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The Impact of Fall Armyworm, Spodoptera frugiperda (J.E. Smith), Feeding and Mechanical Defoliation on Growth and Yield of Rice, Oryza sativa (L.)

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The Impact of Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith), Feeding and Mechanical Defoliation on Growth and Yield of Rice, *Oryza sativa* (L.)

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Abstract

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), (FAW) is a serious pests of many crops, and can be observed feeding throughout the entire growing season on rice, *Oryza sativa*, (L.). A new defoliation based threshold would help rice growers and consultants make more economically sound decisions for FAW. Work from this thesis focuses on determining the amount of damage caused by FAW feeding at different growth stages and effective insecticide seed treatments for controlling this pest.

Field plots were mechanically defoliated to determine grain yield loss across multiple growth stages and defoliation percentages. Results indicated that defoliation in late vegetative and early reproductive growth stages cause appreciable levels of yield loss. Yield loss was associated with large levels of growth recovery, which correlated with yield loss especially in late vegetative and early reproductive growth stages. Defoliating only the flag leaf was found to cause no significant yield loss, even when 100% removed.

Greenhouse studies were conducted to evaluate damage from FAW larval feeding and manual defoliation in a controlled environment. When rice was 100% mechanically defoliated at the 2- to 3- leaf and 2nd- to 3rd tiller growth stages, a yield reduction was observed compared to the untreated control. Larval infestations reached appreciable levels of defoliation only at the 2- to 3- leaf growth stage, where yield loss was observed to be similar to mechanical defoliation.

Choice bioassays were conducted to determine feeding preference from FAW once panicle emergence. Choice bioassays exhibited an increased percent of blank kernels and decrease in yield when FAW only had rice panicles to feed on. Yield reductions were not observed when FAW had the option to feed on the panicle or the flag leaf, but did have a

significant amount of blanked kernels. When only the flag leaf was available to feed on, no differences were observed.

Greenhouse studies were conducted to determine effective insecticide seed treatments for control of FAW. Two insecticide seed treatments (cyantraniliprole and chlorantraniliprole) have the potential to effectively control the FAW. Cyantraniliprole controlled FAW for 49 days after planting, while chlorantraniliprole was still active for FAW at 73 days after planting. Neonicotinoid seed treatments thiamethoxam and clothianidin were not found to be effective for controlling FAW larvae.

These studies provide needed background on the potential impact and control of FAW, and should serve to provide a framework for more effective and economic control of FAW in Arkansas rice.

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Chapter 1- Literature Review

Introduction

Rice, *Oryza sativa*, (L.) is one of the most important crops worldwide. People have gathered and consumed rice for at least 10,000 years (Kovach et al. 2007). Rice is the principal food source for over 40% of the world (Buresh and De Datta 1990). Only 5% of rice that is produced enters the world market while 95% is produced and consumed in Asia (Chang 2000).

The United States planted just under 971,245 ha in 2017 with an average yield of 3265 kg/ha (NASS 2018). Arkansas, California, Louisiana, Mississippi, Missouri and Texas are the leading states in rice production (USDA Census of Agriculture, 2009). About one-half of the U.S. crop is sold domestically and the rest is exported (USDA ERS 2014). The U.S is the major exporter of rice in the world (USDA ERS 2014).

Rice Production in Arkansas

Arkansas planted just over 583,152 ha in 2018 making it the leading producer of rice in the United States producing 48% of the U.S. crop (NASS 2018). It has been the number one rice-producing state in the U.S. since 1973. Rice is currently grown in 40 of the 75 counties mainly on the eastern half of the state. The three largest rice producing counties in Arkansas are Poinsett, Lawrence, and Arkansas (Hardke 2019). Increased yields have been observed over the years due to hybrid rice, new cultivars and better production methods and technologies.

The days to maturity for cultivars planted in Arkansas ranges from 105-145 days from germination to maturity (Moldenhauer et al. 2019). The number of days to maturity depend on the environment and the cultivar planted. Rice has three main growth stages: vegetative, reproductive, and grain filling and ripening. There are also three main components that factor how well rice will yield: number of panicles per unit of land area, average number of grain produced per panicle, and average weight of the individual grains (Hardke 2019). Important

inputs in rice management include fungicides, herbicides, insecticides, soil fertility and fertilization.

The vegetative phase of rice includes seed germination, seedling emergence, pre-tillering (V1-V4), tillering (V5-V13), maximum tillering, and vegetative lag phase. Depending on the cultivar planted (long or short duration) and environmental conditions the vegetative phase can last 47-117 days. The reproductive phase can last anywhere from 19 to 25 days and includes panicle initiation (PI, R0), internode elongation (IE), panicle differentiation (PD, R1), boot (R2), heading (R3), and anthesis (R4). The ripening phase includes the milk stage (R6), soft dough (R6), hard dough (R7), grain drying (R8), and harvest maturity (R9) and can last anywhere between 30-45 days (Moldenhauer et al. 2019 and Hardke 2019).

In Arkansas, 85% of rice is drill seeded with the other 15% being broadcasted. Broadcasted seed is divided into 10% dry seeded, and 5% water seeded. Planting begins around the end of March, with 95% of planting completed by June 1st. When drilled dry-seeded rice reaches the 4-5 leaf growth stage, flooding of the field usually begins. This occurs around the end of May into early June. Harvest begins around the middle of August and continues through early November (Hardke 2019).

Insect Pests of Rice

Farmers must contend with multiple insect pests in rice production in Arkansas. The three major insect pests are: grape colaspis, *Colaspis brunnea* (F.), rice water weevil, *Lissorhoptrus oryzophilus* (Kuschel) and rice stink bug, *Oebalus pugnax* (F.) (Lorenz and Hardke 2013). Minor or occasional pests include greenbug aphid, *Schizaphis graminum* (Rondani), bird cherry-oat aphid, *Rhopalosiphum padi* (L.), sugarcane borer, *Diatraea saccharalis* (F.), rice stalk borer, *Chilo plejadellus* (Zincken), billbugs, *Sphenophorus pertinax ludoviciana* (Chittenden), rice seed

midges, *Chironomus spp.* (Way), chinch bugs, *Blissus leucopterus* (Say), true army worms *Pseudaletia unipuncta* (Haworth) and Fall armyworm, *Spodoptera frugiperda* (Smith) (Lorenz and Hardke 2013).

In Louisiana, foliar pyrethroid insecticides were commonly used to treat rice water weevil, but were found to cause significant damage to crawfish production (Barbee et al. 2010). Currently, insecticide seed treatments are the primary means of control for both grape colaspis and rice water weevil, although a leaf scar based threshold exists for foliar applications to control rice water weevil (Lorenz and Hardke 2013). Since the mid-2000s, an anthranilic diamide (active ingredient: chlorantraniliprole; Dermacor® X-100, DuPont) and two neonicotinoids (active ingredients: thiamethoxam; Cruiser Maxx® Syngenta; clothianidin; Nipsit INSIDE®, Valent) have been used as a seed treatment for control of rice water weevil and are less impactful on crawfish production (Barbee et al. 2010).

Rice stink bug is a pest of rice after panicle emergence, and can cause significant direct and indirect damage until just before the rice plant is harvested (Lorenz and Hardke 2013). According to Swanson and Newsom (1962), the rice stink bug feeds on developing kernels of grasses and rice once the head emerges from the boot.

The potential for economic loss from rice water weevil, grape colaspis, and rice stink bug has been well documented, and control recommendations are available (Lorenz and Hardke 2013).

Biology of Fall Armyworm

Fall armyworm has a complete life cycle of around 30 days during the hot summer months, 60 days in the fall and spring, and 80 to 90 days in the winter in the Southern United

States (Capinera 2008, Luginbill 1928, Vickery 1929). The female moth lays masses of dome-shaped eggs with flat bases that curve upwards to a rounded point at the apex. The eggs measure approximately 0.4 mm in diameter and 0.3 mm in height. The size of FAW egg masses vary, but are typically around 100 to 200 eggs, with one female producing as many as 1,500 eggs over her adult life. Eggs are attached to foliage, followed by deposits of scales in and around the eggs giving it a moldy appearance. Eggs hatch within two to three days in the summer months (Capinera 2008).

According to Capinera (2008), first and second instars of FAW are usually greenish with a black head and have a granular texture. The FAW has six instars and is typically identified by the inverted “Y” on its head. FAW length ranges from 1.7 mm at first instar to 34.2 mm at the sixth instar. Being nocturnal, the larvae move down the canopy toward darker conditions during the brighter times of the day. The larval stage lasts approximately 14 days during the summer, and as long as 30 days during the winter months (Capinera 2008).

Fall armyworm larvae pupate in a cocoon in the soil at a depth of 2 to 8 cm (Capinera 2008). The cocoon is oval shaped, constructed of silk mixed with soil, and is 20 to 30 mm in length. The pupae appear reddish brown and measure approximately 14 to 18 mm long and 4.5 mm wide. The pupal stage lasts approximately 8 to 9 days during the summer and as long as 20 to 30 days during the winter in southern United States. The survival rate of pupae is known to vary considerably from warmer to cooler regions (Capinera 2008).

Adult FAW have a wing span of 32 to 40 mm. The forewings of male FAW moths are more distinctly marked than female FAW moths with white, triangular spots at the tip and near the center of the wing. The hindwings of both sexes have an iridescent silver-white appearance with a dark border. Adults are nocturnal, and most active during warm humid evenings with a

lifespan on average of about 10 days but can last as long as 21 days. The adult female typically lays her eggs within the first five days after eclosion (Luginbill 1928, Sparks 1979).

There are two genetically different strains of the FAW (corn and grass), which also shows differences in host plants (Pashley 1986, Pashley 1988). The grass strain feeds on soybeans and grass crops including bermudagrass and rice, while the corn strain feeds primarily on corn and grain sorghum (Levy et al. 2002). Based on larval and pupal weights, both strains perform equally well when feeding on rice or bermudagrass. In contrast, the corn strain performs better on corn than the grass strain (Pashley 1988, Whitford et al. 1988, Klass et al. 1995). The corn strain is able to utilize corn as a host plant better than the grass strain due to physiological differences between the strains that affect their ability to more efficiently digest corn (Klass et al. 1995).

Pashley and Martin (1987) determined that interstrain mating was complicated and had a significant impact on the biology of this pest. When rice-strain males were bred with corn-strain females, the male offspring were unable to produce spermatozoa. Grass strain males could not successfully mate with corn strain females, but corn strain males could successfully mate with grass strain females (Pashley and Martin 1987).

Migration

Migration patterns of FAW do not appear consistent. Nagoshi and Meagher (2008) found FAW overwinters in Florida and Texas and migrates northward as far as southern Canada during the summer months. One of the earliest studies suggests that the Texas overwintering population migrates northward into Oklahoma and northeasterly into the Mississippi river valley. The Florida overwintering population migrates to northern Florida by early May and into north-central Georgia by June continuing northeasterly into South Carolina on the east side of the

Appalachian mountains (Luginbill 1928). Young (1979) found the Western population of FAW originated from the overwintering population in Texas. However, they were unable to determine whether or not FAW in the Eastern population could have come from either Texas or Florida.

As a result of this apparent discrepancy, Nagoshi and Meagher (2008) advocate migratory testing based on genetic markers, rather than pesticide resistance, while examining corn strain populations in Georgia, Alabama, Louisiana, and Mississippi. They observed that the genetic markers of the FAW populations in Georgia were indistinguishable from the overwintering population from Florida, but that the genetic markers of FAW populations in Louisiana, Mississippi, and Alabama were indistinguishable from the overwintering population from Texas. This suggests that the Texas population migrates farther east than earlier studies suggested, and that there is some overlapping and mixing of the two populations.

Multiple studies have observed that wind patterns largely determine the frequency, intensity, and displacement in the migratory patterns of FAW (Reynolds et al. 2005, Wood et al. 2006, and Chapman et al. 2008). Pair and Westbrook (1995) found weather transfer systems to be the most important climatic factor determining FAW abundance in the United States. Westbook et al. (2016) found projections of air transport trajectory models can be used to determine migratory pathways. Looking at the overwintering sites in Texas and Florida, you can distinguish the FAW migration pathway by the different populations (Westbrook et al. 2016). Texas populations of the FAW resembled the Louisiana and Mississippi populations which infest west of the Appalachian mountains. While the Florida population resembles the Georgia population and infests the Atlantic Coast states (Nagoshi et al. 2008).

Damage

FAW larvae feed on the foliage of rice, and can be found throughout the entire growing season. Large FAW infestations in rice generally occur along levees, field boarders, and in parts of the field where larvae can escape the flood, and can consume high amounts of tissue (Heinrichs et al. 2017). According to Luginbill (1928), the first three instars of larvae skeletonize the leaves of plants, with the first instar rarely eating entirely through a leaf. Fourth to sixth star larvae can defoliate whole plants (Luginbill 1928). According to Heinrichs et al (2017), seedling rice has seen major damage from feeding due to the flood not being applied to the field. Dale (1994) states that young larvae feed on the edge of the leave surfaces towards the midrib. The FAW has the potential to cut seedling plants to the ground, while older plants are only defoliated. Stand loss can be severe in young seedlings if large numbers of armyworms are present (Heinrichs et al. 2017).

