# Impact of *Oebalus pugnax* (Hemiptera: Pentatomidae) Infestation Timing on Rice Yields and Quality

G. A. AWUNI,<sup>1,2</sup> J. GORE,<sup>3,4</sup> D. COOK,<sup>3</sup> F. MUSSER,<sup>2</sup> A. CATCHOT,<sup>2</sup> and C. DOBBINS<sup>3</sup>

J. Econ. Entomol. 108(4): 1739-1747 (2015); DOI: 10.1093/jee/tov123

ABSTRACT Sleeve and large field cage experiments were conducted in Stoneville, MS, in 2010 and 2011 to assess adult rice stink bug, *Oebalus pugnax* (F.), injury in rice. 'Cocodrie' and 'Wells' were infested at bloom, milk, and soft dough stages of panicle development. Twenty rice panicles were infested individually in the sleeve cage experiment as replicates with 0, 1, or 2 O. pugnax in a split-plot, completely randomized design. The large cage experiment had four replications infested with 9 or 18 O. pugnax per square meter over multiple rice panicles in a split-plot, randomized complete block design per cultivar. Caged uninfested controls were included in each experiment. Rough rice yield and percentage of clean, damaged, and blank kernels were evaluated. In both experiments, stage of panicle development impacted grain yield and quality. Yield loss was greatest during the bloom stage, while kernel damage was greatest during the milk and soft dough stages. Rice yield decreased with increased infestation density. Kernel damage increased with increased infestation density. Blank kernels affect yield, while kernel damage affects grain quality. While grain yield is the bottom line, grain quality affects marketability, which directly affects yield profitability. Based on these results, this study considers O. pugnax injury significant in all three stages of panicle development and concludes that a more aggressive threshold is recommended from panicle emergence through soft dough. More research is needed to determine the specific threshold, but it appears to be lower than the current threshold of 5 per 10 sweeps.

**KEY WORDS** rice stink bug, infestation time, infestation density

Weed, insect, and disease pests are limiting factors in rice, *Oryza sativa* (L.), production throughout the world (De Datta 1987, Chaudhary et al. 2002). The rice stink bug, *Oebalus pugnax* (F.), is an important late season pest of rice in all production regions of the United States (Swanson and Newsom 1962, McPherson and McPherson 2000, Way 2003), except California (Gianessi 2009).

*O. pugnax* is a graminaceous feeder (Odglen and Warren 1962), preferring rice over other grasses from heading through grain maturity (Ingram 1927, Douglas and Ingram 1942, Odglen and Warren 1962). The association of *O. pugnax* with rice in the United States was reported by Riley (1882). *O. pugnax* has a needle-like piercing and sucking stylet that constitute the mouth-parts which facilitate feeding. The stylet helps in penetration of cell walls of developing rice kernels by mechanical pressure and discharge of salivary enzymes to help digest and remove sap (Brown 2003). Adults and nymphs feeding similarly and cause both mechanical and chemical damage to rice kernels at the point of feeding.

Rice growth and development has been categorized into four phases: the seedling, vegetative, reproductive, and ripening phases (De Datta 1987, Moldenhauer 2001, Chaudhary et al. 2002). As a late season pest of rice, the reproductive and ripening phases are utmost concern to O. pugnax injury. The reproductive phase includes but not limited to the boot (pre-flowering) and heading (flowering) stages. The ripening phase is divided into the milk, soft dough, hard dough, and grain maturity stages (Chaudhary et al. 2002, Moldenhauer and Gibbons 2003). O. pugnax can cause rice injury from flowering through grain maturation, resulting in direct and indirect losses (Swanson and Newsom 1962, Bowling 1963, Patel et al. 2006). This has created concerns from rice growers in the Mississippi Delta regarding the specific stage of panicle development that is most sensitive to O. pugnax injury. O. pugnax infestation at flowering, grain filling, and ripening stages has been reported to cause blank kernels, partially filled (wrinkled), or discolored grains (Patel et al. 2006, Espino et al. 2007). These situations have resulted in either direct yield loss, indirect yield loss, or both to rice producers. Additionally, O. pugnax fed kernels result in grain deterioration during milling, which ultimately affects grain quality and marketability.

Although previous findings have reported significant yield loss in rice from *O. pugnax* infestations (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1963, Patel et al. 2006, Espino et al. 2007), it is still not clear to what extent *O. pugnax* can impact grain yield

© The Authors 2015. Published by Oxford University Press on behalf of Entomological Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons. org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

<sup>&</sup>lt;sup>1</sup>Department of Plant and Soil Science, Mississippi State University, MS 39762.

<sup>&</sup>lt;sup>2</sup> Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, MS 39762.

<sup>&</sup>lt;sup>3</sup> Delta Research and Extension Center (DREC), Mississippi State University, Stoneville, MS 38776.

<sup>&</sup>lt;sup>4</sup> Corresponding author, e-mail: jgore@drec.msstate.edu.

and quality at each stage of panicle development. Previous evaluations of *O. pugnax* injury with artificial cage infestations resulted in varying conclusions (Ingram 1927, Douglas and Tullis 1950, Bowling 1963) probably because of the asynchronous nature of rice tillers. Recent findings suggest significant yield and quality reduction during the milk and soft dough stages of panicle development (Patel et al. 2006, Espino and Way 2008). The general concept that yield and quality reduction increase proportionally with increased pest populations (Peterson and Higley 2001) has not be well studied with regards to *O. pugnax* infestation relative to panicle development.

To understand the impact of *O. pugnax* injury on grain yield and quality, there was the need to have a comprehensive study from the flowering stage through soft dough stage of panicle development. It is expected that each stage of panicle development and *O. pugnax* density should respond similarly to *O. pugnax* injury. Obviously, many factors contribute to economic losses in rice; however, the stage of panicle development for *O. pugnax* injury could be a key management factor for this pest.

The objectives of the current experiments were to assess *O. pugnax* injury on grain yield and the quality of rice at three stages of panicle development, namely, bloom, milk, and soft dough stages, with varying densities of *O. pugnax* infested concurrently with sleeve cages over individual panicles and large field cages over multiple panicles.

