10-23-2018

Induced resistance in rice, oryza sativa, to herbivores in the southern united states

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INDUCED RESISTANCE IN RICE, *ORYZA SATIVA*, TO HERBIVORES IN THE SOUTHERN UNITED STATES

A Dissertation

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

in

The Department of Entomology

by

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B.S., Purdue University, 2007
M.S., Kansas State University, 2010
December 2018
This dissertation is dedicated to my nieces Emma and Elizabeth Caudill. So that they may always believe in themselves, and know they can accomplish whatever they put their minds to. I am always here for you girls.
Acknowledgments

I would like to thank the Louisiana State University AgCenter, the Department of Entomology, and the faculty and staff at the Crowley Rice Research Station. I would like to acknowledge the Rice Research Board, Louisiana Agricultural Consultants Association, The Young family, and USDA NIFA for financial contributions to this research. I would also like to acknowledge, and thank, my advisor Dr. Michael Stout. Mike was the best advisor I could have hoped for. He provided me with constant support, allowed me freedom in developing my research program, and somehow did not overwhelm me. I’m glad Mike was there to motivate me through the lows as well as celebrating with me during the highs. Additionally, I would like to thank all of my committee members Dr. Jeff Davis, Dr. Blake Wilson, Dr. Kevin McCarter, and Dr. James Cronin. Each of you contributed your time and efforts to help me to shape my career as a graduate student here at LSU, and to grow as a scientist overall. I sincerely appreciate the support. I would also like to thank all of the student workers at Crowley, and at LSU, who assisted me with data collection and processing. I could never have gotten through it all alone. Finally, to my closest of friends and family who kept me going Mom and Dad, Jason and Valerie Caudill, Janet and Neil Rullman, Alana Russell, James Villegas, Rachel Strecker, the Spruell family, Jessica Wesling, Kiki Felis, and many others I cannot name here. Thank you all for your constant and unwavering support and motivation. Now there is a doctor in the family!
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Abstract

The effects of herbivory and chemical applications as potential inducers of resistance to herbivores in rice, *Oryza sativa* L., were explored through a series of field, greenhouse, and laboratory experiments. Studies were conducted to investigate the preference and performance of several herbivore pests of rice with a focus on, *Lissorhoptrus oryzophilus* Kuschel, the rice water weevil (RWW), under conditions where rice plants had been stressed by previous herbivory or chemical applications. The effects of defoliation by *Spodoptera frugiperda* (J.E. Smith), the fall armyworm, on resistance and tolerance of rice to RWW under field conditions were examined. Herbivory by fall armyworm on rice may indirectly affect RWW in rice fields in the southeastern United States via changes in rice resistance and tolerance. Hypotheses were that defoliation by fall armyworm would alter the resistance of rice to subsequent infestation by RWW, and that defoliation would reduce the tolerance of rice to subsequent RWW injury. Additional experiments probed the effects of larval and adult feeding on rice tissues by RWW on subsequent adult RWW preference. Hypotheses were that prior feeding by either stage would alter plant resistance to subsequent adult infestation. These studies provided strong support for larval induced resistance to aboveground conspecific adults, while providing weaker support for adult induced resistance to subsequent RWW adults. Investigations of the effects of methyl jasmonate, a derivative of the plant hormone jasmonic acid, which is involved in the mechanisms underlying induced resistance to herbivores, were also studied at the greenhouse and field level. It was hypothesized that methyl jasmonate seed treatments would increase resistance in rice to RWW. Results indicated that while the compound did not provide protection equal to pyrethroid insecticides, it was capable of inducing resistance to RWW at the cost of delays in plant growth and potential reductions in yield. Finally, herbicide-induced resistance to major herbivores in LA
rice was investigated at the field level. These herbivores included the RWW, a complex of stemborers (Lepidoptera:Crambidae) including *Diatraea saccharalis* (Fabricius), the sugarcane borer, *Chilo plejadellus* (Zincken), the rice stalk borer, and *Eoreuma loftini* (Dyar), the Mexican rice borer, as well as the rice stink bug, *Oebalus pugnax* (Fabricius). Hypotheses were that there would be both direct and indirect effects of herbicides on insect and weed densities in rice, and that these effects would translate to differences in rice yields between treatments. Overall results from this series of experiments indicate that herbicides are capable of altering pest densities of both weeds and insects, and the resulting changes in these communities can have impacts on yields. Collectively, this work documents the potential of plant stressors including herbivory, hormone seed treatments, and herbicide injury to influence resistance of rice to insect herbivores. The broader implications of the work presented are significant due to the potential impact this information may have on IPM and future research. It is important to note that rice in the southern U. S. can be used as a model for developing countries where pest management options are limited and pesticide safety is frequently eschewed.
Chapter 1.

Introduction

More than half the world’s population depends on rice as the main staple of their diet. Nearly 1,000,000 hectares are produced in the United States, with about 161,875 hectares of that being produced in Louisiana (USDA 2018). Many of the insect pests present in Louisiana rice are global pests. Investigating these pests and their ecological interactions with rice plants may benefit growers around the globe. There are three goals of this chapter: first, to introduce several insect pest species in the Louisiana rice agroecosystem (Figure 2.1); second, to discuss host plant resistance; finally, to discuss how induced resistance may be utilized for sustainable pest management. This chapter is intended to aid the reader in understanding why the rice system, and herbivores of rice present in the southern US, are an appropriate system to explore effects of induced resistance on insect communities.

Pest Complex in Louisiana Rice

*Lissorhoptrus oryzophilus* Kuschel, the rice water weevil (RWW), is a coleopteran insect pest in the family Curculionidae. It is native to the United States, but has expanded its range to most temperate rice growing regions of the world (Aghaee & Godfrey 2014). One study in Texas showed that seven percent of field caught females are parthenogenic while invasive populations in California, Europe, and Asia are thought to be made up exclusively of parthenogenetically-reproducing females (Jiang et al. 2008). It is believed that this feature of the insects’ reproductive biology allowed it to become an excellent invader of new territory (Jiang et al. 2008). The insect is semiaquatic; adults are both proficient swimmers and capable of flight. Adults feed on the foliage of rice plants,
consuming small amounts of leaf tissue and causing longitudinal scars, but little economic damage (Smith 1983). While adults are noted in the field after emergence of rice, females only begin oviposition in earnest when the field is flooded (Shang et al. 2004). Depth of flood affects RWW oviposition and preference is shown for plants flooded to 5.1 cm from the base as opposed to 1.3 cm or 10.2 cm (Stout et al. 2002). Plant age is also a determining factor in oviposition with plants in the tillering stage preferred over pre-tillering or reproductive stage plants (Stout et al. 2013). Eggs are laid in the leaf sheath of flooded rice plants. Larvae eclose and make their way to the plant roots, which they both feed upon and utilize for gas exchange via the plants’ aerenchyma cells (Isley, D., & Schwardt, H. 1930). There are four larval instars. Larval feeding reduces plant tillering and shoot growth, as well as root biomass. This feeding activity overall reduces panicle densities and grain weights at harvest (Zou et al. 2004). Pupae continue development while submerged on rice plant roots. Once adults emerge they return to the surface and repeat the life cycle. There are multiple generations per year in the southern US (Aghaee & Godfrey 2014). Host plants include aquatic and semiaquatic plants in the families Poaceae and Cyperaceae (Lupi et al. 2009; Tindall & Stout 2003).

Current methods of control for RWW include insecticides, water management, and host plant resistance. In Louisiana, pyrethroids, neonicotinoids, and chlorantraniliprole are used in management of the RWW with the latter two the most commonly used insecticides (Lanka et al. 2014). Chlorantraniliprole is the most effective of these options, is effective against RWW and stemborers, and is utilized most heavily in areas known to consistently have high densities of these pests (Beuzelin et al. 2016; Lanka et al. 2014). Seed treatments are often preferred to pyrethroids by growers that
rotate rice and crayfish production as this insecticide has significantly lower toxicity to the crayfish (Barbee et al. 2010).

Water management is also an avenue that can be manipulated for cultural control of the RWW. For example, there is evidence that delaying flooding by four weeks compared to normal practices can delay larval infestation and reduce density of RWW larvae (Rice et al. 1999). Thousands of rice varieties have been screened for resistance traits, and while low levels of resistance and tolerance have been noted, there is no variety that can sufficiently protect against damage incurred by the insect (Saad et al. 2018).

*Spodoptera frugiperda* (J.E. Smith), the fall armyworm is a lepidopteran insect in the family Noctuidae. It is native to the Americas, and its’ historic range spans as far south as Argentina. In 2016 the pest was found on the continents of Africa and Europe, and therefore has become a global pest (Goergen et al. 2016). The adults are mottled tan or gray in color, and cause no injury to rice. Adults lay eggs on the foliage of rice plants beginning early in the season, but are not frequently found after the permanent flood has been established. Larvae eclose from the eggs and are voracious feeders. Feeding on rice foliage is occasionally economically damaging, and sometimes necessitates replanting of parts or entire fields. One study showed that there was a correlation between defoliation by fall armyworm and reduction in panicle density and yield (Pantoja & Smith 1986). There are six larval instars before pupation in the soil (Hardke et al. 2015). There are four generations in Louisiana (Capinera 1999). There are two strains of fall armyworm, one of which feeds mostly on rice and forage grasses. The other strain is a pest of many crops including corn, cotton, and sorghum (Acevedo et al. 2018). Management of fall
armyworm in rice currently includes managing nearby grasses, flooding, and chemical insecticides.

Rice is also host to several stem boring species. The insects in this complex include the sugarcane borer, *Diatraea saccharalis* (Fabricius), the rice stalk borer, *Chilo plejadellus* (Zincken), and the Mexican rice borer, *Eoreuma loftini* (Dyar). These borers are lepidopterans in the family Crambidae. Sugarcane borer is native to the western hemisphere, but not to the United States, arriving in Louisiana in 1855 (Capinera 1999). The adults lay flat oval shaped eggs, which are pale white, but change to orange and then black as they develop. Larvae are yellowish and spotted. After eclosion from eggs larvae move to the leaf whorl where they feed within the leaf sheath. The first instars feed externally on foliage until they have molted at least once (Hall et al. 2007). At this point the larvae bore into the stem and proceed through five to seven instars. This larval feeding can be economically damaging in multiple rice stages. In the vegetative stage the larvae can cause unfurled leaves to die resulting in dead-hearts. In the reproductive stage they can cause unfilled grains called whiteheads and overall yield loss (LV et al. 2008). They are capable of overwintering inside the stem of some plants such as sugarcane and rice. In Louisiana there are three to five overlapping generations per year, and due to temperature variation. Host crops in the southern US include rice, sugarcane, and maize, but the insect will also utilize various wild grass species (Roe et al. 1981).

The rice stalk borer is native to North America. Adults are about one inch long with pale wings covered with small dark spots. Eggs are very similar to that of sugarcane borer, flat and oval shaped and pale in coloration. Larvae have a black head capsule and pale yellow body with two pairs of stripes that run along the length of the body. Feeding
is similar in all borer species described here with larvae feeding on the leaf sheath before boring into the stem causing damage to the host plant. Larvae of this species exit the plant stem to pupate. There are two to three generations a year in southern rice (LSU AgCenter 2012).

The Mexican rice borer was first described in the southern US in Arizona, but has expanded its range to Louisiana as of 2008 (Wilson et al. 2015) Adults are light tan and, unlike the two previously described species, lack spots on the wings. Eggs are spherical and are laid on dry leaves. Larvae are light colored and have light colored head capsules. Like the rice stalk borer they have longitudinal stripes along the abdomen. Feeding and injury to the host plant is similar to the two previously mentioned species and, like sugarcane borer pupation, occurs within plant stems (Beuzelin et al. 2016). Possible host plants include rice, sugarcane, maize, sorghum, lemongrass and other large grasses (Hayden 2012). The work by Hayden also notes the expansion of this pest into Florida where rice is grown. As an invasive species, there is some uncertainty regarding potential economic impact of this insect, though it has been predicted to exceed 45 million if rice in the entire state becomes infested (Reay-Jones 2008). Additionally, densities of Mexican rice borer in rice not protected by insecticidal treatments in Louisiana, have already reached levels capable of causing economic damage (Wilson et al. 2015). Monitoring of this insect has also provided evidence for range expansion across the state of Louisiana including the location where field studies in this dissertation took place (Wilson et al. 2017).

Insecticides are the main method of control for borers though they are not entirely effective. Foliar insecticides are most effective before the larvae have entered the stem,
after which point the plant itself protects them from exposure. This feeding behavior, inside plant stems, also makes it difficult for farmers to scout for the pest. However, it was shown that multiple applications of lambda-cyhalothrin provided a $172/ha benefit when applied at the five centimeter panicle and late booting/heading stages in rice against sugarcane borer (Reay-Jones et al. 2007). The insecticidal seed treatment Dermacor-X-100® has also been shown to significantly reduce sugarcane borer densities in both field and greenhouse experiments (Sidhu et al. 2014; Wilson et al. 2015). There are currently no tolerant or resistant varieties which decrease damage to an acceptable level.

*Oebalus pugnax* (Fabricius), the rice stink bug, is native to North America where it is a pest of wheat, sorghum, and rice (McPherson & McPherson 2000). This insect has a hemimetabolous lifecycle including egg, nymph, and adult stages. The nymphs pass through five instars. Both adults and late instar nymphs cause damage to rice by removing liquid contents of developing grains and thereby negatively affecting grain yield and quality. Damage occurs due to the mechanical injury from stylet feeding, removal of grain contents, and the introduction of salivary enzymes and pathogenic microorganisms (Espino et al. 2007; Awuni et al. 2015; Brown 2003). Rice stink bug can cause injury at stages from rice flowering through grain maturation (Awuni et al. 2015). There are as many as six generations per year, though it is not clear how many generations are in rice fields due to the wide range of alternative hosts. These include both graminaceous crops and weeds (McPherson & McPherson 2000). Currently rice stink bug management in Louisiana is reliant on insecticide applications of pyrethroids and neonicotinoids. However, there is already some evidence of resistance to lambda-
cyhalothrin which encourages research to unveil alternative strategies (Blackman et al. 2015).

Figure 1.1. Insect pest complex in Louisiana rice as the season progresses from left to right. In the early season after rice emergence *Spodoptera frugiperda* (J.E. Smith), the fall armyworm can be found in the field. These insects are rarely found after the permanent flood. Adults of *Lissorhoptrus oryzophilus* Kuschel, the rice water weevil (RWW), may be found in the field before flooding, but larvae are present after flooding. The complex of stemborers begins to oviposit after flooding and may be found in fields into the late season. *Oebalus pugnax* (Fabricius), the rice stink bug is found in rice after heading has commenced.

**Host Plant Resistance**

The resistance of crop plants to pests has been of interest to agriculturalists as long as humans have been cultivating plants, but the field of host plant resistance (HPR) has matured greatly over the centuries. In the United States, there were resistant varieties of apple as early as the late 18th to early 19th century (Smith 2005). From there the field
advanced, with publications on HPR showing up in the literature in the 1940’s and the first review by Snelling (1941). In Painter (1951) wrote the book on host plant resistance, greatly expanding the field. Highlights of his research include breeding sorghum resistance to chinch bugs and wheat resistance to Hessian fly, as well as teaching the first class on HPR. Painter was also very interested in determining and defining the mechanisms associated with plant resistance. He developed a trichotomy for categorizing different types of plant resistance, but noted that there were “unclassified resistance mechanisms”, which in his time were very difficult to elucidate. Painter suggested that plants defended themselves via one or a combination of several strategies: tolerance, antibiosis, and antixenosis (i.e., non-preference) (Painter 1951). Tolerance refers to the ability of a plant to endure or compensate for insect damage; in other terms, tolerance involves reduction in fitness loss without negative impacts on the herbivore (Kwok & Laird 2012). Antibiosis refers to plant traits that have negative effects on insect physiology and life history including growth and survival. Antixenosis refers to plant traits that exert negative effects on insect behavior reducing preference for the plant (Smith 2005).

However, modern scientists have argued that these categories of resistance are vaguely delineated. For example, in most cases of plant resistance, there is some combination of antibiosis and antixenosis involved which cannot be truly separated from one another, and there may be varying levels of either with differing repercussions in regard to pest management. These inadequacies have resulted in a push from ecologists toward more descriptive subdivisions that clearly indicate the nature of plant insect interaction, with a smaller push from those in IPM who would like to see a focus on
utility in pest management. This shift in ideology is partly due to an increased interest in the underlying mechanisms of resistance (as in early days mechanistic understanding was thought to be less important), and partly in order to align the basic and applied bodies of literature (Stout 2013). Categories of resistance are therefore better defined along two axes, one including constitutive and induced traits, and the second including direct and indirect traits. These result in four overall categories of resistance: constitutive direct, constitutive indirect, induced direct, and induced indirect (Kwok & Laird 2012). These four axes are in addition to tolerance.

Constitutive resistance refers to traits or biochemical products that are produced in the plant or expressed at some level, in the absence of a biotic or abiotic stressor. The intensity of constitutive traits may change depending upon the plants’ age and ontogeny (Kwok & Laird 2012). These traits can affect arthropods both directly and indirectly. For example, spines and thorns can directly interfere with arthropod feeding behavior, whereas extrafloral nectaries can attract parasitoids, and thereby indirectly cause insect mortality. Underlying defense hormones such as salicylic and jasmonic acid may be constitutively produced as well as they are involved in many non-defense related processes (Creelman and Mullet 1997; Perez et al. 2012). Constitutive resistance may be costly to the plant in situations where there is no environmental threat, and this may be one reason why induced responses evolved (Heil and Baldwin 2002).

Induced resistance refers to traits or biochemical products whose production in the plant begins or is upregulated in the presence of a biotic or abiotic stressor. It was first noted by entomologists in the 1960’s (Beck 1965). Induced resistance, like constitutive resistance, has direct and indirect components. Direct defenses involve the internal
chemistry of the plant producing compounds that deter feeding by herbivores such as toxins and digestibility reducers (Kessler et al. 2004). These defenses can have direct negative effects on behavior, growth, and physiology of pests. Indirect defenses involve the attraction of natural enemies, which can reduce herbivore densities. Induced responses such as the release of volatiles has been shown to reduce herbivory by as much as 90% when direct and indirect components are considered (Kessler & Baldwin 2001). Host plant resistance is very complex, and most plant traits do not fall neatly and consistently into one category or the other. Some constitutively produced compounds can be induced to higher levels in the presence of a stressor. Therefore, a constitutive trait can become an induced trait. Additionally, a specific trait may be constitutive in one species and induced in other species. For example, extrafloral nectaries are constitutive in some plants, but can be induced in others (Wackers and Bonifay 2004). There are many examples of induced plant defenses in literature, and likewise there are many ways these defenses can be triggered including biotic (e.g., herbivory and oviposition) and abiotic (e.g., wind and chemical) stressors.

**Biotic and Abiotic Inducers**

Insect activities that cause injury to plants such as herbivory and oviposition are biotic stressors to plants and can result in induced resistance. In some instances, induced plants produce antifeedants such as glyceollin, a phytoalexin. These compounds can directly affect herbivores. One study showed that glyceollin had a deterrent effect on feeding of two beetle species (Fischer et al. 1990). Another study showed that the squash beetle, *Epilachna borealis*, induced production of cucurbitacins in cucumber, *Cucurbita pepo*. The beetles were able to detect the cucurbitacins and ‘chose’ to avoid leaves with
high levels of the compound. Additionally, it was noted that fitness of the beetle was negatively affected (Tallamy 1985). The plant family Brassicaceae produces glucosinolates, plant secondary metabolites that have been intensely investigated for direct resistance to herbivores, and reviewed by Hopkins et al. (2009). Glucosinolates are one of many compounds that are produced constitutively in the plant, but upon insect attack increase production. Adding to the complexity of HPR, glucosinolate-based defenses appear to protect against generalist herbivores whilst specialists can take advantage of and utilize these compounds (Hopkins et al. 2009).

Induced production of volatile organic compounds (VOCs) has been shown to promote direct and indirect resistance to herbivores. For example, herbivory by larvae of several species, *Heliothis virescens*, *Helicoverpa zea*, and *Manduca sexta*, induce volatile emission in *Nicotiana tabacum*. Some of these volatiles have been determined to be highly repellant to female adults of those species (De Moraes et al. 2001). It is well supported that herbivore induced volatiles decrease oviposition rates and increase egg predation, and that herbivores can utilize volatile cues for orientation toward plants and assessment of plant quality (De Moraes et al. 2001; Kessler & Baldwin 2001; Zakir et al. 2013). Herbivore-induced plant volatiles can protect plants against oviposition by herbivores even if the initial injury was inflicted upon a neighboring plant. It was shown that adult *S. littoralis* avoid oviposition on undamaged cotton and alfalfa plants when neighboring cotton plants have been injured by conspecific herbivores (Zakir et al. 2013).

Insect activities other than herbivory can also induce direct and indirect resistance. Oviposition itself has been noted to induce resistance and can result in a reduction of further colonization by the herbivore, reduced subsequent oviposition, and
release of VOCs which are repellant to adults (Fatouros et al. 2012). One study showed oviposition induced indirect resistance to adult moths, *Chilo partellus*, by a change in green leaf volatiles. This change in volatile composition resulted in attraction of, *Cotesia sesamiae*, a parasitoid wasp showing that these effects can extend to multitrophic levels (Bruce et al. 2010).

VOCs indirectly increase plant resistance by attracting natural enemies and altering multitrophic interactions. There are many studies which show that natural enemies are attracted to herbivore induced volatiles, and that these predators and parasitoids can alter insect densities and the overall composition of insect communities associate with injured plants (Poelman et al. 2012; Dicke & van Loon 2000; Ali et al. 2013).

Abiotic stressors have also been noted to induce resistance to herbivores. Scientists have used synthetic compounds to induce responses in a variety of plants in order to study plant defenses. Wind has been noted to induce resistance to herbivores. Mechanical stimulation of the common bean, *Phaseolus vulgaris* L., by electrical fans induced resistance to the two spotted spider mite *Tetranychus urticae* Koch (Cipollini Jr. 1997). The author states this was due to upregulation of lignin and strengthening of plant cell walls.

**Spatial and Temporal Separation of Inducers**

When herbivores share a host plant, various types of interactions may occur among them. There may be competition, mutualism, amenalism, contramenalism, or commensalism. If insects are on the same host at the same time, but spatially separated, outcomes must be plant-mediated. Likewise, when insects are on the same host at
different times, interactions must be plant-mediated. Such indirect interactions are examples of trait-mediated indirect interactions. These indirect interactions between herbivores are important in structuring communities of organisms associated with plants (Utsumi et al. 2010; Viswanathan et al. 2015). It is vital that these plant-insect interactions be investigated in agricultural systems in order to understand how host plant resistance and specifically induced resistance might be utilized as a tool for IPM.

