Evaluation of Rice Stink Bug, Oebalus pugnax (F.), Damage and Monitoring Techniques in Rice, Oryza sativa L., and Grain Sorghum, Sorghum bicolor (L.)

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Abstract

Rice stink bug, *Oebalus pugnax* (F.), is a serious pest of headed rice, *Oryza sativa* L. and an occasional pest of heading grain sorghum in the Mid-south. Work from this dissertation focuses on resolving gaps in and knowledge of rice stink bug sampling and management, and attempts to create a basis for rice stink bug damage assessment in future studies.

Field experiments were conducted from 2016-2018 to asses variation in sweep net sampling by observing producers, researchers, extension personnel, consultants and their workers. Large levels of variation were found in sweep lengths between observed sweepers and reliability of smaller sweep lengths that were commonly used was evaluated. Controlled sweep lengths of 0.9m, 1.8m, and 3.5m were evaluated and significant differences in rice stink bug collection were observed. Sweep net samples measuring 1.8m or greater per sweep were determined to be accurate and reliable, and therefore are recommended for future sampling.

In 2018, uncaged trials were conducted to relate sweep net samples of 1.8m to direct and indirect yield loss by the rice stink bug. Peck levels and total milled rice were affected by rice stink bug density, whereas data on total head rice and direct yield loss were less clear. These results confirm the validity of the current Arkansas indirect yield loss threshold of 10 rice stink bugs during the second two weeks of heading.

In 2016-2018, insecticide termination timing for rice stink bug in rice was determined based on visual evaluation of percent rice grain maturity (hard dough). Data suggested that rice with low percentages of hard dough kernels were susceptible to indirect yield loss, but applications can be terminated at 60% hard dough if threshold-level populations aren’t present.

Experiments were performed in 2016 and 2017 to determine the amount of yield loss caused to grain sorghum by rice stink bug feeding at different heading growth stages, and to

develop dynamic thresholds for these stages. These data indicate that rice stink bug poses its greatest threat to grain sorghum in the early heading growth stages, and yield loss potential decreases as grain sorghum matures.

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Dedication

This dissertation is dedicated to my wife Sarah Cato. No one person has believed in me more than Sarah, and without her unconditional love and support none of this would have been possible. Sarah literally followed me through rice fields on hot Sunday mornings, and never once complained. Sarah deserves as much credit as anyone for the completion of this degree.

Table of Contents

Chapter 1 - Introduction

The rice stink bug, *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae), is a major pest of rice, *Oryza sativa* (L.), grown in the southern United States (Webb 1920). This stinkbug is oligophagous and feeds upon many cultivated (wheat, *Triticum aestivum* L., grain sorghum *Sorghum bicolor* (L.) Moench, corn, *Zea mays* L., and rice, *Oryza sativa* L.) and uncultivated (barnyardgrass *Echinochloa crus-galli* P.Beauv., cheat *Bromus tectorum* (L.), ryegrass, *Lolium spp.* L., and Johnsongrass, *Sorghum halepense* (L.) Perse.) grass species (Douglas 1939; Odglen and Warren 1962, Awuni et al. 2015a). Rice stink bug prefers to feed on rice and barnyardgrass, and is known to immediately move from alternate hosts once rice or barnyardgrass exert susceptible heads (Rashid et al. 2005, Rashid et al. 2006, Awuni et al. 2015a). Large shifts in occurrence are especially evident once rice fields in the midsouth begin to exert panicles, where rice stink bugs migrate from alternate hosts to preferred hosts due to an attraction to host kairomones (Douglas and Tullis 1950, Rashid et al. 2005, Rashid et al. 2006, Awuni et al. 2015a).

Rice stink bug feeds on the developing kernels of rice and other grasses beginning at the heading phase when the panicle is exerted from the boot until the end of the ripening phase, known as the hard dough growth stage (Swanson and Newsom 1962). Rice stink bug feeds by inserting piercing-sucking stylets into kernels to extract nutrients. Feeding during the flowering stage of rice development often causes blanked kernels and direct rough rice yield loss (Douglas and Tullis 1950, Swanson and Newsom 1962, Espino et al. 2007). This damage is a result of a reduction in the grain content and damage to flowers, where abortion of the flower could lead to a completely blank kernel. Feeding during the milk to soft and hard dough growth stages can result in a loss of quality associated with broken, chalky or discolored kernels (Douglas and Tullis 1950, Swanson and Newsom 1962, Espino et al. 2007). Loss of quality from rice stink bug feeding is caused by an introduction of pathogenic fungi inside of rice kernels. Rice stink bug feeding with piercing-sucking mouthparts leaves the kernels more susceptible to potential fungal invasions (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1963). Discolorations or malformations to rice kernels are collectively known as “peck” or “pecky rice,” and bulls-eye shaped lesions are most indicative of damage that resulted from rice stink bug feeding (Douglas and Tullis 1950, Lee et al. 1993). A high occurrence of pecky kernels can reduce USDA grade. Grade reduction is a response to unappealing discolorations and a potential decrease in the more valuable whole kernels (head rice), as peck is associated with increased kernel breakage during milling (Swanson and Newsom 1962, Bowling 1963, Espino et al. 2007, Hardke and Siebenmorgen 2013). Rice that receives no value reduction when sold is graded at either USDA Grade 1 or Grade 2, meaning that no more than 0.5% or 1.5% of kernels (by weight) are considered damaged (pecky) (Hardke and Siebenmorgen 2013). At 2.5% or 4.0% damaged kernels, rice is considered grade 3 and grade 4, and can incur a deduction of up to $0.003-$0.006/ kg of rice respectively. These reductions in quality could impact producers as much as $34-$68/hectare if assuming potential yield around 10,000 kg/hectare for grade 3 and grade 4 rice respectively.

In Arkansas, rice stink bug is managed with two different thresholds depending upon the growth stages present in each rice field (Lorenz and Hardke 2013). During the first two weeks of heading the action threshold is 5 rice stink bugs per 10 sweeps to prevent direct yield loss, and during the next 2 weeks of heading, the action threshold is 10 rice stink bugs on 10 sweeps to prevent quality loss. Thresholds differ in Louisiana and Mississippi, where the action threshold is 3 rice stink bugs per 10 sweeps during the first two weeks of heading, and Texas currently uses a dynamic threshold that considers cost of application, value of rice, loan value, expected yield, and growth stage (Way and Espino 2012, Catchot et al. 2018, Lorenz and Hardke 2013, Stout and Wilson 2018).

Many studies have sought to quantify the amount of direct and indirect yield loss that rice stink bug may cause. Many studies used cages that only examined the effect of rice stink bug on a single or a few panicles using sleeve cages (Nilakhe 1976, Rashid 2003, Patel et al. 2006, Awuni et al. 2015b). All studies using sleeve cages observed either large amounts of direct or indirect yield loss, depending upon the full scope of each trial. Many studies have also used cages that trap rice stink bug on multiple rice plants, which observed a density to area (length by width) relationship when considering the effect on indirect or direct yield loss (Douglas and Tullis 1950, Odglen and Warren 1962, Swanson and Newsom 1962, Bowling 1963, Espino et al. 2007, Blackman 2014, Awuni et al. 2015b). Douglas and Tullis (1950), Swanson and Newsom (1962), and Awuni et al. (2015b) observed large levels of direct and indirect yield loss with increased density of rice stink bug infestation using large cages. Odglen and Warren (1962), Espino et al. (2007), and Blackman (2014) did not observe direct yield loss through blanked kernels or whole mass loss, but Espino et al. (2007) did observe significant increases in peck with an increase in rice stink bug density using large cages. Two studies have sought to relate damage without the use of cages by monitoring populations using sweep nets and comparing sprayed plots to unsprayed plots (Harper et al. 1993, Tindall et al. 2005). Neither study observed direct yield loss, although both studies did find a relationship between increased peck occurrence and rice stink bug density.

Many of these damage studies have directly related the area of rice in cages to the area being sampled by sweep nets, however, there currently are no recommendations for the area (length x width) that should be sampled with a sweep net. Current sampling recommendations include frequency, at 10 sets of 10 sweeps with a 38cm diameter sweep net,, but the length of the sweep is more ambiguous. Across all rice production states sampling recommendations suggest a 180° sweep, from side to side, with at least 1 step per sweep (Way and Espino 2012, Catchot et al. 2018, Lorenz and Hardke 2013, Stout and Wilson 2018). Consultants and producers have been unhappy with these recommendations for almost 30 years. Multiple studies sought to discover why this sampling regimen is unattractive to stakeholders and provide alternatives (Harper et al. 1990, Espino et al. 2008, Way et al. 2018). Rice is not an easy crop to sample, as movement through fields is made difficult by flooded environments and tillering rice plants. Additionally, threshold studies estimate the amount of area sampled to create a treatment recommendation. This requires a known sampling area (length x width) and a known sampling success within that area (number caught vs. number present). If these two factors are unknown or poorly estimated, inaccurate thresholds will result.

In Arkansas, many insecticide applications are made during the hard dough growth stage due to the high densities of rice stink bug that often move from harvested fields nearby, or as the result of an egg lay from subthreshold populations during the first two weeks of heading. Harper et al. (1993) and Patel et al. (2006) found significantly increased levels of peck due to infestations of rice stink bug at the hard dough growth stage. However, not all kernels on an individual panicle reach the hard dough growth stage at the same time (Counce et al. 2000). Kernels at the tip of each panicle develop first and kernels closer to the base are often at a more susceptible growth stage. Considering that many insecticide applications are made during the hard dough growth stage, and that susceptibility to indirect damage likely decreases as rice plants reach full maturity, an insecticide termination timing is necessary to prevent unwarranted insecticide applications.

Rice stink bug is also known to occasionally be a serious pest of grain sorghum, although they are almost always present in fields to some degree. Hall and Teetes (1982) determined that an insecticide application was warranted when rice stink bug densities reached 4 per head at the milk stage and 8 per head at soft dough. The current rice stink bug threshold for grain sorghum in Arkansas and other states is based solely on work by Hall and Teetes (1982), although thresholds vary slightly from state to state. While the relationship between rice stink bug densities and grain sorghum yield has not been replicated in over 30 years, rice stink bug recommendations in Texas have recently been modified (Texas A&M 2018). Typical grain sorghum prices would yield an economic threshold ranging from 1 rice stink bug per head to 1 rice stink bug per two heads on average, making this threshold much lower than Arkansas's current threshold. Additionally, this threshold does not change in relation to the plant phenology (Texas A&M 2018), although yield loss from rice stink bug varies depending upon the growth stage when feeding occurs (Patel et al. 2006; Espino et al. 2007).

Objectives

1. The objectives of this study were to determine the role that sweep length plays in sampling accuracy and determine the feasibility of using a shorter sweep length (< 3.5m).
2. Estimate direct and indirect yield loss due to different densities of rice stink bug in a defined sampling area of uncaged rice.
3. Determine changes in kernel damage (% peck) of rice plants relative to feeding by a range of densities of rice stink bug during increasing percentages of kernels at hard dough on rice panicles, and create a decision-making protocol to terminate insecticide applications.

4. Determine the amount of yield loss caused to grain sorghum caused by rice stink bug across heading growth stages and develop dynamic thresholds across these growth stages.

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Chapter 2 - Sweep Length Sampling Recommendations for Rice Stink Bug, *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae) in Rice, *Oryza sativa* (L.)

Abstract

The rice stink bug, *Oebalus pugnax* (F.), is a key pest of heading rice in the southern United States. Sweep net sampling is the recommended method for sampling rice stink bug in rice, *Oryza sativa* L., but there currently exists no specific recommendation for sweep length, and a large amount of variation likely exists amongst samplers. The objectives of this study were to determine the role that sweep length plays in sampling accuracy and determine the feasibility of using sweep lengths smaller than 180°. When monitoring sweep lengths by consultants, producers, and researchers, a large amount of variation in sweep length and a significant linear relationship between sweep length and rice stink bug catch per 10 sweeps was observed. Sweep length was then controlled at three levels (0.8m, 1.8m, and 3.5m) and a change from 0.8m-1.8m in sweep length led to an increase on average of 2.28 rice stink bugs per 10 sweeps. These data suggest knowledge of sweep length is vital, and paired with large amounts of observed variation in sweep length, recommending a specific sweep length is ideal. Using Taylor's values, it was determined that 1.8m sweeps resulted in density estimates that were as reliable as 3.5m (180°) sweeps, suggesting a longer sweep length was not necessary. Recommendation of 1.8m sweeps will lead to greater accuracy in relating threshold studies to real-world sampling area, increase adoption rates and confidence in the recommended sampling regimen, and increase accuracy of action threshold decisions being made for rice stink bug.

Introduction

The rice stink bug, *Oebalus pugnax* (F.), is a major pest of rice, *Oryza sativa* L., in the southern United States (Webb 1920). Rice stink bug feeds on the developing kernels of many species of grasses including cultivated species such as rice, *Oryza sativa* L. and grain sorghum *Sorghum bicolor* (L.), as well as many uncultivated species such as barnyardgrass, *Echinochloa* *crus-galli* Beauv and ryegrass, *Lolium spp.* L. (Douglas 1939, Odglen and Warren 1962). The damage that rice stink bug causes is directly dependent upon the plant's growth stage at which feeding begins (Swanson and Newsom 1962, Bowling 1963, Espino et al. 2007). Feeding that occurs from the flowering growth stage through the beginning portion of the milk growth stage can result in direct yield loss from blanked kernels and reduced kernel weights (Swanson and Newsom 1962, Bowling 1963, Espino et al. 2007). Feeding that occurs in the milk, soft dough, or hard dough growth stage, reduces grain quality by increasing the number of broken, chalky, or discolored kernels (Swanson and Newsom 1962, Bowling 1963, Espino et al. 2007, Lorenz and Hardke 2013). This reduced grain quality can result in a lower USDA grade due to potential kernel breakage and because it is less appealing to consumers due to discoloration, leading to a reduction in grain value (Swanson and Newsom 1962, Bowling 1963, Espino et al. 2007, Hardke and Siebenmorgen 2013).

Sampling for rice stink bug is performed using a standard 38cm diameter sweep net, with 10 sets of 10 sweep samples recommended for estimating the density present in a field. In Arkansas, rice stink bug is managed with two different thresholds depending upon the growth stages present in each rice field (Lorenz and Hardke 2013). During the first two weeks of heading the action threshold is 5 rice stink bugs per 10 sweeps to prevent direct yield loss, and during the next 2 weeks of heading, the action threshold is 10 rice stink bugs per 10 sweeps to prevent quality loss. These thresholds were created using cage trials, which is typical of most rice stink bug damage determination work. The damage in a controlled area of rice within the cage is then related directly to the area sampled with a standard 38cm sweep net (Swanson and Newsom 1962, Bowling 1963, Espino et al. 2007, Awuni et al. 2015).

Many threshold studies have directly related the area of rice in cages to the area being sampled by sweep nets, however, there currently are no recommendations for the area (length x width) that should be sampled with a sweep net. Current sampling recommendations include frequency, at 10 sets of 10 sweeps, and the width of the sweep net at 38cm, but the length of the sweep is more ambiguous. Across rice producing states, sampling recommendations are never more specific than suggesting a 180° sweep, from side to side, with at least 1 step per sweep and between sweeps, with many states providing less instruction (Way and Espino 2012, Catchot et al. 2018, Lorenz and Hardke 2013, Stout and Wilson 2018). The original threshold was based on a true 180° sweep (3.5m), as well as the research that determined the number of samples to make an accurate recommendation (Foster et al. 1989, Espino et al. 2008) (Personal communications with M.O. Way). Although a true 180° sweep (3.5m) may have been possible when the recommendations were first made, thicker rice stands and increased tillering in hybrid cultivars makes it more difficult to walk through rice fields at a quick pace. In fact, consultants and producers have been unhappy with these recommendations for almost 30 years. Multiple studies have sought to discover why this sampling regimen is unattractive to stakeholders and identify alternatives (Harper et al. 1990, Espino et al. 2008, Way et al. 2018). At scout schools, field days, in-service training of extension agents, and at the biannual rice college in Arkansas, participants did not adhere strictly to a 180° sweep (3.5m) resulting in a large amount of observed variation between persons in actual sweep length. Many rice stink bug samplers have adopted a shorter sweep length that is personally comfortable. Considering that threshold studies estimate the amount of area sampled to create a treatment recommendation, it is important to standardize the sweep length that growers and consultants are typically using. If the area being sampled by consultants and producers differs from that estimated by researchers when adapting thresholds, inaccurate recommendations will likely result.

The objectives of this study were to determine the role that sweep length plays in sampling accuracy and determine the feasibility of using a shorter sweep length (< 3.5m).

Materials and Methods

Variation in Sweep Length

Eight Arkansas rice fields were sampled in the summer of 2016 using a blocked design with 3-4 replicate blocks per field (transects). At least 3 unique rice sweepers were observed within each transect. Transects were at least 50m apart and 10m from the field edge. The length sampled by sweep net was measured and considered as a potential predictor. Sweepers evaluated for this study included entomology program associates and graduate students, county agents, and Arkansas crop consultants and their employees. Sweep length was determined by following the sweeper and measuring the distance of each sweep from the center of the sweeper's body to the edge of the net when it exited the crop canopy. To record the total sweep length, sweepers took 2 sets of 5 consecutive sweeps, with the distance of a sweep from the left and then the right of the sweeper to the center of the body being recorded at the end of the two sets of 5 sweeps. The sweep lengths measured from the left and right pair of 5 sweeps were added together and considered the total sweep length. The number of rice stink bugs captured from each set of 5 sweeps was also counted and recorded. The total of each replicate was then paired with the total number of rice stink bugs caught from the 2 sets of 5 sweeps.

Data were analyzed using PROC REG (SAS v. 9.4, SAS Institute, Cary, NC) and a general linear model with a normal distribution. The total number of stink bugs sampled was used as the response variable and sweep length as the predictor. Data were found to be nonnormal, and the response was log-transformed after indication from a Box-Cox analysis (Osborne 2010). A significant linear relationship was determined using a t-test at *P*=0.05.

Differences between Controlled Sweep Lengths

Ten Arkansas rice fields were sampled in 2017 using a randomized complete block design with 4-8 replicate blocks (transects measuring at least 110m) and 3 treatments. Within each field three sweep lengths were used: 0.9m, 1.8m, and 3.5m (180° sweep). These lengths were selected as representatives of sweep lengths encompassing what was observed in 2016, with the mean and median of observed sweep lengths equaling 1.87 and 1.99m respectively. The 3.5m sweep contained a large arc (180°), whereas the 0.9m and 1.8m sweep lengths contained minimal arc, and were a linear distanced in front of sweepers as they moved. Two sweepers completed 3-4 subsamples of each sweep length treatment within each transect, and at least 4 transects were completed in each field with over 50m separating each transect. Sweeps were taken slightly in front of the sweeper with the ring of the net positioned with the rice heads in the center, at a quick pace, with at least 1-2 steps being taken between each sweep. In total 922 sets of 10 sweep samples were taken across the ten fields that were sampled. Of those 10 fields sampled, 6 were considered to be at the early growth stage (flowering and milk), and 4 were considered to be at the late growth stage (soft dough and hard dough). When considering cultivar, 5 of the fields were hybrid cultivars and 5 were pureline varieties.

Data were compared using a one-way analysis of variance utilizing PROC GLIMMIX (SAS v. 9.4, SAS Institute, Cary, NC) and a general linear model with a normal distribution, and denominator degrees of freedom were adjusted using a Kenward-Rogers approximation (Kenward and Roger 1997). Sweep length was considered a fixed factor, with the number of rice stink bugs sampled averaged across subsamples within transect being used as the response variable. Additionally, random variables for this analysis consisted of: field, transect nested within field, and sampler nested within each field*transect. Means were then separated using Tukey's HSD post hoc analysis at a significance level of $\alpha=0.05$.