Defoliating pests primarily decrease yield potential of crops by reducing the crops ability to perform photosynthesis (Buntin 1986). FAW infest more than 60 plant species including forage grasses, corn, alfalfa, cotton, soybeans, and most vegetable crops (Flanders et al. 2017). Damage from FAW is usually observed first on bermudagrass. The grass has a thinned-out appearance and develops brown spots from FAW damage. Early damage symptomology can appear similar to drought stress, which is a dark brown coloration or burned out patch resembling dying/wilting leaves along with dark coloration. This patch grows as FAW spreads and consumes more foliage. Large FAW densities have been observed in pastures during droughts due to natural enemies being less active (Loftin et al. 2012). Observations of complete defoliation from FAW have been observed in hayfields and pastures (Loftin et al. 2012). According to Bowling (1978), corn, grain sorghum, and bermudagrass have been the main focus

for research on damage by the fall armyworm in recent years; however, numerous pesticides applications were made in Texas to control dense larval populations of FAW in rice (Bowling 1978).

Marenco et al. (1992) observed that damage to corn is often severe as larvae consume all but the ribs and stalks of the corn plants. The larvae can destroy the growing point of corn by burrowing into the bud or whorl and clipping the leaves. The FAW has also been observed to occasionally feed on the ear of corn. FAW tends to burrow through the side of the ear through the husk, unlike the corn earworm, which tends to eat through the silk at the tip of the ear. In sweet corn, FAW can reduce yields by as much as 5 to 20 percent (Marenco el al. 1992).

Luttrell and Mink (1999) infested cotton with egg masses from the FAW to determine yield reduction from FAW feeding. Survival of the FAW was low at only 0.07% after 3 days of being infested. Additionally, more damage was noticed when third instars were used rather than egg masses. Cage studies were also conducted using 3^{rd}, 4^{th}, and 5^{th} instars to determine damage to the fruiting structures. Damage was highest when infested with the 3^{rd} instars, and there was no significant difference in survival of squares when infested with the 4^{th} and 5^{th} instars. Luttrell and Mink (1999) concluded that fall armyworms do not routinely infest cotton and more research is required to determine how much damage is inflicted to the blooms and bolls.

The effect of FAW feeding on rice may be of economic concern. The FAW has been observed feeding on the foliage as well as the panicle of rice crops (Mitchell et al. 1991). Bowling (1978) observed reductions in rice yields when plants were defoliated at the seedling and tillering growth stages. In the seedling growth stage, a 3% yield loss was observed when defoliated mechanically at 25%, and 8% yield loss at 50% defoliation. During the tillering

growth stage, a 5% yield loss was observed at 25% defoliation, and 12% at 50% defoliation. Rice plants recovered rapidly from defoliation at early vegetative growth stages (Bowling 1978).

According to Luginbill (1928) fourth instars consumed 3.9% more foliage compared to third instars. In cage studies, Pantoja et al. (1986) observed damage to rice as early as 24 h after initial infestation with the upper leaves being affected first, but as the infestation time period progressed, whole plants were consumed. Distribution patterns were uniform across the plot, but the FAW congregated in the shaded areas made by the metal cages. The greater the population density, the more damage was observed to the panicle. Fields with high densities of FAW (215.1 larvae/m^2) were observed to reduce yield as much as 15 to 20 percent. Reduction was not significant until the level reached 215.1 larvae/m^2, at which mean yields were 17% lower than the yields of uninfested cages.

Wu and Baldwin (2010) state that the rice plant can differentiate herbivore attacks from mechanical wounding. The plant does this by recognizing elicitors such as the fatty acid-amino acid conjugates in the oral secretions of the insect. The rice plant has an induced chemical defense when fed upon. The insect's salivary enzymes reduce the effects of plant metabolites when feeding. In response to feeding from an herbivore, rice accumulates higher levels of Jasmonate or defense-related secondary metabolites compared to just mechanical defoliation (Fukmoto et al. 2013 and Shinya et al. 2016), and in return can attract natural enemies.

Control Tactics

Sampling

Sampling of FAW is needed to determine when insecticide applications need to be made. Black light traps and pheromone traps can be used to monitor adult FAW populations. If adult

moths are visible in the black light or pheromone traps, it is recommended to scout the field for eggs and larvae. The current threshold for FAW in rice is to treat when there are six or more armyworms per square foot early in the season, and late in the season when armyworms are found feeding on and damaging the flag leaf. There is currently no formal scouting procedure for armyworms in rice. Rice field edges should be monitored for migration of armyworms from wheat or oat fields. Insecticide treatment should be used before severe damage occurs and reduces rice stand (Hardke 2019). Flooding the field can be an effective control measure for armyworms in rice. A sweep net could also be used to determine the density of the larvae in rice at later growth stages.

Insecticide Control

According to the Arkansas MP-144, insecticide application is warranted for control of FAW in pastures when there are two or more armyworms per square foot, or 1 armyworm per sweep with a 15 inch sweep net (Studebaker et al. 2019). Pyrethroid insecticides work faster than insect growth regulators and are more effective against small and medium-sized caterpillars ($3/4^{th}$ inch). The residual activity of the pyrethroids is less than that of the insect growth regulators. Since the insect growth regulators are slower acting, it is recommended that they should be used when a majority of the caterpillars are small (1/8 to ¼ inch) (Loftin et. al. 2012). Pyrethroids control the grass strain with Diamides controlling both the grass and corn strain. Pyrethroids are more cost effective but should not be used if reinfestation is a possibility.

Insecticide seed treatments that have a long residual activity could potentially reduce the number of insecticides that are applied throughout the growing season (Hardke et al. 2011). According to Pablo et al. (2018), insecticide seed treatments with the active ingredients of thiamethoxam and chlorantraniliprole reduced FAW leaf consumption in soybeans. The

chlorantraniliprole seed treatment had the best results against FAW feeding, and had 100% mortality within 24 hours, while the thiamethoxam seed treatment had 0% mortality. Thiamethoxam reduced FAW damage by 40% in soybeans, while chlorantraniliprole reduced damage by greater than 90%. Thrash et al. (2013) looked into the survivorship of FAW when infested onto soybeans with chlorantraniliprole and cyantraniliprole seed treatments. In the V3 growth stage, populations were reduced to 20 and 50% for chlorantraniliprole and cyantraniliprole, respectively after 4 days. Chlorantraniliprole significantly reduced survivorship after just 3 days compared to the untreated check (Thrash et al. 2013). Hardke et al. (2011) looked into whorl stage corn with seed treatments chlorantraniliprole and cyantraniliprole reducing FAW populations the best. Chlorantraniliprole and cyantraniliprole and high mortaility on FAW through 28 days after treatment when compared to the untreated check (Hardke et al. 2011).

Cultural and Biological Control Techniques

Planting early maturing corn allows the crop to avoid high densities of FAW versus planting later maturing varieties (Mitchell 1978). FAW populations seem to be unaffected by reduced tillage (All 1988). Although several pathogens have been known to reduce FAW larvae abundance in corn, only *Bacillus thuringiensis*is (Bt) is recommended and natural strains need to be on the foliage before larval infestation. Natural strains have not proven as effective as genetically modified strains (All et al. 1996). Infestations of corn ears by Lepidoptera have shown there can be an increase in fungal growth and contaminated ears by mycotoxins such as *Aspergillus flavus* (Widstrom 1979, McMillian 1983, McMillian et al. 1985, Smith and Riley 1992). Alflatoxin contamination of corn ears may be reduced in Bt corn (Williams et al. 1998).

Infestations of FAW in late planted *Bt* corn prevented yield loss by 50% (Buntin et al. 2001, Buntin et al. 2004).

According to Litsinger (1995), there are two types of cultural control practices: primary and secondary. Primary cultural control is done specifically to control insects. This may be done by draining the rice field or planting a trap crop. Secondary practices include normal preparations such as tillage, weeding, and fertilization, which in return reduce pest populations (Litsinger 1995). Planting method of rice plays a role in pest management as well, by determining planting time, plant density, and water level. According to Isely (1941), crop rotation works best against species with limited host ranges, limited dispersal, and long life cycles. Crop rotation is also recommended for armyworm control (Alam and Nurullah 1977). According to Litsinger (1995), early maturing cultivars have a shorter vegetative growth stage, which can negatively affect the armyworm growth and development (Alam and Nurullah 1977). Planting time is also important in crop production. According to Isley (1941), trap cropping relies heavily on that crop attracting the pest away from the main crop. Tillage of the soil before planting has a positive impact on insects by crushing, burying, and exposing pest to desiccation and predators (Litsinger 1995). Weeding is also an important cultural technique. Grasses are beneficial to insects in the rice field by giving them a place to develop (Litsinger 1995). According to Jepson (1954), weeds act as a bridge between the weed host and rice crop. Weeding the rice crop is recommended for control of armyworms (Hutson 1920).

Resistance

There is clear documentation to resistance in Bt crops all around the world (Tabashnik et al. 2013, Huang et al. 2011), including FAW resistance in Puerto Rico to Cry1F in maize (Storer et al. 2010, Storer et al. 2012). Puerto Rico is the only place where field resistance to Bt maize

has been documented for FAW (Vélez et al. 2013). A study was performed by Huang et al. (2014), to determine survival of FAW on Cry1F maize in Louisiana, Florida, North Carolina, and Georgia. The results included survival of FAW on Cry1F maize, which was unexpected. The geographical range remains unknown for FAW resistance in Cry1F maize (Huang et al. 2014).

Fall armyworm collected from corn in North Florida showed resistance to pyrethroids, organophosphates, and carbamates (Yu 1991). The highest levels of resistance were shown in fluvalinate, methyl parathion, and carbaryl, respectively. The broad spectrum resistance observed was due to increased detoxification by microsomal oxidases, and target site insensitivity (Yu 1991). Young (1979) also determined migratory patterns of the FAW based on resistance to carbaryl and methomyl, which are in the carbamate class.

Fall Armyworm

Although major insect pests of rice generally attack at a specific growth stage, and remediation is based on these timings, the fall armyworm feeds on rice across all growth stages.

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) was first recorded as a pest of rice in 1845 in Florida (Luginbill 1928). Recently FAW has been observed more frequently in Arkansas rice fields. The FAW is a polyphagous feeder but prefers feeding on grasses (Heinrichs et al. 2017). Forty-two different plant families are fed on by this pest in North America. While considered a major pest in several crops, the FAW is considered an occasional pest of rice (Heinrichs et al. 2017). With increasing prevalence of this pest in recent years more scrutiny is called for regarding the question of whether or not damage from FAW feeding equates to economic yield loss. Arkansas' current recommendation for FAW in rice is to treat when there are six or more armyworms per square foot. After flag leave emergence, treat when fall armyworms are present and damaging the flag leaf (Lorenz et al., 2019). The purpose of our

study is to determine the impact of FAW feeding at multiple growth stages of rice and to determine if a threshold can be developed to help growers determine when or if there is a need to control this pest.

Chapter 2- Evaluating the Impact of Simulated Fall Armyworm, *Spodoptera frugiperda*, Defoliation on Rice, *Oryza sativa*, Grain Yield

Abstract

Little research has been conducted on the impact of defoliation on rice, *Oryza sativa*, (L.) from the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), (FAW) in Arkansas. A defoliation based threshold would provide growers and consultants with a simple way to make economically sound decisions about controlling FAW. Rice plants were mechanically defoliated at 0, 33, 66, and 100% with a weed eater at 2- to 3- leaf, early tiller (V5-V6), late tiller (V11-V13), and beginning internode elongation (BIE) growth stages. Major grain yield loss was observed in the weed eater field trial at the late tiller and BIE growth stages at both location when defoliation exceeded 33%. At the Pine Tree location, daily growth rate (DGR) increased in the early tiller, late tiller, and BIE growth stages as percent defoliation increased. The same trend was observed for the late tiller and BIE growth stages at the Harrisburg location. At the 2- to 3- leaf growth stage, yield increased as DGR increased at the Pine Tree location. A similar trend was observed at the Harrisburg location for the early tiller growth stage. As DGR increased yield decreased in the late tiller and BIE growth stages at both locations. No yield differences were observed in the scissors defoliation studies.

Introduction

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (FAW), was first described by Smith and Abbot (1797) where it was found feeding on grain sorghum, *Sorghum bicolor* (L.). The first record of it feeding on rice, *Oryza sativa* (L.), was in Florida in 1845 (Luginbill 1928). In recent years, defoliation from FAW has become more prevalent in rice fields across Arkansas. Additionally, the number of hectares affected by FAW could continue to increase due to an increase in row rice production, as opposed to traditionally flooded rice fields. In flood irrigated rice, plants are protected from larvae moving into a field from turn rows and ditches due to their

inability to cross standing water. The only way for FAW to infest flooded fields is for moths to oviposit directly on the rice plants. In contrast, row rice does not have the protection of standing water, so larvae can move directly into rice fields from adjacent hosts. Because Arkansas grows over half of the rice in the U.S., this research has the potential to impact a large amount of hectares (NASS 2017).

Defoliation impacts the yield potential of any crop by limiting the plants ability to perform photosynthesis (Buntin 1986). Previous research has shown that excessive defoliation in rice, even at early growth stages, can impact rice yields. Bowling (1978) observed reductions in rice yields when defoliated at the seedling and tillering growth stages. He found that rice yields, when mechanically defoliated at the seedling growth stage, were reduced by 3% from 25% defoliation, and 8% from 50% defoliation. In the tillering growth stage, a 5% yield loss was observed at 25% defoliation, and 12% at 50% defoliation. In contrast, Taylor (1971), observed yield increases from 50 and 66% defoliation during the tillering growth stage. Medhi et al. (2015) also found that it was advantageous to prune rice up until 100 days after germination.

