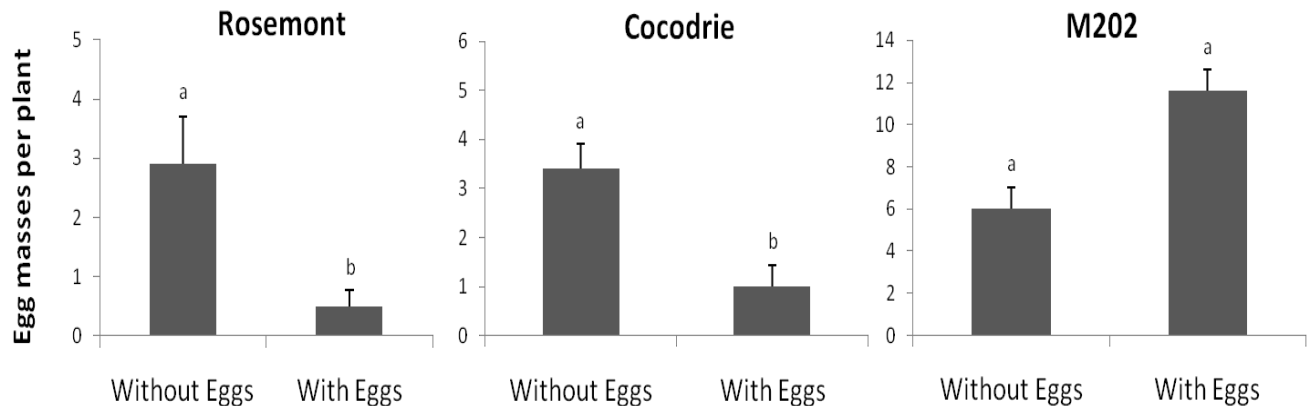
Figure 3.1. Mean number of egg masses per plant ($\pm$ se) from 2009 experiments. Reproductive age *O. sativa* plants were exposed to female *D. saccharalis* over a period of four days. After this period, a new group of female *D. saccharalis* were presented with a choice of plants with or without conspecific egg masses and allowed to oviposit over another period of four days. Means followed by different letters indicate a significant difference ($P \leq 0.05$).

Because the result with 'M202' contradicted results with 'Rosemont' and 'Cocodrie' two additional experiments were conducted using cultivar 'M202.' In the first of these, significantly

more egg masses per plant were oviposited on plants with eggs (10.9 ± 1.6) compared to plants without eggs (4.3 ± 1.1) (Figure 3.2; $F_{1,17}=11.41$, $P=0.004$). In the second experiment, the same response was observed, with more egg masses per plant oviposited on plants with eggs present (8.7 ± 1.5) compared to control plants (5 ± 1.5) (Figure 3.2; $F_{1,5}=15.92$, $P=0.01$).
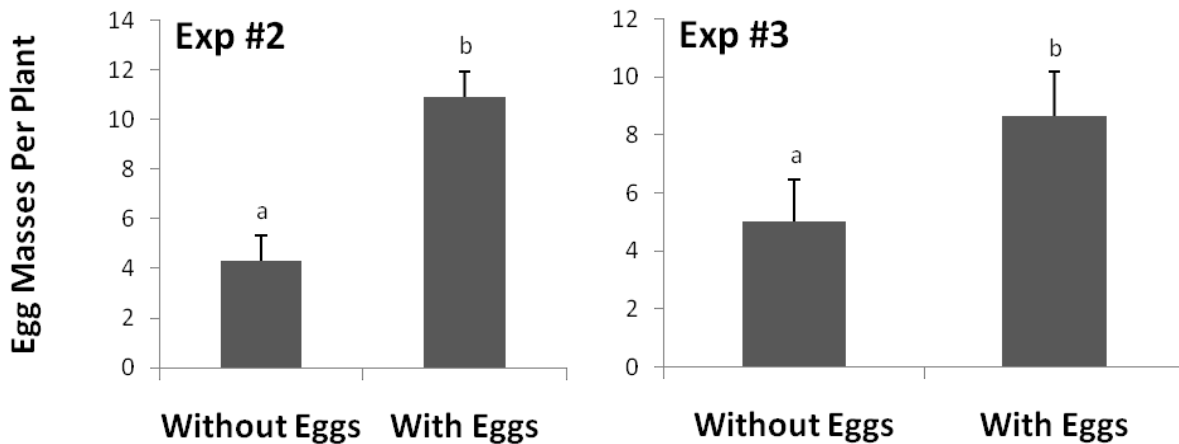
Figure 3.2. Mean number of egg masses per plant (± se) from additional experiments conducted using cultivar 'M202.' Female *D. saccharalis* were given a choice of plants with or without conspecific egg masses and allowed to oviposit over a period of four days. Means followed by different letters indicate a significant difference ($P \leq 0.05$).

In 2010, experiments with 'M202' and other varieties were run in parallel to eliminate sources of variation potentially contributing to the varietal differences observed in previous experiments. Although we were sometimes unable to detect a significant difference between the two treatment groups when using 'M202', 'Rosemont' and 'Cocodrie' in the parallel experiments, trends in the data suggests that plants with egg masses are less preferred for subsequent oviposition in 'Rosemont' and 'Cocodrie.' In the first of the three paired studies, no significant difference was found between the number of egg masses oviposited on either 'Rosemont' control plants or 'Rosemont' plants with egg masses ($F_{1,2}=8.14$, $P=0.1$) or 'M202'

($F_{1,3}=0.11$, $P=0.7$) (Table 3.1). Similarly, no difference was observed in the number of new egg masses on 'M202'plants with or without eggs ($F_{1,3}=1.29$, $P=0.34$). In the second of these paired studies, significantly more egg masses were oviposited on 'Reiho' control plants compared to 'Reiho' plants with egg masses present ($F_{1,2}=20.16$, $P=0.04$). In the last of these paired studies, no difference was observed in the number of new egg masses per plant on plants with or without eggs using 'M202' ($F_{1,2}=4.57$, $P=0.17$) or 'Cocodrie' ($F_{1,2}=2.69$, $P=0.24$). Despite the lack of significance in two of three studies, control plants received 74% and 82% more egg masses on cultivars 'Rosemont' and 'Cocodrie' respectively. Conversely, 'M202' control plants received 30%-47% fewer egg masses per plant compared to the treatment group. The lack of significance observed between treatment groups in these experiments is likely due to an insufficient number of replications in each experiment. A limiting factor in experiments conducted in 2010 was the number of cages used in experiments, as only six or seven were available for use; experiments conducted in 2011 utilized more cages (10 total) and thus more replications.

Table 3.1. Results from 2010 (Reiho, Cocodrie and Rosemont) and 2011 (Priscilla) in which induced responses to *D. saccharalis* ovioposition were examined in 'M202'and other cultivars in simultaneous, parallel experiments. The mean number of egg masses per plant (±SEM) on plants with or without conspecific egg masses is presented. * Indicates a significant difference ($P \leq 0.05$).

M202			Second Cultivar	
Without Eggs	With Eggs		Without Eggs	With Eggs
5.4 ± 0.9	11.6 ± 1.5*	Priscilla*	8.2 ± 1.5	3 ± 0.9
3.5 ± 0.6	5 ± 1.8	Reiho*	9.7 ± 1.5	1.3 ± 1.3
4.7 ± 2.7	9 ± 2	Cocodrie	9.3 ± 4.4	1.7 ± 1.7
11 ± 3.7	10.3 ± 1.7	Rosemont	15.3 ± 3.9	4 ± 0.6

3.4. Discussion

We conducted a series of experiments designed to test the hypothesis that *D. saccharalis* oviposition induces resistance in rice plants to further oviposition. In five experiments with

cultivars 'Cocodrie', 'Reiho' and 'Rosemont' plants with egg masses from prior exposure to stem borers received 71%-86% fewer egg masses compared to plants not previously exposed to *D. saccharalis*. However, the resistance induced by borer oviposition was cultivar-specific: in all experiments using cultivar 'M202' no induced resistance was observed following oviposition. In fact, in two of the first three experiments using 'M202' female *D. saccharalis* chose to oviposit significantly more egg masses on plants with conspecific egg masses. In our 2010 experiments using 'M202,' we found no differences between the number of egg masses per plant on either control plants or those plants with egg masses present, but trends were consistent with the initial experiments using 'M202.'

Our results support the conclusion that resistance following *D. saccharalis* oviposition is consistent with an induced response in the plant. Other interpretations of our results are less plausible than the interpretation that reduction in oviposition is caused by an oviposition-triggered plant response. In particular, the results are not likely to have been caused by insect-derived cues such as marker pheromones, because this interpretation is not consistent with varietal differences. Furthermore, plants from the family Poaceae have been shown to respond to oviposition in at least two other studies. Following oviposition by *S. furcifera*, rice plants produce watery lesions around egg masses that contain an ovicidal compound, benzyl benzoate, in the Japonica-derived cultivar 'Reiho' (Seino et al., 1996; Suzuki et al., 1996), but not in the Indica derived cultivar 'IR24' (Yamasaki et al., 2003), further illustrating a specific response from the host plant that is consistent with induced resistance. Induced resistance following stem-borer (*C. partellus*) oviposition on an African forage grass, *B. brizantha*, has recently been demonstrated. Plants with *C. partellus* eggs were less preferred for subsequent oviposition, and the volatile blend emitted by *B. brizantha* was altered following *C. partellus* oviposition. As a result, plants were more attractive to a parasitoid, *C. sesamiae,* than plants without eggs.

Oviposition induced responses have also been described in a number of dicotyledonous plants, and in some cases, elicitors of responses have been characterized. Jasmonic acid (JA) is a plant hormone that serves as an important signal molecule to mediate the expression of both direct and indirect defenses against herbivory (Browse and Howe, 2008; Thaler et al., 2002). Tooker and De Moraes (2005, 2007) found JA in the eggs and neonates of more than 20 different insect species, including *D. saccharalis*. Salicylic acid (SA) is a potent inducer of pathogenesis-related genes and is associated with resistance to biotrophic pathogens (Glazebrook, 2005). Salicylic acid has been discovered in the eggs of species from at least eight orders of insects (Tooker and De Moraes, 2007). Oviposition by *Pieris* spp. on *Brassica nigra* induces the formation of necrotic lesions at the oviposition site, resulting in egg desiccation and mortality (Shapiro and Devay, 1987). Similarly, *P. brassicae* and *P. rapae* oviposition has been shown to change gene expression in *Arabidopsis thaliana*, resulting in transcriptional responses similar to those of hypersensitive responses to pathogens, including the induction of defense and stress related genes (Little et al., 2007). In *A. thaliana*, oviposition by *P. brassicae* leads to a rapid accumulation of SA at the site of oviposition (Bruessow et al., 2010). Together, these discoveries suggest possible mechanisms for induced resistance in rice. Bruchins are long-chain fatty acid-derived molecules found in the eggs and adults of bruchid beetles that can induce direct defense (Doss et al., 2000). In pea plants (*Pisum sativum*), tumor-like growths are initiated upon oviposition by the pea weevil (*Bruchus pisorum* L.), which elevates the egg from the plant surface, increases the risk of desiccation, predation or dislodgement (Doss et al., 1995, 2000). In the presence of an insect-derived cue, the plants undergo significant changes in response to this elicitor, consistent with the nature of induced resistance.

