Investigation of insect-weed interactions in the rice agroecosystem

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INVESTIGATION OF INSECT-WEED INTERACTIONS IN THE RICE AGROECOSYSTEM

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

Kelly V. Tindall
B.S. Louisiana State University-Shreveport, 1998
M.S. Louisiana State University, 2000
December 2004
For Jordan Grace, Colin, and Christi,
know that you can accomplish anything
ACKNOWLEDGEMENTS

I would like to thank Louisiana State University AgCenter, the faculty and staff of the Department of Entomology, and the personnel of the Northeast, Macon Ridge, and Rice research stations. I would also like to express appreciation to my major professor, Michael Stout, Bill Williams and Roger Leonard for acting as surrogate advisors while I was in North Louisiana, as well as my other committee members, Eric Webster, Jim Ottea, Carl Motsenboker, and Rodrigo Valverde. I would like to thank Drs. Frank Guillot, Jim Fuxa, and Tim Schowalter for guiding our department in my time at LSU. I would like to express special appreciation to James Geaghan for all of his help with statistics; Steve Linscombe for providing me with seed and use of his milling equipment; John Bernhardt for training me to assess pecky rice; Morgan Smith and Todd Bridges at for allowing me to conduct a test at Woodsland Plantation; and Donna Mitten at Bayer CropSciences for her assistance with the glufosinate-tolerant project. I would like to also thank, Boris Castro, Ann Burns, Bruce Copes, Jerry Bartleson, Ryan Pousson and numerous student workers for there assistance, especially, Rita Riggio, not only for her assistance, but also for her friendship. I would to express extreme and sincere appreciation to my friends and families, (all three of them – the Tindalls, Chandlers, and Harrisons), for all their support which was provided in so many ways; without their support, this project would not have been possible. Most importantly, I would like to thank my parents for instilling in me the importance of education and the discipline to pursue this degree. I would like to thank Louisiana Rice Research Board and the Southern Regional IPM Competitive Grants Program for funding this research.
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ABSTRACT

Interactions between graminaceous weed and insect pests of rice and between management practices for these pests were investigated. Studies were conducted to examine preference and performance of rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, on several weeds commonly found in rice fields. Several weeds were more preferred than rice, and larvae were present on several dicotyledonous weeds, suggesting the host range of *L. oryzophilus* is broader than previously thought.

Effects of the presence of barnyardgrass, (*Echinochloa crus-galli* Beauv.), on rice stink bugs, *Oebalus pugnax* F., and *L. oryzophilus* populations in rice fields were investigated. Presence of barnyardgrass and the synchrony of barnyardgrass seed heads and rice panicles influenced *O. pugnax* densities on rice. Barnyardgrass served as a trap crop or as a source of infestation of *O. pugnax* depending on the developmental stage of barnyardgrass relative that of rice. Presence of barnyardgrass had little impact on *L. oryzophilus*. Similar studies conducted with borers and Amazon sprangletop, *Leptochloa panicoides* (Presl.) Hitchc. revealed that injury to rice was greater in weedy plots of rice than in pure plots of rice.

Studies were conducted to determine how density of weeds affected *O. pugnax* populations and how weeds and *O. pugnax* combined to reduce grain yield and quality. Numbers of *O. pugnax* and percentages of filled seeds, pecky rice, and broken kernels increased as weed density increased. Increases were less severe in insecticide-treated than non-treated plots. However, yield losses from weeds and insects were not significantly greater than from weeds alone.
Preference and performance of *L. oryzophilus* on herbicide-treated and non-treated glufosinate-tolerant rice were investigated. Glufosinate applications on glufosinate-tolerant rice reduced weevil oviposition by 30% and reduced larval densities by 20% compared to non-treated glufosinate-tolerant rice. Glufosinate was not toxic to *L. oryzophilus* at rates used in these experiments, and feeding was not deterred by glufosinate, suggesting that glufosinate application induced resistance. Larval densities on glufosinate-treated and non-treated glufosinate-tolerant rice in field experiments did not differ; however, delayed floods reduced numbers of larvae on rice compared to those on early flooded rice.
CHAPTER 1

INTRODUCTION

Weeds and insects often coexist and reduce yields in agricultural systems. Weeds reduce yields by an estimated 12% annually, whereas insects account for a 13% annual reduction in yields in United States agricultural systems (Pimentel 1991). In addition to the individual effects that insects and weeds have on crops, these two types of pests and their management practices can interact and impact crop production. It is the intent of this chapter to discuss pests and pest management strategies in the rice agroecosystem and suggest ways in which weed and insect pests and their management practices can interact.

Pests of the Rice Agroecosystem

Weeds

Nearly 80% of rice grown in the United States is treated with herbicides two or more times in a single season because weed pressure is severe in many rice fields (Crawford et al. 1990). Some of the most common weeds infesting Louisiana rice fields include: red rice, *Oryza sativa* L., barnyardgrass, *Echinochloa crusgalli* Beauv., broadleaf signalgrass *Brachiaria platyphylla* Nash., hemp sesbsnia, *Sesbania exaltata* (Raf.) A.W. Hill., alligator weed, *Alternathera philoxeroides* (Mart.) Griseb., duck salad, *Heteranthera limosa* (Sw.) Willd., and perennial and annual sedges, *Cyperus* spp. (Jordan and Sanders 1999). Floods are applied to assist with weed control; however, water-tolerant and aquatic grasses, sedges, and broadleaf weeds are problematic in rice.

Of the most common weeds found in Louisiana rice fields, red rice is the primary weed pest in southern Louisiana (Crawford et al. 1990). Management of red rice is more complex because of similarities between commercial rice and red rice (Linscombe et al.
1993, Braverman and Linscombe 1994, Williams et al. 2001). In 1964, red rice was first recognized as a weed of rice in both North and South Carolina (Kwon et al. 1991, Sankula et al. 1997a). Many scientific names have been given to red rice (Oryza barthii Chev., O. longistaminate Chev. and Roehr., O. rufipogon Griff., and O. punctata Kotschy ex Steud.); however, in the United States, the accepted name is O. sativa (Kwon et al. 1991). An estimated 75% or more of rice grown in Louisiana is infested with red rice (Sankula et al. 1997b). Red rice reduces yield and quality of rice by $50 million annually in the southern United States (Kwon et al. 1991). Red rice has a red pericarp that requires extra milling to remove. Extra milling causes breakage of commercial rice and reduces yield and quality (Pantone and Baker 1991, Kwon et al. 1991, Dunand 1998). Additionally, red rice is taller and produces more tillers and straw than commercial rice, making it more competitive than commercial rice. Red rice also shatters before harvest (Kwon et al. 1991, Sankula et al. 1997a) and can complicate harvesting due to lodging (Sankula et al. 1997a).

Both red rice and commercial rice produce aryl acylamidase, an enzyme that metabolizes the most common herbicide used in rice, propanil [N-(3,4-dichlorophenyl) propionamide] (Pantone and Baker 1991). The similarities between commercial rice and red rice make red rice difficult to control with herbicides (Linscombe et al. 1993, Braverman and Linscombe 1994, Williams et al. 2001). Therefore, cultural practices, including water-seeding, pinpoint floods, increased seeding rates, or altered seeding dates, have been major tools for red rice management. These practices give commercial rice an early-season competitive advantage over red rice (Dunand 1988, Crawford et al. 1990).

Cultural practices, however, are not 100% effective for red rice control and do not control every weed species present. Therefore cheaper, more specific, and more effective
practices are always needed. A recent development has been the development of herbicide-tolerant rice lines. Herbicide-tolerant rice has been either genetically engineered or selected through mutagenesis to yield herbicide tolerance (Williams et al. 2001). The intent of herbicide-tolerant crops has been to reduce the number of herbicide applications and/or the use of non-environmentally friendly herbicides (Buckelew et al. 2000). However, the primary benefit of herbicide-tolerant rice varieties will be the ability to chemically control red rice without injuring commercial rice. Because herbicide-tolerant rice varieties are tolerant to selected herbicides, the herbicide will not be lethal to commercial rice but will selectively control many weeds of rice, including red rice. Three herbicide-tolerant rice lines have been developed: Roundup Ready™ rice (Monsanto, St. Louis, MO) (glyphosate-tolerant), LibertyLink® rice (Bayer CropScience, Kansas City, MO), (glufosinate-tolerant) (Braverman and Linscombe 1994), and Clearfield Rice™ (BASF, Research Triangle Park, NC) (imidazolinone tolerant) (Williams et al. 2001). The future of Roundup Ready rice is uncertain at this time. Clearfield rice was grown commercially on a large scale for the first time in 2004; LibertyLink rice has received regulatory approval in the United States (USDA 1999a, 1999b; FDA 2000, EPA 2003), but it will not be grown commercially until approval is granted by the European Union. These varieties have been and continue to be examined for their potential in weed control program (Linscombe et al. 1993, Braverman and Linscombe 1994, Croughan 1994, Sankula et al. 1997a, 1997b, Williams et al. 2001, Lanclos et al. 2004). However, there have not been any investigations concerning insect and weed interactions or how the new weed management strategies for herbicide-tolerant rice may affect insect population dynamics.
Rice Water Weevil

In 1881, before rice production became economically important in Louisiana, the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, was first documented as an economically important insect in rice systems near Savannah, Georgia (Isley and Schwardt 1934). The rice water weevil is considered the most important insect pest in Louisiana and throughout United States rice producing states (Smith 1983, Way 1990) and has the potential to be a global threat because it was accidentally introduced into Japan in 1978 (Smith 1983), Korea, Taiwan and mainland China in the 1990’s (Heinrichs and Quisenberry 1999). Adults feed on leaves causing longitudinal scars, which are considered to be of little economic importance. Oviposition does not usually begin until after the fields are flooded (Everett and Trahan, 1967; Muda et al., 1981; Smith, 1983). Females oviposit in the leaf sheaths below the water surface (Everett and Trahan 1967, Raksarart and Tugwell 1975, Smith 1983, Way 1990). Larvae eclose within four to nine days after eggs are laid and migrate to the roots (Everett and Trahan 1967, Raksarart and Tugwell 1975). Larvae feeding on roots cause an average of a 10% loss in yield (Smith 1983) and results in losses up to 50 million dollars (Spradley and Widham 1995) annually if uncontrolled.

Since rice water weevil prefer to oviposit beneath the water surface and are adapted to flooded conditions, native hosts of rice water weevil are thought to be aquatic and water-tolerant grasses (Poaceae) and sedges (Cyperaceae). Isley and Schwardt (1934) and Smith (1983) provide lists of plants known to be hosts for rice water weevil representing 10 genera of Poaceae (17 species) and 3 genera of Cyperaceae (4 species). Of the plant species listed, only 3 species and one genus of grasses (barnyardgrass, fall panicum, *Panicum dichotomiflorum* Michx.; and bermuda grass, *Cynodon dactylon* (L.) Pers.; *Paspalum* spp.)
and one genus of sedges (*Cyperus*) are weeds in rice fields. Numerous water-tolerant and aquatic grass and sedge species are common in rice fields such as barnyardgrass, red rice, broadleaf signalgrass, Amazon and bearded sprangletop (*Leptochloa spp.*), and several *Cyperus spp.* (Smith and Hill 1990, Jordan and Sanders 1999). These plants are potential but unconfirmed hosts of rice water weevil. There is no information on rice water weevil preference for and growth and development on potential hosts, especially common grasses and sedges in rice fields.

Non-chemical methods for rice water weevil control, (i.e., draining/drying fields, host plant resistance, and biological control agents), have been investigated, but have shown little success (Puissegur 1976, Bunyarat et al. 1977, Smith 1983, Way 1990, Thompson et al. 1994, N’Guessan and Quisenberry 1994, N’Guessan et al. 1994, Palrang and Godfrey 1994, Rice 1996, Heinrichs and Quisenberry 1999, Stout et al. 2001, Stout and Riggio 2003). Draining and drying fields until the soil cracks during heavy infestations of rice water weevil larvae may be effective in some cases but is not recommended because of frequent rain and the costs associated with reapplication of herbicide and fertilizer (Way 1990, Thompson et al. 1994). Thousands of rice lines have been screened for resistance to the rice water weevil, but only a few have shown low levels of resistance (N’Guessan and Quisenberry 1994, N’Guessan et al. 1994, Heinrichs and Quisenberry 1999, Stout et al. 2001, Stout and Riggio 2003); however, even the most resistant lines are not immune to rice water weevil damage and, therefore, require additional methods for rice water weevil control. Only a few potential biological control agents (Libellulidae nymphs, *Beauveria bassiana*, a mermithid nematode, frogs, and birds) have been reported, but little is known
about the effects of these biological control agents in the field (Puissegur 1976, Bunyarat et al. 1977, Smith 1983, Palrang and Godfrey 1994, Rice 1996)

Since no successes from non-chemical methods have arisen, chemical control of rice water weevil has been the main method of control. Carbofuran (FMC, Philadelphia, PA) was found to provide effective control in the mid 1960’s and was the primary means of control until the late 1990’s. In 1998, carbofuran lost its registration for rice. New insecticides were introduced for rice water weevil control beginning in 1997 (fipronil, Rhone-Poulenc, Monmouth Junction, NJ; \textit{lambda}-cyhalothrin, Sygenta, Wilmington, DE; \textit{gamma}-cyhalothrin, Dow AgroSciences, Indianappolis, IN; \textit{zeta}-cypermethrin, FMC, Philadelphia, PA; and diflubenzuron, Uniroyal Chemical, Inc., Naugatuck, CT). Fipronil targets larvae, like carbofuran; the remaining compounds, however, target different life stages. Diflubenzuron targets eggs; and the pyrethroids (\textit{lambda}-cyhalothrin, \textit{gamma}-cyhalothrin, and \textit{zeta}-cypermethrin) target adults (Stout et al. 2000). Fipronil was used heavily in the years immediately after the loss of carbofuran; however, fipronil will no longer be available after the 2005 growing season due to voluntary withdrawl from the United States rice market by its manufacturers (B.A. Castro, LSU AgCenter, Department of Entomology, personal communication). Another concern for rice producers is that many rice fields are also used for crawfish production. Both rice water weevils and crawfish are arthropods and have similar physiological responses to these insecticides. Regulatory labels for these insecticides prohibit the use of these insecticides in rice fields that are also used for crawfish production. Therefore, additional methods for rice water weevil management are needed.
Early flooding (i.e., floods applied at the two to three leaf stage) is commonly practiced in southwestern Louisiana where red rice is a severe weed pest because early flooding assists in red rice control. In areas where red rice is a less severe pest, floods are delayed (i.e., floods are applied at the four to five leaf stage or later). The presence of flood also influences rice water weevil oviposition, with more eggs oviposited in leaf sheaths of flooded rice plants than non-flooded plants. Depth of flood also influences oviposition; floods of 10.2 cm were the most preferred when rice water weevils were provided a choice between multiple flood depths (Stout et al. 2002b). Research has also shown that younger plants are more susceptible to rice water weevil injury (Stout et al. 2002a) The time at which permanent floods are applied also affects rice water weevil injury to rice (Rice et al. 1999, Zou et al. 2004). When floods are delayed by two weeks, numbers of rice water weevil larvae on roots were reduced by as much nine times that on roots of early flooded rice (Rice et al. 1999, Zou et al. 2004).

The practice of delaying floods has not been readily adopted because applications of early floods assist in red rice control (Dunand 1988). With the introduction of herbicide-tolerant rice varieties, water management practices will remain an important tool in weed management; however, it will not be as critical because there will be herbicides available that can control red rice. The use of herbicide-tolerant rice varieties may allow producers in southwestern Louisiana, where early flooding is most commonly practiced, the extra benefit of rice water weevil control because delayed flooding may become part of pest management strategies in areas of heavy weed pressure. Since herbicides would be able to control red rice, floods could be delayed for a short time to allow rice plants to become more tolerant to rice water weevil feeding.
Rice Stink Bug

Rice stink bug (*Oebalus pugnax* F.) is another important insect pest in rice in the southeastern United States. Female rice stink bugs lay two rows of barrel-shaped green eggs on plant foliage or panicles (Odglen and Warren 1962). Nymphs and adults remove endosperm from developing rice grains and reduce yield and grain quality. Rice stink bugs pass through five instars, but only late instars and adults are considered economically important. Fifteen to 23 days are required to complete the life cycle from egg to adult, and developmental time can be influenced by temperature and the host on which it was reared (Nilakhe 1975, Naresh and Smith 1983). Rice stink bugs overwinter as adults in October and emerge in April (Nilakhe 1976).

Rice stink bugs are most damaging during the early stages of grain filling (milk stage) and have been shown to cause injury to grain sorghum (*Sorghum bicolor* L.) and wheat (*Triticum aestivum* L.) at the milk stage (Hall and Teetes 1982, Viator et al. 1983). Feeding in the later stages of grain development causes atrophied seeds and reduces the quality of the grains (pecky rice) (Odglen and Warren 1962, Lee et al. 1993). Pecky rice is a broad term used to describe the appearance of discolored kernels that results from a combination of insect feeding and pathogen infection (Tullis 1936, McPherson and McPherson 2000); several pathogens have been isolated from pecky rice kernels (Tullis 1936 [and sources within], Daughtery and Foster 1966, Marchetti and Petersen 1984, Hollay et al. 1987, Lee et al. 1993). Lee et al. (1993) provided evidence of an insect-vector relationship between rice stink bugs and pathogens. Pecky rice and atrophied seeds reduce grain quality because they are more likely to break during the milling process (Douglas 1939, Odglen and Warren 1962, McPherson and McPherson 2000).
While the host range of rice water weevil is limited to habitats with water, rice stink bugs are able to utilize both aquatic and non-aquatic habitats. Rice stink bugs are known to feed on six graminaceous crops and numerous graminaceous weeds, many of which are hosts of rice water weevil (Odglen and Warren 1962, Nilakhe 1976, Naresh and Smith 1984, McPherson and McPherson 2000). Of the known hosts that have been tested, *Paspalum urvillei* Steud., is a preferred host of rice stink bug (Naresh and Smith 1984), and there is evidence that barnyardgrass is also a preferred host (Douglas 1939, Odglen and Warren 1962). Many alternate hosts for rice stink bugs occur either in or near rice fields (i.e., on levees and turn-rows, interspersed among rice or neighboring fields of corn or sorghum).

**Stemborer Complex**

There are three stem boring species known to injure rice in the southern United States, the rice stalk borer, *Chilo plejadellus* Zincken, sugarcane borer, *Diatraea saccharalis* F., and European corn borer, *Ostrinia nubilalis* Hübner (B.A. Castro, LSU AgCenter, Department of Entomology, personal communication). European corn borers were recently added to the list of borers attacking rice after the first severe infestation of European corn borer in rice was documented in 2003 (Castro et al. 2004). Young larvae feed on the inner tissue of leaf sheaths seven to ten days before boring into the stalks. Feeding on plant tissue in the stalks can lead to lodging, deadhearts, whiteheads, and partial whiteheads (Holloway 1928, Castro et al. 2004). Partial whiteheads result from larvae feeding on individual kernels late in panicle development. Whiteheads are caused by feeding on the neck of the panicle, which disrupts translocation of nutrients for proper development. Feeding on the panicle shortly after panicle differentiation leads to no panicle emerging from the stalk. Extensive feeding on rice stems can cause plants to lodge because rice plants are not able to
support their own weight or cause deadhearts (i.e., when plants do not produce panicles). Sugarcane borer can be more devastating to rice than rice stalk borer, and damage can be worse in rice fields in close proximity to corn or sugarcane (*Saccharum officinarum* L.) (Holloway 1928).

These pests are increasing in importance as pests of rice, although little is known about their behavior in rice. Sugarcane borer overwinter as larvae in stalks of graminaceous plants, pupate in early March, and emerge as adults in late March, early April (Fuchs et al. 1979). Overwintering borer populations can be reduced by destruction of overwintering hosts (Rodriguez-Del-Bosque et al. 1995). Sugarcane borer feed on corn, grain sorghum, or sugarcane prior to attacking rice and preference has been shown for sugarcane of the four cultivated hosts (Sosa 1990). Female sugarcane borers reared on rice can lay as many as 239 eggs in her lifetime, and days required to complete its life cycle ranges from 37-88 days, depending on the time of year (Castillo and Villarreal 1989). Plant height, number and length of internodes, stalk diameter, and amount of pubescence effect sugarcane borer damage in rice (Martins et al 1981).

These borers have a fairly large host range that consists primarily of graminaceous plants, including several crops. The host range of sugarcane borer consists of graminaceous plants in the following genera: *Paspalum* spp., *Andropogon* spp., *Panicum* spp., *Sorghum* spp., *Zea mays* L., *Leptochloa* spp., *Hymenachne* spp., *Digitaria* spp., *Eleusine* spp., and *Echinochloa* spp. (Holloway 1928, Bessin and Reagan 1990). European corn borers have a large host range that consists of both broad leaf and graminaceous plants (Peterson 1956, Udayagiri and Jones 1993). Of these plants, *Panicum* spp., *Leptochloa* spp., *Digitaria* spp., and *Echinochloa* spp. are common weeds in Louisiana rice production systems (Smith and
Hill 1990, Jordan and Sanders 1999). *Leptochloa* spp. have been suggested to cause increase in deadhearts of sugarcane plants when sugarcane was grown in weedy plots of sprangletop (Dr. Gene Reagan, LSU AgCenter, Department of Entomology, unpublished data).

**Interactions of Coexisting Pests in Agroecosystems**

Weeds reduce crop yields and quality by competing for nutrients and water. They also may decrease the value and productivity of land, reduce harvesting and processing efficiency, increase cost and labor for control measures, and restrict flow of water to reservoirs, canals, and ditches (Smith and Hill 1990). Losses from insects include defoliation of root or leaf tissue, removal of fluid from phloem and xylem systems, mining of parenchyma tissue, formation of galls, or blemishing the harvested fruit or vegetable (Schoonhoven et al. 1998). Additional problems associated with insects are transmission of plant diseases (Fry 1982), costs involved with insect management, and development of resistance to control measures (Paoletti and Pimentel 2000).


**Associational Resistance and Susceptibility**

Although weeds are potential alternative hosts for insect pests of rice and both occur in rice fields, little information is available to describe the interaction between weeds and insect populations. Several scenarios have been developed to describe how neighboring plants (i.e., weeds) may impact insect behavior relative to a specific plant (i.e., crop plants). Associational resistance or associational plant refuge is a situation in which a plant gains protection from herbivory due to surrounding plant composition (Letourneau 1986, Pfister and Hay 1988, Karban 1997, Hambäck et al. 2000, White and Whitham 2000). Associational resistance may be a result of nearby plants interfering with insects finding hosts, either visually or chemically (Pfister and Hay 1988, Karban 1997, Hambäck et al. 2000).
Neighboring plants have also been suggested to increase densities of predators by creating a more diverse species complex on which to feed and serve as nectar sources for parasitoids in agroecosystems (Ali and Reagan 1985, Chiverton and Sotherton 1991, Gurr and Wratten 1999, Mensah 1999).

Associational susceptibility, associational damage, or shared doom refers to the opposite situation in which herbivory increases due to the presence of nearby plants (Pfister and Hay 1988, Karban 1997, White and Whitham 2000). Nectar sources that lure parasitoids to a field may also attract adult lepidopterans that may produce damaging populations of offspring (Karban 1997). The presence of a more preferred host may attract large numbers of insects. After consuming the more preferred host or the host becomes unsuitable, insects will move to a less preferred host, which may not have been damaged in the absence of the more preferred host. This scenario has been termed the “spillover” effect (White and Whitham 2000).

Frequently, plots with two or more plant species have been shown to have smaller insect populations (e.g., associational resistance) than plots with a single plant species (Letourneau 1986, Pfister and Hay 1988, Andow 1991, Karban 1997, Hambäck et al. 2000, White and Whitham 2000). However, exceptions have been found where increased insect populations were larger in mixed vegetation (e.g., associational susceptibility) (Pfister and Hay 1988, Andow 1991, Karban 1997, White and Whitham 2000). White and Whitham (2000) propose that associational resistance may be more attributed to monophagous insects, whereas associational susceptibility may be more characteristic of polyphagous insects.
Interactions between Management Practices

Besides weeds influencing insect populations, management practices for either pest can interact. Herbicide-insect interactions were first investigated in the 1940’s when higher insect populations were observed following herbicide application. Since that time, scientists have investigated whether herbicides are toxic to insects or alter the biochemistry of a treated plant and indirectly influence insect populations (Ulber 1978/1979, Schaefer et al 1982, Campbell 1988 and sources within, Freemark and Boutin 1995 and sources within). Most work on herbicide-insect interaction was done prior to the 1990’s; however, with the introduction of herbicide tolerant crops, weed management practices may change. Use of herbicide tolerant crops is expected to result in better weed control, which may result in changes in insect pest management practices. Therefore, it is advantageous to understand how weeds influence insects in crop systems.