#### **Materials and Methods**

Experimental Site and Agronomic Practices. Field experiments were conducted with sleeve cages (20 by  $25\,\mathrm{cm}$ ) in one experiment and portable field cages (1.8) by 1.8 by 1.8 m<sup>3</sup>) in another experiment. The experiments were conducted concurrently at the Delta Research and Extension Center in Stoneville, MS, during 2010 and 2011. Two conventional (nonhybrid) highyielding, long grain rice cultivars ('Cocodrie' and 'Wells') were tested with standard agronomic practices for Mississippi drill-seeded rice (Miller et al. 2008). Rice was drill-seeded in both years into fine-textured alluvial soils (Sharkey series; Snipes et al. 2005) at 90 kg/ha in eight row plots (18-cm centers) by 4.57 m in length on 14 April 2010 and 13 April 2011. In total, 80 subplots each that covered 6.6 m<sup>2</sup> each were seeded per cultivar each year. Subplots were separated from each other by 1 m buffers that consisted of bare soil on all sides. The two cultivars were seeded in separate blocks and separated by a 2 m buffer alley. Urea nitrogen (46% N; Cargill LTD, No. 26, Saint-Petersburg, FL) was applied at the rate of 202 kg/ha on 9 June 2010 and 1 June 2011 when rice plants were at the five- to six-leaf stage. Rice plots were flooded immediately after fertilization and the flood was maintained until 2 wk before harvest.

**Insect Collection.** Adult *O. pugnax* were collected from heading grasses in and around Stoneville, MS, with a 38-cm sweep net (BioQuip Products, Rancho Dominguez, CA). The insects were sorted after every 10 sweeps, placed in 29 by 29 by 29 cm<sup>3</sup> Bugdorm

cages (BioQuip Products, Rancho Dominguez, CA) made of white 16 by 24 mesh polypropylene screen. In the laboratory, *O. pugnax* were maintained on 10% sugar solution for at least 12 h prior to infestation.

Stage of Panicle Development for Infestation. The stage of panicle development for O. pugnax infestations followed the timeline proposed by Counce et al. (2000) and Moldenhaur and Gibbons (2003). The two cultivars differed in growth and development resulting in asynchronous infestation of O. pugnax between cultivars in both years. Cocodrie was infested at  $\sim 106$ days after planting (DAP), 113 DAP at milk, and 120 DAP at soft dough stage. Wells was infested  $\sim 1$  wk after Cocodrie at all stages of panicle development in both years. Cages were removed after 1 wk for each stage of infestation. The bloom stage was determined for infestation when 50% of the uppermost spikelets on panicles within subplots began to flower. Similarly, the milk stage was determined for infestation when 50% of developing spikelets were soft and filled with milky substance. At this stage, the milky substance will spray out when pressed between fingers. Infestation at the soft dough stage was when 50% of uppermost kernels of panicles were starchy but firm and soft. Panicles at this developmental stage would bend in an arc-like manner, and the uppermost kernels turning from green to light brown in color.

**Sleeve Cage Infestation.** Adult *O. pugnax* were infested over individual panicles at random in 6.6-m<sup>2</sup> subplots. Treatments were a  $3 \times 3$  factorial arrangement with three infestation timings (bloom, milk, and dough) and three densities of O. pugnax (0, 1, and 2 per panicle) as a split-plot design in a completely randomized design with 20 replications per treatment. Each caged panicle was considered independent and, therefore, a replicate. In all, 60 panicles were infested per cultivar at each stage of infestation timing. Plastic tags ("Snap-on-tag" A.M. Leonard, Inc., Piqua, OH) labelled with date of infestation, infestation timing and O. pugnax density were placed on caged plants for identification at harvest. Sleeve cages were made from 20 mesh polyester-nylon netting with a drawstring to securely close the cage around the plant stem.

In 2010, Cocodrie was infested from 28 July to 3 August for the bloom stage, 4 to 10 August for the milk stage, and 11 to 17 August for the soft dough stage. Wells was infested from 4 to 10 August at the bloom stage, 11 to 17 August at the milk stage, and 18 to 24 August at the soft dough stage. In 2011, Cocodrie was infested from 27 July to 2 August at the bloom stage, 3 to 9 August at the milk stage, and 10 to 16 August at the soft dough stage. Wells was infested from 3 to 9 August at the bloom stage, 10 to 16 August at the milk stage, and 17 to 24 August at the soft dough stage.

At maturity (~18% moisture), panicles were handharvested individually on 10 September 2010 and 8 September 2011, placed in brown paper bags (12.5 by  $27 \text{ cm}^2$  [w by l]), air-dried to 12% moisture in the greenhouse and threshed manually. Resulting kernels per panicle were placed in 37-ml plastic Solo cups (T125 0090 Solo Cup Co., Highland Park, IL) for examination. **Large Cage Field Infestation.** Portable field cages (1.8 by 1.8 by 1.8 m<sup>3</sup>) were used over multiple rice plants with 0, 9, or 18 *O. pugnax* per square meter caged over 3.24-m<sup>2</sup> subplots at three infestation timings during 2010 and 2011. Cage frames consisted of 1.27-cm rigid conduit (Coul, US Listed) frames covered with 20-mesh Lumite screen (Lumite, Inc., Alto, GA) with a zippered opening on one side for access.

Treatments were in a  $3 \times 3$  factorial arrangement in a randomized complete block design with four replications. Infestation timing was one factor at three levels (bloom, milk, and soft dough stages), and infestation density was the other factor at three levels (0, 9, or 18 adult *O. pugnax* per square meter). In all, 36 plots were infested per cultivar weekly.

The 2010 Cocodrie trial was affected by herbicide drift from an adjacent soybean field that severely injured the rice plants and could not be used for infestation. Wells was the only cultivar tested in 2010. The 2010 Wells was infested from 3 to 9 August at the bloom stage, from 10 to 16 August at the milk stage, and from 17 to 23 August at the soft dough stage. In 2011, Cocodrie was infested from 27 July to 2 August at the bloom stage, from 3 to 9 August at the milk stage, and from 10 to 16 August at the soft dough stage. In 2011, Wells was infested from 4 to 11 August at the bloom stage, from 12 to 18 August at the milk stage, and from 19 to 25 August at the soft dough stage.