Indirect resistance following oviposition has also been demonstrated. Following oviposition by the pine sawfly, *Diprion pini*, branches of Scots pine, *Pinus sylvestris*, emit

volatiles, which then attract the egg parasitoid *Chrysonotomyia ruforum*. The induced response was both local and systemic, and could be replicated using exogenous applications of jasmonic acid (Hilker et al., 2002). Elicitors responsible for attracting egg parasitoids in pine and elm have been associated with a secretion coating eggs of *D. pini* (Hilker et al., 2005). The oviduct secretion could also mimic the attraction of egg parasitoids when applied to artificial wounds (Hilker et al., 2005). In another example, oviposition by the southern green stink bug, *Nezara viridula*, has been shown to induce volatile emissions in two species of beans (*Vicia faba* L. and *Phaseolus vulgaris* L.) that attract the egg parasitoid *Trissolcus basalis* (Wollaston) (Colazza et al., 2004).

Our data provide the first example of direct induced resistance in rice following oviposition from a stem-boring pest. Cultivar specific responses to herbivore damage, whether through oviposition or feeding, are important to characterize in order to better understand how rice plants respond to such damage. The cultivar specificity of the response may be an important tool in the study of mechanisms underlying induced responses in rice. With a better understanding of such mechanisms, plant breeders may be able to incorporate them into breeding programs when selecting for resistance to herbivores. Further experiments that are designed to understand the mechanism behind this induced response are an essential first step in this process.

CHAPTER 4. ASPECTS OF THE CHEMICAL ECOLOGY OF THE RICE STINK BUG AND IMPLICATIONS FOR INTEGRATED PEST MANAGEMENT

4.1. Introduction

The rice stink bug, *Oebalus pugnax* (F.) (Heteroptera: Pentatomidae), is the most important late season insect pest of rice, *Oryza sativa* L., in the southern United States (McPherson and McPherson, 2000; Way, 2003). It invades rice fields in large numbers during reproductive stages of grain development (Douglas, 1939; Espino and Way, 2007; Rashid et al., 2006) and removes contents of developing grains. Stink bug feeding can result in losses in yield and reductions in grain quality (Patel et al., 2006a; Smith et al., 1986; Swanson and Newsom, 1962).   Reduction in grain quality from *O. pugnax* feeding is known as ―pecky‖ rice. Pecky rice is a term for the chalky discoloration of rice kernels resulting directly from stink bug feeding and indirectly from the entry of pathogens into feeding sites (Lee et al., 1993; Marchetti et al., 1983). Throughout the southern rice producing regions of the United States, *O. pugnax* feeds on a wide variety of non-crop hosts in and around rice fields and levees throughout the spring and summer (Douglas, 1939; McPherson and McPherson, 2000; Odglen and Warren, 1962) and then enters rice fields when heading (emergence of panicles) occurs. Currently, the only management option for *O. pugnax* is application of broad spectrum, non-selective insecticides.

Members of Pentatomidae produce a variety of chemical compounds in their metathoracic glands, typically a mixture of C6-C10 aldehydes, esters and straight chain alkanes. Contents of Heteroptera metathoracic glands have been shown to serve as a defensive mechanism and to possess alarm functions (Ishiwatari, 1974; Kou et al., 1989; Lockwood and Story, 1987). Furthermore, Lockwood and Story (1985) reported that first instar *Nezara viridula* (L.) use *n*-tridecane as a dual function pheromone, causing dispersal of conspecifics at high

concentrations and aggregation at lower concentrations, and Ishwitari (1976) demonstrated a similar role of (*E*)-2-hexenal on first-instar nymphs of *Eurydema rugosa*. In addition, egg parasitoids have been shown to be attracted to metathoracic gland contents of stink bugs in laboratory olfactometers (Bin et al., 1993; Colazza et al., 1999; Laumann et al., 2009). Understanding the diverse ecological roles of metathoracic gland components may lead to the development of environmentally sound alternatives to insecticides.

Although the metathoracic gland components of several Pentatomid species have been characterized, those of *O. pugnax* have been only partially characterized (Blum et al., 1960). Furthermore, nothing is known about the influence of host plant on the composition of metathoracic gland secretions. Experiments were undertaken to characterize the chemical composition of metathoracic glands of *O. pugnax* and to gain insight into the role host plant has on development time, adult weights and the metathoracic gland contents of *O. pugnax*. In addition, behavioral responses of *O. pugnax* to metathoracic gland extracts were tested using olfactometer bioassays. We also report data from small-plot field experiments investigating the effects of a synthetic mixture of metathoracic gland components on *O. pugnax* behavior

4.2. Materials and Methods

Metathoracic gland contents were collected by pinning a freshly killed adult ventral side up, removing the legs and a portion of the metathorax using fine scissors, and then using a flame-drawn glass capillary pipette to carefully pierce the metathoracic gland. The capillary pipette was then broken in 500μl of dichloromethane in 2ml crimp top vials. Extracts of metathoracic glands were stored at -20°C until chromatographic analysis. Contents of metathoracic glands were analyzed by splitless coupled gas chromatography-mass spectrometry (GC-MS) with a Hewlett Packard 6890 Series GC with an autosampler connected to a Finnigan Trace Mass

spectrometer (electron impact ionization 70eV). The GC was held at 40°C for one minute, then programmed at 10°C/minute to 250°C, held for one minute, and then programmed at 10°C/minute to 325°C, and then held for another five minutes. Injector and transfer lines were set to 250°C and 280°C, respectively. A DB-35ms column (30m x 0.25mm ID, J&W Scientific, Folsom, CA) was used with helium as a carrier gas. Each compound was tentatively identified by comparison of its mass spectrum with a mass spectral database. Identifications were confirmed by comparing mass spectra and retention times to authentic standards injected under the same conditions. (*E*)-2-hexenal, (*E*)-2- heptenal, (*E*)-2-octenal, (*E*)-2-hexenyl acetate, (*E*)-2-octenyl acetate, (*E*)-2-decenal, dodecane and tridecane were purchased from Sigma-Aldrich Co. (St. Louis, MO). In order to analyze the chromatographic data, the relative abundance of each compound was calculated by dividing the total area under each peak by the total area of the most abundant compound (tridecane in all cases).

4.2.1. Influence of Host Plant on Development Time, Adult Weight and Metathoracic Gland Contents

The influence of host-plant on development time and weight as well as metathoracic gland contents of adult *O. pugnax* was investigated in the laboratory. Adult *O. pugnax* were collected in rice fields at the Louisiana State University Agricultural Center Rice Research Station, in Crowley, LA and provided with fresh panicles of barnyardgrass (*Echniochloa crus-galli*), rice, (*O. sativa)* and amazon sprangletop (*Leptochloa panicoides)* under laboratory conditions (14:10 L:D, 28°C± 2°, 38% R.H.) in 60cm x 30cm x 40cm aquaria to generate eggs masses. Egg masses were collected from leaves, stored individually in 5.5cm petri dishes, and allowed to hatch. Upon hatching, first instar nymphs from each egg mass were divided into three equally sized groups of 18-24 immatures, and each group was then randomly assigned to a host plant – *E. crus-galli*, *O. sativa* or *L. panicoides* – and reared until individuals reached adulthood.

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Panicles of each host plant were placed inside a 250mL flask with water to prolong freshness, and then placed in the center of a 60cm x 30cm x 40cm aquarium. Panicles were replaced every other day. Upon eclosion, adults from each of the host plants were sexed and weighed using a Mettler-Toledo XS105 scale (Mettler-Toledo, Columbus, OH). The number of days it took for individuals reared on host plants to reach adulthood was also recorded. The entire experiment was repeated three times, with three separate egg masses. The three experiments served as blocks in the statistical analysis. Development time and adult weight were analyzed using a mixed model of analysis, with host plant as a fixed effects and egg mass (experiment) as a random effect in the model. The influence of host plant on metathoracic gland components was analyzed using a multivariate analysis of variance model, with chemical compound as dependent variables and means were separated using Tukey's HSD (Tukey, 1953).

4.2.2. Olfactometer Bioassays

Experiments were conducted using an olfactometer in the laboratory to determine the behavioral response of *O. pugnax* adults towards metathoracic gland contents. Bioassays were carried out using a 20cm long glass Y-tube olfactometer (ARS-FLA, Gainesville, FL). The two arms measured 10cm in length and 3cm in diameter with a 45° angle between them. At the end of each arm, filter paper with metathoracic gland extracts or test chemicals was placed inside an eight cm long glass adapter and then connected to an air delivery system that provided humidified, charcoal-filtered air to each arm at 300ml/min. Initial experiments found that adult *O. pugnax* encounter difficulties while attempting to walk inside the glass y-tube, so a 15cm x 0.5cm strip of filter paper was placed inside the length of the y-tube to facilitate walking. The olfactometer was placed inside of a 35cm square wooden open-ended box to equalize visual cues between two arms.

A single adult *O. pugnax* was gently introduced into the straight portion of the y-tube apparatus and was given 15min to make a choice between the control and treatment arm of the y-tube. Bioassays were conducted between 1200-1800, and a choice was recorded once an insect walked into an arm 3cm past the y junction and remained there for at least 60 seconds. The position of control and treatment arms were switched after every five individuals tested and a new y-tube was used after 10 individuals were tested. The apparatus was cleaned with fragrance-free detergent, rinsed with acetone, distilled water and then oven dried at 50°C for 48 hours prior to subsequent use. Tests were carried out using the full stink bug equivalent (SBE), 25% SBE and 12.5% SBE, by transferring the appropriate fraction of the dichloromethane extract onto 9cm filter paper and then placing inside one of the adapters. The control treatment consisted of a filter paper treated with the equivalent volume of dichloromethane that was allowed to evaporate prior to use. Data were analyzed by comparing the proportion of responders to each treatment with the hypothesized ratio of 50% response to either treatment or control. Data on the number of responses of adult *O. pugnax* to different SBEs were analyzed by chi-square tests (PROC GLM, SAS Institute, 1999). Non-responders were not included in the statistical analysis.