Direct Effects of Herbicides

Only a handful of studies has examined the direct effect of herbicides on insects. Of these studies, over twenty different species have been examined from 16 different families representing eight orders (Ulber 1978/1979, Schaefer et al. 1982, Campbell 1988 and sources within). Only a few studies have performed bioassays to determine toxic levels of herbicides on insects (Adams 1960, El-Ibrashy and Mansour 1970, Dimetry and Mansour 1975, Tanke and Franz 1978). El-Ibrashy and Mansour (1970) showed that injections of 2,4-D into black cutworm, Agrotis ypsilon Hufnagel, resulted in lower body weight but produced no change in feeding behavior. Dimetry and Mansour (1975) showed that exposure to the herbicides amitrole and naptalam acted as feeding deterrents for Heliothis zea Boddie. The herbicide glufosinate, a glutamine synthase inhibitor, has the potential to
affect insects because glutamine synthase is present in many insect systems, including the nervous system. Contact and oral toxicity of glufosinate has been documented for mites (Ahn et al. 1997) and larger canna leafroller, *Calpodes ethlius* Stoll., larvae (Kutlesa and Caveney 2001), respectively. Glufosinate can also act as a feeding deterrent for certain lepidopteran larvae, such as cotton leafworm, *Spodoptera littoralis* Boisd. (El-Ghar 1994).

In addition to herbicides affecting insect pests, beneficial insects (pollinators and natural enemies, e.g., predators and parasitoids, etc.) have also been shown to be affected by herbicides (Adams 1960, Tanke and Franz 1978). Natural enemies play a special role in agricultural ecosystems. In many cases, insect pests can be controlled by naturally-occurring predator and parasitoid populations. One problem with beneficial insects is that beneficial insects have the propensity to be more susceptible to pesticides than insect pests (van den Bosch 1978). Direct effects of pesticides on beneficial insects can indirectly affect insect pests in the field (Simpson 1961). Because of the importance of beneficial insects, many experiments determining direct effects of herbicides have been performed on natural enemies. Insect responses of beneficial insects ranged from having no effect to being toxic, including decreased reproduction or oviposition and reduced or delayed growth (Adams 1960, Simpson 1961, Tanke and Franz 1978).

As previously mentioned, increased pest populations after herbicide application (insect flares) were the observation that stimulated interest in the area of herbicide-insect interactions. Many things can disturb natural enemy populations such as weather conditions, lack of alternate food sources, cultural practices, insecticide applications, etc. Herbicide applications were also shown to have the potential to disrupt beneficial insects by more than one mechanism. Ladybird beetle larvae (*Coccinellidae* spp.) are predacious and
are frequently used as biological control agents against aphids (Aphididae spp.). Adams (1960) performed topical bioassays using 2,4-D herbicide on larvae from three Coccinellidae species. Applications of 2,4-D resulted in a four-fold increase in mortality as well as increases in developmental time compared to non-treated beetles. Adams and Drew (1965) suggested that the use of 2,4-D may also depress larval activity, reducing the amount of prey consumed. Reduction in the numbers of predators or prey consumed can lead to increased pest densities (Simpson 1961). If the herbicide has a deleterious effect on the beneficial insects, the absence of beneficial insects may indirectly influence pest populations.

**Herbicide-Insecticide Interactions**

Herbicides and insecticides have been shown to interact and affect crop injury or pest control when tank mixed or when applied within a given time frame. Propanil and certain insecticides (i.e., carbofuran, phorate, and dimethoate) when applied in combination have a variety of effects on rice plants, ranging from leaf chlorosis and necrosis to reduced height and yield as opposed to being applied individually separated by appropriate periods of time (Smith and Tugwell 1975, Mukhopadhyay and Sen 1981, Robinson et al. 1982, Marambe 2000). Applications of clomazone with aldicarb (Smith et al. 1994) and terbufos and nicosulfuron (Bailey and Kapusta 1994, Baerg et al. 1996, Williams and Harvey 1996) increased injury to cotton and corn, respectively, as opposed to the herbicide or insecticide applied alone for either combination. Crop injury following applications of primisulfuron and malathion was a result of malathion inhibiting cytochrome P-450, preventing metabolism of primisulfuron (Kreuz and Fonné-Phister 1992).
Joint applications of herbicides and insecticides can impact weed or insect control. Application of propanil and carbaryl or methiocarb was better able to control propanil – resistant barnyardgrass than propanil alone (Daou and Talber 1999, Hoagland et al. 1999). Similarly, chlorosulfuron and malathion enhanced the control of resistant *Lolium rigidum* Gaud. biotypes compared to application of chlorosulfuron alone (Christopher et al. 1994). Conversely, application of glyphosate and insecticides (i.e., chlorpyrifos, fipronil, imidacloprid, or methamidophos) reduced control of *Ipomoea lacunosa* L. compared to application of glyphosate alone (Mascarenhas and Griffin 1997). Additionally, some herbicide-insecticide combinations demonstrate enhanced insect control. Control of *Lygus lineolaris* Palisot de Beauvois and *Heliothis virescens* F. was improved when the insecticides azinphos-methyl and cyfluthrin, respectively, were tank mixed with bromoxynil as opposed to only insecticide (Scott et al. 1996). The herbicide simazine acted as a synergist to permethrin against *Musca domesticus* L. at ratios at least 50:1 (Wilkins and Khalequzzaman 1993).

**Indirect Effects of Herbicides**

Herbicides may indirectly affect insect growth and development via plant mediated responses due to herbicide application. Herbicides act on various systems of plants (e.g., cell division, photosynthesis, and amino acid, lipid, carotenoid, folate and cellulose synthesis, etc.) (Duke 1990). Herbicide-induced changes in a plant may alter the nutritive quality of plant tissues for insects. The following is a discussion of effects of herbicides on plants and how herbicides can alter the nutritive quality of plant material for insects, with an emphasis on herbicides used on herbicide tolerant crops.
Herbicide-Induced Changes in the Plant

Amino Acid Synthesis

Broad spectrum herbicides like glyphosate, glufosinate, imidazolinone and sulfonyleurea herbicides, are targeted for use with herbicide tolerant crops now in development. Glyphosate inhibits 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase in the shikimate pathway. Glyphosate competitively binds to EPSP synthase, prevents binding with phosphoenylpyruvate, leads to an accumulation of shikimate, and reduces concentrations of aromatic amino acids, i.e. phenylalanine, tyrosine and tryptophan (Duke 1990). Sulfonylurea and imidazolinone herbicides act on acetolactate synthase and acetohydroxyacid synthase. These enzymes are involved in reactions of the branched chain amino acid pathway, which is responsible for the production of valine, leucine and isoleucine (Duke 1990). Glufosinate-ammonia interferes with the conversion of glutamate to glutamine by inhibiting glutamine synthase (Vasil 1996). Therefore, applications of those herbicides are likely to reduce the amount of specific amino acids in the plant; lower concentrations of amino acids obstruct protein synthesis and other biochemical reactions in the plant and plant growth ceases (Ashton and Monaco 1991).

Plants provide herbivorous insects with nutrients for normal growth and development. When the nutritive quality of a plant changes due to stress (e.g., herbicide application), insect behavior and/or development may also change. After an insect consumes plant material, it attempts to simultaneously maximize nutrient absorption and reduce toxicity of any allelochemicals. Most insects require 10 essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Essential amino acids are required for normal insect development.
However, development can be enhanced by the presence of additional amino acids in the diet (Dadd 1977). An increase or decrease in amino acid concentrations can alter the ability of insects to digest, solubilize, absorb, and/or assimilate food consumed, as well as alter feeding behavior (Felton et al. 1992, Chapman 1998). Increased levels of amino acids may allow an insect to meet its nutritional needs while consuming smaller quantities of food. If less food is eaten, more food remains, possibly leading to a larger than normal population. Conversely, if essential amino acid concentrations drop, it is likely that insect growth and development will decline, unless the insect compensates. An insect has potential to compensate by consuming more food to meet its nutritional requirements, moving to a more suitable host, or increasing assimilation of nutrients (Chapman 1998). Because the production of five essential amino acids (isoleucine, leucine, tryptophan, phenylalanine, and valine) is inhibited due to glyphosate and imidazolinone herbicides, the suitability of plant tissue may decline for insect herbivores following applications of such herbicides.

Additionally, insects have been shown to have diverse responses to ammonia (Moursi 1970, Moursi and El-Zayat 1975, Hribar and Foil 1992, Hirayama et al. et al. 1996, Taneja and Guerin 1997 Braks et al. 2001). Therefore, an accumulation of ammonia in plant tissues following glufosinate application may affect insects. Hirayama et al. (1996) showed that *Bombyx mori* L. was able to utilize ammonia as a nitrogen source. Ammonia can serve as an attractant for *Triatima infestans* (Klug), *Anopheles gambiae* Giles, and *Hybomitra lasiophthalma* (Macquart) (Hribar and Foil 1992, Taneja and Guerin 1997 Braks et al. 2001). Lastly, ammonia has been shown to be toxic to soil arthropods (Acarina and Collembola) (Moursi 1970) *S. littoris* (Moursi and El-Zayat 1975).
Lipid Synthesis

A group at Cornell University has done extensive work with diamondback moth (*Plutella xylostella* L.) and a thiocarbamate herbicide, S-ethyl dipropylthiocarbamate (EPTC) (Eigenbrode and Shelton 1992; Eigenbrode et al. 1993). As early as 1974, Flore and Bukovac (1974, 1976, 1978) determined that applications of EPTC altered wax composition of *Brassica* spp. and gave leaves a glossy appearance. When screening cabbage(*Brassica oleracea* L.) varieties, glossy leaf surfaces of cabbage were found to be associated with resistance to diamondback moth larvae (Eigenbrode and Shelton 1990, Eigenbrode et al. 1990, 1991). Eigenbrode and Shelton (1992, Eigenbrode et al. 1993) examined the potential of EPTC to create herbicide-induced resistance to diamondback moth larvae in cabbage. Eigenbrode and Shelton (1992) discovered that treatment of cabbage with EPTC conferred resistance to first instar diamondback moth larvae, reducing their survival by 48%. Eigenbrode and Shelton (1992) determined cuticular waxes and wax crystallites of EPTC-treated plants were reduced by 40.6 and 20.8% respectively.

Because EPTC conferred resistance to diamondback moth, Eigenbrode et al. (1993) saw its potential for use in cabbage production for control of lepidopteran pests. The glossy-leaf appearance was exhibited 10 days after treatment with EPTC, and plants remained glossy for up to 30 days. Eigenbrode et al. (1993) compared the efficacy of EPTC to permethrin and EPTC plus permethrin as a means of managing diamondback moth, imported cabbage worm, *Pieris rapae* L., and cabbage looper *Trichoplusia ni* Hübner. Similar numbers of diamondback moth and important cabbage worm were found on EPTC, permethrin, and EPTC plus permethrin treated plants, but numbers on all of these treatments were significantly lower than numbers found on a nontreated check. However, EPTC plus
permethrin appeared to have an additive effect on cabbage looper while neither EPTC nor permethrin had different numbers from nontreated plants. Because of the 10 day induction time, EPTC has potential to control some lepidopteran pests if used in conjunction with a pre-EPTC treatment of permethrin.

**Herbicide-Tolerant Varieties**

Currently, three enzymatic targets for herbicides are used in different herbicide-tolerant rice varieties: glyphosate targets EPSP synthase, imidazolinone/sulfonylurea herbicides target acetolactate synthase and acetohydroxyacid synthase, and glufosinate targets glutamine synthase. Glyphosate-tolerant plants have two forms of EPSP synthase. One form is native EPSP synthase and is sensitive to glyphosate. The other form is the product of an foreign inserted gene that expresses EPSP synthase that is tolerant to glyphosate and active in the shikimate pathway (Padgette et al. 1996). Clearfield Rice™ was the product of selection for a mutation that yields tolerance to imidazolinone herbicides. The mutation allows plants to metabolize herbicide at a higher rate than non-tolerant plants (Croughan 1994). Glufosinate-tolerant lines have a bialophos resistance (bar) gene inserted (Sankula et al. 1997a, 1997b). The bar gene allows a plant to acetylate the free amino group of glufosinate before the herbicide reaches the target site; therefore, normal glutamine synthase activity occurs following herbicide application (Braverman and Linscombe 1994).

Herbicide tolerant varieties are said to be tolerant to their respective herbicides; however, tolerant plants can receive slight injury from herbicide application (Lanclos et al. 2003). Depending on the severity of injury, suitability of tolerant plant tissue may be altered for insect pests. To date, little research has been conducted examining interactions between herbicide use on herbicide-tolerant crops and insects. Buckelew et al. (2000) examined
insect interactions using herbicide-tolerant (e.g., Roundup Ready, LibertyLink, STS [sulfonylurea tolerant] soybeans) and non-tolerant soybeans. Their studies compared insect densities on conventionally treated soybeans, herbicide-tolerant soybeans with appropriate herbicide, and hand-weeded conventional soybeans. Weedy plots generally had higher insect densities; however, the potato leafhopper (*Empoasca fabae* Harris) preferred plots with fewer weeds. Since no differences were found between conventional and transgenic varieties, it was suggested that insect populations were related to weed density and not to use of herbicides on herbicide-tolerant varieties. An additional study (Jeff Ellis, LSU Department of Plant Pathology and Crop Physiology, data not published) indicated no significant decrease of insect densities in Roundup Ready soybeans; however, there was no non-treated transgenic soybean as a control. Only one study has been conducted that examined the impact of herbicide-treated and non-treated transgenic herbicide-tolerant varieties on insects (Bitzer et al. 2002). In that study, differences in populations of Collembola associated with transgenic soybeans resulted from differences in weed cover and degree of soil disturbance, not from herbicide applications or herbicide plant-induced effects following herbicide applications. Whether any herbicide-tolerant rice variety receives injury sufficient to induce changes in plant quality to affect insect populations has not been determined.

**Objectives and Goals**

Rice water weevil, rice stink bug, and sugarcane borer are oligophagous insects and it is uncertain how the presence of weeds will influence their behavior in the rice agroecosystem. The proposed experiments will examine how the presence of weeds affects insect populations in rice fields and how the insect response to presence of weeds, in turn, affects
rice yield and quality. Additional experiments will focus on the effects of herbicide-tolerant rice on rice water weevil populations and how weed management practices influence rice water weevil biology, ecology, and management.

**Objectives**

I. To determine the host range and host suitability of common weeds in rice fields for rice water weevil in greenhouse and field studies.

II. To investigate how the presence of a preferred weed host influences rice water weevil, rice stink bug, and borer populations in rice fields both in small plot and large plot demonstration tests.

III. To quantify the resulting damage to rice in response to changes in insect populations due to the presence of weeds.

IV. To determine direct toxicity, behavior effects, and indirect plant-mediated effects of glufosinate applications on rice water weevils in greenhouse and laboratory studies using a glufosinate-tolerant rice line.

V. To evaluate the interaction between flooding regimes, insecticide applications and glufosinate-tolerant rice in the field for rice water weevil control.

**References**


CHAPTER 2
USE OF COMMON WEEDS OF RICE AS HOSTS FOR THE RICE WATER WEEVIL (COLEOPTERA: CURCULIONIDAE) *

Introduction

The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, is native to North America and is the most important insect pest of rice in Louisiana and throughout U.S. rice producing states (Smith 1983, Way 1990). Rice water weevils are semi-aquatic folivores as adults and aquatic root-feeding herbivores as larvae. Adults migrate to rice fields in early spring and feed on leaves of young rice plants. Oviposition is not initiated in full until fields are flooded because females oviposit in leaf sheaths beneath the water surface (Stout et al. 2002). Larvae eclose, migrate to roots, and feed on root tissue. Larval feeding typically reduces yields 10 to 33%, but yield losses can be as high as 70% when infestations are severe (Anonymous 1994).

Little is known about native and alternate hosts of the rice water weevil. Webb (1914), Isley and Schwardt (1934), and Lange and Grigarick (1959) listed hosts for the rice water weevil from three plant families: Poaceae (9 genera, 14 species), Cyperaceae (3 genera, 4 species), and Onagraceae (1 genus, 1 species). *Jussiaea suffruticosa* L. (Onagraceae) is the only dicotyledonous species listed as a host. However, the rice water weevil is assumed to have a broader host range based on its distribution and biology (Isley and Schwardt 1934). In rice agroecosystems, many weeds co-exist with rice and rice water weevils, but only a few of the most common weeds have been confirmed as hosts for rice

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water weevils. Of the plant species listed as hosts, only a few species of grasses
(barnyardgrass, Echinochloa crus-galli Beauv.; fall panicum, Panicum dichotomiflorum
Michx.; bermudagrass, Cynodon dactylon (L.) Pers.; Paspalum spp.) and one genus of
sedges (Cyperus spp.) are common weeds in Louisiana rice fields. Numerous grasses and
sedges such as red rice (Oryza sativa L.), broadleaf signalgrass (Brachiaria platyphylla
Nash.), Amazon sprangletop (Leptochloa panicoidea [Presl] Hitchc.), rice flatsedge
(Cyperus iria L.), yellow nutsedge (Cyperus esculentus L.), and purple nutsedge (Cyperus
rotundus L.) are common in Louisiana rice fields (Jordan and Sanders 1999) and could serve
as hosts for rice water weevils.

Presence of weeds in an agroecosystem can influence insect populations in a positive
or negative manner. Insect-weed interactions in cotton agroecosystems have received
considerable attention. Weeds on the margins of cotton fields serve as a nectar source for
parasitoids, thereby keeping beneficial insects in the field (Gurr and Wratten 1999).
Additionally, a hemipteran pest of cotton, the tarnished plant bug, Lygus lineolaris (Palisot
de Beauvois), has a large host range, and many weeds associated with cotton are preferred
over cotton (Young 1986). Numerous weeds serve as transitional hosts before plant bugs
move to cotton. Manipulation of weedy hosts along field borders (i.e., application of
pesticides or mowing) can influence tarnished plant bug populations in cotton fields
(Fleischer et al. 1988, Snodgrass et al. 2000). Little comparable data is available about
insect-weed interactions in the rice agroecosystem.

Plants listed as hosts for the rice water weevil by previous authors were plants
infested by larvae either in field or cage studies (Isley and Schwardt 1934). However, there
are no reports indicating that the rice water weevil is able to complete its life cycle on these
hosts. Additionally, there is no information about relative host suitability or preference for hosts. Greenhouse experiments were designed to evaluate adult feeding, ovipositional preference, and larval development of rice water weevils on weeds commonly found in rice fields of Louisiana. Indicators of host quality and suitability were examined, including larval weights and the ability of rice water weevil to develop successfully to adulthood. Weeds were sampled in the field to determine if weeds used in greenhouse studies, as well as other common weeds, were infested with rice water weevil larvae under field conditions.

**Materials and Methods**

**Plant and Insect Material**

Barnyardgrass, fall panicum, yellow nutsedge, red rice, broadleaf signalgrass, Amazon sprangletop, hemp sesbania, *Sesbania exaltata* (Rafin.) Cory, (Azlin Seed Service, Leland, MS), and commercial rice (cv ‘Cocodrie’) were planted separately in 9 cm diameter pots in a greenhouse. Because experiments were conducted when plants were young and because roots were consumed by larvae, plants did not become root bound during these experiments. Potting soil consisted of a 4:2:1:1 mixture (by volume) of soil (commerce silt loam), peat moss, sand, and vermiculite. Fertilizer (0.8 g of 23:12:12 N:P:K) was added to each pot and incorporated into the soil mixture at planting. Natural light was not supplemented. Temperature in the greenhouse ranged from 25° to 35° C. Pots were placed in wooden basins lined with black plastic pond liner and watered daily until used in the experiments.

Rice water weevil adults were collected from rice fields at the LSU AgCenter Rice Research Station, near Crowley, Acadia Parish, LA. Adult weevils were maintained in glass jars with freshly cut rice leaves and a moistened paper towel. Weevils were collected for
each experiment approximately 24 hr before the experiment was conducted. Weevils were used only once and discarded.

**Determination of Rice Water Weevil Preference (Greenhouse Experiments)**

Interactions of the rice water weevil and its putative hosts were examined using a commercial rice cultivar and the seven weed species listed above. Hemp sesbania, the only dicot species in these experiments, was used as a negative control, because a dicot was not expected to be a suitable host. Plants were thinned to three per pot for each plant species. Because plant age may influence insect preference, plant age was maintained constant for all replications. Experiments were initiated when all plants except hemp sesbania and yellow nutsedge possessed three to four leaf fully expanded leaves. Hemp sesbania had four fully expanded leaflets and yellow nutsedge was approximately 28 cm tall when experiments began. Two pots (six plants) of each plant species were placed in each of three to eight cylindrical infestation cages. Cages used for adult rice water weevil infestations were constructed of wire frame and covered with insect screen. Dimensions of the cylindrical cages were 46 cm in diameter and 61 cm in height. Basins were flooded to a depth of 18 cm such that 8 cm of plants were under water. Preliminary tests demonstrated infestation densities of four adults per plant for 4 d (Heinrich et al. 1985) resulted in mortality of barnyardgrass and yellow nutsedge because of severe adult feeding, oviposition and/or larval feeding. Therefore, plants were infested with two adult rice water weevils per plant (96 weevils per cage) for 4 d. Adults were placed in the center of the cage and allowed to move freely throughout the cage. Four days after the initiation of adult infestation, pots were removed from cages and adult weevils were removed from plants.
At the time plants were removed from cages, two plants were destructively harvested from each pot and taken to a laboratory to obtain two independent measures of rice water weevil preference and host suitability. One plant was used to assess ovipositional preference. The second plant was used to determine densities of neonates associated with each plant. The third plant in each pot was left in the greenhouse. Late instar densities from the last plant were determined 21 d after removal of plants from cages. Larvae were mostly third or fourth instars by this time and few pupae were found. Numbers of late instars also provided information on both preference and host quality.

**Oviposition Preference**

Ovipositional preference was determined from one plant in each pot by counting eggs in the leaf sheaths of graminaceous plants (Gifford and Trahan 1969). All plants were bleached in a 75% ethanol/water solution for several weeks before counting eggs. Eggs were counted using a dissecting microscope (40X magnification). Because hemp sesbania does not have a leaf sheath, stems of hemp sesbania were cut longitudinally under a dissecting scope to examine the incision line for eggs. Stems of hemp sesbania are hollow; therefore, stems could be opened easily and flattened. The vascular tissue was separated from the epidermis of the stem. Both vascular and epidermal tissues were examined under the microscope for eggs; however, all eggs found on hemp sesbania were in the epidermal layer of the stem.

**Eclosion of Neonates**

A second plant was used to determine numbers of first instars eclosing from eggs on each plant by placing individual plants in test tubes filled with water (Bowling 1973). Test tubes were housed in an environmentally controlled growth chamber [25 °C, 16:8 (L:D) h].
When larvae eclose, larvae migrate to the roots to begin feeding; consequently, larvae sink to the bottom of test tubes. Water was emptied into a Petri dish and neonate larvae were counted and discarded. To ensure larvae did not remain on roots, plants were shaken vigorously in test tubes filled with water. Each plant was placed back in the test tube and refilled with water. First instars were counted every other day for two weeks.

**Densities and Weights of Late Instars**

Approximately 25 d after initiation of adult infestations, the final plant was removed from each pot and roots and soil of plants were washed into 40 mesh screen buckets (12.7 cm X 17.8 cm) (Smith and Robinson 1982). Buckets were placed in a saturated saline solution, causing rice water weevil larvae to float to the surface. Larvae were collected, taken to the laboratory, and lyophilized for 48h. Larvae from each plant were pooled and weighed to the nearest mg. Total weight was divided by the number of larvae to obtain an average larval weight.

**Data Analysis**

This entire experiment was replicated three times. Replication size differed due to difficulties in synchronizing eight plant species at a single stage of growth. The first replication had eight cages (initiated on May 25, 2001); the second replication had three cages (initiated on June 30, 2001), and the third replication had five cages (initiated on July 15, 2001). There was a total of 32 plants of each species for each parameter measured.

Numbers of eggs, neonate larvae, larvae collected 25 d after adult infestations, and larval weights were analyzed using one-way ANOVA and means were separated using Tukey’s studentized range test (SAS Institute, 1998). Hemp sesbania was not included in
the analysis and data were log transformed before analysis to meet the assumption of normality. Non-transformed means are presented in the results.

Survival of insects from one life stage to the next was determined by comparing the number of insects at each stage in the following manner. When plants were removed to measure each parameter (i.e., number of eggs, neonates or 21 d old larvae), care was taken to trace from which pot data were collected from so that comparisons between life stages could be made for each pot. Survival of insects from the egg stage to late instar stage was assessed by using a t-test to compare egg densities and late instar densities for each pot. Survival of insects on different hosts was compared by examining the proportion of eggs surviving to 21 d old larvae using one-way ANOVA. Means were separated using Tukey’s studentized range test (SAS Institute, 1998). Possible correlation between egg densities and mortality of rice water weevils was examined using Pearson correlation coefficient in PROC CORR in SAS.