Optimum Sample Size

Twenty-four commercial Arkansas rice fields were sampled across 2017 and 2018. Each field was sampled in a least four unique transects, nine meters from the edge of the field. An average of 45 samples (sets of 10 sweeps) were taken evenly across these transects, with 1064 samples taken in total. The number of samples taken per transect and the number of transects varied per field depending upon the size and shape of the field. Samples measured either 1.8 m or 3.5 m in width each, samplers were at least 6 m apart, and samplers worked at a quick pace taking at least 1-2 steps in between each sweep. After each sample the number of rice stink bugs caught per sample were recorded. Fifteen of these fields were considered to be in the early heading growth stage (head emergence to milk), and 9 fields were considered in the late heading growth stage (soft dough to hard dough).

The spatial pattern of rice stink bug across commercial rice fields was explained using Taylor's power law, $s^2 = ax^b$, which relates mean density to variance (Taylor 1961, 1984). In this equation s^2 is the sample variance, x is the sample mean, and a and b are Taylor's coefficients as reported by nonlinear regression of the variance and mean of the sample. A log transformation of the sample mean and variance are traditionally used along with simple linear regression to estimate Taylor's coefficients, however, this was not used in this study because it can overestimate variances at low densities (Wilson et al. 1983, Wilson 1994). Non-linear regression of sample variance and sample mean values was performed using PROC NLIN (SAS v. 9.4, SAS Institute, Cary, NC), with data compared across all 24 fields.

The optimum sample size (*n*) at varying levels of reliability was then determined using Karadinos (1976) equation with modifications by Wilson and Room (1982): $n=t^2_{\alpha/2}D_x^{-2}s^2x^{-2}$. This equation was then modified by substituting s^2 as determined by Taylor's power law, yielding: $n=t^2_{\alpha/2}D_x^{-2}ax^{b-2}$, where $t_{\alpha/2}$ is the standard normal variate for a two-tailed confidence interval at α=0.10, D_x is a measure of reliability using the proportion of the mean equivalent to half the desired confidence interval (0.1, 0.2, or 0.3), and *x* is the mean density. D_x values of 0.1, 0.2, and 0.3 were used to determine estimates of sample size within 10, 20, or 30% of mean using a 90% confidence interval (α=0.1). The mean density (*x*) is typically set to the economic threshold value, however, the threshold for rice stink bug is in flux across many states. Therefore a range of values encompassing rice stink bug thresholds were used (3, 5, 10, and 20 rice stink bugs).

Results

Variation in Sweep Length

Regression analysis indicated a significant linear relationship between sweep length and the number of rice stink bugs sampled (log(y) = 0.19 + 0.49x | SE = 0.11 and 0.06 | t = 8.42 | P < 0.01 | RSE = 0.12) (Figure 2.1). Each addition of 1m in sweep length resulted in an increase of 9.4 rice stink bugs captured in a 10 sweep sample. (Figure 2.1). The mean and median sweep length observed was 1.87m and 1.99m respectively (Figure 2.1).

Differences between Controlled Sweep Lengths

Rice stink bug sample averages were controlled across growth stage and cultivar using a one-way analysis of variance where significant differences were observed between the different sweep lengths tested (F = 125.8; df = 2, 187; P < 0.01) (Table 2.1). The 3.5m sweep length mean was found to be significantly larger than both the 1.8m and 0.9m sample means (Table 2.1).

Additionally, the sweep length of 1.8m sampled significantly more rice stink bugs than the sweep length of 0.9m (Table 2.1).

Optimum Sample Size

Taylor's coefficients are reported for two sweep lengths, one that represents the average sweep length observed in 2016 (1.8m) and one that represents a 180° sweep length as described by Espino et al. (2008) (3.5m) (Table 2.2). The number of 10 sweep samples necessary to obtain a density estimate within 10%, 20%, and 30% ($D_x = 0.1$, $D_x = 0.2$, and $D_x = 0.3$) of the mean was then reported for both the 3.5m and 1.8m sweep lengths (Table 2.3, Figure 2.2). The 3.5m and 1.8m sweep lengths exhibited similar numbers of sample units required to obtain reliable density estimates (Table 2.3, Figure 2.2). When considering a density of 3 rice stink bugs per 10 sweeps, 16 and 17 samples of 10 sweeps would be necessary for the 1.8m and 3.5m sweep lengths respectively to obtain an estimate within 20% of the mean ($D_x = 0.2$) (Table 2.3, Figure 2.2). When density increased to 5 rice stink bugs per 10 sweeps, the number of 10 sweep samples decreased to 9 to obtain an estimate within 20% ($D_x = 0.2$) of the mean respectively for both sweep lengths (Table 2.3, Figure 2.2). Very few sets of 10 sweep samples were necessary when densities reached 10 rice stink bugs per 10 sweeps. At that density, only 4 samples of 10 sweeps were needed at the 1.8m and 3.5m sweep lengths to obtain an estimate within 20% ($D_x = 0.2$ and $D_x = 0.3$) of the mean (Table 2.3, Figure 2.2).

Taylor's coefficients are also reported for subsections of the 1.8m sweep length: all fields from 2017 and 2018 compared together; fields from 2017 and 2018 that were sampled in the flowering-milk growth stage analyzed alone; and fields from 2017 and 2018 that were sampled in the soft-hard dough growth stages analyzed alone (Table 2.2). When considering an average density of 5 rice stink bugs per 10 sweeps, 9 and 11 sets of 10 sweeps would be necessary to obtain an estimate within 20% ($D_x = 0.2$) of the mean for fields sampled in the early and late heading growth stages respectively (Table 2.3, Figure 2.3). At a mean of 10 rice stink bugs per 10 sweeps, 4 and 5 samples would be necessary in the early and late heading growth stages respectively to obtain an estimate within 20% ($D_x = 0.2$) of the mean (Table 2.3, Figure 2.3).

Discussion

Multiple studies have previously determined the validity of 10 sweep samples for the rice stink bug in rice, however, the actual sampled area (length x width) has not been reasonably explored (Foster et al. 1989, Espino et al. 2008). Data from 2016 not only indicated that a large amount of variation existed in the sweep lengths employed by rice stink bug samplers, but it also suggested that variation from these samplers significantly correlated with an increase in the number of rice stink bugs sampled. These data indicate that an increase in sweep length from 1-2m could lead to sample estimations that increased by 300%, which could be the deciding factor for treatment decisions.

Variation in sweep length was found to exist with data from 2016 and indicated that sweep length played a vital role in the number of rice stink bugs caught. However, these data were not able to successfully account for other factors related to sweep net sampling technique such as accuracy in sampling the correct canopy height, walking speed, or sweep net hoop angle. Data from 2017 confirmed that when controlling for these additional factors, sweep length still had a significant effect on the number of rice stink bugs being captured. These data indicate that a change from a 0.8m sweep length to a 1.9m sweep length could lead to an increase in mean sample catch of 2.3 rice stink bugs per 10 sweeps. These results suggest that sweepers who employed the same technique (sweep net hoop angle, walking speed, and correct canopy height) could come to different conclusions for treatment decisions if sweep length differed.

Additionally, almost all economic thresholds that have estimated rice stink bug damage have related insect density and an area of rice being sampled (Swanson and Newsom 1962, Bowling 1963, Espino et al. 2007, Awuni et al. 2015). Considering that the 3.5m sweep investigated by this study has historically been used to create rice stink bug thresholds, researchers are likely overestimating the actual area being sampled, and therefore may be misidentifying the role mean rice stink bug catches play in both direct and indirect yield loss from this pest (Espino et al. 2007, Awuni et al. 2015).

Sampling recommendations for rice stink bug management in Arkansas rice currently include language suggesting 10 samples of 10 sweeps that are 180°. This study determined that 1.8m sweeps were similar in reliability to 180° sweeps (3.5m) when estimating population densities of rice stink bug. Overall low levels of variance and uniform densities were observed in this study when considering both 1.8m sweeps and 3.5m sweeps. Previous studies indicated that populations of rice stink bug exhibited large levels of aggregation as variance levels were much higher (Foster et al. 1989, Espino et al. 2008). It is possible that these differences were observed due to much larger landscape-level effects, as these three studies were performed in three different states with differing agroecosystems. Our study was also performed with only 2 operators taking sweep net samples across 1064 samples in 24 fields, and it is possible that the addition of many more sweep net operators and varied technique by Foster et al. (1989) and Espino et al. (2008) led to larger variance. Other studies have suggested low levels of clustering with stink bug species in specific times of the year, especially when considering native species adapted to the environment and when not sampling edges of the field (Wallner 1987, Reay-Jones et al. 2009, Hahn et al. 2017).

The threshold for rice stink bug in Arkansas is either 5 or 10 rice stink bugs per 10 sweeps (Lorenz and Hardke 2013). Therefore, these data suggest 9 or 4 sets of 10 sweep samples would be necessary to obtain an estimate within 20% of the true mean for these two thresholds respectively. This is much lower than previously observed by Espino et al. (2008), which indicates an optimum sample size of ~50 and ~25 sets of 10 sweep samples being necessary for an estimation within 20% of the mean respectively. If thresholds are as low as 3 rice stink bugs per 10 sweep sample, as currently used by both Mississippi and Louisiana during the first 2 weeks of heading (Catchot et al. 2018, Stout and Wilson 2018), our study indicates that 16 sets of 10 sweep samples would be necessary for a reliable estimate within 20% of the mean. A necessity for an increase in sample size as target density decreased was also observed by both Espino et al. (2008) with rice stink bug and Reay-Jones et al. (2009) with multiple stink bug species in cotton. These differences are likely due to decreased uniformity in rice stink bug populations as density decreases.

These data suggest that knowledge of the sweep length used plays a vital role in estimating the area being sampled in a rice field. Considering both the ambiguity of a 180° sweep length sampling regimen, and the reluctance of producers and consultants to adhere to this regimen, a more specific and less exhausting recommendation needs to be administered (Harper et al. 1990, Espino et al. 2008, Way et al. 2018). Average yields in rice have increased over 37% in the last 30 years alone (NASS 2018), and rice has become more difficult to sample due to increased tillering and overall more rice kernels passing through a sweep net sample. These changes, along with smaller sweep lengths already adopted by consultants and producers, indicate that a more laborious sweep net method is not ideal. Considering these implications, 1.8m sweep lengths are recommended in sets of 10 sweeps per sample based on reliability exhibited by this study, and feasibility by the large decrease in area needed to be sampled.

Results from this study seek to aid the process of relating rice stink bug numbers sampled in rice fields to potential damage that this pest can elicit. Recommending a specific sweep length is the first step to accurately identifying and relating to the area being sampled. Further studies hope to accurately relate 1.8m sweeps and associated rice stink bug catch averages with both direct and indirect yield loss caused by this pest, without using cages that may affect insect behavior.

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Table 2.1 Mean number of rice stink bug, *Oebalus pugnax*, caught per 10 sweep sample using three sweep length treatments across ten rice fields sampled in Arkansas in 2017.

Sweep Length	10 Sweep Catch (Rice Stink Bugs)	Std. Deviation	Std. Error	Sample Size (n)
3.5m	10.6 a	7.0	0.4	46
1.8m	6.7 b	5.2	0.3	46
0.9m	4.4 c	4.1	0.2	46

10 sweep rice stink bug catch averages followed by a different letter are significantly different according to a Tukey HSD at α=0.05

Table 2.2 Coefficients for Taylor's power law and results from non-linear regression sampled across 24 Arkansas rice fields during 2017 and 2018, where a and b are coefficients from non-linear regression and R^2 indicates the model fit and P indicates significance, and n is the number of fields explored.

Sweep Length	Heading Class	Mean	Var.	*a*	*b*	R^2	*P*	*n*	Var. to mean ratio
1.8m	All Fields	8.3	4.7	0.8	0.9	0.96	<0.01	24	0.6
	Flowering-Milk	7.8	3.8	0.9	0.8	0.98	<0.01	15	0.5
	Soft-Hard Dough	9.3	5.9	1.2	0.6	0.97	<0.01	9	0.6
3.5m	All Fields	9.5	4.5	0.9	0.7	0.99	<0.01	9	0.5

Table 2.3 Optimum sample size for thresholds of 3, 5, and 10 rice stink bugs per 10 sweeps for a reliability estimate within 20% of the mean, reported for 1.8m and 3.5m sweep lengths across growth stage and for flowering-milk and soft-hard dough for 1.8m sweeps only.

Sweep Length	Heading Class	Reliability	Threshold	Optimum sample size
1.8m	All Fields	20%	3	16
		20%	5	9
		20%	10	4
	Flowering-Milk	20%	3	19
		20%	5	9
		20%	10	4
	Soft-Hard Dough	20%	3	20
		20%	5	11
		20%	10	5
3.5m	All Fields	20%	3	17
		20%	5	9
		20%	10	4

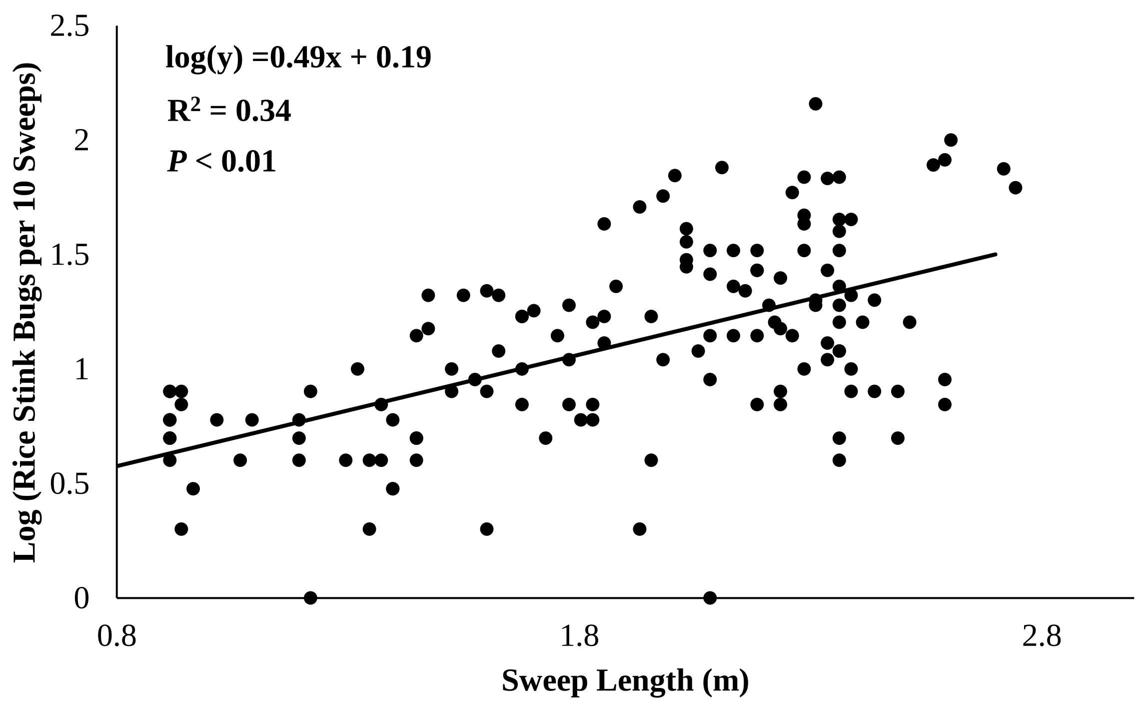

Figure 2.1 Recorded sweep lengths and corresponding log transformed 10 sweep rice stink bug, *Oebalus pugnax*, sample catches from entomology program associates and graduate students, county agents, and Arkansas crop consultants and their employees recorded in Arkansas rice in 2016.

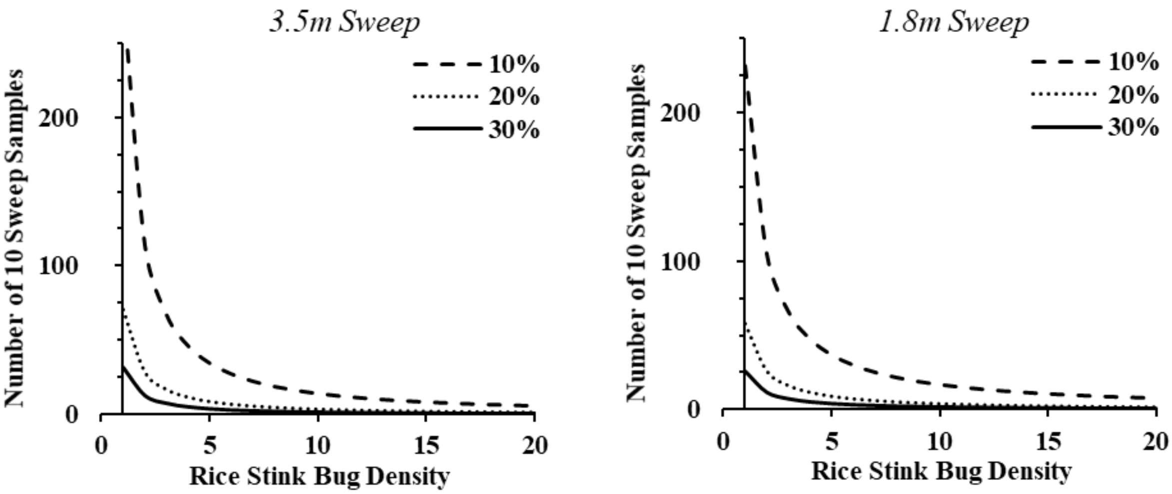

Figure 2.2 Optimum sample size required to obtain a density estimate within 10, 20, and 30% of the mean for 10 sweep samples for rice stink bug, *Oebalus pugnax*, using a sweep length of 3.5m and 1.8m across growth stages in Arkansas rice in 2017 and 2018.

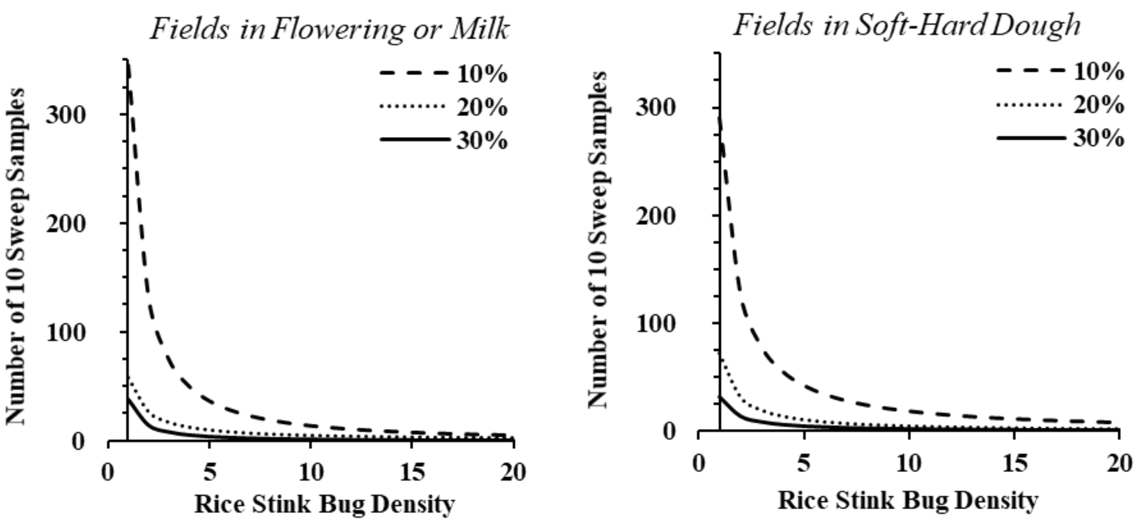

Figure 2.3 Optimum sample size required to obtain a density estimate within 10, 20, and 30% of the mean for 10 sweep samples for rice stink bug, *Oebalus pugnax*, during two different growth stage classes using a 1.8m sweep in Arkansas rice in 2018.