4.2.3. Field Experiments

Two small-plot experiments were conducted to test the effects of *O. pugnax* metathoracic gland contents on behavior of *O. pugnax* in the field. Experiments were conducted at the Louisiana State University Agricultural Center Rice Research Station, in Crowley, LA in 2011. All experiments used the same cultivar of rice, Cocodrie – a widely grown, conventional long grain cultivar – cultivated under standard agronomic practices for drill seeded rice in Louisiana with the exception that no insecticides were applied at any point during the growing season. In

both experiments, plot sizes measured 5.5m long by 1.2m wide. Spacing between plots in the first experiment was 11.9m north-south and 4.9m east-west between plots, and 19.2m north-south and 1.2m east-west between plots in the second experiment. Treatments were assigned to plots according to a completely randomized block design with either five (first experiment) or 10 blocks (second experiment) such that each of the experiments contained10 total plots for each treatment. A synthetic mixture of metathoracic gland components was prepared by first calculating the relative abundance of each chemical by dividing the total area under each peak in the GC analysis by the total area of the most abundant compound. Once relative abundance was calculated, the major components of metathoracic glands -- (*E*)-2-hexenal, (*E*)-2-hexenyl acetate, (*E*)-2-octenyl acetate and tridecane -- were dissolved at their respective ratios into 1500 ml of a 30% ethanol/water solution. Treatment plots were sprayed using a CO_2 powered backpack sprayer at the rate of 56.8liters/0.4ha. Each plot received approximately 130ml of solution. Control plots were sprayed at the same rate with only a 30% ethanol/water solution. Sampling of *O. pugnax* was conducted at 30, 60 and 120 minutes after spraying by making 10 sweeps per plot with a 38cm diameter cloth sweep net (BioQuip, Rancho Dominguez, CA). Data for each of the field experiments was subjected to a repeated measures analysis of variance, with treatment as a fixed effect and plot as a repeated subject using a generalized linear model.

4.3. Results

4.3.1. Influence of Host Plant on Development Time, Adult Weight and Metathoracic Gland Contents

The host plant had a significant effect on duration of *O. pugnax* development (egg to adult) ($F_{2,59}$=27.61, P < 0.0001). Insects reared on *O. sativa* and *E. crus-galli* developed significantly faster than those reared on *L. panicoides* (Table 4.1). There was no difference in

the number of days for males and females to reach maturity ($F_{1,59}=0.14$, P=0.71) and there was

no interaction between sex and host plant ($F_{2,59}=0.28$, P = 0.75).

Table 4.1. Development times (days) to reach adulthood when reared from first instar on one of three different host plants. Means in each column followed by a different letter indicate a statistically significant difference ($P \leq 0.05$).

Host Plant	Male	Female
O. sativa	$18.1 \pm 0.55a$	$18.2 \pm 0.45a$
E. crus-galli	$19.8 \pm 0.7a$	$19.2 \pm 0.58a$
L. panicoides	$22.2 \pm 0.44b$	$22.2 \pm 0.53b$

Host plant significantly affected adult weights ($F_{2,207} = 238.04$, $P < 0.0001$). Insects

reared on *O. sativa* weighed approximately 81% more than those reared on *E. crus-galli* and *L.*

panicoides. Weights of adults reared on *E. crus-galli* and *L. panicoides* did not differ. In

addition, the sex of *O. pugnax* significantly affected weight ($F_{1,207} = 42.95$, $P < 0.0001$; Figure

4.1). Females reared on *O. sativa* weighed significantly more than males reared on *O. sativa*,

and females reared on *E. crus-galli* weighed significantly more than males reared on *E. crus-*

galli. There was no difference in the weights of males and females reared on *L. panicoides*.

Gas chromatographic analysis of metathoracic gland contents showed that *n*-tridecane

was the most abundant compound in all samples. The glandular secretions also contain alkenals

[(*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2-octenal and (*E*)-2-decenal], esters [(*E*)-2-hexenyl acetate

and (*E*)-2-octenyl acetate] as well as another straight-chain alkane, *n*-dodecane (Figure 4.2).
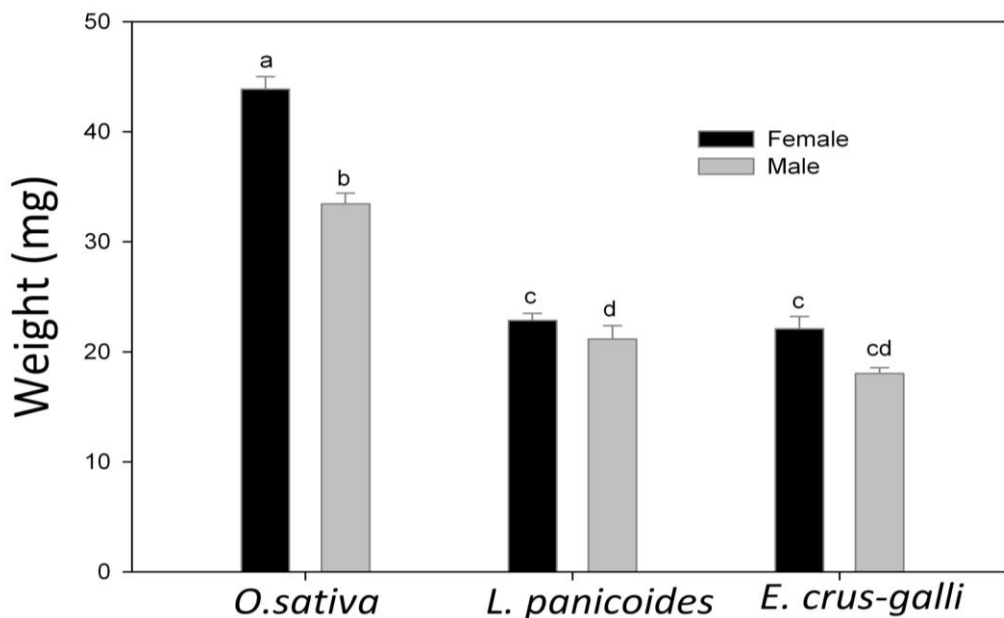
Figure 4.1. Mean weight of adult female and male *O. pugnax* reared on different host plants. Stink bugs were reared from egg to adult on one of three host plants – *Echniochloa crus-galli*, *Leptochloa panicoides* or *Oryza sativa*. Weights followed by different letters indicate a significant differerence ($P \leq 0.05$).

The host plant on which *O. pugnax* was reared had a significant effect on four components of metathoracic glands – (*E*)-2-octenal, (*E*)-2-hexenyl acetate, (*E*)-2-decenal and *n*-dodecane (Table 4.2). The amount of *n*-dodecane was higher in adults reared on *O. sativa* than those reared on *L. panicoides* ($F_{2,59} = 4.62$, P = 0.01), and the amount of (*E*)-2-decenal was significantly higher in *O. pugnax* reared on *E. crus-galli* than those reared on *O. sativa* and *L. panicoides* ($F_{2,59} = 5.24$, P = 0.008). In addition, there was a significant difference in the amount of (*E*)-2-decenal between females (0.04%) and males (0.24%) ($F_{1,59} = 4.61$, P = 0.04). Adults reared on *E. crus-galli* contained 6 and 10 times the amount of (*E*)-2-decenal than adults reared on *L. panicoides* and *O. sativa*, respectively.
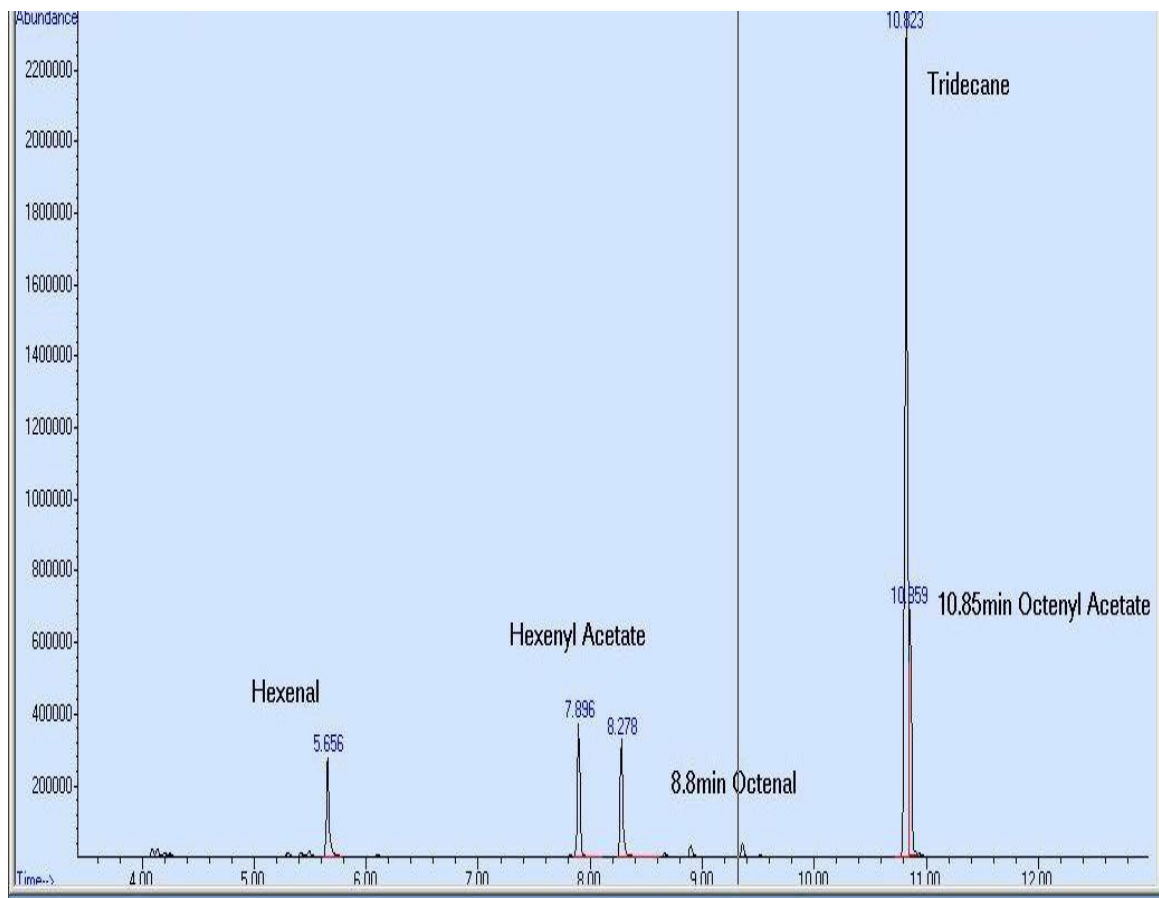
Figure 4.2. Typical chromatogram of *O. pugnax* metathoracic gland samples.