Using mechanical defoliation and larval defoliation methods in research have advantages and disadvantages. Mechanical defoliation saves time and can help to minimize pathogen spread from insect feeding (Baldwin 1990). Additionally, multiple defoliation levels can be applied precisely and evenly across plots. Using larval infestations to defoliate plots can be problematic for multiple reasons such as timing plant growth stage with the appropriate insect growth stage, and high mortality of the insects used in the infestations. However, mechanical defoliation may not represent larval feeding entirely, because leaves are immediately cut to the appropriate defoliation level, whereas larval feeding occurs gradually over time (Rice et al. 1982). Other

studies have shown that plants can react differently to larval defoliation when compared to mechanical defoliation due to enzymes in insect salivary secretions (Wu and Baldwin 2010).

The current action threshold for fall armyworm in Arkansas is based on numbers of larvae per square foot. This can be difficult to determine in rice due to thick stands, large plants, and flooded field conditions. A new threshold based on percent defoliation would be more convenient for the grower or consultant to determine the level of damage in the field. The main objective in this study was to evaluate grain yield loss in rice from simulated FAW defoliation at different growth stages.

Materials and Methods

Defoliation with Battery Powered Weed Eater

Field plots were located at the University of Arkansas Pine Tree Research Station in Colt, Arkansas and the RiceTec field station in Harrisburg, Arkansas in 2018. Rice was drill seeded with a conventional cultivar, Diamond, at Pine Tree, and a hybrid cultivar, Gemini 214 CL, at Harrisburg. The Pine Tree location was planted May 31st, 2018 and the Harrisburg location was planted April 20th, 2018. Plot size differed depending on area at each location. Harrisburg plots were 1.5 meters by 3.0 meters and 1.5 meters by 2.4 meters at Pine Tree. Two factors were used in this study, percent defoliation and defoliation timing. The experimental design was a randomized complete block with a 4 x 4 factorial arrangement of treatments. Eight replications were used at both locations. Rice plants were defoliated 0, 33, 66, or 100%, at the 2- to 3- leaf, early tiller, late tiller, or beginning internode elongation (BIE) growth stage. A battery powered weed eater was used to defoliate designated plots to the appropriate defoliation level at each timing. With plant height taken into account, visual estimations were used to defoliate whole plots to the appropriate defoliation level. The weed eater was controlled by one person, while the

project leader stood back to examine the defoliation, instructing the operator to defoliate more or less to reach the desired level. Entire replications were defoliated by the same person to ensure consistency across all treatments within a replication. For 100% defoliation treatments rice was defoliated to the soil line preflood and defoliated to the water line post flood. After defoliation plant heights were recorded from five randomly selected plants in each plot on the day of defoliation and then 6, 12, and 18 days after defoliation, to measure plant growth. In order to determine the actual percentage a plot was defoliated, the height of the untreated check was recorded and then compared to the height of the defoliated plot. To calculate daily growth rate (DGR), the height of plants at 0 days after defoliation was subtracted from plant height recorded at 18 days after defoliation, then divided by 18, which is the number of days between measurements. Plots were maintained using recommended agronomic practices until harvest (Hardke 2019). At the Pine Tree location there was a zinc deficiency at the third tiller growth stage. Standard agronomic practices were followed and the rice was drained, the soil was allowed to dry, and chelated zinc was applied at 0.45 kg hectare and reflooded. Urea treated with agrotain was applied at 145 lbs/A one day prior to permanent flood establishment at the second and third tiller growth stage on June 14th, 2018 at Harrisburg, and June 20th, 2018 at Pine Tree. Plots were harvested using a plot combine, and grain yield was calculated based on weight and grain moisture for each plot. Data were analyzed with regression analysis (JMP version 14.2.0, Cary, NC) with an alpha level of 0.05. Within the analysis, block was considered a random variable.

Defoliation with Scissors

In 2017, field plots were located at the University of Arkansas Rice Research and Extension Center in Stuttgart, Arkansas. Cultivar Roy J was drill seeded on May 19th, 2017 in

Stuttgart, Arkansas. In 2018, field plots were located at the University of Arkansas Pine Tree Research station in Colt, Arkansas and the Delta Research and Extension center in Stoneville, Mississippi. Diamond was drill seeded on May 2nd, 2018 in Pine Tree and May 10th in Mississippi. Plot sizes were 91.4 cm by 91.4 cm or 0.84 m^2.

The experimental design was a randomized complete block with a 3 x 4 factorial arrangement of treatments. Defoliation timing and percent defoliation were the two factors used in this study. Defoliation levels of 0, 25, 50, or 100% were applied at 2- to 3- leaf, 2nd- to 3rd tiller, or heading growth stages. At the 2- to 3- leaf and 2nd- to 3rd tiller growth stage, all plants within a plot were defoliated to the appropriate level. At the heading growth stage only the flag leaf was removed. In 2018 treatments were added to simulate the clumped distribution commonly seen with natural infestations. In order to do this 5, 10, or 20% of the plants in a plot were defoliated 100% at the 2- to 3- leaf and tiller growth stage, with only the flag leaf being defoliated for the heading growth stage. Scissors were used to defoliate the rice plants in each plot. Plots were maintained using recommended agronomic practices until harvest (Hardke 2019). Urea treated with agrotain was applied at 145 lbs/A at the third tiller growth stage with the flood being applied the following day. The entire plot was then harvested using a plot combine, and yield was calculated based on the weight and grain moisture for each plot. Yields were analyzed as percent of the undefoliated check. Data were analyzed with an ANOVA (JMP version 14.2.0, Cary, NC) with an LSD post hoc analysis with an alpha level of 0.05. Within the analysis, block was considered a random variable.

Results

Defoliation with Battery Powered Weed Eater

A significant interaction was observed between location, defoliation level, and growth stage (F= 3.03, df= 9, 217, P<0.01). Location by growth stage (F= 14.36, df= 3, 217, P<0.01) and location by defoliation level (F= 9.27, df= 3, 217, P<0.01) were significant. A quadratic relationship between percent defoliation and grain yield was observed. As percent defoliation increased, rice yields decreased and the rate of decrease increased. At the Harrisburg location defoliation did not impact yield at the 2- to 3- leaf growth stage (F= 0.99, df= 1, 29, P= 0.32) (Figure 2.1). However, yield reductions were observed from defoliation at early tiller (F= 9.46, df= 1, 28, P<0.01), late tiller (F= 14.98, df= 1, 30, P<0.01), and BIE (F= 24.24, df= 1, 30, P <0.01) growth stages. At the Pine Tree location defoliation did not impact yield at the 2- to 3- leaf (F= 2.22, df= 1, 30, P= 0.15) or early tiller (F= 0.84, df= 1, 29.01, P= 0.36) growth stages (Figure 2.2). Yield reductions were observed from defoliation at the late tiller (F= 71.59, df= 1, 29.02, P<0.01) and BIE (F= 198.26, df= 1, 29.09, P <0.01) growth stages.

At the Harrisburg location, a positive relationship was present between defoliation level and DGR at all growth stages except for the 2- to 3- leaf and early tiller stages (2- to 3- leaf, F= 4.95; df= 1,30; P= 0.03; Early Tiller, F= 0.58; df= 1,30; P= 0.45; Late Tiller, F= 365.26; df= 1,30; P <0.01; BIE, F= 1085.75, df= 1, 30, P<0.01) (Figure 2.3). At the Pine Tree location a negative relationship between defoliation level and daily growth rate was present at the 2- to 3- leaf growth stage (F= 21.95, df= 1, 30, P<0.01) (Figure 2.4). At the early tiller (F= 97.85, df= 1, 29; P<0.01), late tiller (F= 225.41, df= 1, 30, P<0.01), and BIE (F= 147.17, df= 1, 30, P< 0.01) growth stages, defoliation level had a positive relationship with daily growth rate.

At the 2- to 3- leaf growth stage DGR had a positive relationship with yield at the Pine Tree location (F= 6.91, df= 1, 29.62, P=0.01) although no relationship was present at the Harrisburg location (F= 1.5, df= 1, 29.62, P=0.23) (Figure 2.5). The opposite was true at the early tiller growth stage where DGR had a positive relationship with yield at the Harrisburg location (F= 24.72, df=1, 24.77, P<0.01) but no relationship at Pine Tree (F=1.85, df= 1, 22.81, P=0.19) (Figure 2.6). At both the late tiller and BIE growth stages, for both locations, there was a negative relationship between DGR and yield (Late tiller: Harrisburg, F= 24.94, df= 1, 24.94, P= 0.03; Pine Tree, F= 28.23, df= 1, 29.99, P <0.01; BIE: Harrisburg, F= 60.04, df= 1, 26.43, P< 0.01; Pine Tree, F= 23.59, df= 1, 29.97, P< 0.01) (Figures 2.7 and 2.8).

Defoliation with Scissors

For whole plot defoliation treatments, no interactions were observed between location, timing, and defoliation (F= 0.4, df= 4, 4, P= 0.81). No differences were observed for main effects; location (F= 0.44, df= 2, P= 0.64), defoliation timing (F= 1.55, df= 2, P= 0.22), or defoliation level (F= 3.62, df=1, P= 0.06).

For clump defoliation treatments an interaction was observed between location and defoliation timing (F= 3.47, df= 2, P= 0.04). Timing was the only main effect that impacted yield (F= 3.46, df= 2, P= 0.03). Two- to three- leaf (±106.9, 2.79) and tiller growth stages (±102.3, 2.79) were not significantly different. However, the heading growth stage (±95.76, 3.01) yielded significantly lower than the 2- to 3- leaf and tiller growth stages.

Discussion

Previous research investigating the impact of defoliation on rice yields has had differing results among studies. The response of rice in the current trial was highly variable across locations and growth stages. In general, defoliation at any level during the 2- to 3- leaf stage did

not have an impact on rice grain yields in the current study. This is similar to what was observed previously by Taylor (1971) where 50% and 66% defoliation did not impact yields of rice. In contrast, yield losses were observed for 25% and 50% defoliation of rice seedlings in other research (Bowling 1978).

During the early tiller growth stage, grain yield losses were observed at only the Harrisburg location and not the Pine Tree location in the current study. However, defoliation at the late tiller growth stage reduced yields at both locations. In plots defoliated with scissors, no yield differences could be detected from clumped or whole plot defoliation at the 2-3 tiller growth stage. Bowling (1978) observed that 25 and 50% defoliation at the tiller growth stage reduced yield 5% and 12%, respectively. Oyediran and Heinrichs (2002) observed a 40% yield decrease was observed when rice was defoliated 100% 21 days after transplanting, which was likely similar to the early tiller growth stage used in this study. According to Oyediran and Heinrichs (2002), the yield decrease was due to a reduction in tillers and panicles present at harvest. Taylor (1971) found that 50% defoliation at the tillering growth stage actually increased rice yields.

During the BIE growth stage, grain yield losses were observed at both locations in the current study. Rice et al. (1982) found no yield reduction from 25% mechanical defoliation at 3-4 weeks prior to heading, which is similar to the late tiller and BIE defoliation timings in the weed eater studies. However, yield decreases were observed when defoliation was increased to 50 and 100% defoliation at the same growth stages. Taylor (1971) also found that defoliation after the tillering growth stage decreased yields. In addition, plots defoliated with scissors found no reduction in yields occurring during the heading growth stage.

There was the potential that the simulated defoliation methods used in these trials (weed eater versus scissors) may have altered the ability of rice plants to recover. The weed eater gives a torn and shredded appearance, while the scissors cut the plant foliage cleanly. As a result, the use of a weed eater may overestimate the impact of defoliation on rice yields and the use of scissors may underestimate the impact of defoliation. More research is needed to determine the impact of different methods of simulated insect defoliation on rice yields.

DGR was impacted by both the amount of defoliation that the plant incurred as well as the growth stage that the defoliation occurred. The impact of defoliation on DGR at the early growth stages was inconsistent between locations. Some of the factors that may have contributed to the differences include location, planting date, and conventional versus hybrid varieties. However, at the two later growth stages defoliation impacted DGR very similarly at both locations. DGR at late tiller and BIE growth stages increased in conjunction with defoliation in order to compensate for the loss of leaf area.

Between both Pine Tree and Harrisburg locations, yield either had a positive relationship or was not influence by DGR. This indicates that some defoliation at early growth stages can potentially increase a plants growth rate, causing the plant to overcompensate for the injury. At late tiller and BIE growth stages DGR had a negative relationship with yield. Likely, the plant had to increase the amount of photosynthates going into leaf growth, which in turn reduced the amount going into grain production, resulting in reduced yield.

Future research needs to be conducted to determine if different cultivars with different planting dates recover differently without economic damage occurring from defoliation and how the plant recovers in terms of daily growth rate. According to these studies, rice can withstand high percentages of defoliation early on in the growing season without observing an economic

loss. This means defoliation from the FAW may not be as important in earlier growth stages as it is later in the season. The plant seemed to recover fairly fast in terms of daily growth rate for both locations early in the season, while later growth stages did not put foliage back on as efficiently. Pesticide applications may not be as important in earlier growth stages such as the 2- to 3- leaf and early tiller growth stages, but may need to be warranted in later growth stages, especially at high defoliation percentages.