When multiple life stages of conspecific herbivores damage different parts of a plant, there is potential for initial attack by one stage inducing resistance against attacks by the other stage. Herbivory in general may stimulate or inhibit plant growth, change plant nutrient content, and increase allelochemical concentrations. Aboveground herbivory may affect soil biota, and generate belowground responses (Erwin et al. 2014). Belowground herbivory may influence plant physiology, modify allocation patterns, and decrease nutrient absorption. There are several reviews which discuss these spatially separated plant-mediated interactions and several of which provide mechanistic explanations (Erb et al. 2008; Van Der Putten et al. 2001; Wardle et al. 2004; Kant et al. 2015). In many cases belowground herbivory has a negative overall effect on aboveground herbivores whether conspecific or interspecific. Early hypotheses proposed negative effects of aboveground herbivores on belowground herbivores (Masters et al. 1993), but new evidence has provided support for the opposite effect (Johnson et al. 2012; Erwin et al. 2014). In the case of the red milkweed beetle, *Tetraopes tetraophthalmus*, aboveground adult feeding enhanced performance of belowground conspecific larvae, but induced resistance to a foliage feeding lepidopteran species, *Danaus plexippus* (Erwin et al. 2014).
Temporally separated herbivores on the same host plant may also interact indirectly due to changes induced by the earlier attacker (Faeth 1986). Induced responses may persist for as much as two to three weeks before decaying completely (Kaplan & Denno 2007). These effects may have conspecific and interspecific repercussions that result in altered herbivore communities. Indirect induced responses can negatively affect subsequent herbivores by reducing growth, survival, oviposition, and visitation rate, and these responses can overall reduce species richness in a community (Ohgushi 2005). One study utilized laboratory, field cages, and open field plots to investigate the effects of feeding by the planthopper, Prokelisia dolus, on a subsequently arriving plant hopper, P. marginata. The outcome of the study suggested that feeding from the former induced resistance in the plant resulting in reduced body size and survival and prolonged developmental time of the latter. The relationship was not symmetrical however, in that when the insects were introduced to the plant in the reverse order, feeding by P. marginata prolonged development of P. dolus, but had no effect on body size and survival (Denno et al. 2000). This supports statements regarding the intricacy of HPR and the need for additional investigations of specific plant-insect interactions, especially in the agricultural realm.

The Role of Jasmonic Acid in Induced Resistance

The mechanisms underlying the induced responses discussed so far have only been partially elucidated. It is known that oxylipin signaling, including jasmonates and green leaf volatiles, is associated with herbivore-induced responses (Kessler et al. 2004; Tong et al. 2012). It was discovered that plants under attack by insects upregulate production of the hormone jasmonic acid (JA) and its secondary metabolites, which
accumulate in tissues at the site of herbivore attack. Fatty-acid-amino-acid compounds from insects may stimulate the oxylipin signaling in plants (Baldwin et al. 2002), but this is not the only possibility, as injury from insects that do not produce these amino acid conjugates also induce resistance. The subsequent cascade leads to changes in resistance related genes and metabolites. This enhances resistance to insect attack and is one of the underlying mechanisms of induced resistance (Campos et al. 2014). Jasmonates have many functions including defense and protection against biotic and abiotic stress. Jasmonoyl isoleucine (JA-Ile) is the most active form of this hormone and the rapid production and accumulation of this compound in vegetative tissues is reversible (Campos et al. 2014). Biosynthetic intermediates of JA can be biologically active as well including 12-oxyphytodienoic acid (ODPA) and the methyl ester, methyl jasmonate (MJ) (Staswick & Tiryaki 2004). Identification of these intermediates and their functions has opened new avenues of investigation for the pursuit novel pest management strategies.

**Plant Mediated Interactions and Impacts on Pest Management**

Induced responses to herbivory are highly variable across plant families as has been evidenced throughout this chapter. This variability is the impetus for additional studies in individual crop systems and the insect species in those systems. It is also important to note the level of sustainability attainable when utilizing plant-insect interactions and natural plant defenses for management of agricultural crops. While products developed from research regarding induced resistance may not provide the same level of protection, the level of safety to humans and the environment are likely to be much higher than conventional insecticides. Additionally, as noted several times throughout this chapter induced responses in plants are highly complex, and therefore, are
less prone to rapid development of insect resistance to this technology. Finally, products created from enhanced knowledge of HPR combined with conventional products may provide levels of protection that were previously unattainable (Walters et al. 2007).

The use of chemical elicitors is the most promising avenue for incorporation of induced resistance into IPM programs. Jasmonic acid and its derivatives have been tested across an array of plant families for its’ potential to reduce insect pest densities. This includes a series of studies involving foliar applications in tomato, *Lycopersicon esculentum* (Thaler 1999), root drench of tobacco, *Nicotiana attenuata* (Baldwin 1998), and seed treatments of cabbage, *Brassica oleracea* L. (Haas et al. 2018) and cowpea, *Vigna unguiculata* (Pankaj et al. 2013). In each of these cases arthropod and nematode fitness and or density were reduced in plants treated with JA or MJ.

**Examples in Rice**

There was a review published in 2007 covering work in this area up to the mid-2000s, but much has been accomplished since then (Karban & Chen 2007). Feeding by aboveground fall armyworm was shown to have a negative effect on belowground RWW. Root herbivory in turn resulted in decreased fall armyworm growth rate (Tindall & Stout 2001). Additionally, in rice in the southern U.S. it was also shown that, fall armyworm induced direct resistance in rice to conspecific larvae as evidenced by reduced weight gain and growth rate (Stout et al. 2009).

There has been a larger amount of work in rice on VOCs emitted after insect attack. One study identified 196 genes which were upregulated after fall armyworm attack, and of these 18 were involved in volatile synthesis (Yuan et al. 2008). Another study in rice found S-linalool induced by the brown planthopper (BPH), *Nilaparavata*
*lugens*, attracted predators (spiders), parasitoids (*Anagrus nilaparvatae*), and chewing herbivores, but it repelled BPH. This single volatile can therefore alter the insect community in a rice field (Xiao et al. 2012). More recently additional JA-responsive volatile organic compounds have been identified in rice. They are 2,4-heptadienal, β-cyclocitrinal, β-elemene, β-caryophyllene, methyl salicylate, and β-ionone (Tanaka et al. 2014). This study also confirmed results of the previous study with their finding of induced linalool emissions.

Abiotic stressors in rice have also been shown to induce resistance to herbivores. This has been shown in one report involving the herbicide 2, 4-dichlorophenoxyacetic acid. This compound induced resistance in rice to the striped stem borer, *Chilo suppressalis* (Xin et al. 2012). The study involved the linalool synthase promoter, which induced a reaction upstream of the jasmonic acid pathway. Treatment of plants resulted in *C. suppressalis* larvae that gained less mass, and more strongly attracted *Anagrus nilaparvate*, a parasitoid.

**Objectives and Goals**

These insects may induce resistance in the rice plant to both interspecific and intraspecific organisms. A greater understanding of these plant-insect interactions could lead to novel control strategies, which was one motivation for this body of work. The experiments performed and presented in this dissertation were the initial investigations in some insect species while in others they built upon previously completed studies. While many studies of induced resistance have been done in the greenhouse, several of the following objectives have been carried out with field level components. These experiments also investigate major events, such as RWW entrance at flooding, and the
effect of early season insect activity on subsequent mid to late season insect activity. This work was intended to elucidate the consequences of these events at the level of pest management. The hope is that this body of research will contribute to biotechnological approaches to pest control by adding to the knowledge of induced resistance in rice. Following is the list of objectives for this body of work.

I. Determine the effects of factorial combinations of fall armyworm larval feeding and mechanical defoliation on levels of resistance in rice to adult RWW by measuring larval densities later in the season.

II. Determine the effects of RWW adult and larval feeding on levels of resistance in rice to subsequent infestations of RWW adults by measuring oviposition.

III. Determine the effects of methyl jasmonate seed treatments on induced resistance in rice to RWW, and the effects on plant growth characteristics and yield.

IV. Determine if applications from selected different herbicides have effects on mortality of RWW, levels of resistance in rice to several herbivore pests, and indirect effects on herbivores via alterations in weed densities.

References


Chapter 2.


Introduction

Feeding by an herbivore on a plant alters the phenotype of the plant. In many cases, changes in phenotype are manifested as increases in the expression of resistance-related chemical or morphological traits, and consequently as increased resistance of the plant to herbivores. This phenomenon is known as induced resistance. The changes induced by herbivore feeding can be systemic and long-lasting, and thus an herbivore feeding on a plant may interact with other herbivores that subsequently feed on the same plant via induced changes in the shared host plant (Santamaria et al. 2013). Such indirect interactions are important in structuring communities of organisms associated with plants (Utsumi et al. 2010; Viswanathan et al. 2015). For example, in a study of herbivory on *Brassica oleracea* by *Pieris rapae*, early season feeding by *P. rapae* induced responses that caused plants to be avoided by generalist herbivores but preferred by specialist herbivore species later in the growing season. The specialist species affected by early season *P. rapae* feeding included sap suckers and chewing herbivores, and thus early season *P. rapae* herbivory dramatically altered the insect community associated with late season cabbage (Poelman et al. 2008). The implications of indirect interactions in agricultural systems are not wholly understood. Increased understanding of these plant-mediated interactions may lead to the development of novel approaches for managing agricultural pests, including alterations in pest thresholds, and reduction in synthetic insecticide use.
Rice, *Oryza sativa* L., is grown on 161,875 hectares annually in Louisiana, predominantly in the southwestern part of the state (USDA 2018). The complex of herbivores that attack rice represents an ideal system with which to study the importance of plant mediated indirect interactions in agriculture. In particular, *Spodoptera frugiperda* (J.E. Smith), the fall armyworm, is a sporadic early season pest of rice in southwestern Louisiana. Aboveground defoliation by larval fall armyworm can be economically damaging when levels of defoliation exceed 30%, or if severe injury to small seedlings by fall armyworm necessitates replanting (Pantoja & Smith 1986). *Lissorhoptrus oryzophilus*, the rice water weevil (RWW), is the most economically damaging insect pest of rice in the southern United States. Adult RWW feed on foliage of plants both before and after flooding but cause little economic damage. Flooding is a trigger for oviposition in this species (Stout et al. 2002); consequently, larval RWW typically infest roots of rice plants only after flooding. Injury to rice roots by RWW larvae reduces rice tillering and growth, and at harvest reduces panicle densities and grain harvest weights (Zou et al. 2004). The behavioral and temporal patterns of fall armyworm and RWW in rice fields make them likely candidates to interact via induction of plant defenses by fall armyworm defoliation before flooding may affect oviposition and larval feeding by RWW after flooding.

Plant-mediated interactions among aboveground and belowground insects have been documented in numerous plant species, although there is a need to validate laboratory observations of aboveground-belowground indirect interactions under field conditions (Johnson et al. 2012). Prior experiments with the fall armyworm and RWW provide support for the hypothesis that these pests can interact indirectly via changes in
their shared host plant. In greenhouse experiments, defoliation by fall armyworm has been shown to have a plant-mediated effect on the RWW. Hamm et al. (2010) found that defoliation of rice by fall armyworm larvae reduced subsequent oviposition on and emergence of RWW first instars from, defoliated plants. Similarly, severe fall armyworm defoliation of rice plants already infested with RWW larvae reduced weights of those larvae by 48% and densities of larvae by 32% compared to non-defoliated plants. Plants with lower levels of defoliation, however, were as suitable for RWW larvae as undamaged plants (Tindall & Stout 2001). Yuan et al. (2008) reported increased emission of several volatile organic compounds (VOCs) from fall armyworm defoliated rice seedlings, including linalool, which is a known defense-related compound. Increased emission of these types of VOCs following defoliation could impact community structure by attracting parasitoids or other pests.

In addition, defoliation of rice before flooding may affect rice physiology such that defoliated plants are less capable of compensating for injury to roots after flooding. Defoliation of grasses has an overall negative effect on root and shoot biomass (Ferraro & Oesterheld 2002). Defoliation often causes short-term increases in allocation of resources to roots, and these resources can then be used later for regrowth of aboveground portions of the plant (Schultz et al. 2013). In the grass *Calamagrostis epigejos*, for example, production of new leaf area was totally reliant on nitrogen stores remobilized from free amino acids and soluble proteins in the roots (Kavanová & Gloser 2005). As a consequence, defoliation may reduce nitrogen stores in roots and hinder the ability of plants to later compensate for additional herbivore injury. Similarly, root regrowth may rely on mobilization of resources from aboveground portions of the plant.
In corn, *Zea mays*, root regrowth following injury by western corn rootworm larvae relied partly on increased allocation of amino acids from shoots and leaves to roots (Qu et al. 2016). Thus, simultaneous injury of aboveground and belowground portions of a plant may result in competition for resources needed for regrowth. One consequence of combinations of root injury and defoliation may be greater than additive reductions in yield by forcing the plant to utilize vital resources to repair injury in both roots and shoots.

Indirect interactions among organisms, including indirect interactions among aboveground and belowground organisms, may be as important as direct interactions in structuring communities of organisms (Viswanathan et al. 2015; Johnson et al. 2012); however, the importance of indirect interactions in agricultural ecosystems has rarely been investigated. The field experiments described here were designed to investigate a potential plant mediated interaction involving fall armyworm and RWW in drill-seeded rice. The first objective was to determine whether pre-flood defoliation, via fall armyworm feeding or mechanical removal of leaves, would increase plant resistance to larval infestations by RWW after flooding. Based on results from prior experiments conducted in a greenhouse and lab, we hypothesized that FAW, a defoliating insect that sporadically attacks rice before flooding, and RWW, an insect whose larvae infest rice roots after flooding, would interact indirectly as a result of changes in young rice plants induced by FAW or mechanical defoliation before flooding. The second objective was to determine whether mechanical defoliation before flooding would reduce the ability of rice plants to compensate for root injury by RWW larvae, as evidenced by reductions in plant yield. The hypothesis was that reductions in rice growth and yield in plots subjected
to both defoliation and root pruning would be reduced compared to either type of injury alone. Although results varied among experiments and years of this study, the results suggest that defoliation before flooding can alter the resistance and tolerance of rice plants to root injury by RWW larvae after flooding. To our knowledge this is the first test of this latter hypothesis under field conditions.

**Materials and Methods**

**Study Site and Rice Cultivation**

All experiments were conducted at the Louisiana State University Agricultural Center H. Rouse Caffey Rice Research Station, Crowley, Acadia Parish, Louisiana, USA (30.231422°N and -92.379583°W, 7m asl). The soil type at this site is a Crowley silt loam with a pH of 7.1 and 12% organic matter. Fields at this site have been in a rice-fallow rotation for over 30 years. Rice in all experiments was drill-seeded in plots measuring 1.3 m by 5.5 m with seven rows of rice at 17.8 cm spacing, at a rate of 78.5 kg/ha. The rice varieties ‘CL111’, and ‘Cocodrie’ were used in resistance experiments, and rice variety ‘Cheniere’ was used in tolerance experiments. ‘Cheniere’ and ‘Cocodrie’ are long-grain, conventional inbred varieties, while ‘CL111’ is an herbicide-tolerant long-grain inbred variety. Previous research has shown that levels of resistance to RWW in ‘CL111’, ‘Cocodrie’, and ‘Cheniere are similar (Saad et al. 2018). Fertilization, weed, and disease control practices followed recommendations for drill-seeded rice in southwest Louisiana (Saichuk et al. 2014). Dates of planting and fertilization activities can be found in Supplemental Table 2.1.

**Insect Culture and Sampling**

Fall armyworm larvae used in experiments were taken from a laboratory colony. The colony had been initiated from field-collected insects in 2011 with insects added
from the field each year to maintain genetic variability. Larvae were reared on artificial
diet (Southland Products Inc., Lake Village, AR, USA). Rearing conditions were 16
hours light, 8 hours dark. Temperature was maintained at 28.3°C, and relative humidity at
70%.

All experiments relied on natural infestations of RWW, which were present at
high population densities at the experimental site. Adult RWW were present in plots
before flooding while larvae infested roots only after flooding. The procedure for
determining densities of RWW larvae and pupae on roots after flooding involves
removing soil/root core samples from plots and counting larvae and pupae associated
with roots (Stout and Riggio 2003).

**Effects of Fall Armyworm Defoliation on Rice Resistance to RWW**

Three experiments were conducted during the 2015 growing season. The rice
variety ‘Cocodrie’ was used for the first two experiments, while both ‘Cocodrie’ and
‘CL111’ were used for the third. The use of both ‘Cocodrie’ and ‘CL111’ in the third
experiment resulted from an error in assigning treatments to plots. Treatments in these
experiments included a non-defoliated control (CON) and a fall armyworm defoliation
treatment (FAW). When rice in plots had reached the three to four leaf stage, a single
microplot was established in each larger drill-seeded plot by enclosing an area of 0.91 m
with an open-top enclosure constructed of metal flashing. The metal flashing was 20 cm
in height. The microplot is the experimental unit from which data was collected. Tree
Tanglefoot® Insect Barrier (Scotts Company LLC) was applied to the top edge of
flashing to prevent escape of larvae. Microplots assigned to the FAW treatment were
infested by placing approximately 15 to 20 late instars by hand into the microplots, and
by giving three shakes of cheesecloth laden with neonates over plants. Plots were infested for up to fourteen days, after which percent defoliation was estimated and plots were flooded. Defoliation in plots was rated by visual inspection as percentage of total leaf tissue consumed. Flooding killed the majority of fall armyworm; those not killed were removed by hand. Adult RWW naturally present in plots began oviposition at this time. After approximately two weeks, these plots were sampled by coring to determine densities of RWW larvae on roots. A second sampling was performed approximately one week later. Plots were sampled twice to account for the possibility that responses induced by fall armyworm injury declined over time.

Three similar experiments were conducted in 2016 to further investigate the effects of defoliation on rice resistance to RWW. These experiments again employed microplots established in larger plots of drill-seeded rice as described above. The variety ‘Cocodrie’ was used in all experiments. The treatments in these experiments were factorial combinations of mechanical and fall armyworm defoliation: uninjured control (CON), fall armyworm defoliation (FAW) as described above, mechanical defoliation (MECH) as described below, and fall armyworm infestation combined with mechanical defoliation (BOTH).

Mechanical defoliation was accomplished using a gas-powered string trimmer (XT120 John Deere Pro Series). All plots assigned to mechanical defoliation treatments were defoliated by trimming plants above the collar of the third true leaf, which at the time of treatment was approximately half the height of the plant (Counce et al. 2000). This resulted in an approximately 33% reduction in plant biomass. Mechanical defoliation was performed the last day of fall armyworm infestation, and plots were
flooded the following day. Natural infestations of RWW larvae commenced at flooding. Plots were cored to estimate densities of RWW larvae on roots approximately two and three weeks after flooding.

**Impact of Defoliation on Tolerance of Rice to RWW**

Three additional experiments were conducted over the 2015—2016 growing seasons to determine whether artificial defoliation immediately before flooding reduced the tolerance of rice to root injury by RWW larvae. The rice variety ‘Cheniere’ was used in all three experiments, and experimental treatments were initiated when rice had reached the 4-leaf stage. Treatments were factorial combinations of mechanical defoliation and insecticidal seed treatment (to exclude RWW). For the control treatment, CON, plots were not defoliated and RWW larvae were excluded from plots by treating seeds with the insecticidal seed treatment chlorantraniliprole, (Dermacor X-100) (DuPont Crop Protection, Wilmington, DE). For the second treatment, WEEVIL, plots were not mechanically defoliated and rice seeds were not treated with Dermacor X-100. This resulted in belowground infestation by RWW larvae. For the third treatment, MECH, the plots were defoliated with a string trimmer and seeds were treated with Dermacor X-100 to exclude RWW larvae. This resulted in aboveground injury but not belowground root injury. In the fourth treatment, BOTH, plots were mechanically defoliated and were not treated with Dermacor X-100. This resulted in both belowground infestations by RWW larvae and aboveground mechanical defoliation. Mechanical defoliation was performed one day before flooding. Plots were core sampled once at 28 days post flood. When plants reached 100% heading plant height was recorded. Plots were harvested at grain maturity with a mechanical combine. Yields and grain moisture were recorded.
**Statistical Analysis**

All analyses were performed in SAS software version 9.4 (SAS Institute 2013). Means, standard deviations, and standard errors were determined in PROC MEANS. Poisson distribution and log link were used for all count data. All continuous data were tested for normality with PROC UNIVARIATE and the Shapiro-Wilk test ($P < 0.05$). Continuous data were transformed to meet the assumption of normality as needed. There was one data set (yields from the 2015 tolerance experiment) in which deviations from normality could not be corrected. PROC GLIMMIX was used to analyze all count and continuous data as described below.

For the 2015 experiments investigating the effect of defoliation on resistance to RWW, each of the three experiments were analyzed separately due to differences in experimental design. Experiment one employed a completely randomized design with eight replicates. For this experiment, treatment effects were analyzed by generalized linear mixed models, with fall armyworm defoliation as the fixed effect. Experiment two employed a randomized complete block design with six blocks and one replicate per block, whereas experiment three employed a randomized complete block design with two blocks and multiple replications of each treatment within each block. For these experiments, treatment effects were analyzed by generalized linear mixed model, with block as a random effect and fall armyworm defoliation as a fixed effect. Due to constraints in design, in experiment three, the two varieties were forced into separate blocks. Therefore, inferences for this experiment were made only for treatment effects and not across varieties. Means were separated using Tukey’s HSD means separation test ($P \leq 0.05$).
For the 2016 experiments in which the effects of fall armyworm defoliation on rice plant resistance to RWW were investigated, all three experiments employed a randomized complete block design with four blocks and a full factorial of the main effects of fall armyworm defoliation, mechanical defoliation, and their interaction in each block. Count data from the first coring of each experiment (two weeks after flooding) were combined into a single data set, and count data from the second coring (three weeks after flooding) into a second dataset. These two datasets were analyzed separately. Treatment effects were analyzed by generalized linear mixed model using PROC GLIMMIX, with block as a random effect and fall armyworm defoliation, mechanical defoliation, and their interaction as fixed effects. Means were separated using Tukey’s HSD means separation test ($P \leq 0.05$).