At the end of the season, 50 panicles were randomly harvested from each plot by hand on 10 September 2010 and 8 September 2011, placed in brown paper bags (30 by 43 cm<sup>2</sup> [w by l]), air dried in the greenhouse to 12 % moisture and threshed manually. Resulting kernels were placed in 0.47-liter polypropylene containers (F-K Fabri-Kal, Kalamazoo, MI). A 10 g sub-sample of grain was removed and used for characterization of grain quality.

Characterization of Grain Quality. The weights of filled or partially filled kernels was considered yield per panicle. Quality was defined in three categories: clean, damaged, or blank kernels. First, blank kernels were separated from filled or partially filled kernels manually by pressing individual kernels against the thumb and fore-finger. Filled or partially filled kernels were further separated into damaged and clean kernels. Damaged kernels were identified by placing all of the filled kernels on a 40 by 46 cm<sup>2</sup> light table (PORTA-TRACE, Gagne Associates, and Binghamton, NY) that illuminated the kernels from below with a 30-Watt bulb. The light table was placed under a laboratory hood that provided illumination onto the kernels from the top by four 90.44 cm (36 in) 30-Watt cool white fluorescent bulbs (Philips Lighting Company, Burlington, MA). Each kernel was examined for evidence of discoloration as described by Douglas and Tullis (1950) under the following characteristics: 1) shrunken kernels with a circular lesion that may or may not have been discolored, 2) kernels with partial or whole discoloration, and 3) kernels with linear discoloration. Kernels with these characteristics viewed under light through the hull were opaque and did not permit uniform light penetration through the hull at the location of discoloration. In contrast, clean kernels viewed under the light were translucent and permitted uniform light penetration through the hull. Data were expressed as a percentage based on the total number of kernels per panicle in the sleeve cage study or 10g sample in the large cage study for each category.

**Data Analysis.** Statistical comparisons could not be made between cultivars because cultivars were planted in separate blocks. Additionally, development times were asynchronous between cultivars, resulting in  $\sim 1$ wk difference in infestation timings between cultivars. Data were analyzed by cultivar across years. In the sleeve cage experiment, total kernel weight per panicle was used to assess rough rice yield for each cultivar. Data for clean kernels, kernel damage, or blank kernels were converted into percentages based on the respective total number of kernels per panicle. The analysis included year, infestation timing, infestation density, and their interactions as fixed effects in the model. Replication nested in cultivar and replications by infestation timing nested in cultivar were included in the model as random effects. In the large cage experiment, the total kernel weight per 50-panicle sample was used to assess rough rice yield. Data for clean kernels, damaged kernels or blank kernels were converted to percentages based on the total number of kernels in a 10g sample. Year, infestation timing, infestation density, and their interactions were included as fixed effects in the model. Because Cocodrie was used for 1 yr, test (cultivar within a year), replication nested in test, and replication by infestation timing nested in test were included in the model as random effects. Data from the sleeve and large cage experiments were analyzed separately with analysis of variance (PROC MIXED, Littell et al. 1996). Means and SEs were calculated with LSMEANS and separated according to Fisher's protected least significance difference ( $\alpha = 0.05$ ).

## Results

Sleeve Cage Infestation. Yield. The analysis of year by O. pugnax infestation timing by infestation density interaction on total kernel weight per panicle was significant (F = 2.88; df = 10, 623; P < 0.01). Therefore, data were analyzed by year for each cultivar. There was no significant interaction between O. pugnax infestation timing and infestation density for Wells in 2010 (F = 2.03; df = 4, 169; P = 0.09), Cocodrie in 2010 (F = 0.55; df = 4, 114; P = 0.58), or Cocodrie in 2011 (F = 1.39; df = 4, 170; P = 0.24; Table 1). The main effect of O. pugnax infestation timing on total kernel weight was significant for Wells in 2010 (F = 18.19; df = 2, 169; P < 0.01), for Cocodrie in 2010 (F = 4.13; df = 1, 114; P = 0.04), and for Cocodrie in 2011 (F = 4.89; df = 2, 170; P < 0.01; Table 1). Total kernel weight was significantly reduced for infestations during the bloom and soft dough stages compared with infestations during the milk stage in Wells 2010. For Cocodrie, in 2010, total kernel weight was significantly reduced for infestations at the milk stage compared with the soft dough stage. Infestations at the bloom stage were not included in the analysis for Cocodrie in

2010 because of herbicide injury. For Cocodrie, in 2011, total kernel weight was significantly reduced for infestations at the bloom and milk stages compared with infestations at the soft dough stage.

There was a significant effect of  $\overline{O}$ . *pugnax* infestation density on total kernel weight per panicle for Wells in 2010 (F = 49.28; df = 2, 169; P < 0.01), Cocodrie in 2010 (F = 21.41; df = 2, 114; P < 0.01), and Cocodrie in 2011 (F = 23.99; df = 2, 170; P < 0.01; Table 1). In all three experiments, total kernel weight was significantly reduced as *O. pugnax* infestation density increased.

For Wells in 2011, there was a significant (F = 5.32; df = 4, 170; P < 0.01) interaction between infestation timing and infestation density on total kernel weight per panicle (Table 1). Total kernel weight was greater for the uninfested panicles compared with panicles infested with one or two *O. pugnax* at the bloom and soft dough stages. In contrast, total kernel weight for the uninfested panicles was not significantly different panicles infested with one *O. pugnax* at the milk stage. In general, the greatest reductions in total kernel

weights were for *O. pugnax* infestations during the bloom stage compared with the milk and soft dough stages (Table 1).