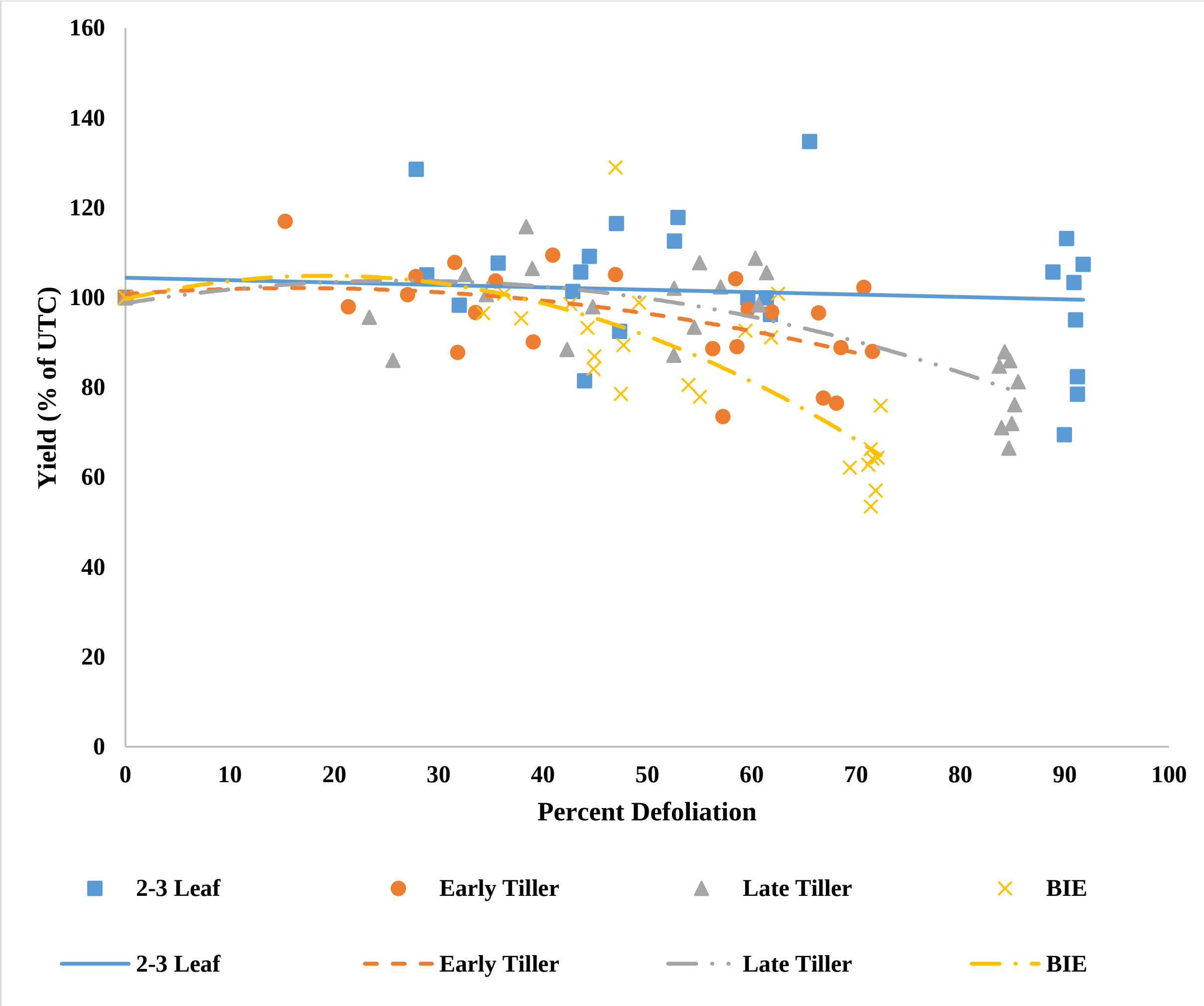

Figure 2.1. Relationship between simulated fall armyworm, *Spodoptera frugiperda*, defoliation on an early planted hybrid cultivar at different plant growth stages and grain yields of rice at the RiceTec location in Harrisburg, AR in 2018.

2- to 3- leaf- (F= 0.99; df= 1, 29; P= 0.32); $100.1 + 0.3612x - 0.00459x^2$

Early Tiller- (F= 9.46; df= 1, 28; P<0.01); $100.8 + 0.1651x - 0.005035x^2$

Late Tiller- (F= 14.98; df= 1, 30; P<0.01); $98.71 + 0.3793x - 0.007145x^2$

BIE- (F= 24.24; df= 1, 30; P<0.01); $99.67 + 0.5412x - 0.01412x^2$

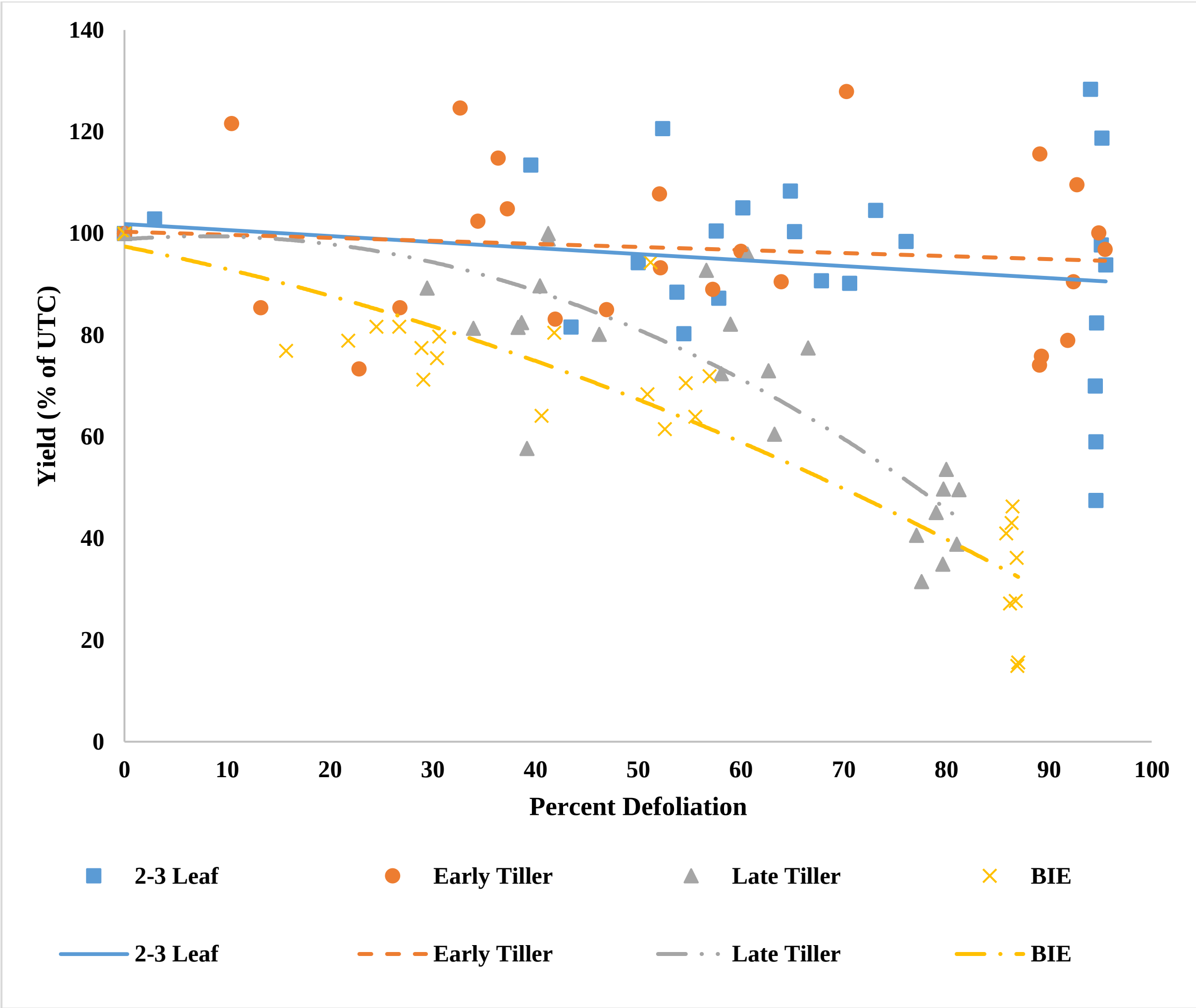

Figure 2.2. Relationship between simulated fall armyworm, *Spodoptera frugiperda*, defoliation on a late planted conventional cultivar at different plant growth stages and grain yields of rice at the Pine Tree location in Colt, AR in 2018.

2- to 3- leaf- (F= 2.22; df= 1, 30; P= 0.15); $100.1+ 0.09501x- 0.002356x^2$

Early Tiller- (F= 0.84; df= 1, 29.01; P= 0.36); $99.79- 0.005134x- 0.000593x^2$

Late Tiller- (F= 71.59; df= 1, 29.02; P= <0.01); $98.84+ 0.1579x- 0.01026x^2$

BIE- (F= 198.26; df= 1, 29.09; P= <0.01); $97.42- 0.4053x- 0.003928x^2$

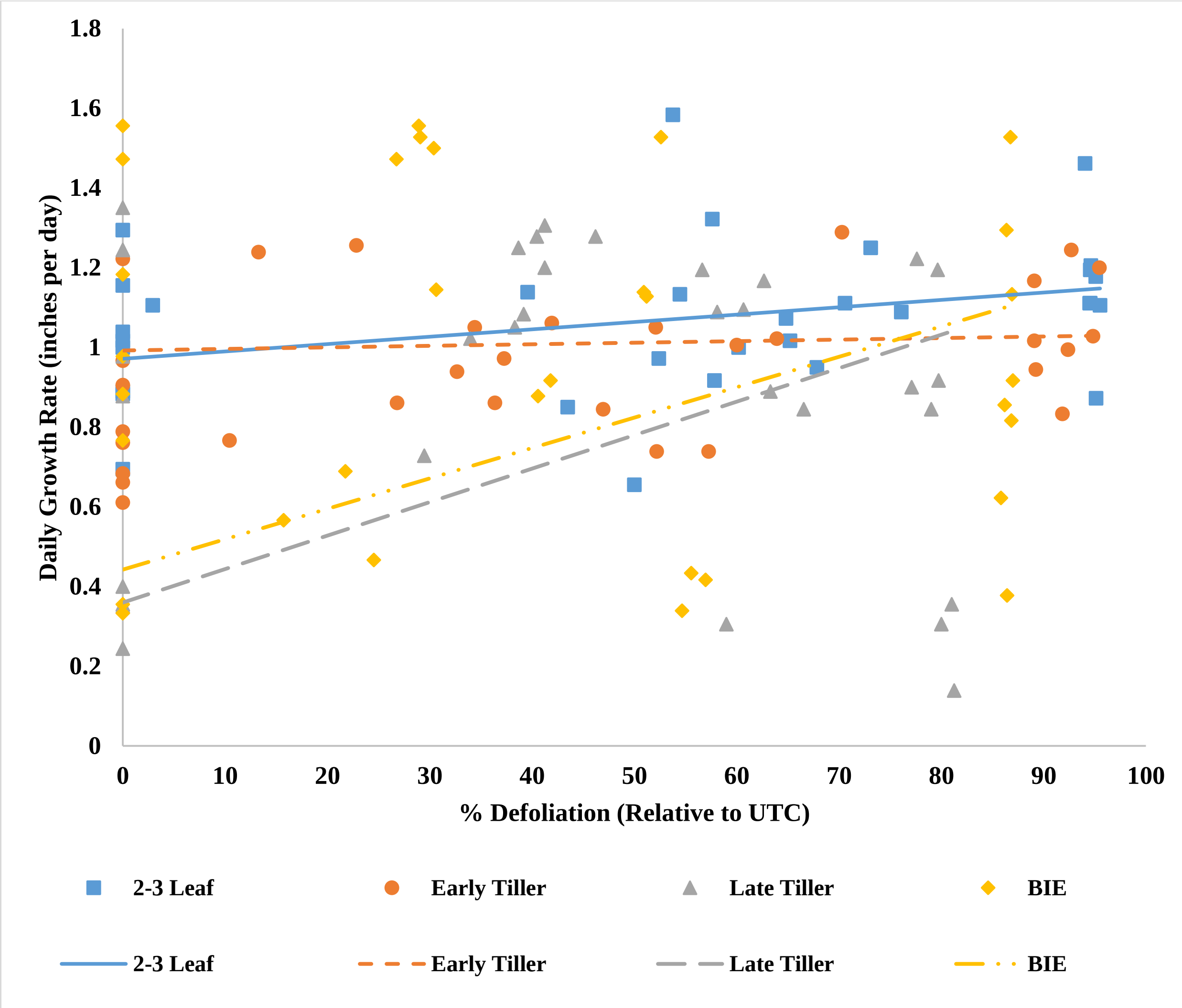

Figure 2.3. Relationship between simulated fall armyworm, *Spodoptera frugiperda*, defoliation on an early planted hybrid cultivar at different plant growth stages and daily growth rates (DGR) of rice over 18 days at the RiceTec location in Harrisburg, AR in 2018.