**Adult Feeding Preference**

Adult feeding preference was examined using rice and the previously listed weed species. Petri dishes (150 mm X 15 mm) were lined with moistened cotton batting. The cotton batting was sectioned into eight quadrants. Each quadrant was randomly assigned plant material from single plant species. Foliage for adults was 4 cm of a leaf blade of the monocot plant species or 4 cm of a hemp sesbania leaflet. Rice water weevil adults were starved for three hours before the experiment to ensure feeding. One adult was placed in the center of each dish and allowed to move freely about the petri dish. After 24 h, adults were removed from dishes, and the length (mm) of feeding scars was measured. Feeding scars of rice water weevil are approximately 1 mm in width, allowing simple calculation of area
consumed. This experiment was conducted three times: 15 adults were used in the first experiment, 25 in the second and 23 in the third. Data were analyzed using one-way ANOVA (completely randomized design) and means were separated by Tukey’s studentized range test (SAS Institute, 1998). Data from hemp sesbania were not included in the analysis and data were log transformed before analysis to meet the assumption of normality. Data are presented as non-transformed means.

**Life Cycle Compatibility**

The following no-choice experiments were performed to determine if rice water weevils are capable of completing their life cycle on the eight plants studied. Six to 12 pots containing one plant of a single plant species were placed in an adult infestation cage (previously described). After 25 d, roots of half the plants of each species were sampled to verify presence of larvae. Foliage of remaining plants was trimmed to remove feeding scars made by adults during the initial infestation. After trimming the foliage, plants were individually covered with cages constructed of wire screen (12.5 cm in diameter and 55 cm in height). Appearance of new feeding scars was used as an indicator that rice water weevil adults had eclosed and completed their life cycle on the host. Because hemp sesbania was shown to be a poor host for adult feeding, non-infested rice plants were placed inside hemp sesbania cages so that any adults emerging from hemp sesbania roots would have a suitable host present on which adults could feed. Rice plants were planted in 50 ml conical vials. One vial with a rice plant was added to each pot of hemp sesbania. These rice plants were placed in vials to prevent larvae present on roots of hemp sesbania from moving to rice and completing their life cycle on rice. Plants were checked every other day for adult feeding scars. The intent of this experiment was to determine whether or not rice water weevil could
complete their life cycle on these various weeds. Therefore, once adult feeding was detected on a plant species, no additional replications were conducted with that plant species. Different methods would be needed to quantify eclosion of adults on each plant species. Two replications were performed with hemp sesbania only because evidence of adult feeding was not observed in the first replication. Table 2.1 shows the number of plants of each species used to determine presence of larval infestations and suitability for insect development.

Field Sampling

Sixteen dicotyledonous and monocotyledonous weed species (Table 2.2) were sampled in rice fields for presence of larvae and pupae on roots. Weeds were sampled throughout the season at several locations. Sample sites included two fields in south Louisiana, both at LSU AgCenter’s Rice Research Station, and three fields in north Louisiana, in Jonesville (Catahoula Parish), Winnsboro (Franklin Parish), and St. Joseph (Tensas Parish). Not all weeds were present at each location. Weeds that were isolated from adjacent plants were sampled to prevent root tissue of other plant species being intertwined with the targeted plant species. Root/soil samples were taken with a metal soil corer (9.2 cm diameter with a depth of 7.6 cm). Roots and soil were sampled for larvae as previously described. This field survey was conducted to determine whether the rice water weevil is able to utilize non-rice hosts under field conditions.

Results

Oviposition Preferences

Densities of eggs oviposited on plants differed among plant species (Table 2.3; $F = 67.34; df = 6, 90; P < 0.0001$). Barnyardgrass, fall panicum, yellow nutsedge, and broadleaf
Table 2.1. Assessment of the ability of rice water weevil to complete its life cycle on commercial rice (Cv. Cocodrie) and seven common weeds in greenhouse experiments. Presence of adult feeding was used as an indicator of completion of life cycle

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Larvae per plant (S.E.)</th>
<th>n</th>
<th>% plants with adult feeding</th>
<th>Development time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice (Cv. ‘Cocodrie’)</td>
<td>10.6 (4.2)</td>
<td>3</td>
<td>100</td>
<td>33-43</td>
</tr>
<tr>
<td>Barnyardgrass</td>
<td>14.3 (2.9)</td>
<td>6</td>
<td>100</td>
<td>28-45</td>
</tr>
<tr>
<td>Fall panicum</td>
<td>7.5 (1.9)</td>
<td>4</td>
<td>100</td>
<td>36-52</td>
</tr>
<tr>
<td>Hemp sesbania</td>
<td>0.0 (0.0)</td>
<td>12</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>Red rice</td>
<td>17.8 (4.5)</td>
<td>6</td>
<td>83</td>
<td>36-52</td>
</tr>
<tr>
<td>Yellow nutsedge</td>
<td>2.0 (2.0)</td>
<td>6</td>
<td>67</td>
<td>35-43</td>
</tr>
<tr>
<td>Broadleaf signalgrass</td>
<td>4.2 (0.7)</td>
<td>6</td>
<td>67</td>
<td>52-58</td>
</tr>
<tr>
<td>Amazon sprangletop</td>
<td>11.4 (2.0)</td>
<td>5</td>
<td>100</td>
<td>36-54</td>
</tr>
</tbody>
</table>

| \(^a\) Number of larvae present when plants were sampled to ensure infestation |
| \(^b\) n = number of plants sampled for both larval infestations and for adult feeding scars |
| \(^c\) [(plants with adult feeding) / (plants sampled)] *100 |
| \(^d\) Number of days from the initiation of the experiment that adult feeding was detected |

signalgrass were more preferred for oviposition than commercial rice. Amazon sprangletop was less preferred for oviposition than commercial rice. There were no significant differences in egg densities on red rice and commercial rice. Hemp sesbania had less than one egg per plant.

**Eclosion of Neonates**

More neonates eclosed from barnyardgrass and yellow nutsedge than from commercial rice (Table 2.3; \( F = 37.62; \) df = 6, 90; \( P < 0.0001 \)). Fewer neonates eclosed from Amazon sprangletop than from commercial rice. Numbers of neonates found on red rice, fall panicum, and broadleaf signalgrass were not significantly different from numbers on commercial rice. Eclosion from hemp sesbania averaged less than one neonate per plant.
**Table 2.2.** Infestation of common monocot (M) and dicot (D) weeds in Louisiana rice fields by rice water weevil larvae and pupae.

<table>
<thead>
<tr>
<th>Weed Species</th>
<th>Plant type</th>
<th>n(^a)</th>
<th>Larvae (SE) per plant</th>
<th>% plants infested with larvae</th>
<th>Pupae (SE) per plant</th>
<th>% plants infested with pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeschynomene indica</em> L., joint vetch</td>
<td>D</td>
<td>11</td>
<td>0.82 (0.35)</td>
<td>45.5</td>
<td>0.09 (0.09)</td>
<td>9.1</td>
</tr>
<tr>
<td><em>Alternathera philoxeroides</em> (Mart.) Griseb., alligator weed</td>
<td>D</td>
<td>41</td>
<td>0.32 (0.10)</td>
<td>22.0</td>
<td>0.00 (0.00)</td>
<td>0</td>
</tr>
<tr>
<td><em>Amaranthus</em> spp., amaranths</td>
<td>D</td>
<td>9</td>
<td>0.42 (0.26)</td>
<td>22.2</td>
<td>0.00 (0.00)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. platyphylla</em> (Griseb.) Nash., broadleaf signalgrass</td>
<td>M</td>
<td>54</td>
<td>0.80 (0.19)</td>
<td>38.9</td>
<td>0.04 (0.03)</td>
<td>3.7</td>
</tr>
<tr>
<td><em>Caperonia palustris</em> (L.) St.-Hil., Texas weed</td>
<td>D</td>
<td>40</td>
<td>1.90 (0.54)</td>
<td>47.4</td>
<td>0.55 (0.14)</td>
<td>34.2</td>
</tr>
<tr>
<td><em>C. dactylon</em> (L.) Pers., bermudagrass</td>
<td>M</td>
<td>39</td>
<td>0.90 (0.28)</td>
<td>35.9</td>
<td>0.00 (0.00)</td>
<td>0</td>
</tr>
<tr>
<td><em>Cyperus</em> spp., sedges</td>
<td>M</td>
<td>52</td>
<td>2.85 (0.47)</td>
<td>75.0</td>
<td>0.15 (0.06)</td>
<td>13.5</td>
</tr>
<tr>
<td><em>E. crus-galli</em> (L.) Beauv., barnyardgrass</td>
<td>M</td>
<td>50</td>
<td>18.12 (2.42)</td>
<td>94.0</td>
<td>0.72 (0.21)</td>
<td>26.0</td>
</tr>
<tr>
<td><em>Eclipta alba</em> (L.) Hassk., Eclipta</td>
<td>D</td>
<td>43</td>
<td>0.81 (0.25)</td>
<td>26.2</td>
<td>0.33 (0.14)</td>
<td>14.3</td>
</tr>
<tr>
<td><em>Heteranthera limosa</em> (Sw.) Willd., duck salad</td>
<td>D</td>
<td>43</td>
<td>0.21 (0.09)</td>
<td>11.6</td>
<td>0.07 (0.07)</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Ipomoea</em> spp., morningglories</td>
<td>D</td>
<td>9</td>
<td>0.67 (0.29)</td>
<td>44.4</td>
<td>0.00 (0.00)</td>
<td>0</td>
</tr>
<tr>
<td><em>Leptochola</em> spp., sprangletop</td>
<td>M</td>
<td>40</td>
<td>6.85 (0.89)</td>
<td>92.5</td>
<td>0.55 (0.20)</td>
<td>22.5</td>
</tr>
<tr>
<td><em>O. sativa</em> L., red rice</td>
<td>M</td>
<td>24</td>
<td>1.96 (0.62)</td>
<td>50.0</td>
<td>0.21 (0.10)</td>
<td>16.7</td>
</tr>
<tr>
<td><em>O. sativa</em> L., commercial rice varieties</td>
<td>M</td>
<td>42</td>
<td>8.30 (1.99)</td>
<td>71.9</td>
<td>0.74 (0.16)</td>
<td>38.6</td>
</tr>
<tr>
<td><em>P. dichotomiflorum</em> Michx., fall panicum</td>
<td>M</td>
<td>7</td>
<td>3.86 (0.88)</td>
<td>100</td>
<td>0.29 (0.29)</td>
<td>14.3</td>
</tr>
<tr>
<td><em>Paspalum dilatatum</em> Poir., dallisgrass</td>
<td>M</td>
<td>3</td>
<td>0.33 (0.33)</td>
<td>33.3</td>
<td>0.00 (0.00)</td>
<td>0</td>
</tr>
<tr>
<td><em>S. exaltata</em> (Raf.) A.W. Hill., hemp sesbania</td>
<td>D</td>
<td>42</td>
<td>0.21 (0.09)</td>
<td>16.7</td>
<td>0.00 (0.00)</td>
<td>0</td>
</tr>
</tbody>
</table>

\(a\) n = number of plants sampled

**Densities of Late Instars**

Fewer late instars were found on roots of yellow nutsedge and broadleaf signalgrass than on roots of commercial rice (Table 2.3; \(F = 14.87; \text{df} = 6, 90; P < 0.0001\)). There were no other significant differences in numbers of late instars on other species compared with numbers found on commercial rice.
Table 2.3. Preference of rice water weevils on commercial rice and seven weed species in greenhouse choice tests. Survival was characterized by examining the number of eggs surviving to larvae 25 days after adult infestation.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Eggs (S.E.) per plant</th>
<th>Neonates (S.E.) per plant</th>
<th>Late instars (S.E.)&lt;sup&gt;a&lt;/sup&gt; per plant</th>
<th>Wt (mg) (S.E.)</th>
<th>Survival&lt;sup&gt;b&lt;/sup&gt; &lt;i&gt;t&lt;/i&gt;, df, &lt;i&gt;P&lt;/i&gt;</th>
<th>Percent survival&lt;sup&gt;c&lt;/sup&gt; (S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice (Cv. ’Cocodrie’)</td>
<td>13.25 (2.14)</td>
<td>9.45 (1.29)</td>
<td>7.16 (0.81)</td>
<td>0.673 (0.084)</td>
<td>2.66, 62, &lt;i&gt;P&lt;/i&gt; &lt;0.0100</td>
<td>54.04 (7.08)</td>
</tr>
<tr>
<td>Barnyardgrass</td>
<td>48.68 (6.13)</td>
<td>17.66 (1.45)</td>
<td>9.47 (0.94)</td>
<td>0.417 (0.044)</td>
<td>4.74, 62, &lt;i&gt;P&lt;/i&gt; &lt;0.0001</td>
<td>19.45 (4.50)</td>
</tr>
<tr>
<td>Fall panicum</td>
<td>62.53 (4.92)</td>
<td>26.87 (5.25)</td>
<td>4.75 (0.66)</td>
<td>0.503 (0.112)</td>
<td>4.26, 61, &lt;i&gt;P&lt;/i&gt; &lt;0.0001</td>
<td>7.59 (2.11)</td>
</tr>
<tr>
<td>Red rice</td>
<td>13.10 (2.34)</td>
<td>6.41 (1.69)</td>
<td>11.19 (1.39)</td>
<td>1.299 (0.215)</td>
<td>0.71, 61, &lt;i&gt;P&lt;/i&gt; &lt;0.4832</td>
<td>85.42 (6.04)</td>
</tr>
<tr>
<td>Yellow nutsedge</td>
<td>99.47 (8.11)</td>
<td>42.41 (5.19)</td>
<td>2.5 (0.81)</td>
<td>0.293 (0.125)</td>
<td>7.60, 62, &lt;i&gt;P&lt;/i&gt; &lt;0.0001</td>
<td>2.51 (1.08)</td>
</tr>
<tr>
<td>Broadleaf signalgrass</td>
<td>31.13 (4.25)</td>
<td>7.94 (1.85)</td>
<td>3.06 (0.41)</td>
<td>0.137 (0.020)</td>
<td>2.75, 61, &lt;i&gt;P&lt;/i&gt; &lt;0.0125</td>
<td>9.82 (4.90)</td>
</tr>
<tr>
<td>Amazon sprangletop</td>
<td>3.48 (1.21)</td>
<td>2.84 (1.15)</td>
<td>6.23 (1.02)</td>
<td>0.859 (0.113)</td>
<td>1.75, 61, &lt;i&gt;P&lt;/i&gt; &lt;0.0878</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Hemp sesbania&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.97 (0.37)</td>
<td>0.94 (0.21)</td>
<td>0.13 (0.59)</td>
<td>0.350 (0.144)</td>
<td>3.80, 62, &lt;i&gt;P&lt;/i&gt; &lt;0.0003</td>
<td>12.90 (4.39)</td>
</tr>
</tbody>
</table>

* means followed by different letters in the same column are significantly different. Means were separated with Tukey’s studentized range test <i>α</i> = 0.05.

<sup>a</sup> Larvae collected 25 d after initiation of adult infestation; 80-90% of larvae were 3<sup>rd</sup> and 4<sup>th</sup> instars

<sup>b</sup> Survival from egg to late instar; differences determined using paired t-tests

<sup>c</sup> (Mean number of late instars / mean number of eggs) * 100

<sup>d</sup> Hemp sesbania data were not included in statistical analysis

Weights of Late Instars

Dry weights of larva feeding on roots of red rice were significantly larger than weights of larvae from commercial rice plants (Table 2.3; F = 16.44; df = 6, 90; <i>P</i> < 0.0001).

Weights of larvae on roots of broadleaf signalgrass were significantly lower than weights of larvae from commercial rice. Larval weights on the remaining plant species were not significantly different from those on commercial rice.
Survival

Paired t-tests were used to assess survival of rice water weevil from egg to late instar on each plant species (Table 2.3). Densities of late instars were significantly lower than densities of eggs on barnyardgrass, commercial rice, fall panicum, hemp sesbania, yellow nutsedge, and broadleaf signalgrass. There was no significant difference in numbers of late instars and eggs on red rice or Amazon sprangletop. The percentage of eggs surviving to late instars was lower on barnyardgrass, fall panicum, yellow nutsedge, and broadleaf signalgrass than on commercial rice (Table 2.3; $F = 45.96; \text{df} = 6, 90; P < 0.0001$). Density of eggs and mortality of rice water weevils were significantly correlated (Pearson correlation coefficient $= 0.8127; P < 0.0263$).

Adult Feeding Preference

Foliage of barnyardgrass received significantly more feeding than all other plant species (Figure 2.1; $F = 7.52; \text{df} = 6, 405; P < 0.0001$). Feeding on the remaining weeds was intermediate and did not differ from feeding on commercial rice. Fall panicum and red rice were significantly more preferred than yellow nutsedge and broadleaf signalgrass. Hemp sesbania had little adult feeding.

Life Cycle Compatibility

Larvae were present on roots of all plant species except hemp sesbania (Table 2.1). With the exception of hemp sesbania, adult feeding was present on over 60% of plants sampled for each plant species, demonstrating that the rice water weevil is able of completing its life cycle on those hosts. Feeding scars were found 28 to 58 d after adult infestations, suggesting that one to two months are required for the completion of the life cycle, depending on the host.
Figure 2.1. Feeding by rice water weevil adults on commercial rice (Cv. ‘Cocodrie’) and common weeds of rice (n = 63). Different letters represent significant differences in amount of feeding between plant species (Tukey’s studentized range test) * Hemp sesbania was not included in the analysis.

Field Sampling

Rice water weevils were able to use plant species other than commercial rice as hosts under field conditions. Larvae were found on all plant species sampled, including dicot plants (Table 2.2). Percent larval infestations ranged from 11 – 47% on dicot weed species whereas infestations on monocot plant species ranged from 33 – 100%. Pupae were found on 65% of plants sampled. Monocot species generally had more larvae and pupae than dicot species.
Discussion

An understanding of interactions among weeds and insects is necessary to develop an integrated management program for these two types of pests. Data presented in this paper provide information on the use of several common weeds in Louisiana rice fields by rice water weevils. The host range of rice water weevil appears to be much broader than previously reported. Several new hosts, all of which are common weeds in rice fields, can be added to the existing list of hosts of the rice water weevil. Broadleaf signalgrass, yellow nutsedge, and Amazon sprangletop are newly documented hosts on which rice water weevils were shown to complete its life cycle. Larvae were also associated with roots of joint vetch, alligator weed, *Amaranthus* spp., Texas weed, eclipta, duck salad, *Ipomoea* spp., and hemp sesbania in field surveys (Table 2.2). Although larvae were found on roots of dicot plants, monocots were more preferred by rice water weevils in greenhouse experiments. These data support previous information that rice water weevils predominately feed on monocot plants (Webb 1914, Isley and Schwardt 1934, Lange and Grigarick 1959).

There are several possible explanations for the presence of larvae on roots of dicot plants in the field. First, it is possible that some rice roots were mixed with roots of sampled weeds, and larvae were actually feeding on roots of rice in the root/soil sample. However, care was taken to sample isolated weeds and prevent rice roots from being included in the sample, so this explanation is unlikely to account for larvae on roots. Second, adults may have oviposited on these dicot plants. This explanation is probable for at least some dicots because rice water weevils oviposited on 25% of hemp sesbania plants presented to adults in greenhouse experiments. Third, adults may have oviposited on monocots, and larvae may have changed hosts underground. Roots of dicot plants may be acceptable hosts for larval
feeding irrespective of their suitability for oviposition. Nordenhem and Norlander (1994) showed that subterranean weevils, *Hylobius abietis* (L.), were able to move 100 mm in sand to relocate to non-inhabited hosts. Laboratory observations have shown rice water weevils are capable of movement on root systems of individual plants (personal observation). However, experiments are needed to characterize larval movement on roots of individual plants or roots of different hosts. Fourth, dicot plants may be more acceptable (either for oviposition or larval feeding) when rice water weevils reach high densities and overcrowding forces competition between weevils. Bigger and Fox (1997) found that diamondback moths, *Plutella xylostella* L., had a broader host range when population densities were high. Rice fields in South Louisiana typically have greater densities than in fields in North Louisiana. Texas weed collected in South Louisiana had an average of 8 larvae per core, but in North Louisiana the average was less than one larva per core. Overcrowding may have caused rice water weevil to move to Texas weed in South Louisiana.

Although field surveys provided supplementary information about host range, temporal and spatial variation in presence of weeds, sampling procedures, and environmental conditions preclude field data from being used to conclusively determine relative host preference. All plant species were not sampled at each field location because they were not present at all locations. In addition, there was considerable variation in collection dates and field locations. The time of season when samples are taken can affect numbers of larvae and pupae present. Some fields were planted with rice seed treated with an insecticide, fipronil. Although fipronil was not applied to seeds of weeds, no research is available to determine the degree to which fipronil leaches into the soil or what effects it
may have on insects on roots of neighboring plants. There was tremendous variation in the field survey; however, the intent of the experiment (i.e. to determine if the plant was used by rice water weevil in the field) was accomplished and showed that roots of most of the common weeds in Louisiana rice fields were infested with larvae. More controlled studies are required to determine the suitability and preference of hosts in the field.

Significant mortality from egg to larval stage was observed on all plant species except red rice and Amazon sprangletop. The average number of eggs, neonate larvae, and late instars for all species combined were 34, 14, and 6, respectively. There were significant differences in mortality when comparing across plant species, and mortality was shown to be strongly correlated to number of eggs oviposited. Although this is not conclusive evidence, it appears that some level of intraspecific competition occurs among rice water weevils. Ideally, investigations of intraspecific competition would be conducted with known densities of larvae; however, currently there are no effective methods for transfer of larvae to root systems. Another possible explanation for mortality is predation of rice water weevils. However, there are few effective predators of rice water weevils (Puissegur 1976), and predators were not present in the greenhouse.

Caswell et al (1973) proposed that insects are more likely to avoid $C_4$ plants than $C_3$ plants because $C_3$ plants are a superior food source for insects. The authors state this hypothesis is not absolute. Rice water weevil would be an exception to the hypothesis because all $C_4$ plants (i.e. barnyardgrass, yellow nutsedge, broadleaf signalgrass, and fall panicum [Elmore and Paul 1983]) were more preferred for oviposition than $C_3$ plants. Although mortality was high on $C_4$ plants, mortality was likely due at least partially to intraspecific competition resulting from high levels of oviposition.
Weeds used in this study were not equally suitable for rice water weevil development. Initial densities of larvae (i.e., numbers of neonates) were similar for commercial rice, fall panicum, red rice, and broadleaf signalgrass. However, larval weights on broadleaf signalgrass were nearly five times lower than larval weights on rice. Thus, broadleaf signalgrass may be less suitable for larval development. Larvae feeding on red rice, a weedy rice with red pericarp, were nearly twice as heavy as those on commercial rice. Although red rice is the same species as commercial rice, it may be a better host for rice water weevil larvae.

Palrang et al. (1994) showed that rice fields grown adjacent to levees with high densities of weeds in California had more rice water weevils present after flooding than fields adjacent to weed-free levees. The authors suggest that weevils emerging from overwintering sites may use weeds on levees as food sources to replenish energy reserves for flight muscle regeneration and eventual flight. This behavior would keep weevils near the field until rice is flooded, at which time weevils could infest rice plants. Therefore, it could be advantageous to have weed-free levees prior to the flooding of rice. Data presented in this paper indicate adult rice water weevils preferred barnyardgrass and showed preferences for fall panicum and red rice over yellow nutsedge and broadleaf signalgrass. Therefore, composition of weeds on levees may be as important as the mere presence of weeds on levees. Additionally, if there are nearby areas, such as ditches or reservoirs, that have standing water with preferred hosts for oviposition, a generation of rice water weevils could develop prior to flooding of rice fields, resulting in more severe infestations. Further investigations are needed to understand interactions between rice water weevils and weeds.
to assist in the development of management strategies to manipulate populations of these pests.

References


CHAPTER 3

EFFECTS OF THE PRESENCE OF BARNYARDGRASS ON RICE WATER WEEVIL (COLEOPTERA: CURCULIONIDAE) AND RICE STINK BUG (HEMIPTERA: PENTATOMIDAE) POPULATIONS ON RICE *

Introduction

Several hypotheses have been developed to describe how neighboring plants influence insect behavior relative to a specific plant. ‘Associational resistance’ refers to an interaction in which a plant gains protection from herbivory due to surrounding plant composition (Andow 1991, Hambäck et al. 2000, White and Whitham 2000). Associational resistance may result from interference by nearby plants with visual or chemical host finding mechanisms of herbivores (Karban 1997, Hambäck et al. 2000) or from increases in densities of predators and/or parasitoids (Gurr and Wratten 1999, Mensah 1999).

‘Associational susceptibility’ refers to the opposite phenomenon in which herbivory increases on a plant due to the presence of nearby plants (Karban 1997, White and Whitham 2000). The presence of a preferred host may attract large numbers of insects. After consuming the preferred host or following changes in host suitability, insects may move to a less preferred host, which may not have been damaged in the absence of the preferred host (Andow 1991, White and Whitham 2000).

