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Impact of Water Management and Agronomic Practices on the Performance of Insecticide Seed Treatments against Rice Water Weevil, *Lissorhoptrus oryzophilus* Kuschel, in Mississippi Rice

Charles Andrew Adams

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Impact of water management and agronomic practices on the performance of insecticide
seed treatments against rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, in
Mississippi rice

By

Charles Andrew Adams

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agricultural Life Sciences
in the Department of Biochemistry, Molecular Biology, Entomology, and Plant
Pathology

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Charles Andrew Adams

2013

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Two field trials were conducted to determine the impact of water management on the efficacy of insecticide seed treatments against rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, in rice at the Delta Research and Extension Center during 2011 and 2012. The performance of thiamethoxam, chlorantraniliprole, and clothianidin was evaluated when the permanent flood was established at different timings (6 and 8 weeks after planting) and the effect of flush number (0, 1, or 2) on seed treatment performance was evaluated. Seed treatment efficacy was not impacted by delayed flooding, but 2 flushes reduced efficacy of some seed treatments.

Experiments were also conducted to determine the impact of reduced seeding rates found in hybrid rice production on the efficacy of insecticide seed treatments targeting rice water weevil. Efficacy was similar when comparing currently labeled rates of thiamethoxam, chlorantraniliprole, and clothianidin with higher rates of these products.

DEDICATION

I would like to dedicate this thesis to my parents, Donny and Donna Adams, and the rest of my family who supported me along the way. Without them this would not have been possible.

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Words cannot express my sincere gratitude towards my advisors Drs. Jeff Gore and Fred Musser who believed in me, and guided me every step of the way through the research and writing of this thesis. Without them this would not have been possible. I would like to give a special thank you to Dr. Don Cook who acted more as an advisor and friend than a committee member, and the rest of my graduate committee Drs. Angus Catchot and Tim Walker for their input into this research and completion of this thesis.

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CHAPTER I
LITERATURE REVIEW

Origin of Rice

Rice, *Oryza sativa* L. is one of the most important cereal crops and feeds more than a third of the world's population (Khush 1997). As many as 80,000 cultivated and wild rice varieties have been collected at the International Rice Germplasm Center of the International Rice Research Institute (IRRI 1978). The genus *Oryza* is believed to have originated about 130 million years ago in Gondwanaland (Chang 1976).

Rice cultivation in the United States originated in 1646 when *O. sativa* was introduced into the James River region of Virginia, and first grown during 1685 in the colony of South Carolina (Gifford and Trahan 1975, Heinrichs 2009). The rice variety, 'Carolina Gold', was introduced into South Carolina when a storm forced a New England ship sailing from Madagascar to harbor in Charleston. Before leaving the port, the captain gave the colonists about 5 kg of rice seed which started the Carolina rice industry. Rice was introduced into Louisiana in 1718, but did not assume importance there until 1887. Commercial rice production in the Sacramento Valley of California began in 1912 (Heinrichs 2009). In Mississippi, commercial rice production began in 1948 in Washington County. Mississippi is the fourth largest rice producing state in the United States following Arkansas, California, and Louisiana, respectively (Buehring 2008).

In 1960, the International Rice Research Institute was created with the focus of developing high yielding varieties. These varieties now constitute 70% of world production. Between 1966 and 1990, rice production doubled due to the large scale adoption of improved varieties. Currently, rice production must increase an additional 60% by 2025 to feed the world's growing population (Khush 1997). Traditional varieties require about 150 days of growth to reach the mature grain stage; whereas, modern varieties can be harvested in as few as 90 days after sowing.

Biology of Rice

Cultivated rice is an annual grass with round jointed culms, flat leaves, and terminal panicles (DeDatta 1981). The vegetative structures of rice consist of roots, culms, and leaves. A branch of the plant bearing the root, culm, leaves, and a panicle, is called a tiller.

Rice has a fibrous root system consisting of seminal roots and secondary adventitious roots (DeDatta 1981). Seminal roots grow out of the radicle and are temporary. Secondary adventitious roots are freely branched and produced from the lower nodes of the young culm. Secondary adventitious roots replace the temporary seminal roots.

Rice growth is characterized by three stages of development that include vegetative, reproductive, and grain filling (DeDatta 1981). Each stage of growth impacts rice grain yield. Factors that determine grain yield are the number of panicles per unit of land area, number of grains per panicle, and the weight of individual grains (DeDatta 1981). These stages may be further broken down based on physiological differences between each stage.

The vegetative stage is divided into four separate phases. They include germination, emergence, pre-tillering, and tillering. Soon after planting, seeds imbibe water, triggering germination. This induces the emergence of the radicle or the coleoptile, depending on environmental conditions. Under aerobic conditions, the radicle will emerge first (DeDatta 1981). Under anaerobic conditions, the coleoptile will emerge first. Germination takes place within a few days at optimum temperatures of 21.1-37.8°C. Temperatures below 21.1°C require a longer period of time for germination (DeDatta 1981).

Seedling emergence (spiking) is the period from emergence to the appearance of the first tiller. At this stage, seedlings develop seminal roots and are dependent on the endosperm for seedling development (DeDatta 1981). Two or more leaves should be fully developed within ten days after spiking. After this period, a new leaf begins developing every 3 to 4 days. Adventitious roots begin replacing the seminal roots (DeDatta 1981). Under favorable conditions, seedlings should emerge within three days; however, unfavorable conditions may impede emergence by 2 to 3 weeks (DeDatta 1981)

The tillering stage follows the seedling stage. It begins with the appearance of the first tiller from the axillary bud on one of the lowermost nodes (DeDatta 1981). Tillers displace leaves as they grow and develop. The number of tillers is directly correlated with grain yield (Kawano and Tanaka 1968, DeDatta 1981). The number of tillers is more influential on grain yield than seeding rate (Miller et al. 1991). During the tillering stage, most of the biomass is produced. Many factors influence tiller production, including cultivar, seeding rate, environment, and soil nutrients (Buehring 2008). Development of secondary tillers begins after the emergence of the primary tillers. At this stage, the plant

begins to rapidly increase in size. After the formation of the primary and secondary tillers, tertiary tillers begin developing. This will continue until the plant reaches its maximum tiller number. Once maximum tiller number is reached, older tillers begin to die and the number of tillers declines and levels off (DeDatta 1981).

The reproductive stage begins at the initiation of panicle development. This occurs just prior to or following the maximum tillering stage. Panicle development is dependent upon environmental conditions (DeDatta 1981). The number of rice grains per panicle is determined during panicle development. This is crucial in maximizing yield. The reproductive stage of rice lasts approximately 30 days. The reproductive stages of rice are classified into six categories: panicle initiation, internode elongation, panicle differentiation, booting, heading, and flowering (DeDatta 1981).

Panicle initiation occurs with the production of the panicle in the main culm. At this stage the nodes of the rice plant are stacked with very little distance between each node. Panicle initiation, also known as the green ring stage, is identified by the presence of a green band just above the top node. This green band is only present for a few days and is the indicator of internode elongation. Panicles form 3 to 4 weeks before they are noticeable in the field and emerge 22 to 33 days after internode elongation (DeDatta 1981). Internode elongation or jointing is recognized after the panicle is produced and the top internode begins to elongate.

Panicle differentiation is a critical time in the reproductive stage. Spikelets become distinguishable and the panicle extends upward inside the flag leaf sheath. The panicle continues to develop slowly. When the panicle has grown to a length of 5 cm, the spikelet primordia differentiate and the number of spikelets is determined (DeDatta

1981). At this time, the panicle is sensitive to environmental conditions that could negatively impact yield. Panicle differentiation occurs when there is 1.27 – 1.9 cm internode elongation on the first panicle (DeDatta 1981). The booting stage is the latter part of panicle differentiation (DeDatta 1981). This occurs approximately 16 days after visible panicle initiation when the sheath and the flag leaf begin to swell (DeDatta 1981). Late boot is when the flag leaf has fully emerged from the culm (DeDatta 1981)

The booting stage is followed by the heading stage. The heading stage is characterized as 10 – 20 percent of panicles emerged from the boot. Grain will mature approximately 30 to 40 days after heading (DeDatta 1981).

Flowering begins with protrusions of the first dehiscing anthers in the terminal spikelets (DeDatta 1981). At this time the panicle stands erect. The panicles begin flowering at the top, middle, and lower thirds, during the first, second, and third day after panicle emergence in tropical environments (Fernandez et al. 1979). Depending on environmental conditions, flowering generally lasts for 6 to 10 days. Rice plants are self pollinated. Pollination occurs from mid-morning until a little past noon. Flowering is negatively impacted by cool wet weather (DeDatta 1981). After the flowering stage, the rice kernel reaches its final dry weight in approximately 35 days (DeDatta 1981).

Rice Production

Recommended seeding rates for drill-seeded cultural systems (the dominant cultural system in rice in the southern United States) range from 56 to 123 kg/ha for conventional (non hybrid) rice (Bond et al. 2005). These seeding rates typically result in seed densities ranging from 278 to 444 seeds per m². Rice hybrids are more vigorous than conventional varieties during vegetative growth. They accumulate more dry matter,

resulting in more spikelets per panicle than conventional varieties (Zhende 1988).

Because of the vigorous growth patterns of hybrid rice, seeding rates are 28-44 kg/ha (Zhende 1988, Bond et al. 2005).

Rice production systems are classified according to ecology in terms of water requirements (Heinrichs 2009). Two major systems of rice cultivation are the dry (upland) system and the flooded system (lowland). In the majority of the world, rice is grown as a lowland crop (Stout et al. 2002b). Human selection and adaptation to diverse environments has resulted in numerous cultivars (Khush 1997). In the United States, rice is grown in Arkansas, California, Louisiana, Mississippi, Missouri, and Texas totaling 1.45 million hectares. Mississippi is the fourth largest rice producing state in the United States averaging 97,000 ha/yr (Miller and Street 2008). Mississippi growers have the option of planting several different rice varieties and hybrids.

Planting dates for rice in the lower Mississippi Delta are from April 1 to May 15 and April 15 to May 15 in the upper Delta (Buehring 2008). Proper flood timing and a good water source are vital for rice growth, weed control, and soil ammonium nitrogen stability. Water needs vary based on soil texture, number and length of irrigation ditches, soil moisture prior to flooding, perimeter levee and irrigation ditch seepage, evaporation, and transpiration by plants (Thomas 2008). In Mississippi, rice is typically planted in a dry seedbed and the permanent flood is not established until the five leaf stage. The time period from planting to permanent flood will vary from 2 to 6 weeks, depending upon environmental conditions. During this time period, flushing with water is typically required. Flushing, a common practice in rice production, is defined as applying water across a planted rice field after planting to facilitate germination and emergence (Koger

et al. 2006). A flush is a form of irrigation where the field is brought to a shallow flood and then drained. Flushing is often needed to alleviate soil crusting, incorporate soil residual herbicides, or irrigate drought stressed seedlings. During hot and dry conditions, it is not uncommon for multiple flushes to be required prior to the application of the permanent flood.

