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Biology, Monitoring, Sampling, and Management of Rice Billbug, Sphenophorus pertinax, in Furrow-irrigated rice

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Biology, Monitoring, Sampling, and Management of Rice Billbug, *Sphenophorus pertinax*, in Furrow-irrigated rice

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by

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Abstract

Furrow-irrigated rice hectares have increased annually for the last five years in the predominant long-grain rice producing states. Initial implementation of the system began in the 1980’s, but lack of rice blast (*Magnaporthe grisea*, Hebert) resistant cultivars made the system a nonviable option for rice producers. Hybridization of rice cultivars with less susceptibility has begun to increase the interest of producers to implement a furrow-irrigated rice production system due to the cost saving opportunities that it may provide.

Elimination of the flood from large sections of the rice fields in a furrow-irrigated system alters management strategies and pest complexes compared to the traditional flooded system. Large portions of these production fields remaining nonaquatic, increasing the susceptibility to previously uncommon pests of flooded rice. Considered a minor pest in traditional flooded systems, rice billbug (*Sphenophorus pertinax*, Chittenden) could become the most detrimental insect of furrow-irrigated rice. Rice billbug feed on the roots and tillers of the rice plants, causing dead tillers and rice panicles to abort, resulting in indirect yield loss.

As the system gains popularity and as hectares increases, questions on management strategies for control of rice billbug has become an issue for producers. Management plans for rice billbug have become a priority in the Mid-Southern rice producing states. The objectives of this research were to further expand knowledge and understanding of the biology of the rice billbug, developing monitoring regimes, and control strategies to suppress rice billbug.

Multiple experiments were conducted from 2019-2022 across the Mid-Southern U.S. to increase knowledge of rice billbug biology and to develop recommendations for control of rice billbug. Surveys, trapping methods, insecticide efficacy, and insecticide application methods were evaluated to create a full management strategy for rice billbug. Volatile semiochemical

extraction was also conducted in the lab to determine the potential for pheromone development for monitoring and control.

Findings in these studies suggest that rice billbug are present across major rice growing states of the southern U.S., and monitoring systems designed for ground active insects can play a role in management and monitoring. These data also suggest the implementation of insecticide seed treatments containing a neonicotinoid in conjunction with a diamide is the most effective strategy for controlling rice billbug. Finally, findings from these studies suggest that there was a semiochemical response observed in rice billbug, but further development is warranted before mass adoption. These data have created a foundation for further studies by researchers across the rice producing states of the United States.

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Chapter 1 - Introduction

Introduction

Arkansas is the largest contributor to U.S. rice, *Oryza sativa* (L.), production, responsible for approximately 50% of the total rice produced and grown in the U.S. (NASS, 2021). In 2019, Arkansas harvested 485,020 hectares of rice, making it the state's second-highest valued commodity (NASS, 2020). Rice is an annual monocot and has a life cycle of 105 to 145 days in standard Arkansas rice cultivars. This range varies based on cultivar and environmental conditions (Moldenhauer et al., 2020).

Traditionally, Arkansas rice production implements flood irrigation primarily to aid in weed control. Flood irrigation can create an anaerobic environment in the soil which can improve nitrogen efficiency, and aid in weed control. Typically, traditional flooded rice systems receive a flood after the 4^{th} or 5^{th} leaf emerges and usually remains flooded until after the rice crop has headed, unless specific scenarios, such as straighthead, require the flood to be removed (Counce et al., 2000). One disadvantage of implementing a traditional flooded rice production system is the necessity to construct levees to manage water depth and uniformly irrigate a field. Recommendations from the University of Arkansas System Division of Agriculture suggest that the difference between elevation of adjacent levees should not exceed more than 2% (Henry et al., 2018). On fields with a steep grade the distance between levees, or bays, must be much closer to properly irrigate a field. Smaller bays are much more difficult to harvest, increasing strain on the machine and in potentially increasing grain losses as the combine harvesters are not able to operate at optimum load. The lack of mobility also results in second cuttings of stubble and increases the amounts of field trash processed through the combine. Most importantly, harvesting smaller rice bays increases the amount of time required to harvest the crop, increasing time and

labor costs (Quick, 2003). Steeper graded fields also require more levees which require more time and fuel, as well as more man hours to install additional levee gates.

Another disadvantage of traditional flooded systems is field preparation before planting. Since traditional flooded rice is commonly planted into a uniform seedbed, this requires extensive tillage and many passes through the field. Consequently, if the field will be rotated to a different crop the following year, tillage will again be required to remove levees and create seedbeds. This has sparked interest in the implementation of the furrow-irrigated rice production system (Hardke et al., 2017).

Furrow-irrigated Rice Production Systems

Furrow-irrigated rice (FIR), commonly referred to as "row rice", is the practice where field preparation of beds and/or furrows run in the direction of prominent field grades and poly tubing is placed on the upper end to flow water downhill (Chlapecka, 2021). This eliminates the number of trips across the field during land preparation, such as levee construction and land leveling. Potential utilization of the previous year's plant beds becomes a viable option and may only require minimal repairs (Chlapecka, 2021). Henry et al. (2018) stated that FIR has increased in popularity in precision leveled scenarios due to the reduction in management costs from reduced equipment passes.

Aside from the benefits from reducing tillage, the implementation of a FIR production system can potentially lower costs by reducing fuel usage from equipment used in establishing and removing levees across the paddy, as well as reduction of passes through the field to create a uniform seedbed. This is especially true in production fields that have significant changes in elevation, requiring more levees and pre-planting soil preparation. Another potential benefit

includes more efficient harvest due to more rapid soil drying and reduction of levees throughout the field (Vories et al., 2002). Lastly, a benefit of FIR production is the reduction in labor costs. Fewer levees throughout the field requires less levees gates to control water levels in the field. Installing levee gates is intensive and a man-powered process, having few to no levee gates creates more time for labor to invest into better rice management practices.

In many FIR scenarios, growers have a levee at the bottom of the field to hold some of the irrigation water. Because of this, FIR fields typically have three management zones, and the size of these zones is determined by the grade of the field. The bottom zone, which is the lowest in elevation and most distal from the irrigation source is more like a traditional flooded system. It usually has a standing flood which can be deeper than that observed in a flooded system. The middle zone has substantial soil saturation and a small presence of standing water. Finally, the top zone of the field, with the highest elevation, has adequate soil moisture, but little to no standing water is present except during irrigation events. As producers seek a more profitable practice, implementation of the FIR system has become more popular. Arkansas has steadily increased its FIR hectares over the past several years. Similarly, demand for information on agronomic and pest management practices in FIR systems has increased.

Experimentation with FIR originally began in the late 1980s, but a lack of cultivars resistant to rice blast (*Magnaporthe oryzae*, Hebert), which is a lesser problem in flood-irrigated rice, made the system unsustainable (Jared, 2019). Rice blast is a fungus that is globally known as one of the most devastating diseases in rice production due to its ability to directly affect the rice panicle (Wamishe et al., 2019). The hybridization of rice and new breeding strategies has aided in the development of more tolerant cultivars to rice blast, which has allowed this system to be profitable to Mid-Southern U.S. rice growers (Jared, 2019). Recommended management

practices for this fungus are cultivar selection, as well as maintaining a flood depth of at least 4 inches (Wamishe et al., 2019). This becomes an issue in FIR due to its lack of flood in the top two management zones of the field. This makes selecting a cultivar resistant to rice blast for FIR production crucial.

Another management practice that must be altered in a FIR production system is fertilizer application timing. Making a nitrogen (N) application before an irrigation event contributes to maximizing FIR yields. Nitrogen management in the traditional flooded system usually involves a single preflood N application followed by a smaller N application during the late-boot stage (Roberts et al., 2018). In the FIR system it is assumed that greater losses of N will occur from increased nitrification-denitrification processes from frequent shifts of wetting and drying cycles (Chlapecka, 2021). Findings from Chlapecka et al. (2021) suggests that there are multiple N programs to maximize all yield parameters in FIR grown on loam soil, as well as a three-way N split recommendation for clayey soils.

One of the major obstacles of rice production is weed management (Lancaster et al., 2018). FIR production systems are more susceptible to yield losses from competition with weeds when compared to traditional flooded systems. Absence of a flood allows the environment to be conducive to highly competitive weeds which can result in 50-91% grain yield losses (Elliot et al., 1984; Fujisaka et al., 1993; Singh et al., 2006), whereas standing water in the field can suppress emergence of many problematic weed species (Tuong et al., 2005; Chauhan and Johnson, 2010). FIR production system's problematic weeds are similar to weed species observed in soybean production system. Palmer amaranth (*Amaranthus palmeri*, Watson) is not considered a major weed in a traditional flooded system, but lack of a flood presence allows it to be the major weed for the FIR system. Several other weed species such as carpetweed (*Mollugo*

verticillata; L.), ground cherry (*Physalis* spp.; L.), and sicklepod (S*enna obtusifolia*; L.) become problems when implementing the FIR system. More species to control results in additional inputs to reduce weed competition (Butts, 2020).

Studies in Arkansas have shown FIR production systems commonly require additional input costs for weed management when compared to the traditional flooded production systems but can still be profitable under water-limiting and topographic situations (Bagavathiannan et al., 2011). Research in Mississippi found an average minimal yield reduction of 57.67 to 41.14 kg ha^{-1} when implementing a FIR production system, although this is location dependent (Golden, 2019). Despite decreased yields, FIR systems may show greater profitability, but additional concerns arise when implementing this production system.

Rice Insect Complex

The three most damaging insect pests of rice in Arkansas, are grape colaspis (*Colaspis brunnea*; L.) (Hemiptera: Chrysomelidae), rice water weevil (*Lissorhoptrus oryzophilus*; Kuschel) (Coleoptera: Curculionidae), and rice stink bug (*Oebalus pugnax*; L.) (Hemiptera: Pentatomidae). Grape colaspis is commonly observed in rice fields that are in rotation with soybean production (Lorenz et al., 2017; Muda et al.,1981). The larvae overwinter deep in the soil and move up in the soil profile to the root zone as temperatures increase. Grape colaspis will feed on the roots of rice plants, as well as other subsoil seedling parts. This feeding will cause severe girdling to the hypogeal stem, causing discoloration, stunting, and wilting that can be mistaken with pathological infections (Lorenz et al., 2017). Cultural control methods for grape colaspis are deep tillage in the late fall and/or early spring, as well as a flush of water on the field to be held for at least 48 hours (Lorenz et al., 2017). Insecticide seed treatments provide the

greatest level of control for grape colaspis, due to the inability of foliar insecticides to reach larvae in the soil. Neonicotinoid seed treatments, such as CruiserMaxx Rice® (Syngenta Corporation, Wilmington, DE) (thiamethoxam, fludioxonil, azoxystrobin and mefenoxam), or NipsIt Inside® (Valent U.S.A. San Ramon, CA) (clothianidin) can maintain successful control of grape colaspis.

When evaluating furrow irrigated production systems, similar control methods are recommended for the control of grape colaspis. The larvae of grape colaspis are notorious for causing substantial stand loss in rice production. Recommendations from *Managing Furrow-irrigated Rice in Arkansas* (2017) suggest increasing the plant population by 10% when compared to the traditional flooded system, due to the lesser success in stand achievement. While reduced populations are already expected, the consequences of not implementing a neonicotinoid insecticide seed treatment in FIR systems can potentially be catastrophic if a grape colaspis infestation occurs. Implementing a neonicotinoid seed treatment will contribute to the protection of the desired plant populations.

Rice water weevil is the most damaging insect pest of Arkansas rice production. Rice water weevil larvae are similar to grape colaspis in regard to larval feeding on the roots of the plant. The rice water weevil adult is a small weevil that will feed on rice foliage creating thin linear leaf scarring. Though this damage appears alarming, no yield effects have been observed from adult rice water weevil feeding (Stout et al., 2002; Shi et al., 2008). Rice water weevil prefers a moist environment and are attracted to open water (Lorenz et al., 2017). Once a rice field is flooded, rice water weevils present emerge from the soil litter and begin to navigate to the field using flight; and begin locating mates for copulation. The flood induces the degradation of indirect flight muscles of rice water weevil (Morgan et al., 1984). Muda et al. (1981) states that

80-90% of rice water weevil adults will become flightless in about 5-7 days after migrating into the production field.

After flood establishment, overwintered females begin to lay eggs. Oviposition occurs 7-14 days after emergence from overwintering sites, and only takes place once a part of the host plant is submerged in water. After eclosion, larvae will then feed inside the leaf sheath and make its way into the root zone, which can lead to stunting and potential death of the plant (Taillon et al., 2013; Zou et al., 2004). Rice water weevils in FIR systems are still threatening, but the design of this production system provide cultural control and condense the density of the infestation to the bottom management zone.

Research pertaining to insect control in FIR is limited, but studies in the Mid-Southern U.S. have increased due to rice producers' interest in the system. Findings from Kelly (2021) in the Mississippi Delta suggested that rice water weevil infestations in the bottom management zone were like those of adjacent flooded fields up to 3 weeks after establishment of a flood, but populations were significantly lower at 4 and 5 weeks after flood establishment. In the top two management zones, rice water weevil densities were significantly lower at all sampling timings when compared to the bottom management zone. These findings correlated to findings from Lorenz et al. (2017), who stated rice water weevil will relocate to more moist environments if soil becomes too dry.

Nonetheless, the implementation of an effective insecticide treatment can keep rice water weevil infestations at a manageable level. Arkansas' Rice Production Handbook states that treating seed with a diamide insecticide such as Dermacor X-100® (chlorantraniliprole) (DuPont; Wilmington, DE) is the most effective product for control of rice water weevil larvae (Lorenz,

2017). More recently, another diamide seed treatment, Fortenza® (cyantraniprole) (Syngenta Corporation, Wilmington, DE) has been labeled for use in rice. Along with diamides, the neonicotinoid insecticide class has been observed to provide control of rice water weevil, however the diamide insecticides have shown more consistent control. Foliar applications can also be made to manage rice water weevil adults and eggs, although this is not the most effective control method. The precise timing of foliar applications is imperative to provide control as well as an economic benefit. Published data on the control of rice water weevil in Arkansas rice is intended for flooded irrigation systems and not specifically a furrow irrigated system. Currently, recommendations for control may not be optimal for use in FIR systems, and further evaluation of control tactics may be warranted.

Rice stink bug is a common and damaging pest in heading rice. Unlike grape colaspis and rice water weevil, rice stink bug feeding is classified as direct damage, feeding directly on the heading rice panicle (Lorenz et al., 2017). This is a large issue in rice production due to the grain being directly consumed and potential for reductions in grain quality. The timing at which the feeding occurs is directly correlated to the type of damage observed. Feeding during the flowering stage will cause grain development to cease and directly affect yield, whereas when feeding occurs during the milk stage it results in losses of all contents within the hull, thus lowering yields. Rice stink bug feeding during and after the soft dough stage will results in 'pecky' rice, which is off colored rice that will lead to quality deductions when sold at market (Lorenz et al., 2017).

Control methods for rice stink bug begins with proper scouting to understand the infestation occurring within the field. Cato (2019) suggests that optimal sampling times for rice stink bug should be conducted during morning, between 8 and 11 a.m. or late in the evening

around 7 to 9 p.m. to best estimate populations. Thresholds for rice stink bug are five or more stinkbugs per 10 sweeps during the first two weeks of heading, and 10 or more during the third and fourth weeks of heading (Lorenz et al., 2017). Another study conducted by Cato et al. (2019) estimates the optimal timing to terminate insecticide applications for rice stink bug is 60% hard dough, unless large densities are present. Pyrethroids such as Warrior II® (lambda-cyhalothrin), Mustang Maxx® (zeta-cypermethrin), and Prolex® (gamma-cyhalothrin) can be applied to aid in the control of rice stink bugs (Lorenz et al. 2017). Several insecticide options exist for rice stink bug control. Researchers hypothesize that similarities in population densities of rice stink bug between traditional flooded and FIR systems would be observed, and control measures previously discussed should be implemented in both rice production systems.

Armyworm Complex

A frequent pest affecting Arkansas rice production is the complex of armyworms that feed on rice. The two species documented in Arkansas rice are the true armyworm (*Pseudaletia unipuncta* (Haworth)) and the fall armyworm (*Spodoptera frugiperda* (J.E. Smith)). The armyworm complex are defoliators that feed on both leaves and stems and can consume all plant material above the soil line, or water line post flood (Lorenz et al., 2017). Though the growing point of rice may not be affected, delays in crop maturity may be observed resulting in management and harvest issues (Lorenz et al., 2017). Recent studies in Arkansas observed in substantial yield losses occurring when defoliation of 66% or greater occurred during the panicle initiation growth stage. Greatest delays in maturity were also observed in rice planted in June (Felts et al., 2020).

Typically, true armyworms are a springtime pest and feed on rice earlier in the season, whereas fall armyworms infest bermudagrass (*Cynodon dactylon*, L.) pastures and rice mid-summer. There are two "host strains" of fall armyworm that are found in the Mid-Southern U.S. They are defined by their preference in host, fall armyworms that are commonly found on larger grasses such as corn (*Zea mays*, L.) and grain sorghum (*Sorghum bicolor*, L.) are designated as the "corn strain". Fall armyworm that feed on smaller monocots like rice and bermudagrass are referred as the "rice strain" (Nagoshi and Meagher, 2008).

Pyrethroid insecticides are known to provide adequate control of armyworms, but populations of the rice strain during the 2021 growing season required a section 18 emergency authorization use due to high amounts of pyrethroid application failures (Aaron, 2021). In previous years, the rice strain of armyworm could be controlled easily with a pyrethroid, but indication of failure during the 2021 growing season suggest that alternative options need to be evaluated (Hightower, 2021). FIR may have an increased threat from armyworm feeding, due to the large portion of the field not submerged in a flood. This allows the caterpillars to feed on greater amounts of the rice plants including the growing point, increasing the possibility of crop destruction in FIR systems.

Rice Billbug

This shift in the production system, a common insect that has not been considered a major rice pest has now become a greater concern. Rice billbug, *Sphenophorus pertinax* (Chittenden), has historically been observed feeding on rice planted on the levees in the traditional flooded system. It is believed that rice billbugs feed on the levees due to flooded rice

being inhospitable for rice billbug feeding and reproduction. As FIR hectare increases across the Mid-Southern U.S., the risk of rice billbug infestations has dramatically increased.

Rice billbug adults are a large glossy black weevil, approximately 2.54 cm long, with a prominent snout for feeding (Chittenden 1905). No published research confirms if this species is the only species of billbug feeding on Arkansas rice. Little information is also known of the true impact of billbug injury to rice, and yield losses that have occurred. Preliminary data from the University of Arkansas System Division of Agriculture is already being assessed to learn more about this pest and assess scouting and management options.

Billbugs are a weevil complex that are generally found across the continental United States and surrounding countries such as Canada and Mexico, and some parts of Asia (Johnson-Cicalese et al., 1990; Reynolds, 2014). The genus of *Sphenophorus* has approximately 74 described species, with 64 of these species being observed in North America (Niemczky and Sheltar, 2000). Documentation of billbug infesting agricultural grasses such as corn and wheat (*Tritcum aestivium* L.), currently no published data documents a weevil in the genus *Sphenophorus* being found on rice. Dupuy and Ramirez (2016) suggest that the species of billbug known as hunting billbug (*Sphenophorus venatus vestitus*, Chittenden) (Coleoptera: Curculionidae) will feed on yellow nutsedge (*Cyperus esculentus* L.), a common weed found in Arkansas rice production (Satterthwait, 1931). Billbugs have also been documented in various turf grasses (Dupuy and Ramirez, 2016). This group of insects is commonly found in thicker grasses that provide shade and allowing them to remain hidden (Kindler and Spomer, 1986). Though specific species of billbug have been documented in various host plants, the host range of billbug feeding is yet to be determined (Dupuy and Ramirez, 2016).

Hosts and Feeding

Dupuy and Ramirez (2016) noted that adults are primarily ground active and are not strong fliers. *Sphenophorus* spp. commonly overwinters in protected areas such as leaf litter, unmanaged turf, or in thicker grasses. Though no publications have suggested overwintering sites for rice billbug, documentation of *Sphenophorus* spp overwintering in heavy grasses and leaf litter below tree lines surrounding production fields have been made. Kindler and Spomer (1986) suggest some species will bury in the soil headfirst, approximately 2.54 cm or less below the soil line. This has been observed with rice billbug, rice samples were taken to better understand the feeding habits in rice fields across Arkansas from 2019-2021. These observations stated rice billbug was commonly located in the roots zone near the soil line.

Life Stages

When female *Sphenophorus* spp are ready to lay eggs, studies have shown stems of well-established grasses, as well as actively growing grasses are preferred for oviposition (Kindler and Spomer, 1986; Vittum et al., 1999; Rondon and Walenta, 2008). The morphological features of rice make it a desirable host for rice billbug, due to its high vigor and tiller development. Once the female *Sphenophorus* spp selects a host for oviposition a small hole is chewed near the crown of the plant and deposits one to three eggs in each opening (Webster, 1982; Satterhwait, 1986). Billbug eggs are typically oblong, glossy, cream colored, and approximately 1-2 mm in length (Kindler and Spomer, 1986). Currently, no observations have been made to record morphological differences in eggs between *Sphenophorus* spp (Richmond et al. 2011).

Generally, there are five larval instars before pupation, though this is species-dependent, and a full cycle through larval stages averages around 30-40 days (Dupuy and Rameriz, 2016).

Larval and pupal identification is difficult but adult identification becomes much easier. Adult billbugs can be identified by their setae, snout length, as well as length of pronotum (Satterthwait, 1931). Common southeastern species such as the hunting and bluegrass billbug commonly remain in the pupal stage anywhere between 3-12 days, then adults will overwinter in protected sites until the following year (Johnson-Cicalese et al., 1990; Watschke et al., 2013).

In more recent years, hunting billbug located in Indiana and North Carolina has been observed to have two overlapping generations throughout the years (Doskocil and Brandenburg 2012; Richmond and Duffy, 2015). Greenhouse studies in Florida have documented up to six generations of *Sphenophorus v. vestitus* in a single year under optimal conditions (Huang and Buss, 2009). Observations of rice billbug have been documented in Arkansas, where larval, pupal, and adult stages of the species were found overwintering below the soil surface in rice fields. This suggests that multiple generations of rice billbug may be possible, but further research is needed to verify how many generations per year occur.

Damage

The most common species of billbug seen in row crop production is the southern corn billbug (*Sphenophorus callosus*, Olivier) (Coleoptera: Curculionidae). This species of billbug is more commonly found on the coastal plains of the Carolinas as well as Georgia (Reisig, 2016). They are more prevalent in fields that have not been rotated out of corn or, sod production, and in fields infested with various sedge species (Oday et al., 1998). The first indication of southern corn billbug feeding is a narrow slit in the side of the young corn tissue slightly below the soil surface. Symptoms from adult billbug feeding on corn are a row of symmetrical holes on an

individual leaf blade, caused by snout insertion and feeding while leaves were rolled within the whorl (Oday et al., 1998).

Severe southern corn billbug injury results in stunted plants as well as plant death (Oday et al., 1998). Similar feeding has been documented on rice plants by Michael Smith of Louisiana State University (1986). Smith documents that the species *Sphenophorus obilitus*, will eat small holes into the stalk and feed on the young tissue inside, leaving a similar series of feeding holes perpendicular to the leaf midrib. Observations published by Smith were limited to rice levees in a traditional flooded rice system. Smith's findings coincide with observations made by the authors recorded white, chlorotic panicles which eventually die found in many FIR fields across the Mid-Southern U.S. Observations have been made by the authors of symmetrical line feeding on rice leaves, rostrum insertions at the base of the tiller, as well as aborted panicle heads and tiller death.

Anecdotal observations made by the authors in a greenhouse setting shows that, leaf feeding is associated with adult feeding. Damage was observed 5-7 days after rice billbug were infested on rice plants. The authors infested rice with male rice billbug to ensure larva damage would not be recorded.

Larval feeding occurs within the rice tiller and the feeding on the juvenile tissue ceases nutrient flow and can cause tiller death. These observations have allowed the authors to hypothesize that larval feeding later in the growing season causes aborted panicle heads. The authors note that billbug feeding is most prominent in the top and middle management zones of FIR where the presence of standing water is limited. Rice billbug has also been found below the soil line in several traditionally flooded rice fields across Arkansas, but these specimens were

immobile and not actively feeding. The authors have hypothesized that rice billbugs are not truly hydrophobic, but control of water level in FIR systems can contribute to the suppression of billbug feeding.