Chapter 3 - Relating Rice Stink Bug, *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae), Sampling to Direct and Indirect Yield Loss in Rice, *Oryza sativa* L.

Abstract

The rice stink bug is the most important pest of heading rice in the southern United States. Although many studies have sought to quantify the amount of direct and indirect yield loss that rice stink bug is capable of causing to rice, no study has directly related rice stink bug densities used to the sampled area in rice fields. The objective of this study was to estimate direct and indirect yield loss due to different densities of rice stink bug in a defined sampling area of uncaged rice. Field experiments were conducted in 2018 across six locations using a randomized complete block design with 4 replicate blocks per location and 4 treatments. Treatment thresholds included: 1) an untreated control, 2) standard threshold of 5 stink bugs per 10 sweeps the first two weeks of heading followed by 10 stink bugs per 10 sweeps the second two weeks of heading, 3) 10 rice stink bugs per 10 sweeps throughout heading, and 4) 20 rice stink bugs per 10 sweeps throughout heading. When considering indirect yield loss, populations averaging 10 rice stink bugs per 10 sweeps yielded peck levels of 1.8% when no insecticide applications were made. A relationship between milled rice yield and peck was also observed, but no significant relationship was observed for head rice yield or direct yield loss. Results from this study confirm the validity of the current Arkansas indirect yield loss threshold of 10 rice stink bugs per 10 sweeps during the second two weeks of heading.

Introduction

The rice stink bug, *Oebalus pugnax* (L.) (Hemiptera: Pentatomidae), is the most important pest of headed rice, *Oryza, sativa* L., in the southern United States (Webb 1920). Rice stink bug is a graminaceous feeder and feeds upon many cultivated (wheat, *Triticum aestivum* L., grain sorghum *Sorghum bicolor* (L.) Moench, and rice) and uncultivated (barnyard grass *Echinochloa crus-galli* P.Beauv., rye grass, *Lolium spp.* L., and Johnson grass, *Sorghum* *halepense* (L.) Perse.) grass species (Douglas 1939, Odglen and Warren 1962, Awuni et al. 2015a). Rice stink bug prefers to feed on rice and barnyard grass compared to other species. It will move from alternate hosts into rice or barnyard grass when panicles begin to emerge (Rashid et al. 2005, Rashid et al. 2006, Awuni et al. 2015a). Large shifts in occurrence are especially evident once rice fields in the midsouth begin to exert panicles (Douglas and Tullis 1950, Rashid et al. 2005, Rashid et al. 2006, Awuni et al. 2015a).

Sampling for rice stink bug is performed using a 38cm diameter sweep net, with 10 sets of 10 sweep samples recommended for estimating the population density present in a field. Rice stink bug in Arkansas is managed with two different action thresholds depending upon the growth stages present in each rice field (Lorenz and Hardke 2013). During the first two weeks of heading, the action threshold is 5 rice stink bugs per 10 sweeps to prevent direct yield loss, and during the next 2 weeks of heading, the action threshold is 10 rice stink bugs per 10 sweeps to prevent quality loss.

Rice stink bug feeds on the developing kernels of rice and other grasses beginning at the heading phase when the panicle is exerted from the boot until the end of the ripening phase, known as the hard dough growth stage (Swanson and Newsom 1962). Rice stink bug feeds by inserting piercing-sucking stylets into kernels to extract nutrients. Feeding during the flowering stage of rice development often causes blanked kernels and direct rough rice yield loss (Douglas and Tullis 1950, Swanson and Newsom 1962, Espino et al. 2007). This damage is a result of a reduction of the grain content and damage to flowers, where abortion of the flower could lead to a completely blank kernel. Feeding during the milk to soft and hard dough growth stages can result in a loss of quality associated with broken, chalky or discolored kernels (Douglas and Tullis 1950, Swanson and Newsom 1962, Espino et al. 2007). Loss of kernel quality caused by rice stink bug feeding makes kernel susceptible to entry of pathogenic fungi (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1963). Bulls-eye shaped lesions are most indicative of damage that resulted from rice stink bug feeding (Douglas and Tullis 1950, Lee et al. 1993). Discolorations or malformations to rice kernels due to stink bug feeding and fungal infection are collectively known as "peck" or "pecky rice." Peck increases the potential that a kernel could break during milling, and a high occurrence of pecky kernels can reduce USDA grade. Grade reduction is a response to unappealing discolorations and a potential decrease in the more valuable whole kernels (head rice) (Swanson and Newsom 1962, Bowling 1963, Espino et al. 2007, Hardke and Siebenmorgen 2013). Traditionally, rice that receives no value reduction when sold in Arkansas is graded at either USDA Grade 1 or Grade 2, meaning that no more than 0.5% or 1.5% of kernels (by weight) are considered pecky (Personal communications with Jarrod Hardke). At 2.5% or 4.0% damaged kernels, rice is considered grade 3 and grade 4, and can incur a deduction of up to $0.003-$0.006 per kg of rice respectively. These reductions in quality could impact producers as much as $34-$68 per ha if assuming potential yield around 10,000 kg per ha for grade 3 and grade 4 rice respectively (Hardke and Siebenmorgen 2013).

Many studies have sought to quantify the amount of direct (weight loss) and indirect yield loss (quality loss) caused by rice stink bug. Some of these studies used sleeve cages that examined the effect of rice stink bug on a single or a few panicles (Nilakhe 1976, Rashid 2003, Patel et al. 2006, Awuni et al. 2015b). All studies using sleeve cages observed either large amounts of direct or indirect yield loss, depending upon the full scope of each trial. Many studies have also used cages that trap rice stink bug on multiple rice plants, which observed a density to area (length by width) relationship when considering the effect on indirect or direct yield loss (Douglas and Tullis 1950, Odglen and Warren 1962, Swanson and Newsom 1962, Bowling

1963, Espino et al. 2007, Blackman 2014, Awuni et al. 2015). Douglas and Tullis (1950), Swanson and Newsom (1962), and Awuni et al. (2015b) observed large levels of direct and indirect yield loss with increased density of rice stink bug infestation using large cages. Odglen and Warren (1962), Espino et al. (2007), and Blackman (2014) did not observe direct yield loss through blanked kernels or whole mass loss, but Espino et al. (2007) did observe significant increases in peck with an increase in rice stink bug density using large cages. Two studies have sought to relate damage without the use of cages by monitoring populations using sweep nets and comparing sprayed plots to unsprayed plots (Harper et al. 1993, Tindall et al. 2005). Neither study observed direct yield loss, although both studies did find a relationship between increased peck occurrence and rice stink bug density.

Damage studies that utilize cages seek to relate the amount of damage present using a known density, number of rice stink bugs per m^2, to the area that is sampled within the field. This requires a known sampling area (length x width) and a known sampling success within that area (number caught vs. number present). Additionally, the area sampled is often based on sweep lengths that researchers use (Rashid et al. 2003, Awuni et al. 2015), whereas a large amount of variation has been observed in Arkansas in the actual area being sampled among consultants, producers, extension, and research personnel. Sampling area and sampling success do not have to be estimated when relating sweep net sample estimates to damage values without the use of cages. Instead, the sampled area used in an uncaged trial can relate directly to the area being sampled in real world situations. Additionally, trials that have utilized uncaged methodologies have not corroborated significant direct and indirect yield loss rates previously estimated by caged trials (Harper et al. 1993, Tindall et al. 2005). These trials however did not report sampling area and did not directly relate samples taken to actual sampling regimens.

It is possible that caged trials are over or underestimating the amount of damage caused by rice stink bug. If inaccurate estimations of rice stink bug density to sample relationships are being made, then rice stink bug thresholds will not be accurate when used to make treatment decisions. Additionally, rice stink bug dispersal behavior between hosts plants and host species could be restricted when caged. Other studies have shown that cages increase growth of host plant species, which could alter the damage relationship that would be seen without cages (Simmons and Yeargan 1990).

The objective of this study was to estimate direct and indirect yield loss due to different densities of rice stink bug in a defined sampling area of uncaged rice.

Materials and Methods

Field experiments were conducted in 2018 across six locations: two near Stuttgart, AR, two near Almyra, AR, one near Conway, AR, and one near Harrisburg, AR. Five of the six locations were located within grower fields, with locations chosen based on presence of rice stink bug in surveyed fields. Agronomic practices across locations were decided by field managers rather than researchers, therefore some differences existed in fertility, cultivar, and pest management. No fungicides or insecticides were applied to the test area before or during initiation of this study unless indicated by the assigned treatment.

A randomized complete block design was utilized with 4 replicate blocks per location and 4 treatments. Treatments utilized were variations of rice stink bug thresholds at 4 levels: 1) an untreated control, 2) standard threshold of 5 stink bugs per 10 sweeps the first two weeks of heading followed by 10 stink bugs per 10 sweeps the second two weeks of heading, 3) 10 rice stink bugs per 10 sweeps throughout heading, and 4) 20 rice stink bugs per 10 sweeps throughout heading. These will be referred to as ‘untreated’, ‘standard threshold’, ‘10 all season’, and ‘20 all season' respectively from this point forward. Plots measured between 4.5m-6.1m in width and were 15.5m in length.

Average rice stink bug density was estimated within each plot once per week from flowering until 60-70% hard dough. Sampling was performed using a 38cm sweep net while utilizing 1.8m sweeps taken at a quick pace with at least 1-2 steps between each sweep. Only the left half of each plot was sampled to estimate the rice stink bug density present, with 2 sets of 10 sweeps taken per plot across the 15.5m length. The right half of each plot was not sampled to minimize yield loss and kernel damage due to sweep net sampling. Treatment decisions were then determined by averaging the number of rice stink bugs captured across all 4 replicate blocks, with 8 samples being used per treatment decision each week. When thresholds were exceeded within a single location, both sampled and harvested sides of all plots with that treatment received an application of lambda-cyhalothrin (LAMBDA-CY® EC, UPI, 630 Freedom Business Center, Suite 402, King of Prussia, PA) at a rate of 0.08 kg ai per ha. Insecticide applications were made using a CO^2 backpack sprayer, calibrated at 93.5 L per ha with Tee-Jet hollow cone TX-6 nozzles.

The portion of each plot that was not sweep net sampled were harvested after kernel moisture averaged less than 20%, as determined by a mini GAC® handheld grain moisture tester (DICKEY-john, 5200 Dickey John Road, Auburn, IL). Each plot had 7 rows (1.33m in width) each 5.5m-7.5m in length that was harvested with a Wintersteiger® classic plot combine (Wintersteiger Inc., 4705 W. Amelia Earhart Drive, Salt Lake City, UT). Harvest yields were estimated by adjusting the harvest weight (kg) to 12% moisture. Dry yields were then converted to kg per ha.

A 700g sample of rough rice was obtained from each plot and was dried to 12% moisture before grain quality analysis. Quality of rice was determined by rating samples using USDA grade standards (Hardke and Siebenmorgen 2013) and by determining both percent total milled rice yield (MRY) and percent whole kernel rice yield (head rice, HRY). Samples of rough rice weighing 100g were taken from each plot sample, dehulled to brown rice, and then sorted in to three subcategories: clean brown rice, peck caused by rice stink bug, and peck caused by other factors. The percent peck caused by rice stink bugs (RSB Peck) was determined using the formula: (weight of peck caused by rice stink bug ÷ weight of total brown rice sample including clean and pecky rice) × 100. A 162g sample of rough rice was milled to produce white rice using a laboratory-scale rice mill (McGill #2, Rapsco, Brookshire, Texas, USA). This value was used to calculate the milled rice yield (MRY) = (milled rice mass ÷ rough rice mass) × 100. The head rice, kernels at least three-fourths of original kernel length, were then separated using a laboratory-scale rice sizing device with a No. 11 grate (Grainman Model 61, Grain Machinery Manufacturing Corp., Miami, Florida, USA). This value was used to calculate head rice yield (HRY) = (head rice mass ÷ rough rice mass) × 100.

Data were compared using a two-way analysis of variance utilizing PROC GLIMMIX (SAS v. 9.4, SAS Institute, Cary, NC) and a general linear model with a normal distribution. Denominator degrees of freedom were adjusted using a Kenward-Rogers approximation (Kenward and Roger 1997). Data were compared using only 3 threshold treatment levels because no 20 all season threshold plot received an insecticide application at any location and were therefore considered untreated. If the two-way interaction of location × threshold was found to be non-significant, main effects alone were explored. Block alone was considered a random variable for the response variable RSB peck. Location and block nested within location were considered random variables for the response variables MRY, HRY, and yield when the treatment main effect alone was explored. Means were then separated using Tukey's HSD post hoc analysis at $P<0.05$.

Data were further analyzed using regression analysis. This was performed using a mixed model in PROC GLIMMIX (SAS v. 9.4, SAS Institute, Cary, NC) with location and block nested within location considered as random variables. Denominator degrees of freedom were adjusted using a Kenward-Rogers approximation (Kenward and Roger 1997). Two predictors were used with these analyses: RSB per Week (the average number of rice stink bugs sampled per week) and RSB peck. Four response variables were also used: RSB peck, MRY, HRY, and yield. For each regression analysis performed, data were separated into two subsets before analysis: plots that received an insecticide application (sprayed) and plots that never received an insecticide application (unsprayed). Although the full data sets were presented for RSB peck, separate regression lines and analysis were used with sprayed and unsprayed plots so that conclusions could be drawn independently. The model for all regression analyses tested was: $y = \beta_0 + \beta_1 x + \mathcal{E}$.

Results

Sampled Rice Stink Bug Averages

All locations received at least one application of insecticide based upon threshold requirements (Table 3.1, Figure 3.1). Of the 6 locations, 4 received insecticide applications to the standard threshold and 10 all season at the same time: Almyra 1, Almyra 2, Harrisburg, and Conway (Table 3.1, Figure 3.1). Almyra 1, Almyra 2, and Conway exhibited threshold level densities in the standard threshold plots and 10 all season plots at the first sampling timing (Figure 3.2). Both Almyra 1 and Almyra 2 never exceeded thresholds in treated plots after the initial insecticide application, however, treated plots in Conway were retreated 2 weeks later (Figure 3.2). Harrisburg only received an insecticide application later at 60% hard dough when rice stink bug densities averaged over 10, but no threshold was exceeded during the 3 previous weeks of sampling (Figure 3.1). The standard threshold plots at Stuttgart 1 exceeded threshold only during the first week of sampling (Figure 3.1). The standard threshold plots at Stuttgart 2 exceeded threshold at the milk/soft dough growth stage and received a single application, and the 10 all season threshold exceeded treatment level and was treated at 60% hard dough (Figure 3.1).

Direct Yield Loss

Utilizing a two-way ANOVA for yield, no significant interaction of threshold treatment × location was observed (F = 0.91; df= 10, 75; P = 0.53). The treatment main effect was not found to be significant for yield using ANOVA (F = 1.07; df= 2, 70; P = 0.35). When utilizing regression analysis, no significant linear relationship (P=0.59) was observed between yield of untreated plots and the number of rice stink bugs sampled per week (Table 3.2).

Indirect Yield Loss - RSB Peck

A significant threshold × location interaction was observed peck (F = 8.84; df = 10, 75; P < 0.01) (Table 3.1). At all locations except Stuttgart 1, untreated plots exhibited significantly more RSB peck than both the standard and 10 all season thresholds (Table 3.1). At the Stuttgart 1 location, untreated plots were not found to exhibit significantly more RSB peck than 10 all season plots, at 1.22% and 0.84% RSB peck respectively. However, untreated plots at Stuttgart 1 did exhibit significantly more RSB peck than the standard threshold plots (Table 3.1). The largest levels of peck were observed at the Conway location, with 3.68% RSB peck in untreated plots compared to 1.41% and 1.18% RSB peck in the standard and 10 all season respectively (Table 3.1).

A significant linear relationship was observed between the average number of rice stink bugs observed per week and RSB peck when considering plots that did not receive an insecticide application (Table 3.3, Figure 3.3). With every 1 rice stink bug averaged per week, an increase in 0.10% RSB peck could be expected (Figure 3.3). At an average of 10 rice stink bugs caught per week, 1.8% RSB peck could be expected. No significant linear relationship was observed between RSB peck and the average number of rice stink bugs sampled per week for treatments receiving an insecticide application (Table 3.3, Figure 3.3). Neither predicted line approached 0% RSB peck when samples averaged 0 RSB per week, as RSB peck was not fully distinguishable from other potential causes.

Milling and Head Yields

No significant treatment × location interaction was observed for MRY (F = 1.27; df= 10, 75; P = 0.26), but the main effect of treatment was found to be significant (F = 10.01; df= 2, 70; P < 0.01). Untreated plots exhibited significantly lower MRY at 69.7% when compared to both the standard and 10 all season treatments at 70.4% and 70.3% respectively (Table 3.2). No significant treatment × location interaction was observed for HRY (F = 0.47; df = 10, 75; P = 0.91), but a significant treatment main effect was observed (F = 4.71; df= 2, 70; P = 0.01). Significantly lower HRY was observed in the untreated compared to the standard treatment at 55.8% and 57.2% respectively, but neither treatment was found to differ from the 10 all season threshold at 56.5% HRY (Table 3.2).

No significant relationship (P=0.07) was observed between RSB peck and MRY for untreated plots (Table 3.3). MRY was also not found to have a significant linear relationship (P=0.48) with the average number of RSB observed each week (Table 3.3). No significant linear relationship was observed between HRY and the average number of rice stink bugs caught per week (*P*=0.88) or RSB peck (*P*=0.97) (Table 3.3).

Discussion

Research on the rice stink bug and its ability to damage rice has taken place since the early 20th century, however, some cage studies may not be accurately relating damage within a confined space to the area being sampled in a rice field. Our study indicates that when using ten 1.8m sweeps to judge rice stink bug density, populations that average 10-15 rice stink bugs per week are capable of significantly lowering grain quality. When populations were left unchecked, 0.1% RSB peck was incurred with every addition of 1 rice stink bug per week. However, RSB peck averages below 0.5% were not observed when no rice stink bugs were present, indicating that RSB peck was not fully distinguishable from other potential causal factors. If a field averaged 5 rice stink bugs and was not sprayed, 1.3% RSB peck could be expected, meaning no USDA grade reductions would be incurred. Awuni et al. (2015b) observed 5-6% RSB Peck using infestations equivalent to 4 rice stink bugs per 10 sweeps when estimating 0.3 m^2 per sweep. Our study indicates that populations around 4 rice stink bugs per 10 sweeps will not elicit levels of peck warranting an insecticide application. However, if fields averaged 10 rice stink bugs per 10 sweeps, the current second two weeks of heading threshold in Arkansas, 1.79% RSB peck could be expected if plots were not treated. When considering peck a field may be graded for, an addition of 1.8% peck by rice stink bug populations alone could result in total peck levels close to or above the 2.5% mark that indicates USDA Grade 3 rice, meaning a loss of around $34 per ha.

Tindall et al. (2005) also observed significant levels of peck in uncaged trials, where averages with no insecticide applications exceeded 9% and 15% across two years of data. This compares to peck levels of 4% and 5% in plots that received insecticide applications, which is much higher than what was observed in our study (Tindall et al. 2005). Rice stink bug densities estimated by Tindall et al. (2005) also averaged lower than what was observed at many of our locations. It's possible that large levels of smut or other disease led to an increased rate of peck by rice stink bug, as evidenced by the large amount of peck in treated plots which would be rated as USDA grade 4 rice. Harper et al. (1993) found similar rates of peck to our study if data were considered across growth stage, with a total peck average of 0.1% expected for density averages across soft dough to grain maturity. It's possible that these differences could be due to sample area size differences, as the sampled area per 10 sweeps was not reported by Harper et al. (1993).