Grain quality (Cocodrie). There was no significant interaction between infestation timing and infestation density for percentage of clean kernels (F = 1.01;df = 2, 290; P = 0.40), percentage of damaged kernels (F = 1.25; df = 4, 290; P = 0.29), or percentage of blank kernels (F = 0.48; df = 4, 290; P = 0.75) per panicle. The main effect of O. pugnax infestation timing was significant for percentage of clean kernels ( $F = \overline{11.98}$ ; df = 2, 290; P < 0.01), percentage of damaged kernels (F = 3.49; df = 2, 290; P < 0.03), and percentage of blank kernels (F = 5.60; df = 2, 290; P < 0.01). The percentage of clean kernels was significantly reduced for infestations at the milk stage compared with infestations at the bloom and soft dough stages (Table 2). The percentage of damaged kernels and blank kernels was significantly greater for infestations at the milk stage compared with infestations at the bloom stage. Additionally, the percentage of blank kernels was

Table 1. Impact of *Oebalus pugnax* infestation density and infestation timing on mean (SEM) total rough rice yield (g per panicle) from sleeve cage infestations conducted in Stoneville, MS in 2010 and 2011

Test <sup>a</sup>	Infestation density <sup><math>b</math></sup>	Bloom	Milk	Soft dough	Mean
Infestation timing					
Wells' 2010	Uninfested	3.2(0.20)	3.5(0.19)	3.0(0.19)	3.2 (0.11)A
	1	1.7(0.12)	2.8(0.19)	2.2(0.16)	2.2 (0.11)B
	2	1.5(0.14)	2.5(0.12)	1.9(0.14)	2.0 (0.09)C
	Mean	2.2 (0.14)b	2.9 (0.10)a	2.4 (0.10)b	
'Cocodrie' 2010	Uninfested	_	2.3(0.10)	2.5(0.13)	2.4 (0.09)A
	1	_	1.9(0.11)	2.2(0.15)	2.1 (0.11)B
	2	_	1.6(0.12)	1.6(0.11)	1.6 (0.08)C
	Mean	_	1.9(0.07)b	2.1 (0.09)a	. ,
'Wells' 2011	Uninfested	3.5 (0.19)a	3.3 (0.23)ab	3.8 (0.21)a	3.6(0.12)
	1	1.9 (0.19)d	2.9 (0.13)bc	2.8 (0.14)c	2.5(0.10)
	2	1.4 (0.15)d	2.8(0.19)c	2.8 (0.22)c	2.3(0.14)
	Mean	2.3(0.16)	3.0 (0.11)	3.1 (0.13)	. ,
'Cocodrie' 2011	Uninfested	2.8(0.18)	3.1 (0.17)	3.2(0.16)	3.0 (0.10)A
	1	2.3(0.15)	2.0(0.22)	2.6(0.14)	2.3 (0.10)B
	2	2.0(0.21)	1.7(0.17)	2.4(0.19)	2.0 (0.11)C
	Mean	2.4 (0.11)b	2.3 (0.13)b	2.7 (0.10)a	

Means followed by a similar uppercase letter or lowercase letter are not significantly different ( $\alpha = 0.05$ ).

<sup>a</sup>Each test represents a cultivar within a year.

<sup>b</sup>Number of O. pugnax infested per panicle in sleeve cages.

Table 2. Impact of *Oebalus pugnax* infestation density and infestation timing on mean (SEM) percentage of clean, damaged, and blank kernels in 'Cocodric' from sleeve cage infestations conducted in Stoneville, MS in 2010 and 2011

Category	Infestation density <sup>a</sup>	Bloom	Milk	Soft dough	Mean
Infestation timi	ng				
Clean	Uninfested	72.0 (3.10)	64.9 (2.75)	67.9 (2.28)	68.3 (0.97)A
	1	60.6 (2.83)	42.9 (3.17)	54.3 (2.18)	52.6 (1.30)B
	2	44.7 (4.93)	31.5(4.04)	43.1 (2.70)	39.8 (1.42)C
	Mean	59.1 (2.33)b	46.4 (1.65)a	55.1 (1.65)b	
Damaged	Uninfested	8.5 (1.61)	7.7 (1.08)	7.6 (0.55)	7.9 (0.35)C
	1	9.1 (1.25)	14.5(1.88)	10.8 (0.93)	11.5 (0.52)B
	2	11.0(1.65)	17.8 (1.65)	14.7 (2.69)	14.5(0.76)A
	Mean	9.6 (0.47)b	13.3 (0.61)a	11.0 (0.60)ab	
Blank	Uninfested	19.5(2.64)	27.4 (2.83)	24.5(2.15)	23.8(1.53)C
	1	30.3 (3.21)	42.6 (3.98)	35.0 (2.26	35.9 (1.92)B
	2	44.2 (4.65)	50.7 (4.29)	42.3 (2.55)	45.7 (2.20)A
	Mean	31.3 (2.43)b	40.2 (2.28)a	33.9 (1.49)b	

Means followed by a similar uppercase letter or lowercase letter are not significantly different ( $\alpha = 0.05$ ).

<sup>a</sup>Number of O. pugnax infested per panicle in sleeve cages.

significantly greater for infestations at the milk stage compared with infestations at the soft dough stage.

There was a significant main effect of *O. pugnax* infestation density on percentage of clean kernels (F = 56.27; df = 2, 290; P < 0.01), percentage of damaged kernels (F = 10.38; df = 2, 290; P < 0.01), and percentage of blank kernels (F = 30.80; df = 2, 290; P < 0.01). *O. pugnax* infestation reduced the percentage of clean kernels, but increased percentage of damaged kernels and blank kernels compared with the uninfested panicles (Table 2). Rice panicles infested with one *O. pugnax* had a greater percentage of clean kernels and blank kernels of damaged kernels and blank kernels compared with panicles infested with two *O. pugnax* (Table 2).

Grain quality (Wells). There was a significant infestation timing by infestation density interaction for the percentage of clean kernels (F = 10.61; df = 4, 348; P < 0.01), percentage of damaged kernels (F = 7.26; df = 4, 348; P < 0.01), and percentage of blank kernels (F = 17.33; df = 4, 348; P < 0.01) per panicle. Significant differences were observed among infestation timings for the percentage clean, damaged, and blank kernels in the caged uninfested panicles, suggesting that the sleeve cages had an impact on those factors (Table 3). Regardless, the percentage of clean kernels decreased as infestation density increased at all infestation timings. Also, the percentage of clean kernels was lower for infestations of one or two O. pugnax at the bloom stage compared with infestations of one or two O. pugnax at the milk and soft dough stages. The percentage of damaged kernels was significantly greater for infestations of one or two O. pugnax at the milk and soft dough stages compared with infestations of one or two O. pugnax at the bloom stage. Additionally, the percentage of damaged kernels was greater at the milk stage compared with the soft dough stage when two O. pugnax were infested per panicle, but now when one O. pugnax was infested per panicle. The percentage of blank kernels was greater at the bloom stage compared with the milk and soft dough stages at all infestation densities. Additionally, the percentage of blank kernels was significantly greater for one or two

*O. pugnax* per panicle compared with the uninfested panicles at all growth stages except one *O. pugnax* per panicle at the milk stage, which was not different than the uninfested panicles at the milk stage.