2- to 3- leaf- F= 4.95; df= 1,30; P= 0.03

Early Tiller- F= 0.58; df= 1,30; P= 0.45

Late Tiller- F= 365.26; df= 1,30; P <0.01

BIE- F= 1085.75; df= 1,30; P <0.01

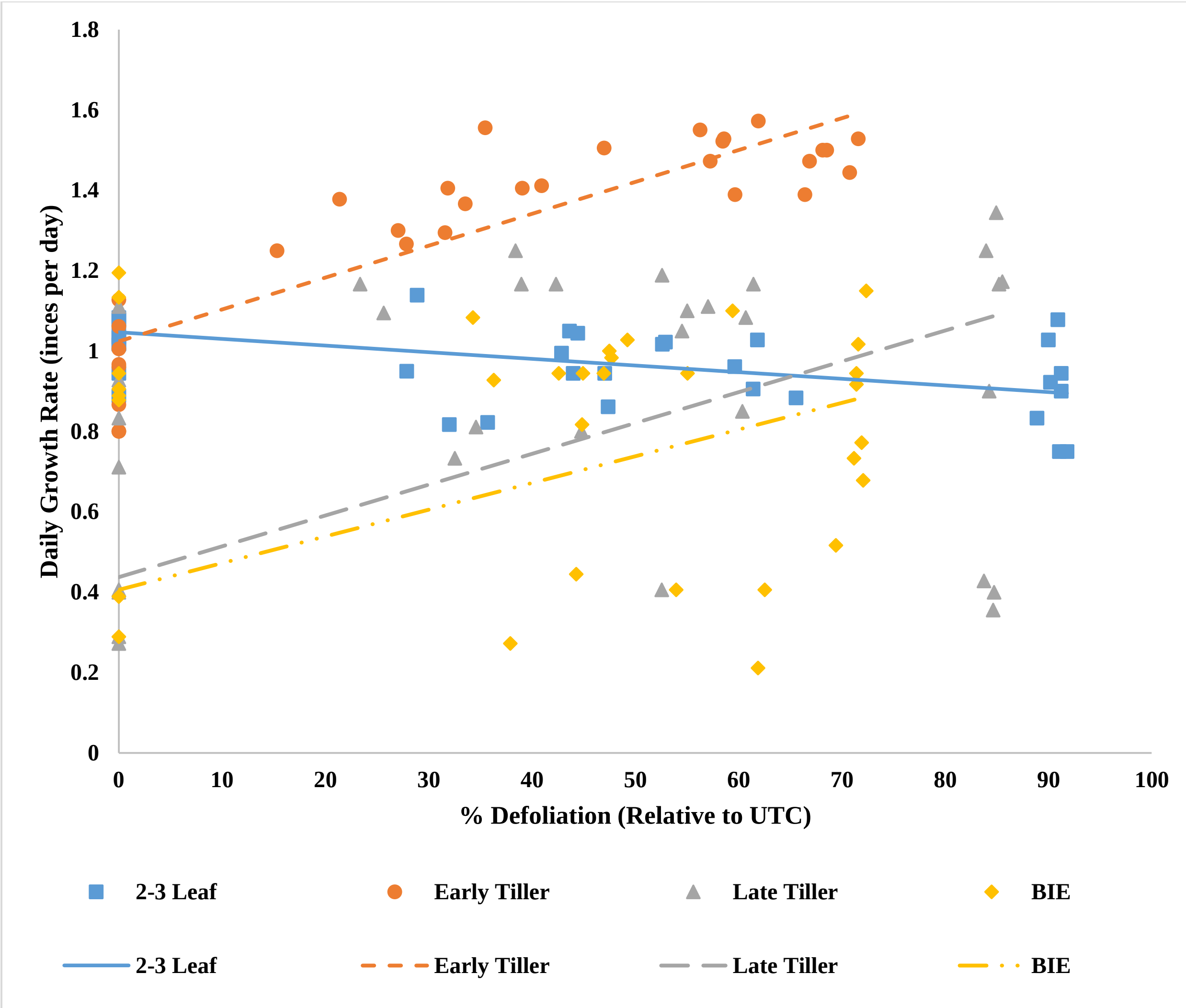

Figure 2.4. Relationship between simulated fall armyworm, *Spodoptera frugiperda*, defoliation on a late planted conventional cultivar at different plant growth stages and daily growth rates (DGR) of rice over 18 days at the Pine Tree location in Colt, AR in 2018.
2- to 3- leaf – F= 21.95, df= 1,30; P= <0.01
Early Tiller – F= 97.85; df= 1,29; P= <0.01
Late Tiller – F= 225.41 df= 1,30; P= <0.01
BIE- F= 147.17; df= 1,30; P=<0.01

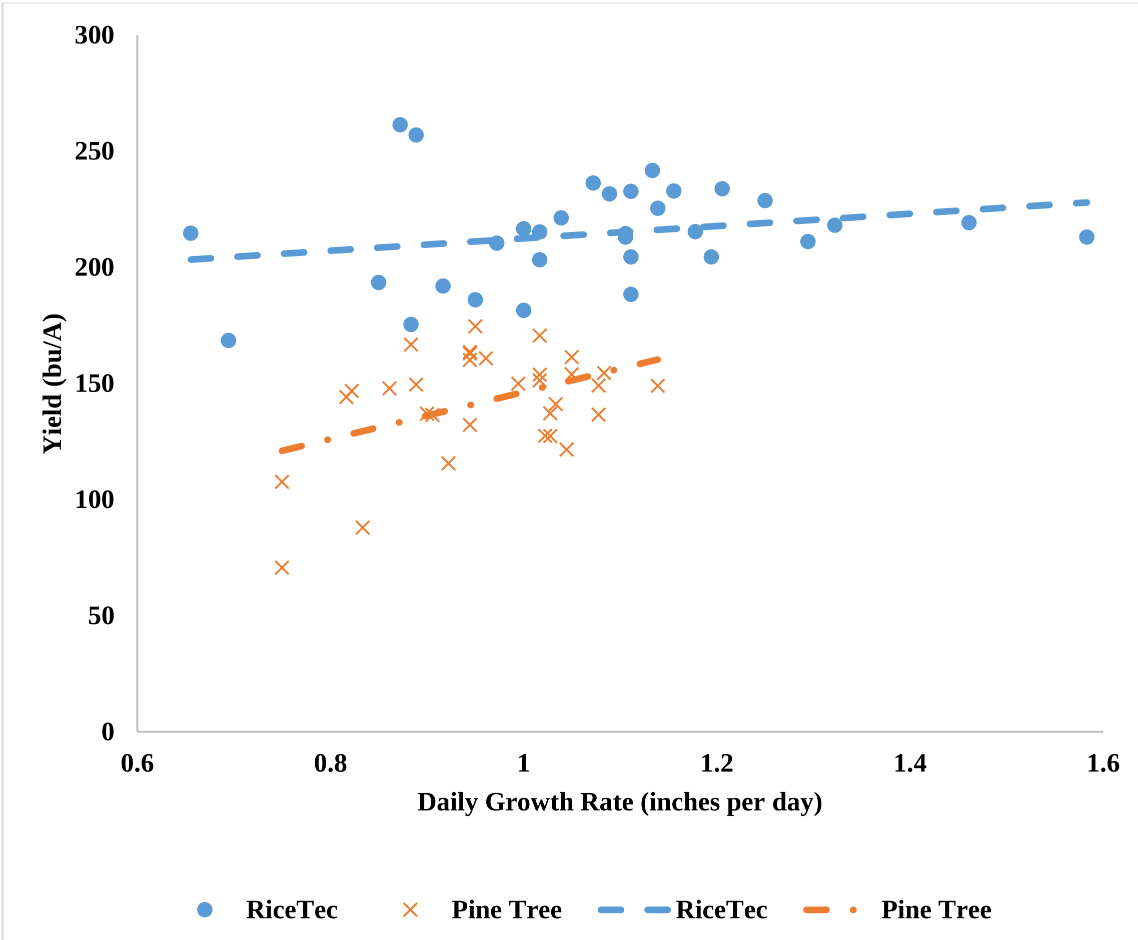

Figure 2.5. Daily growth rate and grain yield after defoliation at the 2- to 3- leaf growth stage for Pine Tree and Harrisburg locations.
Harrisburg – F= 1.5, df= 1, 25.37, P= 0.23
Pine Tree – F= 6.91, df= 1, 29.62, P= 0.01

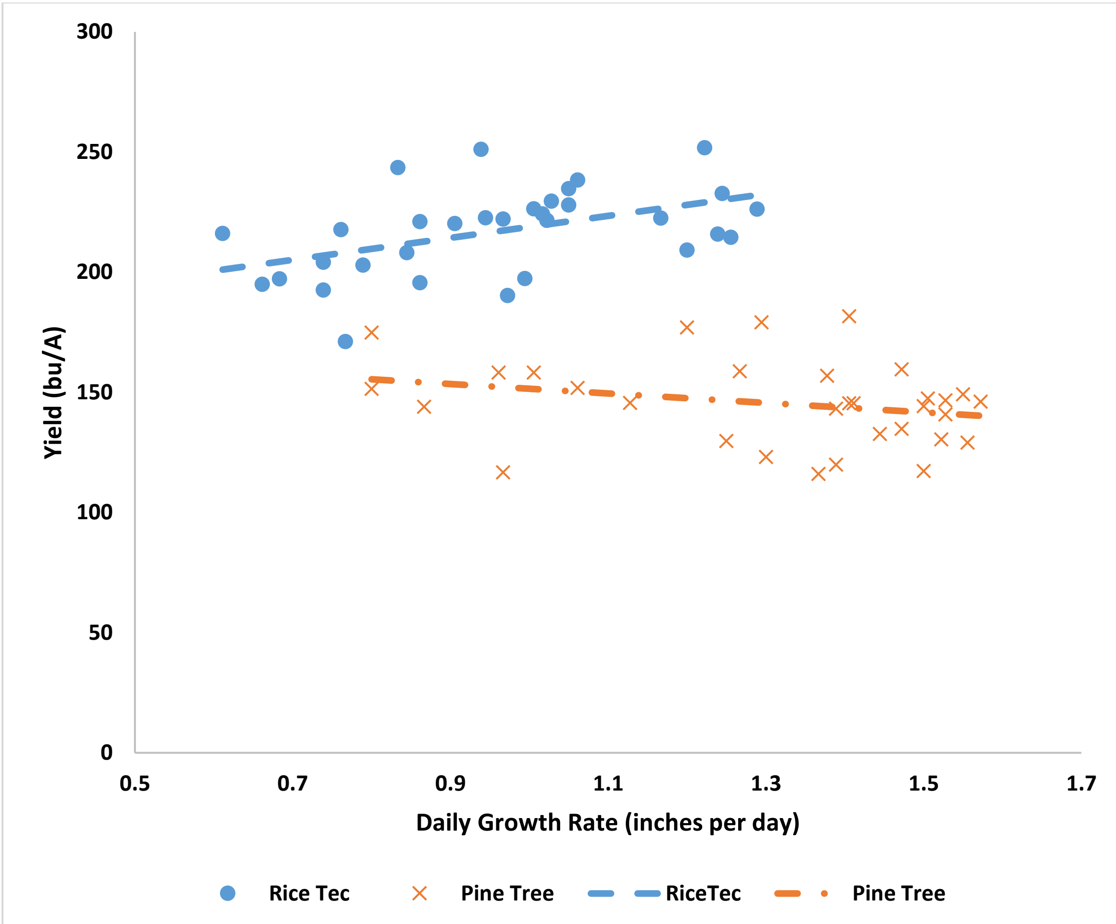

Figure 2.6. Daily growth rate and grain yield after defoliation at the early tiller growth stage for Pine Tree and Harrisburg locations.
Harrisburg – $F= 24.72$, $df= 1, 24.77$, $P< 0.01$
Pine Tree – $F= 1.85$, $df= 1, 22.81$, $P= 0.19$