Currently, there is no consensus for predicting the response of an insect to the presence of mixtures of plants (vegetational diversity). Andow (1991) summarized previous research on insect response to vegetational diversity. This author found that in 59.1% of experiments with monophagous herbivores populations were lower in polycultures than in

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monocultures whereas populations of monophagous herbivores were higher in polycultures than monocultures in only 7.7% of experiments. Only 28.4% of experiments showed polyphagous insects had lower densities whereas 40.3% of studies had higher densities in polycultures than monocultures. Approximately 20% of experiments showed variable responses and 12% showed no difference in populations for both monophagous and polyphagous insects.

The two most important insect pests of rice in Louisiana are the rice water weevil, *Lissorhoptrus oryzae* Kuschel, and the rice stink bug, *Oebalus pugnax* (F.). Adult rice water weevils are semi-aquatic folivores and larvae are aquatic root herbivores. Adult weevils oviposit in leaf sheaths beneath the surface of flood waters. Larvae eclose, migrate to roots, and feed on root tissue (Smith 1983; Way 1990). Pruning of roots by larvae can reduce yields by 10 to 33% but yield loss can be as high as 70% under heavy pressure (Anonymous 1994). Because rice water weevils are aquatic, their known host range is limited to aquatic and water-tolerant plants. Several documented hosts of the rice water weevil are common weeds in rice agroecosystems and rice water weevils prefer many monocot weeds over rice (Tindall and Stout 2003).

While the host range of rice water weevil is limited to habitats with water, rice stink bugs are able to utilize both aquatic and non-aquatic habitats. Rice stink bugs are known to feed on numerous graminaceous weeds as well as six graminaceous crops (Odglen and Warren 1962, Nilakhe 1976, Naresh and Smith 1984, McPherson and McPherson 2000). Many alternate hosts for rice stink bugs occur either in or near rice fields (i.e., on levees and turn-rows, interspersed among rice or neighboring fields of corn or sorghum). Female rice stink bugs lay two rows of barrel-shaped green eggs on plant foliage or panicles (Odglen
and Warren 1962). Nymphs and adults remove endosperm from developing rice grains and reduce yield and grain quality. Rice stink bugs pass through five instars, but only late instars and adults are considered economically important.

Although these two insects do not utilize the same plant tissues, they show similarities in their host ranges and offer a unique opportunity to study insect-weed interactions in rice agroecosystems. Both insects are known to associate primarily with monocot plant species (Odglen and Warren 1962; Tindall and Stout 2003). Recent work has shown that the rice water weevil prefers several common weeds in rice fields over rice, including barnyardgrass, *Echinochloa crus-galli* Beauv. (Tindall and Stout 2003). Additionally, although it has not been adequately tested, there is evidence that suggests rice stink bugs prefer barnyardgrass over rice (Douglas 1939, Odglen and Warren 1962). Therefore, barnyardgrass was chosen to examine the impact of vegetational diversity on populations of rice water weevils and rice stink bugs in rice fields. Based on summaries of Andow (1988, 1991), it was hypothesized that higher numbers of grass-feeding insects would be found in rice grown in association with barnyardgrass (polyculture) compared to whole plots of rice (monoculture).

**Materials and Methods**

Experiments were conducted in northeast Louisiana at the Macon Ridge Research Station, Winnsboro (Franklin Parish), La., during the summers of 2001, 2002, and 2003. The soil at Winnsboro is a loessial upland soil (Gigger silt loam). Experimental design was a randomized block design with three replications in 2001 and four replications in 2002 and 2003. Table 3.1 provides dates of important agronomic practices and data collection.
Table 3.1 Dates of selected agronomic practices.

<table>
<thead>
<tr>
<th>Event</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planting date</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>May 29</td>
<td>May 28</td>
<td>May 27</td>
</tr>
<tr>
<td>Barnyardgrass (seed)</td>
<td>May 29</td>
<td>May 28, June 10</td>
<td>May 27</td>
</tr>
<tr>
<td>Permanent Flood</td>
<td>June 26</td>
<td>July 7</td>
<td>July 11</td>
</tr>
<tr>
<td>90-100% Panicle Emergence</td>
<td>Aug 30</td>
<td>Aug 27</td>
<td>Aug 27</td>
</tr>
<tr>
<td>Rice water weevil sampling dates</td>
<td>July 20</td>
<td>July 25</td>
<td>n/a</td>
</tr>
<tr>
<td>Rice stink bug sampling dates</td>
<td>Aug 9, 17, 24, 31</td>
<td>Aug 7, 15, 24, 28</td>
<td>Aug 9, 18, 28</td>
</tr>
<tr>
<td></td>
<td>Sept 5</td>
<td>Sept 8</td>
<td>Sept 4, 9</td>
</tr>
</tbody>
</table>

Each plot measured 4 m X 3 m and consisted of 20 rows of plants. Treatments consisted of three spatial arrangements of rice and barnyardgrass. Two treatments were mixed plots that differed in placement of barnyardgrass relative to rice. In one treatment, the interior 10 rows were planted with barnyardgrass and the outer five rows on either side were planted with rice. In the other mixed plot arrangement, the interior 10 rows were planted with rice and the outer five rows on either side planted with barnyardgrass. The remaining treatment consisted of both the interior 10 rows and exterior five rows on either side planted with rice. Fields used for this experiment have well-established populations of barnyardgrass and low densities of few other graminaceous weeds. However, to ensure good stands of barnyardgrass, barnyardgrass seed was incorporated into the soil with a rake when rice was planted. In 2001 and 2003, barnyardgrass emerged at the same time as rice; however, in 2002, barnyardgrass emerged approximately one month after rice.

Plots were spaced 4 m apart to isolate them from other plots. Herbicides were used to remove unwanted plants within and between plots. Herbicides used to remove weeds from rice were clomazone (Command®, FMC – 336 g AI/ha) and quinclorac (Facet®, BASF – 420...
g AI/ha), applied preemergence. Additionally, cyhalofop (Clincher®, Dow AgroSciences – 210 g AI/ha) was applied postemergence as needed (POST ASN). Halosulfuron-methyl (Permit®, Monsanto – 53 g AI/ha) was applied POST ASN to rice and barnyardgrass to control broadleaf weeds and sedges. There was no graminaceous weed control in barnyardgrass areas. Bare areas were treated with glyphosate (Roundup®, Monsanto – 2.3 L/ha), and Command, Permit and Facet (at rates previously listed).

Data Collection

Populations of rice water weevil larvae were estimated approximately 30 days after permanent floods were applied in 2001 and 2002 (Table 3.1). Larvae were sampled using a root-soil core sampler (9.2 cm diameter with a depth of 7.6 cm). Soil and larvae were washed from roots of plants into 40 mesh screen buckets. Buckets were placed in a saturated saline solution, causing rice water weevil larvae to float to the surface so larvae could be counted (Smith and Robinson 1982). Six core samples were taken from each plot. When sampling in mixed plots, samples were taken from the barnyardgrass area and the rice areas of each plot. Although data were collected from both the interior and exterior portions of plots, only data from the rice portion of the plot are presented. Whole plots of rice were sampled in the same manner as rice in mixed plots (i.e., 3 root/soil samples were taken from the interior portion and 3 samples from exterior portions of plots).

Rice stink bugs were sampled with a sweep net (15 cm in diameter) in the interior and exterior portions of plots. Forty sweeps were made per plot, 20 from the interior portions of plots and 20 from exterior portions of plots. Sampling on outer margins of plots was conducted by sweeping the length of the plot, 10 sweeps per side. Sampling from the interior portion of plots was accomplished by walking through the middle of the plot,
sweeping across rows. Since plots were sampled twice (both inner and outer portions of plots) and sweeping disturbs insects, sweeps were separated by at least one hour. Sampling for rice stink bugs began approximately two weeks prior to panicle emergence of rice and continued weekly for five weeks.

Data Analysis

For each sample date, numbers of rice stink bugs and rice water weevils were subjected to ANOVA using PROC MIXED in SAS (SAS Institute, 1998) to assess overall effects of treatments (spatial arrangement of rice relative to barnyardgrass). Contrast statements were used to compare densities of insects found on rice from the interior portion of whole plots of rice and insect populations on rice collected from the interior portion of mixed plots. Likewise, contrasts were made between insect populations on rice from exterior portions of whole plots of rice and insect populations on rice collected from exterior portions of mixed plots. Numbers of insects collected were log transformed prior to analysis to meet the assumption of normality. Non-transformed means are presented in the results.

Results

Presence of barnyardgrass did not alter numbers of rice water weevil on rice roots in either arrangement of mixed plots in 2001 or 2002. In 2001, the average density of rice water weevils on rice in the exterior portion of mixed plots was 6.67 larvae per core and 7.89 larvae per core in exterior portions of whole plots of rice ($F = 1.35; df = 1, 28; P = 0.2547$). When rice was located in the interior of mixed plots, the average number of rice water weevils was 4.56 compared with 8.11 found in corresponding portions of whole plots ($F = 2.84; df = 1, 28; P = 0.1029$). The average number of larvae found in 2002 in the exterior area of plots was 1.17 and 1.83 in mixed plots and whole plots, respectively ($F = 60$
There was an average of 1.08 larvae per core found in the interior portion of rice in mixed plots and 0.42 in the interior of whole plots of rice ($F = 1.67; df = 1, 39; P = 0.2035$).

In 2001 and 2003, but not 2002, rice stink bug populations were higher in rice grown in association with barnyardgrass than in rice grown in association with rice at several sampling points (Table 3.2, 3.3, and 3.4). Numbers of adults, nymphs, or total rice stink bugs were equal or numerically or statistically greater in samples from mixed plots than in samples from whole plots of rice in 26 of 30 comparisons in 2001 and 21 of 30 comparisons in 2003. In 2001, significant treatment effects were found only on Aug 24 and Sept. 5 (Table 3.2). Adults and total numbers were at least five times greater in rice in either spatial arrangement of mixed plots on Aug. 24 than in entire plots of rice. Nine times more adults were collected on rice surrounded by barnyardgrass than on rice surrounded by rice on Sept. 5. Similarly, in 2003, numbers of both nymphs and total rice stink bugs were at least 1.5 times greater in both arrangements of mixed plots than in whole plots of rice on Aug. 28 (Table 3.3). On Sept. 4, rice stink bugs were 2.5 to 4 times greater on rice in the interior portions of mixed plots than in whole plots of rice. Numbers of nymphs and total rice stink bugs were at least 2 times greater on rice in interior of mixed plots compared to whole plots of rice on Sept. 9.

Presence of barnyardgrass also influenced densities of rice stink bugs collected from rice in 2002 (Table 3.4). Rice stink bugs were numerically or statistically greater on rice in mixed plots compared to whole plots for the first four sampling dates, with the exception of nymphs being greater in whole plots of rice on Aug. 7 and Aug. 28. On the second sample
Table 3.2. Mean number of rice stink bugs per 20 sweeps found in 2001 on rice when grown in association with barnyardgrass (BYG) compared to numbers found in whole plots of rice.

<table>
<thead>
<tr>
<th>Date</th>
<th>Plant in Interior</th>
<th>Plant in Exterior</th>
<th>Area Sampled</th>
<th>Nymphs ± SE</th>
<th>Adults ± SE</th>
<th>Total ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-9-01</td>
<td>Rice</td>
<td>BYG</td>
<td>Interior</td>
<td>0.33 ± 0.33</td>
<td>1.33 ± 0.88</td>
<td>1.67 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Interior</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>BYG</td>
<td>Rice</td>
<td>Exterior</td>
<td>1.0 ± 1.0</td>
<td>0 ± 0</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Exterior</td>
<td>0 ± 0</td>
<td>0.33 ± 0.33</td>
<td>0.33 ± 0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$F = 0.73; df = 3, 6;$</td>
<td>$F = 2.00; df = 3, 6;$</td>
<td>$F = 1.12; df = 3, 6;$</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$P = 0.5690$</td>
<td>$P = 0.2156$</td>
<td>$P = 0.4122$</td>
</tr>
<tr>
<td>8-17-01</td>
<td>Rice</td>
<td>BYG</td>
<td>Interior</td>
<td>1.33 ± 1.33</td>
<td>1.0 ± 1.0</td>
<td>2.33 ± 2.33</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Interior</td>
<td>0 ± 0</td>
<td>0.33 ± 0.33</td>
<td>0.33 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>BYG</td>
<td>Rice</td>
<td>Exterior</td>
<td>1.33 ± 0.67</td>
<td>1.0 ± 1.0</td>
<td>2.33 ± 1.45</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Exterior</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
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<td>$F = 1.58; df = 3, 6;$</td>
<td>$F = 1.00; df = 3, 6;$</td>
<td>$F = 1.72; df = 3, 6;$</td>
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<td>$P = 0.4547$</td>
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<td>8-24-01</td>
<td>Rice</td>
<td>BYG</td>
<td>Interior</td>
<td>5.0 ± 2.65</td>
<td>3.33 ± 0.88</td>
<td>8.33 ± 2.91</td>
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<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Interior</td>
<td>1.0 ± 1.00</td>
<td>0.67 ± 0.67</td>
<td>1.67 ± 1.67</td>
</tr>
<tr>
<td></td>
<td>BYG</td>
<td>Rice</td>
<td>Exterior</td>
<td>8.33 ± 5.46</td>
<td>6.67 ± 3.28</td>
<td>15.0 ± 8.5</td>
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<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Exterior</td>
<td>1.33 ± 1.33</td>
<td>0.33 ± 0.33</td>
<td>1.67 ± 1.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$F = 2.75; df = 3, 6;$</td>
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<td></td>
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<td></td>
<td>$P = 0.1349$</td>
<td>$P = 0.0004$</td>
<td>$P = 0.0077$</td>
</tr>
<tr>
<td>8-31-01</td>
<td>Rice</td>
<td>BYG</td>
<td>Interior</td>
<td>4.67 ± 4.67</td>
<td>1.67 ± 0.88</td>
<td>6.33 ± 3.84</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Interior</td>
<td>3.0 ± 1.0</td>
<td>3.67 ± 2.73</td>
<td>6.67 ± 2.19</td>
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<tr>
<td></td>
<td>BYG</td>
<td>Rice</td>
<td>Exterior</td>
<td>2.0 ± 1.15</td>
<td>6.0 ± 2.08</td>
<td>8.0 ± 3.0</td>
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<tr>
<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Exterior</td>
<td>1.67 ± 0.33</td>
<td>4.33 ± 3.84</td>
<td>6.0 ± 4.0</td>
</tr>
<tr>
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<td>$F = 0.15; df = 3, 6;$</td>
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<td>$F = 0.27; df = 3, 6;$</td>
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<td>$P = 0.9248$</td>
<td>$P = 0.3033$</td>
<td>$P = 0.8467$</td>
</tr>
<tr>
<td>9-5-01*</td>
<td>Rice</td>
<td>BYG</td>
<td>Interior</td>
<td>24.0 ± 3.79</td>
<td>11.33 ± 2.96</td>
<td>35.33 ± 6.36</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Interior</td>
<td>7.0 ± 2.52</td>
<td>11.33 ± 4.41</td>
<td>18.33 ± 6.64</td>
</tr>
<tr>
<td></td>
<td>BYG</td>
<td>Rice</td>
<td>Exterior</td>
<td>14.0 ± 3.61</td>
<td>34.0 ± 10.54</td>
<td>48.0 ± 14.0</td>
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<td></td>
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<td>Rice</td>
<td>Exterior</td>
<td>16.67 ± 4.7</td>
<td>3.67 ± 3.18</td>
<td>20.33 ± 4.26</td>
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<td>$P = 0.1529$</td>
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</tbody>
</table>

* Treatments consisted of plots of rice and barnyardgrass in the following spatial arrangements: rice in the interior portion of plots, BYG in the exterior portions; rice in the exterior portions of plots, BYG in the interior portion; and rice in both interior and exterior portions of plots

b F-values presented for treatment effects

c bold means denote significance at $P < 0.05$; means separated by contrast statements

* denotes 90-100% panicle emergence of rice.
Table 3.3. Mean number of rice stink bugs per 20 sweeps found in 2003 on rice when grown in association with barnyardgrass (BYG) compared to numbers found in whole plots of rice.

<table>
<thead>
<tr>
<th>Date</th>
<th>Plant in Interior</th>
<th>Plant in Exterior</th>
<th>Area Sampled</th>
<th>Nymphs ± SE</th>
<th>Adults ± SE</th>
<th>Total ± SE</th>
</tr>
</thead>
<tbody>
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<td>8-9-03</td>
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<td>BYG</td>
<td>Interior</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Interior</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>BYG</td>
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<td>Exterior</td>
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<td>Rice</td>
<td>Exterior</td>
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<td>$F = 0.82; df = 3, 9$; $P = 0.5153$</td>
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<tr>
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<td>Exterior</td>
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<td>12.5 ± 3.57</td>
<td>16.50 ± 3.66</td>
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<td>$F = 4.06; df = 3, 9$; $P = 0.0444$</td>
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</table>

$a$ Treatments consisted of plots of rice and barnyardgrass in the following spatial arrangements: rice in the interior portion of plots, BYG in the exterior portions; rice in the exterior portions of plots, BYG in the interior portion; and rice in both interior and exterior portions of plots

$b$ F-values presented for treatment effects

$c$ Bold means denote significance at $P < 0.05$; means separated by contrast statements

* denotes 90-100% panicle emergence of rice.
Table 3.4. Mean number of rice stink bugs per 20 sweeps found in 2002 on rice when grown in association with barnyardgrass (BYG) compared to numbers found in whole plots of rice.

<table>
<thead>
<tr>
<th>Date</th>
<th>Plant in Interior</th>
<th>Plant in Exterior</th>
<th>Area Sampled</th>
<th>Nymphs ± SE</th>
<th>Adults ± SE</th>
<th>Total ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-7-02</td>
<td>Rice</td>
<td>BYG</td>
<td>Interior</td>
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<td>0.75 ± 0.75</td>
<td>1.25 ± 0.95</td>
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<tr>
<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Interior</td>
<td>0 ± 0</td>
<td>0.50 ± 0.29</td>
<td>0.50 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>BYG</td>
<td>Rice</td>
<td>Exterior</td>
<td><strong>0 ± 0</strong> b</td>
<td>0.50 ± 0.50</td>
<td>0.50 ± 0.50</td>
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<td>Rice</td>
<td>Exterior</td>
<td><strong>1.25 ± 0.63</strong></td>
<td><strong>F = 4.71; df = 3, 9; c</strong></td>
<td><strong>P = 0.0304</strong></td>
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<td>BYG</td>
<td>Interior</td>
<td>4.75 ± 3.47</td>
<td>1.25 ± 0.63</td>
<td>6.0 ± 4.02</td>
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<td>Rice</td>
<td>Interior</td>
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<td>0.75 ± 0.75</td>
<td><strong>0.75 ± 0.75</strong></td>
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<td>Exterior</td>
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<td>0.5 ± 0.5</td>
<td>0.5 ± 0.5</td>
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<td>Rice</td>
<td>Exterior</td>
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<td>0 ± 0</td>
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<td><strong>P = 0.0408</strong></td>
<td><strong>F = 1.34; df = 3, 9;</strong></td>
<td><strong>P = 0.3223</strong></td>
<td><strong>F = 4.89; df = 3, 9;</strong></td>
<td><strong>P = 0.0277</strong></td>
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<td>8-24-02</td>
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<td>BYG</td>
<td>Interior</td>
<td>3.5 ± 0.5</td>
<td>5.50 ± 3.07</td>
<td>9.0 ± 3.08</td>
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<td>Rice</td>
<td>Rice</td>
<td>Interior</td>
<td>1.0 ± 0.58</td>
<td>1.0 ± 0.71</td>
<td>2.0 ± 1.08</td>
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<td>Rice</td>
<td>Exterior</td>
<td>3.75 ± 1.65</td>
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<td>Rice</td>
<td>Exterior</td>
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<td><strong>F = 3.25; df = 3, 9;</strong></td>
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<td>8-28-02*</td>
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<td>BYG</td>
<td>Interior</td>
<td>12.75 ± 1.97</td>
<td>5.0 ± 1.15</td>
<td>17.75 ± 1.49</td>
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<td>Rice</td>
<td>Interior</td>
<td>16.0 ± 8.07</td>
<td>0.75 ± 0.48</td>
<td>16.75 ± 7.79</td>
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<tr>
<td></td>
<td>BYG</td>
<td>Rice</td>
<td>Exterior</td>
<td>12.5 ± 4.63</td>
<td>5.25 ± 2.59</td>
<td>17.75 ± 6.14</td>
</tr>
<tr>
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<td>Rice</td>
<td>Rice</td>
<td>Exterior</td>
<td>13.25 ± 4.11</td>
<td>3.25 ± 1.70</td>
<td>16.50 ± 5.56</td>
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<td><strong>F = 3.32; df = 3, 9;</strong></td>
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<td>BYG</td>
<td>Interior</td>
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<td>4.00 ± 1.08</td>
<td><strong>22.75 ± 1.55</strong></td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Rice</td>
<td>Interior</td>
<td><strong>33.25 ± 6.93</strong></td>
<td>1.75 ± 0.48</td>
<td><strong>35.00 ± 6.56</strong></td>
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<td></td>
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<td>Rice</td>
<td>Exterior</td>
<td><strong>34.25 ± 6.30</strong></td>
<td>7.50 ± 2.72</td>
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<td>Rice</td>
<td>Exterior</td>
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<td>3.25 ± 1.31</td>
<td>53.50 ± 7.68</td>
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<td><strong>P = 0.0016</strong></td>
<td><strong>F = 1.16; df = 3, 9;</strong></td>
<td><strong>P = 0.3779</strong></td>
<td><strong>F = 8.17; df = 3, 9;</strong></td>
<td><strong>P = 0.0062</strong></td>
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</tbody>
</table>

* Treatments consisted of plots of rice and barnyardgrass in the following spatial arrangements: rice in the interior portion of plots, BYG in the exterior portions; rice in the exterior portions of plots, BYG in the interior portion; and rice in both interior and exterior portions of plots

b bold means denote significance at P < 0.05; means separated by contrast statements

c F-values presented for treatment effects

* denotes 90-100% panicle emergence of rice
date (Aug. 15), nymphs and total number of rice stink bugs were at least four times greater on rice in mixed plots when rice was in the interior of plots. On Sept. 8, nymphs were 1.5 to 2 times greater on rice in whole plots than on rice in mixed plots (either arrangement). Also, total number of rice stink bugs was 1.5 times greater on rice in whole plots compared to rice in mixed plots on Sept. 8.

Rice stink bugs were also collected from barnyardgrass portions of mixed plots (Figure 3.1). Data were similar for the different treatments; therefore, only one graph is shown to present the trend among treatments. Data are from mixed plots in which barnyardgrass surrounded rice in 2003. Numbers of rice stink bugs differed over time. On Aug. 18, there was an average of 12.75 ± 3.01 rice stink bugs on barnyardgrass. Numbers declined to 5.0 ± 2.35 on Aug. 28 on barnyardgrass, while numbers of on rice increased from 3.23 ± 2.29 on Aug. 18 to 19.75 ± 3.09 Aug. 28. The decrease in numbers of rice stink bugs on barnyardgrass and concurrent increased on rice occurred after 90-100% panicle emergence of rice.

**Discussion**

While presence of barnyardgrass appears to have little impact on populations of rice water weevils, presence of barnyardgrass affected both timing and severity of rice stink bug infestations on rice. Rice stink bug populations were influenced not only by the presence of barnyardgrass but also by the phenology of barnyardgrass relative to rice. Rice stink bugs were more prevalent on rice of mixed plots than pure plots of rice prior to 90-100% panicle emergence of rice in 2001 and 2002. Figure 1 shows that rice stink bugs utilized
Figure 3.1. The total number (±SE) of rice stink bugs on barnyardgrass and rice when rice was in the exterior portion of plots where * denotes 90-100% panicle emergence of rice (2003).

barnyardgrass as a host prior to panicle emergence of rice. Barnyardgrass requires approximately 50-55 days after emergence to produce mature seed (40-45 days after emergence for initiation of seed set and another 8-9 days for development of mature seed) (Swanton et al 2000). Panicle emergence of rice was 90-100% approximately 90 days after planting. Since barnyardgrass produces seed heads before rice, barnyardgrass can serve as a source of rice stink bug infestation in rice (Douglas 1939). Additionally, rice stink bugs may move from barnyardgrass to rice as panicle emergence progresses from 1-100%, causing an earlier infestation than if barnyardgrass was not present.
After panicle emergence of rice, numbers of rice stink bugs collected from rice in the different treatments varied in between years this experiment was conducted. In 2001 and 2003 there were significantly more rice stink bugs on rice grown in association with barnyardgrass than on whole plots of rice. Differences in reproductive development of barnyardgrass and rice allowed rice stink bugs to feed on barnyardgrass at least a month before barnyardgrass began to senesce and rice panicles emerged. Because the host suitability of barnyardgrass declined as rice became a suitable host, it is likely rice stink bugs migrated from barnyardgrass to vulnerable rice causing greater infestations on rice in mixed plots compared to whole plots. Futuyma and Wasserman (1980) reported a similar case with larvae of Alsophila pometaria Harris (Geometridae). Larvae of A. pometaria feed on young foliage of both scarlet oak and white oak; however, budbreak of scarlet oak occurred 10 days prior to budbreak of white oak. Larvae accumulated on scarlet oak because it was an available host. After 10 days of defoliating scarlet oak, white oak produced buds and larvae migrated to the white oak because white oak, the preferred foliage for A. pometaria was present with young leaves.