Large Cage Field Infestation. Yield. There was no significant test (cultivar  $\times$  year) by infestation timing by infestation density interaction for rough rice yield per 50 panicle sample (F = 0.36; df = 8, 81; P = 0.99), so the data were pooled across all tests. There was not a significant O. pugnax infestation timing by infestation density interaction for rough rice yield per 50 panicle sample (F = 0.61; df = 4, 97; P = 0.65; Table 4). The main effect of O. pugnax infestation timing on rough rice yield per 50 panicle sample was significant (F = 18.7; df = 2, 97; P < 0.01; Table 4). Rough rice yields per 50 panicle sample averaged (standard error of the mean [SEM]) 133.7 (7.0) g, 136.3 (7.0) g, and 150.1 (7.0) g for O. pugnax infestations at bloom, milk, and soft dough stages, respectively (Table 4). Infestations of O. pugnax at the bloom and milk stages resulted in lower rough rice yields compared with infestations at the soft dough stage. The main effect of O. pugnax infestation density on rough rice yield per 50 panicle sample also was significant (F = 43.78; df = 2, 97; P < 0.01). Rough rice yields per 50 panicle sample averaged (SEM) 153.5 (7.0) g, 139.8 (7.0) g, and 126.7 (7.0) g for the uninfested, 9 and 18 O. pugnax per square meter, respectively (Table 4). Both levels of O. pugnax infestation density significantly

Table 4. Impact of *Oebalus pugnax* infestation density and infestation timing on mean (SEM) weights (g) of rough rice per 50 panicle sample in large cage experiments averaged across 'Wells' and 'Cocodrie' at Stoneville, MS in 2010 and 2011

Infestation density <sup>a</sup>	Bloom	Milk	Soft dough	Mean
Infestation timing Uninfested	146.8 (3.15)	153.2 (4.41)	160.6 (3.62)	153.5 (2.3)A
9 18	$133.3 (6.17) \\ 120.9 (5.62)$	134.7 (5.45) 121.9 (4.94)	151.4 (3.03) 138.1 (3.02)	139.8 (3.2)B 126.7 (3.0)C
Mean	133.7 (3.4)b	136.3 (3.6)b	150.1 (2.4)a	

Means followed by a similar uppercase letter or lowercase letter are not significantly different ( $\alpha = 0.05$ ).

<sup>*a*</sup>Number of *O*. pugnax infested per m<sup>2</sup> in 1.8 by 1.8 m field cages.

Table 3. Impact of *Oebalus pugnax* infestation density and infestation timing on mean (SEM) percentage of clean, damaged, and blank kernels in 'Wells' from sleeve cage infestations conducted in Stoneville, MS in 2010 and 2011

Category	Infestation density <sup>a</sup>	Bloom	Milk	Soft dough	Mean
Infestation timir	ıg				
Clean	Uninfested	68.3 (2.52)b	71.9 (1.56)ab	77.0 (1.77)a	72.4 (1.18)
	1	35.8 (2.64)f	59.9 (2.13)cd	65.7 (2.82)bc	53.8(1.88)
	2	23.4 (2.39)g	47.1 (2.25)e	57.4 (1.93)d	42.6 (1.81)
	Mean	42.5 (1.31)	59.6 (1.30)	66.7 (1.30)	
Damaged	Uninfested	2.0 (0.32)e	5.2 (0.50)d	4.2 (0.75)de	3.8(0.34)
	1	5.9 (0.87)d	12.1 (0.76)bc	9.8 (1.04)c	9.2(0.56)
	2	5.8 (0.99)d	18.6 (1.32)a	13.2 (1.18)b	12.5(0.83)
	Mean	4.6 (0.53)	12.0 (0.52)	9.1 (0.53)	
Blank	Uninfested	29.7 (2.49)cd	22.8 (1.54)ef	18.8 (1.39)f	23.8(1.14)
	1	58.3 (2.65)b	28.1 (1.98)de	24.5 (2.31)de	36.9 (1.92)
	2	70.8 (2.87)a	34.3 (2.31)c	29.4 (1.58)cd	44.8(2.47)
	Mean	52.9 (2.21)	28.4 (1.20)	24.2 (1.10)	

Means followed by a similar uppercase letter or lowercase letter are not significantly different ( $\alpha = 0.05$ ). "Number of O. pugnax infested per panicle in sleeve cages. reduced rough rice yields compared with the uninfested. Additionally, rough rice yields were significantly lower where *O. pugnax* were infested at 18 per square meter compared with 9 per square meter.

Grain Quality. There was no significant test (cultivar × year) by infestation time by infestation density interaction for percentage of clean kernels (F = 0.96; df = 8, 81; P = 0.47), percentage of damaged kernels (F = 0.87; df = 8, 81; P = 0.55), or percentage of blank kernels (F = 1.02; df = 8, 81; P = 0.43), so data were pooled across all tests. There was no significant O. pugnax infestation timing by infestation density interaction for percentage of clean kernels (F = 0.37; df = 4, 81; P = 0.83), percentage of damaged kernels (F = 0.25; df = 4, 81; P = 0.91), or percentage of blank kernels (F = 0.35; df = 4, 81; P = 0.84).