All three experiments investigating the impact of defoliation on tolerance of rice to RWW employed a randomized complete block design with six blocks and a full factorial of the main effects of mechanical defoliation, Dermacor, and their interaction in each block. Treatment effects were analyzed by generalized linear mixed models using block as a random effect. Fixed effects included defoliation, insecticide treatment, and their interaction. Means of RWW density and height were separated using Tukey’s HSD means separation test ($P \leq 0.05$). Means of yield were separated using Fisher’s protected LSD means separation test ($P \leq 0.05$).

**Results**

**Effects of Fall Armyworm Defoliation on Rice Plant Resistance to RWW**

In 2015, mean percent defoliation in plots infested by fall armyworm before flooding were 21%, 11% and 38%, for the first, second, and third experiments, respectively. Densities of RWW larvae and pupae were numerically lower in fall
armyworm defoliated plots in five of the six core samplings, with the differences between treatments significant in three of them (Figure 2.1). The effects of fall armyworm defoliation on densities of RWW larvae were stronger in the first and third experiments than in the second. In the first experiment, densities of RWW larvae and pupae were significantly lower in fall armyworm defoliated plots in the second core sampling (P=0.012), but not the first (P=0.445). In the third experiment, effects of fall armyworm defoliation on RWW densities were significant in both core samplings (P=0.019; P=0.004). The average densities of RWW larvae and pupae were approximately 25% and 75% lower in fall armyworm defoliated plots in the first and third experiments, respectively, compared to densities in control plots. In the second experiment, no significant effects of fall armyworm defoliation were found in either coring (P=0.445; P=0.167), and average densities of RWW larvae were 27% higher in fall armyworm defoliated plots (FAW) compared to control plots (CON) in this experiment.
Figure 2.1. Effects of pre-flood defoliation by fall armyworm larvae, on densities of rice water weevil (RWW) larvae and pupae after flooding. For the resistance hypothesis, core samples to estimate population densities of RWW after flooding were taken twice, for each of the experiments performed in 2015. Significant differences between means of control (CON) and defoliated (DEF) plots are shown. The * indicates significant difference between control and defoliation means with Tukey’s HSD test at $\alpha<0.05$.

In the three 2016 experiments, mean percent defoliation in plots infested with fall armyworm larvae were 47%, 53%, and 47% for the first, second, and third experiments, respectively. Densities of RWW larvae and pupae were assessed by core sampling at two time points after flooding for each of these three experiments (Figure 2.2). Densities of RWW larvae and pupae were not significantly different among treatments for either the CORE 1 dataset (P=0.347; P=0.588; P=0.844), or the CORE 2 dataset (P=0.315;
P=0.162; P=0.811) (Figure 2.2). For the CORE 1 data set, RWW densities were lowest in plots subjected to fall armyworm infestation (FAW). For the CORE 2 data set, RWW densities were lowest in plots subjected to both fall armyworm and mechanical defoliation (BOTH). RWW densities were intermediate in plots subjected mechanical defoliation (MECH) in both core samplings (Figure 2.2).

Figure 2.2. Effects of pre-flood defoliation by fall armyworm (FAW) larvae, mechanical defoliation (MECH), and the combination (BOTH) on densities of rice water weevil (RWW) larvae and pupae after flooding. For the resistance hypothesis, core samples to estimate population densities of RWW after flooding were taken twice, for each of the experiments performed in 2016. First core samplings were combined, and second core samplings were combined, and analyzed separately.
Impact of Defoliation on Tolerance of Rice to RWW

Dermacor X-100 resulted in an approximately 96% reduction in densities of RWW larvae and pupae when comparing CON and WEEVIL treatments (Figure 2.3; P<0.001), while mechanical defoliation resulted in removal of about 33% of plant biomass (see above). Densities of RWW larvae and pupae were not significantly affected by mechanical defoliation (P=0.478) (Figure 2.3). There was no interaction of mechanical defoliation and insecticide treatment on RWW densities (P=0.993).

Figure 2.3. Effects of pre-flood defoliation (MECH), rice water weevil (RWW) injury to roots (WEEVIL), and the combination (BOTH) on densities of RWW larvae and pupae after flooding. Core samples to estimate population densities of RWW after flooding were taken approximately one month after flooding. Significant differences between means of control (CON), RWW infested (WEEVIL), defoliated (MECH), and RWW infested and defoliated (BOTH) plots in tolerance experiments in 2015 are shown. Bars accompanied by different letters indicate a significant difference with Tukey’s HSD test at α<0.05.
The main effect of mechanical defoliation on rice yields was marginally significant (P=0.051). In plots that were mechanically defoliated alone, yields were reduced by approximately 5% when compared to control plots. The main effect of insecticide on yields, in contrast, was strong and significant (P=0.001). When compared to plots in the CON treatment, yields were reduced by 14.7% in plots with RWW injury alone (WEEVIL). Interestingly, yields were reduced by 29.6% compared to CON yields in plots that received both defoliation and root injury (BOTH), and yields in this treatment were significantly lower than yields from plots subjected to defoliation alone (MECH) or weevils alone (WEEVIL) (Figure 2.4). The interaction between defoliation and insecticide treatment was not significant (P=0.308).
Figure 2.4. Effects of pre-flood defoliation (MECH), rice water weevil (RWW) injury to roots (WEEVIL), and the combination of defoliation and RWW root injury on rice yields (BOTH). Yield was measured at harvest and adjusted for moisture content. Treatments are as follows control (CON), RWW infested (WEEVIL), defoliated (MECH), and RWW infested and defoliated (BOTH). Bars accompanied by different letters indicate a significant difference with Fisher’s LSD test at α<0.05.

In the first 2016 experiment, there was a significant main effect of Dermacor seed treatments on densities of RWW larvae and pupae. Dermacor reduced densities of RWW larvae by approximately 90% when comparing CON and WEEVIL treatments (P<0.001) (Figure 2.5A). There was also a significant main effect of defoliation on densities of RWW (P=0.019), although the Tukey comparison did not show significant differences in RWW densities between CON and MECH treatments. Defoliation did not significantly affect heights of plants at maturity (P=0.131) (Figure 2.5A). There were no significant
interaction between defoliation and insecticide with respect to RWW densities or height (P=0.070; P=0.940).

Figure 2.5. Effects of pre-flood defoliation (MECH), rice water weevil (RWW) injury (WEEVIL), and the combination of weevil injury and defoliation (BOTH) on densities of rice water weevil (RWW) larvae and pupae after flooding. Core samples to estimate population densities of RWW after flooding were taken approximately one month after flooding. Significant differences between means of control (CON), RWW infested (WEEVIL), defoliated (MECH), and RWW infested and defoliated (BOTH) tolerance experiments in 2016 are shown for experiment one (A) and experiment two (B). Bars accompanied by different letters represent means that differed significantly with Tukey’s HSD test at α<0.05

There was no significant effect of defoliation on yield (P=0.152). However, there were significant main effects of insecticide (weevil exclusion) on both plant height and yield (P=0.028; P=0.000), although the Tukey comparison did not show significant
differences in height between CON and WEEVIL treatments (Figure 2.6A). Mean yields in plots in which RWW feeding occurred were significantly lower than yields from plots with no RWW injury (Figure 2.7A). There was a significant interaction of defoliation and insecticide on yield (P=0.016) (Figure 2.7A). This interaction indicates that effects of defoliation were different in the presence and absence of the insecticidal seed treatment. In the presence of the insecticide there was a significant decrease in yield from defoliation (comparing CON and MECH treatments), however, this decrease in yield was not observed in the absence of the insecticide (comparing WEEVIL and BOTH treatments).

Figure 2.6. Effects of pre-flood defoliation (MECH), rice water weevil (RWW) injury (WEEVIL), and the combination of RWW injury and defoliation (BOTH) on average plant height. Height was measured when plants reached 100% heading. Significant differences between means of control (CON), RWW infested (WEEVIL), defoliated
(MECH), and RWW infested and defoliated (BOTH) tolerance experiments in 2016 are shown for experiment one (A) and experiment two (B). Bars accompanied by different letters represent means that differed significantly with Tukey’s HSD test at $\alpha<0.05$

![Bar chart for experiment one (A) showing average yield in kg/plot for different treatments: CON, MECH, WEEVIL, BOTH. Bars accompanied by different letters represent means that differed significantly with Tukey’s HSD test.]

![Bar chart for experiment two (B) showing average yield in kg/plot for different treatments: CON, MECH, WEEVIL, BOTH. Bars accompanied by different letters represent means that differed significantly with Fisher’s LSD test.]

Figure 2.7. Effects of pre-flood defoliation (MECH), weevil injury (WEEVIL), and the combination of RWW injury and defoliation (BOTH) on average yield in tolerance experiments are shown for experiment one (A) and experiment two (B) in 2016. Yield was measured at harvest and adjusted for moisture content. Treatments are as follows control (CON), RWW infested (WEEVIL), defoliated (MECH), and weevil infested and defoliated (BOTH). Bars accompanied by different letters represent means that differed significantly with Fisher’s LSD test at $\alpha<0.05$

In the second experiment of 2016, insecticidal seed treatments reduced RWW densities by approximately 82% when comparing treatments CON and WEEVIL ($P<0.001$), again showing the treatment had its intended effect (Figure 2.5B). Defoliation
did not significantly affect RWW densities (P=0.352) (Figure 2.5B). There was no significant interaction of defoliation and insecticide treatment on RWW densities (P=0.906).

As in the previous two experiments, RWW injury had a greater impact on rice growth and yield than defoliation. The main effect of insecticidal seed treatment on plant height and yield were significant (P=0.004; P<0.001). Again, Tukey comparison showed no significant differences between CON and WEEVIL treatments (Figure 2.6B); Mean yields in plots where RWW feeding occurred were 21.5% lower than controls (Figure 2.7B). Defoliation did not affect plant height at maturity or yield (P=0.716; P=0.120) (Figure 2.6B, and 2.7B), and there were no significant interactions between defoliation and insecticide treatment on height or yield (P=0.470; P=0.506).

Discussion

A total of six experiments were conducted over two growing seasons to test the hypothesis that fall armyworm injury to aboveground portions of rice plants before flooding increases resistance of rice to infestation of roots by RWW larvae after flooding. In 2015, the overall reduction in RWW densities in fall armyworm defoliated plots relative to control plots was 41%, but results varied markedly over the three experiments, with strong reductions in RWW densities in fall armyworm defoliated plots in the first and third experiments (average of 48% reduction in defoliated plots) but no significant reductions in the second experiment. The reasons for this inconsistency among experiments in 2015 are not known, but overall densities of RWW larvae in control plots were approximately 81% lower in the second experiment compared to the first and third experiments. Additionally, plants in the second experiment were older at the time of
infestation with fall armyworm, and the level of defoliation resulting from fall armyworm infestation was lower than in the first and third experiments. Inducibility in plants often decreases with plant age (Kwok & Laird 2012). Young sagebrush plants have been shown, for example, to be better emitters and receivers of plant volatiles after herbivory (Shiojiri & Karban 2006). Also, there is evidence that strength of induction increases with level of defoliation. In soybean, *Glycine max*, strength of induction increased significantly with level of defoliation by Mexican bean beetle larvae (Underwood 2000). In a previous study in rice with fall armyworm and RWW conducted in a greenhouse, only severe defoliation by fall armyworm was found to affect densities and larval weights of RWW on roots (Tindall & Stout 2001).

In contrast to the 2015 experiments, pre-flood defoliation had no significant effects on post-flood infestations of RWW in three experiments conducted in 2016. However, overall densities in fall armyworm defoliated plots were numerically lower in all three experiments. Mean densities of RWW larvae per treatment over the three experiments were 7% to 24% higher in control plots than in plots subject to mechanical defoliation, fall armyworm defoliation, or both types of defoliation. Many factors may have contributed to this year-to-year variation in response to pre-flood defoliation. These factors include planting date, plant age at defoliation or infestation by RWW larvae, level of defoliation, soil type, weather, and rice variety. Many of these factors are not as important under greenhouse conditions, which may explain why the effects of fall armyworm defoliation on RWW were consistently stronger in previous greenhouse experiments (Hamm et al. 2010) than in these field studies.
Another factor potentially contributing to variation in results among years is variation in salivary factors from the fall armyworm. Acevedo et al. (2018) showed that levels of the salivary enzyme phospholipase C are likely responsible for variation in levels of defense induction by different strains of fall armyworm in grasses (Acevedo et al. 2018). The strain of fall armyworm used to infest plots was not determined in either 2015 or 2016, but it is possible that addition of field-collected insects to the colony after experiments were completed in 2015 changed the genetic composition of the colony (and hence the composition of salivary elicitors and effectors possessed by the larvae).

Finally, differences in seasonal RWW densities may also partly explain differences in results between 2015 and 2016. Densities of RWW larvae in control plots were 56% higher in 2016 than in 2015. High density infestations are capable of masking low levels of resistance (Smith 2005), and it is possible that the higher densities of RWW present in 2016 compromised our ability to detect the effect of prior injury on RWW densities.

The mechanisms by which defoliation leads to reduced infestation of rice roots by RWW are not known. Removal of oviposition sites in defoliated plants is, however, an unlikely explanation. RWW adults oviposit in rice leaf sheaths below the water surface (Stout et al. 2002). Mechanical defoliation is as likely as fall armyworm defoliation to affect these oviposition sites, and significant reductions in RWW larval densities were never observed after mechanical defoliation. It is more likely that fall armyworm injury triggered biochemical changes in young rice plants that reduced their suitability as sites for RWW oviposition or hosts for larval survival. Feeding by chewing herbivores is known to induce the jasmonic acid (JA) pathway in rice (Zhou et al. 2009), and
exogenous JA is known to induce resistance to chewing insects in rice (Hamm et al. 2010).

Over the three tolerance experiments, defoliation alone reduced rice yields by approximately 6%. RWW feeding alone, in contrast, reduced yields by an average of approximately 18.5% over all three experiments. This suggests that while rice can often compensate for defoliation, it cannot do the same when faced with root injury by RWW, and that rice is more tolerant of defoliation than root injury. Similarly, Lupinus arboreus was found to be less tolerant of below-ground herbivory by Hepialus californicus than aboveground injury by Orgyia vetusta (Maron 2018). Most interesting, however, were the data on yield losses from plots subjected to combinations of root injury and defoliation (BOTH). Overall yields in this treatment group were the lowest of all treatments in two of the three experiments. Over all three experiments, the combination of RWW feeding and defoliation reduced yields by approximately 25% when compared to controls. This suggests that combinations of aboveground and belowground injury can have greater than additive effects on yield. This aggravated yield loss may have been due to an inability of plants to allocate resources simultaneously to injured leaves and stems and to roots injured by RWW larvae.

The inconsistency in results among experiments in this study highlights the need to conduct experiments under field conditions, where many factors influence the expression of resistance and resistance-related traits, and not just under more controlled conditions in the laboratory or greenhouse. Despite the variability in results, however, these experiments demonstrate that herbivory by fall armyworm on rice may indirectly affect RWW in rice fields in the southeastern United States via changes in rice resistance.
and tolerance: defoliation by fall armyworm may increase the resistance of rice to subsequent infestation by RWW, and defoliation may reduce the tolerance of rice to subsequent RWW injury. Relatively few prior studies have demonstrated indirect interactions under circumstances representative of actual field conditions in commercial agricultural fields. Erb et al. (2011) demonstrated that infestation of maize plants by fall armyworm reduced the colonization and emergence of Diabrotica virgifera virgifera, which, like RWW, is a root feeder. However, this reduction only occurred when fall armyworm arrived on corn plants before D. virgifera (Erb et al. 2011). In another study, parasitism of Ostrinia nubilalis, the European corn borer, was reduced by 98% in maize fields infested with corn rootworm. The authors suggest reduction in plant size and density by rootworms was responsible for the reduction in parasitism (White & Andow 2006).

The importance of indirect interactions in structuring communities of insects on plants is currently a topic of great interest (Stam et al. 2018). This topic has potentially important implications for IPM, for example, when management of an early season pest changes the likelihood or severity of a pest infestation later in the season. Current recommendations call for insecticide treatment for fall armyworm in Louisiana rice when fall armyworm densities exceed one fall armyworm per two seedlings (Saichuk et al. 2014). Pantoja and Smith (1986) observed significant reductions in yield components and yields when levels of defoliation exceeded 33%. The latter results are consistent with the results of our studies, in which removal of approximately one-third of plant biomass in the tolerance experiments resulted in significant loses in yield in one of three experiments. The relatively small and inconsistent increases in resistance to RWW in fall
armyworm defoliated plots probably do not warrant changes to existing thresholds. However, the potential for reduced tolerance to RWW injury in plots previously defoliated by fall armyworm suggests that farmers who do not use measures to manage RWW may need to be more vigilant in their monitoring of both RWW and fall armyworm populations to avoid high potential yield losses associated with the combination of feeding from both species.

References


Chapter 3.

Belowground Larvae of the Rice Water Weevil, Lissorhoptrus oryzophilus, Induce Resistance to Conspecific Adults in Rice, Oryza sativa

Introduction

Plants often respond to attack by herbivores by expressing or producing resistance-related biochemical and morphological traits at higher levels. These responses can occur rapidly within the host plant. In maize, for example, green leaf volatiles were released from leaves immediately after injury by Spodoptera exigua, and sesquiterpenoids were released within four hours of injury (Turlings et al. 1998). Similarly, responses in rice can be very rapid: Hu et al. (2018) observed changes in expression of several genes within 15 to 30 minutes of injury by Chilo suppressalis. Moreover, plant responses can persist for a period of time after the inducing injury and can extend spatially well beyond the injured tissue. Gomez et al. (2010), for example, found that reduction in leaf palatability, induced by Mamestra brassicae in Trifolium repens lasted at least 28 days after initial injury and reductions in palatability were observed in uninjured portions of injured plants. This latter study also noted a time lag between localized and systemic responses in T. repens. Systemic responses to wounding occurred within three days of injury (51 hours), but local responses were more rapid (Gómez et al. 2010). Thus, plant responses near the site of injury may be evident almost immediately but changes in other portions of the plant may not occur until later.

The spatial and temporal dynamics of plant responses to herbivore attack make a wide range of plant-mediated interactions among herbivores on a shared host plant possible. Extremely rapid responses may affect the initial (inducing) attacker as well as
other herbivores feeding on the plant contemporaneously. Inbar et al. (1999) reported that whiteflies, *Bemisia argentifolii*, negatively affected leaf miners, *Liriomyza trifolii*, when the two herbivores fed simultaneously on the same tomato (*Solanum lycopersicon*) plant. The two herbivores interacted directly via exploitation interactions and indirectly via plant-mediated mechanisms. The plant-mediated interactions were likely due to the induction of pathogenesis related proteins by *Bemisia argentifolii* (Inbar et al. 1999). Plant-mediated interactions may also occur between temporally separated herbivores on a shared host plant. Neonates of lepidopteran species frequently contend with plant responses induced during feeding by earlier cohorts of their own species. This was shown to be true of fall armyworm, *Spodoptera frugiperda* (J.E. Smith), on cowpea seedlings. The induced responses negatively affected fall armyworm neonates that fed subsequently on the plant. The dispersing neonates preferred plants which had been damaged four hours previously to plants which had been damaged only one hour before placement of eggs (Carroll et al. 2008).

Extremely rapid induction of defenses may serve as a negative feedback mechanism to reduce the fitness of the attacking herbivore and to limit the size of populations of an herbivore on a plant. An elegant demonstration of this was recently made by Perkins et al. (2013). By carefully monitoring insect movement and spatial patterns of plant chemical responses in *Arabidopsis thaliana*, these authors found that larvae of the generalist moth *Helicoverpa armigera* adjusted their short-term behavior on plants by moving away from induced parts of plants and toward uninduced parts of plants. However, herbivore injury does not always result in induced resistance, and there are many examples of herbivore attack resulting in attraction of, or increased oviposition by,
conspecific insects (Dicke & van Loon 2000). Larvae of the autumnal moth, *Epirrita autumnata* Borkh, for example, induce rapid responses in mountain birch, *Betula pubescens ssp. czerepanovii* (Orlova H’amet-Ahti), that make the injured birch more susceptible to conspecific larvae (Lempa et al. 2004). Therefore, there is a diverse array of induced responses noted across families of plants and the insects they interact with, and this necessitates the investigation of plant-mediated interactions across a diverse array of systems.

A special case of plant-mediated interactions among herbivores involves interactions of root-feeding herbivores with herbivores that feed on above-ground portions of plants. There are dozens of examples in the literature (Erb et al. 2008). Belowground herbivory causes physiological changes in the aboveground portions of plants, for example via the production of toxins, which may directly affect aboveground herbivores, or the production of volatiles which may attract natural enemies and thereby indirectly affect aboveground herbivores (Bezemer & Van Dam 2005). For example, root-feeding larvae of the root fly, *Delia radicum*, induced resistance in *Brassica nigra* L. to the foliage-feeding larvae of the cabbage butterfly, *Pieris brassicae*. The developmental time of the caterpillar was slowed, increasing exposure time to predators. The effect on the caterpillar was likely due to higher glucosinolate levels in the plant (Soler et al. 2005). Another study showed that feeding by western corn rootworm, *Diabrotica virgifera virgifera*, on the roots of maize, *Zea mays*, induced increases in expression of defense-related genes in maize leaves. The resulting compound, 2,4-dihydroxy-7-methoxy-1,4-benzoazin-3-one (DIMBOA), then acted as a feeding deterrent to leaf-feeding larvae of *Spodoptera littoralis* (Erb et al. 2009).
Fewer studies have been conducted using herbivores that feed on both roots and aboveground portions of the same plant at different life stages. One such study using the tree species *Triadica sebifera* showed that root feeding by larvae of *Bikasha collaris* increased feeding by conspecific adults on aboveground portions of plants, but decreased feeding by heterospecific adults of several lepidopteran, coleopteran, and heteropteran species (Huang et al. 2014). However, as stated previously, induced plant responses can be highly variable across species. Therefore, there is further need to investigate indirect effects of belowground herbivores on their aboveground counterparts when multiple stages of an herbivore share the same host plant.