Nutrient Requirements

Flooding of the soil produces changes in soil chemical and biochemical processes that impacts the availability of nutrients (DeDatta 1981). The degree of change is dependent upon the soil type (DeDatta 1981). These changes are greater in soils with low nitrate and manganese dioxide concentrations or with high organic matter (Ponnamperuma 1965). Several factors affect the availability of nutrients. These include soil texture, duration of submergence, and temperature. These factors can strongly influence the availability and concentration of water soluble nitrate, ammonia, iron, manganese, potassium, calcium, magnesium, sulfate, copper, zinc, molybdenum, carbon dioxide and organic acids (Ponnamperuma 1965).

Insects of Rice

The world rice crop is attacked by more than 100 species of insects (Pathak and Khan 1994). Twenty of these species can cause economic damage (Pathak and Khan 1994). Insect pests that cause significant yield losses are stem borers, leafhoppers and planthoppers (direct damage by feeding and transmission of viruses), gall midges, a group of defoliating species (mainly lepidopterans), and a grain-sucking bug complex that feeds on developing grains (Pathak and Khan 1994).

Due to the introduction of high-yielding modern varieties, distinct changes have occurred in the insect pest complex of rice. Several species, once considered minor pests, have become major pests (Pathak and Khan 1994). Until the 1960s, the stem borers were considered the most serious pests of rice throughout the tropics. However, infestations have declined in recent years. In Japan, the population densities of stem borers have steadily declined since the mid-1960s (Pathak and Khan 1994). Other insect pests are reportedly becoming more important on rice in many countries. Examples are thrips species in India and China, rice bug species in Malaysia, and mealybug species in India and Bangladesh. In addition, new pests have been recorded in several areas, including sugarcane leafhopper, *Pyrilla perpusilla* Walker, and rusty plum aphid, *Hysteroneura setariae* Thomas (Pathak and Khan 1994). Another important example is the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, a pest originally distributed in the Mississippi River basin in the USA, but now distributed worldwide. Also, rice water weevil has become the most destructive rice pest in Japan (Pathak and Khan 1994).

Rice Water Weevil

History and Distribution

The rice water weevil is the most widely distributed and destructive early season insect pest of rice, in the United States (Cave et al. 1984, Way 1990, Saito et al. 2005). It has been associated with rice since the introduction of the crop into the United States (Bowling 1957). It was first described by R.I. Sailer, and R.E. Warner, and most recently by G. Kuschel (Grigarick and Beards 1965). In North America, the rice water weevil was first noted on rice near Savannah, Georgia in 1881 (Isely and Schwardt 1934). The rice water weevil was observed at damaging levels during 1904 in Beaumont, Texas by W.D.

Pierce (Newell 1913). During 1909, Newell (1913) found rice water weevil in abundance five km north of Lake Arthur, Louisiana. These weevils were found at a density of one weevil per five to six plants. In 1959, the rice water weevil was first documented in California in a 1036-square km area in the middle of the Sacramento Valley. The rice water weevil distribution currently encompasses all major rice production areas in the United States (Grigarick and Beards 1965).

Males of this species are common in most rice producing states; however, they have not been found in California. This indicates that the weevil was introduced as a parthenogenic female prior to 1959 (Grigarick and Beards 1965). In late May 1976, the rice water weevil was discovered in a 730 ha area in Aichi Prefecture, Japan. The population of rice water weevil in Japan is composed entirely of females, as in California (Saito et al. 2005). The insect is presently regarded as the most destructive rice pest in Japan and the most difficult to control (Pathak and Khan 1994). It is believed to have been accidentally transported to Japan with hay imported from the USA. The rice water weevil has now spread throughout all rice producing regions in Japan and neighboring Asian countries (Saito et al. 2005). Although native to North America, its introduction into some of the major rice-producing regions of Asia has made the rice water weevil a global threat to rice production (Heinrichs and Quisenberry 1999, Stout et al. 2002b, Saito et al. 2005).

Description of Adults

There are two insect groups that live in or near water. Ward (1992) divided these groups based on their respiratory methods and organs. These two groups were categorized as the true aquatic species and semi-aquatic species. The semi-aquatic species

have hydrophobic exocuticles and tracheal systems which are unable to exchange air with water (Ward 1992). Semi-aquatic insects breathe by coming to the surface of the water, living in an air bubble in water, or by utilizing an air supply from the host (Zhang et al. 2006). The rice water weevil is categorized as being semi-aquatic.

Adult rice water weevils are approximately 2.5 to 3.5 mm in length and grayish with a darker area on the dorsum (Saito et al. 2005). They are good swimmers, but are rather sluggish when out of the water (Newell 1913). Although populations of rice water weevils are multivoltine, a single generation usually occurs in rice during a growing season (Shang et al. 2004). Other generations are believed to develop on aquatic and water tolerant grasses (Poaceae) and sedges (Cyperaceae) (Tindall and Stout 2003).

Adult rice water weevils overwinter around the base or crowns of various weeds and grasses or in leaf litter in wooded areas and emerge from overwintering in early spring (Grigarick and Beards 1965, Shang et al. 2004). Regeneration of flight muscles and emergence of weevils from overwintering in spring are dependent on temperature (Zou et al. 2004c). Emergence dates vary from 4 to 20 d after mean temperatures exceeded 15.6°C. After emergence from overwintering, the adults feed on the leaves of rice and other aquatic grasses and sedges in flooded or unflooded fields (Tindall and Stout 2003). Grigarick and Beards (1965) found that weevils begin to feed on uncultivated grasses in March and April and fly to rice fields in April, May, and June. Adult movement occurs during late evening or night and they are attracted to open water (Newell 1913, Isely and Schwardt 1934). Adult feeding activity increases with the flooding of rice fields and rapidly declines to a low level within 3 weeks (Sooksai and Tugwell 1978). Feeding is somewhat aggregated in areas where open water is present.

Adults may fly to hibernation sites as early as July, where they enter diapause and overwinter (Nilakhe 1977, Wu and Wilson 1997).

Presence of the permanent flood is the most important external influence on the interaction between rice water weevil and rice. Adult rice water weevils prefer to feed on plants grown in flooded conditions over plants growing in unflooded conditions (Grigarick and Beards 1965, Stout et al. 2002b). In general, peak oviposition occurs one to two weeks after flooding (Wu and Wilson 1997). Larval populations peak approximately two to three weeks following the peak of adult feeding (Morgan et al. 1989), and approximately three to four weeks following the application of the permanent flood (Thompson and Quisenberry 1995, Zou et al. 2004a).

In early studies, observation of the rice water weevil was difficult because of its small size and its flooded habitat. This led early investigators to make partially erroneous assumptions pertaining to the ovipositional activity of the female and the location of the first stage larva (Bowling 1972). Early investigators reported that the female rice water weevil places the egg longitudinally in the roots of the rice plant (Webb 1914, Isely and Schwardt 1930). More recent studies suggest that the roots of the rice plant are not the preferred site of oviposition. According to Saito et al.(2005), eggs of the rice water weevil are approximately 0.8 mm long and are placed longitudinally inside the leaf sheath. In an ovipositional study by Grigarick and Beards (1965), it was demonstrated that 93% of the eggs were oviposited in the basal half of the submerged portion of the leaf sheath, 5.5% in the submerged upper portion of the leaf sheath, and only 1.5% in the roots. This is supported by Stout et al. (2002b) where weevils showed preference for oviposition at or below the water line in the presence of a flood. However, 1.26% of eggs

that were not laid in the leaf sheath were found to be in the leaf blades. Stout et al. (2002b) reported that no eggs were found in roots, coleoptiles, or culms of plants.

The presence and depth of the permanent flood alters several aspects of oviposition behavior in female rice water weevils, including the occurrence of oviposition, location of oviposition, and number of eggs oviposited (Stout et al. 2002b). The presence of a flood is not required for oviposition to occur, but more eggs are laid in the presence of standing water than in the absence of standing water (Stout et al. 2002b). In addition, depth of the flood influences rice water weevil oviposition. The number of eggs laid in plants in the presence of a 10.2 cm flood was approximately ten-fold greater than that of “saturated soil” and 1.3 cm floods (Stout et al. 2002b). Eggs are laid singly or clustered tightly, and rice water weevil oviposit in leaf sheaths of all ages, but prefer to oviposit in the sheaths of younger leaves (Stout et al. 2002b). Female rice water weevils oviposit approximately 75 to 136 eggs over a 1-2 month period depending on temperature and environment (Grigarick et al. 1976, Stout et al. 2002b).

Larval Development

The larva of the rice water weevil is whitish, elongate, cylindrical, and reaches approximately 8 mm in length in the fourth instar (Isely and Schwardt 1930). The larval stage is spent almost entirely in flooded or water-saturated soils, where they feed on or in the roots of their hosts (Zhang et al. 2006). Larvae and pupae develop in anaerobic conditions and have a well developed tracheal system that is modified for securing an adequate supply of oxygen from the roots of the rice plant (Zhang et al. 2006). The rice water weevil has six pairs of tracheal branches from the main trunk that leads to modified spiracles forming paired dorsal hooks (Isely and Schwardt 1930, Zhang et al. 2006). The

dorsal hooks are believed to aid in locomotion and respiration by penetrating rice root tissues to obtain air (Isely and Schwardt 1930, Zhang et al. 2006). Larvae in flooded soils make temporary chambers next to the rice root (Zhang et al. 2006). Rice has dense aerenchyma in its roots. Larvae use their modified spiracles to pierce the root. Air presumably flows from the root into the chamber forming an air bubble, facilitating respiration (Zhang et al. 2006). The pupae obtain oxygen in much the same way as the larvae.

Larval development has been well studied for rice water weevil. Four, six, and nine days are required for eggs to hatch at 35, 30, and 25°C, respectively (Raksarart and Tugwell 1975). Eclosion does not occur at temperatures above 40°C (Raksarart and Tugwell 1975). Following eclosion, larvae mine the leaf sheath for a short period before migrating down the plant to the roots (Grigarick and Beards 1965, Bowling 1972, Cave et al. 1984, Wu and Wilson 1997). Larval development time is approximately 21 ± 5.26 days (Cave et al. 1984). Estimation of larval duration is 1.20 ± 0.39 days for the first instar, 2.56 ± 0.59 days for the second larval instar, 7.14 ± 2.09 days for the third larval instar, and 10.34 ± 2.19 days for the fourth larval instar (Cave et al. 1984). The cumulative development period for egg, larva, and pupa development is approximately 32 days (Zou et al. 2004c). Egg and larval development require 17-22 days at 26-31°C (Zou et al. 2004c).