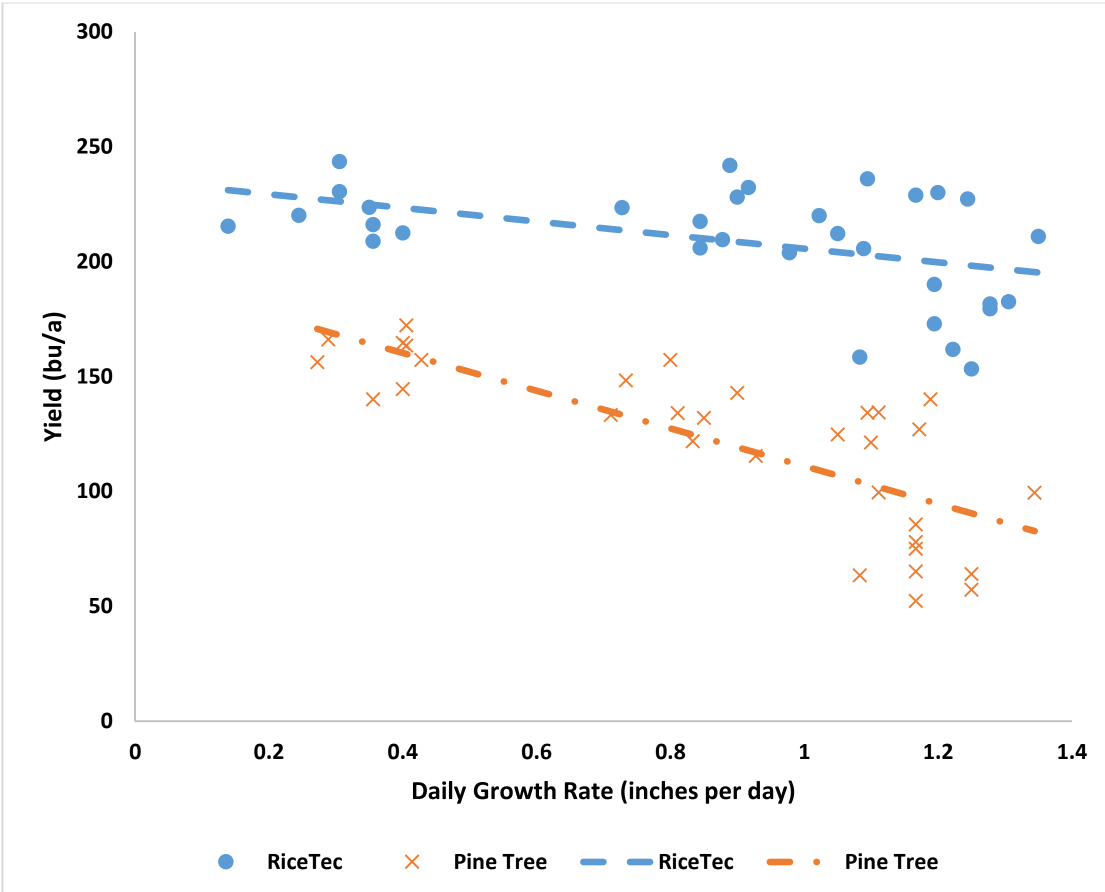

Figure 2.7. Daily growth rate and grain yield after defoliation at the late tiller growth stage for Pine Tree and Harrisburg locations.
Harrisburg – F= 24.94, df= 1, 24.94, P= 0.03
Pine Tree – F= 28.23, df= 1, 29.99, P <0.01

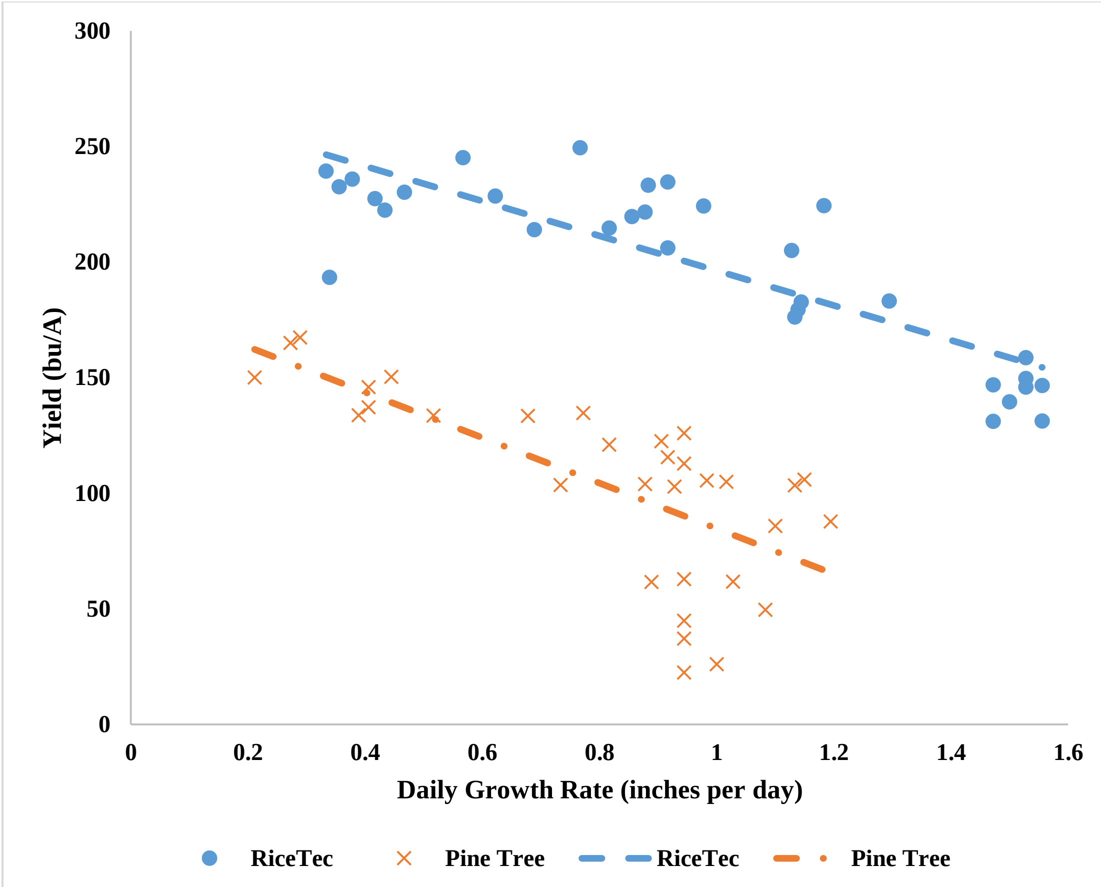

Figure 2.8. Daily growth rate and grain yield after defoliation at the BIE growth stage for Pine Tree and Harrisburg locations.
Harrisburg – $F= 60.04$, $df= 1, 26.43$, $P< 0.01$
Pine Tree – $F= 23.59$, $df= 1, 29.97$, $P< 0.01$

Chapter 3- Evaluating the Effects of Defoliation on Rice and Feeding Behavior of the Fall Armyworm

Abstract

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith), (FAW) has recently become a more common pest of rice in the Mid-South, with infestations being observed in all growth stages of rice. Limited information exists on the impact of defoliation by FAW in rice and the economic impact this defoliation can have on rice. In greenhouse studies, neonate FAW were infested on rice plants at the 2- to 3-leaf, 2^{nd} to 3^{rd} tiller, and heading growth stage. When larvae defoliated rice to 100% at the 2- to 3- leaf growth stage yield reduction was observed compared to the untreated check. No yield differences were observed among defoliation percentages at the 2nd- to 3rd tiller or heading growth stage. Mechanical defoliation was also conducted in the greenhouse, using the same growth stages and defoliation percentages. When rice was 100% mechanically defoliated at the 2- to 3- leaf and 2nd- to 3rd tiller growth stages, a yield reduction was observed compared to the untreated control. Sleeve cages containing either the rice panicle, flag leaf, or panicle and flag leaf were infested with one FAW to determine the potential damage to developing rice panicles. An increase percent of blank kernels and decrease in yield was observed when FAW only had rice panicles to feed on. These and future studies will be helpful in developing a defoliation action threshold for FAW in rice.

Introduction

Rice, O*ryza sativa* (L.), is produced across the world and occupies 9% of arable land. Over half of the world's population consumes rice daily (Heinrichs et al. 2017). In the US, Arkansas is the leading rice producing state with 484,004 hectares planted in 2017, and 1,091,841 hectares total in the US (NASS 2017 and USDA ERS 2017).

Fall armyworm, *Spodoptera frugiperda*, (FAW) (J.E. Smith) has the potential to be an important pest of rice (Pantoja et al. 1986). Luginbill (1928) first reported damage from FAW in

rice in 1845. Severe FAW damage was observed in North Carolina in 1899 (Chittenden 1900, Pantoja et al. 1986). FAW has been documented feeding on both the leaves of rice, as well as, rice panicles (Mitchell et al. 1991). Although much is known about FAW in other crops, including corn, grain sorghum, and soybeans, little research has been done to determine the impact of defoliation from this pest on rice.

Bowling (1978) observed yield reductions in rice when plants were defoliated 25 and 50% at the seedling and tillering growth stages. Pantoja et al. (1986) observed damage to the upper leaves of rice plants 24 hours after infestation with FAW, with whole plants eventually being consumed. Also, saliva from the FAW may play a role in decreasing the plants ability to recover after being fed upon (Wu and Baldwin 2010). Rice accumulates high levels of Jasmonate or defense-related secondary metabolites after herbivory occurs compared to plants that were mechanically defoliated (Fukmoto et al. 2013, Shinya et al. 2016).

The current recommendation for FAW control in rice in Arkansas is to treat when there are six or more armyworms per square foot. Later in the season, treatment is suggested when FAW are present and damaging the flag leaf (Lorenz et al. 2019). The ability of any crop to perform photosynthesis is reduced by defoliating pests, potentially reducing the crops yield potential (Buntin 1986). Applications of insecticides are commonly made for FAW in Arkansas when defoliation is observed in rice, but more information is needed on when economic yield loss occurs from defoliation. An action threshold based on percent defoliation would improve the management of FAW. The objectives of this study were to evaluate defoliation in rice at multiple growth stages, to conduct a choice assay test, and determine the ability of FAW to damage developing heads of rice.

Materials and Methods

Greenhouse Defoliation Studies

Studies were conducted in a greenhouse at the Lonoke Agriculture Research and Extension Center in Lonoke, Arkansas. The rice variety Diamond was planted in 6 inch pots at 10 seeds per pot and thinned to 5 plants after stand establishment. Two pots were used for each treatment within a replication. This study consisted of two factors, percent defoliation and defoliation timing in a randomized complete block design with 4 replications of all treatment combinations. Approximately 100 FAW neonates were placed on rice plants in each pot at the 2- to 3- leaf, 2nd- to 3rd tiller, and heading growth stages. FAW were allowed to feed until 25, 50, or 100% defoliation was reached. A visual representation was used to determine level of defoliation during feeding comparing the pots to the untreated check. Once the appropriate percent defoliation was met, FAW were picked off of the rice plants. Defoliation levels were based on the percent of foliage eaten across the entire plant for 2- to 3- leaf and tiller timings, whereas in the heading growth stage only the flag leaf was assessed.

A separate trial was conducted using mechanical defoliation to assess the impact of defoliation on rice. The methodology used for this trial was similar to the larval infested greenhouse trial except for how defoliation levels were reached. Two factors were used, percent defoliation and defoliation timing, and a randomized complete block design was utilized with 4 replications of all treatment combinations. Rice plants were manually defoliated at the 2- to 3- leaf, 2nd- to 3rd tiller, and heading growth stages. Defoliation percentages were 25, 50, and 100% at each growth stage, with the percent defoliation equaling the percent of the plant defoliated using scissors. At the 2- to 3- leaf and tiller growth stages the whole plant was defoliated, whereas at the heading growth stage only the flag leaf was defoliated.

Once rice plants reached the tiller growth stage in both the larval infestations and manual defoliation trials, pots were placed into a 12.7 cm flood and remained there until harvest. Rice panicles were removed from the plant and weighed to determine yield. Data were analyzed with analysis of variance using a mixed model in JMP (JMP version 14.2.0), with block considered as a random variable. A Tukey's HSD post hoc analysis with an alpha level of 0.05 was utilized to separate means of significant analyses.

Fall armyworm Feeding Choice Assay

Trials were conducted in 2018 at the Rice Research and Extension Center near Stuttgart, Arkansas to determine if FAW panicle feeding affected the yield and number of blank kernels of rice panicles (Figure 3.1). The rice variety Diamond was drill seeded at 34 kg per hectare on April 20th, 2018. Two factors were considered for this study, rice plant part at 3 levels and infestation of FAW, and two site years were performed with the same treatment structure. White insect rearing sleeve cages (20 x 40cm; BioQuip Products, Rancho Dominguez, CA, 90220, USA) were placed on rice plants when panicles were fully emerged at the initiation of anthesis. Sleeve cages were placed around rice panicles, the flag leaf, or the panicle and flag leaf. A single second-third instar FAW was placed in cages that were considered infested. Larvae were allowed to feed for 6 days and mortality was checked every 24 hours and replaced if mortality occurred. For the cages that contained both a flag leaf and panicle, the location of the larva was recorded every 24 hours at 8 am. A total of 10 replications were performed for each of the infested treatments and 5 for each uninfested treatment. Uninfested treatments were caged with no infested larvae.

Plots were maintained using recommended agronomic practices until harvest (Hardke 2019). Cages remained on the plant until harvest then the entire panicle was removed and the

kernels were counted. Filled kernels and blanked kernels were separated and counted. The number of unfilled seed (blank kernels) from each head was then used as a metric to determine the level at which the larva was able to successfully feed on and damage the rice head. Data were analyzed with ANOVA using JMP (JMP, v. 14.2.0. SAS Institute Inc., Cary, NC,) and LSD post hoc analysis with an alpha level of 0.05. Block was considered a random variable. Data were also analyzed with regression analysis in PROC REG, SAS v. 9.4 (SAS Institute, Inc., Cary, NC), with yield (grams) as the response variable and percent blanked kernels as the explanatory variable.

Results

Greenhouse Studies

Larval Infestations

Larval infestations were not very successful with only 25% defoliation being achieved at the 2nd- to 3rd- tiller growth stage and 3% at heading growth stages. A significant interaction between growth stage and percent defoliation was observed when considering yield percent of the UTC for larval infestations (F = 2.82; df= 6, 37; P = 0.02). When considering the 2- to 3- leaf growth stage, a significant effect was observed for levels of defoliation (F = 11.2; df= 3, 9; P < 0.01), and when defoliation was 100%, only 61% of the UTC yield was observed (Table 3.1). No significant effect of defoliation was observed at the 2nd- to 3rd- tiller (F = 1.5; df= 3, 9; P = 0.27) for larval infestations.