The interaction between rice, *Oryza sativa* L., and the rice water weevil (RWW), *Lissorhoptrus oryzophilus* Kuschel, an important pest of rice globally, provides an appropriate system to investigate plant-mediated interactions among herbivores. In particular, both adults and larvae of RWW feed on young rice plants, but they feed on different plant organs. Adults feed on leaves creating longitudinal scars that do not significantly affect yields at harvest (Lupi et al. 2009). Eggs are laid within leaf sheaths by females after plants are flooded. Larvae feed on roots of flooded rice plants, where they pass through four larval instars and a pupal stage in approximately 30 days (Zou et al. 2003). The ecology and population dynamics of this herbivore are such that different cohorts of weevils may infest rice plants over an extended period of time after flooding, and both larval and adult stages may attack a plant at the same time. Therefore, it is possible belowground-aboveground rice-mediated interactions exist between life stages of this insect.
The objective of this study was to investigate whether prior feeding by rice water weevils on rice plants, either feeding by adults on leaves or feeding by larvae on roots, induces resistance to subsequent infestation by the rice water weevil. An effect of adult feeding on subsequent oviposition by adult females would require only a local, relatively short-lived response; the hypothesis for this experiment was that aboveground adult feeding would induce resistance to adults that were subsequently introduced to the plant, and that oviposition by these adults would be reduced when compared to undamaged plants. Additionally, it was hypothesized there would be an effect of variety on RWW preference. An effect of larval feeding on adult oviposition, on the other hand, would require an aboveground-belowground interaction between two life stages of a species; the hypothesis for this objective was that the larval feeding would indeed induce a systemic response resulting in decreased oviposition by adults introduced to larval-infested plants. This work is novel as little is known concerning indirect interactions in this species, *Lissorhoptrus oryzophilus* and important as it provides information on intraspecific competition.

**Materials and Methods**

**Effects of Adult Feeding on Plant Resistance to Subsequent Oviposition**

Two experiments were conducted, one in 2017 and one in 2018, to test the hypothesis that prior feeding by adult RWW on leaves of young rice plants increases the resistance of plants to oviposition by subsequent female RWW. Plants for these experiments were grown under greenhouse conditions in 1.9 L plastic pots (15.25 cm diameter, Atlantis Hydroponics, Atlanta, Georgia). Plants were grown in a commercial soil mix, ShowScape TopSoil (Phillips Bark, Brookhaven, MS). These experiments were performed with two commercial varieties, ‘Jefferson’ and ‘Jupiter’, in 2017 and three
varieties, ‘Jefferson’, ‘Jupiter’, and ‘Cheniere’, in 2018. ‘Jefferson’ and ‘Cheniere’ are long grain varieties whilst ‘Jupiter’ is a medium grain variety. Previous studies have shown ‘Jefferson’ to have low levels of resistance to RWW with densities 7-70% lower than other commercial varieties (Saad et al. 2018). Conversely, the variety ‘Jupiter’ was shown to be susceptible (higher RWW densities compared to most commercial varieties under field condition) (Saad et al. 2018). These are commonly used varieties in Louisiana. Pots were thinned to two plants per pot after three weeks of growth and fertilized with 0.5g Osmocote® (14-14-14 NPK, Marysville, OH). Plants possessed four leaves when used for experiments.

The procedure for establishing groups of plants with different levels of adult injury (leaf scarring) involved confining adult male weevils to rice seedlings under unflooded conditions and allowing adults to feed on leaves for 48 hours. Adult RWW were collected from field plots at the H. Rouse Caffey Rice Research Station Crowley, Louisiana, and maintained in large plastic containers with rice leaves and water. Males were culled from dishes by separating mating pairs *in copula*. Males were confined to plants using cylindrical plastic tubes (8.5 cm in diameter by 23 cm in height) with one end forced into the soil and the other end covered with a mesh lid. Cages also had two mesh-covered holes for air circulation. Both plants growing in the pot were enclosed in the cage. Cages were infested with male RWW at densities of zero, two, and six insects per cage, resulting in six treatments (variety by injury combinations) in 2017 (Jupiter 0, Jupiter 2, Jupiter 6, Jefferson 0, Jefferson 2, Jefferson 6) and nine treatments in 2018 (Jupiter 0, Jupiter 2, Jupiter 6, Jefferson 0, Jefferson 2, Jefferson 6, Cheniere 0, Cheniere 2, Cheniere 6). Cages were removed after two days of adult feeding and adults were
destroyed. The total number of feeding scars found on the two plants in each pot was recorded to ensure caging adults on plants had achieved different levels of injury.

The procedure for comparing the resistance of adult-injured and control plants has been described by (Stout et al. 2002). Briefly, pots with rice seedlings were placed into large mesh infestation cages. Infestation cages were constructed of cylindrical frames made of wire (46 cm in diameter and 61 cm in height) covered with fine mesh screening. Infestation cages with plants were placed in basins lined with heavy plastic pond liner to retain floodwater. One pot of each treatment was placed in each cage. Once cages were flooded (two days after initial infestation with adult males) they were infested with adult RWW mating pairs at a density of three weevils per plant. Basins were flooded so that the lower five cm of each plant were submerged. Adults were allowed to feed, mate, and oviposit for five days, during which time insects had free access to plants of different varieties and adult injury levels. A randomized complete block design was employed. In the experiment in 2017 eight blocks (cages) with one replicate of each treatment (variety and density level combination) were used (six pots per cage). In the experiment in 2018 eight blocks (cages) with one replicate of each treatment (variety and treatment level combination) were used (nine pots per cage). Pots were removed from cages at the end of the infestation period and remaining insects destroyed.

In order to obtain an estimation of oviposition preference in each density-variety combination, plants were gently removed from soil and soil was rinsed from roots. Plants, including roots, were inserted into test tubes filled with water, arranged on test tube racks, and maintained in an insect rearing room for the remaining duration of the experiment. Rearing room conditions were 16:8 L:D, 28.3°C, and 70% relative humidity.
Weevils infesting plants treated in this way eclose from eggs and, after feeding within shoots for a short time, move down to roots. Emergence of first instars from eggs was monitored daily. This was done by vigorously shaking the plant in the test tubes and emptying the water, and any emerged larvae, into a petri dish, where larvae were counted. Counting continued for ten days or until larvae had not emerged for two days in a row.

**Effects of Larval Feeding (belowground) on Resistance to Oviposition by RWW Adults (Aboveground)**

Three experiments were conducted, one in 2016 and two in 2018, to test the hypothesis that prior feeding by larvae of RWW on roots of young rice plants under flooded conditions increases the resistance of plants to oviposition by subsequent female RWW adults. Plants were grown under conditions similar to those described earlier with two exceptions. First, plants were grown in soil taken from fields at the H. Rouse Caffey Rice Research Station and soil was sterilized in an autoclave but not fertilized. Second, only a single variety, ‘Cheniere’ was used. After three weeks of growth, rice seedlings were thinned to one per pot and each plant was infested with 15 rice water weevil larvae, a combination of ten early instars and five late instars. Larvae were collected from infested rice plants by removing entire rice plants from the soil and agitating their roots in water-filled buckets. As larvae were dislodged from roots they floated to the surface and were collected into 30 ml diet cups. Larvae were transferred to roots of potted plants in the greenhouse by saturating soil in pots, making a small indentation with a finger near the base of plants, and placing larvae into the indentation to provide easy access to roots. Larvae were rinsed with a water bottle from diet cups onto the surface of the soil near the indentation and larvae generally burrowed into soil within minutes of transferal. Pots were flooded and larvae were allowed to feed on roots for one week.
After a week of larval infestation, two controls (uninfested) and two larvae-infested pots were placed into mesh infestation cages (previously described). Cages were placed in basins lined with black plastic pond liner and were flooded such that the lower five cm of each plant were submerged. Three adult RWW per plant of mixed sex were added to cages. The adults were allowed to feed, mate, and oviposit four days in 2016 and five days in 2018. Pots were then removed from cages, any insects found were destroyed, and plants were removed from pots and all soil rinsed from roots into mesh-screened buckets. The number of RWW larvae recovered in buckets from the initial infestation was recorded. Additionally, plants which were not included in the second infestation were washed and dried for measures of root and shoot biomass. Plants were then placed into test tubes and emergence of first instars from plants was counted as previously described.

**Statistical Analysis**

All analyses were performed in SAS version 9.4 (SAS Institute). Means, standard deviations, and standard errors were determined in PROC MEANS. The Poisson distribution and log link were used for all count data. Means were separated using Tukey’s HSD means separation test (P<.05). PROC GLIMMIX was used to analyze all count and continuous data as described below.

Treatment effects were analyzed by generalized linear mixed model using PROC GLIMMIX, with block as a random effect and fixed effects variety, density level, and their interaction. Means were separated using Tukey’s HSD means separation test (P ≤ .05). Simple linear regressions (SLR) using PROC REG were performed to reveal the
nature of relationships between amounts of scarring in each variety and the density of RWW immatures.

For experiments testing the hypothesis larval induced resistance to adult RWW a randomized complete block design was also employed. In 2016 eight blocks and three replicates of each treatment (Control and Weevil) within each block were used. In the two experiments in 2018 nine and seven blocks were used respectively, with two replicates of each treatment (Control and Weevil) within each block were used in the analyses. Cages where little or no oviposition was noted were excluded from analysis. For these experiments, treatment effects were analyzed by generalized linear mixed model, with block as a random effect and weevil infestation as a fixed effect.

**Results**

**Effects of Adult Feeding on Plant Resistance to Subsequent Oviposition**

No scars were observed on plants not exposed to adult males. For the plants exposed to two adult males per pot, an average of 14.0 ± 2.8 scars were found per two plants, while the plants exposed to six adult males per pot had an average of 45.4 ± 4.4 scars per two plants.

The number of first instars emerging from plants was not affected by densities of adults (P=0.160). However, analysis revealed a significant main effect of variety and a significant interaction between adult density level and variety (P=0.009; P<0.001) (Figure 3.1). This significance was attributable to differences in densities of RWW immatures recovered from the Jup6 and Jeff0 treatments as well as the Jeff2 and Jeff6 treatments. A SLR investigating the relationship between the number of feeding scars resulting from initial infestation and the number of RWW first instars recovered from test
tubes showed no significant relationship for either Jefferson (P=0.294) or Jupiter (P=0.241) (data not shown).

Figure 3.1. Effects of initial infestation by adult rice water weevil (RWW) on densities of RWW immatures resulting from a second infestation with adults. Significant differences between means of combinations of variety and initial infestation level are shown. Different letters indicate significant differences between control and weevil infested means with Tukey’s HSD test at α<0.05.

For the plants exposed to two adult males per pot, an average of 15.2 ± 1.0 scars were found per two plants, while the plants exposed to six adult males per pot had an average of 48.0 ± 3.0 scars per two plants.

The analysis of numbers of first instars emerging from plants in test tubes showed significant main effects of adult infestation treatment and variety, as well as a significant
interaction between these two main effects (P<0.001; P<0.001; P<0.001) (Figure 3.2). Densities of first instars were significantly lower in treatment Chen6 than all other combinations, and significantly higher in treatment Jup0 than all other treatment combinations. The simple linear regression relating the number of scars to the densities of first instars showed a significant negative relationship for the variety Jupiter (P=0.017) (Figure 3.3) but not for the other varieties (Cheniere P=.203; Jefferson P=0.502; data not shown).

Figure 3.2. Effects of initial infestation by rice water weevil (RWW) adults on densities of RWW immatures resulting from a second infestation by adults. Significant differences between means of all combinations of variety and initial infestation level are shown. Different letters indicate significant differences between control and weevil infested means with Tukey’s HSD test at α<.05.
Figure 3.3. Simple linear regression between the amount of scars on plants in a pot and the densities of RWW immatures recovered from that pot, in variety Jupiter. There was a significant relationship which shows a decrease in the densities of RWW immatures as the amount of scarring increases.

**Effects of Larval Feeding (belowground) on Resistance to Oviposition by RWW Adults (Aboveground)**

In the 2016 experiment, direct infestation of rice seedlings with rice water weevil larvae produced groups of plants with marked differences in root injury: plants not infested with larvae (control) had no larvae on their roots, whereas plants that had been infested had an average of 5.7 ± 1.7 surviving larvae. Both root and shoot biomass were significantly lower in infested pots than in controls (P=0.001; P<0.001) (Figure 3.4).
Approximately 80% fewer first instars emerged from previously infested plants than from control plants (P<0.001) (Figure 3.5).

Figure 3.4. Effects of feeding by rice water weevil (RWW) larvae on biomass of roots and shoots of plants in each pot. Significant differences between means of control and weevil infested pots are shown. Different letters indicate significant differences between control and weevil infested means with Tukey’s HSD test at α<.05.
Figure 3.5. Effects of prior feeding by larvae of the rice water weevil (RWW) on emergence of RWW immatures from plants subsequently infested with adult weevils. Significant differences in the mean numbers of immature larvae emerging from previously infested and non-infested plants were observed. Different letters indicate significant differences between control and weevil infested means with Tukey’s HSD test at α<.05.

In the first experiment in 2018, direct infestation of rice seedlings with rice water weevil larvae produced two groups of plants with marked difference in root injury: the control had no larvae on their roots, whereas plants that had been infested had an average of 6.4 ± 1.0 surviving larvae. The presence of larvae on plants significantly reduced biomass of both roots, but did not significantly affect shoots (P=0.049; P=0.219) (Figure 3.6). When adult weevils were given free access to plants infested or not infested with weevil larvae, they showed a clear preference for ovipositing on control plants.
Approximately 56% fewer first instars emerged from previously infested plants than from control plants (P<.0001) (Figure 3.7).

Figure 3.6. Effects of initial Rice Water Weevil (RWW) larvae feeding, on biomass of roots and shoots of plants in each pot. For the larvae induced resistance hypothesis, biomass from RWW larvae infested and control pots were measured. Significant differences between means of control and weevil infested pots are shown. Different letters indicate significant differences between control and weevil infested means with Tukey’s HSD test at α<.050
Figure 3.7. Effects of prior feeding by larvae of the rice water weevil (RWW), on subsequently infested RWW adults. Significant differences in the mean numbers of immature larvae emerging from previously infested (Weevil) and non-infested (Control) plants were observed. Different letters indicate significant differences between control and weevil infested means with Tukey’s HSD test at α<.05.

In the second experiment in 2018, direct infestation of rice seedlings with rice water weevil larvae produced two groups of plants with marked difference in root injury: control plants had no larvae on their roots, whereas plants that had been infested with 15 larvae had an average of 3.5 ± 0.6 surviving larvae. Biomass data from two control pots and two RWW infested pots showed no significant difference between biomass of roots or shoots (P=.5644; P=.5601). When adult weevils were given free access to plants infested or not infested with weevil larvae no significant difference in larval emergence was observed between previously infested and non-infested pots (P=0.2181). A mean of
6.3 ± 1.5 first instars emerged from control plants, whereas 5.1 ± 1.2 emerged from previously infested plants.

**Discussion**

The experiments reported here provide strong support for the hypothesis that feeding by RWW larvae on roots of rice plants induces systemic resistance to adults of the RWW ovipositing on aboveground portions of the plant, while providing somewhat weaker support for induction of localized resistance by adult RWW against oviposition by subsequent RWW adults. Induction of systemic resistance by RWW larvae agrees with a large body of literature showing belowground injury to induce strong systemic responses which affect aboveground herbivores (Erb et al. 2008). It also supports previous research carried out in rice showing that aboveground chewing herbivores are capable of inducing resistance to herbivores separated in space or time from the inducing herbivore (Tindall & Stout 2001). Feeding by larvae of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), reduced oviposition by RWW on fall armyworm injured plants (Hamm et al. 2010). Similarly, in another study, prior feeding by late-instar fall armyworm induced direct resistance to subsequent conspecific larvae, with larvae fed on plant foliage from injured plants showing overall reductions in growth rate when compared to larvae fed on undamaged plant tissue (Stout et al. 2009). The results in this latter study were weak and variable, which concurs with the results presented here for the experiments involving induction of resistance to RWW oviposition by adult RWW feeding.

Consistent differences in weevil larvae among treatments demonstrate the utility of our infestation procedures. In each of these cases plants in the density level six groups showed significantly lower RWW immature densities than plants in the density level zero
(control) groups. In the second year in rice variety Jupiter, there was a significant relationship which showed densities of RWW immatures decreased with an increasing level of adult feeding scars. While slight differences in the varieties across years are seen, there are no cases in which plants in the density level six groups received significantly higher oviposition than the density level zero (control) groups. This supports the concept of induced resistance, but also suggests that RWW adult feeding is not a strong enough stressor to consistently trigger induced responses.

In experiments that tested effects of belowground larval injury on aboveground adults, successful larval infestations were achieved in two of three experiments. There was an average of six recovered larvae per pot in each of these experiments. This level of infestation resulted in a significant reduction of root biomass in both experiments and a significant reduction of shoot biomass in one experiment. RWW immature densities recovered from injured plants were significantly lower than from plants which were not injured by previous larval feeding. This effect was not seen in the third experiment where larval infestations were not as successful and the number of larvae recovered from the initial infestation was approximately three. Here there was no significant difference seen in root biomass or shoot biomass, and no significant differences were noted in RWW immature densities between injured and uninjured plants. to the failure of root injury to induce resistance against subsequent oviposition by adults may have been a consequence of the need to reach a threshold of injury before defenses are induced (Underwood 2000).

The effect of belowground injury on aboveground herbivores is one area of the literature in which large arrays of responses have been noted, although in many cases belowground feeding has been shown to induce resistance to aboveground foliage feeders
his group of experiments showed that the former is true in the case of rice plant-mediated interactions and the larval and adult stages of RWW.

Further research to elucidate the underlying mechanisms of induced resistance in rice will be required in order to better understand plant-insect interactions in this system. There is evidence that the mechanism is dependent on the jasmonic acid cascade. This is supported by the Hamm et al. (2010) study which also tested applications of exogenous JA for effects on direct induced resistance to RWW. Results showed as much as a 66% reduction in oviposition and an 85% reduction in larval densities in greenhouse experiments. Field trials also resulted in decreased densities of RWW larvae when foliar applications of JA were made in rice prior to flooding. Reductions in oviposition may be due to increased production of toxins in the plant, or due to repellent volatile organic compounds. Volatiles induced by fall armyworm in rice that result in indirect defenses have been identified (Yuan et al. 2008), however, volatiles which result in direct induced resistance, and changes in volatile quantity and composition after injury from RWW have not been identified. Continued investigation in this area would result in increased understanding of induced responses and plant-mediated interactions.

References


Carroll, M.J., Schmelz, E.A. and Teal, P.E.A., 2008. The attraction of Spodoptera frugiperda neonates to cowpea seedlings is mediated by volatiles induced by


Chapter 4.

Seed Treatments using Methyl Jasmonate Induce Resistance to Rice Water Weevil, Lissorhoptrus oryzophilus, and Alter Plant Growth

Introduction

The phytohormone jasmonic acid (JA) has been identified as the primary hormonal mediator of plant responses to insect attack. Perception of herbivore feeding by the plant triggers the rapid accumulation of JA at the site of attack. The biosynthesis of JA from its precursor linolenic acid, through various active intermediates, and culminating in its conjugation with isoleucine (Ile) to form the receptor-active JA-Ile, has been characterized (Campos et al. 2014, Lortzing & Steppuhn 2016). The interaction of JA-Ile with its receptor complex in the nucleus results in a comprehensive transcriptional reorganization in the plant. Changes in gene expression result, in turn, in the local and systemic production of defense-related metabolites by the plant, that cause increases in direct and indirect resistance to insect herbivores (Campos et al. 2014, Dar et al. 2015, Lortzing & Steppuhn 2016). The well-established role of JA in induced plant defense has led to the suggestion that the JA pathway could be manipulated to protect crops from their insect pests (Baldwin 1998; Thaler 1999; Hass et al. 2018).

One way of manipulating the JA pathway in plants is to apply JA or one of its derivatives to plants. Various derivatives and formulations of JA have been tested across an array of plant families to investigate potential uses in integrated pest management (IPM). This includes a series of studies involving foliar applications of JA and methyl jasmonate (MJ, a methylated derivative of JA) in tomato, Solanum lycopersicum (Thaler 1999; Thaler et al. 1996). These studies found that treatment of plants with MJ rendered plants less suitable to insects such as Spodoptera exigua, likely due to an increased
production of phytochemicals resulting from the foliar applications. JA applications were also shown to increase parasitism of caterpillar pests in the field (Thaler 1999). Another study utilized JA seed treatments on cabbage, *Brassica oleracea*, and showed induced resistance to *Plutella xylostella* L. manifested by reduced larval growth rates, and reduced rates of intrinsic growth of *Myzus persicae* populations (Haas et al. 2018). Additionally, a greenhouse study using seed treatments of MJ and JA on several crops including tomato, cowpea, soybean, and wheat, showed a reduction in nematode infection in cowpea and tomato plants (Pankaj et al. 2013). In this study, the germination and plant growth parameters of tomato and wheat were not affected. However, cowpea showed delayed germination and reduced shoot mass, while soybean seeds showed reduced germination. These results show that the JA pathway is highly conserved across plant families and that exogenous jasmonates elicit defense to diverse arthropod and nematode challenges. Potential benefits of elicitors in integrated pest management programs include reductions in pest feeding injury, promotion of natural biological control by induction of volatile organic compounds, reduced environmental contamination, and ease of use, as current water seeding techniques may be applied.

It is important to note, that the benefits of exogenous jasmonate may be counterbalanced by costs, such as reductions in plant growth and fitness, associated with upregulation of the jasmonic acid cascade. For example, drenching roots of native populations of tobacco, *Nicotiana attenuata*, with MJ induced resistance to *Trimerotropis pallidipennis*, the pallid-winged grasshopper. This was likely due to large increases in the production of nicotine, though the plants also suffered a fitness cost of significantly reduced seed production (Baldwin 1998). In another study tomato treated with foliar
applications of JA induced resistance to *Manduca sexta*, but the cost of these defenses resulted in approximately 35% fewer fruits (Redman et al. 2001). While there has been progress in understanding the biochemical crosstalk and other mechanisms responsible for tradeoffs between growth and defense in plants (Guo et al. 2018), there is still much to be learned in this area to determine whether the benefits of jasmonate application to crop plants outweigh the potential costs.

One aspect of herbivore or elicitor-induced resistance for which little information is available, involves the duration of induced resistance after the initial induction (Cipollini Jr. 1997; Haas et al. 2018; Gómez et al. 2010). Induced responses in the herb *Trifolium repens* decayed 28 days after induction by *Mamestra brassicae* larval feeding (Gómez et al. 2010). Another study involving marine algae showed induced responses were sustained for as long as 38 days following mechanical injury (Hemmi et al. 2004). The idea that induced defenses decay over time is plausible, because the most widely accepted hypothesis for the evolution of induced resistance is preservation of plant resources and reduction of fitness costs associated with producing defenses (Heil & Baldwin 2002). In this sense the ability of a plant to upregulate and downregulate defenses would be very advantageous. In the absence of continued injury, and thus the absence of the herbivore associated molecular patterns that trigger JA production, the plant is able to reverse production of plant secondary metabolites. This reduction in defensive compounds may be expected to result in a decay of induced resistance to subsequent herbivores.