Larvae progress through four instars and a pupal stage on roots before emerging as adults (Cave and Smith 1983). Densities of third and fourth instars peak 14-20 days after flooding (Zou et al. 2004c). Isely and Schwardt (1934) reported that there were three instars of rice water weevil with the following head capsule widths: 0.20-0.22 mm

(first instar), 0.33-0.35 mm (second instar), and 0.44-0.45 mm (third instar). Bowling (1972) found some larvae with smaller head capsule widths than those reported by Isely and Schwardt (1934), and concluded that there were four larval instars. Cave and Smith (1983) reported 4 larval instars with the following head capsule widths: 0.16 ± 0.0 mm (first instar), 0.22 ± 0.02 mm (second instar), 0.32 ± 0.02 mm (third instar), and 0.45 ± 0.06 mm (fourth instar). The most damaging larval stages are the third and fourth instars (Wu and Wilson 1997, Stout et al. 2001).

Damage in Rice

Adults feed on the upper surface of the foliage, leaving narrow longitudinal scars parallel to the venation of rice leaves (Sooksai and Tugwell 1978, Cave et al. 1984, Zou et al. 2004a). Adult rice water weevils use their mandibles to remove the upper epidermis and contents of leaf cells (Newell 1913). As feeding progresses, the weevil moves forward towards the apex of the leaf. This results in a feeding scar from 2.7 – 5.1cm in length. The length of the feeding scar is dependent upon the feeding duration. The mandibles do not pierce through the leaf but remove only the epidermis, “skeletonizing” the leaf at the point of feeding (Newell 1913). Feeding scars are more abundant on rice under flooded conditions than under drained conditions. This could be contributed to the increase in nitrogen concentrations in flooded plants. Increased levels of nitrogen are known to increase the suitability and attractiveness of rice foliage for herbivores (White 1984). Economic damage from rice water weevil adult feeding is rare.

Rice plants suffer greater yield losses when rice water weevil infestations occur during seedling development (Wu and Wilson 1997, Stout et al. 2002a, Zou et al. 2004a). Yield losses from larval feeding typically approach 10% but can exceed 25% under

severe infestations (Stout et al. 2000, Stout et al. 2002a, Zou et al. 2004a). Larvae feed primarily on root tissue (Sooksai and Tugwell 1978, Stout et al. 2002a, Stout et al. 2002b), and injury results in stunted seedlings, lodging during harvest, and yield losses of up to 1,123 kg of rough rice per ha (Cave et al. 1984). Larval feeding can severely reduce rice growth, tillering, and yield (Cave and Smith 1983, Grigarick 1984, Hesler et al. 2000, Zou et al. 2004a).

Injury to roots also impacts tiller production directly impacting yield. Tillering ability is influenced by many environmental factors, but the impact of root pruning is not completely understood (Zou et al. 2004a). Greater proportions of tillers abort early in fields with rice water weevil (Zou et al. 2004a). Tillering significantly influences the production of panicles in rice, and is a component of yield (Miller et al. 1991). In the vegetative stage of rice development, removal of root tissues reduced tillering and total shoot biomass by 36% and 35%, respectively (Zou et al. 2004a). Zou et al.(2004a) also found that number of grains per panicle and grain weight were correlated with rice water weevil feeding. Reduction in shoot biomass results in a reduction in total leaf area, total plant photosynthesis, and stem carbohydrate levels (Zou et al. 2004a). The decrease in grain number and weight may be due to lower photosynthetic rates or stored resources in rice water weevil-damaged plants (Zou et al. 2004a).

Management of the Rice Water Weevil

Cultural Management

Historically, management of rice water weevil has been difficult to achieve because of larval adaptations to flooded environments. Control of the rice water weevil has been an issue since the discovery of this pest near Savanna, Georgia in 1881(Isely

and Schwardt 1934). Water management practices influence the incidence and severity of insect pests in rice (Hesler and Grigarick 1992). Many arthropods that could potentially feed on rice cannot tolerate a flooded environment, whereas the rice water weevil thrives under flooded conditions (Pantoja et al. 1993, Rice et al. 1999). Management of the rice water weevil prior to the introduction of insecticides was primarily obtained through the use of field drainage (Newell 1913, Webb 1914, Isely and Schwardt 1934, Morgan et al. 1989, Hesler et al. 1992). Management of the rice water weevil through drainage adds to water management costs, causes damage to the plant, leads to loss of fertilizer, and is not as effective as insecticide control (Newell 1913, Webb 1914, Isely and Schwardt 1934, Whitehead 1954, Morgan et al. 1989, Hesler et al. 1992, Thompson et al. 1994).

Other types of water management strategies have the potential to be effective and economical tactics for rice water weevil management (Quisenberry et al. 1992). Flooding greatly impacts rice water weevil behavior, so the behavior of rice water weevils may be amenable to manipulation by altering water management practices (Stout et al. 2002b). An alternative to drainage for the management of rice water weevil is to delay the permanent flood (Rice et al. 1999). Delayed flood avoids fertilizer and herbicide losses associated with drainage and reflooding of the rice field (Rice et al. 1999). Historically, fields were flooded within 1 week of germination to reduce red rice germination and subsequent infestations. Imidazolinone herbicides provide effective control of red rice. The introduction of imidazolinone resistant rice germplasm to manage red rice, *Oryza punctata* Kotzchy ex Steud., has reduced the need for an early flood and made delayed flooding a more viable practice for rice water weevil control. A 2 to 4 week delay in establishment of the permanent flood can prevent larvae from reaching damaging

densities by approximately one month (Rice et al. 1999). Tolerance of rice to rice water weevil feeding increases with plant age and short delays in establishment of the permanent flood can reduce the impact of infestations on yield (Rice et al. 1999, Stout et al. 2002a, Zou et al. 2004b). Management of the rice water weevil without an insecticide application and with reduced herbicide applications should offset the potential yield loss from delayed flooding (Rice et al. 1999).

Chemical Management

Control with insecticides is currently the primary management strategy for rice water weevil in the U.S. In a study by Whitehead (1954), aldrin, dieldrin, heptachlor, DDT, chlordane, and toxaphene were applied to rice in an attempt to control rice water weevil. The results of this study suggested that low dosages of these insecticides just prior to flooding would give satisfactory control of the rice water weevil. Whitehead (1954) identified four benefits of insecticidal control of rice water weevil compared to field drainage. Insecticidal control proved to be more effective than drainage and drying with no negative impact on yield, insecticidal control was less expensive than draining and reflooding, insecticidal control required less water, reduced mosquito populations. Bowling (1959) showed that insecticides applied as seed treatments, foliar sprays, or mixed with a fertilizer were equally effective for targeting rice water weevil. Lindane, aldrin, and dieldrin provided $\geq 90\%$ control, while thimet provided 50% control of rice water weevil when applied as seed treatments (Bowling 1959). Advantages of seed treatment over foliar application methods of control include lower cost, ease of application, and protection of seed rice during storage (Bowling 1959). During the late

1950's and early 1960's aldrin provided effective control of the rice water weevil as a seed treatment.

Granular carbofuran (Furadan®; FMC Corporation, Philadelphia, Pa.) was used widely and was the predominant control measure of rice water weevil from 1969 to 1998 (Way 1990, Stout et al. 2000). Carbofuran was used as a larvacide and applied approximately 2 weeks after flooding or when larval densities reached threshold. In 1998, the registration for carbofuran was revoked. This led to the registration of lambda-cyhalothrin (Karate® , Syngenta Crop Protection, Greensboro, NC), a synthetic pyrethroid, fipronil (Icon® , Rhone-Poulenc Ag Company, Research Triangle Park, NC), a phenyl pyrazole, and diflubenzuron, (Dimlin® , Chemtura, Middlebury, CT) an insect growth regulator to replace carbofuran for rice water weevil control (Stout et al. 2000).

With the exception of fipronil, carbofuran had a longer residual than these insecticides and controlled subsequent infestations of rice water weevil. However, it was used after the larval threshold was reached, and roots are very susceptible to water weevil damage early in the plants development (Stout et al. 2002a). Diflubenzuron and lambda-cyhalothrin are typically applied within ten days of the established flood as a foliar spray targeting adults and application timing is critical to prevent oviposition. The use of these insecticides before peak adult populations have arrived could leave the plant open for later infestations. Fipronil was used preventatively as a seed treatment. It provided effective control, but was voluntarily discontinued in 2005 (Tindall et al. 2004). These insecticides combined with a delayed flood reduced early weevil infestations and resulted in significantly higher yields (Stout et al. 2000).

Seed Treatments

In recent years, several compounds have been registered as seed treatments for rice water weevil in the southern United States. Seed treatments are applied preventatively and are effective in reducing rice water weevil larvae densities (Lanka et al. 2013) and alleviating timing concerns associated with foliar applications of insecticides. The current insecticidal seed treatments labeled for control of the rice water weevil in Mississippi are thiamethoxam (Cruiser[®] 5FS, Syngenta Crop Protection), clothianidin (NipsIt INSIDE[®], Valent Agricultural Products), and chlorantraniliprole (Dermacor[®] X-100, E.I. DuPont de Nemours) (Catchot et al. 2013).

Thiamethoxam is a neonicotinoid that acts by binding to nicotinic acetylcholine receptors (Maienfisch et al. 2001). Thiamethoxam is systemic and exhibits contact and stomach action (PPDB 2013). Its relatively high water solubility (4100 mg l⁻¹ at 20°C) and translocation within the xylem of plant tissues, make thiamethoxam effective as a seed treatment (Maienfisch et al. 2001, PPDB 2013). Thiamethoxam is a broad spectrum insecticide, showing activity across a wide range of insect pests (Maienfisch et al. 2001, PPDB 2013).

Chlorantraniliprole was introduced in 2007, and was the first reported larvacide in the anthranilic diamide class (Lahm et al. 2007). Chlorantraniliprole acts by activating insect ryanodine receptors. This causes the unregulated release of internal calcium stores causing calcium depletion, resulting in paralysis and death (Lahm et al. 2007).

Chlorantraniliprole has excellent larvicidal activity against many Coleoptera and Lepidoptera pest species (Lahm et al. 2007, Lanka et al. 2013).

Clothianidin, like thiamethoxam, is a broad spectrum synthetic insecticide belonging to the neonicotinoid class. It displays translaminar and root systemic activity and acts as an acetylcholine receptor agonist by binding to nicotinic acetylcholine receptors (Lahm et al. 2007).