The main effect of O. pugnax infestation density was significant for percentage of clean kernels (F = 69.68; df = 2, 81; P < 0.01), percentage of damaged kernels (F = 21.20; df = 2, 81; P < 0.01), and percentage of blank kernels (F = 48.80; df = 2, 81; P < 0.01). The percentage of clean kernels was greater for panicles from the uninfested cages compared to the infested cages when averaged across infestation timings (Table 5). Cages infested with 18 O. pugnax per square meter resulted in a lower percentage of clean kernels compared with cages infested with 9 O. pugnax per square meter. The percentage of damaged kernels was greater when panicles were infested with O. pugnax compared with the uninfested control, but there was no difference between panicles from cages infested with 9 or 18 O. pugnax per square meter. The percentage of blank kernels increased significantly as O. pugnax infestation density increased (Table 5). Panicles from cages infested with 18 O. pugnax per square meter had a significantly greater percentage of blank kernels compared with panicles from all other infestation densities. Additionally, panicles from cages infested with 9 O. pugnax per square meter had a significantly greater percentage of blank kernels compared to panicles from the uninfested cages.

The main effect of *O. pugnax* infestation timing was not significant for percentage of damaged kernels (F=2.48; df=2, 81; P=0.09), but it was significant for percentage of clean kernels (F=7.90; df=2, 81; P=0.01), and percentage of blank kernels (F=6.20; df=2, 81; P=0.01; Table 5). The percentage of clean kernels was significantly reduced for infestations during the bloom stage compared with infestations during the milk and soft dough stages of panicle development (Table 5). The percentage of blank kernels was significantly greater for infestations during the bloom and milk stages compared with the soft dough stage of panicle development (Table 5).

#### Discussion

Knowledge of the growth stages of rice is important for managing the crop. After planting, crop growth stages must be well-timed in relation to water management, application of chemical inputs, and cultural practices, including insect control, to achieve maximum yields. Insect pests constitute a major threat to rice yields (Bowling 1967) and different insect species attack the rice plant at different growth stages (Dale 1994, Mackill and McKenzie 2003). O. pugnax is the single most widespread pest of rice during the reproductive stages and must be closely monitored during the heading and grain filling stages of rice development. The heading stage is identified when portions of a panicle start to emerge from the end of the rice stem. During the heading stage, rice maturity can be further characterized as either flowering stage or grain filling stage based on panicle development. The grain filling stage is further divided into milk, soft dough, hard dough, and physiological maturity of panicle development. Percentages are used to describe stage of panicle development, and 50% panicle development refers to when 50% of rice tillers or panicles have attained a particular developmental stage such as headed, bloom, milk, dough, or maturity.

Counce et al. (2000) reported 10 stages of panicle development based on discrete morphological criteria to include: initiation of the panicle (R0), differentiation of the panicle (R1), formation of the flag leaf collar (R2), exertion of the panicle (heading; R3), flowering

Table 5. Impact of *Oebalus pugnax* infestation density and infestation timing on mean (SEM) percentage of clean, damaged, and blank kernels averaged across 'Wells' and 'Cocodrie' from large cage infestation experiments conducted in Stoneville, MS in 2010 and 2011

Category	Infestation density <sup>a</sup>	Bloom	Milk	Soft dough	Mean
Infestation timin	g				
Clean	Uninfested	75.1 (1.74)	75.4 (2.27)	78.0 (1.66)	76.2 (1.09)A
	9	66.6 (1.93)	68.4 (2.49)	71.9 (1.92)	69.0 (1.25)B
	18	58.4 (3.09)	60.5 (2.22)	64.9 (1.86)	61.3 (1.52)C
	Mean	66.7 (1.74)b	68.1 (1.73)a	71.6 (1.36)a	
Damaged	Uninfested	3.8(0.54)	4.4 (0.63)	3.6 (0.67)	3.9 (0.35)B
	9	5.3 (0.77)	5.6 (0.68)	4.9(0.76)	5.3(0.41)A
	18	5.6(0.61)	5.8 (0.66)	5.6 (0.80)	5.6 (0.39)A
	Mean	4.9 (0.39)ab	5.3 (0.38)a	4.7(0.44)	
Blank	Uninfested	21.1 (1.74)	20.2 (1.78)	18.4 (1.55	19.9 (0.95)C
	9	28.1 (1.93)	26.0 (2.10)	23.2 (1.78)	25.8 (1.06)B
	18	36.0 (3.09)	33.6 (2.64)	29.5 (1.55)	33.1 (1.42)A
	Mean	28.4(1.54)a	26.6 (1.55)a	23 7 (1 19)h	

Means followed by a similar uppercase letter or lowercase letter are not significantly different ( $\alpha = 0.05$ ).

<sup>*a*</sup>Number of *O. pugnax* infested per  $m^2$  in 1.8 by 1.8 m field cages.

or blooming (R4), expansion of grain length and width (R5), expansion of grain depth (R6), dry down of grain (R7), at least a single grain on a panicle matured (R8), and panicle completely matured (R9). The bloom stage correlates with R4, sometimes referred to as anthesis or the flowering stage, and is the beginning of grain formation. The milk stage (R5) is noted when milk-like starch accumulation begins to form the grain in the kernel. The dough stage is separated into soft and hard dough stages. At the soft dough stage (R6), the accumulated starch that forms the grain in the kernel becomes firm, but soft. During the hard dough stage, starch accumulation for the grain remains firm but becomes more brittle than during the soft dough stage.

Two traditional field cage methods were used concurrently in this study; sleeve cages to keep individual O. pugnax adults over individual rice panicles and large field cages over multiple rice panicles. In this study, damaged kernels included shriveled, partially filled, and discolored (pecky) kernels, which affect grain quality and marketability. An examination of the results across both studies indicated rice is most vulnerable to yield losses during the bloom stage. Decreases in grain quality were more common for infestations during the milk and soft dough stages of panicle development. This agrees with previous research where higher percentages of pecky kernels were observed at the milk stage compared with the soft dough stage (Espino and Way 2008). Similarly, Patel et al. (2006) reported significant percentages of pecky kernels at the late milk and soft dough stages of rice. Although no direct comparisons could be made in the current study, Wells has been reported to have moderate susceptibility to O. pugnax injury (Moldenhauer 2001), while Cocodrie is rated as susceptible to O. pugnax injury (Slaton et al. 2000). However, O. pugnax caused significant grain yield loss and damage across infestation timings in Cocodrie and Wells cultivars in both the sleeve cage and large cage experiments.