In order to determine whether the benefits of utilizing elicitors in pest management outweigh the costs in an agricultural system, investigations must be made
into specific crop-insect interactions under realistic (field) conditions. Rice in the southern U.S. is attacked by an array of herbivore pests, the most economically damaging of which is the rice water weevil (RWW), *Lissorhoptrus oryzophilus*. Adults of this species chiefly infest early season rice, and oviposition occurs primarily under flooded conditions (Stout 2002). As a consequence, larvae which feed on the roots of flooded rice plants, are present in high numbers peaking four to five weeks after flooding, and begin to decrease thereafter (Shang et al. 2004). Feeding by larvae on the roots of flooded plants reduces root biomass and ultimately causes yield loss. Yield losses can exceed 20% under heavy pressure (Stout 2002). Therefore, the ability to manage early season infestations would be very beneficial to growers in order to prevent economic losses. Therefore, protection during this early season period is vital. Use of elicitors could greatly reduce the use of the insecticidal seed treatments and foliar insecticides that are currently utilized to manage the pest. Additionally, seed treatment options are currently not available for organic growers, and would also provide a method for water seeding growers to take advantage of this technology.

The objectives of this study were to determine if treating seeds with MJ stimulated the resistance of water-seeded rice to the RWW under field conditions; and, if so, to assess how long the effect lasted and whether the benefits of seed treatment were counterbalanced by simultaneous costs of MJ treatments to plant growth and yield. It was hypothesized, based on prior work in this system that treatment of seeds with MJ would induce defenses to RWW, resulting in reduced larval densities on treated plants in the field. Additionally, it was hypothesized that there would be a tradeoff between plant defense and growth, represented by delays or reductions in rice plant emergence and
heading, and reductions in plant biomass and yield. Finally, it was hypothesized that the effects of MJ would decay over time, resulting in increasing densities of RWW and decreased effects on plant growth over time.

**Materials and Methods**

**Study Site and Rice Culture**

In greenhouse experiments rice seeds were sown in a greenhouse in a soil mix (2:1:1, soil: peat moss: sand) in 15 cm diameter pots at the rate of five seeds/pot. The rice variety ‘Cheniere’, a long-grain conventional inbred variety, was used in all experiments. Seed treatments are described below. Plants were maintained in the greenhouse under standard conditions with ambient lightening at approximately 29.0°C -33.0°C. Plants were watered regularly in order to maintain adequate soil moisture. Plants in pots were thinned to two plants per plot approximately two weeks after planting.

Field experiments were conducted at the Louisiana State University Agricultural Center H. Rouse Caffey Rice Research Station (30.23142°N and -92.379583°W, 7m asl). The soil type at this site is a Crowley silt loam with a pH of 7.1 and 12% organic matter. Fields at this site have been in a two-year rice-fallow rotation for over 30 years. Greenhouse experiments were conducted at a greenhouse on the campus of Louisiana State University.

Rice in field experiments was water seeded. Water seeding involves soaking dry seeds in water for approximately 24 hours, removing seeds from water for another 24 hours to allow germination to begin, and then casting partially germinated seed into flooded fields or plots. Generally, once seeds are cast into a field, flood waters are removed to allow seedlings to establish, and permanent flood is established within a few
weeks of seeding. The seeding rate for field experiments was the equivalent of 200 kg/ha.

**Greenhouse Experiment**

The duration of resistance induced by three concentrations of MJ (0, 2.5 mM, and 5.0 mM) were investigated in a greenhouse experiment. To treat seeds, 160 g of seed were soaked in 250 ml of 0, 2.5 mM, and 5.0 mM solutions of MJ in glass flasks. The solutions were prepared by mixing 250 mL of distilled water, 250 μL of Tween 20 (0.1% v:v) (Polyoxyethylene sorbitan monolaurate, Bio-Rad laboratories, Inc.) and Methyl Jasmonate (Sigma-Aldrich Co.) as needed to achieve the desired final concentration. Flasks containing seeds and treatment solutions were shaken for 24 hours on the laboratory bench at room temperature.

Adult rice water weevils were collected from rice fields at the LSU AgCenter H. Rouse Caffey Rice Research Station in Crowley, Acadia Parish, Louisiana, one day prior to use in experiments. Weevils were maintained from collection until use in plastic containers with water and rice leaves. Infestation cages, which were constructed of cylindrical wire frames (46 cm diam, 61 cm tall) covered with a fine mesh screening, were placed in wooden basins lined with heavy black plastic that allowed plants to be flooded. One pot from each of the three treatments was placed in each of the cages. Separate plants were infested at two time points, fifteen days after planting (early three-leaf stage), and thirty days after planting. For infestations at both plant stages adult weevils were released in cages at a density of four weevils per plant. Basins were flooded to a depth of approximately 24 cm, and weevils were allowed to feed, mate, and oviposit on plants in cages for five days. The design was a completely randomized block design.
in which cages were blocks and individual pots were replicates. There were ten blocks at
the first time point and twelve at the second with one replicate of each treatment in each
block.

Plants were removed from cages and any weevils found on plants were removed.
The densities of first instars emerging from plants were determined by removing one
plant from each pot, carefully washing soil from the roots, and suspending individual
plants in labeled test tubes containing distilled water. Test tubes were arranged on a rack
and placed in a growth chamber (28°C, 14:10 hr L:D). Weevils infesting plants treated in
this manner hatched from eggs, emerged from leaf sheaths, and settled on the bottom of
test tubes. First instars were counted by shaking roots free of larvae, and then pouring
water from test tubes into a Petri dish. Dishes were visually inspected for larvae. 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
recovered for two days in a row.

**Field Experiments**

Two field experiments were conducted, one in the 2017 growing season and one
in the 2018 growing season, to determine the effects of MJ seed treatment on densities of
RWW larvae and rice growth and grain yield. Treatments were factorial combinations of
MJ seed treatment and insecticide application. The control treatment, “Control”,
consisted of plots seeded with rice not treated with MJ or insecticide. For the second
treatment, “Karate”, plots were not treated with MJ but were treated with repeated
applications of a pyrethroid insecticide, lambda-cyhalothrin, within 2 weeks of flooding.
This resulted in plots with reduced populations of RWW, but which were not treated with
MJ. For the third treatment, “MJ”, the plots were sown with rice seeds treated with MJ two days prior but were not treated with insecticide. This resulted in plots in which the effects of MJ on densities of RWW and rice growth and yield could be measured. In the fourth treatment, “Both”, plots were sown with rice treated with MJ and treated with repeated applications of a foliar a pyrethroid insecticide. This resulted in plots in which the effects of MJ on growth and yield could be assessed under reduced RWW pressure.

Field experiments employed a randomized complete block design with four blocks and a full factorial of the main effects of MJ seed treatment, insecticide application, and their interaction in each block.

Seeds were treated with MJ by soaking 160 g of ‘Cheniere’ seeds in the following solutions. Seeds for the “Control” and “Karate” treatments were soaked in 250mL of water and 250 μL of 0.1% Tween 20 (Polyoxyethyle sorbitant monolaurate, Bio-Rad laboratories, Inc.). Seeds for the “MJ” and “Both” treatments were soaked in 250mL water, 250 μL of Tween 20, and 140 μL of Methyl Jasmonate (Sigma-Aldrich Co.) resulting in a final concentration of MJ of 2.5mM. Rice seeds were water seeded into plots by hand two days after being treated with MJ solutions.

Insecticide applications were made using the pyrethroid lambda-cyhalothrin at a rate of .06 kg AI/ha. Foliar applications began one day before flooding and were repeated every four days until a total of five applications were applied. Insecticide was applied using a backpack sprayer pressurized with CO₂ and calibrated to deliver 140 L/ha at 207 kPa through four 1002 flat fan nozzles TeeJet® at 51 cm spacing.

Emergence of seeds was assessed prior to flooding in both 2017 and 2018. In the 2017 experiment, emergence of rice plants was assessed at 14 and 17 days after seeding
by counting plants in one random 0.09 m² quadrat in each plot. No pyrethroid applications had been made at this point, and for this reason only the effects of MJ seed treatment were analyzed. In the 2018 experiment, emergence of rice plants was again assessed by taking stand counts at 12 and 22 days after seeding. No pyrethroid applications had been made at this point, and hence the effect of Karate applications was not analyzed. Emergence was assessed by randomly placing within a plot a wire square with an area of 0.09 m², and counting all emerged plants within the square. One count was conducted for each plot on each of two dates, approximately two weeks and three weeks after seeding.

In 2017 plots were permanently flooded three weeks after seeding, but in 2018, due to cool temperatures, rice could not be flooded until nearly six weeks after seeding. All field experiments relied upon natural infestations of RWW, which are consistently present at high population densities at the experimental site. The procedure used to determine densities of RWW larvae and pupae in plots involve removing root/soil core samples from each plot. Each plot was core sampled on five separate dates in 2017, and on two separate dates in 2018. Sampling was performed from one to five weeks post-flooding. The tool used for removing soil/root cores was a metal cylinder with a diameter of 9.2 cm and a depth of 7.6 cm attached to a handle. This core sampler allowed for equally sized samples of soil and rice roots to be collected. Core samples were rinsed with water under moderate pressure through a metal screen bucket in order to remove soil and dislodge RWW larvae from roots. The bucket was then placed in salt water and floating larvae and pupae were counted (Stout et al. 2002). In the 2018 experiment plots were not flooded until six weeks after seeding. Core samplings were taken 18 and 28
days after flooding to assess effects of MJ, Karate, and their interaction on densities of RWW.

Plant growth was also assessed before and after flooding in both 2017 and 2018. Biomass of roots and shoots were determined by removing whole plants from plots by hand. Plant material was rinsed thoroughly, and plant material was then dried in an oven. Roots and shoots were separated from one another and mass was determined using an electronic scale. Plants for biomass were collected on five separate dates in 2017 and two separate dates in 2018. The first sample was taken within four days of flooding and subsequent samples on the week after. The 2018 experiment was sampled at 35 days and 49 days after seeding to measure root and shoot biomass.

Heading (panicle exertion from the flag leaf (Saichuk et al. 2014)) was visually assessed in each plot on three separate dates in 2017 and two separate dates in 2018. At grain maturity, five individual panicles were collected from each plot. The panicles were threshed, and the weight of grains from the five panicles averaged for a measure of mean panicle grain mass. The first assessment was taken when panicle emergence was noted in control plots, and subsequently three days to one week later. Per panicle and per plant grain mass were also measured. Per panicle grain mass was assessed by collecting five individual panicles from each plot. These panicles were threshed and weighed individually. The masses of all five panicles were then averaged, and the resulting average panicle grain mass was used in analysis. Per plant grain mass was assessed by collecting five plants from each plot. The number of panicles per plant was recorded, and the total grain mass of all panicles on one plant was determined. Dates of all agricultural practices and date of when samples were taken are in Supplemental Table 4.1.
Statistical Analysis

All analyses were performed in SAS software version 9.4 (SAS Institute 2013). Means, standard deviations, and standard errors were determined in PROC MEANS. Poisson distribution and log link were used for all count data. All continuous data were tested for normality with PROC UNIVARIATE and the Shapiro-Wilk test (P<.05). PROC GLIMMIX was used to analyze all count and continuous data as described below.

For the greenhouse experiment investigating the effect of MJ seed treatment on resistance to RWW, effects of MJ seed treatment were analyzed separately for the two plant ages. For this experiment, treatment effects were analyzed by generalized linear mixed models, with MJ seed treatment level as the fixed effect. Means were tested for significant differences using Tukey’s HSD (P < 0.05).

Data from core samples, emergence counts, biomass samples, heading, and harvest mass were analyzed separately. Treatment effects were analyzed by generalized linear mixed models using PROC GLIMMIX, with block as a random effect and MJ seed treatment, insecticide application, and their interaction as fixed effects. Repeated measures were performed in PROC MIXED with fixed effects MJ seed treatment, insecticide application, their interaction, and time as fixed effects. Means were separated using Tukey’s HSD means separation test (P < .05).

Results

Greenhouse experiments

Greenhouse experiments testing the effect and duration of MJ seed treatments on RWW densities show that MJ significantly reduced RWW densities at both high and low concentrations, but that this effect decayed over time. At the first sampling date, 15 days post seed treatment, both low and high concentrations of MJ significantly reduced RWW
densities (P<0.001) (Figure 4.1A). At the second sampling date, 29 days post seed treatment, only the low concentration significantly reduced RWW densities (P=0.005) (Figure 4.1B). However, the reduction in RWW densities in the low concentration (LOW) from the control (CON), at the second sampling date is only half of the reduction seen at the first sampling date.

Figure 4.1. Effects three concentrations of Methyl Jasmonate seed treatments (MJ) on RWW densities in the greenhouse experiment performed in 2018. Means in RWW densities in un-treated (CON), low concentrations 2.5mM (LOW), and High concentration 5mM (HIGH) treatments are shown. Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at α<.05.
Field experiments

There were significantly fewer plants emerged in plots with MJ treated seeds (MJ) than in plots with non-treated seeds (CONTROL) on both the first sampling date (P<0.001) (Figure 4.1A) and the second sampling date (P<0.001) (Figure 4.1B), indicating that seedling emergence was either reduced or delayed by MJ treatment.

Figure 4.2. Effects of treatment of rice seeds with methyl jasmonate (MJ) at a concentration of 2.5 mM, on numbers of rice seedlings emerged in a 0.09 m² area (number of plants ± SE) and non-treated (Control) plots at 14 days after seeding (A), and 17 days after seeding (B) are shown. Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at α<.05.

Root and shoot biomass was measured on five separate dates in the 2017 experiment. Overall, treatment of seeds with MJ reduced both root and shoot biomasses
of rice seedlings, although the effects were not significant on all sampling dates. On the first sampling date (one day after flooding), treatment of seeds with MJ reduced shoot biomass by 34% compared to controls (Table 4.2). There was also a significant interaction of MJ and insecticide on root biomass, although insecticide had only been applied three days prior. On the second sampling date, the main effect of MJ on both root and shoot biomass was significant (Table 4.1); root and shoot biomass was 13% and 49% lower, respectively, in MJ-treated plants than in control plants. On the third sampling date, the main effects of both MJ and Karate on both root and shoot biomass were significant (Table 4.1). At this time point, plants in MJ-treated plots exhibited 26% and 9% reductions in root and shoot biomass, respectively, when compared to controls. Plants in Karate-treated plots and Karate and MJ-treated plots, in contrast, had root and shoot biomasses that were 26% and 4% higher, respectively, than controls. On the fourth sampling date (21 days after flooding) the main effect of MJ on root and shoot biomass was again significant (Table 4.1). At this time plants in MJ treated plots showed 26% and 27% reductions in root and shoot biomass, respectively, compared to controls. At the fifth date there were no significant differences in root or shoot biomass across the treatments (Figure 4.3A and 4.3B).
Table 4.1. Effects of factorial combinations of methyl jasmonate seed treatments (MJ) and insecticide applications (Karate) on rice plant root and shoot biomasses were measured on five separate dates in the experiment performed in 2017. P-values are shown for main effects MJ, Karate, and their interaction on root and shoot biomasses. Where means of plant root and shoot biomass differed significantly, as determined by Tukey’s HSD test at $\alpha<.050$, an asterisk is placed aside the p-value.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Days After Seeding</th>
<th>Days After Flooding</th>
<th>P-Values for Effects on Root Biomass</th>
<th>P-Values for Effects on Shoot Biomass</th>
</tr>
</thead>
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<tr>
<td></td>
<td>MJ</td>
<td>Karate</td>
<td>Interaction</td>
<td>MJ</td>
</tr>
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<td>1</td>
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<td>0.002*</td>
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<td>0.001*</td>
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<td>0.039*</td>
</tr>
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<td>21</td>
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<td>0.020*</td>
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<tr>
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<td>28</td>
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<td>0.438</td>
</tr>
</tbody>
</table>

Figure 4.3. Effects of factorial combinations of methyl jasmonate seed treatment (MJ) and insecticide applications (Karate) on root and shoot biomass of rice plant seedlings. Root and shoot biomasses were measured on five separate dates in the experiment performed in 2017. Average root (A) and shoot (B) masses (grams) in of plants in each
treatment (MJ–treated (MJ), insecticide treated (Karate), seed treatments and insecticide applications (Both), and non-treated plots (Control)), on each date are shown.

The 2017 experiment was core sampled to determine densities of rice water weevil on five dates. A repeated measures analysis of the data over all five sampling dates showed that MJ seed treatments significantly reduced RWW densities (P=0.034). Karate applications also significantly reduced RWW densities (P<.0001), but the interaction of Karate and MJ was not significant (P=0.120). The effect of time was significant (P<0.001), indicating RWW densities changed over the season.

On the first sampling date, there were no significant differences among treatments in RWW densities. The second, third, and fourth core sampling dates showed no main effect of MJ, but Karate significantly reduced RWW densities at each time point (P<0.05). On the fourth core sampling date, the interaction of MJ and Karate was significant (P=0.005). At this time point (21 days after flooding), comparisons of each treatment mean with the control treatment mean showed RWW densities were reduced by 45% in MJ-treated plots, by 64% in plots treated with both MJ and Karate, and by 70% in Karate plots. At the fifth core sampling date, the main effects of both Karate (P=0.001) and MJ (P=0.021) were significant, but the interaction was not significant. At the time of the fifth core sampling (28 days after flooding) average RWW densities in plots which received both MJ and Karate (Both) were nearly 50% lower than RWW densities in control plots, whereas MJ and Karate alone only reduced densities by 14% or 25%, respectively (Figure 4.4; Table2). Moreover, a more comprehensive repeated-measures analysis incorporating data from all sampling dates showed a significant overall effect of
MJ seed treatments. This effect resulted in an approximately 30% overall reduction of RWW immatures relative to controls.

Figure 4.4. Effects of factorial combinations of methyl jasmonate seed treatments (MJ) and insecticide application (Karate) on densities of RWW larvae. RWW densities were determined on five separate dates after flooding in the experiment in 2017. Values shown are larvae and pupae per core sample for each treatment, MJ–treated (MJ), insecticide treated (Karate), seed treatments and insecticide applications (Both), and non-treated plots (Control).

Heading was estimated visually on three dates in the 2017 season (81, 83, and 88 days after seeding), and at each time point there were significant differences among treatments in densities of panicles (panicles per plot). At the first sampling date, heading was significantly lower in MJ-treated plots (P=0.001), but neither the effect of Karate (P=0.172) or the interaction of MJ and Karate was significant (P=0.962) (Figure 4.5). At
the second sampling date, heading was again significantly lower in MJ-treated plots (P<0.001), and a significant main effect of Karate was also observed (P=0.006), with Karate-treated plots having significantly higher densities of panicles than plots in all other treatments. No significant interaction was observed (P=0.474) (data not shown). At the third sampling date, there were significant effects of MJ (P<0.001), Karate (P<0.001), and their interaction (P<0.001) (Figure 4.5).

Figure 4.5. Effects of factorial combinations of methyl jasmonate seed treatment (MJ) and application of insecticide (Karate) on heading (number of panicles per plot ±S.E.) 81 and 88 days after seeding in the experiment performed in 2017. Treatments include MJ–treated (MJ), insecticide treated (Karate), seed treatments and insecticide applications (Both), and non-treated plots (Control). Means are shown for only the the first sample date (A) and the third sample date (B). Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at α<.05.
Results showed no main effect of MJ (P=0.578) on mean panicle grain mass, and there was no significant interaction of MJ and Karate (P=0.666). However, mean grain mass was significantly higher in Karate-treated plots (P=0.001), (Figure 4.6).

Figure 4.6. Effects of factorial combinations of Methyl Jasmonate seed treatments (MJ) and insecticidal applications (Karate) on panicle grain mass in the experiment performed in 2017. Mean panicle masses for MJ–treated (MJ), insecticide-treated (Karate), insecticide and MJ-treated (Both), and non-treated plots (Control) are shown. Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at α<.050.

Emergence was significantly lower in MJ-treated plots at 12 days after planting (P<0.001) (Figure 4.7A), and at 22 days after planting (P<0.001) (Figure 4.7B).
Figure 4.7. Effects of treatment of rice seeds with methyl jasmonate (MJ) at a concentration of 2.5 mM on numbers of rice seedlings emerged in a 0.09m$^2$ (number of plants ±SE) area 12 days after seeding (A) and 22 days after seeding (B). Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at $\alpha<.05$.

Both root and shoot biomasses were significantly reduced by MJ treatment at 35 days after seeding ($P=0.012; \ P=0.002$) (Figure 4.8A and 4.8B) (at this time no Karate applications had been made). At the second sample dates, 49 days after seeding, there had been four applications of insecticide made. There were no significant effects of treatments (MJ and Karate) or their interaction on root or shoot biomass (Root $P=0.742; \ P=0.632; \ P=0.088$) (Shoot $P=0.974; \ P=0.926; \ P=0.161$).
Figure 4.8. Effects of treatment of rice seeds with methyl jasmonate (MJ) at a concentration of 2.5mM on rice root (A) and shoot biomass (B) five weeks after seeding in the experiment performed in 2018. Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at α<.05.

At the first sampling date, no RWW larvae were found in core samples. At the second sampling date, densities of RWW larvae and pupae were significantly lower in Karate-treated plots than in control plots (P<0.001), but neither MJ nor the interaction of MJ and Karate had a significant effect on RWW densities (P=0.656; P=0.439) (Figure 4.9).
Figure 4.9. Effects of factorial combinations of methyl jasmonate seed treatments (MJ) and insecticide applications (Karate) on RWW densities (immature RWW per plot ±SE) in the experiment performed in 2018. Means in RWW densities for treatments MJ–treated (MJ), Insecticide-treated (Karate), MJ and insecticide treated (Both), and non-treated plots (Control) are shown at 28 days after flooding. Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at α<.05.

Heading was estimated on two separate dates, 101 and 108 days after seeding. At the first sampling, percent plants heading was significantly lower in MJ-treated plots, (P<0.001), but no effects of Karate or the interaction of MJ and Karate were observed (P=0.592; P=1.00). Similar results were recorded at the second sampling date, with MJ-treated plots showing significantly lower heading (P<0.001), but no significant effects of Karate or the MJ by Karate interaction (P=0.383; P=0.448) (Figure 4.10).
Figure 4.10. Effects of factorial combinations of methyl jasmonate seed treatments (MJ) and insecticide applications (Karate) on heading 101 and 108 days after seeding in the experiment performed in 2018. Means in panicle densities in MJ–treated (MJ), Insecticide treated (Karate), seed treatments and insecticide applications (Both), and non-treated plots (Control), on the first sample date (A) and the second sample date (B) are shown. Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at α<.05.