Published Monitoring Systems

Billbug adults are known to be ground active, so the most commonly used method for monitoring is linear pitfall traps. The original pitfall design was commonly circular in shape and only covered a small area of the soil surface. This limits the chances of insects being captured in larger areas. Linear pitfall traps were modified from the original design as described by Pausch et al. (1979) to monitor density and timing of insect populations moving across the soil surface (Pausch et al., 1979). Linear pitfall traps are modified to cover a larger surface area and increase likelihood of capturing ground traveling insects. Pitfalls are typically manufactured with commercially available materials such as PVC (polyvinyl chloride) piping, or galvanized metal guttering capped at one end, and a collection jar on the opposite end. Linear pitfalls are typically buried at a slope with the collection jar found at the lowest elevation. Soil removed for trap placement should be used to bury the trap flush with the soil line and placed in between overwintering sites and a desired host. Once trapped inside, the insect will walk downhill and fall into a collection tray (Bradenburg, 2016).

Dupuy and Ramirez (2016) found pitfalls to be the most successful of the tested traps to catch billbug, and Potter (1998) suggests traps should be monitored weekly to aid in pest management decision making. The implementation of linear pitfalls in Arkansas could be a viable strategy to monitor billbug. In a FIR system, the implementation of a linear pitfall needs to be modified to increase trapping efficiency. Aggregated and fallow soil increases the chances of

erosion to occur. Modifications for linear pitfall traps are also needed to withstand frequent irrigation events that are common in FIR systems. Linear pitfalls designed for rice billbug needs should be modified to withstand the management practices necessary from adequate FIR production.

Control Tactics

Currently, limited research has been conducted for control of rice billbug. As stated previously, insecticide seed treatments are currently the best control tactic for other weevil species found in rice production. Floyd et al. (2021) suggests that insecticide seed treatments containing a diamide insecticide significantly increased yield compared to standalone neonicotinoid seed treatments, or rice not receiving an insecticide. Neonicotinoids in conjunction with diamide classes applied as seed treatments were recorded to significantly increase yields, but the insect must ingest the active ingredient to be able to suppress the infestation. Floyd et al. (2021) notes that reductions in damage were observed but not eliminated, implying yield losses could still occur. Further research is needed to investigate other management approaches that can be used in conjunction with insecticide seed treatments to reduce greater levels of damage from rice billbug feeding and maximize yield retention.

Conclusions and Recommendations

Billbug feeding can negatively impact many different types of crops, predominantly monocots. As FIR production systems develop across the Southeastern U.S., there is a need for research to understand all aspects of the rice billbug to develop best management practices. Creating a management plan for a formerly minor insect pest is difficult due to the lack of published data. As rice billbug become more prevalent, and hectare of desirable host plants

continue to increase, it is imperative to develop a monitoring and management plan. Developing a management plan to control billbug in FIR requires foundational research and studies that provide basic information to FIR growers.

Three branches of research needed to be conducted to create a successful management strategy. First, understanding the biology of the rice billbug to know how the species develops, feeds, and reproduces. Secondly, create a successful monitoring and sampling program to be able to observe infestation timing and density. Finally, analyzing cultural and chemical control methods to manage rice billbug. Together, all three components will allow researchers to create recommendations to aid FIR producers in rice billbug control. Research is needed to better understand the rice billbug and its possible effects on rice grown in furrow-irrigated production systems.

As rice hectares continue to shift from the traditional flooded fields to the FIR system, questions will also continue to rise on proper management for rice billbug. Rice billbug has solidified itself as a threat to FIR production, and is classified as major pest status by rice researchers across the Mid-Southern U.S. The objective of this research was to develop a greater understanding of the rice billbug in the landscape, create practical monitoring techniques, and evaluate control tactics for the pest. By completing these objectives an overall management strategy for FIR producers could be established, allowing growers to make management decisions to maximize yield retention.

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Chapter 2 - Biological and Observational Field Notes of Rice Billbug (*Sphenophorus pertinax*) (Coleoptera: Curculionidae) across the Mid-Southern U.S.

Abstract

As furrow-irrigated rice (FIR) production systems gain popularity across the Mid-Southern U.S. due to potential savings in labor costs, alterations in the rice growing environment have made these fields susceptible to rice billbug (*Sphenophorus pertinax*, Chittenden) feeding. In traditional flooded rice systems rice billbug was considered a minor pest. Researchers hypothesize that the species is unable to survive in a flooded environment, therefore it was only able to injure minor unflooded portions of traditional rice fields, such as levees. In FIR systems, substantial portions of the field are never completely flooded, which increases the number of viable host plants for rice billbug. A survey was conducted across 80 locations in throughout Arkansas, Louisiana, Mississippi, and Missouri to find similarities in rice billbug behavior across multiple geographies. The objective of this survey was to determine relationships between alternate hosts and rice billbug infestations, as well as when feeding begins in the growing season and how it affects yield in rice. In this study rice billbug was only observed in Arkansas and Missouri, although reports of rice billbug injury have been made in the other two states. Rice billbugs were more commonly found in FIR production fields where grassy borders were present. Infestations were much less common when borders were vegetation free, or fields were surrounded by other crop fields. If damage was to occur in a field, it was first observed most often during the 3-4 tiller growth stage. As rice plants moved closer to reproduction the chances of an infestation beginning were dramatically reduced. This suggests that rice billbugs were moving into rice and feeding when rice plants first begin to tiller. Billbug damaged plants were also observed to produce more panicles than undamaged plants. This indicates that the rice plants attempted to compensate for the billbug damage. However, undamaged plants continued to produce higher yields.

Introduction

The rice growing states of the Mid-Southern U.S.; Arkansas, Louisiana, Missouri, and Mississippi, produced 77% of total rice harvested nationally in 2021 (NASS, 2021). In recent years, furrow-irrigated rice (FIR) hectares have increased due to it being a less labor-intensive system for some growers. Utilizing the FIR system can reduce the amount of diesel fuel used to till soil and construct levees. The FIR production system commonly utilizes plant beds from the previous growing season with furrows running in the direction of the field grade. These fields are irrigated by watering down the furrows from the area with the highest elevation (Chlapecka et al., 2021). This divides FIR production fields into management zones characterized by the amount of soil saturation present (Kelly et al., 2020). FIR systems commonly have a standing flood in the lower end of the field. How much of the field is flooded is dependent on the grade of the field and if a structure was made to catch water, backing it up into the field.

The alteration of this environment has made it more favorable to uncommon pests in the traditional flooded system. For example, chinch bug (*Blissus leucopterus,* Say) (Hemiptera: Lygaeidae) idea is a pest more commonly seen in other monocot crops such as corn (*Zea mays,* L.). Traditionally, chinch bug was only found in higher areas of fields where flooding is difficult or on levees. Pathologically, rice blast (*Magnaporthe oryzae*, Hebert) can be more favorable in the aerobic conditions of a FIR system. Current recommendations suggest implementing rice blast tolerant hybrids in these scenarios (Wamishe et al., 2019).

In conventionally flooded rice fields, rice billbug (*Sphenophorus pertinax,* Chittenden) (Coleoptera: Curculionidae) typically feeds only where floods do not occur, similar to the previously mentioned chinch bug (Floyd et al., 2019). Because of this FIR has become a favorable host for billbug due to reduced amounts of flood from minimal levees (Dupuy and

Ramirez, 2016). Due to the traditional pest status of rice billbug limited data exists on any aspect of the rice billbug. In 2021, almost 20% of total Arkansas rice production hectares were in the FIR system (Chlapecka et al., 2021). This has raised researchers and producers concerns on how rice billbug should be managed as FIR hectares grow.

Limited research has been conducted on rice billbug biology and monitoring, and more research is needed to understand the impact and yield loss associated with rice billbug in a FIR system. To achieve this, ecological and biological observations are needed to aid in developing management strategies. Understanding the ecology of an insects species is fundamental in determining how to manage a destructive pest (Price, 1997) and as FIR hectares continue to increase a need for ecological understanding of rice billbug is imperative to develop management plans. The objective of this research was to build foundational knowledge of rice billbug ecology and biology, including the relationship between infestations and the surrounding landscape, potential overwintering sites, when feeding starts occurring, and yield loss potential.

Materials and Methods

Billbug Survey

A survey was conducted on 80 FIR fields across the 2019, 2020, and 2021 growing seasons in Arkansas (64), Missouri (8), Louisiana (6), and Mississippi (2). Observations were taken of the surrounding landscape in the four cardinal directions around each field. The total dimensions of the field were gathered to calculate the total area. The fields were then divided into three management zones spanning across the width of the field. The zones are divided by the amount of soil saturation in each zone. The top management zone, closest to the irrigate source had minimal soil saturation, and no standing water visible. The middle management zone was

classified as having heavy saturation but less than 10.2 cm of water in the furrow. Lastly, the bottom management was the portion with the most soil saturation of the production field that consistently had greater than 10.16 cm of water stand in the furrow.

At each location, three pink plastic buckets (31.8 cm x 31.8 cm x 38.1 cm) (Atwoods Ranch and Home; Enid, OK; MFG #50640) were distributed equidistantly on a field edge through the top two-thirds of the field, where billbug damage has been commonly observed. Pink buckets were selected based on preliminary findings of Floyd et al. (2021c) recorded higher catches when using this color. Buckets traps were placed on field edges, preferably where natural grasses and treelines were located. Bucket placement was selected based on hypothesized overwintering sites. The distance between buckets was dependent on field size and ranged from 15.2 m to 45.7 m. Buckets were checked weekly by moving the bucket and checking for rice billbugs underneath. When the natural vegetation under the bucket began to senesce, buckets were moved 1 m from the original placement so that new viable vegetation for rice billbug to feed on was present.

Each week rice growth stage and plant injury distance from field edges were recorded once initial injury from rice billbug feeding was observed. Rice growth stage was recorded based scale documented by Vergara (1991). Fields were surveyed by starting on the edge of the field where buckets were placed and moving towards the center of the field. Visual observations were made to determine if either rice billbugs were present or if feeding had occurred. Rice plants were considered injured if plants had signs of puncture wounds or if the new tiller emerging from the leaf sheath was yellow. The initial plant injury was marked with a 1 m wire flag (Gemplers Farm and Home Supply Co; Janesville, WI; MFG #36VF1-FLOPNK) to be able to identify its location each week. The distance from the plant to possible overwintering sites, such as tree

lines, CRP areas, and areas with grassy vegetation such as bermudagrass (*Cynodon dactyon*, L.) were documented. Each following week, the injured rice plant farthest away from predicted overwintering site was marked and distance from the previous week findings was documented. When new billbug injury ceased to appear farther into the field for two consecutive weeks, final measurements were taken. Distance from the injured plant to the highest grade of the field, as well as the field edge were documented as the final measurement. Distances recorded from the survey were then used to estimate the approximate percentage of the field susceptible to billbug injury.

Species Differentiation

Weevil species were collected from survey locations and brought to the Lonoke Agricultural Extension and Research Center in Lonoke, AR for further evaluation. Species not fitting the taxonomic descriptions of *Sphenophorus pertinax* described by Chittenden (1905) were infested on to rice grown in a greenhouse to determine if they would feed on rice. Specimens were monitored daily to document any potential injury from feeding on rice for seven days. After the seven-day monitoring period, specimens were sent to Mississippi State University taxonomy to correctly identify the species

Threshing Samples

During 2020 & 2021 growing seasons rice samples were taken to determine the potential yield losses associated with rice billbug injury on a per plant basis. Ten fields in Arkansas from the survey were selected based on extensive billbug injury being observed. Locations were also selected to account for different rice growing regions of Arkansas. In the selected locations, ten rice billbug-damaged and ten undamaged plants were collected from the field. Plant samples

were collected by removing the entire plant using a sickle (Forestry Suppliers, Jackson, MS; MFG #33070) to cut the plant at the soil surface. All plant material were placed in a 1/6-barrel sack (American Paper and Twine, Mabelvale, AR; MFG #DRO80091) with the panicles being placed in the bottom of the sack to reduce the potential of losing grain in transport and assigned a specific number to identify the sample. Samples were taken back to Lonoke Agricultural Extension and Research Center in Lonoke, AR and threshed using a Wintersteiger Hedge 16 laboratory thresher (Wintersteiger Inc., Salt Lake City, UT). The number of total panicles and the weight of rice grain threshed from each plant were documented. Statistical analysis was performed using Statistical Analysis Software (SAS inc, Cary, NC) version 9.4 using the PROC GLIMMIX function. Location and year were considered random variables. Treatments were separated using the Multiple Pairwise t-test set at α=0.05.

Overwintering sites

Further evaluations were made during 2020 and 2021 in areas that had the most billbug injury in 2019. In April of 2020 and 2021 prior to planting, observations were made at six locations to document potential over wintering sites. Hypothesized overwintering sites for rice billbug were based on documentation of overwintering sites of insect pests commonly seen in Arkansas rice production. Overwintering sites for rice water weevil (*Lissorhoptrus oryzophilus*, Kuschel) (Coleoptera: Curculionidae) are documented in leaf litter, bunchgrasses, grass clumps, and plant debris in and around rice production areas (Shang et al., 2002). Grape colaspis (*Colaspis* brunnea, L.) (Hemiptera: Chrysomelidae) larvae overwinter in the soil of the production field from the previous year (Lorenz et al., 2017). A square meter was measured in three locations in the production field where highest amounts of rice billbug injury were observed in the previous production season. Additionally, three locations were selected on the

turnrow where bermudagrass was present. The locations selected on the turnrow were parallel to the top management zone of the FIR production field. Lastly, if a tree line was present around the production field, the leaflitter of the tree line was sampled. Each fields soil type was documented prior to sampling. Inside the marked area, soil was removed 5.1cm at a time up to 20.3 cm into the soil. As each section was removed soil was sifted through, and billbugs and life stage were documented.

Damage associated with rice billbug

Observational data was collected in a greenhouse using insect viewing cages at the Lonoke Agricultural Extension and Research Center in Lonoke, AR. RiceTec RT7301 conventional long grain hybrid rice was treated with a base fungicide package consisting of, mefenoxam, azoxystrobin, and fludioxonil. Rice was planted in 10.2 cm x 10.2 cm x 8.9 cm pots (Greenhouse Megastore, Danville, IL; MFG #CN-STD-0400) using a 40/60 mixture of potting soil (The Scotts Miracle-Gro Company- Landscaping, Marysville, OH; MFG #75686300) and a Stuttgart silt loam (fine, smectitic, thermic Albaquultic Hapludalf). Rice was grown until 3-4 tillers had been produced. Rice billbugs were collected from the field and sexed based on guidelines of Chittenden (1905). Female rice billbugs were not used in these studies to decrease the likelihood of larval damage occurring. One male rice billbug was infested per rice plant and a single rice pot was placed in 45.7 cm x 45.7 cm x 30.5 cm insect viewing cage and monitored every 24 hours (Figure 1).

Results and Discussion

Billbug Survey

Across all locations of the billbug survey, 71% of fields had documented billbug injury. Rice billbug damage was observed at survey locations in both Arkansas and Missouri (Table 2.1; Figures 2 and 3), however rice billbug damage was not observed in Louisiana or Mississippi (Figures 4 and 5). In Arkansas, rice billbug was present in 78% of surveyed fields in 2019, 80% of fields in 2020, and 93% of fields in 2021. A total of 81% of fields surveyed in Arkansas had billbug injury. The 64 fields in Arkansas included in the survey were distributed across twelve counties in the state. Billbug damage was observed in 11 of 12 counties surveyed. In Missouri during the 2019 survey, 50% of fields surveyed had injury associated with rice billbug present. Rice billbug was observed at two of the three counties included in the survey.

Across years and locations 82% of fields with a presence of rice billbug, had grassy borders, predominantly containing bermudagrass, or a tree line border. Of the field that were not bordered by a grassy area, only 9% had an infestation of rice billbug. When observing the presence of a tree line on at least one edge of a survey field, rice billbug feeding was observed at 66% of these fields. There were 27 survey fields that production crops surrounded all four sides, and rice billbug damage was observed at only 22% of these fields (Table 2.2).

When locations were separated by soil type, fields with predominantly loamy soil had numerically higher percentage of susceptibility (36%) compared to soils consisting of mostly clay (22%). These observations suggest that rice billbug can distribute father across loam soils compared to clays. The authors hypothesize this may be contributed to the water holding capacity of the soil type. These differences may be because clay soils tend to stay more saturated than loam soils which is not as hostable for rice billbug.

Across all locations, initial signs of plant injury were observed at the 3-4 tiller growth stage (Table 2.3). While first signs of injury were observed at the 5-6 tiller, green ring, and boot growth stages, it was at a much lower percent compared to the 3-4 tiller growth stage. No signs of injury were observed on seedling rice at any location during any year.

Species Differentiation

During the survey, other species were observed in or near rice fields. A total of three different billbug species were identified during the survey. These species included *Sphenophorus pertinax* (Chittenden), *Sphenophorus rectus* (Say) (Coleoptera: Curculionidae), and *Sphenophorus ventatus vestitus* (Chittenden) (Coleoptera: Curculionidae). Of the species collected *S. petinax* represented 95% of the total billbugs collected, while *S. rectus* and *S. ventatus vesititus* represented 3% and 2% of the total billbugs collected, respectively. When placed on rice grown in the greenhouse, only S. pertinax actively feed on the rice. It appears that of the species observed in FIR, only *S. pertinax* is a concern for injuring rice.

Threshing samples

Differences were observed when analyzing total panicles of damaged and undamaged plants (df=1,188; F=57.9; P<0.01) (Table 2.4). Plants with rice billbug injury had a greater number of panicles compared to panicles of undamaged rice plants. Differences were also observed in grams of rice per plant between damaged and undamaged plants (df=1,187; F=129.3; P<0.01). Despite having less panicles, more grams of rice were observed for undamaged plants than rice billbug damaged plants. When analyzing grams of rice per panicle, undamaged plants produced twice as much grain as compared to plants with rice billbug damaged (df=1,187; F=456.0; P<0.01). Data suggests that when rice billbug feeds on rice plants,

rice attempts to compensate for injury by increasing panicle production but cannot produce enough grain to recompense.

Observational Findings

Overwintering sites

Rice billbugs were observed at four of the six locations sampled. At all four locations, rice billbug specimens were found on grassy turnrows, and at two locations rice billbugs were found overwintering in the field (Figures 6 and 7). Where billbug was found, both adults and larva were present in an overwintering state. Adults were found in a range of 6.1 cm to 16 cm below the soil line, and larva were found from 4.8 to 9.4 cm below the soil line. Observations from this survey suggest that rice billbug overwinters close to or in the FIR field from that growing season, and that they can overwinter as larvae, pupae, and adults.

Feeding Patterns

When observing feeding patterns of rice billbug in rice, adults were observed feeding on young rice tillers for a few weeks, before moving into the root system and continuing to feed (Figure 8). Once oviposition occurred, larvae hatched and began feeding inside the tiller between the first joint and the soil line (Figure 9). In the final stages of larval development, rice billbug larvae moved into the root crown where they pupated (Figure 10). Once adults, rice billbugs appeared to remain in the soil until rice began to senesce. Upon senescence, adult rice billbugs exited the field to overwintering sites, larvae and pupae stayed within the root zones plants. Observations can be made at this time by surveying under poly-plastic irrigation tubing. Rice billbug show signs of being thigmotactic, preferring to be hidden in tight spaces for protection.

Life Stages

Though no set number of instar stages have been confirmed for rice billbug, authors have documented changes in development from larvae to adult (Figures 11 and 12). Based on observations during the survey, it appears that rice billbugs do not complete two generations on an annual basis but have a delayed generation later in the growing season. This could explain why it is more common to observe larvae and pupae overwintering in the production fields, compared to adults in bunch grass species on the turn row. “First generation” rice billbug going through a full life cycle lay eggs prior to overwintering. Environmental factors, such as temperature and precipitation at the end of the growing season may dictate the development of second-generation eggs and larvae. Additional observations have been made where larvae are in their final stages before pupation in the root crown, and at the same time newly hatched larvae are also present. This later generation of larvae may be from billbug that overwintered as larvae or pupae, needing finish development mating and oviposition can occur.

Damage Associated with Rice Billbug

References to rice billbug injury are commonly associated with blank panicles (Figure 13). Villegas et al. (2021) refers to early plant death in younger rice plants, but plant death was not documented during this survey. Observations in the insect viewing cages were made every 5 to 7 days after infestation. Puncture wounds were observed at the base of a single tiller where rice billbug had inserted their rostrum into the juvenile tissue inside the outer leaf sheath of rice (Figure 14). This resulted in “tiller death” and has been used by the authors as a sampling variable associated with rice billbug feeding (Floyd et al., 2021a). Tiller death is described as the juvenile tissue within the rice tiller emerging from the joint is yellowish-brown and chlorotic and

dies within 72hrs after feeding. A key characteristic to locate this damage is the outer leaf sheath remains green while new tissue arising from the joint is discolored (Figure 15). An average of 2.3 tillers per plant were damaged by a single billbug in greenhouse cage studies. Additionally, of the tillers fed upon, 82% were secondary tillers and 18% were primary.

Oviposition and Egg Development

Limited information is available on the description of eggs or preferred oviposition sites of rice billbug. Felts et al. (2018) suggests that oviposition occurred in the spring and oviposited at the base of a rice plant. These findings have been confirmed by the authors. In the natural environment, the injury referred to as “tiller death” was observed to be oviposition sites for rice billbug females. Rice billbug eggs were located within the puncture wounds on the base of the rice tillers under the outer leaf sheath (Figure 16). Six viable eggs were found during the survey. These eggs were collected and taken to Lonoke Agricultural Extension and Research Center for photographs and incubation. Rice billbug eggs are capsule shaped and cream in color (Figure 17C). Eggs measured 2mm in length (Figure 17A) and 1mm in width (Figure 17B). Eggs were placed in 10.2 cm x 10.2 cm petri dishes (VWR International, Radnor, PA) and placed in an incubator (Percival I-36VL, Percival Scientific Inc., Boone, IO) at 29.4°celcius. Eggs were checked daily, and larvae hatched from five of the six eggs, averaging 11.4 days after incubation. This does not accurately depict a timeline from oviposition to hatching due to the lack of knowledge of how longs the eggs had been laid upon finding them and collecting them.

Conclusions

Results from the survey have given researchers a greater understanding of rice billbug in the landscape. Knowing the rice growth stage which rice billbug prefer can aid in timing of

management strategies. Pinpointing overwintering locations and additional hosts may help create a management regime. These findings will allow researchers to know when and where to expect billbug moving into the field.

Based on the findings throughout the survey, it is clear that rice billbugs move into FIR fields as tillering begins. At that time, adult rice billbugs are both feeding on tillers and laying eggs. It appears that larval feeding occurring in the roots zone is most likely causing the traditional “white heads” associated with rice billbug. When damaged and undamaged plants were compared to one another, the data suggest that rice attempts to compensate for feeding that occurs during the tillering stages by producing a greater number of tillers. However, this compensation was not sufficient and undamaged plants yielded twice as high as damaged plants. These finding will be used as a foundation to create an action threshold for this pest

Furthermore, additional research is needed to better understand the rice billbug. If FIR hectares continue to grow, the amount of rice susceptible to rice billbug injury will increase. Larger areas of susceptible rice may allow rice billbug populations to expand and potentially increase the pests overall population. This survey clearly shows that *S. pertinax* is a major pest of FIR and will likely become the greatest insect pest of this production system.

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Table 2.1 Percentages of survey fields and counties with billbug injury by state for furrow-irrigated rice fields surveyed from 2019 to 2021.

Survey State	Total Fields	Fields with Billbug Injury (%)	Counties Surveyed	Counties with Billbug Injury (%)
Arkansas	64	81	12	92
Missouri	8	50	3	67
Louisiana	6	0	3	0
Mississippi	2	0	2	0

Table 2.2 Percent of fields surveyed with positive rice billbug injury based on field surroundings for furrow-irrigated rice fields surveyed from 2019 to 2021.

Field Edges	Fields with billbug injury (%)
Production crop only	22
No grassy borders	9
Grassy borders	82
Tree line	66

Table 2.3. Percent of fields surveyed with positive rice billbug injury based on rice growth stage for furrow-irrigated rice fields surveyed from 2019 to 2021.