Management strategies for the rice water weevil in recent years have not been sufficient because targeting adults prior to oviposition was difficult. The incorporation of seed treatments into rice production practices will aid in alleviating some of the previous problems associated with rice water weevil control. However, with the incorporation of cultural practices such as delayed flooding and multiple flushes across a rice field, questions arise about the efficacy and longevity of these seed treatments. In order to answer these questions, field experiments were conducted to determine the efficacy and longevity of seed treatments targeting the rice water weevil under different water management strategies. The objectives of these experiments were:

- I. To determine the impact of delaying establishment of permanent flood on the efficacy of insecticidal seed treatments in rice.
- II. To determine the impact of flushes between planting and permanent flood on the efficacy of insecticidal seed treatments against rice water weevil in rice.
- III. To determine the impact of reduced seeding rates of hybrid rice on the efficacy of insecticidal seed treatments against rice water weevil.

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CHAPTER II
IMPACT OF WATER MANAGEMENT ON EFFICACY OF INSECTICIDE SEED
TREATMENTS IN RICE

Abstract

Two experiments were conducted at the Delta Research and Extension Center in Stoneville, MS during 2011 and 2012 to determine the impact of water management practices on the efficacy of insecticidal seed treatments targeting rice water weevil, *Lissorhoptrus oryzophilus* Kuschel. Larval densities and yield were compared for plots treated with labeled rates of thiamethoxam, chlorantraniliprole, and clothianidin were compared to a untreated control. In the first experiment, seed treatments were subjected to flood initiated at six and eight weeks after planting. Seed treatments significantly reduced larval densities in the eight week flood timing, but not in the six week flood timing. Overall, seed treatments yielded higher than the control. Yields were significantly higher in the 8 week flood timing than the six week flood timing. In the second experiment, the impact of multiple flushes on insecticidal seed treatments was evaluated. Seed treatments and the untreated control were subjected to zero, one, or two flushes with water. All seed treatments reduced larval densities below the untreated control. Significantly fewer larvae were observed in the one and two flush treatments than the zero flush treatment. All seed treatments yielded higher than the untreated control in the zero and one flush treatments. At two flushes, thiamethoxam and clothianidin did not

yield significantly different from the control, while chlorantraniliprole yield was significantly higher than the control. These data suggest that time from planting to permanent flood did not impact the efficacy of seed treatments, but multiple flushes reduced the efficacy of thiamethoxam and clothianidin.

Introduction

The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, is the most widely distributed and destructive early season insect pest of rice, *Oryza sativa* L., in the United States (Cave et al. 1984, Way 1990, Saito et al. 2005). Native to North America (Saito et al. 2005), this insect has been associated with rice since the crop was introduced into the U.S. (Bowling 1957). In 1976, the rice water weevil was accidentally introduced into Japan (Pathak and Khan 1994). It has now spread to all major rice producing regions of Asia. The rice water weevil is regarded as the most destructive and difficult to control pest of rice, and is now regarded as a global threat to rice production (Pathak and Khan 1994, Heinrichs and Quisenberry 1999, Stout et al. 2002b, Saito et al. 2005).

Adult rice water weevils overwinter in bunchgrass or in leaf litter in wooded areas and emerge from overwintering in early spring (Shang et al. 2004). After emergence from overwintering, adults feed on the leaves of rice and other aquatic grasses and sedges in flooded or unflooded fields (Tindall and Stout 2003). Adults feed on the upper surface of the foliage, leaving narrow longitudinal scars parallel to the venation of rice leaves (Sooksai and Tugwell 1978, Cave et al. 1984, Zou et al. 2004b). Feeding by the adult is not economically important.

Oviposition in rice commences upon establishment of the permanent flood (Stout et al. 2002b). Peak oviposition generally occurs one to two weeks after the permanent

flood is established (Wu and Wilson 1997). Rice water weevil adults oviposit in leaf sheaths at or below the water line (Stout et al. 2002b). Following eclosion, larvae mine leaf sheaths for a short period before crawling down the plant to feed on the roots (Grigarick and Beards 1965, Bowling 1972, Cave et al. 1984, Wu and Wilson 1997). Larval and pupal stages of this insect are spent almost entirely in flooded or water-saturated soils, where they feed on or in the roots of their hosts (Zhang et al. 2006). Larvae progress through four instars and a pupal stage on roots before emerging as adults (Cave and Smith 1983).

Feeding by rice water weevil larvae results in stunted root systems, reduced tillering, reduced number of grains per panicle, and reduced grain weight (Zou et al. 2004b). Yield losses from larval feeding typically approach 10%, but can exceed 25% under severe infestations (Stout et al. 2000).

Water management practices have a direct effect on rice water weevil behavior in rice production (Webb 1914, Whitehead 1954, Morgan et al. 1989, Thompson et al. 1994, Rice et al. 1999, Stout et al. 2002b). Presence of the permanent flood is the most important external influence on the interaction between rice water weevil and rice (Grigarick and Beards 1965, Stout et al. 2002b). The rice water weevil is a unique pest because of its ability to thrive under flooded conditions (Pantoja et al. 1993, Rice et al. 1999). Rice is most susceptible to rice water weevil damage in the early stages of development. Delay of the permanent flood in drill seeded rice by two to four weeks may result in reduced rice water weevil densities and reduced yield losses due to rice water weevil feeding. Delay of the permanent flood allows rice plants to develop higher levels

of tolerance to rice water weevil injury by significantly increasing root mass (Rice et al. 1999, Stout et al. 2000, Stout et al. 2002a, Stout et al. 2002b, Zou et al. 2004a).

Seed treatments have recently been registered for control of the rice water weevil in the United States. The current recommended seed treatments for control of the rice water weevil include thiamethoxam (Cruiser[®] 5FS, Syngenta Crop Protection), chlorantraniliprole (Dermacor[®] X-100, E.I. DuPont de Nemours), and clothianidin (NipsIt INSIDE[®], Valent Agricultural Products), (Catchot et al. 2013). Chlorantraniliprole is a member of the anthranilic diamide class of chemistry and received registration in the spring of 2011 (DuPont 2010). Thiamethoxam and clothianidin are members of the neonicotinoid class of chemistry. Thiamethoxam received registration in the fall of 2010 and clothianidin received registration in the fall of 2012 (Syngenta 2010, Valent 2010).

The recent introduction of seed treatments targeting rice water weevil has alleviated timing issues related to foliar insecticide applications. This has resulted in better early season management of the rice water weevil. In rice production, early season insect protection is needed for a longer period compared to corn, *Zea mays* L., cotton, *Gossypium hirsutum* L., and soybeans, *Glycine max* Merrell. These crops only need protection from early season pests for the first two to three weeks of development (Lentz and Tol 2000). Regarding the rice water weevil, oviposition does not occur until flood establishment and maximum larval numbers are not reached until approximately three to four weeks following the application of the permanent flood (Thompson and Quisenberry 1995, Zou et al. 2004a). Therefore, in the instance of a delayed flood, seed treatments targeting rice water weevil must maintain acceptable levels of efficacy up to 7-10 weeks after planting.

During the time from planting to permanent flood, flushing may be required. Flushing, a common practice in rice production, is defined as applying water across a rice field to facilitate germination and emergence (Koger et al. 2006). A flush is a form of irrigation where the field is brought to a shallow flood and then drained. It is not intended to be permanent. Flushing is often needed to alleviate soil crusting, incorporate residual herbicides, or irrigate drought-stressed seedlings. In Mississippi, rice is dry seeded into a firm and flat seedbed. The permanent flood is generally established at the fifth leaf stage in Mississippi. This occurs four to six weeks after planting, depending on environmental conditions. It is not uncommon for fields to be flushed at least once before establishment of the permanent flood. Under hot and dry conditions, as observed in 2011-2012, multiple flushes with water may be necessary to ensure normal growth and vigor. Though flushing is agronomically beneficial in rice production, its effect on the efficacy of insecticide seed treatments has not been studied.

Materials and Methods

Two experiments were conducted in 2011 and 2012 at the Delta Research and Extension Center (DREC) in Stoneville, MS to determine the impact of delayed flooding and the impact of multiple flushes with water between planting and permanent flood on the efficacy of seed treatments against rice water weevil in rice. The soil type at this location for both years and experiments was Sharkey clay (very fine, smectitic, thermic chromic epiaquerts) (www.websoilsurvey.nrcs.usda.gov). The rice variety ‘Cocodrie’ (Linscombe et al. 2000) seeded at 95 kg/ha was used for both experiments in both years.

Seed treatments and their use rates for both studies included thiamethoxam (Cruiser[®] 5FS, Syngenta Crop Protection) at 248 ml/100 kg seed, chlorantraniliprole

(Dermacor[®] X-100, E.I. DuPont de Nemours) at 130 ml/100 kg seed, and clothianidin (NipsIt INSIDE[®], Valent Agricultural Products), at 124 ml/100 kg seed. Seed were treated in a laboratory-scale rotary seed treater prior to planting. Plots in both studies during each year measured 1.73 x 4.57 m and were drill seeded at 89.63 kg/ha.

Impact of Delayed Flood

An experiment was conducted to determine the impact of a delayed flood on the efficacy of insecticidal seed treatments targeting rice water weevil. Treatments were in a split-plot arrangement within a randomized complete block design with four replications in 2011 and eight replications in 2012. The number of replications was increased from four to eight to increase statistical power. The main-plot factor was time to permanent flood and included timings of 6 and 8 weeks after planting. The sub-plot factor was seed treatment and included the three seed treatments and rates previously described plus an untreated control.

Plots were planted 11 May 2011 and 26 Apr 2012 for the 8 week timing, and 24 May 2011 and 10 May 2012 for the 6 week timing. All plots were flooded 30 Jun 2011 and 22 Jun 2012. Planting dates were varied for different flood timings so that all treatments could be flooded at the same time. Rice water weevil adults migrate into fields and begin oviposition upon establishment of the permanent flood (Everett and Trahan 1967, Rice et al. 1999, Stout et al. 2000), and rice water weevil adult densities can vary greatly from week to week. Planting all treatments on the same date and establishing the permanent flood at different dates would likely bias the results because some plots may have been exposed to different levels of rice water weevil densities. Planting at different dates and establishing the permanent flood on the same date ensured that all plots were

exposed to similar densities of rice water weevil. Infestation occurred naturally with the establishment of the permanent flood. Plots were sampled on 1 Aug 2011 and 19 Jul 2012. All plots were mechanically harvested for yield on 6 Oct. 2011 and 20 Sep 2012.

Impact of Multiple Flushes

An experiment was conducted to determine the impact of multiple flushes with water on the efficacy of seed treatments targeting rice water weevil. Treatments were in a split-plot arrangement within a randomized complete block design with four replicates in 2011 and 8 replicates in 2012. The number of replications was increased from four to eight to increase statistical power. The main-plot factor was flush number prior to permanent flood establishment. Treatments included 0, 1, or 2 flushes. The sub-plot factor was seed treatment and included the treatments and rates previously described.