There were no significant differences in panicle density per plant (P=0.789; P=0.817; P=0.904). There were significant main effects of MJ seed treatment and Karate on panicle grain mass (P=0.024; P=0.020). Plots receiving MJ seed treatments had significantly reduced per panicle mass when compared to Karate-treated plots. The interaction was not significant (P=0.633) (Figure 4.11).
Figure 4.11. Effects of factorial combinations of methyl jasmonate seed treatments (MJ) and insecticide applications (Karate) on panicle grain mass at harvest in the experiment performed in 2018. Means in panicle mass in MJ–treated (MJ), Insecticide treated (Karate), seed treatments and insecticide applications (Both), and non-treated plots (Control) are shown. Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at α<.050.

Total panicle mass per plant was also assessed for five plants in each plot. Interestingly, when grain mass per plant was compared across treatments, yield per plant was not affected by any treatment (P=0.512; P=0.456; P=0.991) (Figure 4.12).
Figure 4.12. Effects of factorial combinations of methyl jasmonate seed treatments (MJ) and insecticidal applications (Karate) on plant grain mass at harvest in the experiment performed in 2018. Means in whole plant grain densities in MJ–treated (MJ), Insecticide treated (Karate), seed treatments and insecticide applications (Both), and non-treated plots (Control) are shown. Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at $\alpha<.05$.

Discussion

This is the first study to document MJ induced resistance to rice pest under field conditions. The effects of jasmonic acid and its derivatives on crop plants and their associated herbivores is of interest to scientists and IPM practitioners interested in the development of sustainable pest management solutions. While the effects of JA on plant resistance to insects has been widely studied under relatively controlled conditions (e.g. greenhouse conditions), fewer studies have evaluated the effectiveness of jasmonates as a
management tool under field conditions. This study represents the first attempt to test a field-scale seed treatment of a JA derivative for its potential to protect rice against its insect pests. Based on previous work performed in the greenhouse (Stout unpublished) it was hypothesized that seed treatments of methyl jasmonate (MJ) would induce resistance in rice to the RWW, that the induced effects would alter plant growth, and that the effects of MJ would decay as time from seed treatment increased. The results of this study strongly support each of these hypotheses.

Treating seeds with MJ induced resistance to RWW as evidenced by consistently lower densities of RWW larvae when compared to non-treated plots in both the field and greenhouse. Although only one core sampling in 2017 showed a significant effect of MJ on densities of RWW larvae, in all core samplings the densities of RWW immatures in MJ treated plots were numerically lower than the controls. Results in 2017 were very important as they were the first-field level experiments in rice testing MJ seed treatments as a pest management tool.

The results in 2018 differed somewhat from results in 2017. No significant differences in RWW densities due to seed treatments with MJ were noted. Plant growth in this season was delayed due to cold weather, which created a subsequent delay in flooding. This increased the amount of time between the seed treatments and RWW oviposition. It is likely that the effects of induced resistance in this experiment had decayed by the time RWW were ovipositing in the field. This is supported by our greenhouse experiments. In rice, MJ induced resistance decayed to a non-significant level by 30 days post-seed treatment. Most studies in this area look at induced responses immediately or nearly immediately following a stressor. There is little in the literature
about the duration of responses and this is vital to IPM. This is an important contribution to show that these responses in rice will not be maintained throughout the season and speak to the hypotheses regarding tradeoffs of growth and defense and the evolution of induced resistance (Heil & Baldwin 2002).

It is important to note that Karate treatments were more effective at controlling the pest, but the combination of MJ and Karate can produce a synergistic effect such as the one seen in the fifth core sample of the 2017 experiment where RWW densities in plots which received both MJ and Karate (Both) were nearly 50% lower than RWW densities in control plots, whereas either alone only reduced densities by 14% (MJ) or 25%. Important to note from an IPM perspective, is the fact that plots in the Karate treatment received four to five applications of the insecticide, whereas plots in the MJ treatment received a single application.

The results from both 2017 and 2018 field experiments also strongly support our hypothesis that activating plant resistance would incur costs in the plants. MJ treatment significantly delayed or reduced germination in both 2017 and 2018. MJ treatment also reduced root and shoot mass in the early season, though the plants recovered to a non-significant level later in the season. Heading was significantly delayed in the experiments when compared to non MJ-treated plots (Control and Karate), which is likely an extension of the delay in emergence. It is important to note effects on plant growth in the 2018 field experiment may be confounded with unusually cool spring temperatures which likely exaggerated these effects.

Despite the strong effects on plant growth, yields were not significantly reduced when per plant yield of MJ treated plots were compared to the control; however, plots
which received insecticide treatments (Karate) had significantly higher yields than MJ treated plots. In 2018 whole plant yields were taken and there was no significant effect of any treatment at this level. It is vital to the future of IPM programs that we understand these tradeoffs in order to develop long-term natural solutions for rice pest management.

References


Chapter 5.

Direct and Indirect Effects of Herbicides on Insect Herbivores in Rice, Oryza sativa

Introduction

Practices used to manage weeds in agricultural fields may have unintended effects on insect communities (Hill et al. 2012). Norris & Kogan (2000) reviewed the various interactions that occur between weeds, arthropod pests, and their natural enemies. Their work suggests that interactions between weeds and arthropods can be direct or indirect, can involve trophic cascades, and can be affected by agricultural management practices. Management practices can affect interactions between weeds and arthropods by directly affecting either type of pest, or by altering aspects of crop growth (Smith 1968). Any factor, such as competition with other plants that alters crop growth will affect all species associated with that crop. Norris & Kogan (2000) provide dozens of examples of resource and habitat-driven interactions between weeds and insects, including effects of weed management practices such as tillage and herbicide use.

Chemical herbicides are the most common practice for controlling weeds in agriculture. There are at least three routes by which herbicides may impact insect communities. First, herbicide applications cause direct mortality of insects present in the field at the time of application. Alternatively, changes in insect communities may occur indirectly via herbicide-induced resistance in the agricultural crop. This is possible as herbicides can act as a stressor to crop plants, and may increase expression of defense-related genes, which can in turn alter densities of pest populations on the crop. Finally, herbicides by their intended design alter the composition of plant communities, and due to the fact that weeds serve as alternate hosts for many crop pests, their removal may
have an effect on the density and composition of insect communities. Examples of all three routes are found in the scientific literature and several examples are described below.

Herbicides have previously been shown to cause mortality in insects. For example, it was shown that the herbicide AAnetos L can cause significant mortality to Collembola at low doses (Ahn et al. 2001). Both the active ingredient (2,4,5-T isooctylester) and inert ingredients (formulation) of the herbicide were found to be toxic to the hexapods via direct contact (Eijsackers 1978). The herbicide glufosinate-ammonium was highly toxic to nymphs and adults of the mite species Amblyseius womersleyi Schicha, Phytoseiulus persimilis Athias-Henriot, and Tetranychus urticae (Ahn et al. 2001). Imazethapyr and quizalofop ethyl, active ingredients in the herbicides Pursuit 10EC and Tergasuper 5EC, respectively, were found to significantly reduce survival of Spilarctia obliqua larvae (Gupta & Bhattacharya 2008). The same study showed that 2,4-D was non-toxic to the insect, a finding further supported by Haag (1986). In this latter study, exposure to 2,4-D, diquat, and glyphosate had no effect on survival of the water hyacinth weevils Neochetina eichhorniae and N. bruchi (Haag 1986). However, a more recent study showed that herbicides with 2,4-D and diquat as active ingredients negatively affected survival of both Neochetina eichhorniae (Warner) and Eccritotarsus catarinensis (Carvalho) (Hill et al. 2012). Mortality was as high as 80% for the latter, higher than mortality caused by herbicides containing glyphosate for both insects. This study also noted that surfactants included in formulations increased insect mortality. The diversity of results in the literature suggests that the effects of herbicides on insect mortality are dependent upon active ingredient, formulation, and insect species.
Examples of indirect effects of herbicides are also found in the literature. Herbicide-induced resistance has been shown in at least one report involving 2, 4-dichlorophenoxyacetic acid, which was shown to induce resistance in rice to the striped stem borer, *Chilo suppressalis* (Xin et al. 2012). This study provided evidence that a defensive response triggered by the herbicide and mediated by jasmonic acid and ethylene, resulted in an increase in volatile production and an increase in activities of trypsin proteinase inhibitor. Plants treated with 2,4-D exhibited stronger attraction of *Anagrus nilaparvate*, a parasitoid, and supported lower growth rates of *C. suppressalis* (Xin et al. 2012). In a second study, effects of dicamba on two insect species were investigated. The herbicide was shown to be non-toxic when directly applied to *Helicoverpa zea* or when insects fed on dicamba-treated soybean. However, there were significant negative indirect effects on *Vanessa cardui* larvae which had fed on thistle treated with the herbicide. Larvae fed dicamba-treated leaves had significantly lower larval and pupal masses compared to controls (Bohnenblust et al. 2013). The herbicide application also resulted in reduced plant growth, which may have resulted in limitations in food availability for larvae. An additional example of herbicide-induced resistance involves the herbicide S-ethyldipropylthiocarbamate (EPTC), which was shown to induce resistance in cabbage (*Brassica oleracea* L.) to three insect pests including diamondback moth, *Plutella xylostella* (L.), imported cabbage worm, *Pieris rapae* (L.), and cabbage looper, *Trichoplusia ni* (Hbn.). The herbicide induced a glossy phenotype which reduced feeding by first instars of all three species (Eigenbrode et al. 1993).

Finally, altering the community of weed pests in an agroecosystem can affect insect communities by eliminating alternate hosts for insect pests. Conversely, poor weed
control can lead to poor insect control by providing additional food sources for pests. Herbicide drift from 2,4-D and dicamba have been noted to have an effect on plant and arthropod communities. Field-based experiments carried out over multiple years showed that dicamba caused a decline in forb cover as well as several pest herbivores. Increases in one pest species and one beneficial species were observed (Egan et al. 2014).

The rice agricultural system in the southern United States is well-suited to study these types of interactions. Multiple weed species are present in commercial fields in the southern U.S., including red rice (Oryza sativa L. var sylvatica) (Echinochloa crus-galli) and barnyard grass, which can reduce rice yields by as much as 80% (Webster et al. 2018). Herbicides are applied from before crop emergence to mid-season to manage weed pests as needed, but the majority of herbicide is applied early season, prior to permanent flood. Red rice has long been the primary driver of weed management practices in rice production, and was initially controlled by water management. This changed in 2002 with the introduction of Clearfield® technology for controlling red rice with the use of rice varieties tolerant to Newpath® herbicide (Webster et al. 2018).

Rice agroecosystems in this region experience infestations of insect pests at all rice growth stages, and these herbivores could be affected by herbicides via the three described routes. The major pests in most fields in the southern U.S. are the rice water weevil (RWW), Lissorhoptrus oryzophilus Kuschel, a complex of stemborers, and rice stink bug, Oebalus pugnax (Fabricius). Each of these pests specializes on grasses. The RWW is an early season pest present at the time herbicides are applied and may be directly exposed. Herbicides applied early in the growing season may indirectly affect insect pest densities in rice via induced resistance. These induced effects are likely to
have the most effect on early to mid-season pests like RWW. The alteration of plant communities by herbicides, however, could affect insect pests throughout the entire rice growing season, including mid to late season pests such as the stem borers and rice stink bug.

This study has several objectives. The first objective was to determine if any of seven herbicides commonly used in southern U.S. rice affected RWW mortality via direct exposure, or via feeding assays on herbicide-treated leaf material. The second objective was to determine if these herbicides induced resistance in the rice plant, thereby reducing subsequent pest densities of RWW, stem-boring lepidopterans, or rice stink bug. The third objective was to determine if reductions in density of weeds and alterations in the weed community due to herbicide application altered densities of insect pests. The current study is one of a few that has investigated direct and indirect effects simultaneously.

**Methods and Methods**

**Study Site and Rice Culture**

All experiments were conducted at the Louisiana State University Agricultural Center H. Rouse Caffey Rice Research Station (30.231422°N and -92.379583°W, 7m asl). The soil type at this site is Crowley silt loam with a pH of 7.1 and 12% organic matter. Fields at this site have been in a two year rice-fallow rotation for over 30 years. Rice in all experiments was drill-seeded in plots measuring 1.3 m by 5.5 m with 7 rows of rice at 17.8 cm spacing. Seeding rate was 67 kg/ha. The rice variety ‘CL111’ was used in all experiments. ‘CL111’ is an imidazolinone tolerant long-grain inbred variety developed by the rice breeding program at the H. Rouse Caffey Rice Research Station (Oard et al. 2013). Plots were fertilized with 134kg/ha N applied pre-flood and 67kg/ha P
and K applied in the previous fall. Fertilization and disease control practices followed recommendations for drill-seeded rice in southwest Louisiana (Saichuck et al. 2015). Dates of all planting, fertilization, and flooding activities can be found in Supplemental Table 5.1. Plots were harvested at grain maturity with a mechanical combine and yield was adjusted to 12% moisture.

Adults are present in rice throughout the season, but are more numerous in early season rice (Shang, H. et al. 2004), whereas larvae are only present in high numbers post-flooding, due to the ovipositional behavior of adults (Stout et al. 2002). Larval feeding reduces plant tillering and shoot growth, as well as root biomass. This feeding activity overall reduces panicle densities and grain harvest weights (Zou et al., 2004).

Rice in the southern U.S. is also host to several stem-boring species. The insects in this complex include the sugarcane borer, *Diatraea saccharalis* (Fabricius), the rice stalk borer, *Chilo plejadellus* (Zink), and the Mexican rice borer, *Eoreuma loftini* (Dyar). These insects are present in fields throughout the rice growing season, but they appear to primarily infest mid-tillering and reproductive stages of rice. They may be affected directly by herbicides, but due to the timing of their infestation are more likely to be affected by herbicide-induced effects or by alterations in the weed community. Larval feeding can be economically damaging in multiple rice stages. This damage occurs as the larvae bore into and tunnel through the rice stems. In the reproductive stage, larval injury may cause whiteheads, (panicles with primarily unfilled grains) and overall yield loss (LV et al. 2008).

Rice stink bug is native to North America where it is a pest of wheat, sorghum, and rice (Henry & Froeschener, 1988). This insect enters rice late in the season after rice
has begun heading. It may be affected by changes in the weed community resulting from herbicide applications, but is unlikely to be directly affected by herbivores or by herbicide-induced resistance, as it does not invade fields until long after herbicide applications. Adults and late instar nymphs cause damage to rice by negatively affecting rice yield and the quality of grains (Espino et al. 2007; Awuni et al. 2015; Brown 2003).

**RWW Mortality Experiments**

In order to determine if herbicide treatments had direct effect on RWW survival, adult RWW were exposed by direct topical application and by feeding larvae on leaves treated with herbicides. For both experiments, herbicides were applied at the same rates as field trials (see below). To test the effects of topical exposure, four petri dishes for each herbicide, and four for the control, each containing ten field-collected RWW adults, were placed on the ground and lids removed. Dishes containing weevils were sprayed with herbicides using the same backpack sprayer used in field trials. Mortality was assessed 24-hours after spraying as described below.

To test the effects of a combination of contact and oral exposure to herbicides, weevils were fed on herbicide-treated leaf material immediately after treating plants with herbicides. This was done by spraying one field plot by backpack sprayer with each herbicide at the same rate used in field trials. Rice foliage from these plots was immediately collected by cutting from the plant with scissors and placed in a petri dish for adult feeding. Approximately 20 leaf segments were placed in each dish to ensure no starvation would occur. Ten RWW adults were then added to each dish, with four dishes for each herbicide treatment. The petri dishes were moved into a controlled environment with 16:8 L:D, 22°C, 70% RH until mortality was assessed.
For experiments using both methods of exposure, mortality was assessed one day after herbicide application. Weevils in each petri dish were observed for 15-30 minutes. RWW adults that did not display coordinated movement within that time were removed from the dish, and recorded as dead (Miller et al. 2010).

**Herbicide Treatments**

Herbicides in small-plot field experiments were applied one day prior to flooding, after plants reached the four-leaf stage. This timing of applications does not align with standard timing for weed management practices in rice, which generally include a pre-emergence application followed by one or more post-flood applications at or near the time of flooding. However, this timing of applications was employed to standardize treatment applications, and facilitate comparison of their effects on insects. All herbicides were applied at their highest label rate. Information concerning trade names, active ingredients, modes of action, rates, and typical timing for applications of each of these herbicides is provided in Supplemental Table 5.1. Herbicides were applied using a backpack sprayer pressurized with CO₂ and calibrated to deliver 140 L/ha at 207 kPa through four 1002 flat fan nozzles TeeJet® at 51 cm spacing. Herbicide treatments used in the 2015 experiment included ‘Weed Rhap A-4D’ (2,4-D), ‘Command 3ME’ (Command), ‘Newpath’ (Newpath), ‘Ricebeaux’ (Ricebeaux), and an unsprayed control (Control). These herbicides each have different modes of action. It was hypothesized that different modes of action would show differential effects on weevil mortality and defense induction. Additionally, use of five different herbicides was meant to create variation in levels of weed control.
Treatments in 2016 included all herbicides used in 2015. In addition to these herbicides, the herbicides ‘Propanil 4SC’ (Propanil) and ‘Bolero 8EC’ (Bolero) were also used. The active ingredients in these herbicides are both present in Ricebeaux; therefore, in order to determine if either alone was responsible for the effects of Ricebeaux observed in 2015, both were used in 2016 in addition to Ricebeaux. Information regarding dates of application and flooding can be found in Supplemental Table 5.2.

**Insect Sampling**

All experiments relied on natural infestations of the insects under study. RWW is consistently present at high population densities at the experimental site. The procedure used to determine densities of RWW larvae and pupae in plots involved removing root/soil core samples from each plot at two or more time points from two to six weeks after flooding. The tool used for removing soil/root cores was a metal cylinder with a diameter of 9.2 cm and a depth of 7.6 cm attached to a handle. Each plot was core sampled on four separate dates in 2015, and on two separate dates for each experiment in 2016. The core sampler allowed for equally sized samples of soil and rice roots to be collected. To estimate densities of immature weevils in samples, samples were rinsed with water under moderate pressure through a mesh screen bucket in order to remove soil and dislodge RWW larvae from roots. The bucket was then placed in salt water and floating larvae and pupae were counted (Stout et al. 2002).

Densities of whiteheads found in plots shortly after heading were used to estimate stemborer infestation levels (2016 experiments only). Whitehead counts were made by visual inspections within one week of anthesis, approximately two months after herbicide
application. Populations were a mixture of the three stemborer species mentioned above, but larvae were not identified to species.

The rice stink bug, *Oebalus pugnax* (Fabricius), is consistently present in moderate to high densities in heading rice at the experimental site. The procedure used for estimating rice stink bug density consisted of sweeping with 38 cm sweep nets. The length of each plot was swept with ten consecutive sweeps across the plot and adult and immature bugs captured were counted. The total number of rice stink bug per plot was used for analysis. Sweeps were made within one week of anthesis, approximately two months post-herbicide application.

**Rating of Rice Injury and Weed Control**

Visual assessments of rice injury and weed control resulting from herbicide treatments were made one and three weeks after application of herbicide. Ratings for injury were based on discoloration of foliage and plant height on a scale of 0 to 100, with 0=0 injury and 100=complete plant death. The scale for weed control was also 0 to 100, with control plots representing the standard for zero weed control (Webster et al. 2018).

**Statistical Analyses**

All analyses were performed in SAS version 9.4 (SAS Institute). For RWW mortality experiments each petri dish containing ten weevils was considered a replicate and the percentage of dead weevils in each dish was determined and used for analyses. Mortality data were analyzed by generalized linear mixed models using PROC GLIMMIX, with herbicide treatment as the independent variable. Means were separated using Tukey’s HSD means separation test (P<.05). This analysis tested the hypothesis that exposure to herbicides directly reduced RWW survival.
Each of the three field experiments were analyzed separately. The 2015 field experiment employed a randomized complete block design with five blocks and one replicate of each treatment within each block. Block was used as a random effect and herbicide treatment as a fixed effect in the analysis. A multivariate approach was taken with herbicide treatment as the explanatory variable. Response variables included RWW densities in each of the core samplings (Core1, Core2, Core3, and Core4), ratings of weed density (Weed1 and Weed2), and ratings of rice injury (Injury1 and Injury2). A MANOVA followed by pairwise contrasts of each herbicide treatment to the control were performed in PROC GLM. Results were considered significant when the p-value from Wilk’s Lambda test was less than 0.05. Significant results in the pairwise contrasts indicate that one or more response variables showed significant differences from the control. Response variables significant at the multivariate level were analyzed by univariate generalized linear mixed models in PROC GLIMMIX. Means, standard deviations, and standard errors were determined in PROC MEANS. The Poisson distribution and log link were used for all count data. MANOVA, pairwise contrasts, and the univariate generalized linear model tested the hypotheses that herbicide applications altered densities of RWW via herbicide-induced resistance or alterations in the weed community, but do not allow for these hypotheses to be distinguished.

Herbicide treatments resulted in eight response variables: Injury1, Injury2, Weed1, Weed2, Core1, Core2, Core3, and Core4. Because the relationships between the former four variables and latter four variables were of interest, canonical correlation analysis (CCA) in PROC CANCOR was performed in order to determine if significant relationships existed between the response variables. This type of analysis proceeds by
correlating a linear combination of the first four variables and a linear combination of the second four variables (Akbaş & Takma 2005). In order to do this, the data are transformed to find the optimal linear combination of the variables of each set. These new variables are the canonical variates, and it is the relationship between these variates that is of interest (Hair et al. 1998). The strength of the association between the canonical variates is referred to as the canonical correlation, which is the linear correlation between the canonical variates resulting from the data transformation (Hansen et al. 1998). The first canonical correlation represents the maximum association between variates. This process is iterative and additional transformations are made to produce the second canonical correlation, then the third, and so on. Results for the first canonical correlation of each experiment, its cumulative R-square, and level of significance are presented. Non-significant canonical correlations are not shown. Additional output from this analysis includes the canonical coefficients, correlation loadings, and cross loadings, which show the direction of the linear relationship between each individual variable and the canonical variate it contributes to, as well as correlations between each variable and its paired canonical variate. These values can also provide additional information regarding the amount of variance explained by variates for each variable (Akbaş & Takma 2005). At this point, biological results are inferred from the canonical cross loadings for individual variables from the two sets of variates. CCA provides a large amount of output and therefore, not all output is presented.