Rice Growth Stage	Percent of Fields with Injury (%)
Seedling	0
3-4 Tillers	60
5-6 Tillers	23
Green Ring	9
Boot	8

Table 2.4 Comparision between rice billbug injured and uninjured plants in furrow-irrigated rice fields from surveys conducted in 2020 and 2021.

Plant Type	Mean Total Panicles	Mean Grams per Plant	Mean Grams per Panicle
Uninjured Rice	8.7 b	42.1 a	5.0 a
Injured Rice	11.8 a	24.0 b	2.0 b
df	1, 188	1, 187	1, 187
F	57.9	129.3	456.0
P	<0.01	<0.01	<0.01

Threshed rice samples followed by different a letter are significantly different according to a pairwise t-test at an α=0.05

Figure 1. Insect viewing cages used to monitor rice billbug damage for feeding studies conducted from 2019 to 2021 at the Lonoke Agricultural Research and Education Center (Lonoke, AR) (2019).

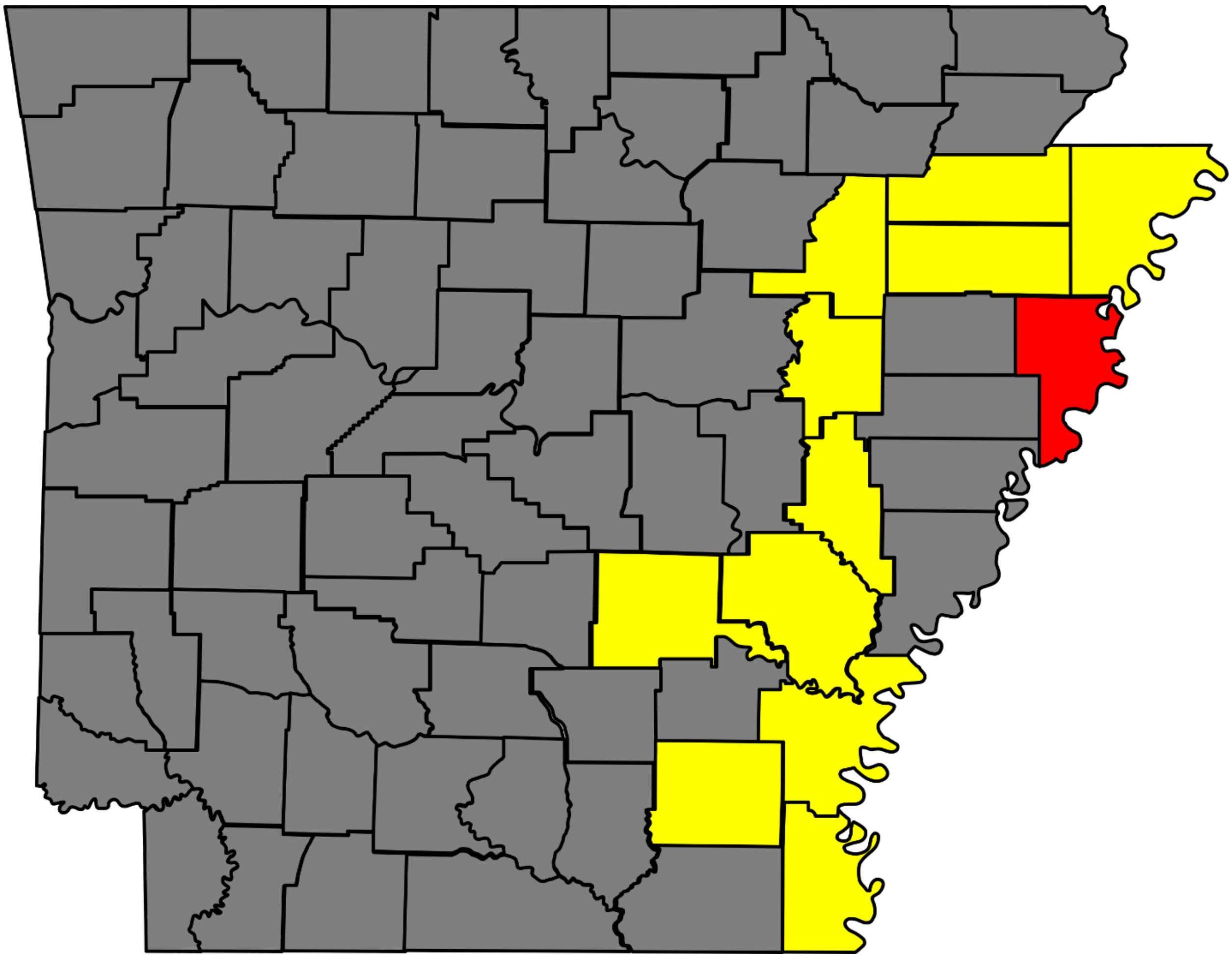

Figure 2. Highlighted counties in Arkansas where rice billbug surveys took place from 2019 through 2021. Counties where billbugs were identified are highlighted in yellow. Counties highlighted in red had no billbug damage in survey fields. Copyright freevectormaps.com

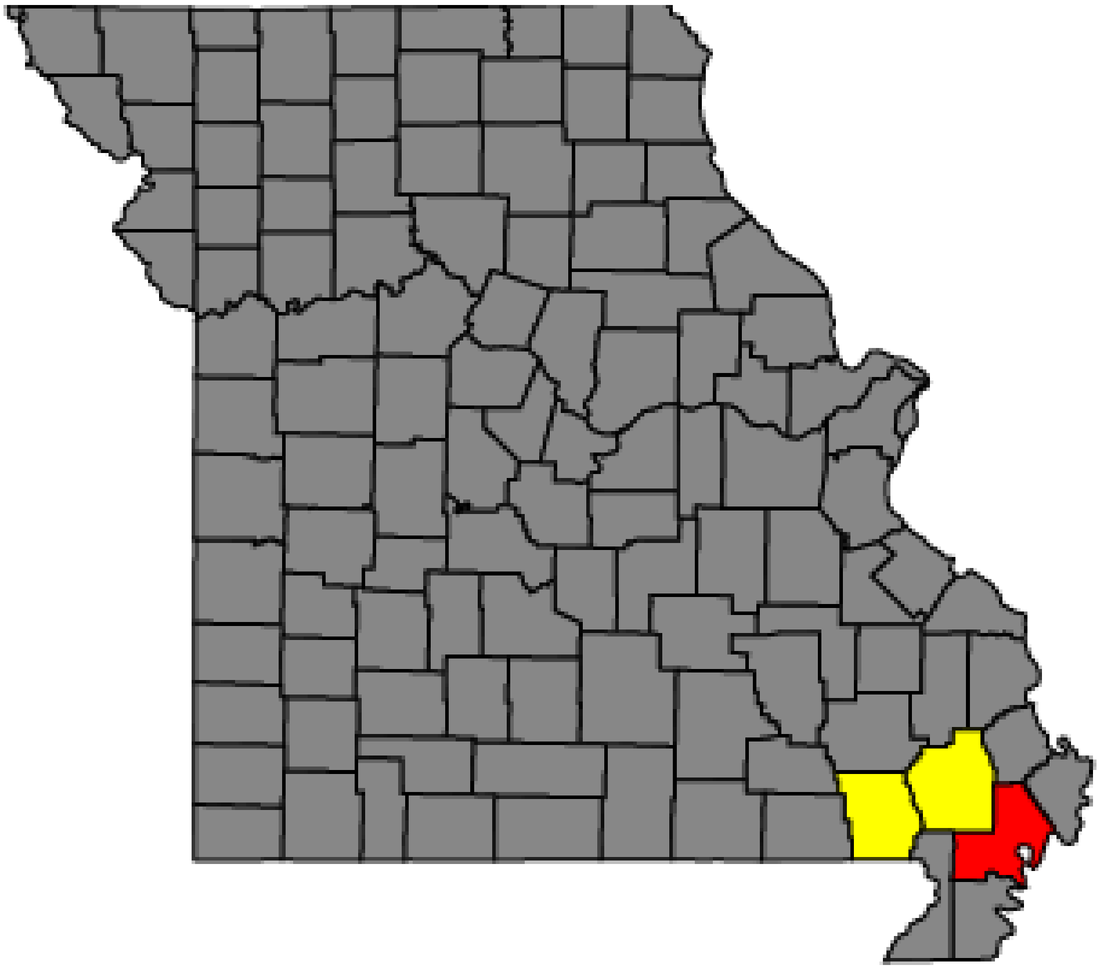

Figure 3. Highlighted counties in Missouri where rice billbug surveys took place from 2019 to 2021. Counties highlighted in yellow signify billbug were identified. Counties highlighted in red had no billbug damage in survey fields. Copyright freevectormaps.com

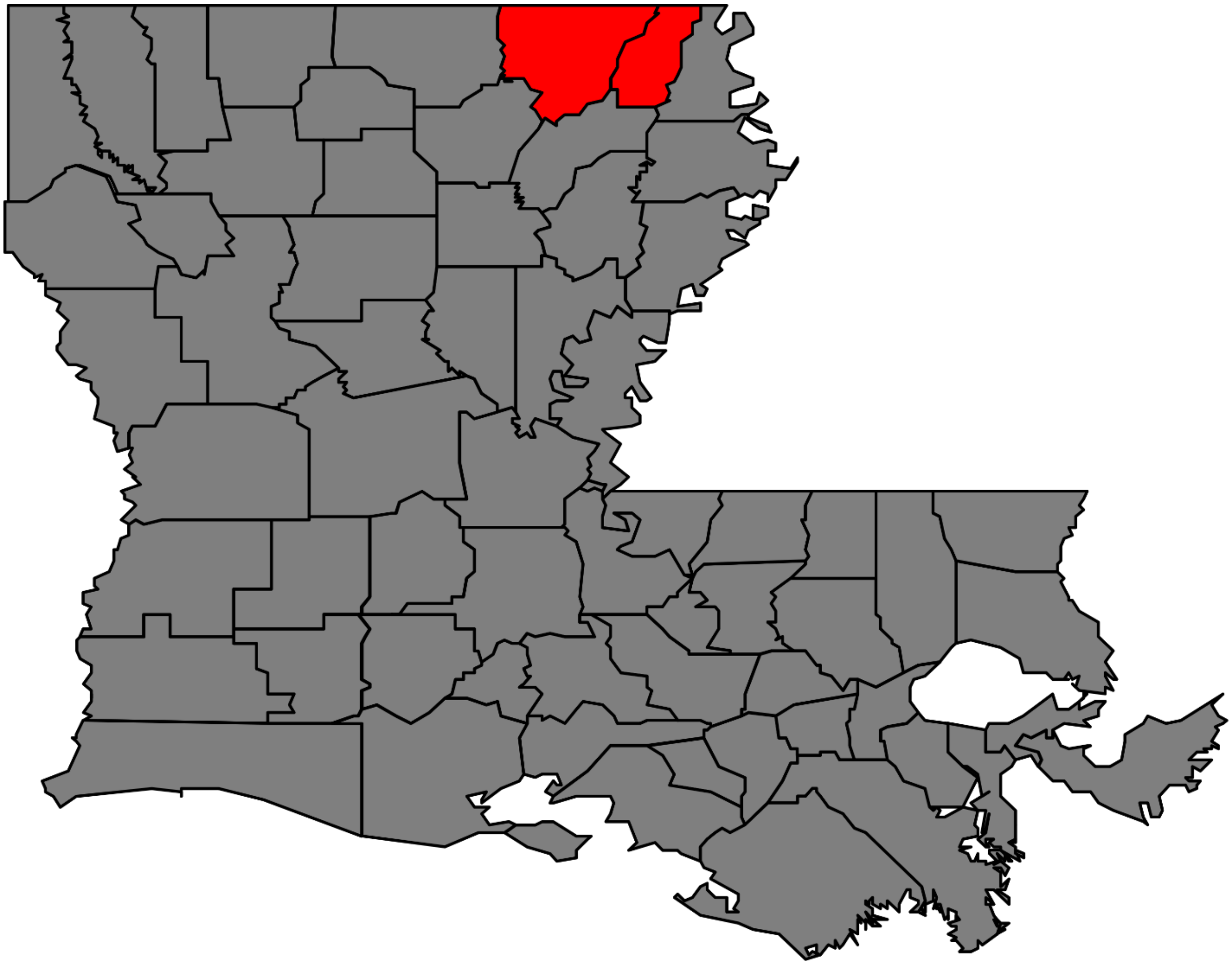

Figure 4. Highlighted counties in Louisiana where rice billbug surveys took place from 2019 to 2021. Counties highlighted in yellow signify billbug were identified. Counties highlighted in red had no billbug damage in survey fields. Copyright freevectormaps.com

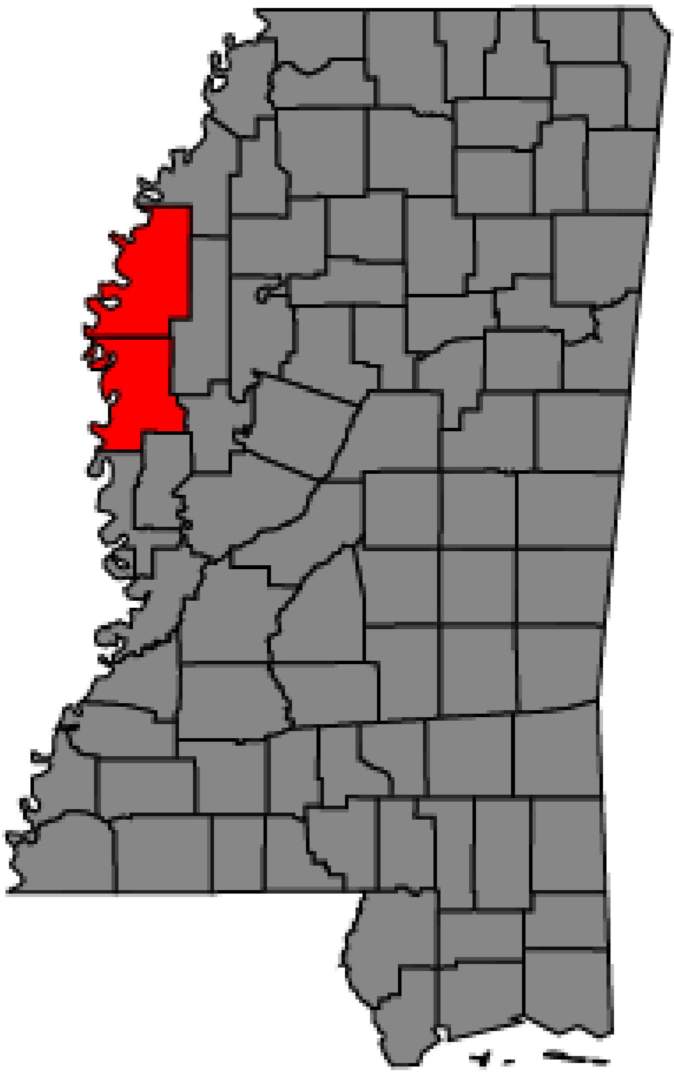

Figure 5. Highlighted counties in Mississippi where rice billbug surveys took place from 2019 to 2021. Counties highlighted in yellow signify billbug were identified. Counties highlighted in red had no billbug damage in survey fields. Copyright freevectormaps.com

Figure 6. Adult rice billbug overwintering underground below bunch grasses on field edge (Almyra, AR) (2020).

Figure 7. Rice billbug larva overwintering underground beneath yellow nutsedge in a furrow-irrigated production field (Macks, AR) (2020).

Figure 8. Adult rice billbug moving below the soil line to begin feeding on the roots (Macks, AR) (2019).

Figure 9. Cross section of a rice tiller with rice billbug larva feeding within rice tiller (McGehee, AR) (2019).

Figure 10. Rice billbug pupae inside root crown of rice plant (Macks, AR) (2019).

Figure 11. Freshly hatched rice billbug larvae, reared from eggs collected in the field and then incubated at the Lonoke Agricultural Research and Extension Center (Lonoke, AR) (2020).

Figure 12. (L to R) Larval and pupal development sampled from furrow-irrigated rice field (Macks, AR) (2019).

Figure 13. Blank rice panicles (white/tan colored) resulting from rice billbug feeding (Almyra, AR) (2021).

Figure 14. Puncture wounds from rice billbug rostrum insertion into base of rice tiller by rice billbug adults (Almyra, AR) (2020).

Figure 15. Characteristic rice billbug damage on young rice from feeding observation studies conducted at the Lonoke Agricultural Research and Education Center (Lonoke, AR) (2019).

Figure 16. Rice billbug egg oviposited into leaf sheath of rice tiller (Macks, AR) (2020).

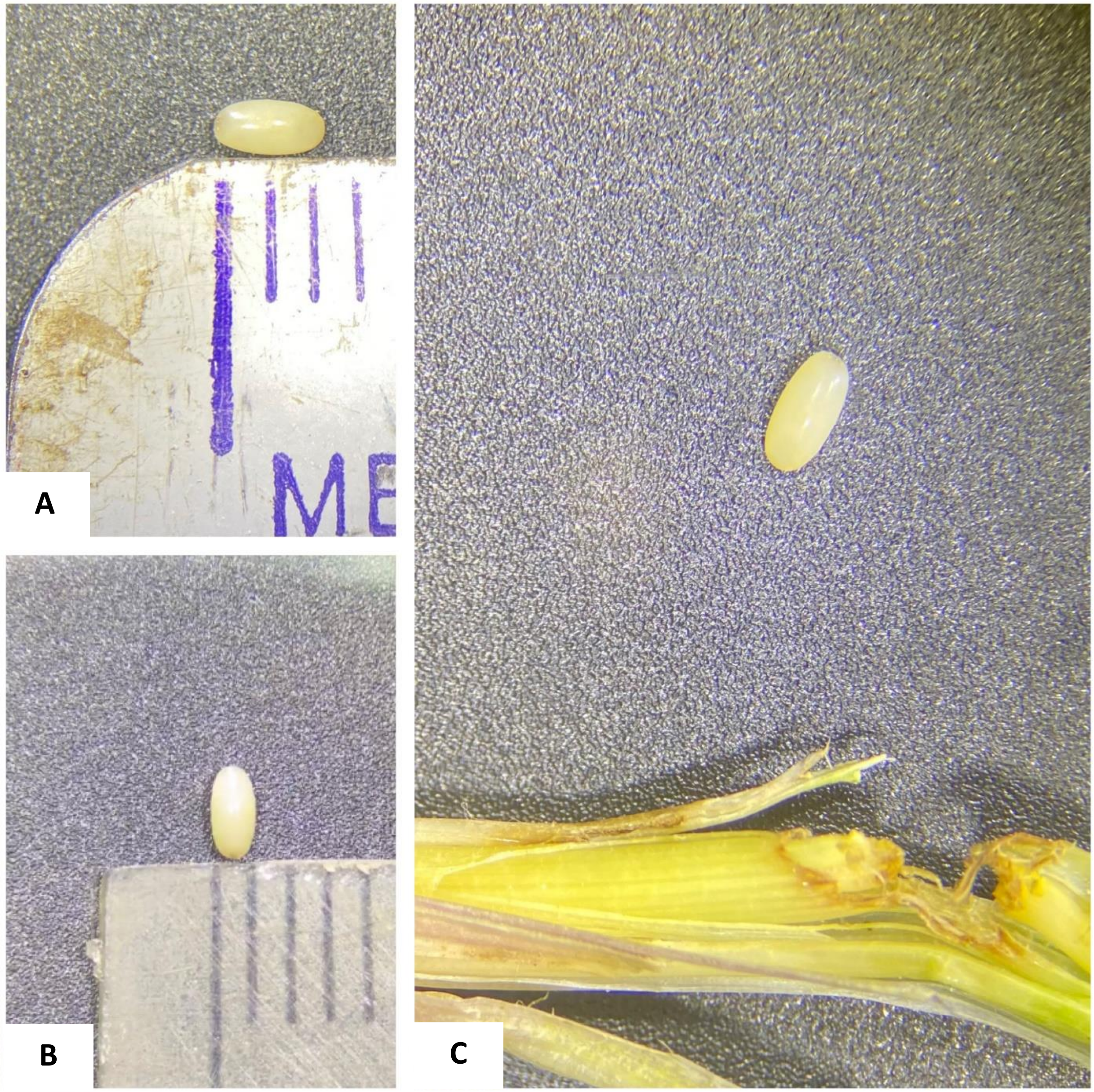

Figure 17. Measurements of rice billbug eggs length (A) and with (B) in millimeters. Extracted billbug egg from rice tiller (C) (Lonoke, AR) (2020).

Chapter 3 - Development of a Monitoring Regime for Rice Billbug, *Sphenophorus pertinax* (Coleoptera: Curculionidae)

Abstract

Furrow-irrigated rice (FIR) production hectares are increasing in Arkansas due to potential cost savings on tillage and levee construction when compared to flood irrigated rice. In a FIR system there is a lack of standing water across the top portion of the field, which increases the fields susceptibility to rice billbug (*Sphenophorus pertinax*). Rice billbugs feed on the roots and tillers of rice plants, causing rice tillers to die and seed heads to abort leading to yield loss. Studies were conducted in three FIR fields in Arkansas to evaluate trapping and monitoring methods for rice billbugs using multiple insect trap styles. Multiple trap styles were used consisting of sticky cards, light traps, flight interception traps, and multiple ground cover methods. Six different colored bucket traps were also tested to document rice billbug response to color. Traps were monitored weekly throughout the growing season to determine the best trap style and when peak populations of rice billbug are in rice. More rice billbugs were trapped with buckets (55% of total rice billbugs caught) than any other trap style. Additionally, no rice billbugs were trapped using flight active traps. Rice billbugs also preferred pink colored buckets compared to all other color options tested. A peak in rice billbug capture occurred from late-May into early-June. During this peak period, close to a 1 to 1 ratio of males to females were observed. Prior to and after the peak, males were the predominate sex observed. It appears that using traps made for ground active insects colored pink is currently the best method for trapping rice billbug. Based on data from these studies, it also appears that there is a window of three to four weeks when both male and female rice billbugs are active in the field.

Introduction

The implementation of an insect monitoring system is one key to a successful IPM program. Monitoring systems can provide information on geographical distribution, effectiveness of control methods, and develop forecasts of potential pest outbreaks (Conway, 1984). Forecasts are a pivotal component of pest management because expected timing and extent of pest attack can make interventions more efficacious (Hill and Waller, 1982). In the Mid-Southern U.S., one of the most utilized insect monitoring systems is for southwestern corn borer (*Diatraea grandiosella*, Dyer) (Lepidoptera: Crambidae). These monitoring systems are designed for conventional (non-*B*t) corn hectares. Corn hybrids containing *B*t genes are protected against corn borers, and monitoring is not required (McLeod and Studebaker, 2003). Using pheromone traps, growers and researchers can predict pest generations and insecticides can be applied when necessary. Corn earworm (*Helicoverpa zea*, Boddie) (Lepidoptera: Noctuidae) is another insect that is monitored extensively with pheromone traps in the Mid-Southern U.S. However, corn earworm is monitored to inform stakeholders that moths are in the area and scouting host crops is warranted, whereas southwestern corn borers traps are used to trigger insecticide applications.

A primary component of insect pest monitoring is an accurate sampling technique to measure the target insect's density within a crop (Dent and Binks, 2020). A large portion of research efforts are directed at the development of sampling techniques and devices for collection. Sampling programs allow researchers to understand baseline ecosystem information by either species abundance or diversity (Hoback et al., 1999). These devices range in levels of complexity from sweep nets and sticky cards to acoustics and thermal imaging (Al-doski et al., 2016). Insect traps are a popular non-labor-intensive method to monitor insects, and have the

ability to collect large amounts of data (Dent and Binks, 2020). Despite insect trapping being the most popular monitoring technique employed by entomologist several problems persist. One of the biggest drawbacks with insect trapping is that values collected are typically relative, which are influenced by environmental factors. Comparison of data from multiple years or regions is difficult if conditions were not similar (Johnson, 1950; Taylor, 1963; Inscoe et al., 1990). Some factors that need to be considered when developing an insect monitoring program are insect locomotion, color preference, and mating patterns (Montgomery et al., 2021).