However, in 2002, significantly more rice stink bugs were collected on rice grown in association with rice after panicle emergence. In 2002, the majority of barnyardgrass germination was delayed in the weedy areas of mixed plots; therefore, 85% or more of the barnyardgrass did not emerge with rice as in 2001 and 2002. Germination was delayed for almost a month, resulting in most barnyardgrass plants being approximately at the same developmental stage as rice. Barnyardgrass is considered to be a preferred host over rice (Douglas 1939, Odglen and Warren 1962) and it was available when rice was present. In 2001 and 2003, there was a decline in numbers of rice stink bugs on barnyardgrass and a
simultaneous increase in numbers on rice. However, in 2002, numbers of rice stink bugs were relatively constant or increased in areas of barnyardgrass (data not shown). Therefore, it is probable that rice stink bugs remained in the barnyardgrass since it was still a suitable host when rice panicles were present. The ability of a preferred host to confine insects is the foundation of trap cropping. Craig (1998) showed that redroot pigweed effectively lured and maintained *Lygus lineolaris* Palisot de Beauvois populations in cotton agroecosystems until cotton was an unattractive host.

There are other possible reasons for the lower numbers of rice stink bugs present in mixed plots compared to whole plots in 2002. Polycultures create a more diverse habitat than monocultures; diverse habitats can cause an increase in populations of beneficial insects (Gurr and Wratten 1999). Although data were not collected to determine if beneficial insects were a factor in this experiment, few rice stink bug adults collected were parasitized (personal observation). Additionally, neighboring plants may visually or chemically interfere with the ability of an insect locating a host; however, there is little evidence in the literature that supports this hypothesis (Andow 1988).

Although the rice water weevil appeared to be unaffected by presence of barnyardgrass, in 2002, there was less than an average of two larvae found per plot. It would be reasonable to assume weevils were not abundant in 2002. However, samples were taken from areas of barnyardgrass in mixed plots. Numbers of larvae on barnyardgrass were similar to those found on rice in 2001 (6.08 ± 2.29 when barnyardgrass was in the exterior of plots and 6.83 ± 1.60 when barnyardgrass was in the interior). When the field was flooded in 2002, the majority of barnyardgrass was ten days old whereas rice was 40 days old. Young barnyardgrass has been shown to be more preferred than rice for adult feeding.
and oviposition in greenhouse studies (Tindall and Stout 2003). Since larvae were scarce in rice, it is probable that rice water weevil were attracted to barnyardgrass with little interest in rice.

Many important insect pests of rice are specialists on grasses. Since there are many important grass weeds in rice production systems and many insects are grass feeders, similar interactions may occur. First, populations of rice water weevil are generally less in north Louisiana compared to populations in south Louisiana. Had these experiments been conducted in south Louisiana, results may have differed. Second, preliminary studies suggest that more damage from the sugarcane borer, *Diatraea saccharalis* F., a generalist feeder of grasses, was present in rice grown in association with Amazon sprangletop (*Leptochloa panicoides* [Presl] Hitchc) (Tindall, unpublished data). Additionally, there are several other grass-feeding insects of rice, rice stalk borer, *Chilo plejedellus* Zincken, fall armyworm, *Spodoptera frugiperda* J.E. Smith, chinch bug, *Blissus leucopterus leucopterus* Say, and grasshoppers (Acrididae), that also may be influenced by the presence of graminaceous weeds.

Thorough investigations of insect-weed interactions can lead to recommendations for cultural practices or pesticide applications that manipulate insects as part of a integrated pest management program. The cotton agroecosystem provides examples of insects pests that have been well-studied with respect to insect-weed interactions. Infestations of *L. lineolaris*, *Heliothis virescens* F., and *Heliocoverpa zea* Boddie can be reduced on cotton as a result of timely destruction of wild hosts (i.e. herbicide applications or mowing) (Snodgrass et al 2000, Stadelbacher 1985, 1987). Additionally, applications of insecticides on wild hosts reduced populations of insects attacking cotton (Stadelbacher 1985, 1987).
Although numerous studies have been conducted investigating insect-weed interactions, the full significance of insect-weed interactions is still poorly recognized in most agroecosystems, including rice. Having a better understanding of the developmental stage of barnyardgrass relative to rice will be important in predicting rice stink bug populations. Recommendations could then be made for timely weed control to reduce rice stink bug infestations. Additionally, grasses along turn-rows or levees may be able to be treated with insecticide to remove local sources of infestation. More data are needed to determine holistic integrated pest management strategies.

References


Craig, C.C. 1998. Development of a trap crop for Lygus lineolaris (Heteroptera: Miridae) and a refuge for production of Heliothis virescens (Lepidoptera: Noctuidae) susceptible to cotton expressing insecticidal proteins. MS Thesis, Mississippi State University, Mississippi State, MS.


CHAPTER 4

EXAMINATION OF THE EFFECT OF THE PRESENCE OF BARNYARDGRASS ON RICE STINK BUG IN A LARGE PLOT DEMONSTRATION TEST

Introduction

Insects and weeds are both important constraints on crop yields in agricultural systems. In addition to their individual effects, insects and weeds may have interactive effects. Presence of weeds may influence the severity of insect infestations by serving as alternate hosts for insect pests (Young 1986, Fleischer et al. 1988, Tindall et al. 2004). Additionally, the presence of weeds has been shown to increase populations of beneficial insects (Ali and Reagan 1985, Gurr and Wratten 1999.). Management practices for weeds may influence management of insects, and vice versa (Fleischer et al. 1988, Snodgrass et al. 2000, Eigenbrode et al. 1993). When herbicides are applied to weeds that serve as alternate hosts for insects, the food source is destroyed, and insects must locate a new host, possibly relocating to a nearby crop (Fleischer et al. 1988, Snodgrass et al. 2000). Despite the potential for interactions between weeds and insects in many agricultural systems, these interactions are often overlooked.

The rice stink bug, Oebalus pugnax F., is an important insect pest of rice, Oryza sativa L., in Louisiana. Both adults and nymphs of the rice stink bug remove endosperm from developing rice grains and reduce yield and grain quality. (Odglen and Warren 1962, McPherson and McPherson 2000). The host range of rice stink bugs consists primarily of monocot plant species. Research has shown that vasseygrass, Paspalum urvillei Steud., (Naresh and Smith 1984) and barnyardgrass, Echinochloa crus-galli Beauv. (Odglen and Warren 1962) are more preferred by rice stink bugs than other plant species. Additionally, previous research suggests that the timing of barnyardgrass heading relative to rice panicle
emergence strongly influences the movement and feeding habits of rice stink bug populations on rice (Tindall et al, 2004). Small plot studies showed barnyardgrass served as a source of rice stink bug infestation when barnyardgrass headed before rice panicles emerge. However, the majority of rice stink bugs remained on barnyardgrass when barnyardgrass seed heads and rice panicles were present at the same time. The hypothesis that the stage of barnyardgrass development influences rice stink bug populations was tested in large scale production system.

**Materials and Methods**

A large plot demonstration test was conducted at Woodsland Plantation in Richland Parish in Northeast Louisiana. Two fields were selected based on the presence of rice and barnyardgrass at appropriate stages of development. One field had barnyardgrass seed heads present approximately 3 to 3.5 weeks prior to rice panicle emergence. The other field had rice panicles and barnyardgrass seed heads present at the same time. Weed infestations were natural infestations throughout the field. Both fields were planted with rice variety ‘Cocodrie’ and were approximately 40 ha in size. These fields were maintained using normal agronomic practices for north Louisiana rice production (Linscombe et al. 1999). The area sampled within each field was approximately 1.2 ha. The field that had barnyardgrass seed heads present prior to panicle emergence of rice was treated with the herbicide, fenoxaprop (Bayer CropScience, 1.1 L / ha) prior to sampling for rice stink bugs.

**Data Collection**

Both adult and nymph rice stink bugs were sampled with a sweep net (15 cm in diameter) weekly for approximately one month. Ten sets of ten sweeps were made randomly in both weedy and weed-free areas of both fields. In weed-free areas, both
barnyardgrass and rice were swept. Weedy areas sampled were at least 10 m away from
weed-free areas when sampling for rice stink bugs.

Data Analysis

Each field was analyzed separately. Analysis of variance was used to compare the
total numbers of rice stink bugs found in weed-free areas of each field to those collected in
weedy areas at each sample date.

Results

In the field that had barnyardgrass seed heads present three and half weeks before
panicle emergence of rice, populations of rice stink bugs were always higher in weedy areas
than in weed-free areas (Figure 4.1). Numbers of rice stink bugs were significantly greater
(3 to7 fold) on weedy rice than on weed-free rice for the first four sample dates prior to
panicle emergence. After rice panicle emergence, numbers of rice stink bugs began to
increase in both weedy and weed-free areas of the rice field; however, numbers of rice stink
bugs were 1.75 times greater in weedy areas than in weed-free areas.

Figure 4.2 represents data collected from the field that had rice panicles and
barnyardgrass seed heads present at the same time. The only significant differences were on
the first two sample dates when numbers of rice stink bugs were 4-8 times greater in weedy
areas than in weed-free areas. By the third sample date, barnyardgrass had senesced, and
numbers of rice stink bug declined in areas of barnyardgrass and increased in weed-free
areas; however, densities of rice stink bugs were similar in weedy and weed-free areas rice
for the remainder of the sample dates.
Figure 4.1. Number of rice stink bugs on weedy or weed-free rice in which barnyardgrass headed before rice. Single arrow shows when rice panicles began to emerge (approximately 5% emergence) and double arrows show when 90-95% emergence of rice occurred. * denotes significant differences between weedy areas and non-weedy areas for each sample date $P < 0.05$. ** denotes marginally significant differences between weedy areas and non-weedy areas for each sample date $P = 0.07$. 
Figure 4.2. Numbers of rice stink bugs on weedy or weed-free areas of a rice field in which rice and barnyardgrass seed heads were present at the same time. Single arrow shows where the majority of barnyardgrass had senesced. * denotes significant differences between weedy areas and non-weedy areas for each sample date $P < 0.05$.

Discussion

Results from this large scale study support findings from small plot studies (Tindall et al. 2004). When seed heads of barnyardgrass were present prior to rice panicle emergence and weeds began to senesce, rice stink bugs populations showed the trend of increasing in weed-free areas of rice. This supports the hypothesis that weeds can be significant sources of rice stink bugs and that the phenology of weeds relative to panicle emergence of rice appears to affect rice stink bug infestations as well.

Data from the field that had barnyardgrass seed heads present three weeks prior to panicle emergence of rice showed that rice stink bugs can be present in rice fields without being damaging to rice. Rice stink bugs were found in areas of barnyardgrass prior to rice
panicles being present. Rice stink bugs feed on rice panicles; if rice stink bugs are present and panicles are not present, damage to rice does not occur.

Figure 4.1 shows that between July 23 and 28, there was a reduction in numbers of rice stink bugs in the weedy areas of the field that had barnyardgrass headed before panicle emergence of rice. This reduction probably resulted from a fenoxaprop application made prior to sampling. This suggests herbicide applications in rice can reduce, but not eliminate, rice stink bug populations due to destruction of hosts (Fleischer et al. 1988, Snodgrass et al. 2000). It is likely that the nutritive quality of barnyardgrass declined, and adults emigrated from the field. The majority of rice stink bugs that were collected after the first date were nymphs until rice panicles emerged. In addition to the nymphs that remained in the field feeding on barnyardgrass, rice stink bugs migrated into the field as rice panicles emerged. As a result more rice stink bugs were present in weedy areas after migration into the field. Since barnyardgrass senesced by panicle emergence, rice stink bugs were likely feeding on rice in weedy areas, whereas, areas of weed-free rice had rice stink bugs primarily from the rice stink bug migration into the field. Therefore, barnyardgrass served as a source of rice stink bug infestation.

More rice stink bugs were collected in weedy areas than weed-free areas when rice panicles and barnyardgrass seed heads were present at the same time. Odglen and Warren (1962) observed similar trends when sampling weedy and weed-free areas. In both studies, when sampling for rice stink bugs, both weeds and rice were sampled. It is not possible to determine the host on which the rice stink bugs were feeding with these sampling techniques. Tindall et al. (2004) suggested that the phenology of the weeds relative to the phenology of rice is important for predicting the host on which rice stink bugs will feed. It
is likely that rice stinkbugs were feeding on barnyardgrass since it is considered a preferred host (Odglen and Warren 1962) and was present in the areas sampled. By Aug. 5, a significant amount of barnyardgrass had senesced and stink bug movement increased with numerically more rice stink bugs being found in weed-free rice than in weedy areas. When rice stink bug numbers increased in rice, rice seeds were developmentally advanced such that they were less vulnerable to stink bug damage.

Data from this study and small plot experiments (Tindall et al. 2004) suggest that timing of weed senescence is important in predicting rice stink bug populations. However, this does not imply that weed-free fields are immune to rice stink bug. Instead, data imply that weedy fields are likely to have earlier infestations than weed-free fields; therefore, weedy feeds should be scouted before panicle emergence of rice to avoid early losses. Further research is needed to determine if more damage occurs from rice stink bugs when barnyardgrass served as its host and barnyardgrass senesced near the time of panicle emergence. Additionally, tests need to be conducted to determine if rice escapes some injury of rice stink bugs if barnyardgrass is a suitable host and present at the same time as rice panicles.

References


CHAPTER 5

COMBINED AND INDIVIDUAL EFFECTS OF GRAMINACEOUS WEEDS AND RICE STINK BUGS ON RICE

Introduction

Weed and insect pests are both important problems faced by rice producers worldwide. Typically research focuses on these pests individually; however, there are numerous ways in which these pests can interact. Insects can feed on the vegetative or reproductive plant tissues of weeds, possibly reducing the seed bank for following years. The presence of weeds can have both positive and negative effects on insect populations (Andow 1991). Weeds can be used as alternate hosts and serve as a source of infestation, or insects may feed on weeds and not damage crop plants. Weeds may also interfere with the ability of an insect to locate the crop plant. Additionally, weeds provide a nectar source for parasitoids and create a more diverse ecosystem with more beneficial insects present to suppress insect pest populations.

The rice stink bug, *Oebalus pugnax* F., is an important insect pest of rice, causing losses of $29-54 per ha. Female rice stink bugs lay two rows of barrel-shaped green eggs that turn red as they mature on plant foliage or panicles (Odglen and Warren 1962, McPherson and McPherson 2000). Early in grain development, nymphs and adults damage rice by removing all the endosperm from kernels, resulting in a non-filled seed. Feeding in the later stages of grain development causes atrophied seeds and reduces the quality of the grains (pecky rice). Pecky rice is a broad term used to describe the appearance of discolored kernels that results from a combination of insect feeding and pathogen infection (Tullis 1936, McPherson and McPherson 2000); several pathogens have been isolated from pecky rice kernels (Tullis 1936 [and sources within], Daughtery and Foster 1966, Marchetti and
Petersen 1984, Hollay et al. 1987, Lee et al. 1993). Lee et al. (1993) provided evidence of an insect-vector relationship between rice stink bugs and pathogens. Pecky rice and atrophied seeds reduce grain quality because they are more likely to break during the milling process (Douglas 1939, Odglen and Warren 1962; McPherson and McPherson 2000).


Previous studies showed that the presence of barnyardgrass in rice fields affected the numbers of rice stink bugs presence on rice (Tindall et al., 2004). Both increases and decreases in rice stink bug densities on rice were detected depending on the phenology of barnyardgrass relative to rice. When barnyardgrass and rice had panicles present at the same time; rice stink bug infestations were lower on rice grown with barnyardgrass than without barnyardgrass. However, when barnyardgrass was senescing during panicle emergence of rice, barnyardgrass served as a source of rice stink bug infestation on the newly emerging rice panicles. If weeds serve as a source of infestation, an increase in rice stink bug damage
may be an indirect effect of the presence of graminaceous weeds. The experiments reported here were designed to examine how varying densities of graminaceous weeds affect rice stink bug populations and to determine if the damage from the combination of rice stink bugs and weeds is greater than damage from weeds alone.

**Materials and Methods**

Experiments were conducted at the Macon Ridge Branch Station, Winnsboro, LA (Franklin Parish) in 2002 and 2003. ‘Cocodrie’ rice was drill seeded into a loessial upland soil (Gigger silt loam) at a rate of 112 kg/ha on May 28, 2002 and May 24, 2003. The drill spacing was 20 cm, and plots consisted of 10 rows that were 4.5 m in length. Each plot was separated by a 2 m weed-free border; weed-free borders were treated preemergence applications of 0.45 kg/ha of quinclorac and 0.55 kg/ha of clomazone. On June 24, 2002 and June 30, 2003, nitrogen in the form of prilled urea was applied at 126 kg/ha immediately prior to the establishment of permanent floods. Rice was flushed as needed.

The experimental design was a completely randomized design with 36 plots that had varying graminaceous weed densities. A range in graminaceous weed densities was established by using various herbicide programs, (herbicide programs consisted of no herbicide, 224.2, 448.3, and 672.5 g ai/ha clomazone applied preemergence, and 448.3 and 672.5 g ai/ha clomazone applied preemergence followed by 213 g ai/ha cyhalofop at the 4-5 leaf rice stage). Broadleaf weeds and sedges were removed by applying 25 g/ha halosulfuron at the 2-3 and 4-5 leaf stages of rice. Approximately two weeks prior to panicle emergence of rice, weed density was estimated for each plot by placing 0.1 m² quadrants over two rows of rice. All vegetation within the 0.1 m² area was removed and taken to the laboratory. Plants were divided into species and counted to determine weed composition and weed density.
Two samples were collected from each plot and averaged to get an estimate of weed density. In 2002, the study area had a natural infestation of barnyardgrass (57%), Amazon sprangletop (10%), and broadleaf signal grass (33%). Weed composition in 2003, consisted of barnyardgrass (38%), Amazon sprangletop (33%), broadleaf signalgrass (8%), and large crabgrass (22%).

After weed composition and density were estimated for each plot, plots were divided into two groups of 18 plots of similar weed density. One group of plots received 672 g/ha lamda-cyhalothrin approximately every 4-5 d after 20% panicle emergence of rice. The lamda-cyhalothrin treatments were used to minimize the effects of rice stink bugs so that the individual effects of weed density could be isolated. Weed density for lamda-cyhalothrin treated plots were 0 -16 weeds/0.1 m$^2$ in 2002 and 0-44.5 weeds/0.1 m$^2$ in 2003; non-treated plots had weed densities ranging from 0-22.5 weeds/0.1 m$^2$ in 2002 and 0-49.5 weeds/0.1 m$^2$ in 2003.

**Data Collection**

**Rice Stink Bugs**

Rice stink bugs were sampled with a sweep net (38 cm in diameter) after 50% panicle emergence. Sampling continued every 5 to 7 d for approximately 3 wk. Samples were averaged to obtain an estimate of rice stink bug populations.

**Percent Filled Seeds**

When rice is harvested with a combine, non-filled seeds are discarded; therefore, prior to harvest, 30 panicles were hand harvested to prevent loss of non-filled seeds. Seeds were also removed from the panicle manually to avoid loss of non-filled seeds. Seeds were then divided into groups of filled and non-filled seeds. A seed was characterized as a non-
filled seed if, when the seed was placed on its tip and pressure was applied with the thumb, the palea and lemma folded easily. Filled seeds were run through an automated seed counter (Count-A-Pak; Seedburo Equipment Co., Chicago, IL). Non-filled seeds were counted manually because they were not detected by the seed counter. Percent filled seeds was determined by the following equation, [(number of filled seeds/total number of seeds)*100].

**Seed Weight**

After seeds were separated into groups of filled and non-filled seed, 100 filled seeds were collected and weighed to the nearest mg.

**Yield**

Yield data were collected using a mechanical harvester on October 2, 2002 and September 30, 2003. Approximately 225 g of seed were collected at harvest to assess pecky rice and milling quality. Yield data were log transformed prior to analysis to meet the assumption of normality.

**Pecky Rice**

Samples of 100 g were collected from mechanically harvested plots. Seeds were run through a McGill Sheller (H.T. McGill Inc., Houston, TX) to remove the paleae and lemmas. Samples were manually sorted to assess pecky rice. Although there are several causes of pecky rice (Tullis 1936, Lee et al. 1993), only pecky rice caused by stink bug feeding was considered in this experiment. Pecky rice associated with rice stink bug feeding exhibit circular spots (Lee et al. 1993). Stink bug pecky rice can range from a small speck that would easily be removed in the milling process to a completely diseased seed. Seeds were considered pecky if there was any amount of stink bug injury present; all other seeds were considered non-pecky. Both pecky and non-pecky rice were weighed and weights
were used to calculate percent pecky rice using \[\text{weight of pecky rice}/(\text{weight of pecky rice} + \text{weight of non-pecky rice}) \times 100\].

**Milling Quality**

Seed samples of 125 g of mechanically harvested seed were run through a McGill Sheller to remove the paleae and lemmas of seeds. Seeds were then run through a McGill Miller (H.T. McGill Inc., Houston, TX) to remove the caryopsis of seeds. Rice was weighed to the nearest 0.01 g. Milled grains were then placed on a machine that shakes kernels down a series of metal plates with depressions that trap broken kernels and allow whole kernels to be collected after being shaken. Whole kernels were then weighed to estimate the milling quality expressed as percent whole kernels \[\text{(Final weight/initial weight)} \times 100\].

**Data Analysis**

All variables were analyzed using analysis of covariance in PROC MIXED (SAS, 1998). Weed density was used as the continuous variable and insecticide application as the categorical variable. The first stage of this analysis determines if there was a significant effect of weed density on the Y-variable measured (i.e., does the slope of the line equal zero?). The second phase of the analysis incorporates the effect of the insecticide application into the analysis and compares the intercepts of the regression lines generated from insecticide treated and non-treated plots (i.e., is there more damage in non-treated plots than treated plots?). The final step of the analysis examining the interaction between weed density and insecticide application and evaluates the slopes of regression lines for both treated and non-treated plots (i.e., is the rate of increase in damage in the non-treated plots greater than the increase in treated plots?). Data from 2002 and 2003 were analyzed together and graphs depict any significant year interactions. Correlation between percent
pecky rice and the milling quality was assessed using Pearson correlation coefficient in PROC CORR in SAS.

**Results**

**Rice Stink Bugs**

In 2002 there were more rice stink bugs present than in 2003; however the results were similar for both years (Fig. 5.1). As weed density increased populations of rice stink bugs in plots increased. Lambda-cyhalothrin effectively suppressed rice stink bug populations; however, complete control was not obtained. In the absence of insecticide, the presence of 1.3 weeds / 0.1 m$^2$ (2002) and 2.3 weeds / 0.1 m$^2$ (2003) caused an increase of one rice stink bug. Additionally, populations of rice stink bugs increased at a greater rate in the non-treated plots than in the treated plots.

**Percent Filled Seeds**

Percentage of filled seeds decreased as weed density increased (Fig. 5.2). A significant effect was observed in both insecticide treated and non-treated plots suggesting that weed density caused direct yield losses in the amount of filled seeds produced. Additionally, there were more non-filled seeds in the non-treated plots compared to treated plots, demonstrating that when not controlled, rice stink bugs reduced the percentage of filled seeds. The insecticide-weed density interaction showed that the rate of decline in percentage of filled seed was more than three times greater in the non-treated rice than in treated rice. In these experiments, a 1% reduction in percent filled seeds occurred when 7.4 weeds / 0.1 m$^2$ were present in insecticide treated plots; whereas, only 2.4 weeds / 0.1 m$^2$ were required to reduce percent filled seeds by 1% in the non-treated plots.
Figure 5.1. Number of rice stink bugs per 20 sweeps on insecticide treated and non-treated rice in response to increasing weed density. 2002 No Insecticide: $y = 34.6063 + 0.7800x$; 2002 Insecticide: $y = 8.7103 - 0.0367x$; 2003 No Insecticide: $y = 15.1257 + 0.4325x$; 2003 Insecticide: $y = 1.1793 + 0.1067x$. Data were analyzed using analysis of covariance; see text for explanation (year - $F = 57.96$; df = 1, 63; $P < 0.0001$; weed density - $F = 31.27$; 1, 63; $P < 0.0001$; year*weed density - $F = 6.30$; df = 1, 63; $P < 0.0147$; Insecticide - $F = 196.22$; 1, 63; $P < 0.0001$; Insecticide*weed density - $F = 3.48$; 1, 63; $P < 0.0669$; Insecticide*year - $F = 19.16$; 1, 63; $P < 0.0001$; Insecticide*year*weed density - $F = 1.94$; 1, 63; $P < 0.1689$).
Figure 5.2. Percent filled seeds in insecticide treated and non-treated plots in response to increasing weed density. No Insecticide: $y = 81.5891 - 0.4800x$; Insecticide: $y = 82.8714 - 0.1355x$. Data were analyzed using analysis of covariance; see text for explanation (year - $F = 2.60; \text{df} = 1, 64; P < 0.1118$; weed density - $F = 22.81; 1, 64; P < 0.0001$; year*weed density - $F = 3.42; \text{df} = 1, 64; P < 0.0692$; Insecticide - $F = 6.38; 1, 64; P < 0.0140$; Insecticide*weed density - $F = 4.72; 1, 64; P < 0.0336$; Insecticide*year - $F = 2.09; 1, 64; P < 0.1527$; Insecticide*year*weed density - $F = 0.00; 1, 64; P < 0.9818$).
**Pecky Rice**

As weed density increased, pecky rice increased (Fig. 5.3). There was 1.6 times more pecky rice in non-treated rice in 2002 than 2003. Pecky rice from non-treated plots was 2.4 (2003) to 3 (2002) times more than from treated plots. Regardless of the year or insecticide application, pecky rice increased at a rate of 1% for every 10 weeds / 0.1 m$^2$.