Plots were planted on 24 May 2011 and 10 May 2012. The first flush was applied on 7 June 2011 and 18 May 2012 followed by the second flush on 15 June 2011 and 13 June 2012. The plots receiving only one flush were flushed at the time of the second flush in the two flush treatment. Rice plots for each flush treatment were planted in separate bays. The last bay (lowest elevation) was flushed two times with the middle bay being flushed one time and the first bay only receiving the permanent flood. Rice water weevil infestations occurred naturally with the establishment of the permanent flood. The permanent flood was established on 30 Jun 2011 and 22 Jun 2012. Plots were sampled four weeks after permanent flood establishment on 1 Aug 2011 and 19 Jul 2012. Plots were mechanically harvested on 6 Oct 2011 and 20 Sep 2012.

Data Collection and Analysis

To determine the effectiveness of the seed treatments in both experiments, two 10 cm diam. x 15.2 cm deep core samples were collected from each plot four weeks after establishment of the permanent flood. Samples included removing upper vegetative growth from plants located in the interior of the plot, then using a modified bulb planter to collect the bottom portion of the plant, its root mass, and the surrounding soil. Approximately three to four plants were removed from each plot for each sample. Samples were taken from the interior rows of each plot. Samples were placed in 3.79-L Ziploc[®] bags and taken to the laboratory to be washed through a series of screens separating larvae from the root mass. Larvae were collected in a 40 mesh screen basket. The basket was placed in a 10% NaCl solution and the number of rice water weevil larvae was determined (Stout et al. 2001). The 10% NaCl changes the specific gravity of the water allowing the rice water weevil larvae to float. At the end of the season, each plot was mechanically harvested with a plot combine. All data were analyzed with analysis of variance using PROC MIXED of SAS (Littell et al. 1996). For both experiments, the main-plot factor, the sub-plot factor, and their interactions were considered fixed effects in the model. Weevil count data were square root transformed to meet model assumptions. Replication nested in year and replicate by water treatment nested within year were random terms in the model, and served as the error terms for the main-plot and sub-plot factors, respectively.

Results

Impact of Delayed Flood

Rice water weevil densities were impacted by an interaction between flood timing and seed treatments ($F = 3.87$; $df = 3, 66$; $P = 0.01$) (Table 2.1). This interaction results from the differences observed between the untreated control in the 6 week flood timing and the untreated control in the 8 week flood timing. There was no significant reduction in larval densities in the seed treatments versus the untreated control in the 6 week flood timing, but the seed treatments significantly reduced weevil densities below the untreated control in the 8 week flood timing (Table 2.1). When the permanent flood was established at 6 weeks after planting, densities of rice water weevil larvae ranged from 14.00 to 16.25 in the treated plots and 20.67 in the untreated plots. In the 8 week flood treatment, densities of rice water weevil larvae ranged from 14.67 to 15.17 in the treated plots compared to 42.42 for the untreated plots.

There was no significant interaction between flood timing and seed treatments for yield ($F = 0.58$; $df = 3, 66$; $P = 0.63$) (Table 2.2). However, flood timing ($F = 9.16$; $df = 1, 11$; $P = 0.01$) and seed treatment ($F = 9.07$; $df = 3, 66$; $P < 0.01$) were both significant factors for yield. Plots flooded at 8 weeks after planting yielded significantly higher than plots flooded at six weeks after planting. Plots flooded at 8 weeks yielded 7723 kg/ha rough rice yield compared to 7330 kg/ha rough rice yield in plots flooded at 6 weeks. Across flood timings, all seed treatments resulted in significantly higher yields than the untreated control (Table 2.2). Yields ranged from 7610 to 7845 kg/ha rough rice yield for the treated plots compared to 6960 kg/ha rough rice yield for the untreated plots.

Impact of Multiple Flushes

There was no interaction between number of flushes and seed treatments was observed for densities of rice water weevil ($F = 0.99$; $df = 6, 90$; $P = 0.44$). However, the main effects of flush number ($F = 8.65$; $df = 2, 20$; $P < 0.01$) and seed treatment ($F = 17.48$; $df = 3, 90$; $P < 0.01$) did significantly impact rice water weevil densities (Table 2.3). Flushing one or more times resulted in significantly lower densities of rice water weevil larvae compared to no flushes. Across all flush treatments, all seed treatments significantly reduced larval densities below the untreated control (Table 2.3). The average numbers of rice water weevil larvae ranged from 14.16 to 14.86 in seed treatment plots compared to 25.03 in the untreated control (Table 2.3).

A significant interaction between number of flushes and seed treatments was observed for yield ($F = 8.14$; $df = 6, 90$; $P < 0.01$) (Table 2.4). All of the treated plots that received 0 or 1 flush yielded significantly more compared to the untreated control that received 0 or 1 flush. Plots treated with chlorantraniliprole that received 2 flushes produced significantly more yield than plots treated with thiamethoxam, clothianidin, or the untreated plots that received 2 flushes. Yields ranged from 7957 to 8127 kg/ha of rough rice for the treated plots that did not receive a flush compared to 6837 kg/ha of rough rice for the untreated control that did not receive a flush. The application of one flush did not negatively impact yield for treated plots. Yields ranged from 7650 to 8064 kg/ha of rough rice for the treated plots that received one flush compared to 6994 kg/ha of rough rice for the untreated control that received one flush. As flush number increased from 0 and 1 to 2, thiamethoxam and clothianidin were negatively impacted based on rough rice yield, producing 7326 and 7402 kg/ha, respectively. Thiamethoxam and

clothianidin were not significantly different than the untreated control receiving two flushes. Plots treated with chlorantraniliprole and receiving 2 flushes were not negatively impacted yielding 8242 kg/ha of rough rice compared to 7465 kg/ha of rough rice for the untreated control that received two flushes. Additionally, no differences were observed for chlorantraniliprole across flush treatments.

Discussion

In Mississippi, the permanent flood is generally established between four and six weeks after planting. Permanent flood establishment at eight weeks is not a common agronomic practice in Mississippi except in instances where rice development is delayed. With the commercialization of Clearfield[®] rice in 2002, the need for early flood establishment for red rice control was reduced (Roel et al. 1999, Bond and Walker 2011). By delaying permanent flood by 2 to 4 weeks, rice plants are allowed to accumulate more biomass and become more tolerant to injury before rice water weevil infestations occur (Rice et al. 1999, Stout et al. 2002a, Zou et al. 2004c).

Water management practices have a strong influence on the relationship between rice and rice water weevil (Hesler et al. 1992), but little information exists about the impact of water management on current seed treatments. In a study by Stout et al. (2001), the efficacy of fipronil (Icon[®], BASF Corporation, Research Triangle Park, NC) was lower in delayed flooded plots than in early flooded plots. It was proposed that the efficacy of fipronil had deteriorated before core samples were collected.

In the current experiment, rice water weevil densities in the untreated plots were higher for the eight week flood timing compared to the six week flood timing. However, Rice et al. (1999), observed that delay of the permanent flood resulted in lower densities

of rice water weevil larvae infesting rice plants. All seed treatments were efficacious at the eight week flood timing and larval densities in the treated plots were lower than those observed in the untreated plots. However, none of the seed treatments provided control at the 6 week flood timing (Table 2.1).

There was no interaction between seed treatment and delayed flood for yield. Rice tolerance to rice water weevil injury increases with plant age (Stout et al. 2002a). Larval densities in the untreated control were higher in the eight week flood timing; however, the untreated eight week flood treatment yielded higher than the six week flood timing. In addition, all seed treatments yielded higher than the untreated control. These data suggest that with an economical weed management strategy, growers can delay the permanent flood and reduce injury from rice water weevil feeding.

During the time from planting to permanent flood, flushing is often required for herbicide activation, to facilitate emergence of seedlings, and to irrigate water stressed seedlings (Roel et al. 1999, Webster and Levy 2009). In hot dry conditions, such as those observed in 2011 and 2012, flushing two or more times prior to establishing the permanent flood is not uncommon.

In this experiment, there was no significant interaction between seed treatment and flush number on rice water weevil densities. Plots that received one flush or two flushes had significantly fewer larvae than plots that did not receive a flush. In both years, plots that did not receive a flush showed severe signs of drought stress. Plots that received one or two flushes were visibly larger and healthier than those that did not receive a flush. The advanced growth stage of these plants could have been mistaken as older less desirable plants for oviposition (Stout et al. 2001). Across all flush treatments,

all seed treatments performed similarly and all reduced rice water weevil densities below the untreated control.

Although no differences were observed in rice water weevil densities among seed treatments, the application of the second flush had a negative impact on yield in the thiamethoxam and clothianidin treated plots. The water solubility of thiamethoxam (4100 mg l^{-1}) and clothianidin (340 mg l^{-1}) (PPDB 2013) may be the reason for the yield losses observed in this study. Yields for plots treated with chlorantraniliprole that received 2 flushes were not significantly different compared to the chlorantraniliprole treated plots that received zero or one flush, suggesting that chlorantraniliprole provided better protection when multiple flushes were applied. This could be contributed to the water solubility of chlorantraniliprole (0.88 mg l^{-1}) (PPDB 2013) being much lower than the neonicotinoids. During a flush, the entire field is brought to a shallow flood with large quantities of water and drained immediately. The additional water may have moved thiamethoxam and clothianidin out of the root zone preventing or reducing uptake by the plants resulting in yield loss.

These data suggest that currently labeled seed treatments reduce rice water weevil densities in conditions that require multiple flushes with water prior to permanent flooding. However, when using thiamethoxam or clothianidin in hot and dry conditions that require 2 or more flushes with water, supplemental applications with a foliar insecticide may be needed to protect rice from subsequent infestations of rice water weevils and protect rice yields.

Table 2.1 Impact of flood timing and insecticide seed treatment on mean (SEM) numbers of rice water weevil larvae per core for 2011 – 2012

Treatment	Mean + S.E. No. Larvae per Core		
	6 week	8 week	Seed Treatment Mean
thiamethoxam	16.25 ± 3.03 b	14.83 ± 2.90 b	15.42 ± 2.06
chlorantraniliprole	14.00 ± 1.20 b	14.67 ± 2.34 b	14.33 ± 1.29
clothianidin	14.00 ± 2.51 b	15.17 ± 2.98 b	14.58 ± 1.91
untreated control	20.67 ± 4.56 b	42.42 ± 11.65 a	31.54 ± 6.52
Mean	16.23 ± 1.54	21.71 ± 3.51	.

Means followed by the same letter are not significantly different ($\alpha = 0.05$).
 Untransformed data presented, but statistics are based on square root transformed data.

Table 2.2 Impact of flood timing and insecticide seed treatment on mean (SEM) rough rice yields in 2011 – 2012.