Overall lower total milling yields (MRY) were observed in untreated plots when compared to plots treated on threshold. Bowling (1963) observed decreases in MRY with increasing rice stink bug populations. Reductions in MRY could be due to the complete breakdown of pecky portions of kernels during milling. No significant association was observed between milled head rice yield (HRY), and the number of rice stink bugs or percent RSB peck by our study. Bowling (1963), Swanson and Newsom (1963), and Espino et al. (2007) observed trends between lowered HRY and increased rice stink bug infestation levels. Considering that our plots were located on grower fields and that rain events prevented timely harvest, moisture at that time of harvest was lower than optimal. It's possible that head yields were lowered overall due to harvesting at low moisture, which could have affected these data (Siebenmorgen et al. 2007).

Many studies have shown the ability of rice stink bug to cause direct yield loss using both sleeve cages and large cages, which has led to a lower threshold often being set in early heading growth stages (Douglas and Tullis 1950, Swanson and Newsom 1962, Nilakhe 1976, Rashid

2003, Patel et al. 2006, Awuni et al. 2015b). No significant association between direct yield loss and the average number of rice stink bugs sampled was observed in this study. Yields could have been affected by insecticide applications made during peak flowering, where physical damage to flowers by droplets may have occurred. Additionally, two locations were not sampled until the milk stage, meaning that yield loss could have occurred at these locations in earlier growth stages. However, uncaged study for rice stink bug has ever observed direct yield loss (Harper et al. 1993, Tindall et al. 2005). Only cages that forced rice stink bug to feed in a confined space with rice plants have observed significant levels of direct yield loss, with many cage studies observing no direct yield loss at all (Odglen and Warren 1962, Espino et al. 2007, Blackman 2014). More work is necessary to determine the level of direct yield loss that the rice stink bug is capable of causing, as this study observed no trend. Uncaged trials will be necessary for determination of direct yield loss, but the location of plots may need to be determined before heading begins so sampling can begin immediately.

Thresholds for Arkansas currently require control at 10 rice stink bugs per 10 sweeps in the last two weeks of heading. This study confirms that controlling populations at this level will prevent appreciable levels of peck. Across all locations applications of insecticides either at the standard threshold or 10 all season threshold yielded peck averages that would incur no quality losses. However, plots at or just above threshold levels that were left unsprayed often exhibited peck that could lead to major losses. Within the last 3-4 years many states have lowered thresholds for indirect yield loss. Data from this study indicates that lower thresholds to prevent additional indirect yield loss are unnecessary. Additionally, samples showed a trend for an increase in rice stink bug population in the hard dough timing, where significant peck can still be caused by large infestations. If applications are made to low pest populations during the milk-soft dough stages, it is possible that additional applications may be necessary during the hard dough growth stages.

This is the first study to directly relate the area sampled within a field to direct or indirect yield loss by the rice stink bug. This study was able to successfully rule out the question of sampling efficiency and estimation of sampled area by directly relating sweep net sample averages to yield loss. Results from this study confirm the validity of the current Arkansas indirect yield loss threshold of 10 rice stink bugs during the second two weeks of heading, but more data is needed to determine the relationship of direct yield loss. We hope that this study will be used as a framework for rice stink bug damage assessment across states for future thresholds.

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Table 3.1 Percent rice stink bug peck (RSB peck) observed in rice across Six locations in Arkansas with corresponding application timings for untreated plots, plots sprayed at the standard threshold, and plots sprayed at 10 rice stink bugs throughout the sampling period (2018).

Location	Threshold	Application Timing	Percent RSB Peck
Stuttgart 1	Untreated	.	1.2 a
	Standard*	Flowering	0.8 b
	10 rice stink bugs	60% Hard Dough	0.8 ab
Stuttgart 2	Untreated	.	1.7 a
	Standard*	Milk/Soft Dough	1.0 b
	10 rice stink bugs	60% Hard Dough	1.5 a
Almyra 1	Untreated	.	2.4 a
	Standard*	Milk	1.4 b
	10 rice stink bugs	Milk	1.4 b
Almyra 2	Untreated	.	2.1 a
	Standard*	Soft Dough	1.2 b
	10 rice stink bugs	Soft Dough	1.2 b
Conway	Untreated	.	3.7 a
	Standard*	Flow/Milk + 40% Hard Dough	1.4 b
	10 rice stink bugs	Flow/Milk + 40% Hard Dough	1.2 b
Harrisburg	Untreated	.	1.4 a
	Standard*	60% Hard Dough	0.9 b
	10 rice stink bugs	60% Hard Dough	1.0 b

Means for Percent RSB peck are significantly different within a location when followed by a different lowercase letter according to a Tukey's HSD post hoc analysis at $P<0.05$.
*Standard rice stink bug threshold is 5 rice stink bugs per 10 sweeps in the first two weeks of heading and 10 rice stink bugs per 10 sweeps in the second two weeks of heading

Table 3.2 Treatment averages for quality and yield measured in percent milled rice yield (MRY), percent head rice yield (HRY), and yield (kg ha^{-1}) analyzed across location for three treatment thresholds in Arkansas rice (2018).

Treatment	MRY	HRY	Yield (kg/ha)	Sample Size
Untreated	69.7 b	55.8 b	10830	48
Standard*	70.4 a	57.2 a	10736	24
10 All Season	70.3 a	56.5 ab	10992	24

Means for MRY, HRY, and Yield are significantly different when followed by a different lowercase letter according to a Tukey's HSD Post Hoc Analysis at $P<0.05$.
*Standard rice stink bug threshold is 5 rice stink bugs in the first two weeks of heading and 10 rice stink bugs in the second two weeks of heading.

Table 3.3 Results of regression analysis across all data utilizing 2 predictors (rice stink bug per 10 sweep sample per week and percentage RSB peck) and 4 response variables (percentage of RSB peck, milled rice yield (MRY), head rice yield (HRY), and yield (kg/ha)) to analyze data from both sprayed and unsprayed plots in rice in Arkansas (2018).

Predictor	**Response**	**Treatment**	**Equation** ($y = \beta_0 + \beta_1 x + \mathcal{E}$)	**Standard Error** (β_0 and β_1)	**RSE***	***T***	**df**	***P***
RSB/ Week	RSB Peck	Sprayed	y = 0.90 + 0.027x	0.15 and 0.02	0.06	1.57	27	*0.13*
		Unsprayed	y = 0.08 + 0.096x	0.18 and 0.01	0.05	8.04	29	*<0.01*
	MRY	Unsprayed	y = 70 – 0.03x	0.83 and 0.05	0.20	-0.70	30	*0.48*
	HRY	Unsprayed	y = 56 – 0.02x	2.71 and 0.12	1.30	-0.16	45	*0.88*
	Yield	Unsprayed	y = 10579 + 19.2x	945 and 35	88306	0.54	45	*0.59*
RSB Peck	MRY	Unsprayed	y = 71 – 0.58x	0.82 and 0.31	0.22	-1.86	40	*0.07*
	HRY	Unsprayed	y = 56 – 0.03x	2.74 and 0.82	1.35	-0.04	45	*0.97*

* Standard error of residual covariance parameter estimate.

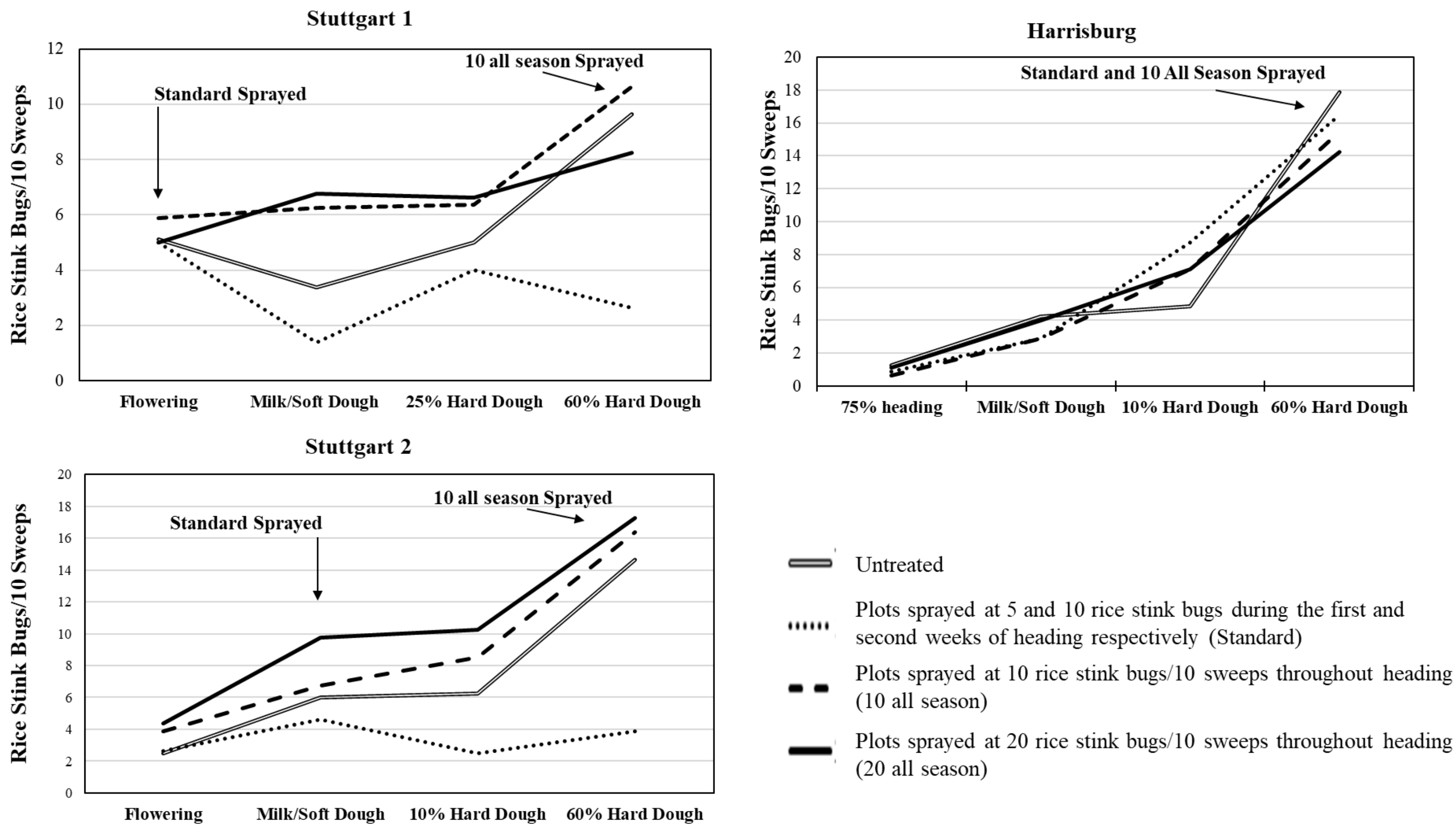

Figure 3.1 Average number of rice stink bugs per 10 sweep sample when sampled each week in uncaged threshold trials for four treatment thresholds at the Stuttgart 1, Stuttgart 2, and Harrisburg locations located in rice fields across Arkansas (2018).

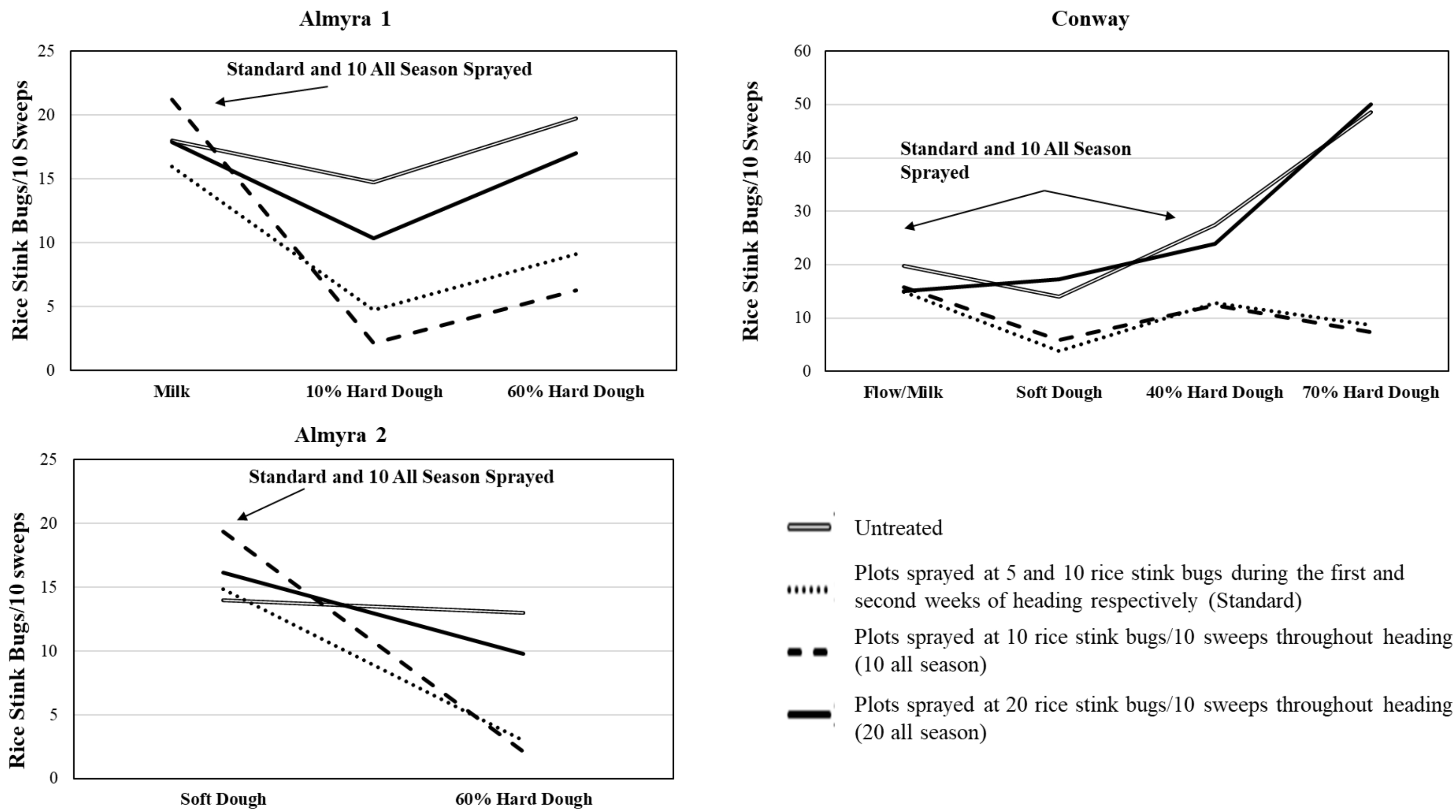

Figure 3.2 Average number of rice stink bugs per 10 sweep sample when sampled each week in uncaged threshold trials for four treatment thresholds at the Almyra 1, Almyra 2, and Conway locations located in rice fields across Arkansas (2018)

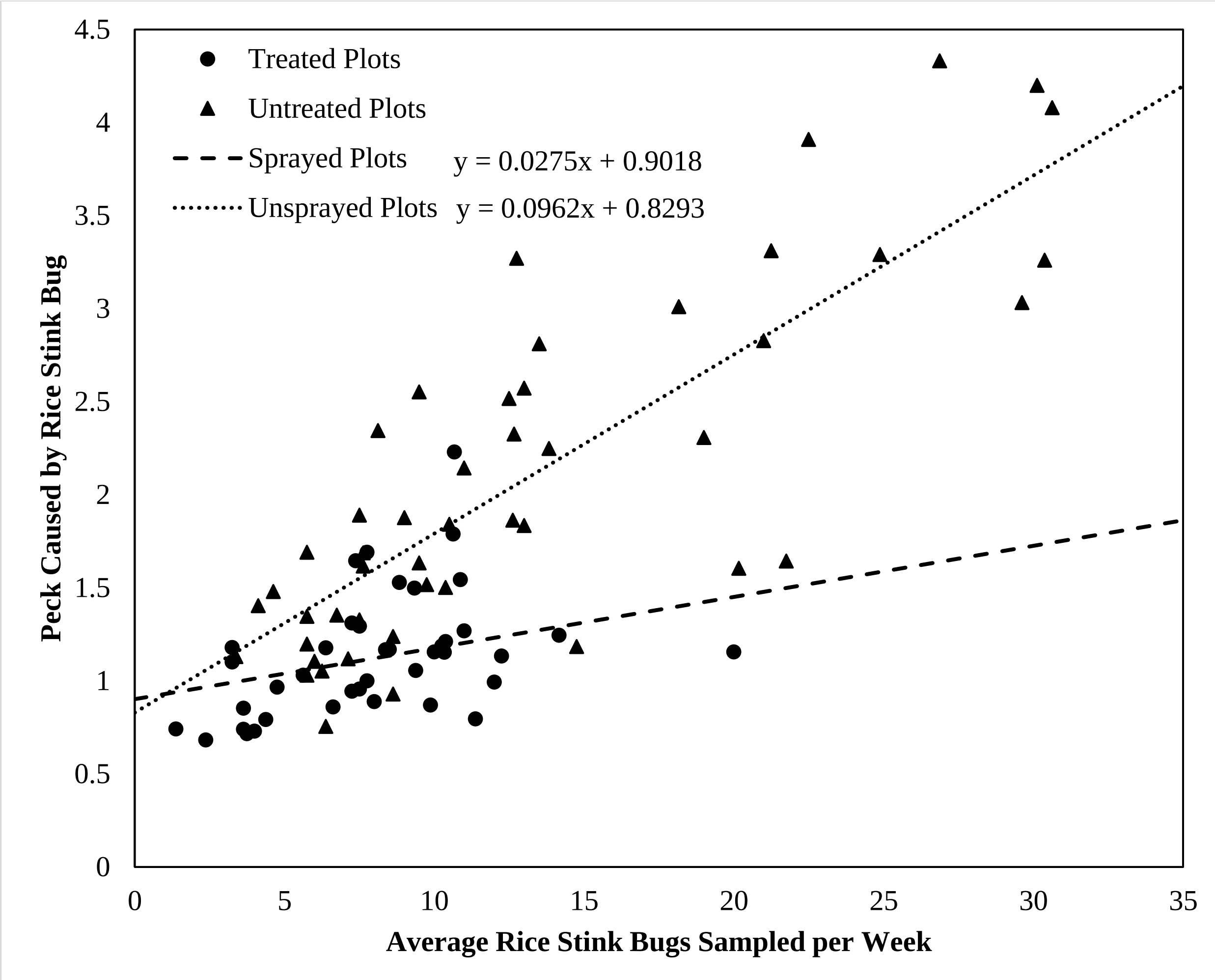

Figure 3.3 Peck caused by rice stink bug (RSB peck) predicted by the average number of rice stink bugs sampled per week in both sprayed (*P*=0.13) and unsprayed plots (*P* <0.01) for plots at 6 locations across Arkansas rice (2018).

Chapter 4 – Timing of Insecticide Termination for Rice Stink Bug, *Oebalus pugnax* (F.), in Rice, *Oryza sativa* L.