**Milling Quality**

Milling quality, expressed as percent whole kernels, revealed that as weed density increased, quality decreased; 5.6 weeds / 0.1 m$^2$ reduced the milling quality by 1% (Fig. 5.4). The percentage of whole kernels was 3.6 and 3.7 lower in non-treated plot than in treated plots in 2002 and 2003, respectively. Percent pecky rice and milling quality were significantly correlated (Pearson correlation coefficient = -0.4924; $P < 0.0001$). Data suggest that for every 1% of pecky rice, milling quality declined by 0.5%.

**Seed Weight**

Seed weights responded differently in 2002 and 2003 (Fig. 5.5). In 2002, seed weights decreased as weed density increased; however, 243 weeds / 0.1 m$^2$ were estimated to reduce seed weight by 1 mg for both treated and non-treated rice. Seed weights were 0.16 mg lower in non-treated rice than treated rice. In 2003, seed weight appeared to increase in response to increasing weed density; the regression equation estimated that 526 weeds / 0.1 m$^2$ were needed to cause an increase of 1mg for both treated and non-treated rice. Seed weights were 0.006 mg greater in the non-treated rice than in the insecticide treated rice. The estimated weed densities required to cause an effect were outside the weed density examined in these experiments.
Figure 5.3. Percent pecky rice in insecticide treated and non-treated plots in response to increasing weed density. 2002 No Insecticide: \( y = 14.69 + 0.09943x \); 2002 Insecticide: \( y = 4.7628 + 0.09943x \); 2003 No Insecticide: \( y = 8.9538 + 0.09943x \); 2003 Insecticide: \( y = 3.7008 + 0.09943x \). Data were analyzed using analysis of covariance; see text for explanation (year - \( F = 10.32; \) df = 1, 63; \( P < 0.0021 \); weed density - \( F = 9.56; \) 1, 63; \( P < 0.0030 \); year*weed density - \( F = 0.56; \) df = 1, 63; \( P < 0.04565 \); Insecticide - \( F = 69.32; \) 1, 63; \( P < 0.0001 \); Insecticide*weed density - \( F = 0.30; \) 1, 63; \( P < 0.5846 \); Insecticide*year - \( F = 6.23; \) 1, 63; \( P < 0.0152 \); Insecticide*year*weed density - \( F = 0.05; \) 1, 63; \( P < 0.8309 \)).
Figure 5.4. Milling quality expressed as whole kernels in insecticide treated and non-treated plots in response to increasing weed density. * Lines for 2002 No Insecticide and 2003 Insecticide overlap. 2002 No Insecticide: y = 84.3231 – 0.1767x; 2002 Insecticide: y = 87.8891 – 0.1767x; 2003 No Insecticide: y = 80.6246 – 0.1767x; 2003 Insecticide: y = 84.3734 – 0.1767x. Data were analyzed using analysis of covariance; see text for explanation (year - $F = 23.83$; df = 1, 61; $P < 0.1118$; weed density - $F = 20.65$; 1, 61; $P < 0.0001$; year*weed density - $F = 3.49$; df = 1, 61; $P < 0.0666$; Insecticide - $F = 16.29$; 1, 61; $P < 0.0002$; Insecticide*weed density - $F = 0.06$; 1, 61; $P < 0.8150$; Insecticide*year - $F = 0.00$; 1, 61; $P < 0.9955$; Insecticide*year*weed density - $F = 0.06$; 1, 61; $P < 0.8042$).
Figure 5.5. Weight of 100 seeds from insecticide treated and non-treated plots in response to increasing weed density. 2002 No Insecticide: \( y = 2.3422 - 0.00412x \); 2002 Insecticide: \( y = 2.1845 - 0.00412x \); 2003 No Insecticide: \( y = 2.1499 + 0.001902x \); 2003 Insecticide: \( y = 2.1434 + 0.001902x \). Data were analyzed using analysis of covariance; see text for explanation (\(\text{year} - F = 9.72; \text{df} = 1, 64; P < 0.0027\); \(\text{weed density} - F = 0.07; 1, 64; P < 0.7869\); \(\text{year} \times \text{weed density} - F = 12.04; \text{df} = 1, 64; P < 0.0009\); \(\text{Insecticide} - F = 12.42; 1, 64; P < 0.0008\); \(\text{Insecticide} \times \text{weed density} - F = 0.51; 1, 64; P < 0.4763\); \(\text{Insecticide} \times \text{year} - F = 17.27; 1, 64; P < 0.0001\) \(\text{Insecticide} \times \text{year} \times \text{weed density} - F = 0.02; 1, 64; P < 0.9019\).
Yield

As weed density increased, grain yields decreased in both 2002 and 2003; however, the loss was more severe in 2002 (Fig. 5.6). Regardless of year, yield losses were approximately 1% per weed / 0.1 m$^2$. Data from the present study showed that yield losses were 163 and 248 kg/ha in 2002 and 2003, respectively. Insecticide had no effect on yield losses.

Discussion

Data from these experiments demonstrate the influence insect-weed interactions can have yield losses and reduction in quality of rice. Increases in weed densities led to increases in densities of rice stink bugs and rice injury. Fewer filled seeds, more pecky rice, and lower milling quality were observed in plots with higher densities of weeds than those with lower densities. Although there was a decrease in filled seeds in plots not treated with insecticide, yield losses were not significantly different than that for insecticide-treated weedy rice, suggesting that weeds are the more important of these two pests with respect to reducing of grain yield losses in these experiments.

The data for percent filled seeds revealed several valuable pieces of information. First, the presence of weeds, irrespective of insects, reduced the amount of filled seeds. Donald and Khan (1996) found similar results with spring wheat; numbers of seeds per spike were reduced as densities of thistles increased. Second, there were more non-filled seeds in plots not treated with insecticide compared to that in treated plots, suggesting that when not controlled, rice stink bugs also reduced the amount of filled seeds. The reduction in percent filled seeds was 3.6 times greater in non-treated rice than in treated rice. Previous
Figure 5.6. Yield loss in response to increasing weed density. 2002: $y = 8.6214 - 0.02978x$; 2003: $y = 9.0414 - 0.02978x$. Data were analyzed using analysis of covariance; see text for explanation (year - $F = 4.80$; df = 1, 63; $P < 0.0322$; weed density - $F = 55.88$; 1, 63; $P < 0.0001$; year*weed density - $F = 0.02$; df = 1, 63; $P < 0.8927$; Insecticide - $F = 2.14$; 1, 63; $P < 0.1482$; Insecticide*weed density - $F = 1.64$; 1, 63; $P < 0.2046$; Insecticide*year - $F = 0.12$; 1, 63; $P < 0.7278$; Insecticide*year*weed density - $F = 0.18$; 1, 63; $P < 0.6690$).
research has shown that rice stink bugs feed on weeds in areas of rice fields that have not yet produced panicles and that this behavior could lead to earlier infestations on rice plants in weedy fields (Tindall et al. 2004). Weeds in this study had seed heads emerge approximately 3.5 weeks before rice panicle emergence, and rice stink bugs were observed in weedy areas of rice prior to panicle emergence of rice. Seed heads of weeds were beginning to senesce as rice panicles began to emerge (personal observation). Therefore, it is likely that instead of rice stink bugs emigrating from weeds to locate a suitable host, rice stink bugs were able to infest adjacent rice plants at vulnerable stages of development, anthesis and early grain filling. These findings support previous results that showed rice infestations of rice stink bugs at anthesis resulted in severe injury and prevented further grain development (Lee et al. 1993). Rice stink bug infestations one day after anthesis reduced filled seeds by approximately 40% (Patel and Stout, unpublished data).

Pecky rice also increased as weed density increased in both insecticide-treated and non-treated rice. In non-treated plots, the increase in pecky rice could be explained by the increase in rice stink bug densities. The increase in pecky rice in plots treated with insecticide, however, was not expected. This result suggests that weeds may also play an important role in the amount of pecky rice detected in these experiments. Weeds may serve as a source of inoculum and their presence may enhance populations of pathogens. A review of published literature on the host range of pathogens isolated from discolored rice kernels showed that 57% of these pathogens are known to infect several genera of common weeds of rice (Table 5.1). Moreover, several pathogens that have been isolated from discolored rice kernels have also been isolated from seeds of *Echinochloa* spp. of weeds.
Table 5.1. List of pathogens that have been isolated from pecky rice kernels and various weeds common to rice fields.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Genera of weeds affected by pathogen</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. alternata</td>
<td></td>
<td>Joshi and Gupta, 1980</td>
</tr>
<tr>
<td>A. padwickii</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. oryzae</td>
<td></td>
<td></td>
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<tr>
<td>Brachysporium</td>
<td>Cynodon spp.</td>
<td>Gupta and Mukerji 1996</td>
</tr>
<tr>
<td>Ceratocystis spp.</td>
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<td></td>
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<tr>
<td>C. miyabeanus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erwinia herbicola</td>
<td></td>
<td>Joshi and Gupta 1980</td>
</tr>
<tr>
<td>Fuscum spp.</td>
<td>Echinochloa spp.</td>
<td></td>
</tr>
<tr>
<td>F. oxysporum</td>
<td>Echinochloa spp.</td>
<td></td>
</tr>
<tr>
<td>Helicoceras oryzae</td>
<td></td>
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<tr>
<td>H. oryzae</td>
<td>Brachiara spp., Paspalum spp.</td>
<td>Artigiani Filho and Bedendo 1995</td>
</tr>
<tr>
<td>Monascus purpureus</td>
<td>Brachiara spp., Echinochloa spp.</td>
<td></td>
</tr>
<tr>
<td>Piricularia spp.</td>
<td>Digitaria spp.</td>
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</tr>
<tr>
<td>Podoniospora spp.</td>
<td></td>
<td></td>
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<tr>
<td>Protopus colorans</td>
<td></td>
<td></td>
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<tr>
<td>Trichoconis caudate</td>
<td></td>
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</tbody>
</table>

a References for these citations include: Tullis 1936 (and sources within), Daughtery and Foster 1966, Marchetti and Petersen 1984, Hollay et al. 1987, Lee et al. 1993.

† Pathogens most frequently isolated from rice kernels; Tullis 1936, Lee et al. 1993.
(Huelma et al. 1996) and *Curvularia lunata*, one of the more commonly isolated pathogens, has also been documented to discolor seeds of an *Echinochloa* sp. (Joshi and Gupta 1980).

Although weed plants in these experiments were not sampled for pathogens, it is likely that these organisms were present on weeds and that rice stink bugs fed on seed heads of weeds infected with pathogens. Several pathogens have been collected from stylets (mouthparts), saliva, and feeding sheaths (saliva remaining at a feeding site) of rice stink bugs (Hollay et al. 1987, Lee et al. 1993); therefore, pathogens may have remained on their stylets after feeding on diseased alternate hosts. After coming in contact with pathogens and moving to rice, rice stink bugs could transfer pathogens when feeding on rice, causing the incidence of pecky rice observed in these studies. Lee et al. (1993) found 13-80% of kernels subjected to simulated rice stink bug feeding in the presence of pathogens were discolored, whereas only 2% of kernels were discolored in the presence of pathogens alone. Therefore, though it is possible for pecky rice to occur in the presence of only a pathogen, penetration of pathogens is enhanced in presence of rice stink bugs. These findings also support the fact that fungicide applications do not reduce the incidence of pecky rice (Lee et al. 1993). If rice stink bugs are a major contributing factor of pecky rice and they are capable of migrating into a field, even if a producer applies a fungicide, pecky rice could be present if rice stink bugs acquired pathogens from alternate hosts some distance away. The phenomenon that infected weeds can serve as a source of inoculum for insect transmitted diseases has been previously documented with black nightshade, *Solanum nigrum* L. and the green peach aphid, *Myzus persicae* (Sulzer.) in small plot studies with bell peppers (Fereres et al. 1996).
Pecky rice is known to affect milling quality of rice (Douglas 1939, Odglen and Warren 1962, McPherson and McPherson 2000); therefore, a negative relationship between these two variables was expected. Data suggest that every one percentage of pecky rice reduced milling quality by half a percent. These data probably underestimate losses because samples examined in these experiments included minor rice stink bug damage that would probably not be considered pecky rice at a commercial mill; kernels with minor injury are less likely to break during the milling quality than those with severe rice stink bug damage.

The effect of weed density on rice yields is well documented (Smith 1988) and yield losses were an expected result. Smith (1988) and Tindall et al. (2003) showed that yields were reduced by 65-71 kg/ha for every one barnyardgrass plant/m$^2$. Experiments relating barnyardgrass density to rice yields have shown yield losses result from a reduction in the numbers tillers, panicles, and seeds per panicle (Tindall et al. 2003). Yield losses from the present study were 163 and 248 kg/ha in 2002 and 2003, respectively. Yield losses from this study are likely higher than those from previous studies since a complex of graminaceous weeds was examined, and not an individual weed species. On the other hand, applications of insecticides to control rice stink bug had no significant effect on grain yield losses. This suggests that, although there was a reduction in percent filled seeds as a result of rice stink bugs, the reduction in filled seeds did not significantly contribute to an overall yield loss. Harper et al. (1993) also found that grain yield losses from rice stink bugs were not significant and that reduction in quality was the major loss attributed to rice stink bugs.

Rice stink bug populations were influenced by weed density; however, rice stink bugs appeared to have a larger effect on quality than on yield of rice. Weed density was shown to have the greatest impact on yield. Available literature suggests that graminaceous
weeds, like barnyardgrass and broadleaf signalgrass, are more competitive with rice prior to
Graminaceous weeds can also interfere with rice during the late season by increasing
numbers of rice stink bugs and may also serve as a source of inoculum of pathogens that
cause to pecky rice. Therefore, weed management throughout the season appears to be
important in order to maximize rice yield and quality.

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CHAPTER 6

INCREASE IN INCIDENCE OF DAMAGE TO RICE BY SUGARCANE BORER, *DIATRAEA SACCHARALIS*, DAMAGE IN THE PRESENCE OF AMAZON SPRANGLETOP, *LEPTOCHLOA PANICOIDES*

Introduction

Three stem boring insects are known to injure rice in the United States, the rice stalk borer, *Chilo plejadellus* Zincken, the sugarcane borer, *Diatraea saccharalis* F., and the European corn borer, *Ostrinia nubilalis* Hübner. European corn borers were recently added to the list of borers attacking rice after the first severe infestation of European corn borer in rice was documented in 2003 (Castro et al. 2004). For all borers in rice grown in the southern United States, eggs are laid on the surface of rice leaves. Larvae eclose, move down the collar into the space between the leaf sheath and the stalk, and begin feeding on leaf tissue for several days. After several days of feeding, larvae bore into rice stalks and continue feeding on the internal tissues. This feeding is responsible for damage to rice in the form of partial whiteheads, whiteheads, deadhearts and lodged plants (Holloway 1928, Castro et al. 2004). The sugarcane borer can be more devastating to rice than rice stalk borer (Holloway 1928); however, no information is available on the impact European corn borer has on rice.

These pests are increasing in importance as pests in rice, although little is known about their behavior in rice (B.A. Castro, LSU AgCenter, Department of Entomology, personal communication). Sugarcane borers overwinter in stalks of graminaceous plants, (Fuchs et al. 1979) and emerging adults seek graminaceous hosts, including sugarcane (*Saccharum officinarum* L.) and corn (*Zea maize* L.). Large populations can build up on sugarcane, corn, and grain sorghum (*Sorghum bicolor* L.) before rice becomes an attractive
host. Thus, infestations in rice fields near sugarcane, corn, and grain sorghum may be more severe than infestations in rice fields distant from large areas of alternate hosts (Holloway 1928). Sugarcane borers prefer sugarcane over corn, grain sorghum, and rice (Sosa 1990). Female sugarcane borers reared on rice lay as many as 239 eggs, and developmental time from egg to adult ranges between 37-88 days, depending on the time of year (Castillo and Villarreal 1989).

Borers of Louisiana rice have a fairly broad host range that consists primarily of graminaceous plants, including several crops. The known host range of sugarcane borer consists of graminaceous plants in the following genera: *Paspalum* spp., *Andropogon* spp., *Panicum* spp., *Sorghum* spp., *Leptochloa* spp., *Hymenachne* spp., *Digitaria* spp., *Eleusine* spp., *Echinochloa* spp. *Oryza* spp., *Saccharum* spp., and *Zea* spp. (Holloway 1928, Bessin and Reagan 1990). European corn borers have a large host range that consists of over 200 hosts including both broadleaf and graminaceous plants (Peterson 1956, Udayagiri and Jones 1993). Of these weedy known to be hosts of borers, *Panicum* spp., *Leptochloa* spp. *Digitaria* spp., and *Echinochloa* spp. are common weeds in Louisiana rice production systems (Smith and Hill 1990, Jordan and Sanders 1999). The presence of *Leptochloa* spp. has been suggested to increase the number of deadhearts of sugarcane plants when sugarcane was grown in weedy plots of sprangletop than in weed-free plots (Dr. Gene Reagan, LSU AgCenter, Department of Entomology, unpublished data).

Previous research suggests that the presence of barnyardgrass seed heads can serve as a source of infestations of rice stink bugs on rice or possibly can act as a ‘trap crop’, confining rice stink bugs, depending on the degree of synchrony of rice and barnyardgrass panicles (Tindall et al. 2004). In the summer of 2003, a field experiment was conducted to
further investigate the interaction between rice stink bug infestations, barnyardgrass, and rice with special reference to the phenology of barnyardgrass and rice. Herbicides were applied to plots to manipulate the phenology of barnyardgrass. However, 90-95% of the weeds that emerged in this experiment were Amazon sprangletop, *Leptochloa panicoides* (Presl) Hitchc., instead of barnyardgrass. When seed heads of sprangletop emerged, many lodged plants, deadhearts and whiteheads were observed in the sprangletop areas of mixed plots. Therefore, rice plots that had an emergence pattern similar to sprangletop were used to investigate the impact of sprangletop presence on borer populations in rice.

**Materials and Methods**

Experiments were conducted at the Macon Ridge Research Station near Winnsboro, LA (Franklin Parish). Rice was planted into a Gigger silt loam at a rate of 100 lb/A. The original experiment was a randomized block design with a factorial treatment arrangement replicated four times. One treatment factor was the spatial arrangement of rice and barnyardgrass. The other treatment was timing of herbicide application. Plots measured 4 m X 3 m and consisted of 20 rows of plants. There were three spatial arrangements of rice and barnyardgrass. In the first arrangement, rice was grown in the absence of barnyardgrass. The remaining arrangements were mixed plots of rice and barnyardgrass. Mixed plots were grown such that the exterior five rows on either side of the plot were rice and the interior 10 rows were barnyardgrass, or the exterior five rows of either side were rice and the interior 10 rows were barnyardgrass. In attempts to manipulate the emergence of barnyardgrass (to promote presence of barnyardgrass seedheads before panicle emergence or at the same time as panicle emergence of rice), glyphosate (2.3 L/ha) was carefully applied to barnyardgrass such that emergence was delayed. However, in several plots, the majority of weeds that
emerged was Amazon sprangletop, and the remaining 5-10% of weeds was barnyardgrass. Three plots of each spatial arrangement of rice and weeds (i.e., three plots of rice in the interior portion of plots and Amazon sprangletop in the exterior portion of plots, three plots of Amazon sprangletop in the interior portion of plots and rice in the exterior portion of plots) were favorable to observe borer damage to rice in the presence of Amazon sprangletop.

Herbicides were used to remove unwanted plants within the areas of rice. To remove grass and broadleaf weeds from rice, clomazone, Command® (FMC) (336 g AI/ha) and quinclorac, Facet®, (BASF) (420 g AI/ha) were applied preemergence (PRE) and postemergence as needed (POST ASN), and cyhalofop, Clincher®, (Dow AgroSciences), (210 g AI/ha) and halosulfuron-methyl, Permit®, (Monsanto) (53 g AI/ha) were applied POST ASN. There was no graminaceous weed control in areas of sprangletop.

**Data Collection**

After whiteheads and deadhearts were observed in Amazon sprangletop areas, sprangletop plants with signs of borer damage were removed from plots, taken to the laboratory, and examined for larvae on August 20. Similarly, after rice panicles emerged, borer damaged plants were taken to the laboratory, examined for larvae, and grouped into categories of damage on September 19. Two sampling methods were employed to sample damage to rice. A 0.1 m² template was placed over two rows of rice and all rice plants were removed and taken to the laboratory. In the laboratory plants were examined individually for sheath lesions, whiteheads, partial whiteheads, and deadhearts. The second method was performed by scouting plots and removing any plants that that had whiteheads, partial whiteheads, and deadhearts. A panicle with three or more non-filled, white rice seeds with
Evidence of borer feeding was considered a partial whitehead. Feeding was considered to be from borers if feeding marks were indicative of caterpillar chewing and frass was present. All larvae were collected from rice and sprangletop plots and were identified to obtain an estimate of the species composition (Peterson 1956).

Data Analysis

Injury (i.e., deadhearts, whiteheads, and partial whiteheads) from borers was analyzed with contrast statements comparing damage found on rice sampled from exterior portion of whole plots of rice to damage on rice collected from the exterior portion of mixed plots (SAS Institute, 1998). Likewise, damage from the interior portion of whole plots of rice was compared to inter portion from the mixed plot of rice. Each sampling method was analyzed separately.

Results

Species Composition

There were 137 borer larvae collected from rice and sprangletop plants. Of those larvae, 97.1% were sugarcane borers. Only 2.2% were European corn borers, and European corn borers were collected only from sprangletop plants. Only one rice stalk borer was collected from rice. As many as 22 first to second instar sugarcane borers were observed on an individual plant.

Damage to Rice by Borers

The amount of borer damage to rice was greater in plots in which sprangletop was present compared to pure plots of rice. Table 6.1 presents data from the sampling method in which random 0.1m² areas were examined. In eight of the 10 comparisons made there was more damage to rice in the mixed plots than in the pure plots of rice. Significant differences
in total damage were detected for both spatial arrangements with sprangletop and rice and in deadhearts in plots that had sprangletop in the interior portion.

Table 6.2 represents data from visual examinations of rice in the mixed and pure plots of rice. Eight possible comparisons were made with this sampling method and all showed that there was more damage to rice in mixed plots compared to rice in pure plots of rice. Marginally significant differences in deadhearts, partial whiteheads, and total damage were found when the spatial arrangement consisted of rice in the exterior portions of plots and sprangletop in the interior portion of plots.

**Table 6.1.** Mean number of plants damaged by borers from borers in 0.1 m² of rice when rice was grown in association with Amazon sprangletop (SPR) compared to numbers found in whole plots of rice. Damage was characterized as deadhearts, partial whiteheads, whiteheads, sheath lesions, and total damage.

<table>
<thead>
<tr>
<th>Plant in Interior</th>
<th>Plant in Exterior</th>
<th>Area Sampled</th>
<th>Deadhearts ± SE</th>
<th>Partial whiteheads ± SE</th>
<th>Whiteheads ± SE</th>
<th>Sheath lesions ± SE</th>
<th>Total ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice SPR</td>
<td>Interior</td>
<td>0.3 ± 0.3</td>
<td>3.0 ± 1.2</td>
<td>0.7 ± 0.7</td>
<td>10.3 ± 1.2</td>
<td>14.3 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Rice Rice</td>
<td>Interior</td>
<td>0 ± 0</td>
<td>0.3 ± 0.3</td>
<td>1.0 ± 1.0</td>
<td>5.0 ± 2.3</td>
<td>6.30 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>SPR Rice</td>
<td>Exterior</td>
<td>4.0 ± 1.5b</td>
<td>1.7 ± 0.9</td>
<td>0.3 ± 0.3</td>
<td>12.0 ± 3.0</td>
<td>18.0 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Rice Rice</td>
<td>Exterior</td>
<td>0 ± 0</td>
<td>2.7 ± 0.9</td>
<td>0 ± 0</td>
<td>5.0 ± 1.7</td>
<td>7.7 ± 2.2</td>
<td></td>
</tr>
</tbody>
</table>

\[ F = 6.85; \quad d_f = 3, 6; \quad P = 0.0230 \]

\[ F = 1.91; \quad d_f = 3, 6; \quad P = 0.2286 \]

\[ F = 0.57; \quad d_f = 3, 6; \quad P = 0.6542 \]

\[ F = 2.80; \quad d_f = 3, 6; \quad P = 0.1311 \]

\[ F = 5.09; \quad d_f = 3, 6; \quad P = 0.0436 \]

\[ a \] Treatments consisted of plots of rice and Amazon sprangletop in the following spatial arrangements: rice in the interior portion of plots, SPR in the exterior portions; rice in the exterior portions of plots, SPR in the interior portion; and rice in both interior and exterior portions of plots.

\[ b \] Bold means denote significance at P < 0.10; means separated by contrast statements.

\[ c \] F-values presented for treatment effects.