Table 4.2. Percentage of compounds in the metathoracic glands of *O. pugnax* relative to the most abundant compound, tridecane. Insects were reared from egg to adult on one of three host plants and metathoracic gland contents were analyzed using coupled GC-MS. Means in each row followed by different letters indicate a significant difference ($P \leq 0.05$).

Compound	Host Plant		
	O. sativa	*E. crus-galli*	*L. panicoides*
(*E*)-2-hexenal	18.3 ± 2.7 a	16.8 ± 5.0 a	17.5 ± 2.5 a
(*E*)-2-heptenal	0.03 ± 0.01 a	0.03 ± 0.02 a	0.02 ± 0.01 a
(*E*)-2-hexenyl acetate	17.8 ± 2.3 a	5.5 ± 1.6 b	11.1 ± 3.5 ab
(*E*)-2-octenal	3.2 ± 0.8 b	5.5 ± 1.9 a	2.8 ± 0.7 b
(*E*)-2-octenyl acetate	27.1 ± 2.1 a	25 ± 3.5 a	35.8 ± 4.4 a
(*E*)-2-decenal	0.03 ± 0.01 b	0.3 ± 0.2 a	0.05 ± 0.01 b
n-dodecane	2.5 ± 0.2 b	1.7 ± 0.2 ab	1.7 ± 0.3 b
n-tridecane	100	100	100

4.3.2. Olfactometer Bioassays

When presented with different concentrations of metathoracic gland secretions, significantly more adults were attracted to the control treatment (77%) than the filter paper treated with 1 SBE ($\chi2 = 9.32$, P = 0.002; Figure 4.3). When the gland secretion was diluted to 1/4 SBE, 55% of adults oriented *O. pugnax* extract ($\chi2 = 0.2$, P = 0.66). When diluted to 1/8 SBE, 65% of adults oriented towards the *O. pugnax* extract ($\chi2 = 5.07$, P = 0.02).
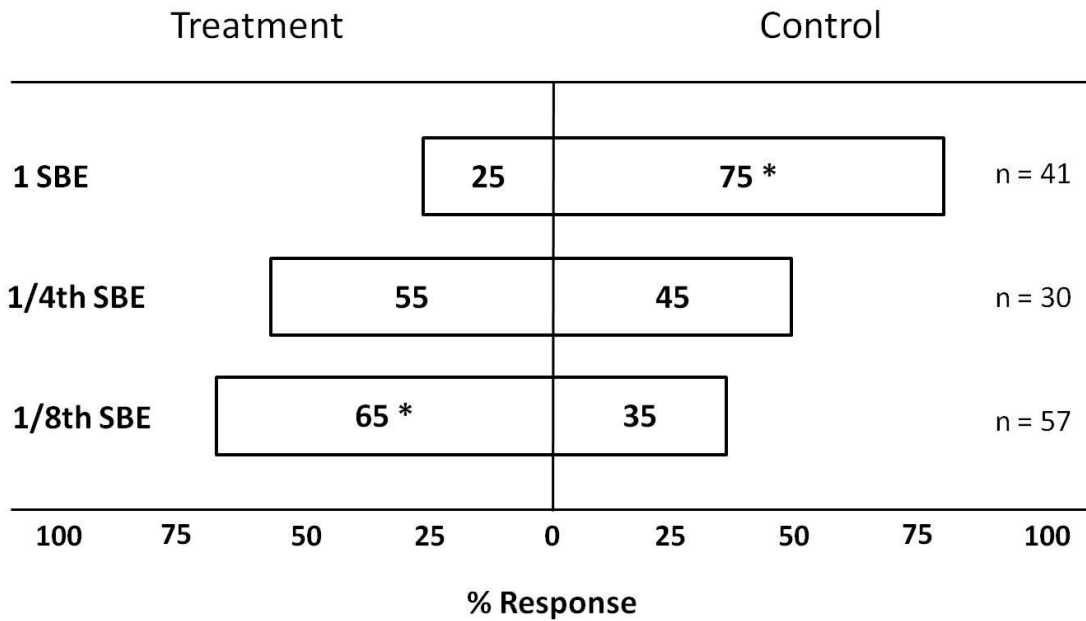
*indicates a significant difference (P ≤ 0.05)

Figure 4.3. Response of *O. pugnax* to crude metathoracic gland extracts in a y-tube olfactometer. A single adult was presented with a choice of one concentration (full, 0.25 or 0.125) of extracts dissolved in dichloromethane and applied to a filter disc or a filter disc treated with an equivalent volume of dichloromethane and given 15 minutes to make a choice. Non- responders were not included in the analysis.

4.3.3. Field Experiments

In two different experiments designed to assess *O. pugnax* response to a synthetic mixture of MTG components, significantly fewer *O. pugnax* were found in plots sprayed with this mixture than those in plots sprayed with control solution. In the first experiment, the repeated measures analysis revealed no significant effect of treatment (F = 1.75, P = 0.20) but a significant effect of time (F = 20.03, P < 0.0001) and no significant interaction between time and treatment

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(F = 1.25, P = 0.298). There was a significant reduction in the number of *O. pugnax* in plots that

received the mixture of MTG glands 60 minutes after spraying, but not 30 or 120

minutes after spraying (Figure 4.4).
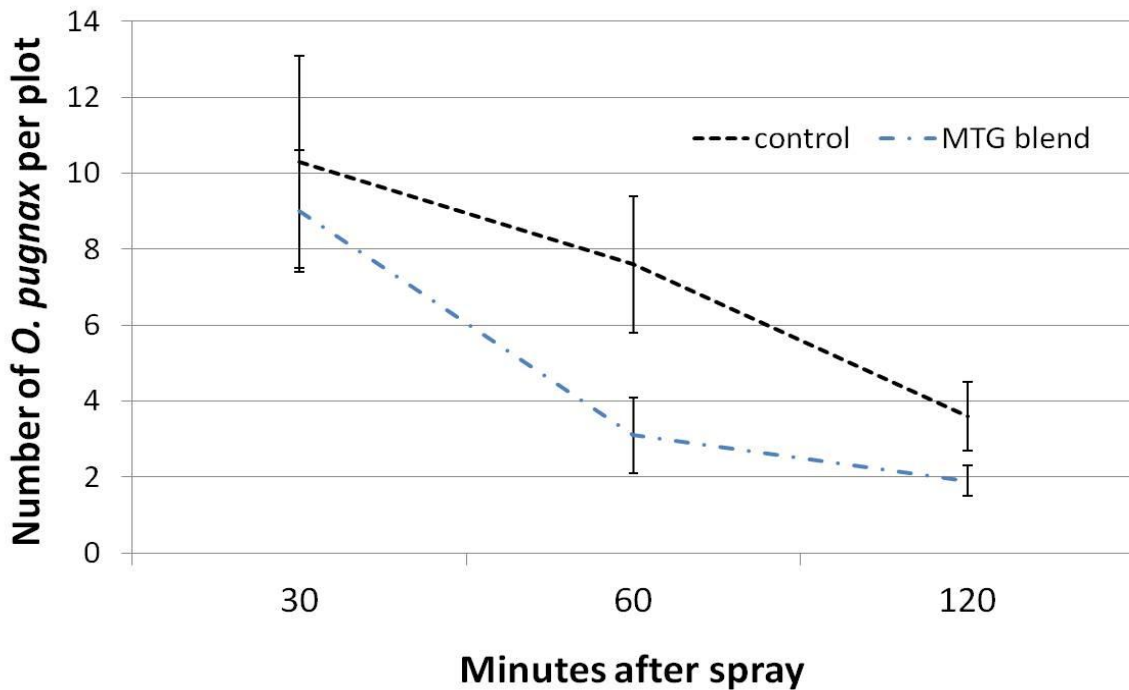
Figure 4.4. First experiment of 2011. Field plots were sprayed with a synthetic blend of the
four most abundant MTG compounds or a control solution (30% ethanol). Plots were sampled
for *O. pugnax* using a sweep net 30, 60 and 120 minutes after spraying.

In the second experiment, the repeated measures analysis revealed no significant effect of

treatment (F = 3.77, P = 0.07) but a significant effect of time (F = 22.34, P < 0.0001) and no

significant interaction between time and treatment (F = 0.53, P = 0.60). There was a significant

reduction in the number of *O. pugnax* in plots that received the mixture of MTG glands 120

minutes after spraying, but not 30 or 60 minutes after spraying (Figure 4.5).
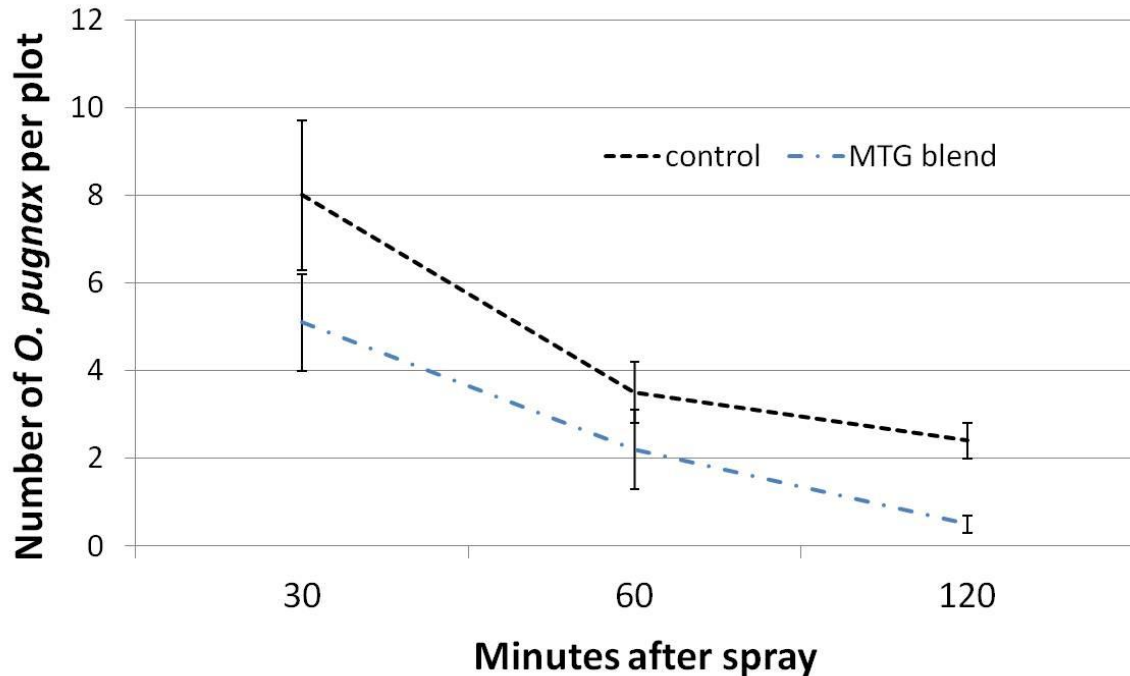
Figure 4.5. Second experiment of 2011. Field plots were sprayed with a synthetic blend of the four most abundant MTG compounds or a control solution (30% ethanol). Plots were sampled for *O. pugnax* using a sweep net 30, 60 and 120 minutes after spraying.