In the Mid-Southern U.S., Arkansas, Louisiana, Missouri, and Mississippi are prominent rice growing states, responsible for 77% of total rice harvested nationally in 2021 (NASS, 2021). Furrow-irrigated (FIR) production has increased in recent years as rice producers seek a reduced labor and more efficient rice production practice. This system has the potential to reduce fuel costs due to reduced tillage and levee construction. Conversely, moving to a furrow irrigated production system has altered the field environment allowing it to be more favorable to non-typical rice pests. Rice billbug (*Sphenophorus pertinax*, Chittenden) (Coleoptera: Curculionidae) is considered a minor rice pest in the traditional flooded system, typically only found feeding on rice planted on the levees. Findings from Dupuy and Ramirez (2016) on closely related billbug species stated that billbugs typically prefer higher plant density to remain hidden from predators. Without the presence of a flood and high plant density for cover, FIR has become a favorable host for rice billbug. Very little research has been conducted on rice billbug biology and monitoring, and fundamental research is needed to understand the impact and yield loss associated with rice billbug in a FIR system.

Limited research has been published on the rice billbug and none refer to monitoring regime. Understanding insect locomotion can provide information to implement correct

placement and style of insect collection devices. In the Mid-South, the most common weevil species in rice is rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) (Coleoptera: Curculionidae). Rice water weevil's primary locomotion method is flight, using the trajectory provided from trees to disperse into flooded rice. Based on rice water weevil collection data from Zou et al. (2004) using light traps, and an understanding of their attraction to open water, researchers have hypothesized that reflection of moonlight on standing water in the paddy is a potential trigger for migration into the field. The preferred way of locomotion for rice billbug needs to be solidified to manufacture a proper collection trap.

Trap coloration can also contribute to a successful trapping regime. Influence of trap color and trap placement have been explored for economically important pests. (Hoback et al., 1999). Prokopy et al. (1983) state that insects respond to color is based on trap position, ground composition, quality of wavelength hitting the trap, and lastly the physiological state of the insect. Anecdotal observations suggest that pink may attract rice billbug. Rice billbug have been found on beehives that were painted pink, so that agriculture pilots could easily identify the hives and mitigate insecticide exposure. Further research was needed to confirm this observation and determine if rice billbug shows preference to colored traps. The objective of these experiments was to develop a monitoring regime for rice billbug.

Materials and Methods

Site Description and General Agronomic Management

An experiment was conducted at twelve FIR locations across Arkansas during the 2019-2021 growing seasons, however data was only collected from one location in Jackson County Arkansas for three consecutive growing seasons. This was due to no rice billbugs being present

at the other nine locations. RiceTec [RT] CLXL745 hybrid (2019), RT7301 (2020), and RT7321FP (2021) were selected for their high rice blast resistance and were planted at a rate of 24.7 kg/ha. All other crop management practices, excluding insect management, were conducted based on University of Arkansas' System Division of Agriculture furrow-irrigated rice Handbook suggestions (Hardke and Chlapecka, 2019).

Trap Design

Eight styles of traps were evaluated to determine the best method for monitoring rice billbug entering the field, these included: colored buckets, pitfall traps, several ground cover methods, flight interception traps, light traps, sticky cards, and pyramid traps. Traps were placed on the turnrow, in between overwintering sites, such as tree lines or turnrows with uniform bunchgrasses and a furrow-irrigated rice field. All traps were placed in an area where shade could not compromise quality of wavelength on to the traps. Monthly, grasses around traps were controlled mechanically with a string trimmer (Echo-USA; Lake Zurich, IL) (PAS-225VP) to provide better visibility of traps by removing weeds in circumference of 2 m around each trap.

Bucket

A series of six buckets (31.8 cm x 31.8 cm x 38.1 cm) (Uline; Pleasant Prairie, WI) were placed on the rice field edge separating the overwintering site from the production field. Six colors; pink, green, blue, orange, yellow, and gray, were placed in randomized order along the tree line and were replicated four times at each location. Buckets were moved laterally each week to allow fresh grass to remain under the buckets. Each bucket was checked weekly, and specimens were collected from the grass under each bucket (Figure 1). All buckets regardless of color were treated as one treatment, when analyzing differences in trap style.

Pitfall Trap

Four linear pitfall traps were buried in the plant bed closest to the turn row, with the top of the trap level with the soil surface. The design was modified from the original design by (Pausch et al. 1979). Pitfall traps were made from 10.2 cm PVC pipe that was 1.2 m in length and with a 3.8 cm slit cut in the top and capped at one end. The other end was equipped with a plastic collection container. Modifications to the original design were warranted due to the soil being disturbed from tillage practices, and loss of soil sediment from irrigation events would compromise the traps efficacy. Aluminum ramps were constructed to match the length of the PVC pipe and then treated with a spray rubber sealer to provide grip for insects. The ramps were then installed on both sides of PVC pipe prior to deployment into the field. Linear pitfalls were buried at a slight angle where the lowest point of the grade leads to the collection container and, ramps were adjusted to be flush with the soil line and were readjusted weekly if required. Insects that fall into the trap are forced to travel into the collection container (Figure 1.2).

Ground Cover Methods

A series of different materials were placed along the field edge and monitored weekly to determine if the billbug adults would seek cover under the materials. A 2.4 m x 2.4 m tarp was spread tightly and staked into the ground on top of the soil surface of the turn row. Multiple pieces of plywood, in 0.9 m x 0.9 m sections, were placed on turn rows as well as 1.2 m segments of 10.2 in PVC pipe sections that were painted pink.

Flight Interception Trap

Additionally, two flight interception traps were constructed and placed at each experiment location to account for billbug using flight to enter the field (Figure 1.3). Reports of

species like rice billbug have been observed as weak fliers Traps were designed so, if flying, a billbug would hit the trap screen and fall into the collection trough below. A screen approximately 2.3 m in height and 1.1 m in width was placed in between assumed overwintering sites and production fields. Each trap was equipped with a collection trough placed on each side of the screen, containing a non-toxic pink propylene glycol solution.

Light Trap

Another trap implemented was a universal light trap (2851A) (BioQuip, Rancho Domingo, CA) containing a halo fluorescent black light. Bulbs were controlled by photoelectric sensors that respond to changes in sunlight. Photoelectric sensors were connected to a deep cycle marine battery, which provided sufficient power between collections. Batteries were replaced and recharged weekly throughout the experiment. The bucket was modified with an aluminum funnel to collect specimens within the bucket, and a non-toxic pink propylene glycol solution was placed inside the bucket. Two light traps were placed at each location.

Sticky Cards

Four replications of sticky cards were placed on a wooden post at 0.9 and 2.1 cm from the soil surface and were distributed evenly throughout the top two-thirds of the field. Yellow 15.2 cm x 30.5 cm (GL-1080) and orange 22.9 cm x 38.1 cm (GL 1060) sticky cards (Great Lakes IPM, Vestaberg, MI) were placed on alternating posts. Sticky cards received additional applications of insect collection adhesive. Sticky cards were replaced weekly.

Pyramid Traps

Two black pyramid insect traps (GL 5000) (Great Lakes IPM, Vestaberg, MI) were placed along the field edge. The traps were made of black corrugated plastic triangles standing

1.2 m in height and staked into the soil. Pyramid trap design is intended to lure insects upward once they land on the trap. A plastic collection jar at the top of the trap encloses insects inside until collection counts can be taken.

Data Collection

Traps were checked weekly for sixteen consecutive weeks starting the first week in May through the last week in August. Rice billbug collections dramatically decreased for the month of August; data was analyzed using only the first twelve weeks to properly analyze collection numbers.

Specimens were sexed by guidelines established by Chittenden (1905), then collected and stored for greenhouse experiments. During collection, total number of rice billbugs and sex ratio were documented for each individual trap. Total collection for each week were dated and utilized in observations for a better understanding of when rice billbug moves into production fields. Propylene glycol solution was replaced in flight interception collection troughs, and traps were readjusted to contain fresh grass under them for a viable food source.

Statistical Analysis

A randomized complete block design was utilized in these experiments. In the color experiments each color was replicated four times. When analyzing trap design replications varied, but all treatments covered equal amounts of surface area. Statistical analysis was completed using the PROC GLIMMIX procedure in SAS (v. 9.4, SAS Institute Inc., Cary, NC). Site year and replication were treated as random effects. Data were pooled over all locations and years and means separated using Multiple Pairwise t-Tests with significance level at $\alpha=0.05$.

Results

Color Preference Analysis

No interaction was observed between color and environment in this study (df=10,15; F=211.3; *P*=0.07). When analyzing main effects, differences were observed when analyzing color preference in the trap study (df=5,71; F= 2.8; *P*=0.02) (Table 3.1). Buckets that were colored pink had consistently greater numbers of rice billbug gathered under them than all other color variations aside from gray. No difference was observed for the color gray between all other colors.

Trap Style Analysis

No interactions were observed between trap style and environment (df=16,41; F=198.76; *P*=0.09), When analyzing different methods of trapping rice billbug differences between traps were observed (df=8,16; F=13.8; *P*<0.01) (Table 3.2). Bucket traps and collection troughs generated the highest numerical percentage of billbug specimens collected, and responsible for 84% of all specimens collected. Traps designed for ground active insects collected 99% of the total rice billbugs trapped for all site years. This observation agrees with findings that were made in the color preference experiment for rice billbug. Collections traps designed for flight-active insects were not efficacious by intended design. Every billbug specimen collected in traps designed for flight were found hiding under the trap. No billbugs were ever found inside the light trap but were rather found underneath the collection bucket. These data suggest collections made with traps designed for ground active insects are better for monitoring billbug than those designed for more flight prone insects. These findings suggest that rice billbugs are likely crawling to infest rice fields rather than flying.

Sex Ratio

No interaction was observed between rice billbug sex and site-year (df=2,55; F=0.27; P=0.8). Differences were observed when analyzing sex of rice billbug collected in trapping studies (df=1,59; F=5.29; P=0.03). More rice billbug males (μ=6.6; SEM=1.2) were trapped than females (μ=3.6; SEM=1.3).

When analyzing data of sex ratio collection across sampling times male rice billbugs (df= 1,59; P=0.02; r^2=0.7) were predominantly collected prior to highest weekly rice billbug catches for all three years of the study (Figure 6). Female rice billbug (df= 1,51; P=0.02; r^2=0.6) correlations trended similarly, but at a lesser numerical value (Figure 7). When observing the sex ratio over time of the trials, weeks resulting in the highest total rice billbug collections the ratio between male and female was almost a 1:1 ratio. An influx of female rice billbugs collecting from traps began the last week in May for four consecutive weeks, and last documented female billbug collected was the first week in July.

Sample timing

A relationship was observed between trap collection and sample timing (df=22,35; P<0.01; r^2 *=0.7)*. Regression analysis suggest that billbug numbers peaked around the week 24 (2nd week in June) (Figure 6). Year was significant in rice billbug sample timing (df=2,35; F=37.6; P<0.01). Billbug specimen collected in 2021 (μ=7.0; SEM 1.6) were higher compared to 2020 (μ=4.8; SEM 1.6) and 2019 (μ=3.58; SEM 1.59). No differences were observed between rice billbug collections when comparing 2019 and 2020.

Discussion

Implementation of insect traps has been a significant contributor to successful pest management decisions (Trematerra 2013). Successful trapping can help determine proper timing

of insecticide applications. When developing a trap to collect rice billbug, data from these studies suggest the color pink should be utilized. Several weevil species have been documented to respond to different colors. Leggett and Cross (1978) documented boll weevil (*Anthonomus grandis,* Boheman) (Coleoptera: Curculionidae) responded to trap color with-out the presence of pheromones. Research from Gadi and Reddy (2014) stated that sweetpotato weevils (*Cylas formicarius*, F.) (Coleoptera: Brentidae) preferred red traps over any other color tested, and the lightest shade of red was selected the most frequently. These findings concur with results from the rice billbug color preference experiments.

These data suggest that the traps should be designed for ground active insects. These findings concur with the results of Kindler and Kinbacher, (1982) and Tashiro and Personius (1970) who state that the genus *Sphenophorus* are predominantly ground active. Crawling appears to be the predominant means of locomotion for rice billbug however, during these studies rice billbug was observed flying short distances on occasion.

Results and observations from analyzing the sex ratio during the monitoring period indicate that male rice billbugs are moving into the production fields prior to females. These findings are in agreeance with those of Bandeira et al. (2021) stating that in most weevil pests, aggregation pheromones are produced by the males to signal both male and female weevils. This could explain the gradual incline in male rice billbug collected in traps followed by all female rice billbugs being collected in a more restricted four-week window.

Conclusions

As FIR hectares continue to increase, and rice billbug populations become more prevalent, trapping regimes will need to continue to be refined. Research is needed to determine

if an insect pheromone in conjunction with traps could be used to properly monitor rice billbug populations. Successful trapping of rice billbug traps could also lead to more precise foliar insecticide applications, increasing their overall efficacy. Several observational findings from these experiments should be considered when monitoring for rice billbug. First, bunch grasses should always be under traps that are not buried to soil level. Rice billbugs appear to like to be hidden, having living plants under the traps allows billbugs to be hidden and increases the likelihood of the specimen remaining under the trap. Slightly shifting the trap to fresh grass approximately every ten days allows traps to remain viable throughout the trapping timetable.

Another observation made was rice billbug adults show preference to subsoil environments. Many specimens were collected in holes dug under the trap. A researcher monitoring rice billbug should consider thoroughly scouting under traps and making sure specimens are not attempting to move subsurface. A significant observation made while conducting this experiment is the possibility of utilizing poly-plastic irrigation tubing as a monitoring tool for rice billbug. The greatest number of billbugs collected throughout this study came from lifting irrigation tubing and collecting specimens from underneath. Data from the trapping studies show that rice billbug prefer to be hiding in protected sites. Many times, billbug were not found in the traps, but rather hiding beneath them. This could be due to their glossy exterior in sunlight making them easier to be seen by predators. Poly-plastic irrigation tubing is one of the most common irrigating methods in FIR. The tubing stretches across the entire upper management zone, providing a large refuge for rice billbug. This observation was first made in 2021 when the last location of the trapping test was conducted. Continued research should include a trap utilizing irrigation pipe as a monitoring method.

Overall, data collected from these experiments have created a foundation for rice billbug monitoring in the future. Pin-pointing rice billbug primary locomotion and preferred colors has created a baseline for research to discover the most efficacious monitoring regime. Future research on billbug monitoring should consider ground active insect trap designs along with the implementation of the color pink for collection optimization.

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Table 3.1 Mean number of rice billbug, *Sphenophorus pertinax*, caught weekly using six different bucket trap colors in Jackson County Arkansas during 2019-2021 growing seasons.

Bucket Color	Mean Billbug Caught Weekly
Pink	11.2 a
Gray	6.8 ab
Orange	5.0 b
Green	4.7 b
Yellow	4.2 b
Blue	3.8 b
df	5,7
F	2.8
P	0.02

Weekly rice billbug catch averages followed by different a letter are significantly different according to a pairwise t-test at an α=0.05

Table 3.2 Mean number of rice billbug, *Sphenophorus pertinax*, caught weekly using eight different trap styles in Jackson County Arkansas during 2019-2021 growing seasons.

Trap Style	Mean Billbug Caught	Percentage of Total Catch (%)
Buckets+	75.0 a	55
Trough+ (Flight Interception)	41.0 b	29
Pitfall+	11.3 c	14
Tarp+	2.0 d	1
Ground Cover Material+	1.3 d	<1
Light Trap*	0.6 d	<1
Flight Interception*	0.0 d	0
Sticky Cards*	0.0 d	0
Pyramid Trap*	0.0 d	0
df	8,16	-
F	13.75	-
P	<0.01	-

Weekly rice billbug catch averages followed by different a letter are significantly different according to a pairwise t-test at an α=0.05
*Indicates trap was designed for flight active insects
+Indicates trap was designed for ground actives insects

Figure 1. Bucket traps placed along the turnrow of a furrow-irrigated rice field (Arkansas County, AR) (2019).

Figure 2. Linear pitfall trap installed in a furrow-irrigated rice field to monitor for rice billbug (Jackson County, AR) (2021).

Figure 3. Flight interception traps and collection troughs on the edges of a furrow-irrigated rice field to monitor for rice billbug (Jackson County, AR) (2019).

Figure 4. Bucket light traps, and sticky cards being placed on the edge of a furrow-irrigated rice field (Jackson County, AR) (2019).

Figure 5. Example of pyramid traps used to monitor for rice billbug in experiments conducted from 2019-2021.

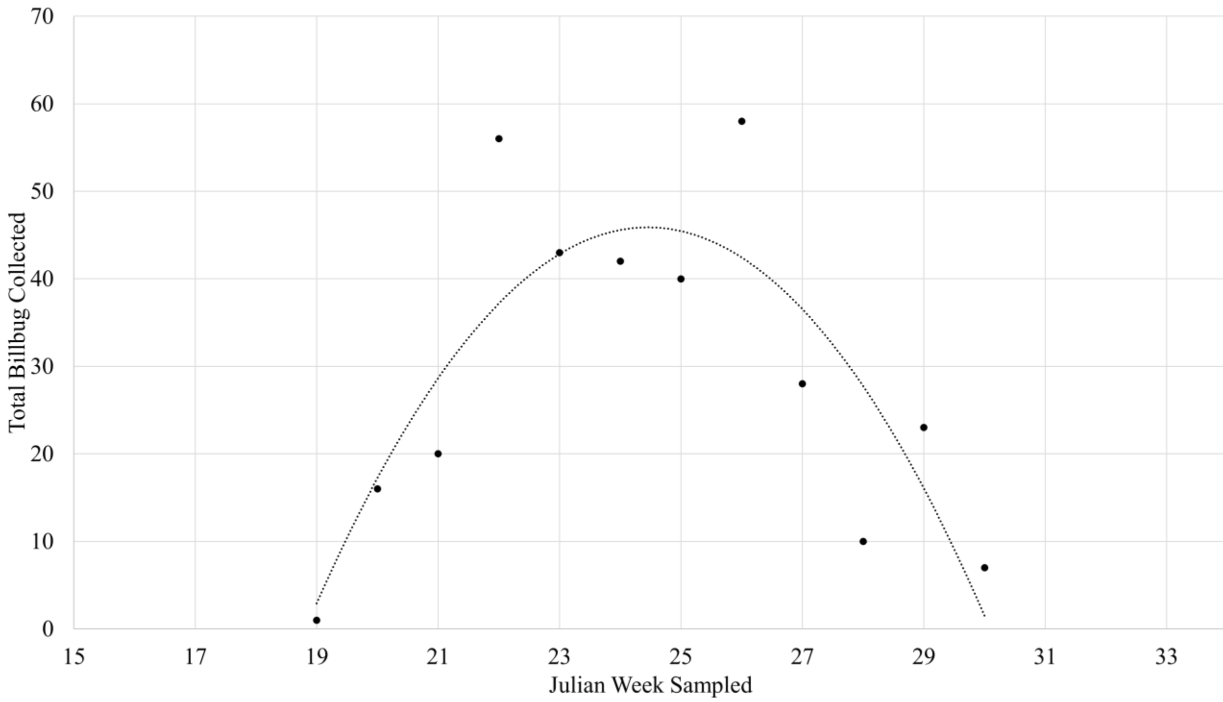

Figure 6. Quadratic regression for sampling timing by total billbug caught for trapping experiment conducted from 2019-2021.

The equation is $y=1.44x^2 +18.64x + (-14.27)$ with a *P*-value <0.01. y=total billbug collected and x=sample timing. Sample timing began the first week in May and continued for twelve consecutive weeks ending during the 4th week in July.

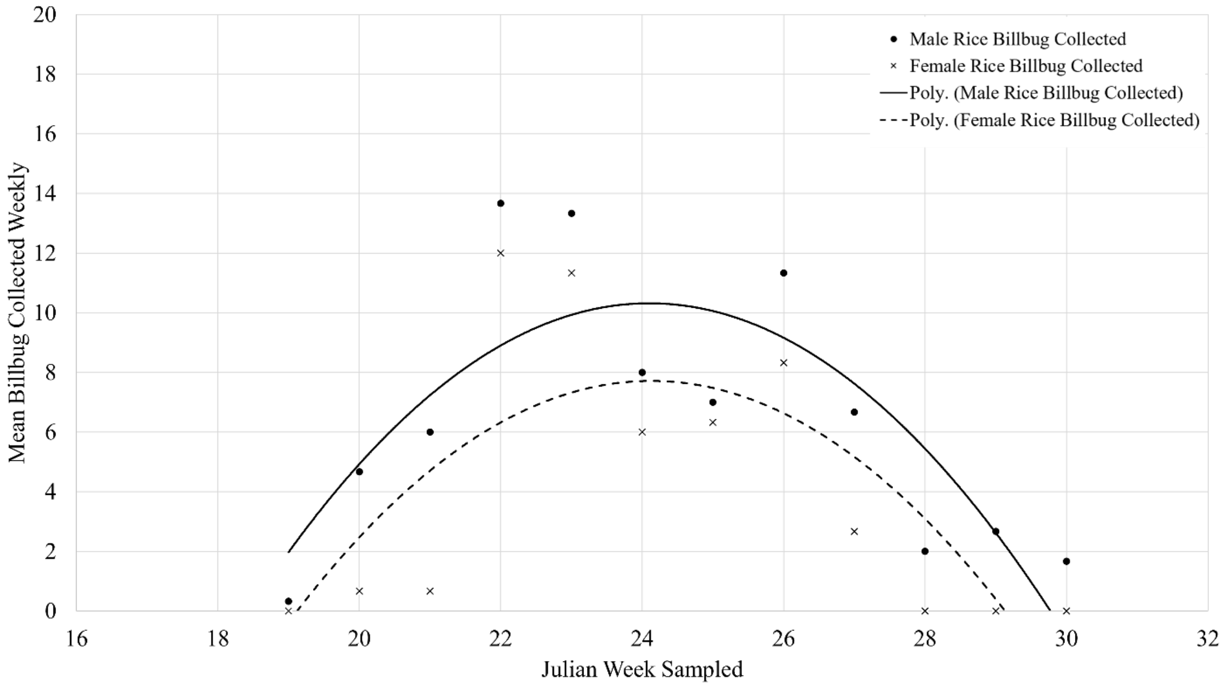

Figure 7. Quadratic regression for sampling timing by each sex for trapping experiment conducted from 2019-2021.

The equation for male rice billbug collections is= $-0.32x^2 +3.91x + (-1.62)$ with a *P*-value =0.02. y=total billbug collected and x=sample timing by Julian calendar week. Sample timing began the first week in May and continued for twelve consecutive weeks ending during the 4th week in July.

The equation for female rice billbug collections is= $-0.30x^2 +3.77x + (-3.84)$ with a *P*-value =0.01. y=total billbug collected and x=sample timing by Julian calendar week. Sample timing began the first week in May and continued for twelve consecutive weeks ending during the 4th week in July.

Chapter 4 – Efficacy of Insecticides for Control of Rice Billbug, *Sphenophorus pertinax* (Coleoptera: Curculionidae)

Abstract

Arkansas rice producers have increased furrow-irrigated rice (FIR) production hectares to reduce labor and tillage. The elimination of a flood across the field has made rice more susceptible to rice billbug (*Sphenophorus pertinax*). Rice billbug feeds on the roots and tillers of rice plants, causing tiller death and aborted heads, ultimately resulting in indirect yield loss. Multiple experiments were conducted during the 2020-2022 growing seasons to evaluate application methods, timing, and efficacy, of selected insecticides for control of rice billbug. Insecticide seed treatments, foliar insecticide applications, and insecticide coated urea fertilizer were all evaluated to determine the best application method to manage rice billbug. Two sampling methods were tested to correlate rice billbug damage to grain yield. When rice reached panicle internode elongation, rice was sampled by counting total tillers and damaged tillers in 1.5 row meters per plot. After panicle emergence, the number of blank heads per 1.5 row meter within a plot were also recorded. A relationship was observed with all tested variables and grain yield. Confirming both tiller as well as blank heads need to be addressed when assessing billbug injury. Plots with a seed treatment containing a neonicotinoid in combination with Fortenza®, as well as Dermacor® in combination with CruiserMaxx®, resulted in yields greater than the untreated check or CruiserMaxx® alone. None of the tested foliar insecticides were successful in reducing tiller damage associated with billbug feeding. Results from these studies suggest using an insecticide seed treatment containing a diamide currently provides the greatest suppression for rice billbug.