Treatment	Mean + S.E. Yield (Kg/Ha)		
	6 week	8 week	Seed Treatment Mean
thiamethoxam	7464 ± 185	7919 ± 229	7692 ± 151 a
chlorantraniliprole	7727 ± 155	7964 ± 163	7846 ± 113 a
clothianidin	7288 ± 182	7932 ± 297	7610 ± 183 a
untreated control	6844 ± 200	7077 ± 119	6960 ± 116 b
Mean	7330 ± 100 b	7723 ± 116 a	.

Means followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 2.3 Impact of flush number and seed treatment on the mean (SEM) number of rice water weevil larvae per soil core 2011-2012.

Seed Treatment	Mean + S.E. No. Larvae per Core			Mean
	0 Flush	1 Flush	2 Flushes	
thiamethoxam	19.25 ± 2.74	12.92 ± 2.32	11.33 ± 1.43	14.50 ± 1.38 b
chlorantraniliprole	17.00 ± 2.12	12.83 ± 2.80	12.64 ± 2.02	14.53 ± 1.33 b
clothianidin	16.92 ± 2.46	13.83 ± 3.24	13.83 ± 1.71	14.86 ± 1.45 b
untreated control	32.58 ± 9.71	22.67 ± 5.71	19.76 ± 1.89	24.42 ± 3.86 a
Mean	21.44 ± 2.73 a	15.56 ± 1.92 b	14.39 ± 0.97 b	.

Means followed by the same letter are not significantly different ($\alpha = 0.05$)

Untransformed data presented, but statistics are based on square root transformed data.

Table 2.4 Impact of flush number and seed treatment on the mean (SEM) rough rice yields 2011-2012

Seed Treatment	Mean + S.E. Yield (Kg/Ha)			Mean
	0 Flush	1 Flush	2 Flushes	
thiamethoxam	8083 ± 299 ab	7836 ± 372 ab	7326 ± 171 c	7749 ± 173
chlorantraniliprole	8127 ± 258 ab	8064 ± 342 a	8242 ± 140 ab	8147 ± 146
clothianidin	7957 ± 330 ab	7650 ± 385 b	7402 ± 135 c	7669 ± 174
untreated control	6837 ± 246 c	6994 ± 276 c	7465 ± 83 c	7094 ± 131
Mean	7751 ± 158	7636 ± 178	7608 ± 85	.

Means followed by the same letter are not significantly different ($\alpha = 0.05$)

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CHAPTER III

EFFICACY OF INSECTICIDAL SEED TREATMENTS IN HYBRID RICE

Abstract

New technologies are currently available for rice producers in the U.S.. Hybrid rice and the introduction of seed treatments targeting rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, have altered the landscape of rice production. Hybrid rice is planted at a lower seeding rate than conventional varieties. The effect that a reduced seeding rate has on seed treatments has not been studied. During 2011 and 2012, an experiment was conducted at seven locations to determine the relationship between the low seeding rates used in hybrid rice, and seed treatments as measured in rice water weevil densities and yield. Labeled rates of thiamethoxam, chlorantraniliprole, and clothianidin were compared to higher rates of these products to determine if labeled rates provide an acceptable level of control of the rice water weevil. Study locations were divided into low, moderate, and high groups based on rice water weevil larval densities. All seed treatments and seed treatment rates reduced rice water weevil densities. However, there was no observed yield or economic benefit from the use of an insecticidal seed treatment in areas of low pressure. Differences in yield were observed among seed treatments and seed treatment rates in moderate and high pressure locations, and all seed treatments yielded better than the untreated plots, but these differences were not always economical. All seed treatments showed an economic advantage in areas of high weevil pressure and

there were no differences between seed treatment products or rates, suggesting that currently labeled seed treatment rates in hybrid rice are effective for rice water weevil management.

Introduction

Rice, *Oryza sativa* L., is an important crop for global food production. As a result, China and other countries began focusing on increased rice production per hectare in the 1970s. Hybrid rice was developed to address the need for increased production per unit of land area (Li and Yuan 2000). Rice hybrids consistently outperform inbred cultivars in tropical and sub-tropical environments (Yang et al. 2007, Bueno et al. 2010). This is done through utilization of heterosis, where hybrid rice accumulates more biomass prior to flowering and quicker than inbred cultivars (Li and Yuan 2000, Bueno et al. 2010, Bond and Walker 2011). Characteristics of hybrid rice include increased tillering, panicle length, and spikelet number per panicle; resulting in approximately 15 to 25% yield increases over conventional inbred lines (Zhende 1988, Li and Yuan 2000, Bond and Walker 2011). Grain yield is the product of dry matter accumulation. Higher grain yields in hybrid rice are due to an increased accumulation of dry matter in the early and middle stages of development (Zhende 1988, Yamauchi 1994). Conventional rice production relies on an accumulation of assimilates after heading for yield (Zhende 1988). Hybrid rice currently accounts for >50% of the production area in China (Yuan 2003). It was commercialized in the U.S. during 2000 and accounted for approximately 25% of rice production by 2010 (Bond and Walker 2011). Hybrid rice requires a lower seeding rate than conventional inbreds because of its high tillering capacity. The seeding rate for

hybrid rice production is 28-44 kg/ha, and 56-123 kg/ha for conventional rice (Bond et al. 2005).

The rice water weevil, *Lissorhoptus oryzaophilus* Kuschel, is the most widely distributed and destructive early season insect pest of rice in the U.S. (Cave et al. 1984, Way 1990, Saito et al. 2005). It is regarded as the most destructive and difficult to control insect pest of rice, and is now regarded as a global threat to rice production (Pathak and Khan 1994, Heinrichs and Quisenberry 1999, Stout et al. 2002, Saito et al. 2005).

Adult rice water weevils overwinter in bunchgrass or in leaf litter in wooded areas and emerge from overwintering in early spring and feed on foliage, leaving narrow longitudinal scars parallel to the venation of rice leaves (Sooksai and Tugwell 1978, Cave et al. 1984, Shang et al. 2004, Zou et al. 2004). This damage is only economically important under severe infestations (Stout et al. 2009).

Rice water weevil adults oviposit in leaf sheaths at or below the water line (Stout et al. 2002, Stout et al. 2009). Oviposition commences when the permanent flood is established and peaks 1 to 2 weeks after flooding (Wu and Wilson 1997). Following eclosion, larvae mine leaf sheaths for a short period before crawling down the plant to feed on the roots (Grigarick and Beards 1965, Bowling 1972, Cave et al. 1984, Wu and Wilson 1997). Yield losses from larval feeding typically approach 10%, but can exceed 25% under severe infestations (Stout et al. 2000). Feeding by rice water weevil larvae reduces root tissue, growth, tillering, and yield (Sooksai and Tugwell 1978, Cave and Smith 1983, Grigarick 1984, Hesler et al. 2000, Stout et al. 2002). The reduction in rice growth and tillering is especially important in hybrid rice production because of its dependence on rapid growth during the vegetative stage in order to increase dry matter

accumulation (Zhende 1988). Feeding by rice water weevil larvae also results in a reduction in shoot biomass, resulting in an overall reduction in total leaf area, total plant photosynthesis, and stem carbohydrate levels (Zou et al. 2004). This is important because of the low seeding rates used in hybrid rice production. Hybrids depend on tiller production to obtain the desired panicle densities, whereas conventional rice production relies on higher seeding rates to achieve desired panicle densities (Zhende 1988).

Insecticide seed treatments have recently been labeled for rice water weevil control. These seed treatments provide effective control of the rice water weevil in the early developmental stages of rice. The currently labeled insecticidal seed treatments for control of the rice water weevil in Mississippi are chlorantraniliprole at 98-390 ml/100kg seed (Dermacor[®] X-100, E.I. DuPont de Nemours), clothianidin at 125 ml/100kg seed (NipsIt INSIDE[®], Valent Agricultural Products), and thiamethoxam at 248 ml/100kg seed (Cruiser[®] 5FS, Syngenta Crop Protection) (Catchot et al. 2013). Thiamethoxam and clothianidin are applied at fixed rates per seed. Chlorantraniliprole is applied as a rate range based on seeding rate. Because rice water weevils are attracted to thin stands, the low seeding rates associated with hybrid rice can increase the susceptibility to damage by the rice water weevil (Stout et al. 2009). The production of primary and secondary tillers is vital to overall yield in hybrid rice, as tillers account for 85-90% of productive panicles (Bond et al. 2008). In contrast, tiller production only accounts for 30-40% of productive panicles in conventional rice (Zhende 1988). Hybrid rice production systems may be more susceptible to rice water weevil injury than conventional rice production systems because rice water weevil impacts early season tiller production (Stout et al. 2009). The reduced seeding rate also results in a reduction in the amount of active ingredient applied

per hectare with seed treatments that have a fixed rate per unit of seed. The objective of this study was to determine the impact of reduced seeding rates on the efficacy of insecticide seed treatments in rice by comparing the labeled rate of each insecticide to higher rates.

Materials and Methods

An experiment was conducted from 2011-2012 over seven locations (Table 3.1) throughout the Mississippi Delta to determine the efficacy of insecticide seed treatments in hybrid rice production systems. Currently labeled rates of insecticides were compared to higher rates of labeled insecticides to determine their effectiveness at lower seeding rates used in rice hybrid production. Treatments included thiamethoxam at 248 ml/100 kg seed and 587 ml/100 kg seed, chlorantraniliprole at 326 ml/100 kg seed and 390 ml/100 kg seed, and clothianidin at 125 ml/100 kg seed and 260 ml/100 kg seed. Rice hybrids were planted during the normal planting window for Mississippi (Table 3.1). The rice hybrid XL723 was used at all locations during both years. The experiment was conducted as a randomized complete block design with seven treatments replicated four times at each location. Seeds were treated in a laboratory-scale rotary seed treater prior to planting. Plot sizes in all experiments were 1.73 X 4.57 m and were drill seeded at 29 kg seed/ha. All agronomic practices were conducted based on Mississippi State University Extension Service recommendations (Buehring 2008).

Data Collection and Analysis

To determine the effectiveness of the seed treatments, two 10 cm diam. x 15.2 cm deep, core samples were collected from each plot at four weeks after establishment of the

permanent flood (Table 3.1). Samples included removing upper vegetative growth from a random plant on an interior row in the plot. A modified bulb planter was used to collect the bottom portion of the plant, its root mass, and the surrounding soil. Because of the low seeding rate, only one plant was removed from each plot for each sample. Samples were taken from the center rows of each plot then placed in 3.79-L. Ziploc[®] bags and transported to the laboratory to be washed through a series of screens separating larvae from the root mass. Larvae were collected in a 40 mesh screen basket. The basket was placed in a 10% NaCl solution and the number of rice water weevil larvae was determined (Stout et al. 2001). At the end of the season, each plot was mechanically harvested with a plot combine.