4.4. Discussion

The rice stink bug, *O. pugnax* (F.), is the most injurious late season pest of rice in the southern United States (Grigarick, 1984; McPherson and McPherson, 2000). Currently, the only viable control option for this pest is the use of insecticides, often broad-spectrum pyrethroids, and alternative management tactics are needed. Volatile chemicals found in the metathoracic and dorsal abdominal glands of Pentatomids are known to play important roles in the behavior and ecology of these insects, and it therefore it may be possible to exploit these chemicals for novel management tactics. In this study, laboratory experiments were designed to characterize the composition of metathoracic gland contents of stink bugs reared on three different hosts and to assess behavioral responses of *O. pugnax* to metathoracic gland contents at different concentrations. Field experiments were then conducted to determine whether a mix of synthetic gland components could influence rice stink bug behavior in small field plots.

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The defensive chemistry of Pentatomids in general has been well characterized (Blum, 1981; Eisner et al., 2005), but the contents of *O. pugnax* metathoracic glands have only been partially characterized. Blum et al. (1960) reported two major components from *O. pugnax* metathoracic glands ((*E*)-2-heptenal and *n*-tridecane). Our results expand on this prior work by showing that *O. pugnax* metathoracic glands contain several other short-chain alkenals, esters and alkanes. Chromatographic results indicate that the metathoracic gland contents of *O. pugnax* are similar to other Pentatomids (Borges and Aldrich, 1992; Gilby and Waterhouse, 1967; Kou et al., 1989; Pareja et al., 2007, Zarbin et al., 2000).

As has been previously shown (Naresh and Smith, 1983), the host plant used to rear *O. pugnax* to adults had a significant impact on development time and adult body weights. *O. pugnax* reared on *O. sativa* developed on average one to two days faster than those reared on *E. crus-galli* and *L. panicoides*. Moreover, females reared on *O. sativa* weighed nearly twice as much as females reared on *E. crus-galli* and *L. panicoides*. It is likely that the increased body weight of adults reared on *O. sativa* translates into increased survival and reproduction. Because these two non-crop hosts often are found in and around rice fields, the development and survival on different hosts can impact the populations of *O. pugnax* moving from senescing hosts into flowering rice fields. This, in turn, can have implications for the management of *O. pugnax* in and around rice fields, as *O. pugnax* is known to invade rice fields en masse once flowering begins.

The marked influence of host plant on weight and development time in this insect led us to hypothesize that host plant would also influence metathoracic gland chemistry. This is an aspect of Pentatomid metathoracic gland and defensive chemistry that has not been previously investigated.. For those insects that sequester compounds from plants, the host-plant on which the insect feeds has been shown to influence defensive chemistry (Duffey, 1980; Hartmann et al.,

2004; Kopf et al., 1998; Nishida, 2002; Rowell-Rahier et al., 1991; Triponez et al., 2007). There is also evidence demonstrating that geographical differences, often associated with shifts in host-plant use, can alter defensive chemistry (Kopf et al., 1998; Triponez et al., 2007; Wahlberg, 2001). Although Pentatomids are not known to sequester compounds from their host our results demonstrate that feeding on different host plants leads to quantitative shifts in at least four compounds.

The metathoracic gland contents of other Pentatomid species have been shown to function as both aggregation and alarm pheromones. Kou et al. (1989) reported that the metathoracic gland secretions of *Erthesina fullo* Thunberg functions as an alarm pheromone that is concentration dependent. When tested at a concentration of one stink-bug equivalent from males, 100% of male *E. fullo* avoided the olfactometer arm with the secretions. When males were confronted with 10^{-1} individual equivalent, only 50% of males avoided the metathoracic gland extract, and 25% avoided the extract when a 10^{-3} individual equivalent was used. Lockwood and Story (1987) found that metathoracic gland secretions of adult *N. viridula* act as an alarm pheromone. They also reported that two components of the alarm pheromone, (*E*)-2-hexenal and (*E*)-2-hexenyl acetate, significantly increased directed movement during the first minute of exposure. Similarly, Ishiwatari (1974) showed that (*E*)-2-hexenal had an alarm effect on three different Pentatomid species. Our results using different concentrations of metathoracic gland extracts show a similar pattern. Adult *O. pugnax* clearly avoided the olfactometer arm that contained 1SBE, but when the concentration was reduced to 1/8th SBE, a significant number of adults moved towards the arm containing metathoracic gland extract. James et al. (1996) demonstrated increased attraction of *Biprorulus bibax* Breddin to individual trees baited with (*E*)-2-hexenal in citrus orchards. Results from our small-plot field experiments also demonstrate increased movement from treated plots in response to an

application of a synthetic mixture containing major metathoracic gland components. The concentration of individual components in our field experiments was intentionally designed to elicit an alarm response in *O. pugnax* rather than to serve as an attractant. Additional research is needed to better understand the relationship between concentration and activity and the effective use of metathoracic gland chemicals in novel management strategies.

The defensive role of Pentatomid metathoracic glands has been described (Aldrich, 1988; Gunawardena and Bandumathie, 1993; Krall et al., 1999; Staddon, 1979). However, natural enemies of Pentatomids, especially parasitoids, are also known to eavesdrop on their prey by exploiting gland contents as cues for host location. Mattiaci et al. (1993) showed that (*E*)-2-decenal, isolated from the metathoracic gland of *N. viridula*, stimulates oviposition in the egg parasitoid *Trissolcus basalis*. Additionally, the egg parasitoids *T. basalis* and *Telenomus podisi* have been shown to use compounds from stink bug defensive secretions to orient towards hosts (Borges and Aldrich, 1994; Borges et al., 1997, 2003; Conti et al., 2004; Laumann et al., 2009). This could have implications for biological control of *O. pugnax*, as parasitization of eggs by *T. podisi* has been identified as the main factor responsible *O. pugnax* egg mortality in and around rice fields (Sudarsono et al. 1992).

In addition to being used as allomones and kairomones, metathoracic glands may also serve to protect Pentatomids from pathogen infection. Borges et al. (1993) demonstrated that (*E*)-2-decenal and (*E*)-2-hexenal, both common Pentatomid defensive compounds, have anti-microbial properties, inhibiting the germination and germ tube development of the entomopathogen *Metarhizium anisopliae*. Similarly, data from our laboratory (Milks and Hamm, unpublished data) demonstrate that metathoracic gland chemicals can affect the *in vitro* growth and proliferation of *Beauveria bassiana* and *M. anisopliae,* as well as kill conidia of both pathogens. In aerations of the rice stalk bug, *Tibraca limbativentris.* these components were not

detected (Borges et al., 2006), and it is worth noting that this species appears to suffer higher infection rates from *M. anisopliae* than the neotropical stink bugs *Euschistus heros, N. viridula* or *Piezodorus guildinii* (DaSilva Martins et al., 2004; Sosa-Gómez & Moscardi, 1998), species in which these compounds have been detected (Fucarino et al., 2004; Pareja et al., 2007; Zarbin et al., 2000). Field evaluations *B. bassiana* for control of *O. pugnax* in rice fields showed limited efficacy (Patel et al., 2006b). Therefore, the presence or absence of these compounds may play an important role in mediating the success of biological control efforts using entomopathogenic fungi.

The biological activity of defensive compounds from Pentatomid metathoracic glands has the potential to be exploited for several promising and novel management strategies. Defensive compounds can be used as allomones, to aid in sampling and detection, elicit aggregation and/or dispersal behavior, or as kairomones, used to attract natural enemies. In natural environments, the distinction between the two may not be as clear as in the laboratory, and future research designed to better understand the multifunctional roles defensive secretions have will aid in the development of innovative management tactics.

CHAPTER 5. DIVERGENT EFFECTS OF PHENOLIC ACIDS ON THE GROWTH OF FALL ARMYWORM (*SPODOPTERA FRUGIPERDA*) AND SUGARCANE BORER (*DIATRAEA SACCHARALIS*)

5.1. Introduction

Phenolic compounds are a diverse group of secondary metabolites and are widespread in plants (Waterman and Mole, 1994). Studies on the activity and role of phenolic compounds have shown a wide range of effects on insects. Early research stressed the growth reducing properties of phenolics towards different insects, and phenolics were thought to function as broad-spectrum defenses to which insects would be unable to develop counter-adaptations to (Feeny, 1970, 1976; Rhoades and Cates, 1976). Later research challenged this generalization by demonstrating a wide-range of effects of dietary phenolics, including acute toxicity (Lindroth and Peterson, 1988; Johnson, 1999), feeding deterrence (Jones and Klocke, 1987), inhibition of digestion and growth (Isman and Duffey, 1982; Summers and Felton, 1994), phagostimulation (Bernays et al., 1991) and nutritive effects (Bernays et al., 1983). In some cases, minor differences in chemical structure are associated with divergent biological effects (Ayers et al., 1997; Lindroth and Peterson, 1988), while in some cases the phenolic compound can have different effects on different insects (Ikonen et al., 2001). Many plants respond to herbivory and other biotic or abiotic stresses by producing elevated levels of phenolics.