Abstract

The stages of rice grain maturity that are most susceptible to rice stink bug, *Oebalus pugnax*, damage have been identified; however, the stage at which they are no longer capable of causing appreciable damage during grain maturity is unclear. The objective of this study was to determine the susceptibility of rice to rice stink bug feeding at different levels of grain maturity and determine an insecticide termination timing. Rice stink bug damage was examined using five levels of grain maturity described as percent of kernels reaching mature straw coloration referred to as hard dough (20, 40, 60, 80, and 100%) across a range of infestation levels using single panicle sleeve cages and large cages. Hybrid and pureline cultivar rice panicles at 20, 40, and 60% hard dough were found to be susceptible to indirect yield loss, as two rice stink bugs per panicle resulted in over 7% peck. In large cage trials, 25 rice stink bugs caused 0.7%-1% peck to hybrid and pureline rice plots at 20% hard dough. Much less damage was found once rice reached 60% hard dough, where peck averages only reached 0.4%. Decreased damage at 60% hard dough was validated using uncaged trials where 0.4% additional peck was observed in unsprayed plots. These data indicate that rice in the early stages of hard dough is susceptible to large levels of indirect yield loss, but unless significant densities of rice stink bug are present at 60% hard dough, no more applications are necessary at this timing.

Introduction

The rice stink bug, *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae), is a major pest of rice, *Oryza sativa* L., grown in Arkansas and many other southern states (Webb 1920). The rice stink bug feeds on the developing kernels of rice and other grasses beginning at the heading growth stage when the panicle is exerted from the boot until the end of the ripening phase, known as hard dough (Swansom and Newsom 1962). Rice stink bug prefers to feed on rice and barnyardgrass, *Echinochloa crus-galli* P.Beauv, and is known to immediately move from alternate hosts once rice or barnyard grass exert susceptible heads (Rashid et al. 2005, Rashid et al. 2006, Awuni et al. 2015a). Large shifts in occurrence are especially evident once rice fields in the Mid-South begin to exsert heads, where rice stink bugs migrate from alternate hosts due to an attraction to preferred host kairomones (Douglas and Tullis 1950, Rashid et al. 2005, Rashid et al. 2006, Awuni et al. 2015a). Rice stink bug infests rice fields before and throughout heading growth stages, with a large increase in density often observed when heading begins and when rice fields are close to full grain maturity. Sampling for rice stink bug is performed using a 38cm sweep net, with 10 sets of 10 sweep samples recommended for estimating the population density present in a field. In Arkansas rice stink bug is managed with two different action thresholds depending upon the growth stages present in each rice field (Lorenz and Hardke 2013). During the first two weeks of heading the action threshold is 5 rice stink bugs per 10 sweeps to prevent direct yield loss, and during the next 2 weeks of heading, the action threshold is 10 rice stink bugs to prevent indirect yield loss (peck).

Feeding by the rice stink bug during emergence and flowering, can cause blanked kernels and direct rough rice yield loss (Douglas and Tullis 1950, Swanson and Newsom 1962, Espino et al. 2007). Rice stink bug feeds by inserting piercing-sucking stylets into kernels to extract nutrients. This damage results in a reduction of the grain content and damage to flowers, where abortion of the flower could lead to a completely blank kernel. At the later stages of heading, milk through soft and hard dough, feeding by the rice stink bug is associated with broken, chalky, or pecky kernels. Pecky kernels caused by feeding of the rice stink bug leaves the kernel to be more susceptible to invasion by fungi (Ryker and Davis 1938, Douglas and Tullis 1950, Swanson and Newsom 1962, Espino et al. 2007). Greater than 2.5% pecky kernels by weight can reduce the USDA grade resulting in a price dockage. This is a response to unappealing discolorations and a potential decrease in the more valuable whole kernels (head rice), as peck is associated with increased kernel breakage during milling (Swanson and Newsom 1962, Bowling 1963, Espino et al. 2007, Hardke and Siebenmorgen 2013). Traditionally, rice that receives no value reduction when sold in Arkansas is graded at either USDA Grade 1 or Grade 2, meaning that no more than 0.5% or 1.5% of kernels (by weight) are considered pecky (Personal communications with Jarrod Hardke). At 2.5% or 4% peck, rice is considered grade 3 and grade 4, and can incur a deduction of up to $0.003-$0.006 per kg of rice respectively. These reductions in quality could impact producers as much as $34-$68 per ha. This assumes potential yield around 10,000 kg per ha for grade 3 and grade 4 rice, respectively.

Rice is most susceptible to quality loss from rice stink bug feeding during the milk and soft dough stages of grain development (Espino et al. 2007). High densities of rice stink bug are also commonly found in fields at the hard dough growth stage (R7-R9), 17 or more days after anthesis (Counce et al. 2000, Patel et al. 2006). Harper et al. (1993) and Patel et al. (2006) found increased levels of peck due to infestations of rice stink bug at the hard dough growth stage (R6-R7). Kernels at the tip of each panicle mature first and kernels closer to the base are often at a more susceptible growth stage. This gradient of maturity is often present across a rice field and it is possible that rice stink bug is causing damage to immature kernels rather than hard dough kernels. When a rice plant is at hard dough, not all kernels on each panicle are at the hard dough growth stage (Counce et al. 2000). In Arkansas, many insecticide applications are made during hard dough due to high densities of rice stink bugs moving from adjacent harvested fields, or as the result of an egg laying from subthreshold populations during the first two weeks of heading. When plants mature the percent of straw-colored kernels increases, and the average percent of visually mature kernels (straw-colored kernels) could be used to decide when to terminate insecticide applications. This timing is important because consultants and producers typically encounter issues concerning re-entry intervals (REI) and pre-harvest intervals (PHI) when they apply insecticides late in hard dough, but because of the fear of losses due to peck, many potentially unnecessary applications are made (Gus Lorenz – Personal Observations).

The objective of this study was to determine changes in kernel damage (% peck) of rice plants relative to feeding by a range of densities of rice stink bug during increasing percentages of kernels at hard dough on rice panicles, and create a decision-making protocol to terminate insecticide applications.

Materials and Methods

Field cage trials were conducted at the University of Arkansas System Division of Agriculture Rice Research and Extension Center (RREC) near Stuttgart, Arkansas. Rice plots consisted of a hybrid cultivar (RT XP753) and a pureline cultivar (Diamond). Plots measured 1.78 x 1.6m using a 19cm drill spacing and 8 rows in total. Standard agronomic practices were used to maintain these plots.

Adult rice stink bugs collected with a 38cm diameter sweep net from heading rice and weedy grasses were utilized for both types of caged trials. To ensure viability, rice stink bug adults were placed in 30cm mesh rearing cages (1466AV, BioQuip Products, Rancho Dominguez, CA, USA) provisioned with fresh heading grass material, moist paper towels, cotton balls soaked in sugar water (200g sugar per 3.8L of water) and maintained at 24°C for at least 24h prior to releasing healthy adults into either sleeve or large cage trials to minimize using damaged rice stink bugs.

Sleeve Cages

In 2016 and 2017, the experimental design included all combinations of 3 treatment factors: infestation density × infestation timing × rice cultivar (10 replicates per year). The density of rice stink bugs in each sleeve cage was either 0 or 2. Timing releases of stink bugs into sleeve cages occurred when rice panicles inside cages had one of five percentages of kernels at hard dough noted as straw-colored kernels (20, 40, 60, 80, 100%). These treatments were conducted with both a hybrid (RT XP753) and pureline (Diamond) rice cultivar. Panicles for this experiment were chosen based on an estimation of their individual percentage of kernels at hard dough and randomly assigned 0 or 2 rice stink bugs per sleeve cage. No single rice plant received more than one sleeve cage.

Applications of lambda-cyhalothrin (Lambda-CY® EC, UPI, 630 Freedom Business Center, Suite 402, King of Prussia, PA) were applied at a rate of 0.08 kg ai per ha using a backpack sprayer when panicle heading initiated and continued weekly until one week prior to sleeve cage placement. These pesticide applications ensured that rice panicles in plots did not accumulate high percentages of rice stink bug caused kernel peck before enclosure in cages. Sleeve cages used were white insect rearing sleeves, 20 × 40 cm (BioQuip Products, Rancho Dominguez, CA 90220, USA). A bamboo rod was used to hold the sleeve cage and rice plant up due to the weight of the cages, and the cage and rice plant were zip-tied to the bamboo pole. Sleeve cages were placed around panicles when their estimated treatment stage began (20, 40, 60, 80, or 100% hard dough), then rice stink bugs were added immediately afterword and infestation dates are reported (Table 4.1). Dead rice stink bug adults within sleeve cages were replaced 24h after introduction, and then every 48h after that to maintain stinkbug levels to designated levels.

At harvest, panicles contained inside the sleeve cages were removed, put in paper bags, and placed in a dryer until moisture reached 12%. Panicles were removed from the paper bags. Rough rice kernels from each panicle were de-hulled. Brown rice was observed with a light box to determine percentage peck per panicle. Samples were sorted by peck damage and a determined percentage of pecky kernels by weight was then determined. The percent peck caused by rice stink bug (% RSB Peck) was determined using (weight of peck caused by rice stink bug ÷ weight of total brown rice sample including clean and pecky rice from other causes) × 100.

Data were compared using a two-way analysis of variance, PROC GLIMMIX, SAS v. 9.4 (SAS Institute, Inc., Cary, NC) and a general linear model with a normal distribution. Denominator degrees of freedom were adjusted using a Kenward-Rogers approximation (Kenward and Roger 1997). RSB peck was adjusted using Abbott's Formula (Abbott 1925), where the average RSB peck found within the corresponding year × cultivar combination was subtracted from the RSB peck found within each cage containing rice stink bugs. Cultivar × infestation timing combinations were then compared between adjusted infested cages. Additionally, RSB peck was log transformed after adjusted using Abbot's formula, although data will be presented with non-log transformed values for ease of interpretation. The year in which the experiment was conducted was considered a random variable. Means were separated using Tukey's HSD post hoc analysis (P=0.05).

Large Cages

Rice plot size was reduced to 0.9m × 0.9m and enclosed in a screen cage (1.8m × 1.8m × 1.5m) two weeks prior to emergence of the panicle from the boot. Cages were sprayed with lambda cyhalothrin at a rate of 0.08 kg ai per ha to kill any rice stink bug already present. A preventive fungicide application was made using Quilt Xcel (propiconazole + azoxystrobin, Syngenta Corporation, P.O. Box 18300, Greensboro, NC 27419) at 0.19 kg ai propiconazole per ha and 0.21 ai azoxystrobin per ha. The application of insecticide and fungicide occurred before rice panicles emerged, and cages remained closed until infestations began. The hard dough growth stage within each cage was determined to be the estimated percent of straw-colored kernels present on at least half of the panicles within each plot.

Three treatment factors included: number of rice stink bugs released into each cage (0, 13, or 25), percent of kernels at hard dough when rice stink bugs were released (20, 40, 60, or 80%), and rice cultivar (RT XP753 or Diamond). Four replications were performed in 2016 for each combination of infestation level × infestation timing × cultivar using a randomized complete block design. In 2017, the same experimental design was utilized, with only 3 replications of the hybrid cultivar. Two untreated cages were utilized per replication (untreated checks) in both years. Rice stink bugs fed from timing of release at the appropriate percentage hard dough until harvest, and infestation dates are reported (Table 4.1). Cages were removed prior to harvest and the entire plot was harvested, weighed, and placed in a dryer until 12% moisture.

Treatment effects on quality of rice was recorded as percentages of total milled rice yield (MRY) and total head rice yield (HRY) for each rice sample per cage using USDA grade standards (Hardke and Siebenmorgen 2013). Samples of rough rice weighing 100g were taken from each harvested plot, dehulled to brown rice, and then sorted into three subcategories: clean brown rice, peck caused by rice stink bug, and peck caused by other factors. The percent peck caused by rice stink bug (% RSB Peck) was determined using the formula: (weight of peck caused by rice stink bug ÷ weight of total brown rice sample including clean and pecky rice) ×

100. Each 162g sample of rough rice was milled to white rice using a laboratory-scale rice mill (McGill #2, Rapsco, Brookshire, Texas, USA). The whole kernel rice or head rice, kernels at least three-fourths of original kernel length, were then separated from the white rice sample using a laboratory-scale rice sizing device with a No. 11 grate (Model 61, Grain Machinery Manufacturing Corp., Miami, Florida, USA). Total milled rice yield (MRY) = (milled rice mass ÷ rough rice mass) × 100. Total head rice yield (HRY) = (head rice mass ÷ rough rice mass) × 100.

Data were compared using a three-way analysis of variance utilizing PROC GLIMMIX (SAS v. 9.4, SAS Institute, Cary, NC) and a general linear model with a normal distribution. Denominator degrees of freedom were adjusted using a Kenward-Rogers approximation (Kenward and Roger 1997). RSB peck was adjusted using Abbott's Formula (Abbott 1925), where the average RSB peck found within the corresponding year × cultivar combination was subtracted from the RSB peck found within each cage containing rice stink bugs. Cultivar × infestation timing combinations were then compared between adjusted infested cages. Data were compared first using cultivar × infestation timing × infestation level, but if no significance was found two-way interactions were explored. If a single factor was not included in any significant interaction, main effects alone were explored. Year and block nested within year were utilized as random variables. Response variables used were RSB peck, MRY, HRY, and yield. Means were then separated using Tukey's HSD post hoc analysis at P=0.05.

Uncaged Hard Dough Confirmation

Field experiments were conducted in 2018 in a rice planting in Stuttgart, AR and one in Harrisburg, AR. Agronomic practices at both locations were decided by field managers rather than researchers, therefore these rice fields differed in fertility, rice cultivar, and pest management. No fungicides or insecticides were applied by producers in proximity of any experimental rice plot while this study was in progress or immediately prior to initiation. There were two treatments arranged in a randomized complete block design (4 replicates near Stuttgart and 8 replicates near Harrisburg): plots (5.5m x 15.5m) that received an insecticide application when at 60% hard dough versus unsprayed rice plots. Both sprayed and unsprayed plots at both Stuttgart and Harrisburg averaged over 10 rice stink bugs at 60% hard dough when insecticide applications were made.

Rice stink bug density was estimated within each plot at 60% hard dough. Sampling was performed using a 38cm diameter sweep net, conducting 1.8m sweeps taken at a quick pace with at least 1-2 steps between each sweep. Only the left half of each plot was sampled to determine the rice stink bug density present, with 2 sets of 10 sweeps taken per plot across the 15.5m length. The right half of each plot was not sampled to minimize yield loss due to sweep net sampling. Sprayed plots received an application of lambda-cyhalothrin at a rate of 0.08 kg ai per ha. Insecticide applications were made using a CO^2 backpack sprayer, calibrated at 92.5 L per ha with Tee-Jet hollow cone TX-6 nozzles. Both sampled and harvested sides of the plot were treated with insecticide.

Plots were harvested once moistures reached 20% grain moisture, as determined by a mini GAC® handheld grain moisture tester (Dickey-john, 5200 Dickey John Road, Auburn, IL). A Wintersteiger® classic plot combine (Wintersteiger Inc., 4705 W. Amelia Earhart Drive, Salt Lake City, UT) was used to harvest 7 rows in the right side of each plot (1.33m width x 5.5m-7.5m length). Quality of rice was determined from a 700g sample of rough rice as described for large cages.

Data were compared using a one-way analysis of variance utilizing PROC GLIMMIX (SAS v. 9.4, SAS Institute, Cary, NC) and a general linear model with a normal distribution. Denominator degrees of freedom were adjusted using a Kenward-Rogers approximation (Kenward and Roger 1997). Location and block nested within location were considered random variables. Response variables used were percent RSB peck, MRY, and HRY. Means were then separated using Tukey's HSD post hoc analysis at P=0.05.

Results

Sleeve Cages

A significant two-way interaction was observed between cultivar × infestation for RSB peck (F = 6.08, df = 4, 186, P < 0.01). The hybrid and pureline cultivars both exhibited large amounts of peck at 20%-60% hard dough, ranging from 17.4%-10.2% and 7.8%-3.4% at those stages respectively (Figure 4.1). The hybrid cultivar infested with 2 rice stink bugs at 20% hard dough yielded significantly more peck than any other infestation timing × infestation level combination (Figure 4.1). Hybrid rice infested with rice stink bugs from 20%-60% hard dough exhibited significantly more peck than both cultivars at 80% and 100% hard dough (Figure 4.1).

Large Cages

The cultivar × infestation timing × infestation level interaction was significant when considering RSB peck (P=0.01), but was not significant for MRY, HRY, or yield (Table 4.2). Infestation timing × infestation level was then compared within cultivar. The infestation timings of 20% and 40% hard dough with 25 rice stink bugs exhibited RSB peck within the pureline cultivar at 1.1% and 0.6% respectively (Figure 4.2). Cages containing pureline rice infested at 20% hard dough with 25 rice stink bugs exhibited significantly more peck than all other pureline infestation timing × infestation level combinations except 25 rice stink bugs at 40% hard dough

(Figure 4.2). The infestation timings of 20% and 40% hard dough with 13 and 25 rice stink bugs respectively exhibited 0.7% RSB peck (Figure 4.2). No hybrid cultivar infestation timing × infestation level was found to have more RSB peck than any other hybrid cultivar combination (Figure 4.2).

The infestation timing × cultivar and cultivar × infestation level interactions were not significant for MRY, HRY, or yield (Table 4.2). MRY exhibited a significant infestation timing main effect (F = 14.95, df = 4, 180, P<0.01) but no infestation timing main effect was found to be significant for HRY or yield (Table 4.3). Cages infested at 20% hard dough exhibited MRY values of 72.0 and were found to be significantly lower than all other infestation timings except 40% hard dough (Table 4.3). MRY at 40% hard dough was not found to be significantly different than any other infestation timings (Table 4.3).

Uncaged Hard Dough Confirmation

Sprayed plots that had lambda-cyhalothrin applied at 60% hard dough differed significantly from unsprayed plots in observed percentages of RSB peck and MRY, but not in HRY (Table 4.4). Unsprayed plots (1.2%) had significantly higher RSB peck than did sprayed plots (0.8%) (Table 4.5). The MRY observed in uncaged plots was significantly lower by 0.4% compared to that for insecticide sprayed plots (Table 4.4).

Discussion

Research on rice stink bug quality losses have focused on the milk and soft dough stage where rice is most susceptible to indirect yield loss, however, many insecticide applications are made to rice at the hard dough growth stage. Data from sleeve cages indicate that a large difference in susceptibility is present based on the percent of visual hard dough. When 60% or less of panicles exhibited straw colored kernels, over 3% peck could still be caused by rice stink bug infestations, which would be considered USDA Grade 3 Rice (Hardke and Siebenmorgen 2013). Awuni et al. (2015b) observed 14.7% peck when two rice stink bugs were released into panicles at the soft dough growth stage. Patel et al. (2006) observed over 10% peck when two rice stink bugs were introduced to each panicle at the end of the soft dough stage (17 days after anthesis: R6-R7), and 7% peck when additional panicles were infested just 4 days later (R7). It's likely that both timings were still below 40% visual hard dough. Overall more peck was observed with hybrid panicles than pureline panicles in our study, possibly because hybrid panicles are larger and contain more kernels. It's also possible that two rice stink bugs per panicle are limited by space, meaning panicles with more kernels could allow for more opportunity to cause damage. This could also explain why the large levels of peck found at 20% and 40% hard dough resulted in similar values to soft dough panicles from pureline cultivars utilized by Awuni et al. (2015b).

Large cage studies allow for a density per area relationship to be established, whereas sleeve cages can only indicate relative susceptibility. When rice stink bugs were released into both pureline and hybrid rice, over 0.7% RSB Peck at 20% hard dough was observed for 25 rice stink bugs. This indicates that in the early stages of hard dough, panicles are still susceptible to levels of peck that could incur quality and economic losses when combined with other factors or feeding at previous growth stages. Peck values were much lower than what was observed in sleeve cages for corresponding infestation timings, but infestation level was much lower in large cages as 0.81m^2 plots contained over 300 panicles each. In cages, Awuni et al. (2015b) observed 4.9% and 5.9% damaged kernels at 9 and 18 rice stink bugs/m^2 respectively when infestations occurred in the soft dough growth stage. This compares to 16 and 31 rice stink bugs per m^2 used by our study, where a maximum of 1% peck was observed during hard dough.