All data were analyzed with analysis of variance using PROC MIXED of SAS (Littell et al. 1996). To separate locations by weevil densities, a pooled analysis was conducted with the untreated controls. In the pooled analysis, location was considered a fixed effect. Replicate nested in year was the random effect. Means and standard errors were calculated with LSMEANS and separated according to Fisher's Protected Least Significant Difference ($\alpha = 0.05$). Locations were then classified based on significant differences in mean rice water weevil densities in the untreated controls. After initial analysis of all pooled locations, locations were classified and grouped according to density of rice water weevil larvae in untreated plots (Table 3.1). Each class was then independently analyzed. Seed treatment was considered as a fixed effect in the model. Replication nested in location was random and served as the error term.

An economic analysis was conducted to determine the economic benefit of seed treatments in hybrid rice under varying levels of rice water weevil pressure. The analysis

conducted was based on returns above expected expenses and based on a one-year, short-run decision (Hood 2011). The budget did not account for cost of land, management, or general farm overhead (Hood 2011). Estimates were calculated on the cost per ha for growing straight levee rice that was flood irrigated at 27 ha-cm, in the Mississippi Delta, for the 2012 growing season. The budget was based on conventional rice production and did not take into consideration the added expense of hybrid rice seed, or seed treatment costs. The budget allotted one foliar application of lambda-cyhalothrin. Lambda-cyhalothrin is labeled for rice water weevil control. However, this cost was left unchanged as lambda-cyhalothrin is also used to manage rice stink bug, *Oebalus pugnax* F., infestations later in the season when rice water weevils are not economically important. In the spring of 2013, seed treatment prices were obtained from multiple chemical and seed distributors in the Mississippi Delta. The averages of these prices were used for the economic analysis. Seed treatments were converted to metric measurements, and their 2013 costs are as follows: thiamethoxam (\$ 0.34/ml), chlorantraniliprole (\$ 0.36/ml), and clothianidin (\$ 0.25/ml). Seed prices were set at 80 dollars/ha. The market value for rough rice yield was based on a five year average and set at 14.54 dollars/cwt (NASS 2013). Special considerations were taken for the cost of hauling and drying of additional grain. All specified expenses were deducted from the gross income to obtain net return of rough rice yield on a per ha basis. All data were analyzed with analysis of variance using PROC MIXED of SAS (Littell et al. 1996). Net economic return was evaluated based on rough rice yields in each class. Each class was analyzed independently. Seed treatment was considered as a fixed effect in the model. Location nested in replication was random in the model, and served as the error term.

Results

Pooled Analysis to Classify Locations

Differences in rice water weevil densities were observed among locations ($F = 67.22$; $df = 6, 18$; $P < 0.01$) (Table 3.1). Rice water weevil densities in the untreated control at the Tunica location was significantly higher than all other locations, and was classified as high (80.75 larvae/core) (Table 3.1). The Bolivar 3, Washington 1, and Washington 3 locations had rice water weevil densities significantly higher than Bolivar 1, Bolivar 2, and Washington 2. Bolivar 3, Washington 1, and Washington 3 were classified as moderate (21.75-32.00 larvae/core). Bolivar 1, Bolivar 2, and Washington 2 were classified as low (0.75-8.00 larvae/core).

Low Rice Water Weevil Pressure

Three locations were classified as being under low rice water weevil pressure (Table 3.1). All seed treatments had significantly fewer larvae per core than the untreated control except for the low rate of clothianidin ($F = 2.39$; $df = 6, 54$; $P = 0.04$). Yield ($F = 0.42$; $df = 6, 54$; $P = 0.86$) and economic return ($F = 0.98$; $df = 6, 54$; $P = 0.45$) did not differ from the untreated control in low pressure areas (Table 3.2). Numbers of rice water weevil larvae ranged from 0.50 to 1.80 in the treated plots compared to 3.0 in the untreated plots. Yields ranged from 14067 to 14652 kg/ha rough rice in the seed treated plots compared to 14174 kg/ha rough rice in the untreated plots. Net economic returns ranged from 1038 to 1178 dollars/ha for the treated plots compared to 1139 dollars/ha for the untreated plots.

Moderate Rice Water Weevil Pressure

Three locations were classified as having moderate rice water weevil densities (Table 3.1). Seed treatment had a significant effect on numbers of rice water weevil larvae ($F = 13.25$; $df = 6, 60$; $P < 0.01$) (Table 3.3). All seed treatments had significantly lower densities of rice water weevil larvae than the untreated control. The high rate of chlorantraniliprole had significantly fewer larvae (6.45 larvae/core) than all other treatments except for the low rate of chlorantraniliprole (7.64 larvae/core) (Table 3.3). Numbers of rice water weevil larvae ranged from 11.36 to 11.72 in all other treated plots compared to 24.91 in the non treated plots.

The use of a seed treatment also had a significant impact on yield ($F = 10.71$; $df = 6, 60$; $P < 0.01$) (Table 3.3). All seed treatments yielded significantly higher than the untreated control. The high rate of thiamethoxam yielded significantly higher than the labeled rate of thiamethoxam resulting in 13394 kg/ha rough rice compared to 12631 kg/ha rough rice yield, respectively. The high rate of chlorantraniliprole and clothianidin did not yield significantly different than the high rate of thiamethoxam, resulting in 13263 kg/ha of rough rice and 13143 kg/ha rough rice yield, respectively. The high rate of chlorantraniliprole and clothianidin did not yield significantly higher than the labeled rate of chlorantraniliprole and clothianidin. Yield for the labeled rates of chlorantraniliprole and clothianidin were 12614 kg/ha rough rice compared to 12499 kg/ha rough rice yield, respectively. All treated plots yielded significantly higher than the untreated control producing 10803 kg/ha rough rice yield.

The increase in yield from seed treatment resulted in a significant increase on the net economic return ($F = 7.12$; $df = 6, 60$; $P < 0.01$) (Table 3.3). All seed treatments

resulted in significantly higher net economic returns than the untreated control. However, no seed treatments provided significantly higher returns than other seed treatments. Net economic returns ranged from 882 to 975 dollars/ha for seed treatments compared to 698 dollars/ha for the untreated control.

High Rice Water Weevil Pressure

One location was classified as having high densities of rice water weevil (Table 3.1). A significant effect of seed treatment was observed for densities of rice water weevil larvae ($F = 11.40$; $df = 6, 18$; $P < 0.01$) (Table 3.4). All seed treatments resulted in significantly lower densities of rice water weevil larvae than the untreated control. The use of the high rate of chlorantraniliprole resulted in significantly fewer larvae than all other seed treatments (20.75 larvae/core) except for the high rate of clothianidin (32 larvae/core). Among all other seed treatments, numbers of rice water weevil larvae ranged from 41.00 to 49.50 in the treated plots compared to 80.75 in the untreated plots.

A significant effect of seed treatment was observed for yield ($F = 6.68$; $df = 6, 18$; $P < 0.01$) (Table 3.4). All of the seed treatments resulted in significantly higher yields compared to the untreated control. No significant differences were observed among seed treatments for yield. Yields ranged from 10511 to 11150 kg/ha rough rice in the treated plots compared to 8445 kg/ha rough rice in the untreated.

A significant effect of seed treatment was observed on net economic returns ($F = 5.93$; $df = 6, 18$; $P < 0.01$) (Table 3.4). All of the seed treatments resulted in significantly higher net economic returns than the untreated control under high rice water weevil infestations. There were no significant differences among seed treatments observed for

net economic returns. Net economic returns ranged from 623 to 704 dollars/ha for the treated plots compared to 384 dollars/ha for the untreated plots.

Discussion

The rice water weevil is the most widely distributed and destructive early season insect pest of rice, in the United States and is currently a global threat to rice production with its introduction into the major rice-producing regions of Asia (Bowling 1957, Cave et al. 1984, Way 1990, Heinrichs and Quisenberry 1999, Stout et al. 2002, Saito et al. 2005). Hybrid rice is becoming increasingly important for global food production (Li and Yuan 2000). The production of primary and secondary tillers is vital to overall yield in hybrid rice production (Bond et al. 2008). Damaged root systems from rice water weevil larval feeding results in reduced numbers of tillers, panicles, grains per panicle, and grain weight (Bowling 1972, Zou et al. 2004). Low seeding rates can increase the susceptibility of rice to damage by the rice water, and hybrid rice may be more susceptible to rice water weevil injury than conventional rice production systems because rice water weevil impacts early season tiller production, (Stout et al. 2009).

Previous studies have shown the relationship between low seeding rate and rice water weevil (Thompson and Quisenberry 1995, Stout et al. 2009). In this study, seed treatments were exposed to a wide range of rice water weevil densities. Though not common throughout Mississippi, high rice water weevil densities, such as those observed in Tunica, do occur. In 2011, rice water weevil numbers were unusually high in various locations throughout the Mississippi Delta. Typically rice water weevil densities fall into the low and moderate classes described in this study. Across all seed treatments, seed treatment rates, and all levels of infestation, insecticidal seed treatments reduced weevil

densities by approximately 58%. Comparing labeled rates to the higher rates, seed treatments reduced weevil densities by approximately 55% and 62%, respectively.

Yield losses from rice water weevil feeding exceeding 20% have been reported in the southern United States (Stout et al. 2000). Yield and net economic losses were directly related to increased rice water weevil densities. The observed benefit of a seed treatment increased with increased densities of rice water weevil pressure. All seed treatments and seed treatment rates reduced rice water weevil densities below the untreated control. However, as rice water weevil densities increased from low to moderate to high, yield increases of 2%, 16%, and 22%, respectively, were observed in the treated plots over the untreated plots. A similar trend was observed for net economic returns. The net economic return was -1.5%, 24%, and 42%, respectively, when treated plots were compared to the untreated control within each level of infestation. Though rice water weevil densities were reduced, there was no observed benefit from the use of an insecticidal seed treatment where rice water weevil pressure was low. The overall yield increase of 1.6% observed in low weevil pressure did not yield enough to justify the cost of the seed treatment. Predicting weevil infestations prior to planting is difficult, so a seed treatment is recommended in all rice producing areas of Mississippi. Under moderate and high weevil pressure, currently labeled rates of insecticides performed as well as the higher rates. At moderate densities of rice water weevil there were some observed differences in yield; however, these differences in yield only covered the cost of the increased rate of the seed treatments and no economic differences were observed among rates on net economic return. These data suggest that currently labeled rates of

insecticidal seed treatments are effective in management of the rice water weevil in low seeding rate production systems.

These data also suggest that seed treatments, although currently the best available option, do not provide absolute protection against rice water weevil. Foliar applications of lambda-cyhalothrin and diflubenzuron are effective in reducing rice water weevil densities and protecting yields if the application is timed in relation to rice water weevil oviposition (Stout et al. 2000). In areas with historic rice water weevil pressure, the use of an insecticide seed treatment, in combination with early monitoring of rice fields and the timely application of a foliar insecticide may be the most effective method for rice water weevil management.