The fall armyworm, *Spodoptera frugiperda* J.E. Smith, is a highly polyphagous insect that has been recorded on over 60 plant species from at least 23 plant families (Johnson et al., 1987; Pashley, 1988). However, its favored hosts are grasses, and it is an important pest of several graminaceous crops, including rice, corn, sorghum and forage grasses (Chang, 1986; Pashley, 1993). The sugarcane borer, *Diatraea saccharalis* (F.), is a major agronomic pest in the southeastern United States. Holloway et al. (1928) reported more than twenty host plants for *D. saccharalis*. In addition to sugarcane, it is an economically important pest of graminaceous crops

such as corn, rice and sweet sorghum (Roe et al., 1981). Both of these insects commonly encounter high concentrations of phenolic compounds when feeding on grasses. Several compounds, namely ferulic acid and *p*-coumaric acid, are commonly found in plants and are particularly common in grasses, where they can occur in concentrations as high as 0.05%-0.1% dry weight (Classen et al., 1990; Wu et al., 2001). Thus, these insects might be expected to possess adaptations to high levels of phenolic compounds in their food.

This study was conducted to determine the effects of phenolic compounds on the larval growth of these two species. Four phenylpropanoid compounds that differ slightly in their chemical structures were used (ferulic acid, *p*-coumaric acid, caffeic acid and cinnamic acid) at different rates in diet incorporation experiments. In this paper, we report that minor structural differences leads to dramatic effects on the growth of *D. saccharalis* and *S. frugiperda*: the addition of hydroxyl groups to the basic cinnamic acid structure leads to an increase in weights of immature *D. saccharalis*, while decreasing weights of immature *S. frugiperda*.

5.2. Materials and Methods

5.2.1. Insects

Fall armyworm larvae were from a colony maintained on commercially available artificial diet (fall armyworm diet, Southland Products, Lake Village, AR) under laboratory conditions (14L;10D, 28°C ± 2°C, 38% R.H. ± 2% R.H.). The colony originated from larvae collected from bermudagrass pastures in the summer of 1997 and thus likely belong to the rice strain of the fall armyworm (Pashley, 1988) and were maintained on a commercially available diet. Larvae collected from rice fields and pastures were added to the colony periodically to maintain genetic diversity. Adult *D. saccharalis* were also obtained from a laboratory colony maintained on artificial diet (sugarcane borer diet, Southland Products, Lake Village, AR). The colony originated from larvae collected in rice fields near Crowley, LA in 2005. Insects

collected from rice fields are added annually to the colony to maintain genetic variability.

5.2.2. Estimation of Phenolic Acids In Rice Plants

Estimates of total phenolic contents and levels of individual phenolic acids in rice tissues were made using spectrophotometric and chromatographic procedures. To estimate total phenolic levels, fresh plant material was collected from the Louisiana State University Agricultural Center Rice Research Station, in Crowley, LA. Twenty plants that showed evidence of stem-borer infestation (whiteheads, feeding lesions, or frass from stems) were cut at the water line and returned to the laboratory, where stems were cut into ca. 7.5cm pieces, weighed and extracted in 15mL of 50% methanol for 48hrs. Stems designated as controls were sampled and extracted in the same manner as damaged plants, with the exception that plants from which these stems were taken had no signs of stem-borer feeding. Total phenolics in samples were assayed using Folin-Ciocalteu method and a Shimadzu UV-1601 spectrophotometer was used to measure absorbance a 720 nm (Stout et al., 1998). A standard curve was constructed using ferulic acid, and estimates of total phenolic levels were expressed as nmoles ferulic acid equivalents per gram fresh weight of stem tissue.

Plants used for identification and quantification of individual phenolic compounds were grown under greenhouse conditions (14L:10D at 29°C ± 3°C) and were harvested 21-25 days after planting. Plants were cut at the soil line and oven dried at 40°C for 48hrs. Once dried, plants were weighed and ground using analytical Wiley mill (General Electric). Ground plant material was then placed in 40ml of 50% methanol for 48hrs, after which the solvent was filtered under vacuum to remove all plant material. The methanol was then removed using a rotary evaporator at 40°C. Once the methanol had evaporated, 40ml of deionized water was added to the remaining aqueous fraction, shaken for five minutes, and then 40ml of hexane was added and then again shaken for five minutes. The hexane layer was then discarded using a

separatory funnel, leaving the aqueous fraction behind. To this fraction, 40ml of ethyl acetate was added. The following day, the aqueous fraction was discarded using a separatory funnel and the ethyl acetate fraction was vacuum filtered over a bed of sodium sulfate to remove any remaining water. The ethyl acetate fraction was then evaporated to dryness under a vacuum at 50°C. Once dry, 1ml of 100% methanol was added to the dried residue and stored at -20°C for chromatographic analysis.

Individual compounds were identified and then quantified using a Dionex HPLC system (Dionex Corp., Sunnyvale, CA). The mobile phase consisted of 0.05% acetic acid (A) and 100% methanol (B) and initial conditions were 80% A: 20% B. Flow rate was 1.0ml/min and was passed through a Supelco Discovery HS C18 100 x 4.6mm 5µm column (Sigma-Aldrich, St. Louis, MO). Absorbance at 320nm was monitored on a photo-diode array detector for 40 minutes per sample. The mobile phase was ramped from initial conditions to 20% A: 80% B over 20 minutes, held for 10 minutes and then returned to initial conditions in another five minutes. Individual phenolic compounds were identified by matching retention times and spectra to authentic standards, which were purchased from Sigma-Aldrich (St. Louis, MO). An external calibration curve was constructed for each compound using six concentrations for each of the compounds.

Total phenolic levels from field-grown plants (nm ferulic acid equivalents per gram fresh weight of stem tissue) were subjected to an analysis of variance, with treatment (stem-borer infested or uninfested) as a fixed effect. Means were separated using Tukey's HSD (Tukey, 1953).

5.2.3. Diet Incorporation Studies

The effects of caffeic acid, cinnamic acid, *p*-coumaric acid and ferulic acid on larval growth of fall armyworms and sugarcane borers were tested by incorporating the compounds

into artificial diet at six concentrations: 0, 0.1, 0.25, 0.5, 1 and 2% dry weight. Commercially available diet mixtures (either ‒sugarcane borer diet‖ or ‒fall armyworm diet‖ from Southland Products Inc., Lake Village, AR) were prepared by mixing 100g of appropriate diet, 519ml boiling water, 4.3250ml of linseed oil and one of the six concentrations of each phenolic compound into a 1L plastic bowl and blended using a hand blender (Rival IB954W, Jarden Corp., Providence, RI) until the diet was thoroughly mixed. Once mixed, approximately 2mL of diet was dispensed into 1oz plastic cups, and sixty insects were reared at each dosage and all concentrations were divided into three randomized blocks. Each block occupied one of three locations within a larger rearing room, where conditions were maintained at 14L;10D, 28°C ± 2°C, 38% R.H. ± 2% R.H.   After 10 days of feeding, *S. frugiperda* larvae were removed from diet and individually placed into empty cups, left to starve for three hours and then weighed to the nearest 0.1mg. After 21 days of feeding, *D. saccharalis* larvae were removed from diet and individually placed into empty cups, left to starve for three hours and then weighed to the nearest 0.1mg. Experiments for each compound were repeated twice, and data from each experiment was pooled to generate a single mean for each compound tested.

Data from diet incorporation experiments were analyzed as randomized block design, with block and experiment as random effects and treatment as a fixed effect. This mean was then used in the analysis of variance to compare insect weight from each treatment as a percentage of control weight. Mean weights were separated using Tukey's HSD (Tukey, 1953).

5.3. Results

Total phenolic levels were significantly higher in stems from plants not infested with *D. saccharalis* than in stems from borer-infested plants ($F_{1,34} = 4.75$, $P = 0.03$). The mean for undamaged plants was 5301.57 nmoles ferulic acid equivalents per gram fresh weight of stem tissue and the mean for damaged plants was 4257.33nm ferulic acid equivalents per gram fresh

weight of stem tissue. Chromatographic analysis of greenhouse plants indicate that *O. sativa* seedlings contain approximately 0.001% dry weight ferulic acid, cinnamic acid and caffeic acid and as much as 0.004% dry weight of *p*-coumaric acid.

The addition of caffeic acid to diet had no effect on *D. saccharalis* larval weight ($F_{5,555}$ = 0.95, P = 0.45) but had a significant impact on *S. frugiperda* larval weight ($F_{5,591}$ = 95.18, P < 0.0001). Dietary concentrations as low as 0.25% caffeic acid significantly reduced *S. frugiperda* larval weight when compared to the 0.1% rate and untreated controls (Table 5.1; Figure 5.1).

The addition of cinnamic acid significantly reduced larval weight of *D. saccharalis* ($F_{5,533}$ = 30.67, P < 0.0001) and *S. frugiperda* ($F_{5,655}$ = 5.86, P < 0.0001). However, the degree to which cinnamic acid reduced *D. saccharalis* larval weight was much higher than observed for *S. frugiperda*. Fall armyworm larvae maintained their weight, relative to controls, with the exception of those fed a 2% cinnamic acid diet, while *D. saccharalis* larvae weight began to decline at the 1% concentration (Figure 5.2).

When p-coumaric acid was incorporated into diet, a significant reduction in larval weight was found for *D. saccharalis* ($F_{5,242}$ = 3.89, P = 0.002) and *S. frugiperda* ($F_{5,690}$ = 25.16, P < 0.0001). Although the overall reduction of *D. saccharalis* was significant, larval weight began to increase as the rate of *p*-coumaric acid increased from 0.5% to 2%. The mean weight of *S. frugiperda*, however, decreased at every concentration tested.