Cage size and screen mesh size have been reported to adversely affect plant yield in multiple crops (Walker 1990, Buntin 2001). Cages used for artificial infestations have been reported to reduce solar radiation necessary for photosynthetic activity, air movement, and precipitation that can impact grain development and yield (Litsinger 1991, Woodford 1973, Hand and Keaster 1967). The sleeve cages used in these experiments appeared to impact grain quality differently at different infestation timings based on differences in damaged kernels in the caged uninfested treatment among the different growth stages. The large cages did not appear to have a measurable effect on yields or grain quality in this experiment. Regardless of these known factors, this study demonstrated that the density of O. pugnax infestation and stage of panicle development that an infestation occurs can significantly reduce grain yield and quality.

Rice generally reaches the soft dough stage  $\sim 3$  wk after flowering at traditional seeding rates. Additionally, the introduction of high-value seeds such as Clearfield varieties (Roel et al. 1999) and hybrid rice (Li and

Yuan 2000) makes management of O. pugnax more difficult because of the overall reduction in seeding rates. In particular, hybrids are generally planted at  $\sim$ 24 kg/ha compared with conventional cultivars that are planted at 84 to 112 kg/ha. At very low seeding rates, the production of tillers becomes more important to maximize yields. Because hybrids produce more active tillers than inbred cultivars such as Cocodrie and Wells, the panicle development stages are often more asynchronous within an individual plant and field. As a result, a longer time may be required for the majority of kernels in hybrid rice to reach the soft dough stage. Because of that, the current threshold should be based on the stage of physiological maturity of a certain percentage of the panicles in a field. Depending on the type of conventional cultivar and active tiller density, panicle exertion may take up to 4-5 d to attain 50% bloom, or 7-12 d to attain 50% milk stage (Moldenhauer and Gibbon 2003). Again, depending on the number of active tillers, approximately  $\geq 3 \text{ wk}$  may be needed to attain the 50% soft dough stage after heading. This suggests thresholds should be based on stage of grain development rather than weeks of heading as is currently used in several states (Catchot et al. 2013, Studebaker et al. 2015).

Although grain yield loss may be a major concern to rice producers when measuring the impact of *O. pugnax* in rice, grain quality is equally important to rice producers, rice millers, and consumers because of its impact on marketability. Rice quality is impacted by shriveled grain and kernel discoloration from *O. pugnax* feeding. Grain quality has an indirect impact on producer yield and income. The U.S. rice grading system may not accept milled rice with >0.5% damaged kernels into grade 1 (U.S. Department of Agriculture-Federal Grain Inspection Service [USDA-FGIS] 2009). Therefore, rice consignments with damaged kernels greater than this grade may receive lower premiums, or may be rejected by consumers.

Economic thresholds have been developed to mitigate the damaging effects of O. pugnax in rice. This threshold justified the cost of control measures when five adult O. pugnax per 10 sweeps are collected with a 38-cm-diameter sweep net during the first 2wk of heading rice and ten adult O. pugnax per 10 sweeps at later development stages (Catchot et al. 2013). The current economic threshold used in Mississippi rice production is a modification of one established by McIlveen et al. (1981). The overall impact of O. pugnax in the current study was consistent over all experiments. Because yield and quality losses were comparable between stages in the large field cages, one may argue that the treatment threshold should be constant from bloom to soft dough stages for Mississippi rice. Based on the assumption that 10 sweeps will sample  $2.9 \text{ m}^2$  (1 sweep = 76 cm in length by 38 cm in width), O. pugnax densities used in the large field cage experiment were comparable with densities of four and eight bugs per 10 sweeps, comparable with the thresholds of 5 and 10 O. pugnax per 10 sweeps. The difficulty in maintaining this action threshold is the asynchronous kernel maturity within a panicle which may directly

impact the nature of panicle maturity within a tiller, and the subsequent rice injury from *O. pugnax* feeding.

The feeding activity of *O. pugnax* in rice is a complex process because of the variability in development of rice panicles among tillers, and kernels within panicles. The current experiment showed that the greatest yield loss occurs during the bloom stage, but damage in kernel formation is greatest during the milk and soft dough stages. This probably reflects the variability in the panicle, kernel, or both development and maturity between or within panicles. This study recommends a more aggressive action threshold for *O. pugnax* from bloom through the soft dough stage of panicle development because of the yield losses observed in the large cage experiments where the densities were estimated to be four and eight *O. pugnax* per 10 sweeps.

#### Acknowledgments

We are grateful to the Mississippi Rice Promotion Board, Mississippi State University (MSU), and the Mississippi Agricultural and Forestry Experiment Station (MAFES) for funding this research. We are also thankful to the faculty and staff of Delta Research and Extension Center, MAFES, and MSU for their technical support.

### **References Cited**

- Bowling, C. C. 1963. Cage tests to evaluate stink bug damage in rice. J. Econ. Entomol. 56: 197–200.
- Bowling, C. C. 1967. Insect pests of rice in the United States. pp. 551–570. In. The Major Insect Pests of the Rice Plant. Proceedings of a symposium at the International Rice Research Institute, September 1964. The Johns Hopkins Press, Baltimore, MD.
- Brown, M. W. 2003. Characterization of stink bug (Heteroptera: Pentatomidae) damage to mid- and late-season apples. J. Agric. Urban Entomol. 20: 193–202.
- Buntin, G. D. 2001. Techniques for evaluating yield loss from insects, pp. 23–41. In R.K.D. Petersen and L. G. Higley (eds.), Biotic Stress and Yield Loss. CRS Press, New York. NY.
- Catchot, A. L., B. Adams, C. Allen, J. Bibb, D. Cook, D. Dodds, J. Gore, R. Jackson, B. Von Kanel, E. Larson, et al. 2013. 2013 insect control guide for agronomic crops. Mississippi State University Extension Service Publication 2471, 99 pp. Mississippi State, MS.
- Chaudhary, R. C., J. S. Nanda, and D. V. Tran. 2002. Guidelines for identification of field constraints to rice production. Food and Agriculture Organization of the United Nations, Rome.
- Counce, P. A., T. C. Keisling, and A. J. Mitchell. 2000. A uniform, objective, and adaptive system for expressing rice development. Crop Sci. 40: 436–443.
- Dale, D. 1994. Insect Pest of the Rice Plant Their Biology and Ecology, p. 363–485. In E. A. Heinrichs (ed.), Biology and management of rice insects. Wily Eastern Ltd., Delhi, India.
- De Datta, S. K. 1987. Principles and Practices of Rice Production. The International Rice Research Institute. Los Baňos, the Philippines. Robert E. Krieger Publishing Company. Malabar, FL.