Mechanical Defoliation

A significant interaction was observed between growth stage and percent defoliation (F = 6.07; df = 6, 31; P < 0.01). The defoliation main effect was observed to be significant for the 2-

to 3- leaf (F = 7.09; df= 3, 7; P = 0.02) and 2nd- to 3rd- tiller (F = 107.3; df= 3, 9; P < 0.01), but no significant effect was observed at the heading growth stage (F = 2.9; df= 3, 9; P = 0.09) (Table 3.1). At the 2- to 3- leaf and 2nd- to 3rd- tiller growth stages, yield was observed to be 54% and 40% of the UTC at 100% defoliation respectively. The lowest observed yield at the heading growth stage was 82% of the UTC.

Choice Assay Test

A significant interaction between infestation and plant part was observed (F = 14.3; df = 3, 85; P < 0.05) when considering the percent of blanked seed. When FAW larvae were only infested on flag leafs, no significant decrease in yield (F = 0.16; df = 1, 28; P = 0.69) or percent blanks (F = 0.001; df = 1, 28; P = 0.97) was observed (Table 3.2). When larvae were infested on rice panicles alone, a significant decrease in yield (F= 4.7; df= 1, 28; P= 0.04) and increase in the percent of blank kernels (F = 9.6; df = 1, 28; P < 0.01) was observed. No significant difference in yield was observed for cages containing both the head and flag leaf (F= 0.59; df = 1, 27; P = 0.45), but cages with FAW exhibited a larger percent of blank kernels compared to uninfested cages. (F= 6.0; df = 1, 27; P = 0.02). FAW larvae were observed to be feeding on the flag leaf 29% of the time, feeding on the rice head 33% of the time, and were observed on the mesh sleeve cage 38% of the time. When data was pooled across all three plant part treatments, a significant linear relationship was observed between the yield from each panicle and the proportion of blanks (R^2 = 0.63), suggesting percent blanks explained a large percent of variation in yield (Y = 6.0 – 6.1x; F = 144; df = 1, 87; P < 0.01; Root MSE = 0.9) (Figure 3.2).

Discussion

In our studies, mechanical defoliation and larval infestation resulted in similar levels of yield loss at the 2- to 3- leaf growth stage for similar levels of defoliation. Although they were

separate studies and cannot be directly compared, the studies using mechanical defoliation and larval infestation produced similar results for yield loss. Little yield loss was observed due to defoliation at 25% or 50%, but large amounts of yield loss at around 40% of the UTC was observed when plants were 100% defoliated. A large amount of yield loss was also observed at the 2nd- to 3rd- tiller growth stage, with manual defoliation resulting in up to 60% yield loss. Lv et al. (2010) observed that mechanical defoliation did not impact rice yield at the 4-leaf growth stage, and defoliation resulted in increased tillers. Lv et al. (2010) suggested that rice plants may fully recover from low to moderate levels of defoliation by producing additional tillers. Although we did not record changes in tiller production, we did observe significant levels of yield reduction at growth stages before and after what was observed by Lv et al. (2010). Once rice plants were in the heading growth stage, we observed little effect on panicle yield from manual defoliation of flag leaves, even when flag leaves were completely removed.

Rice was manually defoliated and subjected to larval feeding to achieve defoliation rates that are commonly observed in Arkansas. Defoliation levels from FAW larvae were only achieved in the earliest growth stage tested, and larval infestations only achieved 25% in the 2nd- to 3rd- tiller growth stage and 3% in the heading growth stage. Rice et al. (1982) concluded that there were differences in yield comparing mechanical defoliation with larval feeding. Larval defoliation by armyworm of 25-30% resulted in as much as 50.2% yield reductions. Yield reduction was not observed with mechanical defoliation at 25%, but was significant at 50 and 100% on 83 day old plants (Rice et al. 1982). When mechanically defoliated, all leaves are cut to an appropriate defoliation level immediately, while larval feeding is gradual over time and may not encompass every leaf (Rice et al. 1982). In our study it took up to 12 days to achieve 100% defoliation with larval infestations, whereas manual defoliation was completed almost

instantaneously and recovery started immediately. Additionally, Wu and Baldwin (2010) found that rice plants can distinguish herbivore feeding from mechanical defoliation by recognizing elicitors such as the fatty acid-amino acid conjugates from insect saliva. If a plants response to this feeding is only induced when herbivory occurs, yield loss from mechanical defoliation could differ from that caused by FAW feeding. However, real-world defoliation levels are difficult to achieve with artificially infested larvae, and we were not able to achieve defoliation levels above 30% at 2^{nd}- to 3^{rd}- tiller and 3% at heading. Baldwin (1990) observed similar limitations and summarized the advantages of mechanical defoliation over larval infestations from previous studies, which included time savings and minimized pathogen spread from insect feeding. Where we were able to achieve defoliation, we did not observe differences in yield loss from the two techniques. However, our study did not consist of a direct comparison of FAW feeding and mechanical defoliation, and therefore is limited in this scope.

Although no reduction in yield loss was observed when flag leaves were completely removed at 100% defoliation, feeding on developing rice panicles has been observed (Figure 3.1) (Mitchell et al. 1991). Feeding on the rice head during the anthesis stage could lead to completely blanked kernels, and clipping of entire seeds could occur during the grain fill growth stages. We observed an increase in blank kernels when FAW larvae were infested on rice panicles alone and when allowed to choose between the flag lead and the rice panicle. This only translated to yield loss when the panicle alone was available for feeding. We also did not observe any differences in the location of the larvae during morning feeding hours with the option of the flag leaf or rice panicle. This suggests that leaf feeding at this growth stage may not be especially preferred. Campos et al. (2012) observed the most attractive plant parts of newly hatched FAW were the leaves and bracts of cotton, followed by the carpel wall of bolls and

squares, suggesting that foliage was preferred over reproductive tissue. Pannuti et al. (2015) observed that the diet of first instar larvae could influence feeding choice in later instars. Considering that we used 3rd instar larvae reared on corn grit, our data could be influenced by a diet that previously included reproductive material.

Results from this study are important in understanding the role that FAW presence and feeding plays in rice production in Arkansas. Manual defoliation of rice under highly controlled conditions led to large levels of yield reduction in early vegetative growth stages, and appears to be very similar to what is caused by larval infestations. However, more work is needed to understand the impact of FAW to later vegetative growth stages. Additionally, feeding during reproductive growth stages could cause damage beyond the defoliation that was observed, which was a key point in old thresholds and scouting. Although defoliation of the flag leaf may be indicative of FAW presence, larvae are likely also feeding on rice panicles. This suggests that thresholds during the rice heading and grain fill growth stages should not rely on defoliation but instead on the number of larvae present. These data along with other studies will lead to more effective treatment decisions made for FAW in rice.

Table 3.1. Rice yield relative to the untreated treatment for greenhouse studies with larval infestations of FAW and mechanical defoliation at three different growth stages of rice infested in 2018.

		Larval Infestations	Mechanical Defoliation
Growth Stage	**Defoliation Level**	**% of UTC (SEM*)**	**% of UTC (SEM*)**
2-3 Leaf	0%	100 (0) a	100 (0) a
	25%	92 (4.6) a	90 (7.2) ab
	50%	99 (5.0) a	94 (9.5) a
	100%	61 (7.6) b	54 (6.4) b
		***P*<0.01**	***P*=0.02**
2nd-3rd Tiller	0%	100 (0) a	100 (0) a
	25%	93 (5.8) a	93 (3.8) a
	50%	.	69 (4.6) b
	100%	.	40 (1.6) c
		***P*=0.27**	***P*<0.01**
Heading	0%	.	100 (0) a
	25%	.	100 (5.8) a
	50%	.	82 (5.3) a
	100%	.	93 (5.5) a
			***P*=0.09**

Yields followed by a different letter are significantly different according to Fisher's LSD post hoc analysis at α=0.05.
*Standard error of the mean.
Target defoliation levels of 50 and 100% for the 2nd- to 3rd- tiller and 25, 50, and 100% at the heading growth stages were not achieved.

Table 3.2. Results from a feeding choice bioassay at Stuttgart, AR in 2018 with yield weight (grams) and percent blanked kernels as a response to rice plant parts infested or non-infested with a single third-instar FAW larvae.

Plant Part		**Grams (SEM*)**	**Percent Blanks (SEM*)**
Flag Leaf	Infested	4.7 (0.2) a	16 (1.0) a
	Non-Infested	4.9 (0.3) a	16 (1.7) a
Head	Infested	3.2 (0.4) a	47 (6.3) b
	Non-Infested	4.6 (0.5) b	19 (1.5) a
Head+Flag Leaf	Infested	4.8 (0.3) a	25 (2.5) b
	Non-Infested	5.1 (0.4) a	16 (1.4) a

*Weight (grams) and Percent Blanks within a single plant part followed by a different letter are significantly different according to Fisher's Protected LSD post hoc analysis at α=0.05.
*Standard error of the mean.

Figure 3.1 Late instar fall armyworm larvae observed to be feeding on the flowers of a recently emerged rice panicle. Larvae were observed to feed in this way on many occasions, and could be observed to move to new kernels immediately once the exposed flower part was completely consumed. A single larvae was observed to ingest flower parts of over 10 flowers in only a few minutes.

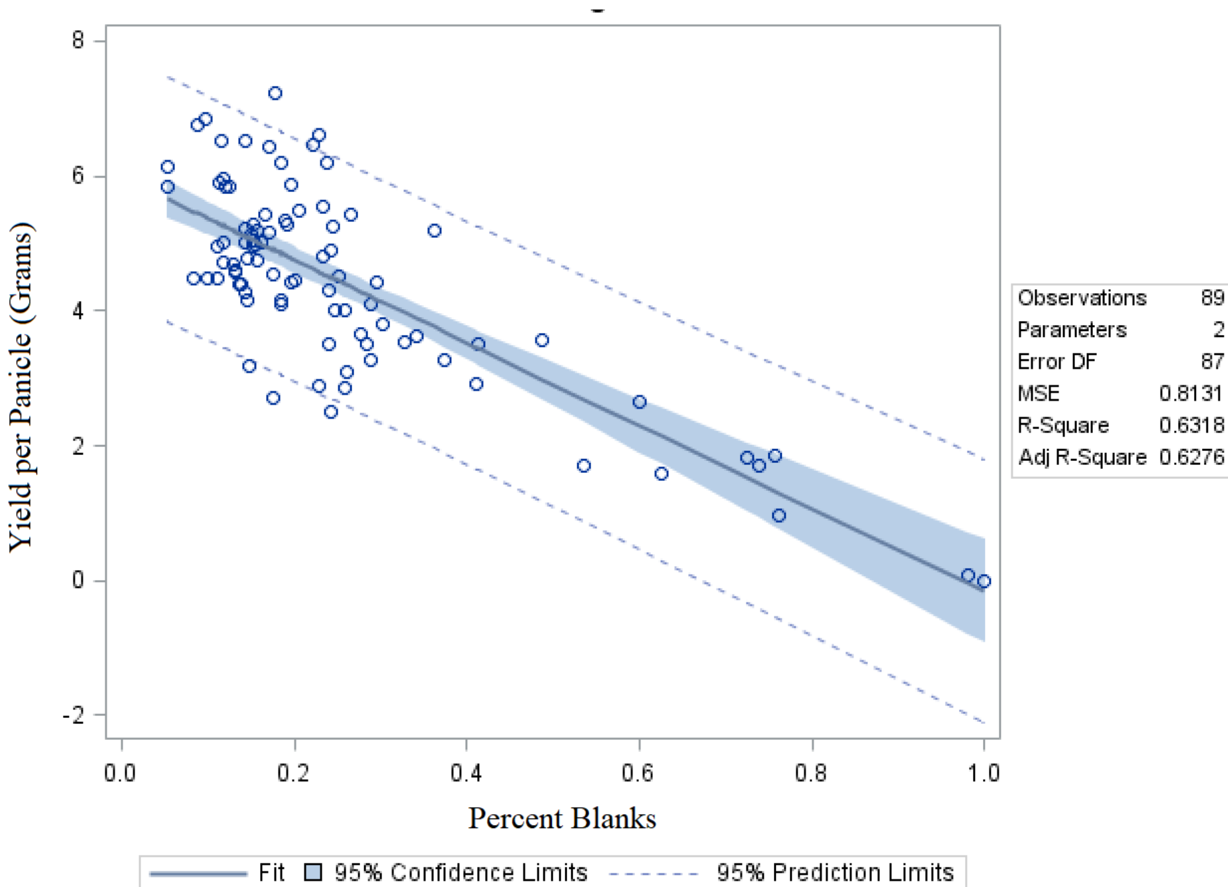

Figure 3.2. Correlation of percent blank kernels and yield in grams for each panicle in the choice assay from the summer of 2018 at Stuttgart, AR, with data from all plant parts assayed.