Table 3.1 Planting dates, sampling dates, and classification of locations for experiments evaluating insecticidal seed treatment efficacy on hybrid rice in 2011 and 2012.

County	Planting Date	Core Sample Date	Classification	Larvae Per Core ¹	Yield Kg/Ha ¹
Bolivar 1	2 April 2012	21 June 2012	Low	1.00 ± 0.58 c	15,110 ± 1483
Bolivar 2	12 April 2011	21 June 2011	Low	0.75 ± 0.75 c	13,520 ± 226
Bolivar 3	9 April 2011	22 June 2011	Moderate	22.75 ± 2.11 b	11,910 ± 306
Washington 1	26 April 2012	20 June 2012	Moderate	21.75 ± 5.62 b	8,794 ± 598
Washington 2	11 May 2011	6 July 2011	Low	8.00 ± 0.58 c	14,109 ± 282
Washington 3	2 April 2012	22 June 2012	Moderate	32.00 ± 1.00 b	12,002 ± 425
Tunica	24 May 2011	22 June 2012	High	80.75 ± 5.29 a	9,039 ± 655

¹Data presented as the mean (SEM) number of rice water weevil larvae in the untreated control for each location.

Table 3.2 Impact of insecticidal seed treatment rates in hybrid rice on mean (SEM) number of rice water weevil larvae per core, grain yields, net economic return under low weevil pressure.

Treatment	Rate ¹	Mean \pm S.E. ²			
		No. Larvae/Core	Yield kg/ha	Net Return \$/ha	
thiamethoxam	248	1.4 \pm 0.62 b	14303 \pm 455	1119 \pm 59	
thiamethoxam	587	1.3 \pm 0.56 b	14067 \pm 552	1038 \pm 72	
chlorantraniliprole	326	1.00 \pm 0.45 b	14218 \pm 454	1091 \pm 59	
chlorantraniliprole	390	0.50 \pm 0.16 b	14652 \pm 430	1138 \pm 56	
clothianidin	124	1.80 \pm 0.80 ab	14577 \pm 413	1178 \pm 60	
clothianidin	260	1.30 \pm 0.68 b	14617 \pm 462	1168 \pm 60	
untreated control	-	3.00 \pm 1.15 a	14174 \pm 455	1139 \pm 60	

¹Rates are given in ml formulated product per 100 kg seed.

²Means followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 3.3 Impact of insecticidal seed treatment rates in hybrid rice on mean (SEM) number of rice water weevil larvae per core, grain yields, net economic return under moderate weevil pressure.

Treatment	Rate ¹	Mean \pm S.E. ²			
		No. Larvae/Core	Yield kg/ha	Net Return \$/ha	
thiamethoxam	248	11.72 \pm 2.69 b	12631 \pm 311 bc	901 \pm 41 a	
thiamethoxam	587	11.36 \pm 2.32 b	13394 \pm 166 a	950 \pm 22 a	
chlorantraniliprole	326	7.64 \pm 1.08 bc	12614 \pm 284 bc	882 \pm 37 a	
chlorantraniliprole	390	6.45 \pm 1.90 c	13263 \pm 192 ab	956 \pm 25 a	
clothianidin	124	11.72 \pm 2.12 b	12499 \pm 318 c	906 \pm 41 a	
clothianidin	260	11.64 \pm 1.67 b	13143 \pm 374 abc	975 \pm 49 a	
untreated control	-	24.91 \pm 2.43 a	10803 \pm 538 d	698 \pm 70 b	

¹ Rates are given in ml formulated product per 100 kg seed.

² Means followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 3.4 Impact of insecticidal seed treatment rates in hybrid rice on mean (SEM) number of rice water weevil larvae per core, grain yields, net economic return under high weevil pressure.

Treatment	Mean \pm S.E. ²			
	Rate ¹	No. Larvae/Core	Yield kg/ha	Net Return \$/ha
thiamethoxam	248	49.50 \pm 5.42 b	10,511 \pm 316 a	623 \pm 41 a
thiamethoxam	587	41.00 \pm 9.75 bc	11,073 \pm 161 a	646 \pm 21 a
chlorantraniliprole	326	42.00 \pm 5.26 bc	10,722 \pm 580 a	634 \pm 76 a
chlorantraniliprole	390	20.75 \pm 1.25 d	11,150 \pm 716 a	680 \pm 94 a
☉ clothianidin	124	41.50 \pm 8.51 bc	10,904 \pm 301 a	697 \pm 39 a
clothianidin	260	32.00 \pm 4.45 cd	11,070 \pm 379 a	704 \pm 50 a
untreated control	-	80.75 \pm 5.30 a	8,445 \pm 154 b	384 \pm 19 b

¹ Rates are given in ml formulated product per 100 kg seed.

² Means followed by the same letter are not significantly different ($\alpha = 0.05$).

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APPENDIX A
BIOASSAY TO DETERMINE EFFICACY AND LONGEVITY OF INSECTICIDAL
SEED TREATMENTS IN RICE

In the summers of 2011 and 2012, bioassays were conducted using first instar fall armyworm larvae and rice water weevil adults to determine the presence or absence of insecticides within the plant and to determine the longevity of seed treatments against rice water weevil under multiple flushes. Bioassays were conducted using thiamethoxam (Cruiser[®] 5FS, Syngenta Crop Protection) at 248 ml/100 kg seed, chlorantraniliprole (Dermacor[®] X-100, E.I. DuPont de Nemours) at 130 ml/100 kg seed, and clothianidin (NipsIt INSIDE[®], Valent Agricultural Products), at 124 ml/100 kg seed and compared to an untreated control. Leaf samples were obtained from the flush number experiment described in CHAPTER II of this manuscript.

Plant tissue for the bioassays was removed from treated plots and untreated plots after emergence and each subsequent week until mortality was no longer observed. Leaf samples were collected one week prior to the application of the first flush treatment in order to obtain a baseline measurement. Treatments were arranged as a randomized complete block design. Leaf material was collected from the uppermost completely unfolded leaf. The leaf material was placed in 1-oz solo cups with a water agar solution to prevent desiccation of the leaf material. Each solo cup contained one leaf blade and one first instar fall armyworm larva for chlorantraniliprole treated plots and one rice water weevil adult for thiamethoxam and clothianidin treated plots. A total of 10 leaf samples were collected per treatment for a total of 240 leaves tested for each fall armyworm bioassay and 360 leaves tested for each rice water weevil bioassay. Mortality was measured 3, and 5 days after infestation.

Larvae from a laboratory colony of fall armyworm were used in the bioassays. During 2011 substantial mortality of fall armyworm was observed on leaves from the

chlorantraniliprole treated plots for approximately 30 days after planting. However, as the study progressed control mortality sharply increased to 60-100%. The bioassay was conducted for three more weeks after the observed increase in the untreated control and control mortality remained high. The same bioassay was conducted in 2012 with 60-100% control mortality observed during the first week. The study was conducted three additional weeks, but control mortality remained at unacceptable levels.

There are multiple reasons for lack of success with this study. Changes were made to the methods in order to determine what factor was causing the high control mortality. Bioassays were conducted without using a water agar solution; larvae were allowed to feed on diet for 2 days prior to infestation, and the bioassay was stored in the laboratory vs. in the rearing lab. None of these changes to the methods provided acceptable answers as to what happened. Another hypothesis as to the cause of the high mortality is that the colony had been laboratory culture for 35 generations and it was no longer capable of feeding on plant tissue.

Rice water weevil adults were collected nightly from a black light trap located next to flooded rice fields at the Delta Research and Extension Center in Stoneville, Ms. Each morning the contents of the black light trap were taken to the laboratory for sorting. Adult rice water weevils were collected using fine paint brushes that were dipped in water to facilitate collection. The rice water weevils were then taken into the insectary and placed on unflooded living rice plants in plastic containers. Rice water weevils were allowed to feed on rice plants for at least 24 hours prior to testing to reduce control mortality. Bioassay methods were as described above.

Rice water weevil numbers were high in 2011. It was not uncommon to collect rice water weevils by the thousands with the black light trap and many bioassays could be conducted. However, it was difficult to differentiate between living and dead rice water weevils because rice water weevil adults are fairly lethargic in the absence of water and during the day. This resulted in unacceptable levels of control mortality. Different methodology was used to differentiate between living and dead adults. These include placing in room temperature water, water heated to 37.78°C, using a hot plate at 37.78°C and through observation without disturbance. However, no acceptable levels of control mortality were observed in this study. In 2012, rice water weevil numbers were too low to conduct the bioassays and the project was terminated.

APPENDIX B

MICRO-PLOT STUDY TO DETERMINE THE IMPACT OF TIME FROM
PLANTING TO PERMANENT FLOOD ON THE EFFICACY OF
INSECTICIDAL SEED TREATMENTS IN RICE

In the summers of 2011 and 2012 micro-plot studies were conducted at the Delta Research and Extension Center in Stoneville, MS to determine the impact of time from planting to permanent flood on the efficacy of insecticidal seed treatments in rice. Rice was planted as micro-plots (19 in X 20 in) in metal pans buried in the field. The metal pans were intended to better regulate water levels in each of the plots. Plots were planted mid-late May as a factorial treatment arrangement in a randomized complete block design with four replications. The first factor was seed treatment. Seed treatments included thiamethoxam (Cruiser[®] 5FS, Syngenta Crop Protection) at 248 ml/100 kg seed, chlorantraniliprole (Dermacor[®] X-100, E.I. DuPont de Nemours) at 130 ml/100 kg seed, and clothianidin (NipsIt INSIDE[®], Valent Agricultural Products), at 124 ml/100 kg seed and compared to an untreated control. The second factor was flood timing of 4, 6, and 8 weeks after planting.

At the time of permanent flood, cages were placed over each individual plot. These cages were infested with 40 adult rice water weevils collected from a black light trap within one week after flooding, and were removed two weeks after infestation. One 4-inch diameter core sample was collected from the middle row of each plot three weeks after infestation. These samples were washed through a series of screens and rice water weevil larvae were collected in a 40 mesh screen basket. The basket was placed in a 10% NaCl solution and the number of rice water weevil larva was determined.

In 2011, rice water weevil numbers were high and proper infestations were able to be made. However, there was much difficulty in managing water levels due to excessive rain and/or heat. Prior to establishing the permanent flood excessive rainfall caused premature flooded conditions and pans had to be drained daily. Following the permanent

flood hot and dry conditions made maintenance of the permanent flood difficult. Because of this results were inconclusive in 2011. In 2012, efforts were made to alleviate some of the problems found in 2011 with little success. Also, rice water weevil numbers were too low to achieve acceptable levels of infestation. Because of these problems and lack of reliable results the project was terminated in Jun 2012.