Uncaged trials were performed to determine if there is a decrease in susceptibility to indirect yield loss at 60% hard dough and relate peck values to actual sweep net samples. An increase of 0.4% RSB peck was observed when comparing unsprayed plots to insecticide sprayed plots. This indicates that if 10-15 rice stink bugs are present when fields average 60% visual hard dough, application of an insecticide may be warranted. Harper et al. (1993) observed slopes of 0.03% and 0.02% peck per rice stink bug at R7 and R9 respectively. This equates to an addition of 0.03% and 0.02% at the two stages of hard dough when considering 10 rice stink bugs, which are lower than rates observed in our study. Observed differences could be due to sweep length, as Harper et al. (1993) did not report the area sampled within the field.

Relating damage rates from relative samples in the uncaged trial to absolute densities used by the large caged trial is difficult. Infestation rates included absolute densities of 16 and 31 rice stink bugs/m^2. When sampling with a sweep net, a relative density is observed, which differs from the absolute density based on sampling success. If it’s assumed that 25% of rice stink bugs are caught within a sampled area, infestation levels would equate to 4 and 7.8 rice stink bugs per m^2. Considering a 1.8m sweep length and 38cm sweep net, 6.8m^2 is sampled during a 10-sweep sample unit, meaning that our infestations levels equate to 27 and 53 rice stink bugs per 10 sweeps. These levels are 2 and 4 times larger than what was observed in the uncaged trial. However, a maximum of 0.4% RSB peck was observed at both infestation levels for 60% hard dough. This suggests that other factors may impact results when comparing rates from confined cages to actual in field samples.

Panicles at the hard dough growth stage are highly susceptible to rice stink bug damage when visual estimations range from 20%-60%. Large amounts of peck were observed using sleeve cages and large cages for both pureline and hybrid cultivars. However, these damage rates could not be successfully related to in-field samples. When utilizing uncaged trials with real-world sampling area, rice at the 60% hard dough growth stage is still susceptible to low levels of peck when threshold is exceeded. However, if rice stink bug densities are under threshold at this timing, it's likely that no further insecticide application is necessary. Both sleeve cage and large cage studies indicate that susceptibility of rice is greatly lowered after 60% hard dough, meaning damage rates will decrease from what was observed by the uncaged trial.

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Table 4.1 Dates when cages of both a pureline (Diamond) and a hybrid cultivar (RT XP753) were infested with live rice stink bugs for both sleeve and large cage trials performed in Stuttgart, Arkansas in 2016, 2017, and 2018 where cages were infested based upon the estimated percent of visually hard dough kernels existing on panicles.

Trial Type	Year	Cultivar	Infestation Timing	Infestation Date
Sleeve Cages	2016	Pureline	20% Hard Dough	9/20
			40% Hard Dough	9/20
			60% Hard Dough	9/20
			80% Hard Dough	9/23
			100% Hard Dough	9/23
		Hybrid	20% Hard Dough	8/15
			40% Hard Dough	8/15
			60% Hard Dough	8/18
			80% Hard Dough	8/26
			100% Hard Dough	8/26
	2017	Pureline	20% Hard Dough	8/15
			40% Hard Dough	8/15
			60% Hard Dough	8/19
			80% Hard Dough	8/25
			100% Hard Dough	9/6
		Hybrid	20% Hard Dough	8/19
			40% Hard Dough	8/25
			60% Hard Dough	8/25
			80% Hard Dough	9/7
			100% Hard Dough	9/7
Large Cage	2017	Pureline	20% Hard Dough	8/24
			40% Hard Dough	8/28 – 9/6
			60% Hard Dough	9/6 – 9/14
			80% Hard Dough	9/14
		Hybrid	20% Hard Dough	8/18 – 8/21
			40% Hard Dough	8/21 – 8/24
			60% Hard Dough	8/28 – 9/6
			80% Hard Dough	9/6 – 9/8
	2018	Pureline	20% Hard Dough	8/14
			40% Hard Dough	8/17
			60% Hard Dough	8/24
			80% Hard Dough	8/31
		Hybrid	20% Hard Dough	8/10
			40% Hard Dough	8/14
			60% Hard Dough	8/17
			80% Hard Dough	8/24

Table 4.2 One, two or three-way analyses of variance of treatment effects on rice stink bug (RSB) feeding damage of rice kernels recorded as percent RSB Peck, milled rice yield (MRY), head rice yield (HRY) and Yield of rice plant plots exposed to various treatment combinations: large screen cages (1.8 m x 1.8 m x 1.5 m) or uncaged; two rice cultivars (RT XP753 or Diamond); one of four rice panicle growth stages when 2 RSBs were released into rice plots; and rice plots unsprayed or sprayed with insecticide (lambda cyhalothrin) at threshold of 10 RSBs per 10 sweeps at 60% hard dough in 2018 in Stuttgart, Arkansas.

Factors Explored	Response Variable	*F*	df	*P*
Large Cages Cultivar × Infestation Timing × Infestation Level	Percent RSB Peck	3.77	3, 102	0.01
	MRY	2.05	4, 180	0.08
	HRY	0.59	4, 181	0.67
	Yield	0.96	4, 181	0.43
Large Cages Infestation Timing × Cultivar	MRY	0.63	4, 180	0.64
	HRY	0.46	4, 181	0.77
	Yield	1.03	4, 181	0.39
Large Cages Infestation Timing × Infestation Level	MRY	0.84	4, 180	0.50
	HRY	0.78	4, 181	0.54
	Yield	0.32	4, 181	0.87
Large Cages Cultivar × Infestation Level	MRY	0.01	2, 180	0.99
	HRY	0.04	2, 181	0.97
	Yield	0.37	2, 181	0.69
Uncaged Sprayed vs. Unsprayed	Percent RSB Peck	24.45	1, 21	<0.01
	MRY	6.52	1, 14	0.02
	HRY	0.91	1, 21	0.35

Table 4.3 Effects of releasing 13 or 25 rice stink bugs (RSB) per screen cage (1.8 m x 1.8 m x 1.5 m) enclosing rice plots at one of four panicle growth stages on total milled rice yield (MRY) and total head rice yield (HRY) across two years (2016 and 2017) and across two cultivars (RT XP753 and Diamond) in Stuttgart, Arkansas.

Infestation Timing	MRY	HRY
Untreated	72.58 a	59.31
20% Hard Dough	71.95 b	58.47
40% Hard Dough	72.26 ab	58.46
60% Hard Dough	72.70 a	59.31
80% Hard Dough	72.60 a	59.17

Response variable values followed by different lower-cased letters are significantly different according to Tukey's HSD at α=0.05.

Table 4.4 Differences in rice stink bug (RSB) feeding damage recorded as % RSB Peck, total milled rice yield (MRY), and total head rice yield (HRY) in uncaged rice plots (5.5 m x 15.5 m) either Unsprayed or Sprayed with insecticide (lambda cyhalothrin) when panicles exceeded threshold of 10 RSBs per 10 sweeps at 60% hard dough in 2018 in Stuttgart (N = 4 blocks) or Harrisburg, Arkansas.

Treatment	N	RSB Peck	MRY	HRY
Sprayed	12	0.8 (0.1) b	69.9 (0.3) a	54.3 (1.3)
Unsprayed	12	1.2 (0.1) a	69.5 (0.3) b	53.4 (1.5)

Response variable values followed by a different lower-cased letter are significantly different according to Tukey's HSD at α=0.05.

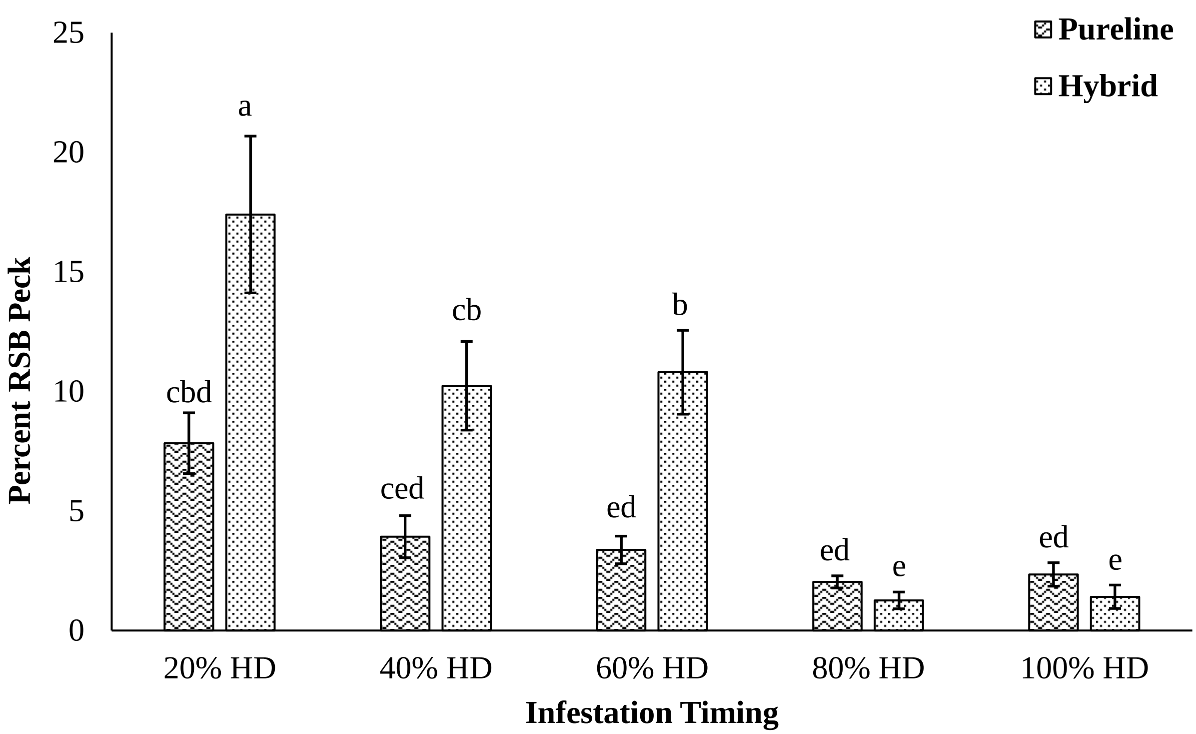

Figure 4.1 Comparison of rice stink bug (RSB) feeding damage of rice kernels (% RSB Peck) from harvested panicles from either of two rice cultivars, hybrid (RT XP753) or pureline (Diamond), enclosed in screen sleeve cages exposed to 2 RSBs per cage released at one of five panicle growth stages and allowed to feed until harvest in 2016 and 2017 in Stuttgart, Arkansas. Average % RSB Peck values followed by a different lower-cased letter are significantly different according to Tukey's HSD at $P<0.05$.

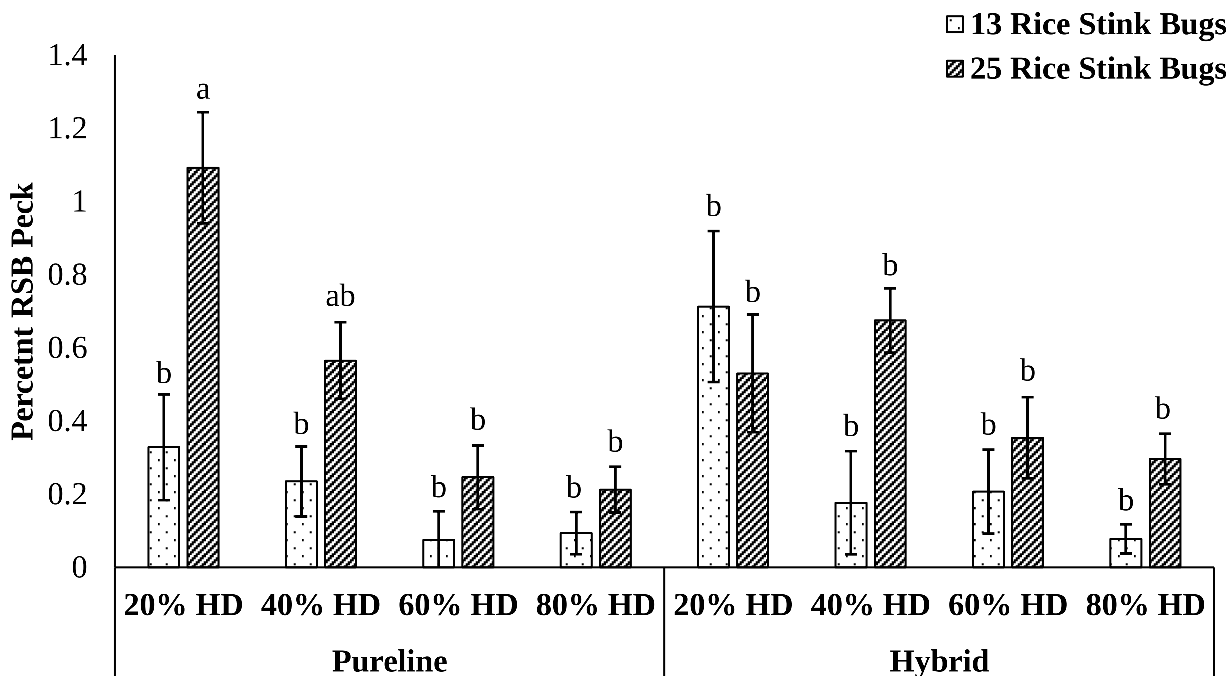

Figure 4.2 Percentage of rice kernels with rice stink bug (RSB) feeding damage (% RSB Peck) harvested from two rice cultivars (RT XP753 or Diamond) enclosed in separate large screen cages (1.8m x 1.8m x 1.5m) exposed to 13 or 25 rice stink bug (RSB) released into screen cages at rice panicle growth stages of 20, 40, 60 or 80% hard dough in 2016 and 2017 at Stuttgart, Arkansas. Average % RSB Peck values within each cultivar followed by a different lower-cased letter are significantly different according to Tukey's HSD at *P*<0.05.

Chapter 5 - Assessment of Rice Stink Bug, *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae), Damage to Grain Sorghum, *Sorghum bicolor* (L.).

Abstract

The rice stink bug, *Oebalus pugnax*, damages the developing kernels of grain sorghum and many other grasses when it feeds. Although studies have shown that rice stink bug can cause appreciable damage to grain sorghum, there is a lack of information regarding the effect of rice stink bug on most grain sorghum heading growth stages. The objectives of this study were to determine the amount of yield loss caused to grain sorghum by rice stink bug feeding at different heading growth stages, and then to develop dynamic thresholds for these stages. Damage to grain sorghum was assessed by confining rice stink bug to sorghum heads using sleeve cages. Five infestation densities (0, 1, 2, 5, 10, and 20 rice stink bugs per head) were applied across four stages of head development (head emergence, flowering, soft dough, and hard dough) at 3 regions (Northeast, Central, and South) in Arkansas. Rice stink bug caused the most damage at head emergence and flowering, at 17% and 11% of yield lost per rice stink bug respectively. Only 4% yield loss per rice stink bug was observed in soft dough, and no significant damage was observed at hard dough. Dynamic thresholds were created for head emergence, flowering, and soft dough growth stages to cover a range of control costs ($15-$40 per ha) and grain value ($40-$315 per tonne). Rice stink bug poses its largest threat to grain sorghum in the early heading growth stages, and the potential for yield loss decreases as grain sorghum matures.

Introduction

The rice stink bug, *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae), is a major pest of rice, *Oryza sativa* (L.), grown in the southern United States (Webb 1920). This stinkbug is oligophagous and feeds upon many cultivated (wheat, *Triticum aestivum* L., grain sorghum *Sorghum bicolor* (L.) Moench, corn, *Zea mays* L., and rice, *Oryza sativa* L.) and uncultivated (barnyardgrass *Echinochloa crus-galli* P.Beauv., cheat *Bromus tectorum* (L.), ryegrass, *Lolium* *spp.* L., and Johnsongrass, *Sorghum halepense* (L.) Perse.) grass species (Douglas 1939; Odglen and Warren 1962). Rice stink bug feeds on the developing kernels of rice and other grasses beginning at the heading phase when the panicle is exerted from the boot until the end of the ripening phase, known as the hard dough growth stage (Swansom and Newsom 1962). Feeding by the rice stink bug in the flowering stage of rice often causes blanked kernels and direct rough rice mass loss (Swanson and Newsom 1962; Bowling 1963; Espino et al. 2007). Feeding during the milk to soft and hard dough growth stages is associated with broken, chalky or pecky kernels.

Grain sorghum is commonly grown in rotation with other crops in Arkansas. The area planted across the state has exceeded 160,000 ha in recent years when economic conditions have been favorable (NASS 2017b). Although grain sorghum in Arkansas is often perceived by producers as a crop with few insect issues where the need for scouting is minimal, the introduction of sugarcane aphid, *Melanaphis sacchari* (Zehntner, 1897), into Arkansas during the 2013-2014 growing season made scouting a necessity for a successful crop (Seiter et al. 2015). This, coupled with an increase to 182,000 ha planted in 2015, and relatively high grain sorghum prices (NASS 2017b), led many producers to intensively scout their grain sorghum. The University of Arkansas Cooperative Extension Service now recommends sorghum to be scouted for sugarcane aphid at least 2 times per week, and this is deemed necessary for much of the growing season (Seiter et al. 2015). Although producers and consultants following these guidelines are searching for early invasions of sugarcane aphid, many occasional or secondary pests are commonly found to be present. From 2013-2015, sorghum scouting and management of insects increased dramatically due to the sugarcane aphid, and many reports of high levels of rice stink bug were received by the University of Arkansas Cooperative Extension Service. Although rice stink bug is rarely controlled in grain sorghum in Arkansas, it is a key pest of heading rice.

In grain sorghum, rice stink bug is sampled by shaking heads into a bucket or net, and the average number of rice stink bugs per head is determined by sampling heads from 10 locations throughout a field (Studebaker et al. 2011). Levels of rice stink bug in grain sorghum fields are occasionally observed above the recommended threshold for Arkansas of five rice stink bugs per head from flowering to soft dough (Studebaker et al. 2011).

The potential for damage in grain sorghum by rice stink bug and other stink bug species was assessed in Texas by Hall and Teetes (1982) who determined that an insecticide application was warranted when rice stink bug population levels reached four per head at the milk stage and eight per head at soft dough. These relationships were determined by infesting rice stink bug only at the milk and soft dough stages, and no infestations were made during head emergence, flowering, or hard dough growth stages. The current rice stink bug threshold for grain sorghum in Arkansas and other states is based solely on this work, although thresholds vary slightly from state to state. While the relationship between rice stink bug population densities and yield has not been replicated in over 30 years, rice stink bug recommendations in Texas have recently been modified (Texas A&M 2018). Texas now includes a dynamic threshold which incorporates a recalculation of Hall and Teetes's (1982) data along with estimations of the number of heads/acre (Texas A&M 2018). Typical grain sorghum prices would yield an economic threshold ranging from one rice stink bug per head to one rice stink bug per two heads on average, making this threshold much lower than Arkansas's current threshold. Additionally, this threshold does not change based on the reproductive growth stage of grain sorghum (Texas A&M 2018).

Past research indicates that both Arkansas's and Texas's thresholds have scientific basis, but the question of whether these thresholds are applicable is still left unanswered for producers and consultants across the grain sorghum growing regions. There is also no published information regarding the effect of rice stink bug on grain sorghum at the head emergence, flowering, and hard dough growth stages, although rice stink bug is capable of damaging rice at these stages (Patel et al. 2006). The first objective of this study was to determine the amount of yield loss caused to grain sorghum across heading growth stages, including previously unstudied early heading growth stages. The second objective of this study was to develop dynamic thresholds across these growth stages.