While the CCA is a very informative and appropriate analysis for this experimental design, biological effects are difficult to portray visually. Due to this weakness, and in order to provide additional support for the results of the CCA, simple
linear regressions (SLR) were performed using PROC REG to reveal the nature of direct relationships between variables of interest. These regression analyses were performed under circumstances in which neither variable was measured without error because they were both response variables of the herbicide treatments. The CCA and SLR analyses test potential effects of both induced resistance and altered weed community. In each analysis (CCA and SLR) the level of injury to rice and level of weed control can be compared to the subsequent densities of RWW. As CCA and SLR analyses utilize only the response variables in the dataset there is no analysis of effects of herbicide treatments involved in either. One outlier was removed from the CCA in 2015.

The two field experiments conducted in 2016 followed the same experimental design, with the addition of two treatments and two blocks in each treatment for a total of seven blocks in each experiment, and one replicate of each treatment in each block. Borers and rice stink bug were also monitored in these experiments, and per plot yield was recorded. Analyses were performed as described above with the addition of three response variables, whitehead densities (WH), rice stink bug densities (RSB), and plot yield (YLD). MANOVA, pairwise contrasts, and the univariate generalized linear model test the hypotheses that herbicide applications altered RWW densities, borer densities, rice stink bug densities, and yield via induced resistance or alterations in weed communities, but do not allow for these hypotheses to be distinguished. Due to borers and rice stink bug being late seasons pests that occur more than eight weeks after herbicide, the altered weed community hypothesis is more likely in these cases.

CCA was performed as described above with one group including response variables (Injury1, Injury2, Weed1, and Weed2) and the second group including response
variables (Core1, Core2, WH, RSB, and YLD). One outlier was removed from the first experiment in this year. SLR was performed as described above for both experiments in 2016 even though the CCA was only marginally significant in the second experiment in this year (2016).

**Results**

**RWW Mortality Experiments**

Direct topical application of herbicides to RWW adults in petri dishes caused substantial mortality of adults (F=16.68; df=6,21; P<0.0001). Mortality was higher than in controls for all herbicide treatments. The herbicides 2,4-D, Bolero, Propanil, and Ricebeaux caused 100% mortality. The herbicides Command and Newpath caused 60—70% mortality, significantly higher than the control, but mortality caused by Command was significantly lower than most of the other herbicides.

Feeding assays in which RWW adults were fed rice leaves immediately after treating leaves with herbicide showed a significant effect of herbicide treatment on mortality (F=4.67; df=6,21; P=0.004), but only the herbicide Bolero caused significant mortality of adults (Figures 5.1A and 5.1B).
Figure 5.1. Mean mortality of RWW adults due to (A) direct topical application of herbicides to RWW adults and (B) due to contact with/consumption of herbicide-treated leaf material. Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at α<.050.

**Field Experiments**

For the 2015 experiment, the MANOVA testing the overall effect of herbicide treatment on response variables was significant (F=10.37; df=28, 51.9; P<0.001), with all contrasts of individual herbicide treatments to the control significant as well (2,4-D F=17.9; df=7,14 P<0.001; Command F=23; df=7,14; P<0.001; Newpath F=9.38; df=7,14; P<0.001; Ricebeaux F=65.15; df=7,14, P<0.001).

Univariate analysis of the first rice injury rating at seven days post application (Injury1) showed a significant effect of herbicide treatment (F=11.56, df=4,20; P<0.001). Plots treated with Ricebeaux were severely injured at seven days post application, but
injury in plots treated with the other herbicides did not differ significantly from control plots (Table 5.1). No injury was observed at the second rating (Injury2), indicating plants in all treatments recovered from injury incurred by all herbicide treatments by 21 days post application.

Table 5.1. Mean injury ratings (% injury ± S.E.) at two time points post-treatment (7 days post application and 21 days post application) for the field experiment in 2015 and the two field experiments in 2016 (2016(1) and 2016(2)). Values in the same column accompanied by different letters represent means that differed significantly with Tukey’s HSD test at α<.050. *N/A = Treatment was not applied in that year.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>2015 experiment</th>
<th>2016 experiment 1</th>
<th>2016 experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Injury1 7 d post-application</td>
<td>Injury1 21 d post-application</td>
<td>Injury2 7 d post-application</td>
</tr>
<tr>
<td>Control</td>
<td>0±0a</td>
<td>0±0</td>
<td>0±0a</td>
</tr>
<tr>
<td>2,4-D</td>
<td>10.00±4.18a</td>
<td>0±0</td>
<td>34.29±2.97b</td>
</tr>
<tr>
<td>Bolero</td>
<td>N/A*</td>
<td>N/A*</td>
<td>12.87±2.86a</td>
</tr>
<tr>
<td>Command</td>
<td>7.00±2.55a</td>
<td>0±0</td>
<td>2.14±2.14a</td>
</tr>
<tr>
<td>Newpath</td>
<td>1.00±1.00a</td>
<td>0±0</td>
<td>9.29±3.52a</td>
</tr>
<tr>
<td>Propanil</td>
<td>N/A*</td>
<td>N/A*</td>
<td>12.86±5.22a</td>
</tr>
<tr>
<td>Ricebeaux</td>
<td>21.00±2.45b</td>
<td>0±0</td>
<td>27.86±3.43b</td>
</tr>
</tbody>
</table>

Univariate analysis revealed significant weed control on the first rating (Weed1) (F=188.5; df=4,20; P<0.001) (Table 5.2) with weed control in all herbicide treatments differing significantly from the control. This indicates the herbicide treatments were effective at altering the density and potentially the composition of the plant community.

Weed control was significantly better in plots treated with Newpath and Ricebeaux than
in plots treated with 2,4-D and Command. The effect of herbicide treatments on weed control was also significant on the second rating date (Weed2) (F=48.11; df=4,20; P<0.001) (Table 5.2). Plots treated with all herbicides showed significantly higher weed control than controls. Newpath and Ricebeaux continued to provide more effective control than 2,4-D and Command.

Table 5.2. Mean weed control (% control ± S.E.) at two time points post-treatment (7 days post application and 21 days post application) for the field experiment in 2015 and both experiments in 2016 (2016 (1) and 2016 (2)). Values accompanied by different letters represent means that differed significantly with Tukey’s HSD test at α<0.05. *N/A = Treatment was not applied in that year.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>2015 experiment</th>
<th>2016 experiment 1</th>
<th>2016 experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weed1 7 d post-application</td>
<td>Weed2 7 d post-application</td>
<td>Weed1 21 d post-application</td>
</tr>
<tr>
<td>Control</td>
<td>0±0a</td>
<td>0±0a</td>
<td>0±0a</td>
</tr>
<tr>
<td>2,4-D</td>
<td>36.00±1.00b</td>
<td>61.00±7.31b</td>
<td>32.86±12.86ab</td>
</tr>
<tr>
<td>Bolero</td>
<td>N/A*</td>
<td>N/A*</td>
<td>54.29±9.97bc</td>
</tr>
<tr>
<td>Command</td>
<td>31.00±4.30b</td>
<td>48.00±6.25b</td>
<td>15.71±8.69ab</td>
</tr>
<tr>
<td>Newpath</td>
<td>84.00±1.87c</td>
<td>84.00±3.67c</td>
<td>54.29±9.97bc</td>
</tr>
<tr>
<td>Propanil</td>
<td>N/A*</td>
<td>N/A*</td>
<td>48.57±11.00b</td>
</tr>
<tr>
<td>Ricebeaux</td>
<td>91.00±4.00c</td>
<td>85.00±4.47c</td>
<td>75.71±2.97c</td>
</tr>
</tbody>
</table>
Densities of RWW did not differ significantly among treatments for any of the core samplings, although the overall treatment effects at three and five weeks post-flooding were marginally significant (Core1 F=1.53; df=4,20; P=0.230; Core2 F=2.47; df=4,20; P=0.077; Core3 F=1.12; df=4,20; P=0.373; Core4 F=2.61; df=4,20; P=0.066). On the second coring date, densities in Ricebeaux-treated plots were 47% lower than controls, and on the fourth coring date densities of weevils in Ricebeaux- and Command-treated plots were 41 and 43% lower than in control plots, respectively (Figure 5.2A and 5.2B).

Figure 5.2. Mean densities (± S.E.) of RWW larvae and pupae in each treatment three weeks after flooding (A) (Core sampling 2) and five weeks after flooding (B) (Core sampling 4). Only marginally significant differences were noted, (Core2 F=2.47; df=4,20; P=0.077) (Core4 F=2.61; df=4,20; P=0.066). However, the number of larvae and pupae recovered in plots treated with Ricebeaux were numerically about half of the control.
The value for the first canonical correlation was 0.839 and was significant (F=2.53; df=12, 45.27; P=0.012), with 94.13% of variance explained by the two variates.

The canonical cross loading values indicate that, in three of four core samplings (Core1, Core2, Core4), injury measured at seven days post-application (Injury1) were negatively related to RWW densities (Table 5.3).

Table 5.3. Canonical cross loading values for three field experiments, 2015—2016. Negative values for three of four RWW core samples in 2015 indicate an overall decrease in RWW densities as injury to rice and weed control increased. In 2016 (experiment one) negative values for injury and one of two weed ratings, and positive values for RWW densities and yield, indicate that densities of RWW and rice yields increased as levels of injury to rice decreased and densities of weeds increased. However, under the same circumstances, borer and rice stink bug densities decreased. In 2016 (experiment two) positive values for injury and negative values for weed control compared to positive values for all insect sampling variables indicate densities of insects increased as levels of injury to rice increased and densities of weeds increased. N/A=no data for that variable in that experiment.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>2015</th>
<th>2016 (1)</th>
<th>2016 (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury 1</td>
<td>0.637</td>
<td>-0.3453</td>
<td>0.547</td>
</tr>
<tr>
<td>Injury 2</td>
<td>N/A</td>
<td>-0.2532</td>
<td>0.466</td>
</tr>
<tr>
<td>Weed 1</td>
<td>0.702</td>
<td>-0.1256</td>
<td>-0.072</td>
</tr>
<tr>
<td>Weed 2</td>
<td>0.726</td>
<td>0.4455</td>
<td>-0.307</td>
</tr>
<tr>
<td>Core 1</td>
<td>-0.572</td>
<td>0.0315</td>
<td>0.306</td>
</tr>
<tr>
<td>Core 2</td>
<td>-0.559</td>
<td>0.3205</td>
<td>0.024</td>
</tr>
<tr>
<td>Core 3</td>
<td>0.187</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Core 4</td>
<td>-0.369</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>WH</td>
<td>N/A</td>
<td>-0.2621</td>
<td>0.288</td>
</tr>
<tr>
<td>RSB</td>
<td>N/A</td>
<td>-0.1466</td>
<td>0.171</td>
</tr>
<tr>
<td>YLD</td>
<td>N/A</td>
<td>0.395</td>
<td>-0.552</td>
</tr>
</tbody>
</table>

SLR showed that the relationship between the weed control rating at seven days post-application (Weed1) and densities of RWW on the first core sampling date (Core1) was significant (F=7.23; df=1.23; P=0.013) (Figure 5.3). The density of RWW immatures
decreased as the density of weeds decreased. The same relationship was observed among densities of RWW immatures in the first two core samplings (Core1 and Core2) and weed control rating at 21 days post-application (Weed2) (Core1 F=4.38; df=1,23; P=0.048; Core2 F=3.75; df=1,23; P=0.065), although the relationship between RWW densities at the second core sampling and weed density at 21 days post-application was only marginally significant.

Figure 5.3. Regression showing the relationship between percent weed control and immature RWW density. As densities of weeds decreased, densities of immature RWW also decreased.
In the first experiment of 2016, the MANOVA testing the overall effect of herbicide treatment on response variables was significant ($F=3.47; df=54, 126.97; P<.0001$), with all pairwise contrasts of herbicide treatments to the control significant as well ($2,4$-D $F=11.15; df=9,24; P<0.0001$; Bolero $F=12.64; df=9,24; P<.0001$; Command $F=3.12; df=9,24; P<0.0001$; Newpath $F=8.19; df=9,24; P<0.0001$; Propanil $F=10.97; df=9,24; P<0.0001$; Ricebeaux $F=20.34; df=9,24; P<0.0001$).

Univariate analysis of injury at seven days post-application (Injury1) showed a significant effect of herbicide treatment ($F=15.43; df=6,24; P<0.0001$). This result was due to severe injury caused by 2,4-D and Ricebeaux (Table 5.1). Injury at 21 days post-application (Injury2) was also affected by treatment ($F=12.08; df=6,24; P<0.0001$). 2,4-D-, Bolero-, and Ricebeaux-treated plots again exhibited significantly higher amounts of injury than the control (Table 5.1).

Herbicide applications reduced weed pressure, indicating the herbicide treatments were effective and altered the plant community. Herbicide application had a significant effect on weed density at seven days post-application (Weed1) ($F=8.19; df=6,42; P<0.0001$) (Table 5.2). Percent weed control in plots treated with Bolero, Newpath, Propanil, and Ricebeaux was significantly different from the control. Ricebeaux-treated plots showed significantly higher weed control than 2,4-D and Command-treated plots. Herbicide application also had a significant effect on weed density at 21 days post-application (Weed2) ($F=19.40; df=6,42; P<0.0001$). Plots treated with Bolero, Command Newpath, Propanil, and Ricebeaux showed significantly higher weed control when compared to control plots (Table 5.2). Plots treated with Newpath, Propanil, and
Ricebeaux exhibited significantly higher control than plots treated with 2,4-D and Command (Table 5.2).

Pre-flood application of herbicides resulted in significant differences in densities of RWW (Core1 *F*=2.63; df=6,35; *P*=0.033; Core2 *F*=7.08; df=6,35; *P*<0.0001). On the first core sampling date (Core1), densities of RWW were lowest in Ricebeaux-treated plots and highest in Propanil-treated plots. Mean densities in these treatments were significantly different from each other, but not from the control (Figure 5.4A). On the second core sampling date (Core2) plots treated with 2,4-D had significantly fewer RWW immatures than all other treatments including the control (Figure 5.4B).

Figure 5.4. Mean densities of RWW immatures in herbicide-treated and untreated (control) plots on the first core sampling where significant difference in means exist between herbicide treatments but not the control (A) and the second core sampling where 2,4-D-treated plots showed significantly lower RWW densities than all other treatments.
Whitehead counts (WH) also differed significantly among herbicide treatments ($F=2.46; \text{df}=6,42; \text{P}=0.039$). Plots treated with Ricebeaux ($2.57\pm0.43$ WH) showed the lowest densities of whiteheads, and densities in these plots were significantly lower than in Command-treated plots ($6.29\pm1.23$ WH), which had the highest densities. Neither herbicide treatment was significantly different from the control ($3.71\pm0.75$ WH). Rice stink bug densities were not significantly affected by herbicide applications ($F=2.04; \text{df}=6,42; \text{P}=0.081$). Finally, yield (YLD) was significantly affected by herbicide treatments ($F=2.69; \text{df}=6,42; \text{P}=0.027$). Bolero-treated plots ($9.79\pm0.25$ kg/ha) had the lowest yield and Command-treated plots ($12.49\pm1.38$ kg/ha) the highest. These treatments differed from each other, but not from the control ($11.48\pm0.16$ kg/ha).

The first canonical correlation was 0.630 and was significant ($F=2.07; \text{df}=20,100.45; \text{P}=0.001$), with 48.2% of variance explained by the two variates (Table 5.3). The canonical cross loading values indicated that a lack of injury (Injury1 and Injury2) had a positive impact on densities of RWW (Core1 and Core2), which agrees with results from 2015. Conversely, this lack of injury had a negative effect on whitehead and rice stink bug densities (WH and RSB). The relationship between insect densities and weed densities was more complicated. Canonical cross loading values show a lack of weed control at seven days post-application (Weed1) had a positive impact on RWW densities (Core1 and Core2), but negatively affected densities of WH and rice stink bug. The value for weed control measured at 21 days post-application (Weed2) indicates effective weed control had a positive effect on RWW, but negatively affects densities of
whiteheads and rice stink bug. Whether weed control was positive or negative, WH and rice stink bug densities had a negative relationship. The weight of the value for Weed2 is the heaviest in the loading indicating that positive weed control (reduced weed density) results in reductions in WH and rice stink bug densities later in the season. Finally, canonical cross loading values indicated that a lack of injury and positive weed control positively affect yield (Table 5.3).

Further SLR was performed on this data set in order to confirm the relationships suggested by the CCA. Generally, the SLRs showed that RWW densities increased as weed density decreased. Densities of RWW in the second core sampling (Core2) and percent weed control at 21 days post-application (Weed2) were significantly positively correlated (F=12.88; df=1,44; P=0.008), showing that densities of RWW increased with decreasing weed density (data not shown). At both dates of injury rating (Injury1 and Injury2), yield decreased as injury to rice increased (Injury1 F=7.55; df=1,47; P=0.009; Injury2 F=12.13; df=1,47; P=.001) (Figure 5.5).
Figure 5.5. Regression showing the relationship between percent injury to rice (% Injury) and yield (kg/plot). The two lines show that as percent injury per plot increased the average yield per plot decreased.

In the second experiment in 2016, the MANOVA was significant (F=4.68; df=54,177.96; P<0.0001), and all pairwise contrasts of herbicide treatments to control were significant as well (2,4-D F=17.50; df=9,34; P<0.0001; Bolero F=14.25; df=9,34; P<0.0001; Command F=17.27; df=9,34 P<0.0001; Newpath F=15.75; df=9,34; P<0.0001; Propanil F=16.45; df=9,34; P<0.0001; Ricebeaux F=17.58; df=9,34; P<0.0001).

Univariate analysis of injury at seven days post-application (Injury1) showed a significant effect of herbicide treatment on injury (F=29.58; df=6,42; P<0.0001). This significance was due to the relatively severe injury caused by 2,4-D (Table 5.1). The
effect of herbicide treatment on injury at 21 days post-application (Injury2) was also significant (F=2.59; df=6,42; P=0.031) with plots treated with 2,4-D continuing to exhibit significant injury relative to the control (Table 5.1).

Herbicide treatment had a significant effect on weed control at seven days post-application (Weed1) (F=27.67; df=6,42; P<0.0001) (Table 5.2). All herbicide treatments provided significantly higher levels of weed control when compared to the control treatment. The 2,4-D treatment resulted in significantly higher weed control than the control, but control in 2,4-D treated plots was significantly lower than in all other herbicide-treated plots. Herbicides also had a significant effect on weed control at 21 days post-application (Weed2) (F=28.84; df=6,42; P<0.0001) (Table 5.2). Herbicides provided significant weed control at 21 days post-application when compared to the control. At 21 days post-application, 2,4-D and Command provided significantly higher weed control than the control group, but significantly lower control than Bolero, Newpath, Propanil, and Ricebeaux (Table 5.2).

Univariate analyses of the effects of herbicide treatments on densities of RWW showed that herbicides did not significantly affect densities of RWW at the first core sampling (F=1.22; df=6,42; P=0.3175) (Figure 5.6A), but there was an effect at the second core sampling (F=3.68; df=6,42; P=0.005). Bolero-treated plots had significantly higher densities than 2,4-D, Command, Newpath, and Ricebeaux-treated plots but densities in Bolero-treated plots did not differ from densities in control plots (Figure 5.6B).
Figure 5.6. Mean densities of RWW immatures in herbicide-treated and untreated (control) plots on the first core sampling where there are no significant differences in means between treatments (A) and the second core sampling where 2,4-D-treated plots showed significantly lower RWW densities than Bolero-treated plots (B). Bars accompanied by different letters represent means that differed significantly by Tukey’s HSD test at α<.050.

There was an overall effect of herbicide on whitehead count (F=2.35; df=6,42; P=0.048), but numbers of whiteheads per plot were very low and no significant differences were found using the Tukey means comparison procedure. Rice stink bug densities were significantly affected by herbicide treatments (F=2.94; df=6,24; P=0.018). More rice stink bug were captured from plots treated with 2,4-D (8.43±2.03 RSB) and Bolero (8.29±2.11 RSB) than plots treated with Ricebeaux (3.89±0.77 RSB) although rice stink bug densities in all of these treatments did not differ from the control.
Yield (YLD) was not significantly affected by herbicide treatments (F=1.12; df=6.42; P=0.365).

The first canonical correlation was 0.643 and was marginally significant (F=1.60; df=20, 133.61; P=0.061), with 78.8% of variance explained by the two variates. Canonical cross loading values indicate that injury (Injury1 and Injury2) and weed density (opposite sign of Weed1 and Weed2) had a positive impact on densities of insects (Core1, Core2, WH, RSB) but a negative impact on yield (YLD) (Table 5.3).

SLR was performed on this data set in order to confirm the relationships suggested by the CCA. Two significant relationships were found between ratings of injury to rice and insect densities. RWW densities (Core1) (F=4.07; df=1,47; P=.050), and whitehead densities (WH) (F=3.91; df=1,47; P=0.054) were positively correlated with injury (Injury1) to rice. There were no significant regressions between weed and insect densities. Results showed that yield was significantly negatively correlated with injury at both the first (Injury1) and second ratings (Injury2) (F=12.09; df=1,47; P=0.001; F=8.59; df=1,47; P=0.005)(data not shown), indicating that rice injury contributed to yield loss in this experiment. Finally, overall yield was positively correlated with percent weed control at 21 days post-application (Weed2) (F=4.94; df=1,47; P=0.031) (Figure 5.7), indicating that yield increased as weed density decreased.
Figure 5.7. Simple Linear Regression comparing the weed density at 21 days post-application to the average yield per plot.

Discussion

Applications of herbicides may affect herbivores in agroecosystems directly, by toxifying them, or indirectly, by inducing plant defenses or altering the availability of alternate plant hosts. Overall, results from this series of experiments indicate that herbicides are capable of altering pest densities of both weeds and insects, and the resulting changes in these communities can have impacts on yields.

The first objective of this study was to determine if applications of seven herbicides commonly used in rice in the southern U.S. caused mortality in RWW adults that were exposed by topical application or by feeding on leaves that had been treated with herbicides. This level of direct and sustained contact with herbicides is unlikely in a
field setting, as RWW adults are capable of flight, are excellent swimmers, and are thereby able to avoid direct exposure. The feeding assays with herbicide-treated foliage likely better represent the type of exposure to which RWW adults are subjected in a field setting. RWW adults that were fed on rice treated with herbicides for the most part did not experience significant mortality. The exception being RWW adults that were fed on rice treated with the herbicide Bolero experienced significant mortality when compared to non-treated rice tissue. In spite of this, the effect of consuming Bolero-treated rice tissue on RWW adults did not translate to a reduction of immature RWW densities in that treatment group in these field trials. In fact, Bolero-treated plots showed a trend toward higher densities of RWW immatures than the control plots. Thus, while herbicides may cause direct mortality of adults, this did not translate into reduced larval populations later in the season. This is likely due to the natural timing of infestation. Fewer adult weevils are present in fields before flooding, as they are still migrating from overwintering sites, and most arrive after flooding and after herbicide applications (Shang, H. et al, 2004).