Table 5.1. Mean weight of *D. saccharalis* and *S. frugiperda* fed on varying concentrations of four different phenolic compounds. Insects were allowed to feed on diet containing phenolic acids and weighed after 21 days (*D. saccharalis*) or after 10 days (*S. frugiperda*). Means in each column followed by a different letter indicate a significant difference in larval weight ($P < 0.05$).

| Compound	Dry Weight (%)	Mean Weight (mg)	
		D. saccharalis	*S. frugiperda*
Caffeic acid	0	121.0 ± 3.5a	160.4 ± 8.1a
	0.1	117.4 ± 3.2a	143.7 ± 8.4a
	0.25	117.2 ± 4.14a	107.5 ± 6.2b
	0.5	118.3 ± 5.2a	77.3 ± 5.0c
	1	128.5 ± 7.7a	39.2 ± 23.8d
	2	115.5 ± 3.6a	26.3 ± 0.002d
Cinnamic acid	0	100.3 ± 3.5a	244.1 ± 12.7a
	0.1	101.5 ± 4.2a	251.0 ± 14.0a
	0.25	95.5 ± 3.3a	246.7 ± 13.8a
	0.5	87.9 ± 3.5a	238.2 ± 12.2a
	1	73.8 ± 3.73b	237.2 ± 11.7a
	2	51.3 ± 2.9c	175.2 ± 10.8b
p-Coumaric acid	0	64.0 ± 3.8a	224.7 ±12.2a
	0.1	58.1 ± 4.4a	191.6 ± 12.0b
	0.25	71.0 ± 5.8a	186.6 ± 11.3b
	0.5	45.5 ± 3.5b	175.9 ± 9.6b
	1	60.2 ± 5.4a	142.4 ± 7.9c
	2	66.2 ± 4.3ab	138.1 ± 9.1c
Ferulic acid	0	117.6 ± 4.1a	224.7 ± 16.1a
	0.1	112.8 ± 4.4a	231.3 ± 16.6a
	0.25	108.1 ± 4.8ab	218.3 ± 16.0a
	0.5	103.4 ± 8.1abc	220.6 ± 15.8a
	1	90.2 ± 4.4bc	215.3 ± 16.0a
	2	90.9 ± 4.5c	217.0 ± 15.7a
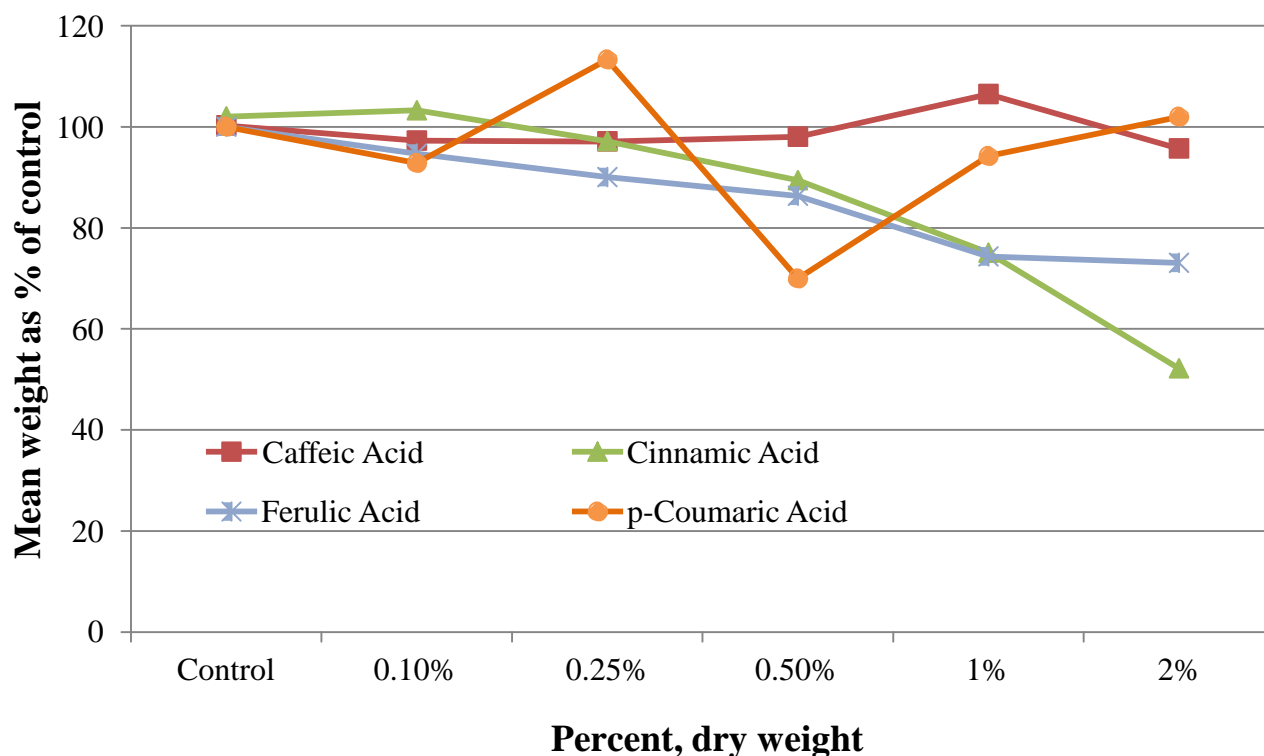
Figure 5.1. Mean weight of 21d old *D. saccharalis* larvae reared on diet that incorporated four different phenolic compounds at one of six rates. Mean weight at each dietary concentration is expressed as a percentage of *D. saccharalis* fed diet with no phenolic compound added.

The addition of ferulic acid to diet significantly decreased *D. saccharalis* larval weight ($F_{5,242} = 3.89$, $P = 0.002$) but had no effect on *S. frugiperda* ($F_{5,667} = 0.42$, $P = 0.83$). At concentrations of 1% and 2% *p*-coumaric acid, the mean weight of *D. saccharalis* was 61-66% of control weights, respectively (Figure 5.2).

5.4. Discussion

Insects that feed on grasses are exposed to high levels of phenolic compounds. Levels of ferulic and *p*-coumaric acid are especially high in grasses. Levels of *p*-coumaric acid found in rye have been found to range from 4-7mg/100g (Andreasen et al., 2000). The most abundant hydroxycinnamic acid derivative found in cereals is ferulic acid (Kim et al., 2006; Lachman and Capouchova, 2006). In wheat, ferulic acid concentrations can range as high as 0.5% (w/w) dry

matter and in barley, up to 0.14% (w/w) (Bunzel et al., 2001).   Grass-feeding insects might therefore be expected to possess adaptations to reduce the potential negative effects of phenolics. In the experiments reported here, we compared the effects of four phenylpropanoid compounds on the larval growth of two economically important grass feeding insects, the fall armyworm and the sugarcane borer. The four phenylpropanoids were found to affect the two species differently; furthermore, minor differences in chemical structure were associated with markedly different effect on the same species.
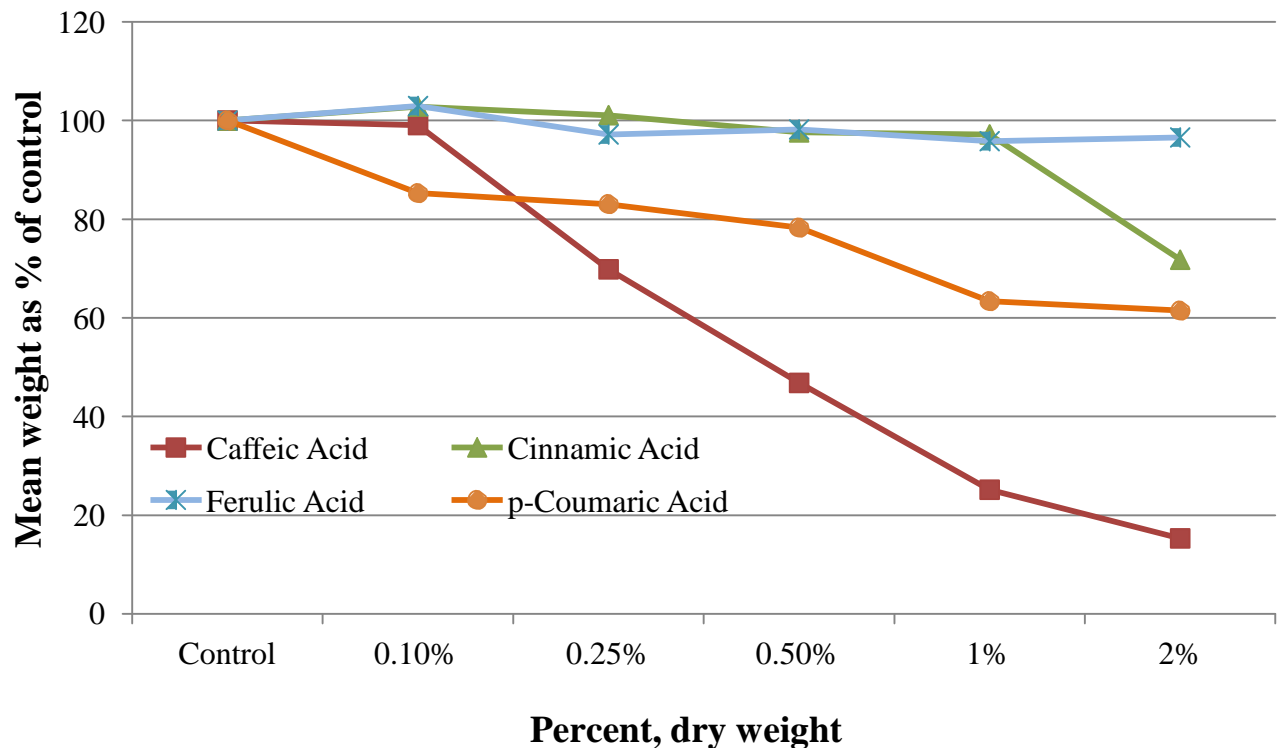

Figure 5.2. Mean weight of 10d old *S. frugiperda* larvae larvae reared on diet that incorporated four different phenolic compounds at one of six rates. Mean weight at each dietary concentration is expressed as a percentage of *S. frugiperda* fed diet with no phenolic compound added.

The effects of ferulic acid and *p*-coumaric acid on *S. frugiperda* clearly demonstrate that small differences in chemical structure can have large effects on the growth-reducing properties of phenolics. These two compounds differ only by the presence of a methoxy group at the meta position (see Figure 5.3) of the aromatic ring, yet ferulic acid had no effect on larval growth while *p*-coumaric acid significantly reduced *S. frugiperda* larval weight. However, both ferulic acid and *p*-coumaric acid had a significant impact on *D. saccharalis* larval growth.

Similalry, caffeic acid and p-coumaric acid differ only by the presence of a hydroxyl group at the meta position of the aromatic ring in caffeic acid. Despite their similarities, these compounds had different effects on *D. saccharalis*. Caffeic acid had no effect on larval growth, yet *p*-coumaric acid significantly reduced *D. saccharalis* weights. In contrast, both compounds had a significant impact on the larval weight of *S. frugiperda*.

Comparisons of the effects of these four compounds on the growth of these two grass-feeding species clearly demonstrate that the two species metabolize these compounds differently. Although caffeic acid differs from ferulic acid by the substitution of a methoxy group with a hydroxyl group at the meta position of the aromatic ring, the effects on *D. saccharalis* and *S. frugiperda* are quite different. Caffeic acid had no effect on *D. saccharalis* growth but significantly decreased *S. frugiperda* larval weights. Similarly, ferulic acid had no effect on *S. frugiperda* but significantly reduced *D. saccharalis* larval weights.