- Douglas, W. A., and J. W. Ingram. 1942. Rice-field insects. USDA Circular No. 632.
- Douglas, W. A., and E. L. Tullis. 1950. Insects and fungi as causes of pecky rice. USDA. Tech. Bull. 1015: 1–20.
- Espino, L., and M. O. Way. 2008. Attractiveness of stages of rice panicle development to *Oebalus pugnax* (Hemiptera: Pentatomidae). J. Econ. Entomol. 101: 1233–1237.
- Espino, L., M. O. Way, and J. K. Olson. 2007. Most susceptible stage of rice panicle development to *Oebalus pugnax* (Hemiptera: Pentatomidae). J. Econ. Entomol. 100: 1282–1290.
- Gianessi, L. 2009. The benefits of insecticides use: Rice. CropLife Foundation. Crop Protection Research Institute. Washington DC. (www.croplifefoundation.org)
- Hand, L. F., and A. J. Keaster. 1967. The environment of an insect field cage. J. Econ. Entomol. 60: 910–915.
- Ingram, J. W. 1927. Insects injurious to the rice crop. Farmer's Bulletin 1543.
- Li, J., and L. P. Yuan. 2000. Hybrid rice. Genetics, breeding and seed production. Plant Breed. Rev. 17: 15–158.
- Litsinger, J. A. 1991. Crop loss assessment in rice, pp. 1–65. In E. A. Heinrichs and T. A. Miller (eds.), Rice insects: Management strategies. Springer Series in Experimental Entomology, New York, NY.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models, p. 633. SAS Institute Inc., Cary, NC.
- Mackill, D. J., and K. S. McKenzie. 2003. Origin and Characteristics of U. S. Rice Cultivars. *In C. W. Smith and R. H. Dilday* (eds.), RICE: Origin, History, Technology, and Production. John Wiley and Sons, Inc.
- McIlveen, G., C. C. Bowling, and B. M. Drees. 1981. Rice insect control. Entomology notes. Texas Agricultural Extension Service 22: 1.
- McPherson, J. E., and R. M. McPherson. 2000. Stink bugs of economic importance in North America North of Mexico. CRC Press, Boca Raton, FL.
- Miller, T., J. Street, N. Buehring, D. Kanter, T. Walker, J. Bond, M. Silva, L. Pringle, J. Thomas, J. Damicone, et al. 2008. Mississippi's rice grower's guide. Mississippi State University Extension Service. Mississippi State University. Publication No. 2255.
- Moldenhauer, K. 2001. United States Patent. Patent No. U. S. 6,281,416 B1.
- Moldenhauer, K., and J. H. Gibbons. 2003. Rice morphology and development. *In* C. W. Smith and R. H. Dilday (eds.), Rice: Origin, History, Technology, and Production. Wiley, NJ.
- Odglen, G. E., and L. O. Warren. 1962. The rice stink bug, *Oebalus pugnax* (F), in Arkansas, pp. 1–23. Arkansas Agricultural Experimental Station Report Series. University of Arkansas, Fayetteville, AR.
- Patel, D. T., M. J. Stout, and J. R. Fuxa. 2006. Effects of rice panicle age on quantitative and qualitative injury by the rice stink bug (Hemiptera: Pentatomidae). Fla. Entomol. 89: 321–327.
- Petersen, R.K.D., and L. G. Higley. 2001. Biotic Stress and Yield Loss. CRS Press, New York, NY.
- Riley, C. V. 1882. Oebalus pugnax. "Entomologist's Report." United States Department of Agriculture Annual Report, page 138.
- Roel, A., J. L. Heilman, and G. N. McCauley. 1999. Water use and plant response in two rice irrigation methods. Agric. Water Manag. 39: 35–46.
- Slaton, N., K. Moldenhauer, C. Wilson, R. Cartwright, J. Gibbons, B. Koen, R. Norman, J. Bernhardt, F. Lee, and J. Robinson. 2000. Rice Information No. 147. Cooperative Extension Service. University of Arkansas Division of

Agriculture, U. S. Department of Agriculture and County Governments Cooperating.

- Snipes, C. E., S. P. Nichols, D. H. Poston, T. W. Walker, L. P. Evans, and H. R. Robinson. 2005. Current agricultural practices of the Mississippi Delta. Mississippi Agricultural and Forestry Experiment Station (MAFES). Mississippi State University. Bulletin 1143.
- Studebaker, G., J. Davis, J. D. Hopkins, D. R. Johnson, K. Loftin, G. Lorenz, N. Seiter, P. Spradley, J. Zawisłak, D. T. Johnson, et al. 2015. 2015 Insecticide Recommendations for Arkansas. University of Arkansas Division of Agriculture Publication MP144, 298 pp. Fayetteville, AR.
- Swanson, M. C., and L. D. Newsom. 1962. Effects of infestation by the rice stink bug, *Oebalus pugnax*, on yield and quality in rice. J. Econ. Entomol. 55: 877–879.

- **USDA-FGIS. 2009.** United States Standards for Rice. USDA -GIPSA. Federal Grain Inspection Service (FGIS). Washington, DC.
- Walker, P. T. 1990. Quantifying insect populations and crop damage, pp. 55–65. In Crop loss assessment in rice. International Rice Research Institute, Manila, Philippines. Food and Agriculture Organization.
- Way, M. O. 2003. Rice arthropod pests and their management in the United States, pp. 437–456. *In. C. W. Smith and R. H. Dilday (eds.)*, Rice: Origin, History, Technology, and Production. Wiley, NJ.
- Woodford, J.A.T. 1973. The climate within a large aphid-proof field cage. Entomol. Exp. Appl. 16: 313–321.

Received 17 April 2013; accepted 21 April 2015.