Chapter 4. Examining Different Insecticide Seed Treatments for Controlling Fall Armyworm, *Spodoptera frugiperda*, in Rice

Abstract

In Arkansas, insecticide seed treatments are used in rice to control grape colaspis and rice water weevil. Control of caterpillar pests has also been documented with some of these seed treatments, but residual control of these seed treatments for caterpillar pests have not been tested in rice. Rice was treated with four different insecticide seed treatments (chlorantraniliprole, clothianidin, thiamethoxam, and cyantraniliprole) plus a fungicide only to determine efficacy and residual control of fall armyworm (FAW) throughout the growing season. Chlorantraniliprole and cyantraniliprole reduced FAW populations and were found to be the most effective seed treatments 3, 5, and 7 days after infestation (DAI) at the 2- to 3- leaf stage and 5 and 7 DAI at the 2^{nd}-3^{rd} tiller growth stage. In the heading growth stage, only chlorantraniliprole provided control of FAW 7 DAI. Both chlorantraniliprole and cyantraniliprole greatly reduced percent defoliation compared to all other seed treatments.

Introduction

Treating seed with insecticide seed treatments has become the standard for controlling early season insect pests in agriculture (Hodgson et al. 2012, Nuyttens et al. 2013). Neonicotinoids are the most widely adopted class of insecticides used as insecticide seed treatments; however, diamide insecticides have been documented to provide exceptional control against lepidopteran and some coleopteran pests (Sparks 2013, Douglas and Tooker 2015, Roditakis et al. 2015). Diamide insecticides affect insect ryanodine receptors and cause uncoordinated muscle contraction and prolonged calcium channel opening, which leads to death (Ebbinghaus-Kintscher et al. 2006, Teixeira and Andaloro 2013).

The fall armyworm, *Spodoptera frugiperda*, (J.E. Smith) (FAW), is a polyphagous feeder and is considered a serious pest of maize, pasture grasses and several other crops, but prefers

feeding on grasses (Heinrichs et al. 2017). FAW was first recorded as a pest of rice, *Oryza sativa* (L.), in 1845 in Florida (Luginbill 1928). Recently FAW has been observed more frequently in Arkansas rice production throughout the whole growing season, but is only considered an occasional pest of rice (Heinrichs et al. 2017). Large FAW infestations generally occur along levees, field boarders, and in parts of the field where larvae can escape the rice flood, and can consume high amounts of plant tissue (Heinrichs et al. 2017). Luginbill (1928), documented that fourth to sixth instar larvae can destroy rice plants by completely defoliating plants.

Insecticide seed treatments that have long residual activity could potentially reduce insecticide applications (Hardke et al. 2011). Studies conducted by Thrash et al. (2013 and Pablo et al. (2018) in soybeans concluded that chlorantraniliprole provided control of FAW through much of the growing season. According to Pablo et al. (2018) FAW leaf consumption in soybeans was reduced by thiamethoxam and chlorantraniliprole seed treatments. Thrash et al. (2013) also observed mortality of FAW from cyantraniliprole in V3 soybeans. In corn, foliar applications of chlorantraniliprole and cyantranilprole reduced FAW survivorship up to 28 days after treatment (Hardke et al. 2011).

Two neonicotinoid (clothianidin and thiamethoxam) and two anthranilic diamide (chlorantraniliprole and cyantraniliprole) seed treatments were compared to fungicide only treated seed to determine efficacy and residual control of FAW in rice. The objective of this study was to examine the use of insecticide seed treatments for control of the FAW in rice and to determine how long these seed treatments will provide control of FAW.

Materials and Methods

A greenhouse study was conducted to determine the efficacy and residual control of insecticide seed treatments for FAW at the Lonoke Agriculture Research and Extension Center

in Lonoke, Arkansas. Rice (Cultivar: Diamond) was treated with 4 different insecticide seed treatments: chlorantraniliprole (0.14 kg/cwt), clothianidin (0.05 kg/cwt), thiamethoxam (0.10 kg/cwt), and cyantraniliprole (0.11 kg/cwt). Rice seed also had a fungicide package (mefenoxam [0.01 kg/cwt], fludioxonil [0.001 kg/cwt], azoxystrobin [0.03 kg/cwt], sedaxane [0.0009 kg/cwt]) for all insecticide seed treatments and the untreated control. Ten seeds were planted in 6-inch pots containing field soil (80%) and top soil (20%). After stand establishment, pots were thinned to 3 plants per pot. At the 2- to 3- leaf, 2^{nd}-3^{rd} tiller, and heading growth stages, 15 second-instar FAW were placed on the soil of each pot. Three replications were completed for each seed treatment and untreated check. An 18x16 in mesh screen was used to make cages to fit around each pot. Staples were used to seal the cage shut on the side, with the top being rolled down and closed with rubber bands at each end. A wooden dowel was used to keep the cage from falling over. Two rubber bands were used at the bottom to ensure the cage was flush with the pot. Cages were left closed after infestation until all larvae had died or pupated. Mortality counts were taken daily until all larvae had died or pupated. A visual percent defoliation rating was taken 7 days after infestation.

Once the rice reached the 2^{nd}- to 3^{rd}- tiller growth stage, pots were placed in tubs and flooded. Pots were taken out of the flood once it reached the appropriate growth stage for infestation. Data were analyzed with an ANOVA in PROC GLIMMIX (SAS v. 9.4, SAS Institute, Cary, NC) and LSD post hoc analysis with an alpha level of 0.05. Within the analysis, block was considered a random variable.

Results

A three-way interaction was observed among days after infestation (DAI), growth stage, and seed treatment (F = 2.5; df = 8, 148; *P* = 0.01) for percent mortality of FAW. Growth stage

was removed from the model and data was reanalyzed by growth stage. An interaction between DAI and seed treatment was observed at the 2- to 3- leaf (F =30.1; df = 4, 8; $P < 0.01$), 2^{nd}- to 3^{rd}- tiller (F = 20.3; df = 4, 8; $P < 0.01$), and heading (F = 12.5; df = 4, 8; $P < 0.01$) growth stages for percent mortality of FAW. Days after infestation (DAI) was removed from the model and data was reanalyzed by growth stage and DAI. An interaction was observed between growth stage and seed treatment (F = 31.1; df = 8, 28; $P < 0.01$) for percent defoliation. Growth stage was removed from the model and data was reanalyzed by growth stage.

2- to 3- Leaf

Clothianidin had a higher percent mortality (F = 5.1; df = 4,8; $P = 0.02$) than chlorantraniliprole, cyantraniliprole, and thiamethoxam at 1 DAI, but was not different than the fungicide only. At 3 DAI (F = 7.9; df = 4, 8; $P < 0.01$), 5 DAI (F = 25.8; df = 4,8; $P < 0.01$), and 7 DAI (F = 24.4; df = 4,8; $P < 0.01$), chlorantraniliprole and cyantraniliprole both had higher percent mortality than all other seed treatments, but were not different from one another (Table 4.1). Chlorantraniliprole and cyantraniliprole had less defoliation (F = 221.8; df = 4,8; $P < 0.01$) than all other seed treatments, and thiamethoxam had less defoliation than the fungicide only and clothianidin (Table 4.2).

2^{nd}- to 3^{rd}-Tiller

Chlorantraniliprole, cyantraniliprole, and thiamethoxam had higher percent mortality (F = 4.5; df = 4, 8; $P = 0.02$) than clothianidin or the fungicide only at 1 DAI. No differences among seed treatments (F = 1.2; df = 4, 8; $P = 0.36$) were observed at 3 DAI. At 5 DAI (F = 28.0; df = 4, 8; $P < 0.01$) and 7 DAI (F = 32.2; df = 4, 10; $P < 0.01$) chlorantraniliprole and cyantraniliprole both had higher percent mortality than all other treatments, but were not different from one another (Table 4.1). Chlorantraniliprole and cyantraniliprole had less defoliation (F = 59.4; df =

4, 8; $P < 0.01$) than all other seed treatments, and thiamethoxam had less defoliation than the fungicide only and clothianidin (Table 4.2).

Heading

No differences among seed treatments were observed at 1 DAI (F = 0.00; df = 4, 8; P = 1.0) and 3 DAI (F = 0.73; df = 4, 8; P = 0.6) for percent mortality of FAW. At 5 DAI (F = 14.5; df = 4, 8; $P < 0.01$) and 7 DAI (F = 9.3; df = 4, 10; $P < 0.01$). Chlorantraniliprole had higher percent mortalitity than all other seed treatments (Table 4.1). Chlorantraniliprole and cyantraniliprole had less defoliation (F = 7.1; df = 4, 8; $P < 0.01$) than all other seed treatments (Table 4.2).

Discussion

Minimal mortality was observed at all growth stages, and for all seed treatments 1 day after infestation (DAI). Similar results were also observed for 3 DAI for the 2nd- to 3rd- tiller and heading growth stages. At 5 and 7 DAI increased mortality was observed for all seed treatments. In general, mortality for all seed treatments increased as DAI increased. Only chlorantraniliprole and cyantraniliprole had 80% or higher mortality of FAW at any growth stage. At 3 DAI or after clothianidin was never different than the fungicide only. Thiamethoxam was only different from the fungicide only at the 2nd- to 3rd- tiller 5 DAI. At all growth stages, chlorantraniliprole and cyantraniliprole greatly reduced percent defoliation compared to the other seed treatments. Hardke et al. (2011) observed high mortality rates of FAW from 3 DAI to 28 DAI for chlorantraniliprole and cyantraniliprole in whorl stage corn. Thrash et al. (2013), observed reductions in FAW survival on V3 soybeans 3 DAI for chlorantraniliprole, with cyantraniliprole significantly reducing populations 4 DAI. Pablo et al. (2018) observed 100% mortality of FAW after 24 hours of being infested on soybeans with a chlorantraniliprole seed treatment.

Anthranilic diamide seed treatments in rice have the ability to provide long residual control of FAW compared to commonly used neonicotinoid seed treatments. In our study chlorantraniliprole and cyantraniliprole were the only two seed treatments to provide adequate control of FAW at all growth stages. At the 2^{nd}- to 3^{rd}- tiller growth stage, chlorantraniliprole and cyantraniliprole were still providing sufficient control of FAW, which was 49-56 days after planting. At the heading growth stage, chlorantraniliprole still provided 80% control of FAW, which was 73-80 days after planting. The data shows that at the 2- to 3- leaf and 2^{nd}- to 3^{rd}- tiller growth stages, cyantraniliprole and chlorantraniliprole have a residual activity to effectively reduce FAW survival. These data also suggest chlorantraniliprole has enough residual activity to reduce populations at the heading growth stage. Cyantraniliprole's residual activity decreases faster than chlorantraniliprole, and no longer provides control of FAW at the heading growth stage.

Table 4.1. Fall armyworm, *Spodoptera frugiperda*, mortality 1, 3, 5, and 7 days after being infested (DAI) on rice with two anthranilic diamide seed treatments and two neonicitinoid seed treatments test at three different growth stages in a greenhouse trial performed in 2018.

Growth Stage (DAP*)	Seed Treatment	Percent Mortality 1 DAI[ƚ]	3 DAI	5 DAI	7 DAI
2-3 Leaf (13-20)	UTC	4 ab	9 b	20 b	27 b
	chlorantraniliprole	0 b	24 a	67 a	87 a
	cyantraniliprole	2 b	27 a	62 a	80 a
	clothianidin	9 a	9 b	16 b	36 b
	thiamethoxam	0 b	11 b	24 b	42 b
		***P*=0.02**	***P*<0.01**	***P*<0.01**	***P*<0.01**
2nd-3rd Tiller (49-56)	UTC	0 b	7	11 c	24 c
	chlorantraniliprole	4 a	7	53 a	84 a
	cyantraniliprole	7 a	13	44 a	91 a
	clothianidin	0 b	7	16 c	27 c
	thiamethoxam	4 a	9	27 b	49 b
		***P*=0.02**	***P*=0.36**	***P*<0.01**	***P*<0.01**
Heading (73-80)	UTC	2	8	16 c	36 b
	chlorantraniliprole	2	13	49 a	80 a
	cyantraniliprole	2	13	29 b	48 b
	clothianidin	2	9	18 bc	33 b
	thiamethoxam	2	9	20 bc	31 b
		***P*=1.0**	***P*=0.60**	***P*<0.01**	***P*<0.01**

Mortality counts within a single growth stage and DAI combination followed by a different letter are significantly different according to Fisher's LSD post hoc analysis at α=0.05.
*DAP=Days after Planting
ƚDAI= Days after Infestation

Table 4.2. Percent defoliation from fall armyworm, *Spodoptera frugiperda*, feeding on rice seed treated with two anthranilic diamide seed treatments and two neonicitinoid seed treatments with infestations occurring at three different growth stages in a greenhouse trial performed in 2018.

Growth Stage	Seed Treatment	Percent Defoliation
2-3 Leaf	untreated check	100 a
	chlorantraniliprole	7 c
	cyantraniliprole	5 c
	clothianidin	100 a
	thiamethoxam	52 b
		***P*<0.01**
2nd-3rd Tiller	untreated check	62 a
	chlorantraniliprole	7 c
	cyantraniliprole	8 c
	clothianidin	58 a
	thiamethoxam	37 b
		***P*<0.01**
Heading	untreated check	28 a
	chlorantraniliprole	5 b
	cyantraniliprole	8 b
	clothianidin	20 a
	thiamethoxam	20 a
		***P*<0.01**

Defoliation percentages within a single growth stage followed by a different letter are significantly different according to Fisher's LSD post hoc analysis at $\alpha=0.05$.

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