Introduction

Over the past five years, furrow-irrigated rice (FIR) hectares have been increasing in Arkansas (Hardke and Chlapecka, 2019). In this production system, standing water is not present across a large portion of the field. This change has altered the pest complex for rice. For example, in a traditional flooded rice system rice billbug (*Sphenophorus pertinax*, Chittenden) (Coleoptera: Curculionidae), has been considered a minor insect pest, typically only feeding on rice plants found on levees. Rice billbugs are restricted to rice on the levees in these fields because the flood impedes their ability to feed. Because FIR has changed irrigation practices, these fields are now susceptible to rice billbug injury.

Prior to 2018, essentially no research had been conducted on rice billbug, due to its inability to distribute into rice planted in the traditional paddy system which made it a pest of minimal concern. Developing best management practices for rice billbug in FIR is imperative as the popularity of this production system continues to increase. Currently, limited research has been conducted to determine the efficacy of insecticides or the best delivery methods for control of rice billbugs in furrow-irrigated rice.

Insecticide Seed Treatments

Rice insects are primarily managed through chemical control methods in the U.S., though alternative cultural or biological control methods are being assessed (Way 1990). In Arkansas, two of the three major rice insect pests, rice water weevil (*Lissorhoptrus oryzophilus*, Kuschel) (Coleoptera: Curculionidae) and grape colaspis, (*Colaspis brunnea*, F.) (Coleoptera: Chrysomelidae) feed on rice prior to heading. In Arkansas, an estimated 70% of all rice hectares

are threatened by infestations of grape colaspis and almost all rice hectares are threatened by rice water weevil infestations (Lorenz, 2017).

Various forms of insecticide seed treatments have been used to control rice water weevils (Bowling, 1968) and are currently the most reliable method of controlling these pests. During 2020, approximately 85% of Arkansas rice hectares implemented an insecticide seed treatment to provide protection of seedling and mid-season rice (Bateman et al., 2020). Currently, the most used seed treatments in rice belong to the neonicotinoid insecticide class, including thiamethoxam (CruiserMaxx®, Syngenta Crop Protection AG, Basel, Switzerland) and clothianidin (NipsIt Inside®, Valent U.S.A., San Ramon, California). These seed treatments are used on 95% of hybrid and 65% of pureline hectares in Arkansas (Bateman et al., 2020).

In 2010, chlorantraniliprole (Dermacor X-100®, Corteva Agrisciences Johnston, IA), a diamide insecticide seed treatment, was approved for use in rice. Another diamide, cyantraniliprole (Fortenza®, Syngenta Crop Protection AG, Basel, Switzerland), was approved for use in 2017 for use as a seed treatment in rice. This class of chemistry has a longer residual than neonicotinoids, increasing the ability for growers to maintain control of rice water weevil regardless of planting date. In 2020, surveys from Mid-Southern U.S. rice researchers stated that approximately 10% of hybrids and 2% of pureline hectares in Arkansas were treated with a diamide seed treatment (Bateman et al., 2020). This low adoption rate is likely because of the increased cost of diamide seed treatments over neonicotinoid seed treatments. Neonicotinoid seed treatments also provide the better control of grape colaspis in rice than diamide seed treatments, but have reduced rice water weevil efficacy under severe infestations (Hummel et al., 2014, Wilson et al., 2021). In addition to insect control, other benefits from insecticide seed

treatments on rice have also been observed. Taillon et al. (2016) found that insecticide seed treatments on rice improved stand and 80% of the time increased yields.

Due to the inconsistent rice water weevil control with neonicotinoids, but the need to manage grape colaspis, research has been conducted on combining seed treatments from both classes. McPherson et al. (2019) and Wilson et al. (2021) observed more consistent control of rice water weevil and increased yields when comparing combinations compared to stand alone seed treatments. Plummer et al. 2020 observed a 1919.9 kg/ha yield increase when using combinations under high densities of grape colaspis and rice water weevil. Felts et al. (2018) found that combinations of neonicotinoids and diamide seed treatments resulted in higher yields than standalone insecticide seed treatments in FIR that was infested with rice billbug. This suggests that there may be some level of rice billbug control with insecticide seed treatments.

Foliar Applications of Insecticides

In the southern U.S. rice producing states, foliar insecticide applications are utilized to control both major and minor pest of rice (Hummel et al., 2014). Rice stink bug, *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae), is the most common insect controlled with foliar insecticide applications in Arkansas, with 40% of all hectares receiving at least one foliar application to control this pest in 2020 (Bateman et al., 2020). Lambda-cyhalothrin is the primary insecticide used to control rice stink bug, and limited alternatives are currently available. Rice water weevil can also be managed with foliar applications of insecticides such as lambda-cyhalothrin by targeting adults, but control is often inconsistent. Foliar insecticides are also used to control most lepidopteran pests such as fall armyworm (*Spodoptera frugiperda*, J.E. Smith) (Lepidoptera: Noctuidae), and true armyworm (*Pseudaletia unipuncta*, Haworth) (Lepidoptera: Noctuidae) in rice.

Due to a dearth of published data on billbug, a need to evaluate various foliar insecticides at multiple application timings is needed. Foliar applications of diamide products have been used for successful control of species of billbug commonly found in turf. Findings from Reynolds and Brandenburg (2015) show that diamide applications made before oviposition were able to suppress billbug damage in turfgrasses. In rice, a study in Louisiana found that rice billbug was suppressed from a clothianidin (Belay®, Valent U.S.A., San Ramon, California) application at early boot stage (Villegas et al., 2021).

Insecticide Coated Urea

Applications of nitrogen (N) are crucial to maximizing rice yields (Norman et al., 2003; Walker et al., 2008; Mandana et al., 2014; Chlapecka et al., 2021). This vital nutrient is also one of the easiest elements that can be lost in the soil profile, due to ammonia volatilization and nitrification-denitrification in rice soils. When implementing a FIR production system, nutrient requirements are slightly more intensive, with up to five applications of supplemental nitrogen being warranted depending on the grade of the field (Hardke et al., 2017, Chlapecka et al., 2021).

Coating granular fertilizers with pesticides could be a potential carrier to overlap residual pesticides. In rice, granule fertilizers have been documented as the best carrier for the herbicide bensulfuron methyl (Londax®, UPL, Cary, NC) for control of ducksalad (*Heteranthera limosa)* ((Sw.) Willd) (Braverman, 1995). In Texas, an 80% reduction in carmine spider mite (*Tetranychus cinnabarinus,* Boisduval) (Trombidiformes: Tetranychidae) populations was observed from insecticide coated fertilizer applications (Ridgway et al., 1967). Previous studies in Texas have also stated decreases in rice water weevil larval numbers from fipronil (Icon®, BASF Corporation, Florham Park, New Jersey) coated fertilizer (Way and Wallace, 1996).

There are several advantages of utilizing pesticide coated fertilizers in an IPM regime with the primary advantages being reduced application costs and lessening soil compaction, by eliminating additional trips across the field. A possible risk of coating urea with an insecticide is localizing the insecticide to the sub-soil as the plants uptakes nutrients from the roots. This could possibly restrict the amount of active ingredient needed to kill insects feeding above the soil surface. Rice billbug adults oviposit into the base of the rice tiller and then move into the soil to feed on the roots. Once hatched, the larvae will feed into the soft juvenile tissue of the rice plant ceasing nutrient flow and overall growth and development. The larvae will hollow out the tiller base and feeding into the crown of the rice plant near the rootzone. This makes rice billbug more difficult to control with contact insecticides, and systemic insecticides may prove more successful.

The efficacy of insecticide coated urea efficacy in rice may be greater when applied to early and mid-season rice. The ability to frequently apply insecticides can allow for overlapping residuals, which may protect the plants for an extended time throughout the growing season. In FIR production systems, Chlapecka et al. (2021) found that three way splits of nitrogen during midseason, depending on soil type, is best strategy to maximize yield. Using fertilizer applications as a carrier for insecticides could decrease application costs by eliminating a separate application for just the insecticide.

Published sampling methods for billbug species

The ability to assess a pest population is critical to developing pest control strategies, development of sampling methods and measures that allow researchers to predict a value loss to the crop (Pedigo and Buntin, 1994). When conducting foundational research on an insect, one of

the biggest concerns is creating a sampling procedure to accurately assess their level of impact on the crop.

Several other species in the genus *Sphenophorus* are commonly found in turfgrasses and sod production throughout the United States (Johnson-Cicalese et al., 1990; Dupuy and Ramirez, 2016).

The hunting billbug, *Sphenophorus venatus vestitus* (Chittenden) is considered the most damaging insect pest of sod production. The use of linear pitfall traps for ground active adults and soil cores for larval stages allows aggregated portions of production fields to be managed with insecticides (Gireesh et al., 2021). Though larval stages are the most damaging, concerns about destructive sampling negatively impact playing surfaces (Dupuy and Ramirez, 2016).

Limited research has been conducted on the development of a sampling strategy for rice billbug. Studies in Louisiana suggest that damage from rice billbug can result in early plant death and blank panicles (Villegas et al., 2021). Damage ratings taken in this study were done by recording the number of blank heads per plot. This does not account for the initial feeding seen prior to heading. Observations made by Floyd et al. (2021a) document that early season feeding results in tiller death, and blank panicles sampling alone does not accurately account for this. To properly assess yield losses, it is hypothesized that both tiller damage and blank panicles should be documented.

Materials and Methods

Site Description

All experiments were conducted during the 2020, 2021, and 2022 growing seasons at nine site-years (location by year). Only four of the nine locations had signs of billbug injury and

were used in the analysis. All field information such as planting date, soil type, seeding rate, and hybrid selection can be referenced in Table 4.1. Plot sizes for all studies were 16 rows on 19 cm spacing by 5 m. Fertility, irrigation timings, and herbicide selection for all site-years were based on recommendations from the Arkansas Furrow-Irrigated Rice Handbook (Hardke and Chlapecka, 2019).

Data Collection

Data collection was the same for all experiments. Two sampling methods were evaluated to measure damage associated with rice billbug feeding. For the first sampling method, the total number of uninjured and rice billbug injured tillers was recorded for all plants in 1.5 meters of row per plot at 1.3 cm internode elongation. For the second sampling method, the total number of uninjured panicles and blank panicles were recorded for 1.5 meters of row per plot at the R9 growth stage. Once rice reached harvest maturity, one of the center two beds of each plot was harvested using a Wintersteiger (Wintersteiger AG, Austria) plot combine, equipped with a Harvest Master (Juniper Systems, Logan UT) weight and moisture system. Rice yield was adjusted to 12% moisture content prior to statistical analysis.

Statistical Analysis

All experiments were arranged as a randomized complete block with four replications. Statistical analysis was completed using the PROC GLIMMIX procedure in Statistical Analysis Software (v. 9.4, SAS Institute Inc., Cary, NC). Site-year and replication were treated as random effects. Data were pooled across all locations and years. Means were separated using Multiple Pairwise t-Tests at α=0.05 unless another procedure was specified.

An interaction was observed between treatment and site-year in multiple experiments for multiple sampling methods. Upon further analysis, it was determined that site-year JC-3 followed opposing trends from the other site-years. This was due in large part to a minimal amount of rice billbug presence. This site-year was removed from the data set and all final analysis was conducted on the remaining three site-years.

Evaluation of Insecticide Seed Treatments in Furrow-irrigated rice for Control of Rice Billbug (*Sphenophorus pertinax*).

Field Study

An experiment was conducted to determine the efficacy of insecticide seed treatments for rice billbug control. All rice was treated with a base fungicide package consisting of sedaxane, mefenoxam, azoxystrobin, and fludioxonil. A total of ten treatments were used in this study. Base insecticide seed treatments consisted of thiamethoxam, clothianidin, chlorantraniliprole, and cyantraniliprole. Combinations of base treatments were also included in the study, as well as rice treated with just the base fungicide treatment as an untreated check (Table 4.2).

Greenhouse study

A greenhouse experiment was conducted from 2019-2022 at the Lonoke Agricultural Extension and Research Center in Lonoke, Arkansas to determine efficacy and time to death for rice billbugs exposed to multiple insecticide seed treatments. RiceTec RT7301 conventional long grain hybrid rice was treated with four different base seed treatments. Thiamethoxam, clothianidin, chlorantraniliprole, cyantraniliprole, and a base fungicide package consisting of azoxystrobin, mefenoxam, and fludioxonil as an untreated check. Five rice seeds were planted in 10.2 cm x 10.2 cm x 8.9 cm pots (Greenhouse Megastore, Danville, IL) using a 40/60 mixture of

potting soil (The Scotts Miracle-Gro Company- Landscaping, Marysville, OH) and a silt loam field soil (fine, smectitic, thermic Albaquultic Hapludalf). Once rice plants reached 3 tillers, rice plants were placed in insect viewing cages to acclimate to environment for 48 hours prior to rice billbugs being placed into the cages. This experiment was replicated 10 times with three subsamples per replication.

Rice billbugs were collected from multiple rice fields and sexed based on guidelines of Chittenden (1905). Prior to infestation, male billbugs were isolated for 24 hours to increase likelihood of feeding. One male rice billbug was infested per rice plant and rice pots were placed in 45.7 cm x 45.7 cm x 30.5 cm insect viewing cages and monitored. Specimens were rated for mortality every 72 hours by removing the pot from the viewing cage and observing the rice billbug for movement in the pot. Specimens that were deceased, or lethargic (antennal and appendage response limited) were considered dead. Days to mortality were documented on all pots, up to 40 days after infestation. Data was analyzed using Kaplan-Meier survival curves in JMP 15. Differences in treatment were determined using the Multiple Pairwise t-Tests at α=0.05 in SAS version 9.4.

Evaluation of Foliar Insecticides for Management of Rice Billbug

To determine the efficacy and residual control of foliar insecticides, an experiment was conducted at a total of three locations, spanning across the growing seasons of 2020-2022 implementing a hybrid cultivar. A total of 15 treatments consisting of multiple foliar insecticides as well as insecticide seed treatments were arranged as a randomized complete block design with 4 replications. Three different foliar insecticides were evaluated in this study: lambda-cyhalothrin (Warrior II®, Syngenta Crop Protection AG, Basel, Switzerland), lambda-cyhalothrin + thiamethoxam (Endigo ZCX®, Syngenta Crop Protection AG, Basel, Switzerland), and

chlorantraniliprole (Prevathon®, FMC Corporation, Philadelphia, PA) were independently applied at four timings. The foliar insecticide timings were at planting, 80 to 100% emergence, first tiller, and 4-5 tillers. Additionally, two insecticide seed treatment combinations and a fungicide only seed treatment were evaluated as a comparison to the foliar insecticides. More information on the treatments evaluated are listed below (Table 4.3).

Evaluation of Insecticide Coated Urea for Control of Rice Billbug

An experiment was conducted to determine the efficacy of insecticide coated urea for control of rice billbug. All plots were planted with a hybrid rice cultivar treated with a fungicide seed treatment consisting of mefenoxam, azoxystrobin, and fludioxonil. Multiple insecticides were coated on urea and applied to furrow-irrigated rice plots at multiple timings. Products coated on urea included clothianidin (Belay®, Valent U.S.A.) at 328.7 ml ha^{-1}, cyantraniliprole (Fortenza®, Syngenta Crop Protection AG, Basel, Switzerland) at 241.1 ml ha^{-1}, and zeta-cypermethrin (Mustang®, FMC Corporation, Philadelphia, PA) at 314.2 ml ha^{-1}. Urea applications were made at three timings: 4-5 leaf, 7-10 days after 1^{st} application, and 7-10 days after the second application. These application timings are common in a furrow-irrigated setting (Hardke and Chlapecka, 2019). At each application timing, all plots received an application of urea. Urea (46-0-0 [N-P_2O_5-K_2O]) applications were at a rate of 127 kg ha^{-1} (Table 4.4).

Evaluation of Insecticide Efficacy and Application Methods for Suppression of Rice Billbug

Multiple insecticide seed treatments as well as foliar insecticides were evaluated for the control of rice billbug (Table 4.5). Eight treatments in this study received only an insecticide seed treatment. Base treatments of thiamethoxam, clothianidin, and chlorantraniliprole were used as seed treatments in this study. Additionally, combinations of base treatments as well as base

treatments in conjunction with cyantraniliprole were evaluated. Two treatments received both a neonicotinoid insecticide seed treatment, either clothianidin or thiamethoxam, followed by a foliar application of chlorantraniliprole. Furthermore, a treatment only receiving a foliar application of chlorantraniliprole was included in this study. Lastly, an untreated check was included for comparison that only had a base fungicide seed treatment.

Determining yield loss potential from rice billbug injury

Variables of total tillers, tiller injury, percent tiller damage, blank heads, and percent blank heads were all analyzed for their relationship to grain yield. All relationships across all site-years and experiments were analyzed using the PROC CORR procedure in Statistical Analysis Software (v. 9.4, SAS Institute Inc., Cary, NC). If significant correlations were present, regression analysis was performed using PROC GLIMMIX in Statistical Analysis Software (v. 9.4, SAS Institute Inc., Cary, NC) with site-year and replication considered random variables.

Results

Evaluation of Insecticide Seed Treatments in Furrow-irrigated rice for Control of Rice Billbug (Sphenophorus pertinax).

Field Trial

No interactions were observed between treatment and site-year with respect to all sampling variables aside from grain yield (Table 4.6). This was influenced due to higher yields at the JC-2 location. Because this was the only interaction observed, only main effects will be discussed. Differences were observed among treatments for tiller injury, percent tiller injury, and grain yield, and among site-years for all variables (Table 4.6).

No differences were observed among treatments for total tillers per plant, mean blank heads, or percent blank heads, however differences were observed in tiller injury per plant, percent tiller injury per plant, and grain yield (Table 4.7). Treatments receiving clothianidin plus cyantraniliprole, clothianidin plus chlorantraniliprole, and cyantraniliprole alone reduced tiller injury and percent tiller injury below that of the untreated control. All insecticide seed treatments yielded greater than the untreated control. Plots receiving a neonicotinoid in combination with a diamide resulted in significantly greater yields when compared to plots receiving only one insecticide product and the combination of neonicotinoids. No differences were observed between treatments receiving both a neonicotinoid and diamide seed treatment combination. Treatments receiving only one insecticide product as a seed treatment was not different regardless of insecticide class. No differences were observed when utilizing combinations of neonicotinoids compared to a single neonicotinoid treatment.

Differences between site-years were observed for all variables (Table 4.8). For both total tillers per plant and mean tiller injury, the JC-2 location was higher than all other locations. Both the JC-1 and JC-2 locations had higher percent tiller injury than the JC-4 location. Mean blank heads, percent blank heads, and grain yield were all higher at the JC-2 locations as compared to the JC-1 and JC-4 locations.

Greenhouse Study

Differences were observed among insecticide seed treatments in the greenhouse trial (df=15; x^2 = 54.2; P<0.01). Rice billbugs infested on insecticide treated rice had quicker mortality than rice not treated with insecticide. Rice treated with the diamide chlorantraniliprole had the fewest numerical days to mortality (μ=12.4) but was not different when compared to the other diamide seed treatment cyantraniliprole (μ=13.77). Regardless of product both diamide

seed treatments resulted in fewer days to mortality compared to the neonicotinoid seed treatments thiamethoxam (μ=20.9) and clothianidin (μ=20.9). No differences were observed between neonicotinoid seed treatments. Rice billbug placed on rice treated with a diamide seed treatment died on average of 8.3 days quicker compared to rice treated with a neonicotinoid. No mortality was observed from any specimen that was placed on rice only treated with a fungicide seed treatment.

Evaluation of Efficacy of Foliar Insecticide for Suppression of Rice Billbug

No interactions were observed between site-year and treatment (Table 4.9) or between application timing and insecticide product (Table 4.10). No differences were observed among treatments for any variable tested except grain yield. Insecticide seen treatment combinations yielded higher than any other treatment in the study. Differences were also observed between site-years (Table 4.12). For all variables except grain yield, the JC-2 location was higher than the JC-1 or JC-4 locations. No differences were observed among the JC-1 or JC-2 locations with respect to grain yield, however both locations were higher than the JC-4 location.

Evaluation of Insecticide Coated Urea for control of Rice Billbug

No interactions were observed between site-year and treatment (Table 4.13) or treatment and application timing (Table 4.14) when analyzing any dependent variable in this study. No differences were observed among treatments for total tillers, mean tiller injury, percent tiller injury, mean blank heads, or percent blank heads, but differences were observed among treatments for grain yield (Table 4.15). Chlorantraniliprole coated urea applied at the third timing had higher yields than all other treatments except the chlorantraniliprole coated urea applied at the second timing. All treatments except clothianidin and zeta-cypermethrin coated urea applied

at the first timing and zeta-cypermethrin coated urea applied at the third timing yielded higher than the untreated control. No differences were observed among locations for total tillers per plant, but for all other variables the JC-2 location was higher than the JC-1 location (Table 4.16).

Evaluation of insecticide efficacy and application methods for suppression on rice billbug

No interactions were observed between site-year and treatment for any variable except grain yield (Table 4.17). This was influenced due to minimal rice billbug presence at the JC-2 location. Because this was the only interaction observed, only main effects will be discussed. No differences were observed among treatments for any variable except grain yield (Table 4.18). Clothianidin plus chlorantraniliprole, thiamethoxam plus chlorantraniliprole, clothianidin plus cyantraniliprole, and thiamethoxam plus cyantraniliprole yielded higher than the untreated control. Clothianidin and Chlorantraniliprole alone were the only treatment to show no differences in yield compared to the untreated control. Differences were observed among site-years for all variables in the analysis (Table 4.19). The JC-2 location had higher tiller per plant, mean tiller injury, percent tiller injury, mean blank heads, and percent blank heads. No differences were observed between the JC-1 and JC-2 location for grain yield, however the JC-2 location yielded higher than the JC-4 location.

Determining yield loss potential from rice billbug injury

A relationship (r=0.19) was observed between mean total tillers and grain yield (Figure 4.1). As total tillers per plant increased yield increased as well. A negative relationship (r=0.50) was observed between mean tiller damage and yield (Figure 4.2). Yield increased until mean tiller injury reached three to four damaged tillers per plant then started to decrease. A similar relationship (r=0.14) was observed for percent damaged tillers and grain yield (Figure 4.3). Yield

generally increased until thirty percent damaged tillers occurred. At this point yield started decreasing. A negative relationship (r=0.25) was observed between mean blank heads and grain yield (Figure 4.4). Yield increased until 10 blank heads per plant was observed and then yield began to decrease. A similar relationship (r=0.25) was observed for percent blank heads and grain yield (Figure 4.5). Yield increased until 20 to 25% blank heads was observed at which point yield began to decrease.

This indicated that both mean tiller injury and blank heads should both be used to aid in determining yield loss from rice billbug injury. Converting billbug injury into percentages may lower overall relationship due to the variability in number of tillers between plants. This can increase the range of damage that does not properly depict rice billbug injury.

Discussion

Billbug Injury

Across all studies, sampling both tiller injury and blank heads correlated with rice grain yield. Independently, the insecticide seed treatment combination study provides enough data to verify this method needs to be refined. Currently, published research on rice billbug does not address tiller damage from rice billbug feeding. These data suggest that there is sufficient evidence that rice billbug feeding is causing substantial injury prior to rice heading.

Despite differences in blank head rates found by Villegas et al. (2021), results from these studies state no differences in blank heads regardless of experiment or treatment. Numerical findings from this study show that blank head sampling should be used when assessing billbug damage, but it should not be the only sampling method performed. In the studies performed by Villegas et al. (2021) the untreated check resulted in an average of 34 blank heads per 0.0011

hectare plots. This level of damage is far less than to the level of injury observed in discussed research. In Arkansas, rice billbug injury is documented at a much greater level. Across all untreated plots sampled for blank heads the average was 4 per 1.5 meter of row, estimating a total of 256 blanks heads per 0.0011 hectare. The number of blank heads from rice billbug feeding observed in Arkansas are 7.5-fold compared to studies documented in Louisiana, and no differences were observed among treatments.

Sampler variability is a major issue in sampling for rice billbug injury, primarily due to misidentification of billbug injury. No research has been conducted on the biology and feeding habits of rice billbug prior to findings of Floyd et al. (2021b), so education on how to assess feeding is limited. Increased exposure to rice billbug feeding should reduce sampler variability over time. Injured tiller sampling also requires rigorous manual labor and is very time consuming, requiring the samples to be removed from each individual plot and brought out of the field for inspection.