**Discussion**

The presence of Amazon sprangletop in proximity to rice increased borer damage to rice. Since over 95% of borers found in plants sampled were sugarcane borers, it is likely that observed patterns (i.e., more damage in rice with sprangletop vs pure stands of rice)
were the result of effects on sugarcane borer behavior. Increase in incidence in borer
damage in the presence of sprangletop was observed using two different sampling methods.

Similar results have been observed in other crop production systems, including in
sugarcane with sugarcane borers and rice with rice stink bugs. More deadhearts were
observed in weedy plots of a *Leptochloa* sp. and sugarcane than in weed-free sugarcane (Dr.
Gene Reagan, unpublished data). Graminaceous weeds also have been shown to increase
rice stink bug populations and their damage to rice (Tindall et al. 2004). The impact of
weeds on rice yields is often thought to result primarily form competition early in the season
presence of weeds impact on insect populations have not often been considered. These
finding suggests that late-season graminaceous weed control is important to reduce insect
damage associated with alternate hosts influencing insect populations since more borer
damage was seen in the presence of sprangletop than in its absence.

*Table 6.2.* Mean number of plants damaged by borers observed when visually examining
entire plots of rice when rice was grown in association with Amazon sprangletop (SPR)
compared to numbers found in rice of whole plots of rice. Damage was characterized as
deadhearts, partial whiteheads, whiteheads, sheath lesions, and total damage.

<table>
<thead>
<tr>
<th>Plant in Interior</th>
<th>Plant in Exterior</th>
<th>Area Sampled</th>
<th>Deadhearts ± SE</th>
<th>Partial whiteheads ± SE</th>
<th>Whiteheads ± SE</th>
<th>Total ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>SPR</td>
<td>Interior</td>
<td>4.3 ± 1.5</td>
<td>19.0 ± 2.1</td>
<td>10.7 ± 2.4</td>
<td>34.0 ± 5.6</td>
</tr>
<tr>
<td>Rice</td>
<td>Rice</td>
<td>Interior</td>
<td>3.0 ± 2.1</td>
<td>13.3 ± 2.2</td>
<td>5.7 ± 3.3</td>
<td>22.0 ± 5.0</td>
</tr>
<tr>
<td>SPR</td>
<td>Rice</td>
<td>Exterior</td>
<td>12.3 ± 4.6</td>
<td>22.7 ± 3.5</td>
<td>11.0 ± 3.6</td>
<td>46.0 ± 10.6</td>
</tr>
<tr>
<td>Rice</td>
<td>Rice</td>
<td>Exterior</td>
<td>1.0 ± 0.6</td>
<td>15.0 ± 3.1</td>
<td>6.3 ± 0.9</td>
<td>22.3 ± 2.7</td>
</tr>
</tbody>
</table>

*F* = 3.50; *c*  
\(df = 3, 6\);  
*P* = 0.0895  

*F* = 3.61;  
\(df = 3, 6\);  
*P* = 0.0847  

*F* = 1.23;  
\(df = 3, 6\);  
*P* = 0.3779  

*F* = 4.11;  
\(df = 3, 6\);  
*P* = 0.0667  

\(a\) Treatments consisted of plots of rice and Amazon sprangletop in the following spatial
arrangements: rice in the interior portion of plots, SPR in the exterior portions; rice in the
exterior portions of plots, SPR in the interior portion; and rice in both interior and exterior
portions of plots.

\(b\) Bold means denote significance at *P* < 0.10; means separated by contrast statements.

\(c\) *F*-values presented for treatment effects.
Both sampling methods detected significant differences in damage to rice grown with or without sprangletop. However, the variables that were different from one another were not consistent between sampling methods. More research needs to be conducted to determine if smaller areas can be sampled to obtain accurate estimates of borer damage.

Previous studies have shown that the presence of weeds may assist in predicting insect populations. Rice stink bugs were shown to feed on weeds prior to panicle emergence of rice and move to rice after panicles emerged (Tindall et al. 2004); therefore, weedy fields need to be scouted prior to panicle emergence to reduce damage to rice. Sprangletop plants produced seed heads approximately three and a half weeks prior to panicle emergence to rice, and severe injury was observed in sprangletop areas of mixed plot. Larvae collected were similar in size regardless of the host from which they were collected. Therefore, borer damage observed in sprangletop plants was likely to estimate the damage to rice that would be apparent after panicles emerge. Although, damage to sprangletop may predict damage to rice, treatment measures would likely be too late since damage to sprangletop plants and rice likely occurred simultaneously. The behavior of stem boring insects is such that larvae stay within the plant on which it is feeding, as opposed to insects like rice stink bugs that move about panicles freely. Therefore, sprangletop damage would not be a good indicator to predict borer damage to rice. Once damaged seedheads are seen on sprangletop plants, damage to rice is likely to have already have occurred.

Data presented are from a single year; therefore, caution must be used before extrapolating these results. However, these findings warrant additional research to verify the effects observed in this study. Results from Tindall et al. (2004) and this study suggest
that season-long graminaceous weed control may be important in reducing damage and loss in grain quality due to the presence of weeds influencing insect populations.

References


CHAPTER 7

EVALUATION OF THE POTENTIAL ROLE OF GLUFOSINATE-TOLERANT RICE IN INTEGRATED PEST MANAGEMENT STRATEGIES FOR RICE WATER WEEVIL (COLEOPTERA: CURCULIONIDAE)*

Introduction

Nearly 80% of rice, Oryza sativa L., grown in the United States is treated with herbicides two or more times in a season to manage weed pests (Crawford et al. 1990). Weed control is complicated by the fact that the primary weed pest of rice, red rice, (O. sativa L.), is conspecific with commercial rice; therefore, no herbicides are available that control red rice that do not also harm commercial rice. Red rice infests 75% or more of the acreage of rice grown in Louisiana (Sankula et al. 1997) and is estimated to cause losses in rice yield and quality by $50 million annually in the southern United States (Kwon et al. 1991). Red rice has a red pericarp that requires extra milling to remove, and extra milling causes breakage of commercial rice grains (Dunand 1988; Kwon et al. 1991; Pantone and Baker 1991). Since red rice is difficult to control with herbicides, producers rely on cultural practices such as water seeding, early flooding, increased seeding rates, and early planting dates to give commercial rice a competitive advantage over red rice (Dunand 1988; Crawford et al. 1990).

Although herbicide-tolerant varieties have been adopted in several crops (James 2003), only recently has an herbicide-tolerant rice variety been released. Some producers planted an herbicide-tolerant variety, Clearfield Rice™ (BASF, Research Triangle Park, NC) in 2003 that was selected for resistance to imidazolone herbicides through a combination of mutagenesis and conventional breeding. Another herbicide-tolerant variety,

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LibertyLink® rice with the genetic locus LLRICE62 (Bayer CropScience, Kansas City, MO), was developed through recombinant DNA technology to be tolerant to glufosinate-ammonium; however, LibertyLink® is not yet commercially available. Rice plants with event LLRICE62 were found to be agronomically, environmentally, and nutritionally equivalent to commercial rice varieties and to pose no health concerns (USDA 1999a, 1999b; FDA 2000). Bayer CropScience received a label from the Environmental Protection Agency (EPA 2003) for glufosinate use on LibertyLink® rice varieties. Although all United States governmental agencies have approved the glufosinate-tolerant technology, it is not expected to be commercially released until 2007. There are currently no data available examining how use of glufosinate-tolerant rice technology affects the most destructive insect pest of rice in the United States, the rice water weevil, Lissorhoptrus oryzophilus Kuschel.

Glufosinate-tolerant rice confers resistance to the herbicide glufosinate-ammonium via insertion of the bialophos resistance (bar) gene (Sankula et al. 1997). Glufosinate inhibits glutamine synthase, thereby hindering the conversion of glutamate to glutamine in susceptible plants (Duke 1990; Dröge-Laser et al. 1994). Inhibition of glutamine synthase in susceptible plants leads to rapid accumulation of ammonia, disruption of chloroplasts, and termination of photosynthesis and photorespiration; however, the most detrimental consequence is inhibition of ribulose bisphosphate carboxylase/photorespiration (Vasil 1996). Presence of the bar gene permits transformed plants to metabolize glufosinate into inactive by-products, allowing an adequate amount of uninhibited glutamine synthase for normal metabolic function.

Applications of several herbicides to plants have been shown to alter host quality for herbivores (Campbell 1988). Herbicide-induced changes include increased nitrogen content
and altered wax composition, changes which result in improved growth rates and reduced feeding of herbivores, respectively (Ishii and Hirano 1963; Eigenbrode and Shelton 1992). Although herbicide applications are not lethal to herbicide-tolerant varieties, injury can still occur (Lanclos et al. 2003). Depending on the severity of injury, the nutritive quality of treated plant tissues for insects may be altered (Campbell 1988). In addition to herbicide-induced effects, direct herbicide toxicity can prolong insect development, increase mortality (Adams 1960), and alter feeding behavior (Dimetry and Mansour 1975). Specifically, the herbicide glufosinate has the potential to affect insects because glutamine synthase is present in many insect systems, including the nervous system. Contact and oral toxicity of glufosinate has been documented for mites (Ahn et al. 1997) and Calpodes ethylius Stoll., (Lepidoptera: Hesperiidae), larvae (Kutlesa and Caveney 2001), respectively. Glufosinate can also act as a feeding deterrent for certain lepidopteran larvae, such as cotton leafworm, Spodoptera littoralis (Boisd.), (Lepidoptera: Noctuidae), (El-Ghar 1994).

Use of herbicide-tolerant rice varieties may offer producers the additional benefit of rice water weevil control. Rice water weevil adults feed on rice leaves and oviposit in the leaf sheath beneath the water surface (Smith, 1983; Rice et al. 1999). Larvae eclose, migrate to roots, and feed on root tissue. Larvae feeding on roots cause an average of a 10% loss in yield and result in losses of up to 50 million dollars (Smith 1983, Spradley and Widham 1995) annually if not controlled.

Rice is more vulnerable to rice water weevil infestations when permanent floods are established at the 2-3 leaf stage (early flood) as opposed to the 4-5 leaf stage (delayed flood). Delayed floods have shown to cause significant reductions in larval populations due to interference with preference of rice water weevils to oviposit below the water surface
(Rice et al. 1999, Stout et al. 2001). When floods were delayed, rice yields were higher than when floods were applied early (Zou et al. 2004). Prior to the availability of herbicide-tolerant rice varieties, floods have been applied early to the majority of rice acreage to assist in red rice management. However, with the introduction of herbicide-tolerant varieties, delayed flooding may be adopted as part of pest management strategies.

Glufosinate-tolerant technology can potentially affect management of the rice water weevil in three ways: by direct exposure of adults to glufosinate, indirectly through an herbicide-induced plant response, or indirectly by allowing floods to be delayed. Greenhouse studies were conducted to determine if glufosinate-treated and non-treated LLRICE62 rice were similar in their susceptibilities to infestations of rice water weevil. Additionally, the preference of rice water weevil adults for glufosinate-treated and non-treated LLRICE62 rice plants was examined. Direct toxicity of glufosinate to rice water weevil adults was also tested. Field experiments were conducted to examine effects of the use of glufosinate-tolerant technology on rice water weevils under field conditions.

**Materials and Methods**

**Plant and Insect Material**

Glufosinate-tolerant rice with event LLRICE62 and its parent line ‘Bengal’ were used in a greenhouse on the LSU campus in Baton Rouge, LA. Seedlings were grown in 9 cm diameter pots using a 4:2:1:1 mixture of soil (sterilized Commerce silt loam), peat moss, sand, and vermiculite. Fertilizer (0.8 g of 23:12:12 N:P:K per pot) was incorporated into the soil mix at planting. Pots were placed in wooden basins lined with black plastic pond liner and watered as needed. Temperature in the greenhouse ranged from 25 °C to 35 °C. Natural light was not supplemented.
Breeder seed of the medium grain variety, Bengal, and two lines derived from Bengal, LL-401 and LL-001, containing transformation event LLRICE62 were obtained from Dr. Steve Linscombe (Rice Research Station, Louisiana State University AgCenter). LL-401 and LL-001 had been selected for similarity to the parent variety, Bengal and for tolerance to the herbicide, glufosinate. Neither transformed lines segregated for glufosinate tolerance in the T3 generation, as demonstrated by application of glufosinate thus, exhibiting homozygosity for the genetic locus containing the bar gene. Further purification and multiplication of the lines produced the breeder seed used for this study. Both transformed lines with event LLRICE62 are shorter than the parental variety Bengal, but the difference in height is not sufficient to move the plant variety protection height classification from short to semidwarf (PVP Office 1998). The greenhouse studies conducted in 2001 used LL-401. Preference tests for treated foliage in 2002 and field studies were conducted using LL-001.

Rice water weevil adults used in greenhouse studies were collected from rice fields at the Louisiana State University AgCenter Rice Research Station in Crowley, LA. Weevils were transported, housed in glass jars, and provided freshly cut rice leaves and a moistened paper towel. Adults were used for experiments within 24 hours of collection and discarded after use in an experiment. Rice water weevils were of various ages since they were field collected and pass through at least one and a half generations per growing season. Field experiments were conducted using natural infestations of rice water weevils.

**Transgenic and Herbicide-Induced Effects**

Four separate greenhouse experiments were conducted to investigate the suitability of glufosinate-treated and non-treated glufosinate-tolerant rice for rice water weevils. For each experiment, treatments included glufosinate-tolerant rice sprayed with commercially
formulated glufosinate at recommended field rates (401 g AI/ha); glufosinate-tolerant rice without herbicide; and Bengal rice (parent line) without herbicide. Glufosinate was not applied to Bengal because glufosinate at the rates uses is lethal to Bengal rice. Glufosinate was applied at the 2-3 leaf rice stage followed by a second application 7 - 10 days later. Glufosinate was applied using a back-pack sprayer pressurized with CO$_2$ and calibrated to deliver 140 L/ha at 207 kPa through four 1002 flat fan nozzles on 51 cm spacing. Important dates of experimental procedures and numbers of plants assessed each time the experiment was repeated are presented in Table 7.1.

**Table 7.1.** Important dates and experimental information for each replication of greenhouse studies conducted in Baton Rouge, LA in 2001 that investigated the effect of the glufosinate-tolerant rice on rice water weevil densities.

<table>
<thead>
<tr>
<th>Herbicide applications$^b$</th>
<th>I$^a$</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3 leaf</td>
<td>May 19</td>
<td>June 16</td>
<td>June 30</td>
<td>July 20</td>
</tr>
<tr>
<td>4-5 leaf</td>
<td>May 28</td>
<td>June 23</td>
<td>July 8</td>
<td>July 27</td>
</tr>
<tr>
<td>Initiation of adult infestation and flood</td>
<td>May 30</td>
<td>June 25</td>
<td>July 10</td>
<td>July 29</td>
</tr>
<tr>
<td>Termination of adult infestation</td>
<td>June 3</td>
<td>June 29</td>
<td>July 14</td>
<td>Aug 2</td>
</tr>
<tr>
<td>Root wash</td>
<td>June 22</td>
<td>July 16</td>
<td>Aug 2</td>
<td>Aug 21</td>
</tr>
<tr>
<td>$n^c$</td>
<td>16</td>
<td>24</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

$^a$ Replication I examined larval populations; replications II, III, IV examined both larval populations and numbers of eggs oviposited in leaf sheaths of rice plants.

$^b$ Glufosinate was applied at a rate of 401 g AI/ha at both timings of application.

$^c$ $n =$ total number of plants per treatment from which data was collected.

Plants were thinned to one plant per pot in the first experiment and two plants per pot in the remaining experiments. Experiments were initiated by spraying herbicide on transgenic plants, always when the plants were at the 2-3 leaf stage. Care was taken when watering plants to avoid removal of residual herbicide on foliage. Two days after the second herbicide application, four pots of each treatment were placed in cylindrical cages constructed of wire frame and insect screen (46 cm in diameter and 61 cm in height and flooded to a
depth of 18 cm). Two adult rice water weevils per plant were released into each cage. Weevils were allowed to feed, mate, and oviposit for four days before adult infestations were terminated by removing plants from cages, discarding weevils found on plants, and placing plants in plastic-lines basins maintained at a flood depth of 6.4 cm above the soil line.

In the second, third, and fourth experiments, one plant from each pot was removed immediately after terminating adult infestations. These plants were used to evaluate rice water weevil ovipositional preference by counting eggs laid in the leaf sheaths of each plant. Plants were bleached in a 75% ethanol/water solution for several weeks (Gifford and Trahan 1969). Rice water weevil eggs were counted by viewing leaves under a dissecting microscope.

Twenty-one days after termination of adult infestation, plants not used for egg counts were removed and their roots and associated soil were washed into sieves consisting of galvanized buckets with a 40 mesh screen bottom (Smith and Robinson 1982). Screen buckets were placed in saturated saline solution, and the number of larvae floating to the surface was recorded. Larvae were collected, taken to the laboratory, frozen, lyophilized, and weighed to the nearest mg.

Treatment effects on egg densities, larval densities, and mean larval weights were analyzed using one-way ANOVA. Means were separated using Tukey’s studentized range test. All analyses were performed using PROC MIXED of SAS (SAS Institute 1998). The dates on which experiments were conducted and the cage in which plants were placed were used in the RANDOM statement.
Direct Herbicide Effects

**Adult Feeding Preference for Glufosinate-Treated and Non-Treated Foliage**

During the summer of 2002, adult feeding preference for glufosinate-treated and non-treated foliage was examined using LL-001 plants as described above. Adults were presented with approximately 5 cm of foliage from transformed rice removed from plants at the 4-5 leaf stage and dipped into commercially formulated glufosinate (401 g AI / ha = 0.0089 M) or dipped into water (control). Petri dishes (100 mm X 15 mm) were lined with cotton batting. Each dish received one piece of treated and non-treated foliage and one adult weevil. Weevils were starved for three hours to ensure feeding before being placed in the center of a petri dish equidistant from the two pieces of foliage and allowed to move freely about the dish. Twenty-four hours after initiation of feeding, weevils were removed from dishes and feeding scars were measured in mm on both pieces of foliage. Scar left by rice water weevil feeding are approximately 1 mm in width, allowing easy calculation of area consumed. Thirty-three adult rice water weevils were assessed for feeding preference. Differences in area consumed on treated and non-treated foliage were analyzed using PROC TTEST (SAS Institute 1998).

**Herbicide Bioassay**

In 2002, toxicity of technical grade and commercial formulations of glufosinate to rice water weevil was assessed. Ten concentrations of commercially formulated glufosinate were used: 0.6 (pure product), 0.044, 0.04, 0.036, 0.031, 0.027, 0.022, 0.018, 0.0089 (recommended rate) and 0 M. Concentrations of technical grade glufosinate were: 0.5, 0.4, 0.3, 0.25, 0.2, 0.125, 0.1, 0.0625, 0.05, 0.03125, 0.025, 0.0078, 0.0039, 0.00156, 0.00129, and 0 M. Technical grade glufosinate is water-soluble and reaches saturation at 0.5M.
Therefore, water was used as the solvent for both forms of glufosinate. Adult weevils were completely submerged in each concentration for three seconds, removed and placed in 473 ml paper cartons lined with moistened cotton batting and covered with cheesecloth. Weevils were provided freshly cut rice leaves daily, and water was added to cotton batting every other day for 9 days. Rice water weevils were considered dead if they were not able to right themselves within 5 minutes of being placed on their dorsal surface. Approximately 50 weevils were used for each concentration of each herbicide formulation. Mortality was rated at 24 hours and every 48 hours after for eight additional days. Data were analyzed using PROC PROBIT in SAS (SAS Institute 1998) to determine the LC$_{50}$ for each formulation.

**Field Studies**

Experiments were conducted to determine if the use of glufosinate-tolerant rice would affect densities of rice water weevil in the field. Experiments were conducted in 2002 and 2003 in north Louisiana at the Macon Ridge Research (Winnsboro, LA, Franklin Parish) and Northeast Research (St. Joseph, LA, Tensas Parish) Stations. Rice seeds were drill-seeded at a rate of 112 kg/ha into a loessial upland soil (Gigger silt loam) or an alluvial flood plain soil (Sharkey clay) in Winnsboro and St. Joseph, respectively. Nitrogen, in the form of prilled urea, was applied at 126 kg/ha the day permanent floods were established. Dates of important agronomic practices and experimental information are presented in Table 7.2.

Experimental design was a split plot with a randomized block structure for both the main plot and subplots. There were four replications each year at each location with the exception of St. Joseph in 2002 when there were three replications. The main plot treatment
Table 7.2. Important dates of agronomic practices for field studies examining the effect of the glufosinate-tolerant rice technology on rice water weevil densities.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Planting date</td>
<td>May 17</td>
<td>May 30</td>
<td>May 28</td>
<td>May 24</td>
</tr>
<tr>
<td>Herbicide applications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-3 leaf</td>
<td>June 3</td>
<td>June 20</td>
<td>June 12</td>
<td>June 11</td>
</tr>
<tr>
<td>4-5 leaf</td>
<td>June 14</td>
<td>July 6</td>
<td>June 24</td>
<td>June 25</td>
</tr>
<tr>
<td>Permanent flood&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early flood</td>
<td>June 5</td>
<td>June 24</td>
<td>June 16</td>
<td>June 13</td>
</tr>
<tr>
<td>Delayed flood</td>
<td>June 18</td>
<td>July 10</td>
<td>June 26</td>
<td>June 29</td>
</tr>
<tr>
<td>Rice water weevil sampling dates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early flood</td>
<td>July 1</td>
<td>July 21</td>
<td>July 9</td>
<td>July 7</td>
</tr>
<tr>
<td>Delayed flood</td>
<td>July 10</td>
<td>Aug 4</td>
<td>July 14</td>
<td>July 24</td>
</tr>
</tbody>
</table>

<sup>a</sup> Two timings of flood were studied: early flood established at the 2-3 leaf stage of rice and delayed flood established at the 4-5 leaf stage of rice.

was time of flood based on the developmental stage of rice; floods were established either at the 2-3 leaf stage (early flood) or the 4-5 leaf stage (delayed flood). The six subplot treatments were factorial arrangements of insecticide application and the combination of rice variety and the herbicide used on the variety. Seeds were either treated with the insecticide fipronil (56 g AI/ha) or non-treated. The variety and herbicide combinations were LL-001 treated with glufosinate, LL-001 with conventional herbicide, and Bengal with conventional herbicide. Glufosinate (401 g AI/ha) was applied twice, once at the 2-3 leaf stage followed by an application at the 4-5 leaf stage, in plots assigned to receive glufosinate. In plots assigned to the conventional herbicide treatment, propanil (3.4 kg AI/ha) and quinclorac (420 g AI/ha) were applied at the 2-3 leaf stage followed by molinate (3.8 kg AI/ha) and propanil (3.8 kg AI/ha) at the 4-5 leaf stage. Herbicides were applied using a back-pack
sprayer pressurized with CO₂ and calibrated to deliver 140 L/ha at 207 kPa equipped with a four-nozzle (1002 flat fan) boom at 51 cm spacing.

Insect densities were assessed by removing three root/soil samples from each plot using a metal soil corer (9.2 cm diameter with a depth of 7.6 cm). Roots and soil were washed in screen-bottom buckets as previously described. Larval samples were collected 3-3.5 weeks after permanent floods were established on the dates shown in Table 7.2. Treatment effects on larval density were analyzed using ANOVA (PROC MIXED of SAS). Year and location were used as RANDOM effects. Means were separated using Tukey’s studentized range test (SAS Institute 1998). Contrast statements were also employed to examine the effect of herbicides and variety on numbers of rice water weevil larvae.