Materials and Methods

Site Description

Field experiments were conducted at three locations in 2016 and 2017 for a total of six site years: the Northeast Research and Extension Center near Keiser, AR; the Lon Mann Cotton Research Station near Marianna, AR; and the Southeast Research and Extension Center near Rohwer, AR. At each location, a commercial grain sorghum hybrid (Pioneer® 84P80, Dupont, Pioneer®, Johnston, IA, USA) was planted using standard agronomic practices (Espinoza and Kelley 2018). All plots were scouted and treated as necessary for natural insect infestations: Flupyradifurone, (Sivanto® 1.67 SL, Bayer CropScience, Research Triangle Park, NC, USA) was applied to sorghum at four of the site years to manage sugarcane aphid infestations at a rate of 0.058 kg ai per ha. These applications of flupyradifurone were made prior to boot stage when artificial rice stink bug infestations had not yet been established. Applications of chlorantraniloprole (Prevathon®, FMC Corporation, Philadelphia, PA) at a rate of 0.052 kg ai per ha were made at each site in 2017 to control corn earworm larvae, *Helicoverpa zea* (Boddie), as emergence of the heads began.

Rice Stink Bug Collection and Handling

Adult and late instar rice stink bug nymphs were collected using sweep nets from both heading weedy grasses and cultivated rice. To insure viability of the individuals for the study, rice stink bugs were moved into the laboratory to be kept in 30cm mesh rearing cages (1466AV, BioQuip Products, Rancho Dominguez, CA, USA) containing fresh heading grass material, a moist paper towel, and a petri dish containing cotton balls soaked in sugar water at a rate 200g of sugar per 3.8 liters of water. These were then held at 24° C for at least 24 hours prior to their use in field experiments to insure only healthy rice stink bugs were used for infestations.

Experimental Setup

Field Experiments used a randomized complete block design with 10 replicate blocks and 20 treatments. The experimental unit was the panicle of a single grain sorghum plant enclosed by a sleeve cage. Cages were placed in a large planted area of grain sorghum at each location, and each replication was blocked within a planted row. Treatments were arranged using a 5 × 4 full factorial each year the study was performed. In 2016, the densities of 0, 2, 5, 10, and 20 rice stink bugs per panicle were used, and rice stink bug densities of 0, 1, 2, 5, and 10 rice stink bugs per panicle were utilized in 2017. These infestation densities were infested at four growth stages: head emergence, flowering, soft dough, hard dough. For each combination of infestation density and growth stage, 10 replications were performed, except for emergence in the southeast region which was only replicated 5 times for each infestation density in 2016 only. Additionally, 20 rice stink bugs per head was only included in the experiment in 2016 due to the large amounts of damage observed at 10 rice stink bugs, and 1 rice stink bug per head was included in 2017 to better understand low levels.

Cage Design and Usage

White insect rearing sleeves, 20 × 40 cm (1460W, BioQuip Products, Rancho Dominguez, CA 90220, USA) were used to confine rice stink bugs to the grain sorghum heads. All cages were installed at head emergence, considered as the point when 10% of the flowers on the head had open anthers and there was enough space between the bottom portion of the sorghum head and the flag leaf to allow for the cage to be tied to the plant without inhibiting growth. Rice stink bugs were then added to each cage when the assigned stage was reached within the cage. For head emergence, rice stink bugs were infested within 24 hours of placing cages on the grain sorghum. For the flowering growth stage, rice stink bugs were infested when 60-75% of the grain sorghum flowers on the head had gained an orange coloration and were likely pollinated. The growth stage of soft dough and hard dough stage infestations were judged based on the grain coloration and consistency of surrounding, uncaged heads of grain sorghum plants not used in this study. Mortality was checked 24 hours after rice stink bugs were released into cages and every 48 hours after the initial 24 hours until the next growth stage timing was reached. Any dead rice stink bugs were during these inspections for the first two weeks after introduction, and rice stink bug populations were then allowed to propagate unchecked through the remaining growth stages until harvest.

When all grain sorghum cages reached harvest maturity, stalks were cut below the cages using shears and then the head and sleeve cage together were placed in a gallon-sized freezer bag. Bags were stored in a freezer at -4° C for at least two weeks to ensure mortality of stink bugs. Cages were removed and panicles were then hand-threshed and the weight of both the empty panicle and the threshed grain was obtained. Gross seed weight was then adjusted for the relative size of the panicles using the equation: adjusted gross seed weight = (gross seed weight /

threshed panicle weight) × (mean threshed weight of panicles) (Hall and Teetes 1982). To better illustrate differences between treatments, the adjusted gross seed weight was then converted to "percent of uninfested control." The percent of uninfested control was calculated by (Adjusted seed weight of the panicle in question) / (Mean adjusted seed weight for the control in the same site year and infestation timing).

Data Analysis

Data were compared using a two-way analysis of variance utilizing PROC GLIMMIX (SAS v. 9.4, SAS Institute, Cary, NC) and a general linear model with a normal distribution. Denominator degrees of freedom were adjusted using a Kenward-Rogers approximation (Kenward and Roger 1997). Site year and block within site year were considered random variables, with infestation density, growth stage, and infestation density × growth stage being considered fixed effects. Means were then separated using Tukey's HSD post hoc analysis at P=0.05.

Data were further analyzed using regression analysis. This was performed using a mixed model in PROC GLIMMIX (SAS v. 9.4, SAS Institute, Cary, NC) with site year and block considered as random variables. Denominator degrees of freedom were adjusted using a kenward-rogers approximation (Kenward and Roger 1997). Adjusted yield in grams of each harvested sorghum head was square root transformed to normalize the data, and was then utilized as the response variable with the number of rice stink bugs added as the explanatory variable. The polynomial predictor of x^2 was used in the model, and was found to be significant in all cases. The model for all regression analyses tested was: $y = \beta_0 + \beta_1 x + \beta_2 x^2 + \mathcal{E}$.

Economic Threshold Calculations

An economic threshold for rice stink bug in grain sorghum was created by first determining a gain threshold (Pedigo et al. 1986). The gain threshold was calculated by dividing the cost of control by the prospective value of the grain, which yielded the number of bushels that warranted an application for rice stink bug. This value was then compared to the slope of the line within each growth stage, after it was converted to tonnes per ha using $((x^2/1000) \times 185{,}250) / 1000$ which brings the slope value from sqrt(grams)/sorghum head/rice stink bug to tonnes/hectare/rice stink bug using 185,250 heads per hectare as an estimate. Considering that a polynomial relationship existed at each of the three growth stages where significant levels of damage were observed, the entire polynomial equation was used when creating the threshold. The damage caused per rice stink bug found on each head was determined using the polynomial equation found for each growth stage in Figure 5.2 with (β_1×rice stink bug number) + ((-1×(β_2^2) × rice stink bug #)), after converting β-values to tonnes per hectare. This accounted for the polynomial nature of the damage curves, where the rate of increase in damage decreased as more rice stink bugs were added to each sorghum head. The amount of damage caused by each rice stink bug found per head on average for each of the growth stages was then compared to the grain threshold for each combination of control cost and grain value, which yielded the Economic Injury Level (EIL) (Pedigo et al. 1986). The EIL value was then multiplied by 0.75 to determine the Economic Threshold (ET), or the point in which rice stink bug needed to be controlled to prevent damage equal to the control costs (Pedigo et al. 1986).

Results

Regression Analysis

There was a significant quadratic relationship between rice stink bug density per cage and grain sorghum yield (Table 5.1, Figure 5.1). Direct reductions in adjusted grain weight, after square root transformation, by this pest were significantly associated with increases of rice stink bug infestation densities at the head emergence, flowering and soft dough growth stages, but not at hard dough (Table 5.1, Figure 5.1). Each addition of rice stink bug to a single sorghum head during the head emergence growth stage exhibited a decrease in 7.0g [$(6.36 - 0.58)^2$] of adjusted grain weight, but a significant positive polynomial slope of 0.02 decreased the rate of yield loss as the number of rice stink bugs present increased (Table 5.1, Figure 5.1). Less yield loss was observed in the flowering growth stage, where 4.86g [$(6.59 - 0.38)^2$] adjusted grain weight was lost per rice stink bug (Table 5.1, Figure 5.1), and a positive polynomial slope of 0.001 decreased the rate of adjusted grain weight loss as the number of rice stink bugs increased. Rice stink bug introduction in soft dough exhibited the lowest amount of yield loss, at 1.95g [$(7.05 – 0.14^2$] per added rice stink bug, and the strong polynomial slope of 0.001g relative to total grain weight loss suggested that high numbers of rice stink bug would decrease loss rates significantly (Table 5.1, Figure 5.1).

Economic Threshold

Dynamic economic thresholds were created for infestations that began at the head emergence, flowering, and soft dough growth stages, but a threshold was not created for the hard dough growth stage since no significant damage was observed (Table 5.2, Table 5.3, Table 5.4). For the emergence growth stage, if control costs are $20 dollars per ha and the value of the grain is $160 per tonne, then this economic threshold would recommend a control be initiated at 2 rice stink bugs per head (Table 5.2). This compares to 4 and 29 rice stink bugs per sorghum head in flowering and soft dough respectively (Table 5.3, Table 5.4). Control decisions based on these economic thresholds can fluctuate from 0.6 to 5 at emergence, 2 to 11 at flowering, and 11-98 rice stink bugs per head at soft dough depending upon a range of crop value from $40-$320 per tonne with the same control cost (Table 5.2, Table 5.3, Table 5.4). If a control tactic that costs $35 per ha was utilized, the economic threshold for $160 per bushel grain sorghum would vary from 3 rice stink bugs in emergence, 7 rice stink bugs in flowering, and 52 rice stink bugs per head in soft dough (Table 5.2, Table 5.3, Table 5.4). These data are based on an assumption of 185,250 sorghum heads per hectare, and ET's will need to be adjusted if the head number deviates drastically.

Discussion

Economic thresholds for rice stink bug in grain sorghum have been available since the mid-1980's, but the experiments used to compute these thresholds were limited to the milk and soft dough growth stages (Hall and Teetes 1982). Our study suggests that rice stink bug infestations in the head emergence, flowering, and soft dough growth stages can cause significant levels of yield loss. Yield loss was most pronounced when infestations began in the head emergence and flowering growth stages, where 17% (6.8g) and 11% (4.82g) of potential yield was lost with each increase of one rice stink bug, respectively. This compares to yield losses no greater than 2.5% and 1.3% per one rice stink bug when added during the milk and soft dough growth stages, respectively (Hall and Teetes 1982). Rice stink bug damaged the earlier stages of grain maturation at much higher rates. Rice stink bug damaged and subsequently blanked the flowers of the developing kernels of sorghum, rather than just reducing the weight of the grain which would be the typical damage expected from infestations at milk to hard dough.

This phenomenon is also observed in other grass crops such as rice, where an increase in blanked kernels could possibly lead to a decrease in overall weight (Swanson and Newsom 1962,; Bowling 1963, Espino et al. 2007, Awuni et al. 2015).

Approximately 4% (1.94g) yield loss per rice stink bug was observed when the infestation occurred at soft dough, which was larger than the 1.3% yield loss previously observed (Hall and Teetes 1982). Both studies observed a quadratic polynomial relationship between yield loss and rice stink bug infestation density, but Hall and Teetes (1982) observed a quadratic relationship that suggested increasing rates of damage as rice stink bugs per head increased. Data from our study suggests a negative quadratic relationship where increasing numbers of rice stink bug decrease the rate of yield loss at all three growth stages explored. These findings suggest that a crowding effect was observed where infestation densities above 10 rice stink bugs per head likely led to increased competition for feeding spaces. This is opposed to data from Hall and Teetes (1982) which did not suggest a vertex where yield loss rate would be inhibited for both milk and soft dough, although they did test up to 16 rice stink bugs per head at both milk and soft dough.

This study indicates that separate thresholds are necessary for head emergence, flowering, and soft dough growth stages. Using a gain threshold of control cost / grain value, head emergence thresholds were much lower than the current threshold in Arkansas of 5 rice stink bugs per head during the flowering growth stage at all grain values, except when grain value is very low or control costs exorbitantly high (Studebaker 2011). At a grain value of $160 per tonne, this threshold would be 3 rice stink bugs too high in head emergence, 1 rice stink bug too high in flowering, and 24 rice stink bugs too low in soft dough. This suggests that for a common economic situation the previous threshold was relatively accurate at flowering, but inaccurate at the emergence and soft dough growth stages. Results from this study will allow more accurate decision-making across these growth stages. For instance, most applications recommended under the previous threshold after the soft dough stage has been reached are likely to be unnecessary. Although previous thresholds indicated 5 rice stink bugs at soft dough and 16 rice stink bugs at hard dough (Studebaker 2011), the new dynamic thresholds indicate that it is very unlikely that control will be economical at soft dough, and no is not economical at hard dough. These thresholds use an estimate of 185,250 sorghum heads per hectare to estimate total weight loss from each rice stink bug per head. If large differences in heads per acre exist then rice stink bug numbers within the dynamic threshold charts can be converted with the following equation: Threshold value × (1 / (# of Sorghum Heads / 185,250)).

A number of differences were observed between the thresholds currently in use in Texas and other states and the thresholds proposed by this study. The original thresholds for grain sorghum were created using an estimation of potential yield for the field (Hall and Teetes 1982). The new threshold does not consider potential yield/hectare necessary, and instead only uses an estimation of tonnes lost/hectare/1 rice stink bug. Newer thresholds currently in use in Texas also no longer consider potential yield and instead only use pounds/acre lost, but large differences in damage potential still exist between those and the thresholds presented by this study (Texas A&M 2018). When considering a control cost of $8 and $4 per bushel grain sorghum at 75,000 heads/acre (equivalent to $40 per tonne grain sorghum and $20 per ha application cost at 185,250 heads per hectare), the Texas A&M calculator estimates that an average of 0.5 rice stink bug per head would be the economic threshold (Texas A&M 2018). Even if this infestation began in the head emergence growth stage, thresholds created through our study would not recommend applying an insecticide until two rice stink bugs per head, and four rice stink bugs per head in flowering. The Texas A&M thresholds are still based off of the original data from Hall and Teetes (1982), and therefore large differences are expected.

Landscape-level differences by state may also lead to different effects by rice stink bug on grain sorghum for each state or area. In Arkansas, the large area of cultivated rice serves as a sink for adult rice stink bug, which are highly attracted to heading rice (Rashid et al. 2005, NASS 2017a). Infestations can be severe if grain sorghum begins to head just before any rice in the area enters head emergence, but populations may also emigrate as soon as nearby rice becomes attractive. However, if infestations begin from head emergence to flowering, significant damage will likely be inflicted before emigration occurs. These landscape-level factors must be considered on an area-by-area basis. In many of the Texas grain sorghum growing regions, grain sorghum is more likely to be a sink for rice stink bug, and rice stink bug is possibly a much more serious pest of grain sorghum in those growing regions. Rice stink bugs are likely to inhabit many of those fields from head emergence to hard dough with little emigration except to move to other grain sorghum fields.

This study indicates the potential for damage in grain sorghum if rice stink bug is not controlled throughout the heading growth stages. Dynamic thresholds created from this data indicate that rice stink bugs pose the largest threat in the early heading growth stages when developing flowers of the grains are being fed on, and yield loss potential decreases as the sorghum grains mature. When combined with knowledge of the landscape, management of rice stink bug will be more accurate and efficient across all grain sorghum growing regions when utilizing these new dynamic thresholds.

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Table 5.1 Regression analysis indicating amount of yield loss in sqrt(grams) caused by each rice stink bug, *Oebalus pugnax*, found per grain sorghum head when infested at 4 heading growth stages: head emergence, flowering, soft dough, and hard dough, for grain sorghum planted at 3 locations across Arkansas in 2016 and 2017.

Growth Stage	Equation ($y = \beta_0 + \beta_1 x + \beta_2 x^2 + \mathcal{E}$)	Standard Error	RSE	x			x^2		
				T	df	*P*	*T*	df	*P*
Head Emergence	$y = 6.36 - 0.58x + 0.02x^2$	$\beta_0 = 0.76$, $\beta_1 = 0.05$, $\beta_2 = 0.002$	0.21	-12.2	238	*P<0.01*	6.56	239	*P<0.01*
Flowering	$y = 6.59 - 0.38x + 0.001x^2$	$\beta_0 = 0.61$, $\beta_1 = 0.04$, $\beta_2 = 0.002$	0.16	-9.2	263	*P<0.01*	4.65	263	*P<0.01*
Soft Dough	$y = 7.05 - 0.14x + 0.001x^2$	$\beta_0 = 0.80$, $\beta_1 = 0.04$, $\beta_2 = 0.002$	0.18	-3.2	263	*P=0.02*	2.38	263	*P=0.02*
Hard Dough	$y = 6.74 + 0.008x - 0.003x^2$	$\beta_0 = 0.75$, $\beta_1 = 0.04$, $\beta_2 = 0.002$	0.14	0.2	291	*P=0.84*	-0.15	291	*P=0.88*

Variables were found to have a significant linear relationship with yield loss within each growth stage according to a T-test at *P*=0.05.

Table 5.2 Dynamic economic threshold for rice stink bug infestations sampled in grain sorghum during the head emergence growth stage, with crop values and control cost taken in to account.

	Rice Stink Bugs / Head					
	Control Costs ($/ha)					
Crop Value ($/tonne)	**15**	**20**	**25**	**30**	**35**	**40**
40	5	6	8	9	11	13
80	2	3	4	5	5	6
120	2	2	3	3	4	5
160	2	2	3	3	3	3
200	2	2	2	2	2	3
240	1	2	2	2	2	2
280	1	2	2	2	2	2
320	0.6	1	2	2	2	2

Table 5.3 Dynamic economic threshold for rice stink bug infestations sampled in grain sorghum during the flowering heading growth stage, with crop values and control cost taken in to account.

	Rice Stink Bugs / Head					
	Control Costs ($/ha)					
Crop Value ($/tonne)	**15**	**20**	**25**	**30**	**35**	**40**
40	11	15	19	23	26	30
80	5	8	9	11	13	15
120	4	5	6	8	8	10
160	3	4	5	6	7	8
200	2	3	4	5	5	6
240	2	3	3	4	5	5
280	2	2	3	4	4	5
320	2	2	2	3	4	4

Table 5.4 Dynamic economic threshold for rice stink bug infestations sampled in grain sorghum during the soft dough heading growth stage, with crop values and control cost taken in to account.

Crop Value ($/tonne)	Rice Stink Bugs / Head Control Costs ($/ha)					
	15	**20**	**25**	**30**	**35**	**40**
40	98	150	*	*	*	*
80	44	60	78	98	122	150
120	29	38	49	60	71	84
160	21	29	36	44	52	60
200	17	23	29	35	41	47
240	14	18	23	29	33	38
280	11	16	20	24	29	32
320	11	14	17	21	24	29

*Asterisks indicate that RSB cannot cause enough damage to warrant an insecticide.