Sampler variability is also an issue when sampling blank heads from rice billbug, due to the limited exposure researchers have had. Education covering the difference between blank heads caused by other pests such as rice stalk borer (*Chilo plejadellus*, Zink) (Lepidoptera: Crambidae) and rice billbug should be considered. As rice billbug research and its exposure to the Mid-Southern U.S. rice industry continues to increase the level of variability should reduce over time. Timing of rating could be a key factor in accurately assessing blank head sampling. Observations that were made during these studies suggest that blank head sampling should be done as soon as plots reach 100% heading in the field. Sampling later, when rice begins to senesce, becomes more difficult to distinguish between panicles that are blank and those that are maturing.

Overall, further research needs to be assessed to streamline rice billbug injury assessments. Creating a sampling regime utilizing both injured tiller and blank head rates is warranted to accurately assess rice billbug losses.

Grain Yield

Regardless of the study, one control measure stood out. When rice was treated with both a neonicotinoid and a diamide seed treatment it, most frequently outyielded any other treatment in the study. All seed treatments increased grain yield compared to the untreated check. These data suggest that in areas where FIR producers are faced with multiple major pests in FIR systems utilizing insecticide seed treatment combinations could provide protection against multiple insect pests.

Multiple studies that have evaluated foliar insecticides for control of rice billbug indicate they are not a viable option. Applying a foliar insecticide at planting, or as rice plants emerge, may be too early in the growing season. Floyd et al. (2021c) observed that rice billbug moved into fields as rice began tillering. Pyrethroid products such as lambda-cyhalothrin are contact insecticides with short residual control, and if infestations have not begun, little to no control can be expected. Applying a systemic product such as thiamethoxam or chlorantraniliprole could provide residual protection to reduce injury from rice billbug. Chlorantraniliprole is not currently labeled for use after rice has emerged. The lack of approved insecticides in rice doesn’t allow many options for FIR producers to control rice billbug without implementing an insecticide seed treatment. Further research is needed to pinpoint what application timing and product would be the most successful in controlling rice billbug in FIR systems

Data from the coated urea trial suggests that this application method is not suitable for rice billbug suppression. This is likely due to the tested products not being suitable for rice billbug suppression or that this application method doesn't provide the needed coverage to protect the plant. The "pre-flood" application of urea typically occurs at the 4 to 5 leaf stage in rice which may be prior to billbug migration into the production fields. The "mid-season" fertilizer application typically occurs after tiller establishment, and the absence of standing water and slope may warrant up to 5 applications of nitrogen. This may contribute to control of billbug in the field, but if oviposition has occurred, larvae are most likely already established in the protected sites of the rice plant, resulting in a poor control. Further research should be conducted to analyze if applying a long residual insecticide to urea and applying it during the "preflood" application along with an insecticide seed treatment could increase suppression of rice billbug. Testing assorted products and timings could result in increased insecticide efficacy.

When comparing insecticides and application methods, insecticide seed treatments containing both a neonicotinoid and diamide insecticide performed better than a neonicotinoid seed treatment plus a foliar diamide application. It was also observed that applying a neonicotinoid seed treatment followed by a diamide overspray resulted in lower yields compared to any plot containing a combination seed treatment. Timing may have influenced the foliar spray, so further research is needed to analyze multiple insecticide seed treatment combinations in conjunction with a range of foliar application timings.

Overall, an insecticide seed treatment combination containing neonicotinoid and a diamide resulted in the greatest suppression against rice billbug and retained the greatest rice yield.

Conclusion

Overall, the utilization of insecticide seed treatments appears to be the most efficacious strategy for suppressing rice billbug populations. Seed treatments containing diamide insecticides provided greater control than those only consisting of a neonicotinoid. This agrees with finding from Plummer et al. (2020) where insecticide seed treatment combinations provided the greatest control of rice water weevil. Increased control from the addition of a diamide was hypothesized to be due to both rice water weevil and rice billbug both belonging in the family *Curculionidae*. In Arkansas, where producers are faced with multiple major insect pests such as rice water weevil and grape colaspis, insecticide seed treatments are already implemented to control these pests. Findings from Plummer et al. (2020) suggests that combinations of multiple insecticide classes on seed has successfully controlled rice water weevil. Findings from this study show similar benefits for suppressing rice billbug. This indicates that producers who already implement combinations of insecticide seed treatment, will have to make no major adjustments if shifting to a FIR system. These data suggest that insecticide seed treatments should be recommended to suppress rice billbug populations and retain yield.

Foliar insecticide results show that more research needs to be conducted to discover the most efficacious application timing. Currently, there is no clear timing that would optimize rice billbug control with a foliar application. A stipulation with foliar control of billbug is the limited number of insecticide options available for control. Results indicate that contact insecticides such as pyrethroids are not a viable option to control rice billbug. Findings from Floyd et al. (2021b) indicate that rice billbugs are predominantly ground active. This could make contact insecticides less efficacious due to protection provided from the plant canopy. Systemic insecticides could result in better control, but limited options are available for use in rice. Results from these studies would suggest that foliar insecticides should not be the primary control tactic for suppressing rice

billbug. Foliar insecticides may be considered as a secondary option, but more research is required before foliar applications can be recommended.

Potential to suppress rice billbug with insecticide coated urea suggests there may be potential but is not currently recommended. In rice production, nitrogen application research has been a primary focus to maximize production. There are multiple scenarios to apply nitrogen, but rates are typically adjusted and not application timing. This makes it more difficult to use urea as an application method despite cost savings due to timeline of rice billbug infestation. If rice billbug does not infest a field close to a urea application timing, little to no control can be expected. Research needs to continue to examine multiple timings and products to assess if urea can be used as a viable application method.

Sampling Methods

No one sampling method appeared to be the best option for monitoring rice billbug. Contrary to research by Villegas et al. (2021) solely sampling blank heads does not accurately depict rice billbug injury. Rice billbug feeding begins as rice plants begin to tiller and feeding causes tiller death prior to the plant reaching internode elongation. This injury results in tiller being unable to produce a head. Feeding by adults' results in tiller death 5-7 days after. Females will then oviposit, and larva will hatch and feed close to the soil line, primarily feeding between where tillers emerge from soil surface and the first joint. Observations by the author hypothesize that blank head injury occurs from secondary feeding because one larvae is too large to feed within the tiller. Once this occurs, larvae move into the root zone and feed on the base of rice tillers. At this point, internode elongation has likely occurred, and panicles are beginning to develop within the tiller. Secondary feeding would cease nutrient and water uptake to the developing panicle causing a blank head. So, it is hypothesized sampling solely blank heads does

not account for billbug injury prior to internode elongation. Rice billbug research needs to sample both damaged tillers and blank heads to account for all rice billbug injury. The creation of a sampling regime where damaged tillers could be assessed in a non-destructive manner may be beneficial due to the ability to have an absolute value of damaged tillers and blank heads for a single plant. Currently, sampling tiller injury in a destructive manner inhibits acquiring an absolute value for injury, due to one the plant is destroyed one cannot assess how many blank heads would be achieved by the plant. Blank head samples are currently being acquired from sampling another section of the plot, and an absolute value cannot truly be achieved.

Overall, based on injury and yield losses assessed in this study, rice billbug has a high likelihood of gaining major pest status in FIR production systems. This should encourage researchers to explore multiple control and sampling options to suppress rice billbug. Currently, findings from these studies would suggest that insecticide seed treatments containing both a neonicotinoid and a diamide insecticide class should be recommended to suppress rice billbug and retain a greater amount of yield. However, more research on rice billbug control needs to be conducted.

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Table 4.1. Location, soil types, cultivar, and planting dates for rice billbug experiments conducted in Jackson County, Arkansas from 2020-2022.

Site-year	Year	Latitude Longitude	Hybrid Cultivar	Soil Type	Planting Date
JC-1	2020	35.6099150 -91.3479780	RTXP753XL	Egam silt loam	4/16
JC-2	2021	35.6099150 -91.3479780	RT7321FP	Egam silt loam	5/14
JC-3	2021	35.5971799 -91.3494070	RT732FP1	Amagon and Forestdale silt loam	5/14
JC-4	2022	35.6099150 -91.3479780	RT7301	Egam silt loam	5/14

Table 4.2. List of IRAC codes, active ingredient, trade name, and product rates for treatments included in rice billbug insecticide seed treatment studies conducted in Jackson County, Arkansas from 2020 to 2022.

IRAC Code^	Active Ingredient	Trade Name	Rate (ml/100 kg seed)
4a	Thiamethoxam	CruiserMaxx Rice®	455
4a	Clothianidin	NipsIt Inside®	124
28	Chlorantraniliprole	Dermacor®	325
28	Cyantraniliprole	Fortenza®	226
4a + 4a	Thiamethoxam + Clothianidin	CruiserMaxx Rice® + NipsIt Inside®	455 + 124
4a + 28	Thiamethoxam + Chlorantraniliprole	CruiserMaxx Rice® + Dermacor®	455 + 325
4a + 28	Thiamethoxam + Cyantraniliprole	CruiserMaxx Rice® + Fortenza®	455 + 226
4a + 28	Clothianidin + Chlorantraniliprole	NipsIt Inside® + Dermacor®	124 + 325
4a + 28	Clothianidin + Cyantraniliprole	NipsIt Inside® + Fortenza®	124 + 326
N/A	Untreated	Untreated	N/A

^Denotes Mode of action classification given by the Insecticide Resistance Action Committee

Table 4.3. List of IRAC codes, active ingredient, trade name, application timings and product rates for treatments included in the rice billbug foliar insecticide studies conducted in Jackson County, Arkansas from 2020 to 2022.

IRAC Code^	Active Ingredient	Trade Name	Timing	Rate
3a	Lambda-cyhalothrin	Warrior II®	at Planting*	55 (ml/ha)
4a	Thiamethoxan Lambda-cyhalothrin	Endigo ZCX®	at Planting*	148 (ml/ha)
28	Chlorantraniliprole	Prevathon®	at Planting*	591 (ml/ha)
3a	Lambda-cyhalothrin	Warrior II®	80-100% Emergence+	55 (ml/ha)
4a 3a	Thiamethoxan Lambda-cyhalothrin	Endigo ZCX®	80-100% Emergence+	148 (ml/ha)
28	Chlorantraniliprole	Prevathon®	80-100% Emergence+	591 (ml/ha)
3a	Lambda-cyhalothrin	Warrior II®	1st Tiller~	55 (ml/ha)
4a 3a	Thiamethoxan Lambda-cyhalothrin	Endigo ZCX®	1st Tiller~	148 (ml/ha)
28	Chlorantraniliprole	Prevathon®	1st Tiller~	591 (ml/ha)
3a	Lambda-cyhalothrin	Warrior II®	4-5th Tiller§	55 (ml/ha)
4a 3a	Thiamethoxan Lambda-cyhalothrin	Endigo ZCX®	4-5th Tiller§	148 (ml/ha)
28	Chlorantraniliprole	Prevathon®	4-5th Tiller§	591 (ml/ha)
4a 28	Thiamethoxam + Chlorantraniliprole	CruiserMaxx Rice® + Dermacor®	N/A	455 + 325 (ml/100 kg)
4a 28	Thiamethoxam + Cyantraniliprole	CruiserMaxx Rice® + Fortenza®	N/A	455 + 226 (ml/100 kg)
	N/A	Untreated	N/A	N/A

^Denotes Mode of action classification given by the Insecticide Resistance Action Committee
*Denotes application dates were May 14 in 2021 and May 14 in 2022
+Denotes application dates were May 14 in 2021 and May 14 in 2022
~Denotes application dates were June 7 in 2021 and June 2 in 2022
§Denotes application dates were June 16 in 2021 and June 27 in 2022

Table 4.4. List of IRAC codes, active ingredient, trade name, application timing, and product rates for treatments included in rice billbug insecticide coated urea studies conducted in Jackson County, Arkansas from 2020 to 2022.

IRAC Code^	Active Ingredient	Trade Name	Application Timing	Insecticide Rate (ml/ha)
4a	Clothianidin	Belay®	1st N Application*	328.7
3a	Zeta-Cypermethrin	Mustang®	1st N Application*	241.1
28	Cyantraniliprole	Fortenza®	1st N Application*	314.2
4a	Clothianidin	Belay®	2nd N Application+	328.7
3a	Zeta-Cypermethrin	Mustang®	2nd N Application+	241.1
28	Cyantraniliprole	Fortenza®	2nd N Application+	314.2
4a	Clothianidin	Belay®	3rd N Application~	328.7
3a	Zeta-Cypermethrin	Mustang®	3rd N Application~	241.1
28	Cyantraniliprole	Fortenza®	3rd N Application~	314.2
N/A	N/A	Untreated	N/A	N/A

^Denotes Mode of action classification given by the Insecticide Resistance Action Committee
*Denotes application dates were June 3 in 2020 and June 6 in 2021
+Denotes application dates were June 15 in 2020 and June 18 in 2021
~Denotes application dates were June 25 in 2020 and June 27 in 2022

Table 4.5. List of IRAC codes, active ingredient, trade name, application timings, and product rates for treatments included in rice billbug insecticide and application method studies conducted in Jackson County, Arkansas from 2020 to 2022.

IRAC Code^	Active Ingredient	Trade Name	Rate
4a	Thiamethoxam*	CruiserMaxx Rice®	455 (ml/100 kg)
4a	Clothianidin*	NipsIt Inside®	124 (ml/100 kg)
28	Chlorantraniliprole*	Dermacor®	325 (ml/100 kg)
28	Chlorantraniliprole~	Prevathon®	1460.8 (ml/ha)
4a + 28	Clothianidin* + Chlorantraniliprole~	NipsIt Inside® + Prevathon®	124 (ml/100 kg) + 1460.8 (ml/ha)
4a + 28	Thiamethoxam* Chlorantraniliprole	CruiserMaxx® + Prevathon®	455 (ml/100 kg) + 1460.8 (ml/ha)
4a + 28	Thiamethoxam* + Chlorantraniliprole	CruiserMaxx® + Dermacor®	455 + 325 (ml/100 kg)
4a + 28	Clothianidin* + Chlorantraniliprole	NipsIt Inside® + Dermacor®	124 + 325 (ml/100 kg)
4a + 28	Thiamethoxam* + Cyantraniliprole*	CruiserMaxx Rice® + Fortenza®	455 + 226 (ml/100 kg)
4a + 28	Clothianidin* + Cyantraniliprole*	NipsIt Inside® + Fortenza®	124 + 226 (ml/100 kg)
4a + 4a	Clothianidin* Thiamethoxam*	NipsIt Inside® + CruiserMaxx Rice®	124 + 455 (ml/100 kg)
N/A	N/A	Untreated	N/A

^Denotes Mode of action classification given by the Insecticide Resistance Action Committee
*Denotes insecticide was applied directly to rice seed
~Denotes insecticide listed was applied on to rice foliage

Table 4.6 Main effects and interaction statistics for multiple rice billbug sampling methods and grain yield for insecticide seed treatment studies conducted in Jackson County, Arkansas from 2020-2022.

Effects	Treatment (T)			Site-Year (YR)			T x YR		
	df	**F**	***P***	**df**	**F**	***P***	**df**	**F**	**P**
Total Tillers	9,2164	1.4	0.2	2,2164	512.3	<0.01	18,2146	0.5	0.9
Mean Tiller Injury	9,2164	2.2	<0.01	2,2146	57.4	<0.01	18,2146	1.0	0.5
Tiller Injury (percent)	9,2164	2.3	0.01	2,2164	17.9	<0.01	18,2146	1.2	0.3
Mean Blank Heads	9,146	0.6	0.8	2,146	52.9	<0.01	18,146	0.6	0.9
Blank Heads (percent)	9,146	0.4	0.9	2,146	45.1	<0.01	18,146	0.6	0.8
Grain Yield	27,146	2.0	<0.01	2,146	34.6	<0.01	18,146	2.1	0.01

Data was analyzed in PROC GLIMMIX using the multiple Pairwise *t* test at α=0.05.

Table 4.7. Insecticide seed treatment control of rice billbug based on multiple sampling methods and grain yield for studies conducted in Jackson County, Arkansas from 2020-2022.

Treatment Name	Total Tillers	Mean Tiller Injury	Tiller Injury (percent)	Mean Blank Heads	Blank Heads (percent)	Yield (kg/ha)
Thiamethoxam	7.8	2.0 ab	18.4 abc	4.2	9.7	9219.7 b
Clothianidin	8.1	2.2 a	20.1 a	3.1	8.2	9218.7 b
Chlorantraniliprole	7.5	1.6 bdc	17.1 abc	2.9	7.6	9537.9b
Cyantraniliprole	7.1	1.5 d	15.2 c	3.3	8.2	9591.3 b
Thiamethoxam + Clothianidin	7.7	1.9 abc	17.1 abc	4.7	11.8	9232.8 b
Thiamethoxam + Chlorantraniliprole	7.5	2.0 ab	18.7 ab	3.7	8.6	10057.6 a
Thiamethoxam + Cyantraniliprole	7.4	1.9 abcd	17.9 abc	3.6	9.6	10116.6 a
Clothianidin + Chlorantraniliprole	7.4	1.6 dc	15.6 bc	3.6	8.4	9998.7 a
Clothianidin + Cyantraniliprole	7.7	1.5 dc	15.2 c	3.7	9.2	10079.8a
Untreated	7.7	2.01 ab	20.2 a	4.2	10.80	8426.7 c
df	9, 2164	9, 2164	9, 2164	9, 146	9, 146	27, 146
F	1.4	2.2	2.3	0.6	0.4	2.0
P	0.2	<0.01	0.01	0.8	0.9	<0.01

Treatments with the same letter are not significantly different according to multiple Pairwise *t* test at α=0.05.

Table 4.8. Site-year analysis of insecticide seed treatment studies for multiple rice billbug sampling methods and grain yield for studies conducted in Jackson County, Arkansas from 2020-2022.

Site-year	Total Tillers	Mean Tiller Injury	Tiller Injury (percent)	Mean Blank Heads	Blank Heads (percent)	Yield (kg/ha)
JC-1	8.0 b	2.2 b	26.0 a	4.4 b	12.0 b	9313.7 b
JC-2	12.5 a	2.9 a	22.1 a	6.8 a	15.2 a	9978.0 a
JC-4	6.1 c	1.0 c	13.0 b	1.0 c	1.6 c	8872.9 c
df	2,2164	2,2164	2,2164	2,146	2,146	2,146
F	512.3	57.4	17.9	52.9	45.1	36.4
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Location information can be found in Table 4.1

Treatments with the same letter are not significantly different according to multiple Pairwise *t* test at α=0.05

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Table 4.9. Main effects and interaction statistics for multiple rice billbug sampling methods and grain yield for foliar insecticide studies conducted in Jackson County, Arkansas from 2020-2022.

Effects	Treatment (T)			Site-Year (YR)			T x YR		
	df	F	*P*	df	F	*P*	df	F	P
Total Tillers	14,104	0.9	0.6	2,104	46.6	0.8	58,104	0.6	0.9
Mean Tiller Injury	14,104	1.5	0.1	2,104	1.9	<0.01	58,104	1.1	0.4
Tiller Injury (percent)	14,104	1.1	0.4	2,104	48.7	<0.01	58,104	0.6	0.8
Mean Blank Heads	14,104	1.1	0.4	2,104	56.4	<0.01	58,104	1.5	0.1
Blank Heads (percent)	14,104	0.8	0.7	2,104	2.5	<0.01	58,104	1.2	0.3
Grain Yield	14,104	7.5	<0.01	2,104	41.9	<0.01	58,104	1.1	0.4

Data was analyzed in PROC GLIMMIX using the multiple Pairwise *t* test at α=0.05.

Table 4.10. Main effects and interaction statistics for application timing and insecticide product for multiple rice billbug sampling methods and grain yield for foliar insecticide studies conducted in Jackson County, Arkansas from 2020-2022.

Effects	Application Timing (T)			Insecticide Product (PR)			T x PR		
	df	F	*P*	df	F	*P*	df	F	P
Total Tillers	3,104	1.0	0.4	3,104	1.2	0.3	6,104	0.6	0.7
Mean Tiller Injury	3,104	0.2	0.9	3,104	0.7	0.6	6,104	0.2	0.9
Tiller Injury (percent)	3,104	1.1	0.3	3,104	0.8	0.6	6,104	0.3	1.0
Mean Blank Heads	3,104	1.1	0.4	3,104	0.1	1.0	6,104	0.7	0.6
Blank Heads (percent)	3,104	0.2	0.9	3,104	0.2	0.9	6,104	0.4	0.9
Grain Yield	3,104	0.2	0.9	3,104	0.3	0.8	6,104	0.4	0.9

Data was analyzed in PROC GLIMMIX using the multiple Pairwise *t* test at α=0.05.

Table 4.11. Foliar insecticide control of rice billbug based on multiple sampling methods and grain yield for studies conducted in Jackson County, Arkansas from 2020-2022.

Treatment Name	Application Timing	Total Tillers	Mean Tiller Injury	Tiller Injury (percent)	Mean Blank Heads	Blank Heads (percent)	Yield (kg/ha)
Lambda-cyhalothrin	at Planting	9.9	1.8	14.8	3.0	7.5	8872.9 bcd
Thiamethoxan Lambda-cyhalothrin	at Planting	8.9	1.4	12.1	3.2	8.0	8873.9 bcd
Chlorantraniliprole	at Planting	9.6	1.7	14.4	3.5	8.4	8870.8 bcd
Lambda-cyhalothrin	80-100% Emergence	10.4	1.7	13.9	3.3	7.8	8863.8 bcd
Thiamethoxan Lambda-cyhalothrin	80-100% Emergence	9.2	1.6	13.0	3.6	8.0	8840.6 bcd
Chlorantraniliprole	80-100% Emergence	10.4	2.0	18.4	3.4	8.4	8921.5 bc
Lambda-cyhalothrin	1st Tiller	9.7	1.5	13.2	3.3	7.9	8903.6 bc
Thiamethoxan Lambda-cyhalothrin	1st Tiller	9.4	1.2	10.9	3.5	8.0	8639.4 cde
Chlorantraniliprole	1st Tiller	10.2	1.4	11.2	3.6	10.7	9182.9 bc
Lambda-cyhalothrin	4-5th Tiller	9.8	1.3	10.8	3.1	7.9	8356.1 e
Thiamethoxan Lambda-cyhalothrin	4-5th Tiller	10.5	1.3	10.4	2.5	6.1	9106.3 bc
Chlorantraniliprole	4-5th Tiller	10.3	1.6	12.9	2.5	5.8	9406.7 b
Thiamethoxam Chlorantraniliprole	N/A	10.7	2.0	15.0	2.3	4.9	10309.2 a
Thiamethoxam Cyantraniliprole	N/A	10.3	1.8	15.2	3.3	7.8	10427.7 a
Untreated	N/A	9.9	1.6	12.2	4.0	9.6	8436.8 de
df		14, 104	14, 104	14, 104	14, 104	14, 104	14, 104
F		0.9	1.5	1.1	1.1	0.8	7.5
P		0.6	0.1	0.4	0.4	0.7	<0.01

Treatments with the same letter are not significantly different according to multiple Pairwise *t* test at α=0.05

Table 4.12. Site-year analysis of foliar insecticide studies for multiple rice billbug sampling methods and grain yield for studies conducted in Jackson County, Arkansas from 2020-2022.

Site-year	Total Tillers	Mean Tiller Injury	Tiller Injury (percent)	Mean Blank Heads	Blank Heads (percent)	Yield (kg/ha)
JC-1	8.1 b	3.6 b	16.9 b	4.0 b	12.9 b	9358.8 a
JC-2	11.0 a	6.4 a	22.3 a	7.2 a	16.6 a	9489.0 a
JC-4	8.4 b	1.0 c	11.3 c	2.0 c	4.2 c	8805.7 b
df	2,104	2,104	2,104	2,104	2,104	2,104
F	46.6	1.9	48.7	56.4	2.5	41.9
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Location information can be found in Table 4.1

Treatments with the same letter are not significantly different according to multiple Pairwise *t* test at α=0.05

Table 4.13. Main effects and interaction statistics for multiple rice billbug sampling methods and grain yield for insecticide coated urea studies conducted in Jackson County, Arkansas from 2020-2022.