The activity of cinnamic acid and *p*-coumaric acid was also shown to differ between *D. saccharalis* and *S. frugiperda*. The only difference between the two chemicals is the lack of a hydroxyl group at the para position of the aromatic ring in cinnamic acid. Cinnamic acid had a significant effect on *S. frugiperda* only at a concentration of 2% dry weight whereas the effect on *D. saccharalis* became significant at concentrations of 1-2%. The effect of *p*-coumaric acid was significant at every level tested on *S. frugiperda*, yet had no effect on *D. saccharalis* until

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concentrations reached 0.5%.

The concentrations of phenolic compounds used in these diet incorporation studies are consistent with estimates of the levels of phenolics in cereal leaf and stem tissues in this study and previous studies (Dimberg et al., 2005; Hernanz et al., 2001; Kovacova and Malinova, 2007). In another study, total phenols in *O. sativa* were measured following infestation by the Asian gall midge, *Orseolia oryzae* (Wood-Mason) and found to range between 0.18mg/g to 0.5mg/g dry tissue (Amudhan et al., 1999). The amount of total phenolics from rice bran reported by Onofre and Hettiarachchy (2007) average 8.85mg/g dry tissue. In our study, the amount of individual phenolic compounds from 21d *O. sativa* seedlings range from 0.002mg/g to 0.07mg/g dry weight. However, most phenolics exist in a bound form, and in oats and wheat, up to 70% of total phenols are in this form (Adom and Liu, 2002). Additionally, extraction procedures, plant age and cultivar can influence the amount of recoverable phenols. Nonetheless, the extraction procedure utilized in our experiments allowed us to characterize phenolic levels and to use them at biologically relevant concentrations in our diet incorporation experiments.

Data from this study are consistent with data from previous studies showing that minor differences in chemical structure of phenolics may significantly alter the biological activities on insects (Ayers et al., 1997; Lindroth and Peterson, 1988). The differences in biological activity may be due to differences in affinity for specific target sites or to alterations in interactions with detoxifying enzymes. Thus, further comparative studies of the effects of these phenylpropanoid compounds on these two grass-feeding species may provide insights into the mode of action and ecological roles of phenolics.
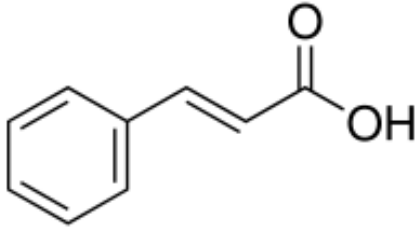
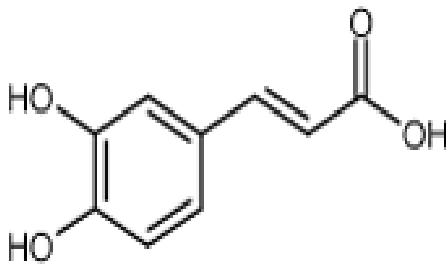a) Cinnamic acid   b) *p*-Coumaric acid
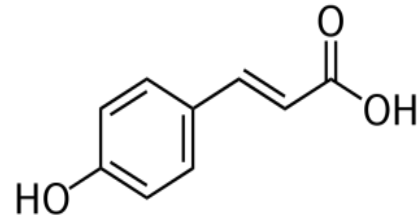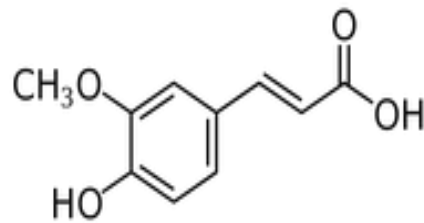
c) Caffeic acid   d) Ferulic acid

Figure 5.3. Structures of four related phenylpropanoid compounds. a) Caffeic acid; b) Cinnamic acid; c) *p*-Coumaric acid; and d) Ferulic acid

SUMMARY

A combination of laboratory, greenhouse and field experiments were undertaken in order to develop novel management strategies for key insect pests of rice in Louisiana. Because of the semi-aquatic nature of rice production, the integration of management tactics is particularly important to reduce undesirable consequences of an over-reliance on insecticides and to maintain valuable aquatic resources. Preserving natural resources is an essential aspect of farming that has traditionally been inherent in all that practice agriculture, and is a responsibility that all involved in agriculture must share in.

The rice water weevil is currently the most serious insect pest of rice in the United States. The potential for economic loss is a perennial concern for rice producers in the southwestern region of Louisiana, which is also the most intensively cultivated area of rice production in the state. In addition, crawfish production and rotation in conjunction with rice farming in the southwestern region presents a unique challenge when applying broad-spectrum insecticides. Newly labeled neonicotinoids and anthranilic diamides, often applied as a seed coating, have the potential to be much safer towards non-target invertebrates. However, these chemicals are always present, regardless of the pest density. In some cases, for example, when pests are not at an economically damaging level, there is no reason to treat, and therefore no reason for a seed treatment. Because of this, seed treatments for *L. oryzophilus* control may not always be the best management option.

Years of laboratory research have characterized the phenomenon of induced resistance, yet little of this has been conducted on agricultural crops, especially cereal grains such as rice (Karban and Chen, 2007). The use of elicitors, such as JA, has been shown to induce resistance in *O. sativa* in greenhouse experiments (Stout et al., 2009). Continued experiments using

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exogenous JA in field experiments resulted in a transient but significant decrease in the number of *L. oryzophilus* larvae per plant (Chapter 2), and highlighted the role of using elicitors in agricultural settings. Perhaps just as significantly, this work demonstrated the importance of timing to stimulate resistance at appropriate times in the life cycles of the pest and crop (Stout et al., 2002a).

The use of host-plant resistance is also a key concept in integrated pest management. Experiments conducted to initially evaluate the role of *O. sativa* cultivar on *D. saccharalis* oviposition behavior yielded unexpected results. One cultivar, ‗M202‗, became more attractive to ovipositing *D. saccharalis* females following oviposition by the same species. None of the other cultivars tested ('Reiho,' Cocodrie,' 'Rosemont' and Priscilla‗) followed this trend. Plants have been shown to respond to herbivore oviposition (Hilker and Meiners, 2002, 2006) and one cultivar of rice responds to oviposition by the whitebacked planthopper, *Sogatella furcifera*, by producing an ovicidal substance at the oviposition site (Seino et al., 1996; Suzuki et al., 1996; Yamasaki et al., 2003). In this case, the mechanism underlying this induced response has been characterized, and can be used in further breeding programs. Although the mechanism(s) responsible for the results in Chapter 3 are unknown, continued research into host-plant resistance may provide a framework for which host-plant responses can be incorporated into a cultivar development program for *D. saccharalis.*

The role and utility of metathoracic gland secretions from *O. pugnax* was investigated as a basis for developing novel management strategies (Chapter 4). In other Pentatomid species, volatile chemicals found in the metathoracic and dorsal abdominal glands are known to play important roles in the behavior and ecology of these insects, and therefore it may be possible to exploit these chemicals for innovative management tactics. Crude gland extracts were shown to

clearly possess a concentration-dependent response, with a high concentration (one adult equivalent) deterring adults and a low concentration ($1/8^{th}$ adult equivalent) attracting adults. Chromatographic analysis of MTG contents expanded on the work of Blum et al. (1961) by characterizing additional trans-2-alkenals and *n*-alkanes, as well as identifying two additional esters. The role of host-plant on the MTG secretions had a qualitative influence on the chemical composition, as well as exerting a significant effect on the development time and weights of adults. Four of the most abundant MTG compounds were then mixed in their respective ratios and applied to small-plots of *O. sativa*. Plots that received a solution of these compounds were found to have reduced numbers of *O. pugnax* than control plots in two different experiments. The results and outcome of these experiments opens up new avenues of applied research for unique approaches to the management of *O. pugnax*.

In response to herbivory, many plants produce elevated amounts of secondary compounds, such as phenolic acids. The structure-activity relationship between four commonly occurring phenolics in rice – ferulic, *p*-coumaric, cinnamic and caffeic acids – was examined on two different insects, *D. saccharalis* and *S. frugiperda*. Initial work was carried out to quantify the total amount of phenolic compounds in rice grown under field conditions, and further experiments quantified individual levels of these compounds in greenhouse grown rice (Chapter 5).

The results of diet incorporation experiments show divergent effects of these compounds on the two insects used. The incorporation of caffeic acid negatively affected the growth of *S. frugiperda* but had no effect on *D. saccharalis*. In contrast, ferulic acid negatively affected the growth of *D. saccharalis* but had no effect on *S. frugiperda*. The only difference between these two chemicals is the presence of a methoxy group, and illustrates how the structure of phenolics

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can have widely divergent effects on different insects. It is important to understand how these compounds interact between rice and its herbivores, as cereals have been shown to possess high levels of phenolics; wheat has been shown to contain as much as 0.5% dry weight of phenolics (Benzel et al., 2001). The importance of how different insects are impacted by phenolics may also provide insight into the metabolism and detoxification of such compounds.

The overall goal of this research was to investigate how rice and insects interact and building upon this knowledge, develop unique management strategies. The contributions of this research include the first application of an elicitor in a field setting to induce resistance in rice as well as demonstrating the phenomenon of induced susceptibility in rice cultivar ‚M202.' This induced susceptibility has the potential to provide a mechanistic understanding of how plants respond – or in this case – what plants don't do in response to oviposition, often the first step in herbivory. Moreover, this research has demonstrated how the presence, or absence, of simple molecular groups attached to larger phenolic compounds can have widely differing effects on two herbivores. The chemical ecology of *O. pugnax* described in this research has the greatest potential to be incorporated into management strategies, and is an ongoing area of research that I plan to continue.

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VITA

Jason Charles Hamm was born in 1975 in Topeka, Kansas. After graduating Shawnee Heights High School in Tecumseh, Kansas, in 1994, he enrolled at the University of Kansas. He obtained his Bachelor of Science in systematics and ecology from the University of Kansas in December of 1999, and upon graduation, worked as a hazardous materials chemist for Clean Harbors Environmental Services, Inc. out of Lenexa, Kansas. Less than a year later, he married Netkamol Pakwhan in Kansas City, Missouri, on November 25, 2000. In August of 2001, he began working as a research assistant under Drs. Steve Ashe and Michael Engel in the Entomology Division of the University of Kansas Natural History Museum and Biodiversity Research Center, where he helped to create an online database of the entire bee collection. In August of 2004, he began graduate studies in Department of Entomology at Louisiana State University under Dr. Michael J. Stout, and is currently completing the requirements for the Doctor of Philosophy and plans to pursue a career in research and teaching.