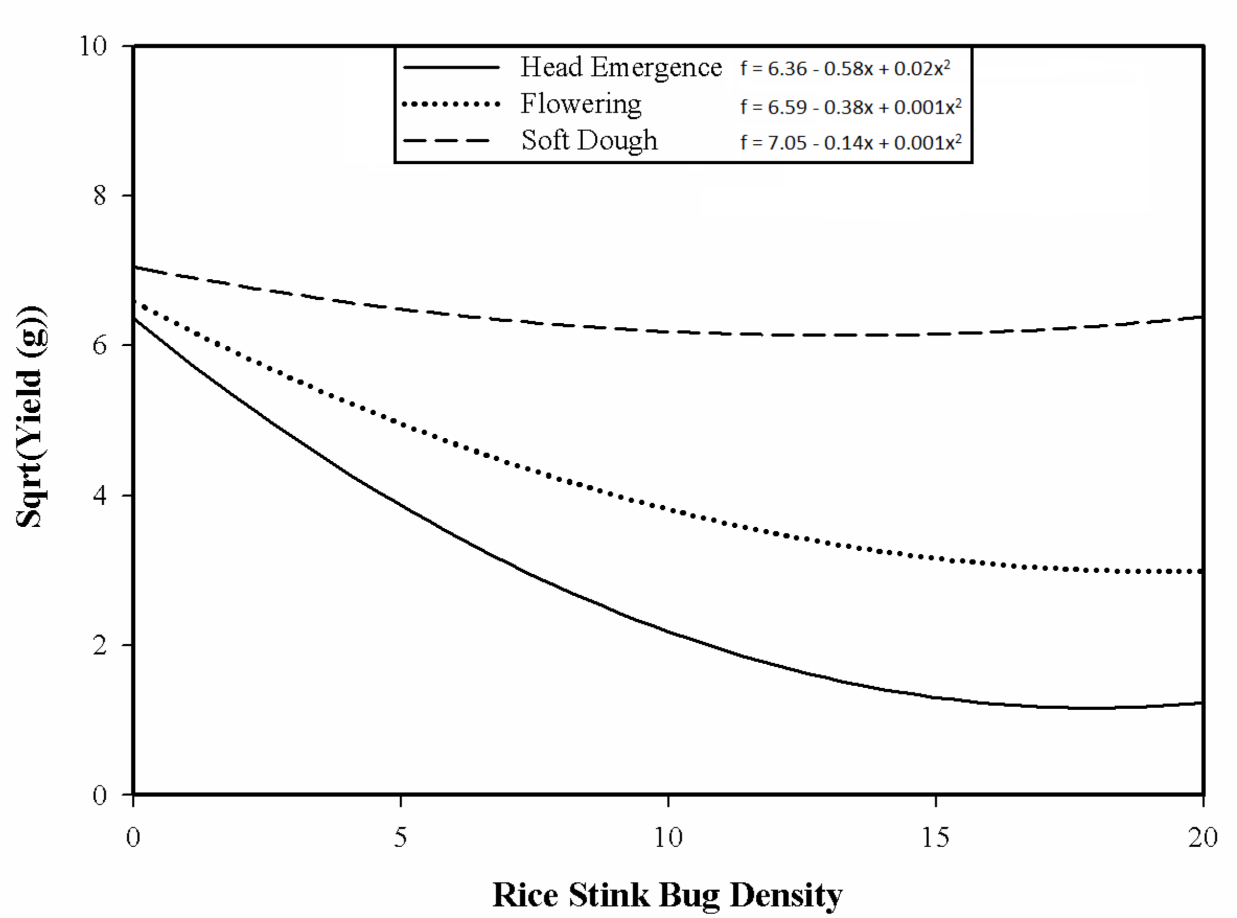
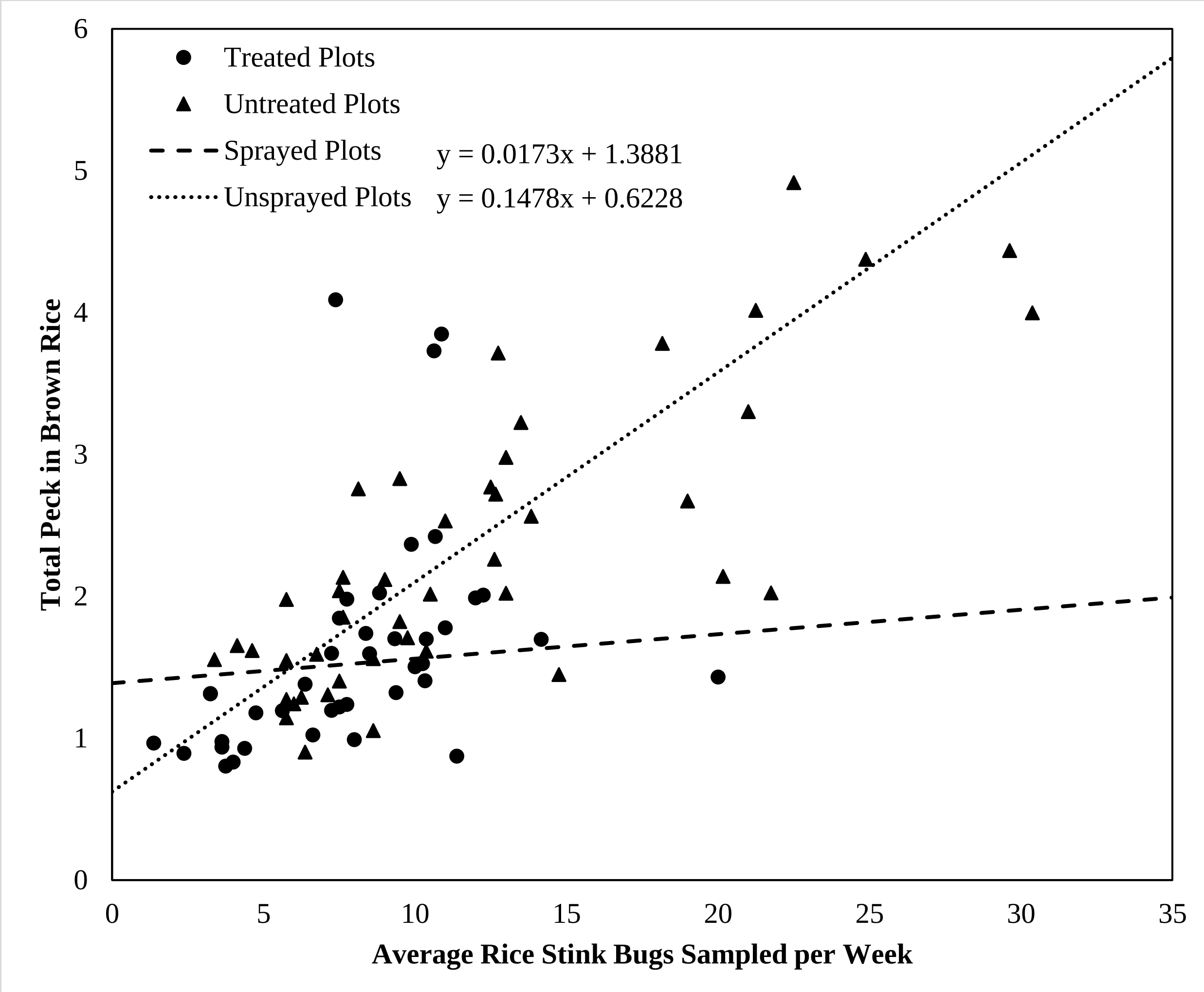


Figure 5.1 Decrease in yield, sqrt(g), as rice stink bug infestation increases in each of three grain sorghum heading growth stages: emergence ($P<0.01$), flowering ($P<0.01$), and soft dough ($P=0.02$), with a quadratic relationship denoted by second order polynomial slope in each line formula for emergence ($P<0.01$), flowering ($P<0.01$), and soft dough ($P=0.02$).

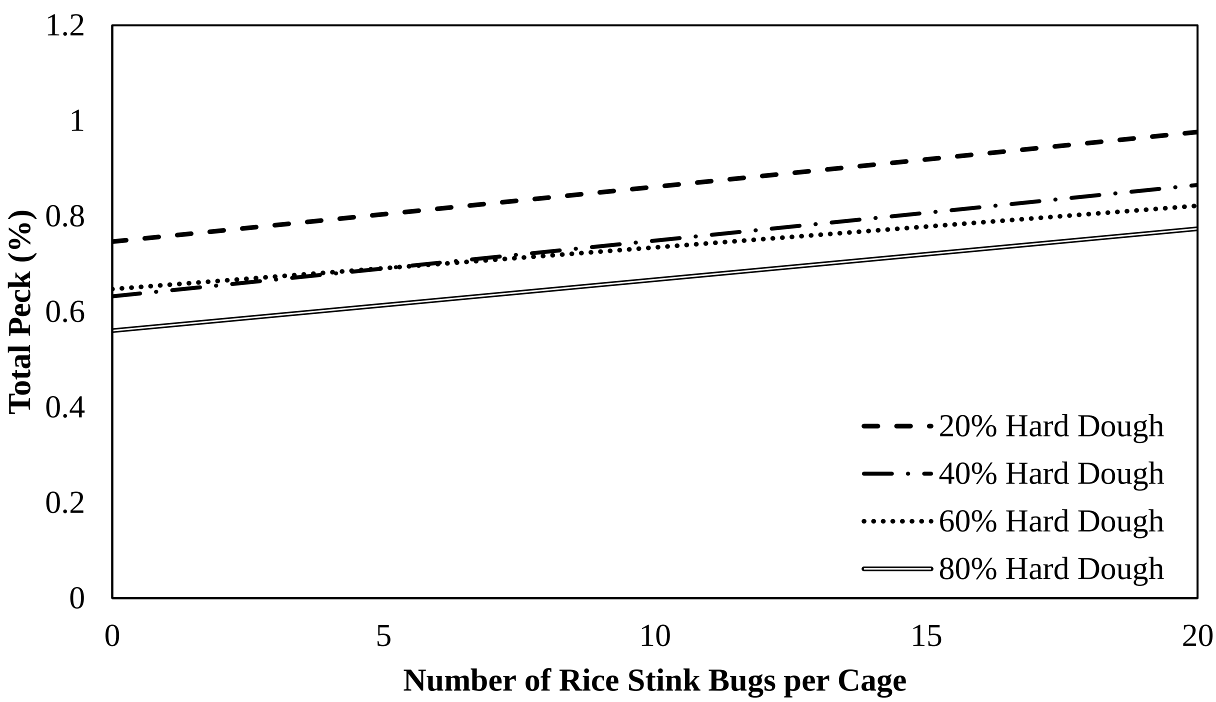
Appendix



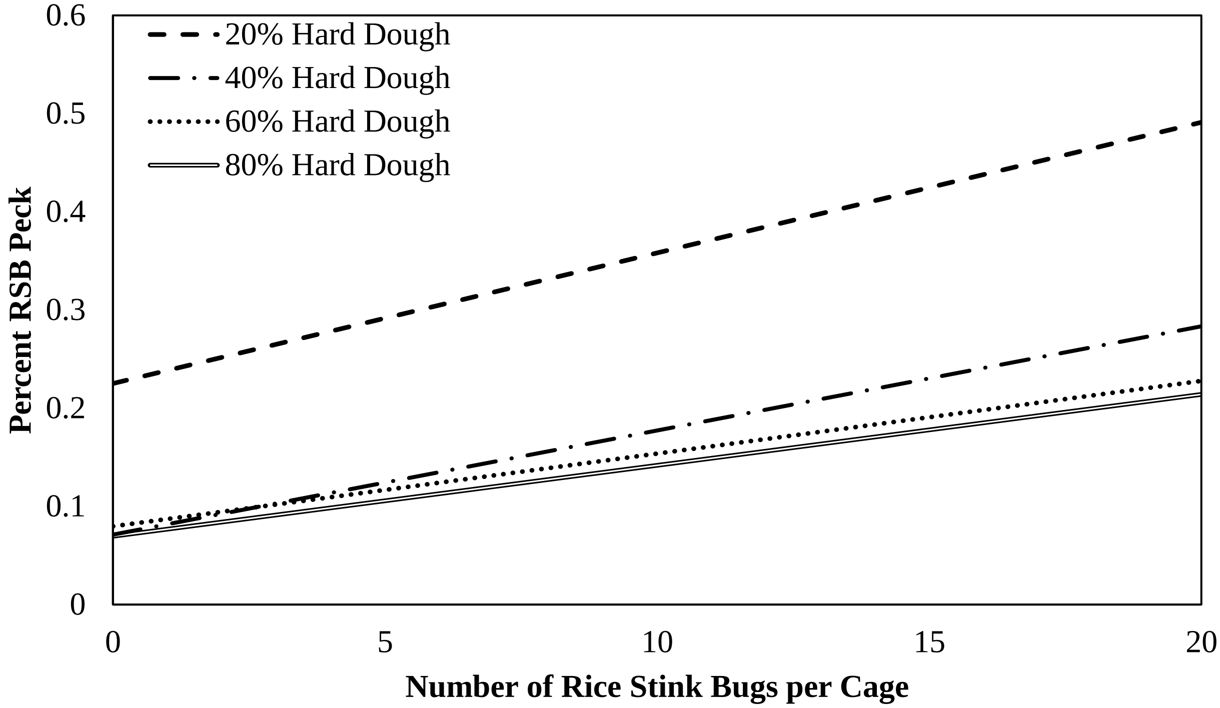
Appendix 3.1 Total peck observed in brown rice predicted by the average number of rice stinkbugs sampled per week in both sprayed (*P*=0.64) and unsprayed plots (*P*<0.0001) for uncaged trials across 6 locations in eastern and central Arkansas sampled in 2018.

Appendix 4.1 Regression equations that predict rice stink bug (RSB) percentage feeding damage to rice kernels as log (RSB Peck) versus x = 0, 13 or 25 RSBs released per large screen cage (1.8 m x 1.8 m x 1.5 m) during each of four rice panicle growth stages across two rice cultivars (RT XP753 or Diamond) and two years 2016 and 2017 in Stuttgart, Arkansas.

Predictor	**Timing**	**Equation** ($y = e^{\beta 0 + \beta 1x + \varepsilon}$)	**Standard Error** (β_0 and β_1)	**RSE**	***T***	**df**	***P***
Log (RSB Peck)	20% Hard Dough	$y = 10^{-0.62 + 0.016x}$	0.44, 0.008	0.02	2.02	24	*0.05*
	40% Hard Dough	$y = 10^{-1.05 + 0.027x}$	0.50, 0.009	0.03	2.82	14	*0.01*
	60% Hard Dough	$y = 10^{-1.05 + 0.021x}$	0.36, 0.008	0.02	2.80	14	*0.01*
	80% Hard Dough	$y = 10^{-1.10 + 0.022x}$	0.31, 0.005	0.01	4.35	14	<*0.01*



Appendix 4.2 Total peck observed in large cages infested at four estimated hard dough timings based on % hard dough (HD) (straw-colored) kernels as predicted by the number of rice stink bugs added to each cage for trials performed in Stuttgart, Arkansas in 2017 and 2018.

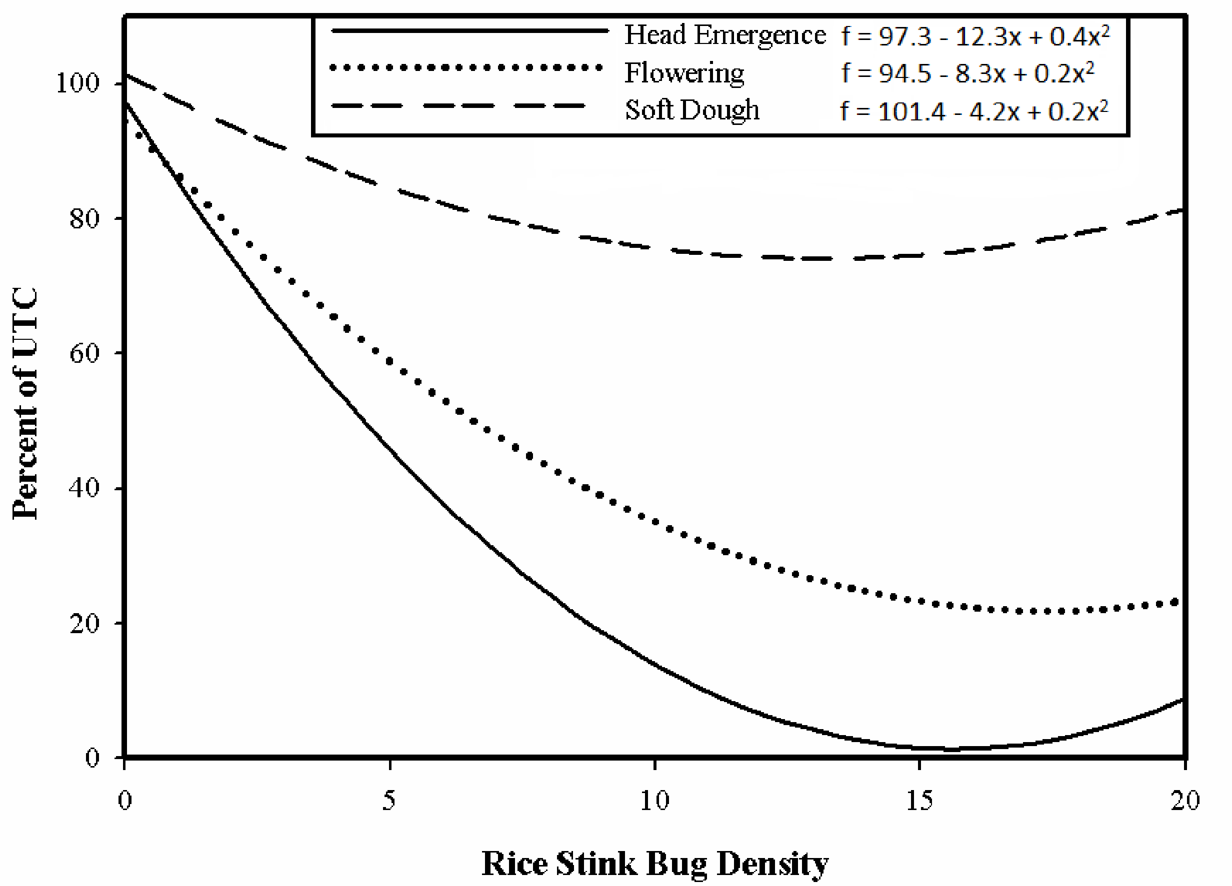


Appendix 4.3 RSB peck observed in large cages infested at four estimated hard dough timings based on % hard dough (HD) (straw-colored) kernels as predicted by the number of rice stink bugs added to each cage for trials performed in Stuttgart, Arkansas in 2017 and 2018.

Appendix 5.1 Sleeve cage study comparing infestation densities of rice stink bug within and across four grain sorghum heading growth stages: head emergence, flowering, soft dough, and hard dough, using yield percent* of uninfested combinations.

Growth Stage	Rice Stink Bug Density	Yield Percent of Uninfested* (n)
Head Emergence	0	100a EF (60)
	1	87a EF (30)
	2	74a DEF (60)
	5	43b BC (60)
	10	19cd AB (60)
	20	11d A (30)
Flowering	0	100a EF (59)
	1	79ab DEF (30)
	2	73ab DE (60)
	5	57bc CD (60)
	10	38cd BC (60)
	20	27d AB (30)
Soft Dough	0	100a EF (60)
	1	99a F (30)
	2	96a EF (59)
	5	82a EF (60)
	10	76a DEF (60)
	20	82a DEF (30)
Hard Dough	0	100a EF (59)
	1	97a EF (30)
	2	100a EF (60)
	5	102a F (60)
	10	101a EF (60)
	20	105a EF (30)

*Yield Percent of Uninfested is statistically different across growth stage combinations when followed by a different uppercased letter according to Tukey's HSD post hoc analysis at P<0.05.
*Yield Percent of Uninfested is statistically different within growth stage combinations when followed by a different lowercased letter according to Tukey's HSD post hoc analysis at α=0.05.



Appendix 5.2 Decrease in yield relative to the uninfested as number of rice stink bugs increase in each of three grain sorghum heading growth stages.