Effects	Treatment (T)			Site-Year (YR)			T x YR		
	df	F	*P*	df	F	*P*	df	F	P
Total Tillers	9,30	0.9	0.6	1,30	0.8	<0.01	25,94	2.0	0.9
Mean Tiller Injury	9,30	0.4	0.2	1,30	1.9	<0.01	25,94	0.4	0.8
Tiller Injury (percent)	9,30	0.4	0.4	1,30	48.7	<0.01	25,94	0.6	0.7
Mean Blank Heads	9,70	1.1	0.6	1,70	47.4	<0.01	25,94	1.5	0.1
Blank Heads (percent)	9,70	0.8	0.3	1,70	5.84	<0.01	25,94	1.2	0.8
Grain Yield	9,70	2.5	0.02	1,70	5.63	<0.01	25,94	2.1	0.4

Data was analyzed in PROC GLIMMIX using the multiple Pairwise *t* test at α=0.05.

Table 4.14. Main effects and interaction statistics for insecticide treatment and application timing for multiple rice billbug sampling methods and grain yield for insecticide coated urea studies conducted in Jackson County, Arkansas from 2020-2022.

Effects	**Treatment (T)**			**Application Timing (A)**			**T x A**		
	df	**F**	***P***	**df**	**F**	***P***	**df**	**F**	**P**
Total Tillers	9,30	8.5	0.9	3,30	4.1	0.1	12,30	4.3	0.3
Mean Tiller Injury	9,30	1.7	0.1	3,30	37.4	0.4	12,30	5.1	0.1
Tiller Injury (percent)	9,30	1.9	0.4	3,30	2.4	0.2	12,30	1.8	0.5
Mean Blank Heads	9,70	1.3	0.3	3,70	3.5	0.7	34,94	8.7	0.1
Blank Heads (percent)	9,70	0.9	0.5	3,70	90.2	0.1	34,94	9.9	0.3
Grain Yield	9,70	2.17	0.06	3,70	77.5	0.3	34,94	71.0	0.3

Data was analyzed in PROC GLIMMIX using the multiple Pairwise *t* test at α=0.05.

Table 4.15. Insecticide coated urea control of rice billbug based on multiple sampling methods and grain yield for studies conducted in Jackson County, Arkansas from 2020-2022.

Treatment Name	Application Timing	Total Tillers	Mean Tiller Injury	Tiller Injury (percent)	Mean Blank Heads	Blank Heads (percent)	Yield (kg/ha)
Clothianidin	**1st**	10.9	1.6	10.6	3.1	7.2	9376.0 cde
Zeta-Cypermethrin	**1st**	11.1	1.7	11.4	3.3	6.6	9310.5 de
Chlorantraniliprole	**1st**	11.0	1.9	12.9	2.6	5.7	9268.2 bcd
Clothianidin	**2nd**	12.5	2.0	10.6	3.0	5.9	9886.2 bcd
Zeta-Cypermethrin	**2nd**	10.7	1.8	13.9	1.9	4.7	10010.8 abc
Chlorantraniliprole	**2nd**	10.8	1.6	11.2	3.4	7.4	10118.1 ab
Clothianidin	**3rd**	10.4	1.6	12.2	2.5	5.7	9765.7 bcd
Zeta-Cypermethrin	**3rd**	11.4	1.7	14.4	4.1	9.4	9581.2 bcde
Chlorantraniliprole	**3rd**	11.8	1.5	8.7	1.8	4.3	11078.9 a
Untreated	**N/A**	10.6	1.5	9.7	2.2	4.9	9020.0 e
df		9, 30	9, 30	9, 30	9, 70	9, 70	9,70
F		0.9	0.4	0.4	1.1	0.8	2.5
P		0.6	0.9	0.9	0.4	0.7	0.02

Treatments with the same letter are not significantly different according to multiple Pairwise *t* test at α=0.05.

Table 4.16. Site-year analysis of insecticide coated urea studies for multiple rice billbug sampling methods and grain yield for studies conducted in Jackson County, Arkansas from 2020-2022.

Site-year	Total Tillers	Mean Tiller Injury	Tiller Injury (percent)	Mean Blank Heads	Blank Heads (percent)	Yield (kg/ha)
JC-1	11.9 a	4.0 b	17.1 b	2.0 b	9.2b	9056.2 b
JC-2	12.4 a	5.9 a	21.4 a	5.0 a	15.0a	9774.1 a
df	1,30	1,30	1,30	1,70	1,70	1,70
F	0.78	1.9	48.7	47.4	5.84	5.63
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Location information can be found in Table 4.1
Treatments with the same letter are not significantly different according to multiple Pairwise *t* test at α=0.05

Table 4.17. Main effects and interaction statistics for multiple rice billbug sampling methods and grain yield for insecticide and application method studies conducted in Jackson County, Arkansas from 2020-2022.

Effects	Treatment (T)			Site-Year (YR)			T x YR		
	df	**F**	***P***	**df**	**F**	***P***	**df**	**F**	**P**
Total Tillers	11,82	1.3	0.2	2,82	54.7	<0.01	22,82	1.30	0.2
Mean Tiller Injury	11,82	1.2	0.3	2,82	0.01	<0.01	22,82	1.5	0.1
Tiller Injury (percent)	11,82	1.0	0.5	2,82	47.1	<0.01	22,82	0.43	1.0
Mean Blank Heads	11,82	1.8	0.1	2,82	88.4	<0.01	22,82	1.5	0.6
Blank Heads (percent)	11,82	1.9	0.1	2,82	101.0	<0.01	22,82	1.2	0.3
Grain Yield	11,82	2.8	<0.01	2,82	4.8	<0.01	22,82	2.3	<0.01

Data was analyzed in PROC GLIMMIX using the multiple Pairwise *t* test at α=0.05.

Table 4.18. Insecticide and application method control of rice billbug based on multiple sampling methods and grain yield for studies conducted in Jackson County, Arkansas from 2020-2022.

Treatment Name	Total Tillers	Mean Tiller Injury	Tiller Injury (percent)	Mean Blank Heads	Blank Heads (percent)	Yield (kg/ha)
Thiamethoxam*	9.9	1.8	15.5	3.0	8.4	8955.5 bcd
Clothianidin*	9.6	1.8	17.3	4.2	12.3	8865.8 bcd
Chlorantraniliprole*	8.9	1.6	15.6	4.3	11.8	9345.8 bc
Chlorantraniliprole~	10.4	2.2	19.9	2.9	6.7	8618.8 de
Clothianidin* + Chlorantraniliprole~	9.6	1.8	16.8	3.7	9.0	8897.1 bcd
Thiamethoxam* + Chlorantraniliprole	8.0	1.1	11.4	3.1	7.8	9413.3 b
Thiamethoxam* + Chlorantraniliprole	10.0	1.8	16.9	2.7	7.2	10327.4 a
Clothianidin* + Chlorantraniliprole	9.0	1.4	15.1	3.3	9.1	9764.2 a
Thiamethoxam* + Cyantraniliprole*	9.9	1.6	15.7	5.2	12.6	10130.2 a
Clothianidin* + Cyantraniliprole*	10.3	1.9	16.3	1.9	4.7	9956.9 a
Untreated	9.4	1.5	15.9	3.2	7.5	8395.4 e
Df	11, 82	11, 82	11.82	11, 177	11, 177	33, 141
F	1.3	1.2	1.0	1.8	1.9	2.8
P	0.2	0.3	0.5	0.1	0.1	<0.01

Treatments with the same letter are not significantly different according to multiple Pairwise *t* test at $\alpha=0.05$

*Denotes insecticide was applied directly to rice seed

~Denotes insecticide listed was applied on to rice foliage

Table 4.19. Site-year analysis of insecticide and application method studies for multiple rice billbug sampling methods and grain yield for studies conducted in Jackson County, Arkansas from 2020-2022.

Site-year	Total Tillers	Mean Tiller Injury	Tiller Injury (percent)	Mean Blank Heads	Blank Heads (percent)	Yield (kg/ha)
JC-1	9.1 b	3.1 b	16.9 b	1.9 b	12.9 b	9358.8 ab
JC-2	12.0 a	7.1 a	22.3 a	7.1 a	16.5 a	9489.0 a
JC-4	8.	2.0 b	11.3 c	2.3 b	4.2 c	8805.7 b
df	2,82	2,82	2,82	2,82	2,82	2,82
F	54.7	0.1	47.1	88.4	101.0	4.8
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Location information can be found in Table 4.1
Treatments with the same letter are not significantly different according to multiple Pairwise *t* test at α=0.05

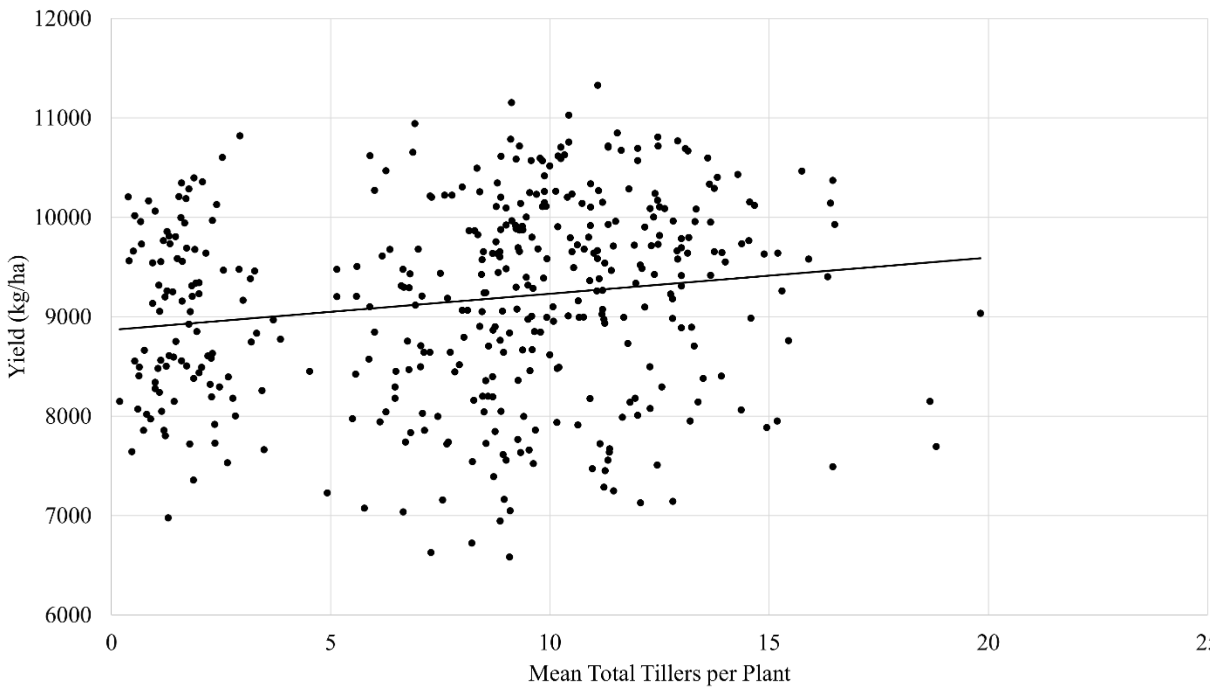

Figure 4.1. Linear regression for mean total tillers per plant by grain yield across multiple rice billbug experiments conducted in Jackson County, Arkansas from 2020-2022.
The equation is y=0.72x+175.16 with a *P*-value <0.01. y=grain yield and x=mean tillers per plant.

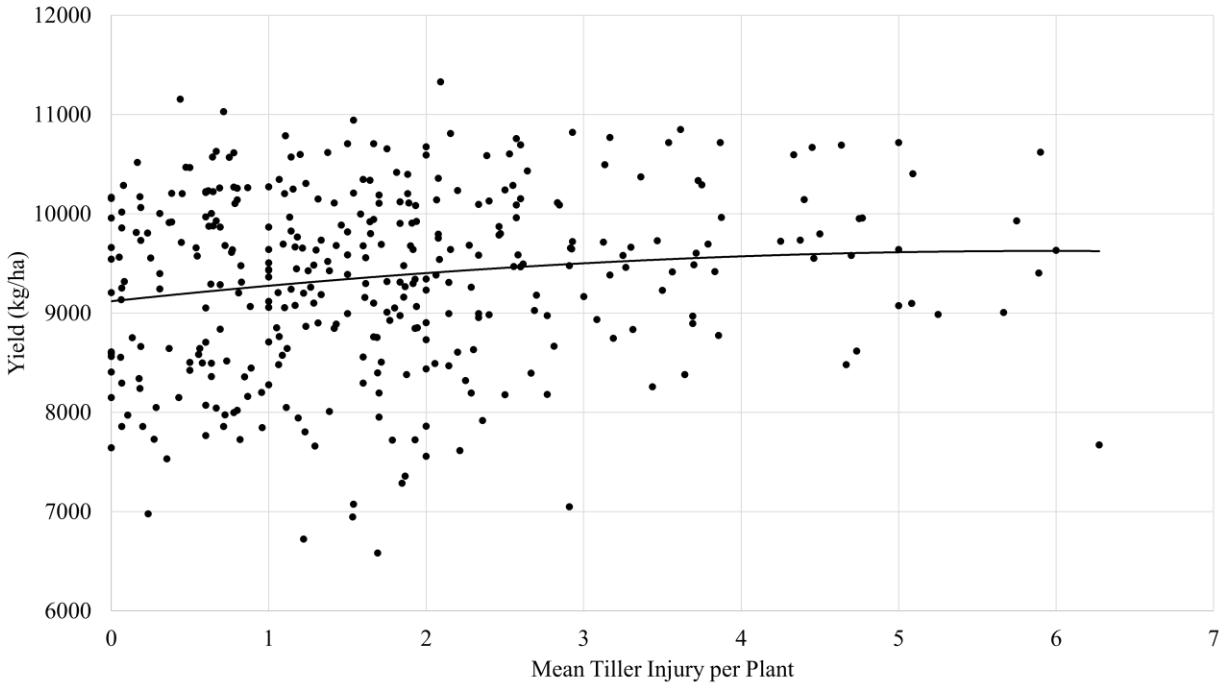

Figure 4.2. Quadratic regression for mean tiller injury per plant by grain yield across multiple rice billbug experiments conducted in Jackson County, Arkansas from 2020-2022.
The equation is $y= -14.35x^2+170.60x + (9119.0)$ with a *P*-value <0.01. y=grain yield and x=percent tiller injury per plant.

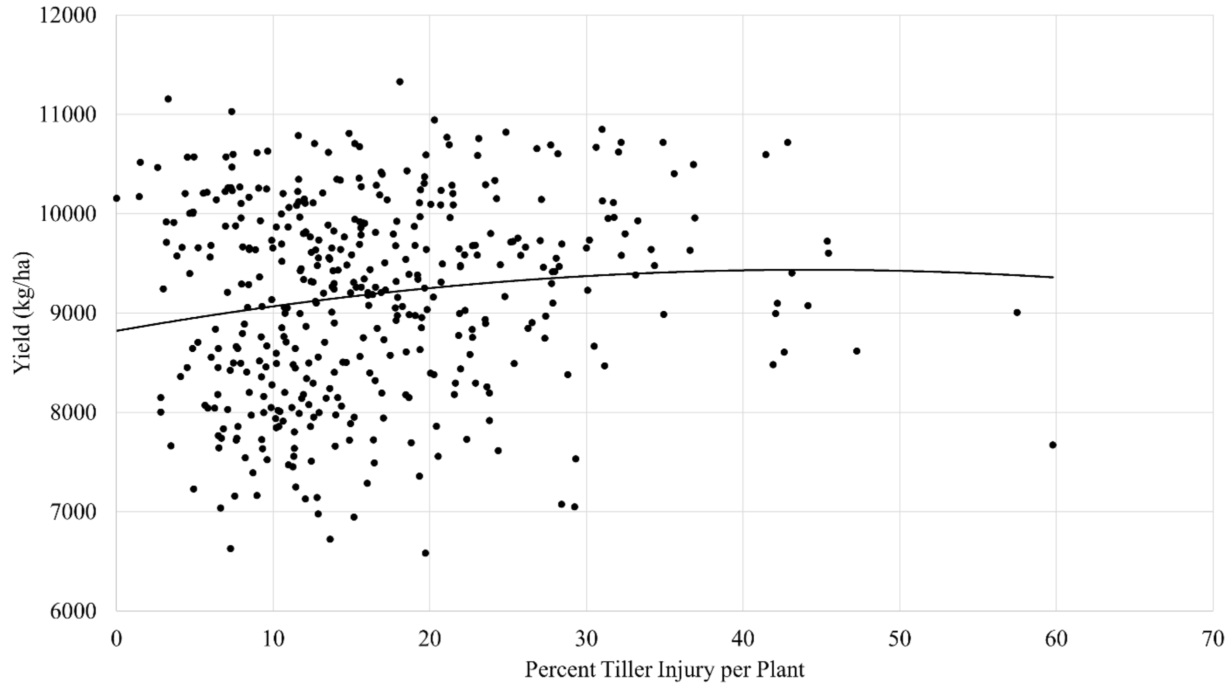

Figure 4.3. Quadratic regression for percent tiller injury per plant by grain yield across multiple rice billbug experiments conducted in Jackson County, Arkansas from 2020-2022.
The equation is $y= -0.32x^2+27.84x + (8820.5)$ with a *P*-value <0.01. y=grain yield and x=percent tiller injury per plant.

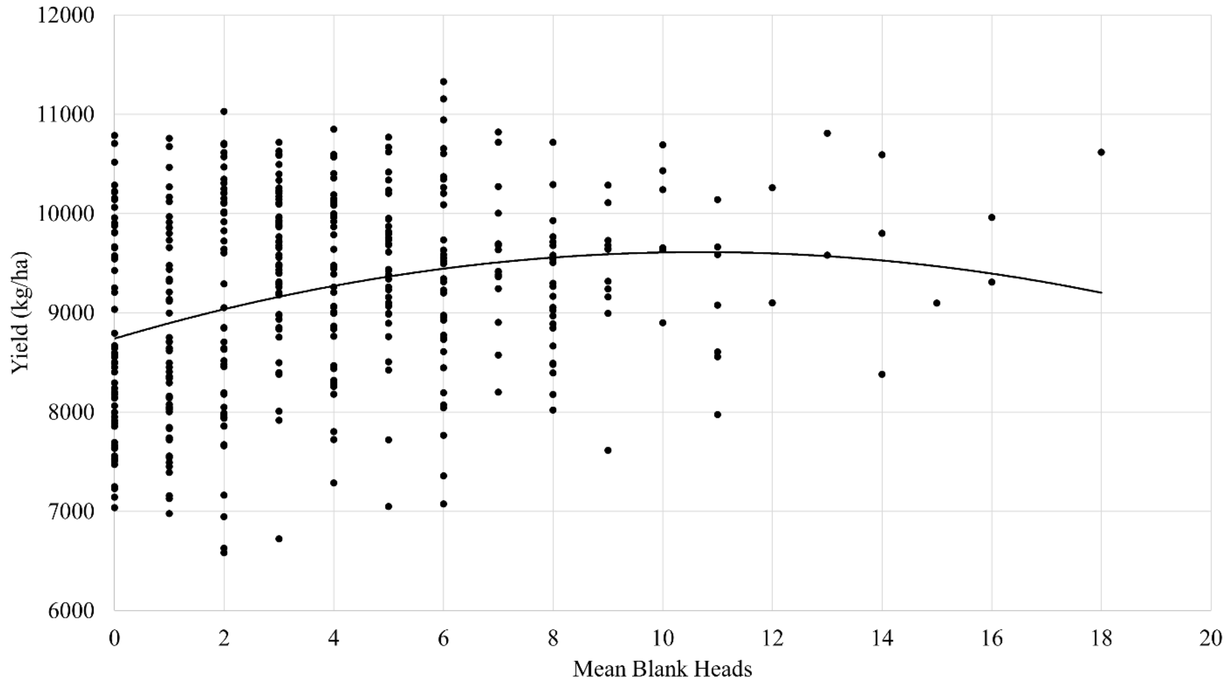

Figure 4.4 Quadratic regression for mean blank heads per sample by grain yield across multiple rice billbug experiments conducted in Jackson County, Arkansas from 2020-2022.
The equation is $y=- -7.64x^2 + 163.22x +(8740.3)$ with a *P*-value <0.01. y=grain yield and x=mean blank heads per sample.

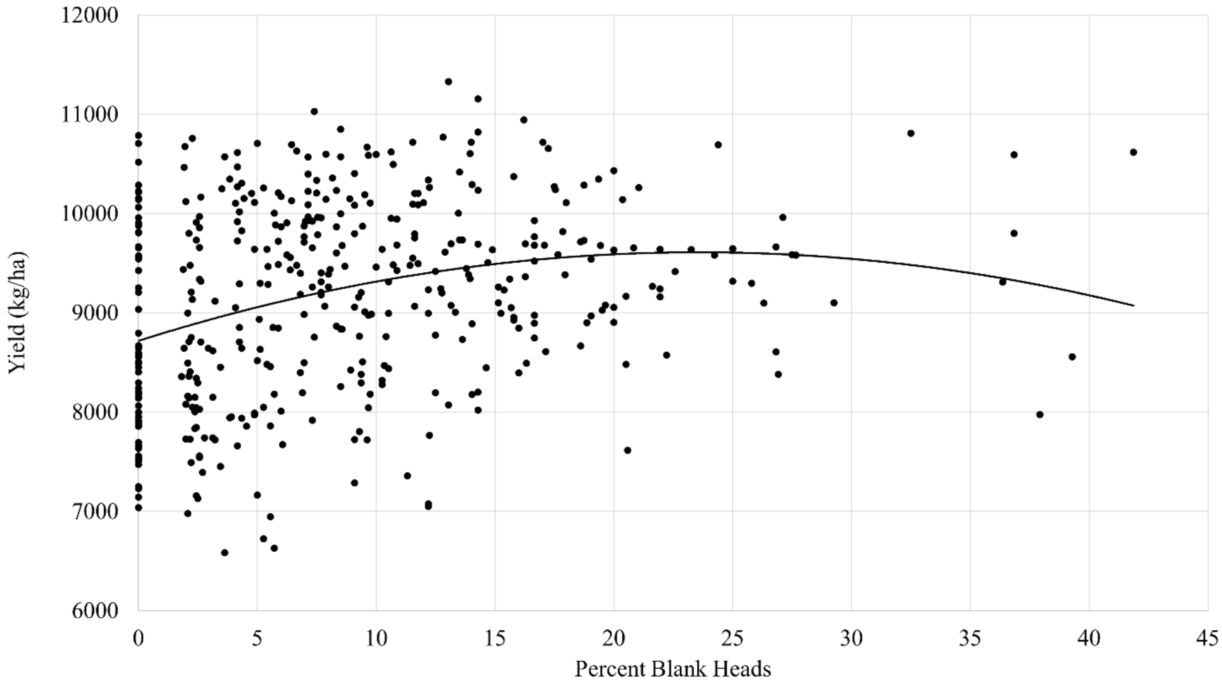

Figure 4.5. Quadratic regression for percent blank heads per sample by grain across multiple rice billbug experiments conducted in Jackson County, Arkansas from 2020-2022.
The equation is y= -1.61x^2 +75.7 + (8718) with a *P*-value <0.01. y=grain yield and x=percent blank heads per sample

Chapter 5 - Extraction and Evaluation of Rice Billbug (*Sphenophorus* pertinax) (Coleoptera: Curculionidae) Insect Lures using Olfactory Techniques

Abstract

The utilization of semiochemicals such as insect pheromones has proven successful in the eradication regimes for U.S. agriculture in the last half century. The boll weevil as well as the pink bollworm have received the status of eradicated by the U.S. Department of Agriculture. Insect pheromones can allow producers to make timely applications to reduce threatening levels of potential pests. The rice billbug, (*Sphenophorus pertinax,* Chittenden), has recently becoming a concern to Mid-Southern U.S. rice producers implementing a potential cost saving, production system. Furrow-irrigated rice hectare has continued to increase for the last five years. Lack of research on the rice billbug has risen concerns as the furrow-irrigated rice system gains popularity. Further understanding, and evaluations of control tactics have become a priority for entomologist in rice growing regions of the U.S. Extraction and implementation of pheromone targeted for rice billbug, could allow timely control measures to take place and potentially reduce the amount of injured rice across the field. In this study, volatile semiochemicals extraction, and Y-tube choice tests were conducted at the University of Arkansas Department of Entomology in Fayetteville, AR. Volatiles were extracted from rice billbug populations caught during the 2021 and 2022 growing season across the state of Arkansas. Three different chemicals (volatiles and closely related compounds) were evaluated for their attractancy to male and female rice billbugs. Findings from these preliminary studies shows that a significant response to tested semiochemicals was observed. Blends A (4-Methyl-2-pentanol) and B (2,6-Dimethyl-4-heptanol) were more frequently selected compared to blend C containing tert-Butyl hydroperoxide. Blend C also suggested signs of repellency in males, and higher attraction in females. However, further research is needed in this direction as developing a semiochemical-

based effective monitoring regime could potentially aid growers in economically and efficiently controlling rice billbug in future crops.