Results

Transgenic and Herbicide-Induced Effects

More eggs were found on glufosinate-tolerant rice not treated with glufosinate than on glufosinate-tolerant rice treated with glufosinate or on the non-treated parent line, non-treated ‘Bengal’ (Table 7.3). Fewer larvae were present on roots of glufosinate-treated glufosinate-tolerant plants than on non-treated glufosinate-tolerant rice 25 days after infestation. Numbers of larvae on non-treated glufosinate-tolerant rice and non-treated ‘Bengal’ rice did not differ. Although larvae feeding on non-treated glufosinate-tolerant rice were 1.5 times larger than those on glufosinate-tolerant rice and 2 times larger than those feeding on ‘Bengal’, differences were not statistically significant.
Table 7.3. Mean number of eggs and larvae per plant and mean larval weights of rice water weevils when adult weevils were presented a choice of glufosinate-tolerant rice (LL-401) treated with glufosinate, glufosinate-tolerant rice (LL-401) with no herbicide, and the parent variety ‘Bengal’ with no herbicide in greenhouse experiments in Baton Rouge, LA, 2001.

<table>
<thead>
<tr>
<th>Variable Measured</th>
<th>Eggs (SE)</th>
<th>Larvae (SE)</th>
<th>Larval wt mg (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL-401 + glufosinate</td>
<td>32.8 (2.9) b</td>
<td>10.5 (0.8) b</td>
<td>0.83 (0.12) a</td>
</tr>
<tr>
<td>LL-401 no glufosinate</td>
<td>47.0 (6.0) a</td>
<td>13.3 (0.9) a</td>
<td>1.27 (0.37) a</td>
</tr>
<tr>
<td>‘Bengal’ no glufosinate</td>
<td>31.9 (2.9) b</td>
<td>12.9 (1.0) ab</td>
<td>0.63 (0.06) a</td>
</tr>
<tr>
<td>n b</td>
<td>56</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>F; df; P &lt;</td>
<td>F = 4.26; df = 2, 158;</td>
<td>F = 3.33; df = 2, 188;</td>
<td>F = 2.19; df = 2, 187;</td>
</tr>
<tr>
<td></td>
<td>P = 0.0158</td>
<td>P = 0.0380</td>
<td>P = 0.1151</td>
</tr>
</tbody>
</table>

*a Means in the same column followed by different letters are significantly different. Means were separated by Tukey’s studentized range test $\alpha = 0.05$.

*b n = number of plants / treatment; the n value represents of three replications of this experiment for the “egg” variable and four replications for the remaining variables.

Direct Herbicide Effects

Adult Feeding Preference of Glufosinate-Treated and Non-Treated Foliage

The amount of foliage consumed by adult rice water weevils on glufosinate-treated LL-001 foliage (10.03 mm$^2 \pm 3.72$) and non-treated foliage (5.91 mm$^2 \pm 2.48$) did not differ ($F = 0.92; df = 55.7; P < 0.3359$). This result suggests that adult rice water weevils showed no preference for glufosinate-treated or non-treated rice foliage.

Herbicide Bioassay

The LC$_{50}$’s for technical grade and commercially-formulated glufosinate were 0.03119 and 0.01946 M, respectively (Table 7.4). Confidence intervals overlapped for commercially-formulated and technical grade glufosinate, meaning that the LC$_{50}$’s were similar for both formulations.

Field Studies

Table 7.5 presents the mean rice water weevil larval densities for field experiments in which interactions among timing of permanent flood, insecticide, and the combinations of
Table 7.4. LC$_{50}$ for rice water weevil adults exposed to commercially formulated or technical grade glufosinate.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$n$</th>
<th>Slope ± SE</th>
<th>LC$_{50}$ (95% CL) M</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial formulation</td>
<td>512</td>
<td>1.2038 ± 0.3064</td>
<td>0.0195 (0.008 – 0.030)</td>
<td>0.0059</td>
</tr>
<tr>
<td>Technical Grade</td>
<td>809</td>
<td>0.5836 ± 0.1009</td>
<td>0.0312 (0.013 – 0.062)</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Table 7.5. Evaluation of the glufosinate-tolerant rice (LL-002) technology and potential interactions between insecticide use and flooding regimes on rice water weevil larvae compared to a conventional rice line$^a$.

<table>
<thead>
<tr>
<th>Insecticide$^b$</th>
<th>Stage at flood$^c$</th>
<th>Variety + Herbicide$^d$</th>
<th>Larvae ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Insecticide</td>
<td>2-3 leaf</td>
<td>LL + glufosinate</td>
<td>6.76 ± 1.43</td>
</tr>
<tr>
<td>No Insecticide</td>
<td>2-3 leaf</td>
<td>Beng + Conv Herb</td>
<td>6.25 ± 1.40</td>
</tr>
<tr>
<td>No Insecticide</td>
<td>2-3 leaf</td>
<td>LL + Conv Herb</td>
<td>6.03 ± 1.37</td>
</tr>
<tr>
<td>No Insecticide</td>
<td>4-5 leaf</td>
<td>LL + glufosinate</td>
<td>3.25 ± 1.39</td>
</tr>
<tr>
<td>No Insecticide</td>
<td>4-5 leaf</td>
<td>Beng + Conv Herb</td>
<td>3.22 ± 1.38</td>
</tr>
<tr>
<td>No Insecticide</td>
<td>4-5 leaf</td>
<td>LL + Conv Herb</td>
<td>2.28 ± 1.38</td>
</tr>
<tr>
<td>Insecticide</td>
<td>2-3 leaf</td>
<td>LL + glufosinate</td>
<td>1.08 ± 1.39</td>
</tr>
<tr>
<td>Insecticide</td>
<td>2-3 leaf</td>
<td>Beng + Conv Herb</td>
<td>1.06 ± 1.39</td>
</tr>
<tr>
<td>Insecticide</td>
<td>2-3 leaf</td>
<td>LL + Conv Herb</td>
<td>1.01 ± 1.43</td>
</tr>
<tr>
<td>Insecticide</td>
<td>4-5 leaf</td>
<td>LL + glufosinate</td>
<td>1.00 ± 1.39</td>
</tr>
<tr>
<td>Insecticide</td>
<td>4-5 leaf</td>
<td>Beng + Conv Herb</td>
<td>0.88 ± 1.40</td>
</tr>
<tr>
<td>Insecticide</td>
<td>4-5 leaf</td>
<td>LL + Conv Herb</td>
<td>0.85 ± 1.38</td>
</tr>
</tbody>
</table>

$^a$ Data are from field experiments conducted in 2002 and 2003 in Winnsboro and St. Joseph, LA. See text for further explanation of experimental design.

$^b$ Icon applied as a seed treatment at a rate of 56 g AI / ha.

$^c$ Developmental stage of rice when the permanent flood was applied.

$^d$ Variety and herbicide examined, glufosinate-tolerant rice treated with glufosinate (401 g /ha) (LL+glufosinate), glufosinate-tolerant rice treated with conventional herbicides (LL+Conv Herb), or the parent line, Bengal, treated with conventional herbicides (Beng+Conv Herb). Conventional herbicides were propanil (3.4 kg/ha) and quinclorac (420 g/ha) applied at the 2-3 leaf stage of rice followed by molinate (3.8 kg/ha) and propanil (3.8 kg / ha).

variety and herbicide use were examined. The effect of flooding was marginally significant with $3.7 \pm 1.3$ larvae found on roots of early flooded rice and $1.9 \pm 1.3$ ($df = 1, 4$; $F$-val = 125
6.28; \( P-val = 0.0663 \) found on roots of late-flooded rice. There were four times more rice water weevil larvae collected from rice not treated with insecticide \((4.6 \pm 1.2)\) than from insecticide-treated rice \((1.0 \pm 1.2)\) \((df = 1, 126; F-val = 145.64; P-val = <0.0001)\). The effect of the combination of variety and herbicide was not significant \((F = 0.82; df = 2, 126; P = 0.4434)\). The interaction of time of flood and insecticide was significant \((F = 8.06; df = 1, 126; P = 0.0053)\). There were significantly more larvae present on roots of early flooded rice not treated with insecticide \((6.3 \pm 1.3)\) than on roots of any other insecticide flood combination. Late flooded rice with no insecticide had significantly more larvae \((2.9 \pm 1.3)\) than early or late flooded rice treated with insecticide \((1.1 \pm 1.3 \text{ larvae and } 0.9 \pm 1.3 \text{ larvae, respectively})\); numbers of larvae in insecticide treated plots were not significantly different from one another. All other interactions were not significant \((flood*combination of herbicide and variety - F = 0.26; df = 2, 126; P = 0.7747; insecticide*combination of herbicide and variety - F = 0.47; df = 2, 126; P = 0.6287; insecticide*flood*combination of herbicide and variety \(F = 0.66; df = 2, 126; P-val = 0.5162\)). Contrast statements did not reveal significant effects of variety or herbicide on larval densities \((variety - F = 0.66; df = 1, 126; P = 0.4187; \text{herbicide} - F = 1.59; df = 1, 126; P = 0.2103)\).

**Discussion**

Greenhouse experiments showed that glufosinate-tolerant rice with the \textit{bar} gene not treated with glufosinate was more preferred for oviposition than the non-transformed parent line, Bengal. However, levels of susceptibility to oviposition were restored to levels similar to that of the parent line after glufosinate applications were made. Larval densities on glufosinate-tolerant rice treated with glufosinate in field experiments did not differ from those on the parent line or glufosinate-tolerant rice treated with conventional herbicides.
However, delaying floods for approximately two weeks from the 2-3 leaf stage until the 4-5 leaf stage and the use of insecticide both reduced larval infestations of rice water weevil.

Many differences between transformed plants and their parent lines have been documented. The majority of these differences have been agronomic traits. Lanclos et al. (2003) reported that two transformed glufosinate-tolerant lines of rice (BNGL HC-11 and BNGL HC-62) were shorter and had lower grain moistures at harvest compared to their parent line when glufosinate was not applied. An additional transgenic rice line, CPRS PB-13, was taller and produced lower yields than its parent line in the absence of glufosinate application. Similarly, Brandle and Miki (1993) found yields were lower from non-treated sulfonylurea-tolerant transgenic tobacco, *Nicotiana tabacum* L. (Solanaceae), plants compared to non-transformed control plants. Several explanations have been given for the observed differences in agronomic traits between transformed plant lines and their non-transformed parent lines. The first possibility is somaclonal variation (i.e. cytogenic or phenotypic) occurring as a result of tissue culture used during the transformation process. (Jain 2001; Bhat and Srinivasan 2002). Choi et al. (2000) found that variation was amplified in transgenic barley, *Hordeum vulgare* L. (Poaceae), compared to non-transgenic barley in tissue culture. Second, the insertion of genes into the genome during transformation is a random process; therefore, differences between transformed and non-transformed plants could be due to insertional effects (Bhat and Srinivasan 2002). For example, if the *bar* gene was inserted in a region involved in resistance to rice water weevil oviposition, resistance expression could be altered. Third, a change in an agronomic trait may be selected for during the breeding process following transformation as exemplified by the shorter stature of glufosinate-tolerant lines LL-401 and LL-001 than the parent line Bengal.
Differences other than agronomic traits between transgenic varieties and their parent lines have been documented. The *uidA* (*gus*) gene that encodes for β-glucuronidase activity and the *nptII* gene encoding the neomycin phosphotransferase II are commonly used as marker genes and are considered to have neutral effects on other organisms. However, Colorado potato beetles, *Leptinotarsa decemlineata* (Say), (Coleoptera: Chrysomelidae), consumed potatoes, *Solanum tuberosum* L. (Solanaceae), transformed with *uidA* and *nptII* genes at a higher rate than the non-transformed parent. Colorado potato beetles fed foliage from transformed plants were also larger and more tolerant to starvation conditions than those fed foliage from non-transformed potato (de Turck et al. 2002). In addition, a cotton, *Gossypium hirsutum* L. (Malvaceae), variety, Paymaster 1560B, with the *cry 1Ac* gene for insect resistance expressed lower levels of resistance to root-knot nematode relative to its parent line PM 1560 (Colyer et al. 2000); however, the mechanism associated with this loss of resistance has not been examined. Symptoms of rice blast disease (*Magnaporthe grisea*) and sheath blight (*Rhizoctonia solani*) were less common on glufosinate-tolerant rice; however, the reductions were attributed to toxic effects of glufosinate to the pathogens and not to the variety of rice (Uchimiya et al. 1993; Tada et al. 1996).

Data showed ovipositional preference for non-treated LL-401 to be higher than that for glufosinate-treated LL-401 or its parent line; however, the data were inconsistent with respect to larval densities. Few experiments that include a non-treated transgenic control have examined the effects of herbicide-tolerant transgenic varieties on insects, although this type of evaluation of agronomic characteristics of transgenic varieties is required by governmental agencies. Such studies have shown that glufosinate applications to BNGL HC-11, BNGL HC-62, and CPRS PB-13 at various timings produced shorter plants and
increased number of days required to reach 50% panicle emergence relative to their respective non-treated controls (Lanclos et al. 2003). Herbicide applications to transformed (sulfonylurea-tolerant) tobacco lines increased sucker growth by 1.7 to 8.5 times, depending on the line, herbicide, and herbicide rate (Brandle and Miki 1993). Tobacco yields were also reduced in this experiment and were attributed to increases in sucker weight. Only one study has been conducted that examined the impact of herbicide-treated and non-treated transgenic herbicide-tolerant varieties on insects (Bitzer et al. 2002). In that study, differences in populations of Collembola associated with transgenic soybeans, Glycine max (L.) Merr. Fabaceae. resulted from differences in weed cover and degree of soil disturbance, not from herbicide applications or herbicide plant-induced effects following herbicide applications.

In an attempt to determine the difference in number of eggs oviposited in glufosinate-treated and non-treated rice plants LL-401 following glufosinate applications, contact bioassays and preference tests were conducted to investigate direct herbicide toxicity and deterrence due to the presence of herbicide. The LC$_{50}$ from the dose response bioassay with commercially formulated glufosinate was two times greater than the concentration used in greenhouse experiments. Additionally, rice water weevils were not used in greenhouse experiments until two days after plants were treated with glufosinate; hence, rice water weevil exposure to glufosinate was limited to foliar residues. Therefore, glufosinate was probably not toxic to adult weevils at the level adults were exposed. Additionally, since weevils were able to survive three seconds of complete submersion in solutions of herbicide at the recommended rates, a greater exposure than they would ever encounter in the field, it is unlikely that the herbicide will directly impact rice water weevil populations in the field.
Although larvae of *S. littoralis* consumed less glufosinate-treated foliage than non-treated leaves (El-Ghar 1994), data from the adult feeding preference tests with the rice water weevil showed that glufosinate did not influence adult feeding of weevils. The lack of direct effects of glufosinate on rice water weevil feeding or survival argues for the idea that differences in egg and larval densities resulted from herbicide-induced changed in rice resistance to rice water weevil.

Campbell (1988) reviewed the effects of herbicides and plant growth regulators on plant quality for herbivorous insects. Results varied with insect and plant species and chemical used. Forty-one percent of the experiments reviewed showed increases in insect populations due to herbicide-induced effects, whereas 24% of the experiments showed decreases in populations. No herbicide-induced effects were seen in 35% of the experiments. Factors thought to contribute to observed effects ranged from destruction of alternate hosts to increases in nitrogen and sucrose levels and protein content. Eigenbrode and Shelton (1992) suggested that reductions in cuticular waxes and wax crystallites following application of the herbicide S-ethyl dipropylthiocarbamate to cabbage, *Brassica oleracea* L. (Brassicaceae) induced resistance to first instars of *Plutella xylostella* L., (Lepidoptera: Plutellidae). Since glufosinate applications have several injurious effects on susceptible plants (Vasil 1996), if 100% of glufosinate is not metabolized and a small amount reaches the target site, it is possible that changes in host quality following application deterred rice water weevils from ovipositing in leaf sheaths of glufosinate-treated transformed rice plants.

Although variety and herbicide effects were found in greenhouse experiments, no such effects were detected in the field. This could be a result of having more controlled
environmental conditions in greenhouse experiments than in field experiments. Additionally, since time of flood and use of insecticide strongly affected rice water weevil densities, herbicide and variety effects may have been masked by delaying floods or insecticide use. Previous studies have shown that delaying floods by two to four weeks (i.e., four to six weeks after planting) led to a reduction in number of rice water weevil larvae present on roots of rice plants (Rice et al. 1999, Zou et al. 2004). In areas of severe red rice infestations, delayed floods have not been an option since early flooding, which is necessary to prevent yield losses (Dunand 1988; Crawford et al. 1990). With the use of herbicide-tolerant varieties, producers can adopt the practice of delaying floods.

Although greenhouse experiments suggested there may be small differences between glufosinate-treated and non-treated glufosinate-tolerant rice, treated glufosinate-tolerant rice was similar to its parent line in its resistance to the rice water weevil. Field data suggest that rice water weevil populations will not be directly affected by the event LLRICE62. However, there is potential to alter flooding regimes when using the glufosinate-tolerant technology, and those changes may also aid in rice water weevil control. Therefore, results from greenhouse experiments appear to be inconsequential and glufosinate-tolerant rice would be well received by producers for both weed control and rice water weevil suppression. Additionally, having the glufosinate-tolerant technology will provide producers with an alternative to imidazolinone-tolerant rice varieties, thereby delaying the development of herbicide resistance in red rice.
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CHAPTER 8

SUMMARY AND CONCLUSIONS

Rice, *Oryza sativa* L., is an important commodity in the southern United States and is a valuable plant commodity in Louisiana. Both weeds and insects interfere with rice production and are estimated to reduce yields annually by 17% and 8%, respectively. Although weeds and insects have been shown to interact in agricultural systems, little work has been conducted to determine how insects, weeds, and management practices for weeds and insects interact in rice. The two major insect pests of rice in Louisiana are the rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) and the rice stink bug (*Oebalus pugnax* F.). Borers, (i.e., rice stalk borer, *Chilo plejadellus* Zincken, sugarcane borer, *Diatraea saccharalis* F., and European corn borer, *Pyrausta nubilalis* Hübner.), are becoming pests of increasing importance. The goal of this research was to investigate interactions between graminaceous weed pests and insect pests of rice (i.e., rice water weevil, rice stink bug, and sugarcane borer) and interactions between management practices for weeds and insects in rice.

Little is known about the host range, feeding preference, and performance of the rice water weevil. Prior research indicates that rice water weevils feed primarily on monocotyledonous plants, and many monocot weeds occur in rice fields. Host utilization of the rice water weevil was evaluated on rice, cv. ‘Cocodrie’, and seven weeds commonly found in rice fields in preference and life cycle compatibility tests in the greenhouse. Barnyardgrass, *Echinochloa crus-galli* Beauv., yellow nutsedge, *Cyperus esculentus* L., broadleaf signalgrass, *Brachiaria platyphylla* Nash., and fall panicum, *Panicum dichotomiflorum* Michx., were more preferred for oviposition than rice. More neonate
larvae eclosed on barnyardgrass and yellow nutsedge than eclosed on rice. Densities of late
instars feeding on roots of yellow nutsedge and broadleaf signalgrass were significantly
lower than densities on rice. Barnyardgrass was also more preferred for adult feeding than
rice and all other weeds. Rice water weevils were able to complete their life cycle on all
plants examined except hemp sesbania, *Sesbania exaltata* (Rafin.) Cory, the only
dicotyledonous plant species tested in the greenhouse. Field surveys confirmed larvae
infested roots of all weed species sampled in the greenhouse, as well as several other weed
species. Many of the plants infested with larvae were dicotyledonous plants, suggesting that
the host range of rice water weevil is much broader than previously reported. Several new
hosts were added to an existing list of host plants for the rice water weevil.

A few host range and feeding preference studies have been conducted previously
with the rice stink bug. Similarities exist in the host ranges of the rice stink bug and rice
water weevil. Therefore, a common preferred host, barnyardgrass, was selected to examine
how the presence of barnyardgrass affected densities of these insects on rice under field
conditions. Data showed that, not only did the presence of barnyardgrass influence rice
stink bug densities on rice, but also the synchrony of barnyardgrass seed heads and rice
panicles was found to be important in understanding the rice stink bug-weed interaction.
There were up to four times as many rice stink bugs on rice when barnyardgrass was not
present than when barnyardgrass was present. In this case, barnyardgrass appeared to serve
as a trap crop when seed heads were present at the same time as rice panicles. However,
barnyardgrass can serve as a source of infestation of stink bugs if barnyardgrass produces
seed heads prior to rice panicle emergence and begins to senesce at the time panicles
emerge. Densities of rice stink bugs were as many as nine times greater on rice grown in the
presence of barnyardgrass than on rice grown in the absence of barnyardgrass. Large plot demonstration tests conducted at Woodsland Plantation (Richland Parish) in weedy and weed-free areas of rice fields supported these findings from small plot studies. The presence of barnyardgrass appeared to have little impact on rice water weevil. Similar studies were conducted with the sugarcane borer and the weed, Amazon sprangletop, *Leptochloa panicoides* (Presl.) Hitchc. Experiments showed that injury to rice (i.e., deadhearts, whiteheads, and partial whiteheads) was greater in weedy plots of rice than in pure plots of rice. These findings suggest that weeds can impact insect populations; therefore, timely weed control may assist in insect management.

Since the presence of barnyardgrass was shown to have the potential to increase rice stink bug populations, field experiments were conducted to investigate the relationships between weed density, rice stink bug populations, and damage to rice caused by the combination of rice stink bugs and weeds. Graminaceous weeds examined were barnyardgrass, Amazon sprangletop, broadleaf signalgrass, and large crabgrass, *Digitaria sanguinalis*, (L.). Rice seed weight, percent filled seed, percent pecky rice, milling quality, and yield were measured. Data showed that one to two weeds / 0.1m$^2$ was associated with an increase of one rice stink bug per plot. Weeds served as hosts of rice stink bugs prior to panicle emergence of rice; consequently, rice stink bugs infested rice early in the grain filling process and reduced the percentage of filled seeds. Ten weeds / 0.1m$^2$ caused a one percent increase in pecky rice, and for every percent pecky rice, milling quality was reduced by 0.5%. Plots not treated with insecticide had significantly more non-filled seeds, pecky rice, and broken kernels than treated plots. Neither weeds nor insects at the densities observed in this test appeared to affect seed weight. Rice stink bug damage did not
significantly contribute to yield losses greater than weeds in the absence of rice stink bugs. Rice stink bugs had more of an affect on the quality of rice rather than yields. Results reported here suggest that late season weed control may be important in terms of indirect losses in grain quality associated with increased populations of rice stink bug.

The impact of an herbicide-tolerant rice variety was assessed for its resistance to rice water weevil and its place in current integrated pest management strategies. Greenhouse experiments were conducted to evaluate the resistance of a glufosinate-tolerant rice variety and its glufosinate-susceptible parent line, ‘Bengal’, to the rice water weevil in the presence and absence of glufosinate applications. Greenhouse studies demonstrated that, in the absence of glufosinate, glufosinate-tolerant rice had 30% more rice water weevil eggs than ‘Bengal’ rice, the parent line, or glufosinate-tolerant treated with recommended rates of commercially formulated glufosinate. Applications of glufosinate to glufosinate-tolerant rice resulted in a 20% reduction in rice water weevil larval densities compared to non-treated glufosinate-tolerant rice. The LC$_{50}$ dose response and behavioral effects of glufosinate on adult rice water weevils were also studied. The LC$_{50}$ of glufosinate against adult rice water weevils was nearly two times the concentration recommended for application to glufosinate-tolerant rice. There was no difference in the amount of leaf area consumed by adult rice water weevils on glufosinate-treated and non-treated foliage. The absence of direct toxicity of glufosinate to rice water weevil at recommended glufosinate use rates and lack of behavioral effects suggest that the reduction in rice water weevil densities observed following glufosinate applications resulted from herbicide-induced plant resistance. Field studies investigated the impacts of glufosinate-tolerant rice on rice water weevil management in the presence and absence of glufosinate under early and delayed
flood conditions. Field experiments showed that neither rice variety nor herbicide use affected larval densities; however, delaying flood and insecticide effectively reduced numbers of rice water weevil larvae.

Findings presented here show that there are many ways in which insect-weed interactions occur in rice agroecosystems. Results suggest that graminaceous weeds can attract rice stink bugs into fields prior to panicle emergence of rice; therefore, rice stink bugs can be present in rice fields as rice panicles emerge, the stage at which rice stink bugs are most damaging. Consequently, weedy fields should be scouted earlier and more actively for rice stink bugs than weed-free fields to minimize rice stink bug damage.

Prior to these studies, late-season weed control for graminaceous weeds was not considered to be of economic importance since the majority of graminaceous weeds are early-season competitors. Data reported here suggest that presence of graminaceous weeds late in the growing season may affect grain quality and increase insect infestations and damage. Additional research needs to be conducted to examine the economic aspects of late season control for losses due to quality and insect pressure.

With the advent of herbicide-tolerant rice varieties, red rice control can be accomplished by chemical means. This should allow for some flexibility in flooding regimens. Being able to delay floods by 10-14 days should assist in reduction in numbers of rice water weevil and their damage. This research offers valuable information in the area of insect-weed interactions as well as laying a foundation for the development of a multi-disciplinary integrated pest management program for rice.
APPENDIX

LETTER OF PERMISSION FOR CHAPTERS 2, 3, AND 7

Dear Ms. Tindall,

The Entomological Society of America grants you permission to use the articles cited below as part of your dissertation for Louisiana State University.


Sincerely,

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