The second objective was to determine if these herbicides induced resistance to herbivores thereby reducing subsequent pest densities. Of the herbivores studied, herbicide-induced resistance is most likely to affect RWW which are present in high numbers over the four weeks after flooding (Shang et al. 2004). Stemborers and rice stink bug are present in high numbers later in the season and are less likely to be affected by herbicide-induced resistance, which likely decays over time. Evidence from this study supports a weak, but relatively consistent induction of defenses to RWW. Control plots not treated with herbicides had higher average densities of RWW immatures in six out of eight core samplings than in plots treated with herbicides. However, in only one of eight
core samplings were RWW densities in herbicide-treated plots significantly lower than controls. This was noted in 2,4-D-treated plots.

There were also instances in which rice water weevil densities in herbicide-treated plots differed from one another, but not from densities in control plots. This may be related to different levels of injury to rice caused by different herbicide treatments. It is known that in some cases the levels of induction of plant defenses are correlated with the amount of injury a plant receives (Underwood 2000). This study noted that decreases in RWW densities were correlated with increases in plant injury. In field trials, herbicide treatments 2,4-D, Bolero, and Ricebeaux consistently caused significant injury to rice plants. There were two instances in which RWW densities in 2,4-D-treated plots were significantly lower than densities in other herbicide-treated plots. Additionally, Ricebeaux-treated plots had lower RWW densities than most other treatments in almost every core sampling throughout the three experiments. CCA suggested a significant relationship may exist between injury and RWW density. SLR supported this trend, although not at a significant level. In three of four cases in 2015, injury showed a negative effect on RWW densities, but there were no cases in 2016 where this relationship was significant and no trend was evident. In one case in the second experiment of 2016, there was a significant relationship, the relationship between injury and RWW densities were positive. Notably, injury ratings were at or below 1.43% for five of the six herbicides, and therefore, do not represent a case where injury could have induced resistance.

Stemborers and rice stink bug enter the field much later than the RWW and are therefore less likely to be affected by induced resistance, as these effects are known to
decay over time (Gómez et al. 2010). This was supported by results as the only significant relationship between injury ratings and whitehead density occurred in 2016 where the injury rating was virtually zero, and densities of rice stink bug showed no significant relationships with early season injury. Overall, results indicate that the herbicides used in this study may have induced low levels of resistance to the RWW but not to the other insect pests. It may be that high levels of herbicide-induced resistance were not triggered by the low levels of injury observed in this study. Low levels of injury from the herbicides used in this study were expected, as these herbicides were developed with the intent of causing as little injury to rice as possible.

The third objective of this study was to determine if changes in weed densities due to herbicide applications altered densities of subsequent insect pest populations. All of the herbivores investigated can utilize weedy grass species present in rice fields as hosts. It was expected that densities of stem borers and rice stink bug would be most affected by changes in weed densities, as they are not present early in the season when most herbicide applications are made and when herbicide-induced resistance is expected to be strongest, but are present mid- to late-season well after herbicide applications when differences in weed densities are most pronounced. Results provide weak evidence that alterations in weed densities had effects on pest densities. In field experiments in 2015 and in the second experiment in 2016, CCA and simple linear regression analyses showed that RWW densities were affected by the level of weed control, with RWW densities negatively affected by increasing weed control (decreasing weed density) and positively affected by decreasing weed control (increasing weed density). Evidence for an effect of weed densities on RWW densities from the first 2016 experiment was mixed, as the
canonical cross loading values for Weed1 and Weed2 were not consistent. RWW densities appear to be positively affected by both increases and decreases in weed control. In the second experiment, the CCA was only marginally significant and the SLRs were non-significant, suggesting no relationship between RWW densities and weed densities. As RWW densities generally decrease in rice as the season progresses this was not surprising.

Differences in stemborer and rice stink bug densities were also noted, related to changes in weed densities. In the first experiment of 2016 there were significant differences in densities of whiteheads among herbicide treatments. Command-treated plots had the lowest weed control rating (aside from the control at zero) at seven days post-application (Weed1) and highest WH count, while Ricebeaux-treated plots had the highest weed control rating, and the lowest number of whiteheads, suggesting that plots with higher weed densities had higher densities of borers. The relationship was not observed, however, using data from the second weed rating (Weed2) at 21 days post-application. The CCA, which does not take herbicide treatments into account, showed relatively consistent results between the two experiments in 2016. In the first experiment of 2016, regardless of whether correlation cross loading values for weed control (Weed1 and Weed2) were positive or negative the values for WH and RSB were negative. Alternatively, in the second experiment in 2016 decreasing weed control (increasing weed density) had a positive effect on both WH and rice stink bug densities. This means in three of four comparisons, WH and rice stink bug densities are positively correlated with increasing weed densities. This is further supported by SLRs which, while not significant, showed that at three of four weed control ratings, there was a trend toward an
increase in whiteheads as weed density increased. In all three experiments, at five out of six weed control rating dates, all herbicides were shown to be capable of significantly controlling weed pests. Therefore, overall weed densities in the study were low, and this may have inhibited investigation of whether changes in weed densities due to herbicide applications altered densities of subsequent insect pest populations.

In addition to the primary objective of investigating herbicide effects on densities of insects’ pests, yields were taken and differences observed among herbicide treatments. Results of SLR in both experiments in 2016 showed a significant negative relationship between level of injury to rice and yield. This suggests that herbicide injury may have contributed to yield reduction. In the second experiment of 2016, injury was the greatest in plots treated with 2,4-D, from 13-22%, and yield in 2,4-D-treated plots was the lowest of all groups. The first experiment of 2016 also showed that Bolero-treated plots had significantly lower yields than Command treated plots. This may be due to the fact that Bolero treated rice received as much as 85% more injury than Command in this experiment. Injury to rice and subsequent reduced yields may also be explained by the high rates applied and inappropriate timings of applications. In the second experiment of 2016, the SLR showed a significant increase in yield with increasing weed control. This may be due to a decrease in plant competition, but may also be attributed to lower insect pest densities in plots with lower weed densities. The combination of herbicide injury and insect herbivory has previously been shown to have negative effects on plant health. When herbicide-stressed volunteer potatoes (considered to be weeds), experienced herbivory from Colorado potato beetles, *Leptinotarsa decemlineata*, weed management
was more effective than compared to control of weeds which were not herbicide stressed (Williams et al. 2004).

While there have been many studies investigating the effects of herbicides on insect communities, the vast majority concern direct effects on insect mortality or fecundity and indirect effects due to changes in weed populations (Sharma et al. 2018). There have been very few studies which investigate changes in plant resistance due to herbicide applications, and of those that have resistance to pathogens has been investigated more frequently. Specifically in rice, this may be because it has been noted that herbicide applications induce systemic acquired resistance (SAR) via the salicylic acid (SA) pathway and not the jasmonic acid (JA) pathway (Chen et al. 2017). The SA pathway is more strongly associated with pathogen resistance while the JA pathway is more strongly associated with resistance to chewing herbivores (Kunkel & Brooks 2002). The literature is not completely devoid of investigations of induced resistance to herbivores. One study provided evidence that a defensive response triggered by the herbicide 2,4-D and mediated by jasmonic acid and ethylene resulted in an increase in volatile production and an increase in activities of trypsin proteinase inhibitor. Plants treated with 2,4-D exhibited stronger attraction of Anagrus nilaparvate, a parasitoid, and supported lower growth rates of C. suppressalis (Xin et al. 2012). Another study reported that 2,4-D induced susceptibility in corn, Zea mays, to two insect and one pathogen pest (Oka, I.N., Pimentel 1976). Additionally, studies investigating herbicide-induced stress in the plant and subsequent effects of herbivory have been investigated. Phosphonate herbicides have been shown to have a dual effect as insecticides to the black bean aphid, Aphis fabae, when applied systemically in broad bean, Vicia faba (Lipok
There are few if any other studies which have combined investigations of all three routes by which herbicides may alter insect communities. There is a need for additional studies investigating direct and indirect effects simultaneously in order to make clearer deductions.

The objectives put forth in this study have not yet been adequately tested, but results presented here give credence to the ideas of direct mortality from herbicide applications, and herbicide-induced resistance to insect herbivores. When results of all three objectives are considered together it appears chemical herbicide application in rice agroecosystems has effects on weed and insect pest densities. It agrees with the large amount of literature which shows 2,4-D can induce plant-mediated responses which have effects on insect communities. This study overall reinforces the importance of appropriate weed management in rice agricultural systems, as increases in weed and insect densities can negatively affect yield, and improper use of herbicides can injure crops with the same result.

References


Williams, M.M., Walsh, D., and Boydston, R. 2004. Integrating Arthropod Herbivory and Reduced Herbicide Use for Weed Management America Linked references are available on JSTOR for this article: Integrating arthropod herbivory and reduced herbicide use for weed management. *Weed Science*, 52(6), pp.1018–1025.


Chapter 6.

Conclusion

Rice, *Oryza sativa* L., is a vital commodity and contributor to the economy of the southern United States as well as other regions of the world. Insect herbivores cause extensive damage to rice crops worldwide, reducing income of rice growers and overall food supply. The insect pest complex in rice in the southern U.S. is currently managed mainly by insecticide applications with potentially dangerous repercussions for human health and the environment. The most economically damaging of these insects is the rice water weevil (RWW), *Lissorhoptrus oryzophilus*. In an effort to develop non-insecticidal options for managing the RWW and other pests, the research presented here focused on plant-mediated effects on the RWW. Novel strategies to combat RWW and other insect pests of rice are urgently needed. The main objectives of the experiments reported were to investigate the occurrence of induced resistance in rice, to better understand the nature of plant-mediated interactions in this agroecosystem, and to determine the potential utility of induced resistance for pest management of rice.

Within all ecosystems organisms interact with each other and their environment. These interactions are complex, often involving multiple organisms interacting directly and indirectly with one another, as well as abiotic factors. Linkages between insects associated with the same host plant may have important implications for pest management, as plant-mediated effects have been shown to alter communities of associated herbivores. Insect herbivores are capable of inducing defensive responses in plants, which may directly alter subsequent herbivore densities by the production of toxic compounds and antifeedants. Temporally and spatially separated herbivores may also be
indirectly affected by plant-mediated responses, such as the attraction of natural enemies via volatile organic compounds. Understanding the nature of plant-mediated interactions between pests in the rice agroecosystem is of paramount importance to the realization of utilizing induced resistance as a strategy for pest management in rice.

The first objective of this body of work was to determine the effects of factorial combinations of fall armyworm, *Spodoptera frugiperda* (J.E. Smith), larval feeding and mechanical defoliation on levels of resistance in rice to RWW and tolerance to defoliation. This was achieved by measuring larval densities and grain yields. Determining the potential for early season herbivores to induce resistance to early and mid-season herbivores was of major interest as there are many temporally separated insects in this agroecosystem. While the literature provides evidence for induction of resistance by fall armyworm, only a handful of studies had investigated the effects of upregulated plant defenses on RWW densities. Here it was shown that fall armyworm feeding can induce resistance to RWW in rice, although the effects were inconsistent. Additionally, it was determined that defoliation may reduce the tolerance of rice to the RWW. Defoliation by fall armyworm did not have negative consequences for the rice plant, but could induce resistance, indicating that growers may be inclined to ignore low infestations of this insect in hope of taking advantage of this natural plant resistance. However, there was some evidence that defoliation may reduce tolerance to RWW infestations. The main outcome from this series of experiments is that the plant-mediated interactions between fall armyworm and RWW were not as strong as anticipated. The results indicate somewhat weak and inconsistent induction of defenses to RWW, and potential decreases in tolerance due to defoliation, therefore, it is unlikely fall armyworm
rice water weevil interactions will play a major role in pest management of rice in the southern US.

The second objective was to investigate plant-mediated interactions among life stages of the RWW on seedling rice. This was accomplished by determining the effects of feeding by adult RWW (on leaves), and larval RWW (on roots), on levels of resistance in rice to subsequent infestations of RWW adults. Oviposition by these adults was measured to assess levels of resistance in rice. This objective was not geared directly at pest management but at understanding the ability of the plant to be induced using the major pest RWW as a model. The experiments were designed to answer questions about inducibility potential and duration in rice, which are vital to overall understanding of induced resistance in rice. It was discovered that both larvae and adults of RWW induce resistance in the plant, although larvae induced a consistently strong systemic response, while adults induced weak localized responses. These results support previous research completed in the area indicating belowground herbivory results in drastic changes in plant physiology.

The third objective was to determine the effects of methyl jasmonate (MJ) seed treatments on levels of resistance in the rice plant to RWW, and additionally to determine if the seed treatments altered plant growth and yield. The role of jasmonic acid in inducing plant resistance to herbivores has been intensely investigated, but there is still much to learn. There is evidence to suggest plants will suffer growth and fitness costs when this pathway is upregulated. It is important to weigh the costs and benefits in numerous cropping systems in order to determine the utility of elicitors as a tool for integrated pest management. This body of work showed that seed treatment of jasmonic
acid induced defenses to the RWW, that these effects decayed over time, and that there were alterations in plant growth, namely delayed emergence and heading and decreased root and shoot biomass. Very few studies have been performed at the field level utilizing jasmonate applications for insect management, and to the authors knowledge these were the first performed in rice. These results contribute to the literature concerning effects of methyl jasmonate on herbivore populations and plant growth in a rice agroecosystem. This method of utilizing MJ could be a great early season option for water seeding and organic growers.

The fourth and final objective was to determine if applications of seven different herbicides had direct or indirect effects on populations of pests in small rice plots. Direct effects were measured by assessing mortality of RWW after direct application or feeding on rice leaves immediately after herbicide treatment. Indirect effects of herbicides on plant-mediated resistance, and indirect effects of herbicides on herbivores via alterations in weed densities, were assessed by sampling herbivore densities throughout the season. Results from herbicide trials performed over two years indicate that herbicides are not likely to cause high direct mortality to RWW present during or immediately after application. Additionally, the herbicides tested were not strong inducers of resistance in rice to insect herbivores, but clearly showed that alterations in the weed communities could affect insect communities and rice yield. This indicates that proper weed management is vital to rice production as insect pests tend to increase with increasing weed populations. The combination of weed competition and insect damage were likely the cause for reductions in yield.
The overall conclusion is that the linkages between insect herbivores via the rice plant are not all that strong in this agroecosystem. While it is possible to induce resistance in rice via herbivory, the plant-mediated effects on herbivores are weak and inconsistent. Alternatively, the phytohormone, methyl jasmonate, was noted to consistently induce defenses in rice to RWW, within one month of application. However, the resulting densities of RWW on MJ-treated plants were significantly higher than insecticide-treated plants, indicating that these defenses were also somewhat weak. Nevertheless, it is possible induced defenses may be taken advantage of and utilized to the benefit of integrated pest management. Knowledge of plant insect interactions and use of elicitors can be incorporated into management programs to reduce the current costs of pest control. There are still hurdles to overcome and barriers in this area. For example, there is still a major lack of knowledge of mechanisms of induced resistance in rice, and the true costs of upregulation of defense pathways for the plant, and how these induced responses may affect other organisms associated with the plant such as pollinators. Future research in this area will be vital to understanding ecological interactions and the implications of induced resistance for pest management and development of new strategies.
Appendix

Supplemental Table 2.1. Dates of planting, fertilization, fall armyworm infestation, mechanical defoliation, flooding, cores one and two, height and harvest are provided for each experiment.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Year</th>
<th>Planting Date</th>
<th>Fertilization Date</th>
<th>FAW Infestation Date</th>
<th>Mechanical Defoliation Date</th>
<th>Defoliation Visual Rating Date</th>
<th>Flooding Date</th>
<th>Core 1 Date</th>
<th>Core 2 Date</th>
<th>Height Measurement Date</th>
<th>Harvest Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance</td>
<td>2015</td>
<td>25-Mar</td>
<td>12-May</td>
<td>29-Apr</td>
<td>N/A</td>
<td>12-May</td>
<td>14-May</td>
<td>3-Jun</td>
<td>5-Jun</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Resistance</td>
<td>2015</td>
<td>30-Mar</td>
<td>1-Jun</td>
<td>20-May</td>
<td>N/A</td>
<td>1-Jun</td>
<td>2-Jun</td>
<td>18-Jun</td>
<td>24-Jun</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Resistance</td>
<td>2015</td>
<td>4-May</td>
<td>9-Jun</td>
<td>29-May</td>
<td>N/A</td>
<td>8-Jun</td>
<td>10-Jun</td>
<td>25-Jun</td>
<td>29-Jun</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Resistance</td>
<td>2016</td>
<td>23-Mar</td>
<td>5-May</td>
<td>27-Apr</td>
<td>5-May</td>
<td>6-May</td>
<td>25-May</td>
<td>1-Jun</td>
<td>N/A</td>
<td>1-Jun</td>
<td>N/A</td>
</tr>
<tr>
<td>Resistance</td>
<td>2016</td>
<td>29-Mar</td>
<td>11-May</td>
<td>29-Apr</td>
<td>11-May</td>
<td>11-May</td>
<td>31-May</td>
<td>8-Jun</td>
<td>N/A</td>
<td>8-Jun</td>
<td>N/A</td>
</tr>
<tr>
<td>Resistance</td>
<td>2016</td>
<td>12-Apr</td>
<td>24-May</td>
<td>18-May</td>
<td>25-May</td>
<td>25-May</td>
<td>26-May</td>
<td>13-Jun</td>
<td>20-Jun</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Resistance</td>
<td>2016</td>
<td>9-May</td>
<td>2-Jun</td>
<td>25-May</td>
<td>2-Jun</td>
<td>3-Jun</td>
<td>23-Jun</td>
<td>28-Jun</td>
<td>N/A</td>
<td>27-Aug</td>
<td>N/A</td>
</tr>
<tr>
<td>Tolerance</td>
<td>2015</td>
<td>4-May</td>
<td>9-Jun</td>
<td>8-Jun</td>
<td>N/A</td>
<td>10-Jun</td>
<td>14-Jul</td>
<td>x</td>
<td>N/A</td>
<td>4-Aug</td>
<td>11-Aug</td>
</tr>
<tr>
<td>Tolerance</td>
<td>2016</td>
<td>29-Mar</td>
<td>11-May</td>
<td>8-Jun</td>
<td>N/A</td>
<td>12-May</td>
<td>13-Jul</td>
<td>x</td>
<td>31-Aug</td>
<td>8-Sept</td>
<td>17-May</td>
</tr>
</tbody>
</table>

Supplemental Table 4.1. Dates of agricultural practices for the 2017 and 2018 field experiments including, seed treatment, sowing, flooding, insecticide foliar sprays, and harvest. Also shown are dates of sampling for emergence, biomass, core samples for rice water weevil, and heading.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Seed Treatment</th>
<th>Water Seeding</th>
<th>Emergence</th>
<th>Flooding</th>
<th>Foliar Sprays</th>
<th>Biomass</th>
<th>Core Sampling</th>
<th>Heading</th>
<th>Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>25-Mar</td>
<td>27-Mar</td>
<td>9-Apr</td>
<td>4-May</td>
<td>4-May</td>
<td>8-May</td>
<td>22-May</td>
<td>6-Jul</td>
<td>6-Aug</td>
</tr>
</tbody>
</table>

Supplemental Table 5.1. Information regarding herbicides including trade name, active ingredient, mode of action, labeled rate range, rate used, whether a surfactant was used, and proper times for application.
<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Active Ingredient</th>
<th>Mode of Action</th>
<th>Rate Range</th>
<th>Rate Used</th>
<th>Surfactant Y/N</th>
<th>When to Apply</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weed Rhal A-4D</td>
<td>Dimethyamine salt of 2,4-dichloro-phenoxoacetic acid</td>
<td>Growth Regulator</td>
<td>1-2.5 pints/acre</td>
<td>2.5 pints/acre</td>
<td>Y</td>
<td>4 weeks prior to planting or late tillering stage</td>
</tr>
<tr>
<td>Command 3ME</td>
<td>Clomazone</td>
<td>Pigment Inhibitor</td>
<td>.4-.6lbs/acre</td>
<td>.8lbs/acre</td>
<td>Y</td>
<td>Prior to planting to 7 days after planting</td>
</tr>
<tr>
<td>Bolero SE</td>
<td>Thiobencarb</td>
<td>Photosynthesis</td>
<td>2-4 pts/acre</td>
<td>4 pts/acre</td>
<td>N</td>
<td>Prior to planting not past 2-3 leaf stage on wet soil only</td>
</tr>
<tr>
<td>Newpath</td>
<td>Ammonium salt of imazethapyr</td>
<td>ALS Inhibitor</td>
<td>4-6 floz/acre</td>
<td>6 floz/acre</td>
<td>Y</td>
<td>2-5 leaf stage; Requires two applications for control</td>
</tr>
<tr>
<td>Propanil 45C</td>
<td>3’,4’-Dichloropropanilide</td>
<td>Lipid Synthesis</td>
<td>3-6qts/qcre</td>
<td>6qts/qcre</td>
<td>N</td>
<td>1-4 leaf stage</td>
</tr>
<tr>
<td>Ricebeaux</td>
<td>Propanil and Thiobencarb</td>
<td>Lipid Synthesis</td>
<td>3-5.3 qts/qcre</td>
<td>5.3qts/qcre</td>
<td>N</td>
<td>1-6 leaf stage</td>
</tr>
<tr>
<td>Low Foam</td>
<td>Alkyl Polyoxyleylene Ether</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Supplemental Table 5.2. Dates of agricultural practices for the experiment in 2015 and both experiments in 2016, including, planting, fertilization, herbicide application, flooding, rating for injury to rice, rating for control of weeds, core samples for rice water weevil, whitehead sampling, rice stink bug sampling, and harvest.
VITA

Emily Clare Kraus, born in Lawrenceburg, Indiana, received a bachelor’s degree from Purdue University in Entomology. She immediately continued in academia pursuing her Master’s of Science at Kansas State University. She then served in the United States Peace Corps in Senegal, West Africa. Upon completing her service she worked for Monsanto in St. Louis, Missouri for one year before returning to academia. She is a candidate for a Doctorate in Entomology at Louisiana State University. Upon completion of her doctorate she will lead her own research program in sustainable agriculture.