Introduction

Food safety, higher input costs, environmental conservation, and resistance management are the main contributing factors to a major shift in the pest management paradigm over the last few decades. The shift has gone from a calendar-based and broad-spectrum insecticide application to using a more integrated and high-efficacy approach (Witzgall et al., 2010). This shift has caused the integration of conventional, behavioral-based, biological, and cultural pest management strategies into IPM programs becoming increasingly important in 21st-century IPM systems. The implementation of semiochemicals such as insect pheromones is a major component of monitoring and managing target pests in agricultural crops (Tewari et al., 2014). Mating disruption, mass trapping, attract and kill, and push-pull are all dependent on the use of insect pheromone to successfully fulfill their strategy.

Insect Pheromones

Insect pheromones are described as volatile organic molecules of low molecular weight that draw out behavioral responses from individuals from the same species and can aid in communication from the same or opposite sex of a species (Phillips, 1997). Pheromones can be divided into several subcategories based on the nature of the compound and the action they trigger between emitters and receivers (Law and Regnier, 1971; Ginzel, 2010). Pheromones can be categorized as sex, alarm, aggregation, anti-aggregation, oviposition-deterring, and trail pheromones (Tewari et al. 2014). Releaser pheromones (alarm pheromones) will cause an immediate change in receiver behavior. Primer pheromones can be relatively slower and longer-term physiological changes (Law and Regnier, 1971; Ginzel 2010).

Generally, insect pheromones are produced by specialized exocrine glands associated with insect cuticle (Law and Regnier, 1971; Ayasse et al., 2001; Billen and Morgan, 2019). Though commonly produced by the cuticle, pheromones have been proven to be developed by other structures of the insect. This has been documented in one of the most notorious agricultural pests the boll weevil (*Anthonomus grandis,* Boheman)(Coleopter: Curculionidae) which biosynthesis of an aggregation pheromone occurs in the gut tissue (Taban et al., 2006). The family Curculionidae, commonly referred to as weevils, is a group of phytophagous insects and many are involved in the destruction of field and orchard crops (Tewari et al., 2014). In weevil pests (Coleoptera: Curculionidae), the pheromones are produced by the males to attract both male and female and are commonly referred to as aggregation pheromones (Bandeira et al. 2021). Ambrogi et al. (2009) state that predominant volatile compounds consist of secondary alcohols, aldehydes, (di)ketones, hydroxyesters, and hydroxyethers. Pheromones, both sex, and aggregate, as well as plant volatiles have been identified for 29 and 36 weevil species, respectively. The implementation of an aggregation pheromone of insects, specifically in Curculionidae is a vital tool for early detection of infestations and was one of the most important components of the Boll Weevil Eradication Program of the United States (Piñero et al., 2012; Tewari et al., 2014).

Sex Pheromones

Most used for the order Lepidoptera, sex pheromones act as signaling to attract potential mates over long distances. Chemoreceptor sensilla are highly sensitive and can facilitate low concentrations of sex pheromone in the environment (Law and Regnier, 1971). Time of day, weather, and availability of host plants are all factors that govern the release of sex pheromones

(Law and Regnier, 1971). Insects, both larval and adult stages, can segregate chemicals released from host plants and use them as a forerunner for sex pheromones (Landolt and Phillips, 1997).

Aggregation Pheromones

Aggregation pheromones are used as intraspecific signals that relay group formation and mating at a desired food source (Tinzaara et al., 2002). This classification of pheromone is observed in boll weevil, where chemicals released from males and plant volatiles attract adults to synthetic compounds (grandlure) placed in traps along cotton fields (Hardee et al., 1972; Hardee et al., 1974; Magalhães et al., 2018).

Pheromone Testing with Y-tube bioassays

A relatively simple but very effective method for testing synthetic compounds for pheromone testing is using a Y-tube choice test with or without olfactometer. The original concept of the olfactometer was to create an apparatus in which repellents and attractants could accurately be determined in a laboratory setting, then tested in the field. This could reduce time and expenses when using trial and error methods in the field (McIndoo,1926). The general concept of the Y-tube choice assays is to place an attractant in one arm of the Y-tube and control in the other. While in Y-tube olfactometer, in addition to above, the tube is airtight with charcoal filtered air being pushed through the two arms and pulled through the trunk of the "Y" This allows sole concentrations of the tested volatile to be tested and reduces the risk of air contaminates from outside the olfactometer. The tested specimen will then be placed at the trunk of the "Y" and the specimen will then travel to its desired volatile. Selected arms and time it would take to select an arm may be to dilution of the compound being tested. Bioassays using these methods can aid researchers in not only selecting the correct chemical compound for in-

field testing, but also the concentration at which it should be applied for optimum results. For instance, successful results with “Y” tube olfactometer testing of boll weevil volatiles lead to the use of synthetic aggregate pheromone that are still being used decades later. Olfactometer tests are not restricted to testing solely pheromones. Plant volatiles and other semiochemicals can also be tested to monitor responses from target species. Volatile organic compounds (VOC’s) play a large role in plant-insect ecological interaction, in particular herbivores that use VOC’s to locate its preferred hosts. A paper published by Magalhães et al. (2018) breaks down the implementation of olfactometer to analyze different compounds that help locate desired hosts plants. Different growth stages and injured and uninjured plants were sampled to observe what compounds were most efficacious for attracting cotton boll weevil. Results allowed researchers to conclude that identification of cotton compounds were significantly quicker on plants in vegetative plants when compared to reproductive plants. Significantly higher amounts of VOC’s were identified at 24-48 and 72-96 hours when vegetative plants were observed. This observation is interesting knowing the feeding habits of the cotton boll weevil, the insect targets fruiting structures of the cotton plant. So quicker responses to VOC’s from vegetative stages of cotton tell the story that cotton boll weevil uses these VOC’s to identify locations of the plants. The cotton boll weevil will then find a haven until the host plant is in a desired stage for feeding. This is a complex of higher learning in insects that the general population does not expect from insects. It seems that the high mixtures of VOC’s provide a more quantity than quality.

In the reproductive stages it appears to accomplish the opposite. Bioassays results show that six of the compounds show behavioral responses to adult boll weevils. These six compounds in combination can show statistically similar reactions when compared to natural reproductive stage cotton plant volatiles. These seem to go for more of a quality than quantity approach.

Based on the paper, it appears that these six compounds all result in a similar reaction. Observations led the reader to believe that the quality of the compounds gives the insect a more desirable response in that the desired host plant has now reached a point where it can be fed on.

Guerra (1968) published research explaining a method to bioassay sex pheromones for pink bollworm control. Results from this study show one of two theories. First, that the extract from virgin female pink bollworm moths act as aphrodisiac while simultaneously as a true attractant. Secondly, the extract taken from the female were attracted by the compound, and but another less volatile substance is what truly triggered the mating responses.

Stipulations of Olfactometer Testing

Olfactometer have resulted in economic successes over the last several decades, resulting in billions of dollars retained by producers. Though, the success has been very beneficial to some target species, there are stipulations. Several studies that have had successes in lab scenarios but once put in the field, the repellants lose their efficacy. Chamberlain (1959), observed several chemicals commonly used as repellants were not optimal for repellency in agriculture scenarios. Results stated that temperature had no effect of tested compounds, and gustatory materials had greater effects on agricultural pests than olfactory. These data suggest that concentrations of olfactory chemical need to be greater concentrations to not be diluted by other chemicals in the atmosphere.

All in all, testing olfactory chemicals for control of insects is a successful method in responses to successful IPM practices. Though successful, most practices have an Achilles heel. Not all insects, can be triggered by olfactory chemicals, and testing needs to be conducted to assure if more in depth olfactory research is required. A positive outlook is that olfactory testing

can be useful in discovering that if a target species is stimulated by olfactory compounds. Regardless of success, olfactory compound testing is a foundation when researching new insect species and new practical IPM practices.

Using Semiochemicals to Monitor Rice Billbug

In furrow-irrigated rice (FIR) rice billbug (*Sphenophorus pertinax*, Chittenden) (Coleoptera: Curculionidae) has achieved major pest status. Limited control measures have been observed to suppress rice billbug injury and retain yield. Findings from Floyd et al. (2021a) suggests that insecticide seed treatment combinations can suppress rice billbug populations and reduce injury. Findings from Floyd et al. (2021b) state that controlling rice billbug with a foliar application has not been successful. The authors suggest that application timing may be an issue in control failures with a foliar spray. The timing of rice billbug movement into the field has been refined in findings by Floyd et al. (2021c) but researchers suggest more investigation is warranted. In Arkansas, the implementation of pheromone in southwestern corn borer monitoring regimes allows growers and researchers to predict generations and target insecticide applications based on trap collection (McLeod and Studebaker, 2003). Researchers have hypothesized the utilization of rice billbug pheromone or related volatiles to target timely applications of foliar insecticides. If successful, the use of rice billbug semiochemicals could be a reliable source for FIR growers to make economically sound decisions on insecticide application. The objective of this experiment is to determine if some volatile compounds can be promoted into a rice billbug monitoring regime and deem development necessary.

Materials and Methods

Initial semiochemical blend testing

Sampling and Extraction

Rice billbug were collected from sites around Arkansas and placed in separate containers after being sexed in the field. Rice billbug were sexed using guidelines provided by findings of Chittenden (1905). The collected specimens were placed in a freezer to kill specimens before extractions. Once 400 billbugs of both male and female billbugs were collected the abdomen of both sexes were removed from head and thorax. The segments of the billbug were submerged in a glass vial containing a hexane solvent (5 ml) for 10 minutes, and then billbug segments were removed (Figure 1) from the extract. Each sex had eight vials of both, head+ thorax and abdomen solution. After extraction, all samples were filtered for any particles/insect body wax. These samples were analyzed using a matrix-assisted laser desorption/ionization (MALDI) mass spectrometry at the Arkansas Statewide Mass Spectrometry Facility. Volatile chemical compounds in samples were identified based on prominence. The three most prevalent compounds or related chemicals were evaluated for their attractancy to male and female rice billbugs.

Y-tube Bioassay

Behavioral assays were tested using a standard glass Y-tube to determine responses of rice billbug to volatile chemical compounds. In each arm of the Y-tube, a rubber septum loaded with 10 µl of the volatile compound (lure) was tested. Initial testing was conducted with no air flow being pushed through the Y tube. Rubber septum with volatile compound was placed in one trunk of the Y tube and untreated in the opposing trunk. Six rice billbug adults were placed at the trunk of the tube and sealed inside (Figure 1). Billbugs were placed in the tube for 10 min and first choice and residence time were noted. In this study, results were recorded in three categories: chose compound, chose control or did not choose. Each option was assigned a

number for analysis. Three chemical compounds used in this study were (a): 4-Methyl-2-pentanol (Blend A), (b): 2,6-Dimethyl-4-heptanol (Blend B), and (c) tert-Butyl hydroperoxide (Blend C) (Table 5.1). These compounds were the most prevalent chemicals or similar to chemicals identified in the extraction process. These compounds were tested on both male and female separately. Six runs of this experiment were conducted for each compound and tested on both sexes of rice billbug. Individually, a male or female billbug were placed at the base of the trunk of the Y-tube. Billbugs were placed in the tube for 10 min and first choice and residence time were recorded. Each weevil was tested only one time and rubber septum lures were replaced after 6 runs. When transitioning from male to female testing, the olfactometer were cleaned thoroughly using an acetone solution to prevent any residual sensory chemicals for the opposing sex. The arm on which a chemical was placed in a previous run were changed to eliminate the risk of directional bias. A total of 36 billbugs, were tested at each run. Data were analyzed using the SAS v9.4. Initial runs of these experiments were analyzed using the proc freq function, and significant response to blends were testing using fisher's exact test α=0.05.

In field evaluation of billbug lures

A single location in Arkansas county, Arkansas was selected for this trial due to high collection numbers earlier in the growing season. Traps were placed in the top management zone of the production field. All three blends were soaked into a cork covered with a small plastic container. These blends were deployed on top of a metal wire approximately 45 cm above the soil line. The wire was placed in the center of poly plastic irrigation tubing cut to cover $0.093m^2$ of soil area. The number of billbugs collected, and gender were documented daily for one week. All semiochemicals trapping systems were analyzed in the SAS 9.4v program across all locations.

Results

Initial semiochemical blend testing

(a) Male Response

A total of 150 male billbugs were tested in these experiments. Frequency of response to selected semiochemical blends can be found in Table 5.2. A significant response to blends was observed in this study (n=150; df=4; $P<0.01$). When analyzing the frequency of specimen that preferred the blend compared to the untreated check, blend A and B were most frequently selected at 40% and 42%, respectively. In contrast, blend C only had a 17.8% response by male billbugs. When analyzing solely blend, blend A, had a 37.5% response by male rice billbugs, while only 2.1% responded to the control. When testing blend B, male rice billbugs were more frequently making a choice compared to other blends. Blend B had a slightly higher frequency of response of 39.6% to the blend, but the control was selected more frequently (8.3%) when compared to blend A. Observations from the blend C experiment indicate that possible repellency was observed. The untreated control was twice as likely to be selected (35.2%) than blend C (14.8%). Regardless of blend, the most frequently recorded observation was male billbug did not choose. Over half of the billbugs tested for each chemical aggregated at the end of the y-tube and did not make a choice in the allotted time frame.

(b) **Female Response**

A total of 90 female billbugs were tested in these experiments, and the frequency of response to selected semiochemical blends are stated on Table 5.2. A significant response to blends was observed in this study (n=90; df=4; $P<0.01$). When analyzing response to blend A, 36.7% of females tested responded to the blend compared to the control (26.7%). However, blend B had slightly less response of 27.3% compared to the response from blend A. Blend B

also had less of a response compared to its control. Blend C was more often selected by females (39.4%) than blends A or B. When testing females only 26.7% did not make a choice.

(c) Pooled Response

Data from both the male and female experiments were pooled and a total of 240 billbug specimens were tested. A significant response to blends (n=240; df=4; $P<0.01$) was also observed when combing the data sets (Table 5.3). When pooled, blend A was the most frequently selected (37.2%) compared to both blend B (35.9%) and C (26.9%). Independently, blend A was also more than twice as likely to be selected (37.9%) when compared to its control (11.5%). The untreated control was less frequently selected in the blend A runs (11.5%) compared to control selected in blends B (24.4%) and C (34.5%) Blend A also had the highest frequency of billbug specimens not making a choice (51.3%).

In-field trial

No rice billbug specimens were found during the first five days of sampling. On day six, three male billbugs were found. One was collected from a trap containing blend A and two were found on traps containing blend B.

Discussion

Y-tube bioassay

Based on results from initial y-tube bioassays, male rice billbugs were more responsive to blends A and B. Blend compared to blend C appears to result in a repellency effect. On the contrary, females more frequently responded to blend C than A or B. This could indicate that chemical compounds in blend C or other closely related compounds may be used for signaling to

female rice billbug. This could aid in the development of a viable semiochemical to implement into a rice billbug trapping regime. However, further research is needed in this direction. Lures developed from such blends could be used to monitor female rice billbug populations prior to oviposition. If successful, insecticides applications could be initiated based on trap capture of female rice billbugs. Effective control of female rice billbugs prior to oviposition could reduce the amount of injury observed in FIR fields.

Depending on the stability of volatile chemicals in field environment, Blends A or B may be a viable option for producers wanting to adapt the FIR system in the following growing season. Blends A or B could allow a grower to observe if rice billbug populations are present in the area, deeming management decisions for rice billbug control necessary. It should also be noted that semiochemicals used in lab studies are occasionally not suitable for experiments in larger landscapes. Larger amounts of samples should be tested in the lab to monitor response to semiochemicals before blends are developed to withstand harsh factors of the uncontrolled environment. However, further research is needed to determine stability and longevity of these blends when deployed in field environment.

More infield trials are needed to verify if these blends are applicable in a non-controlled landscape. Based on results from the in-field trial readers would assume that these blends are not a feasible monitoring tool, but environmental conditions occurred that may have affected this trial. During the field trial, a precipitation event occurred less than 48 hours after semiochemical was placed in the field. Due to the simplicity of the traps, it is highly likely that the blends were compromised, and negatively affected the efficacy of the lures in attracting billbugs. Before a second initiation of the trial could occur, the producer harvested the field and traps had to be removed.

Conclusions

These data suggest similar findings to Giblin-Davis et al. (1996), that states semiochemicals released by males are aggregation pheromones that are attractive to both sexes. The release of chemical blend A or B was more frequently responded to by males. When blend C was used females were more frequent in responding to the variable. This could explain observations made in the field. Typically, it's been observed that males enter FIR fields first and an increase in females is observed later in the season. This could be the release of ter-Butyl hydroperoxide by males to attract female rice billbugs. Results from these experiments indicate that using semiochemical volatiles for monitoring rice billbug populations in FIR fields are a possibility, but more studies should be conducted, and more data should be collected to refine the best blend and determine the most efficacious strategy to deploy these volatiles in field environments. Using the volatile-based lures to monitor females to determine when to make an insecticide application prior to oviposition has the potential to reduce rice billbug injury, but currently no foliar insecticides are successfully controlling rice billbug. Based on successful management strategies currently available, blends from this study should be used as an awareness tool upon further refining. It could allow growers who are interested in implementing the FIR production system to observe if rice billbug is a threat on their operation. This could lead to proper management decisions, such as efficacious seed treatment packages being put in place prior to planting. As rice billbug research continues, semiochemical and other volatile organic compounds should be continually tested to discover their fit in a successful management regime.

In conclusion, the utilization of pheromones and other semiochemicals for rice billbug management could be a vital tool in managing rice billbug in the future, but further development is needed to distinguish proper semiochemical blends along with productive trapping systems.

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Table 5.1. Description of chemical blends used in bioassay experiments.

Blend Title	Chemical
A	4-Methyl-2-pentanol
B	2,6-Dimethyl-4-heptanol
C	tert-Butyl hydroperoxide

Table 5.2 Frequency table for initial lures y-tube bioassays. Male rice billbug response to billbug lure blends.

Response	**Blend**			
	A	**B**	**C**	**Total**
Did not Choose	29	25	27	81
Frequency	19.3	16.7	18.0	54.0
Row %	35.8	30.9	33.3	
Column %	60.4	52.1	50.0	
Chose Variable	18	19	8	45
Frequency	12.0	12.7	5.3	30.0
Row %	40.0	42.2	17.8	
Column %	37.5	39.6	14.8	
Chose Control	1	4	19	24
Frequency	0.7	2.7	12.7	16.0
Row %	4.2	16.7	79.2	
Column %	2.1	8.3	35.2	
N				150
df				4
X^2				<0.01
P				<0.01

Data was analyzed in PROC GLIMMIX using the Fisher's exact test at α=0.05.

Table 5.3 Frequency table for initial lure y-tube bioassays. Female rice billbug response to billbug lure blends.

Response	**Blend**			
	A	**B**	**C**	**Total**
Did not Choose	8	15	10	33
Frequency	8.9	16.7	11.1	36.7
Row %	24.2	45.5	30.3	
Column %	26.7	50.0	33.3	
Chose Variable	11	9	13	33
Frequency	12.2	10.0	14.4	36.7
Row %	33.3	27.3	39.4	
Column %	36.7	30.0	43.3	
Chose Control	11	6	7	24
Frequency	12.2	6.7	7.8	26.7
Row %	45.8	25.0	29.2	
Column %	36.7	30.0	23.3	
N				90
df				4
X^2				0.30
P				<0.01

Data was analyzed in PROC GLIMMIX using the Fisher's exact test at α=0.05.

Table 5.4 Frequency table for initial lure y-tube bioassays. Total rice billbug response to billbug lure blends.

Response	**Blend**			
	A	**B**	**C**	**Total**
Did not Choose	40	31	34	105
Frequency	16.7	12.9	14.2	43.8
Row %	38.1	29.5	32.4	
Column %	51.3	39.7	40.5	
Chose Variable	29	28	21	78
Frequency	12.08	11.7	8.8	32.5
Row %	37.18	35.9	26.9	
Column %	37.18	35.9	25.0	
Chose Control	9	19	29	57
Frequency	3.75	7.9	12.1	23.8
Row %	15.79	33.3	50.9	
Column %	11.54	24.3	34.5	
N				240
df				4
X^2				<0.01
P				<0.01

Data was analyzed in PROC GLIMMIX using the Fisher's exact test at α=0.05.

Figure 1. Separating rice billbug head and thorax from abdomen to begin insect volatile extraction process (Fayetteville, AR) (2020).

Figure 2. Vials containing either head and thorax or abdomen of rice billbugs for separated for semiochemical extraction (Fayetteville, AR) (2021).

Figure 3. Six male rice billbugs placed in Y-tube to observe response to semiochemical blends (Fayetteville, AR) (2022).

Chapter 6 – Conclusions

Prior to this research little to no information on rice billbug (*Sphenophorus pertinax*) (Coleoptera: Curculionidae) was available on this insect, and producers already utilizing the furrow-irrigated rice (FIR) system had no insight on how to manage this pest. As FIR gains popularity, growers will need options to manage this pest. Observations from this research have proven that rice billbug will be the major insect pest of FIR systems. Because of the information obtained through these studies, FIR producers will have a better understanding of where rice billbug is most likely to occur and how to manage this pest.

During the survey conducted in FIR from 2019 to 2021, multiple observations were made on rice billbug that had not been previously documented. Rice billbugs were found in almost every county surveyed and a large percentage of the fields surveyed. This suggests that rice billbug will readily seek out FIR and that no FIR locations are immune to infestation. This work also documents the relationship between grassy turnrows or fields that were near tree lines. Fields in these situations have much higher probability of having a rice billbug infestation. Most rice, whether FIR or traditional flooded systems, are rotated on a yearly basis in Arkansas. Based on the finding that rice billbug was overwintering in the field, it is imperative to rotate to decrease the likelihood of rice billbug infestations. While this may help to decrease the probability or severity of infestations, observations were made from 2019 to 2022 where fields moving into a FIR system for the first time were still infested with rice billbug.

Growers do have the ability to monitor for this pest using pink buckets. While this program performed the best in the studies discussed here, overall, it is inefficient. It performed better than any other method tested in these studies, yet during the survey rice billbug were located in multiple fields when no rice billbugs were found under the bucket. Preliminary work on pheromones to be used in monitoring rice billbug shows some promise. With more refinement

in both the pheromone and traps, a successful monitoring system can be established. Currently growers should evaluate their poly-plastic irrigation pipe as soon as rice begins to tiller and when it begins to senesce. Observations made at the end of the 2021 growing season showed that rice billbug will readily crawl under the irrigation pipe as they move into and out of FIR fields.

The major take-away from all the studies conducted from 2019 to 2022 is that rice billbug is the major insect pest of FIR. The one control tactic that performed better than all others was the use of a neonicotinoid seed treatment in conjunction with a diamide seed treatment. This combination performed better than foliar sprays, insecticide coated urea, or single insecticide seed treatments. Even though combinations of insecticide seed treatments performed better yield wise than all other treatments, damage still occurred in these plots. As of now there is no control method available to eliminate rice billbug injury. However, if growers are going to have FIR fields, that should be encouraged to use a combination of insecticide seed treatments.

Overall, findings from this research positively impacts the FIR industry across the Mid-Southern U.S., with the goal of keeping FIR producers profitable. Though this research is pivotal, expansion of these findings is required. This research was conducted to be a foundation on which innovation and experimentation could be forged. As rice billbug awareness becomes more prominent, an increase in questions and concerns is inevitable. Further development of rice billbug management strategies is imperative to keep FIR producers profitable for years to come.