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# Comparison of the effects of neonicotinoids and pyrethroids against *Oebalus pugnax* (Hemiptera: Pentatomidae) in rice

Bryce Blackman<sup>1</sup>, Srinivas Lanka<sup>2</sup>, Natalie Hummel<sup>3</sup>, Mo Way<sup>4</sup> and Michael Stout<sup>2\*</sup>

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## Abstract

The rice stink bug, *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae), is an economically important late-season pest of rice in the southern United States. Stink bug feeding results in yield reduction and discounted purchase price due to broken or discolored ("pecky") rice grains. The primary tactic for *O. pugnax* management is the application of insecticides once adults reach an action threshold. Recent surveys show that pyrethroids are preferred by southern U.S. rice farmers over all other insecticides to reduce *O. pugnax* densities. However, preliminary tests in 2009 suggested resistance to pyrethroids may be developing in an *O. pugnax* population in Texas, where applications are more frequent than in other rice-growing areas. This study compared the effects of pyrethroids and neonicotinoids on *O. pugnax* behavior and mortality in the laboratory and in a number of field experiments conducted between 2011 and 2014. Results from these experiments showed that control of *O. pugnax* given by the neonicotinoid, dinotefuran, was similar to that given by pyrethroids in the laboratory and field. Results from small-plot field studies were influenced by movement of adult rice stink bugs from surrounding untreated plots, and the data from commercial-scale trials and from sampling of nymphs in small plots may provide more useful information on the efficacies of insecticides. Two experiments provided limited evidence for longer residual activity of dinotefuran compared to the pyrethroid  $\lambda$ -cyhalothrin, and a laboratory study showed that both insecticides reduced feeding activity of rice stink bugs. Tests also confirmed the increased tolerance of a Texas population of rice stink bugs to  $\lambda$ -cyhalothrin, suggesting the need for insecticides with different modes of action in the *O. pugnax* management program.

Key Words: dinotefuran; lambda-cyhalothrin; rice stink bug

## Resumen

El chinche de arroz, *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae), es una peste de importancia economica al fin de la temporada del arroz en el Sur de los Estados Unidos. El daño causado por este insecto se presenta en forma descolorida ("pecosa") o en grano trizado. Este problema entonces resulta en la reducción de producción y por ende en un descuento en el costo de este arroz. La táctica principal para el manejo de *O. pugnax* es la aplicación de insecticidas en cuanto los adultos hayan alcanzado una umbral de acción. Cuestionarios recientes muestran que los piretroides son preferidos por los agricultores de arroz en el Sur de los Estados Unidos mas que otros insecticidas para reducir densidades *O. pugnax*. Sin embargo, experimentos preeliminares realizados en Texas en el 2009 sugieren un desarrollo en resistencia a piretroides en una población *O. pugnax* en donde aplicaciones son mas frecuentes que en otros sembríos de arroz. Este estudio realizado en algunos experimentos de laboratorio y campo entre los años 2011 y 2014 compara los efectos en el comportamiento y mortalidad de piretroides y neonicotinoides sobre *O. pugnax*. Los resultados de laboratorio y de campo de estos experimentos muestran que el control de *O. pugnax* dados por neonicotinoides, dinotefuran fue parecida a la de los piretroides. Los resultados de sembríos pequeños que fueron influenciados por el movimiento del chinche de arroz adulto fue desde el alrededor de sembríos sin tratamiento, exámenes de escala-comercial, y de experimentos con ninfas en sembríos pequeños. Estos resultados pueden proveer mas información que puede ser usada en cuanto a la eficacia de insecticidas. Dos experimentos proporcionaron evidencia limitada de actividad larga de dinotefuran en comparación con pyrethroid  $\lambda$ -cyhalothrin, y un experimento de laboratorio demostró que los dos insecticidas disminuyeron la actividad de alimentación del chinche de arroz. Exámenes también confirman el incremento en tolerancia de parte de una población de *O. pugnax* de Texas para con  $\lambda$ -cyhalothrin lo cual sugiere la necesidad adicional de insecticidas en el manejo del programa *O. pugnax*.

Palabras Clave: dinotefuran; lambda-cyhalothrin; chinche de arroz

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The rice stink bug (RSB), *Oebalus pugnax* (Fabricius) (Hemiptera: Pentatomidae), is an economically important late-season pest of rice (*Oryza sativa* L.; Poales: Poaceae) in the southern United States (Riley

1882; Ingram 1927; Douglas and Tullis 1950; Lee et al. 1993). Rice stink bug adults emerge from overwintering in the spring, and populations pass through multiple generations on graminaceous weeds before

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moving into rice fields after panicle emergence (Way 2003). Nymphs and adults feed on developing rice grains from anthesis until grain hardening. Feeding increases the incidence of unfilled, broken, and discolored grains known as “pecky” rice in milled grain (Helm 1954; Swanson and Newsom 1962; Bowling 1963; Harper et al. 1993; Tindall et al. 2005; Patel et al. 2006; Espino et al. 2007). Peck in rice samples can result in reduced purchase price and loss of income for producers.

Insecticides labeled for use against rice stink bug before 2013 consisted of pyrethroids, organophosphates, and carbamates. Insecticides in the pyrethroid class have been labeled for rice stink bug management for more than 15 yr (EPA 1997; Schultz 2004; Delta Farm Press 2004). Recent surveys show that  $\lambda$ -cyhalothrin (Karate Zeon®, Syngenta Crop Protection, Greensboro, NC) and z-cypermethrin (Mustang® Maxx, FMC, Research Park Triangle, NC) are the primary products used against rice stink bugs in Louisiana and Texas (Blackman et al. 2014). However, recent acute toxicity assays on rice stink bugs from several states have detected possible resistance to pyrethroids in a south Texas rice stink bug population that typically receives multiple applications of pyrethroid insecticides (Miller et al. 2010b).

The organophosphates, malathion and methyl parathion, are less expensive than pyrethroids and can be applied closer to the time of harvest, factors that have contributed to their continued use against rice stink bugs. However, the Environmental Protection Agency (EPA) has rescinded the labels for methyl parathion products in rice effective on 31 Dec 2013, and the products are no longer available for use in the United States. The continued use of malathion is also in question because it has been shown to be significantly less effective against rice stink bugs than pyrethroids (Johnson et al. 2003; Blackman et al. 2012).

With the removal of methyl parathion, the ineffectiveness of malathion, and indications of increased tolerance of rice stink bugs to pyrethroids, a new class of insecticides is needed to give producers additional options for rice stink bug management and prevent selection for pyrethroid-resistant populations. The EPA issued a full label for the neonicotinoid insecticide dinotefuran (Tenchu® 20SG, Mitsui Chemical Agro, Inc., Tokyo) (Table 1) to be used against rice stink bugs in rice in Louisiana and Texas in 2013, after several years of Section 18 Emergency Exemptions for the insecticide. Neonicotinoids act at nicotinic acetylcholine receptor sites in insects and are especially effective against piercing-sucking insects such as rice stink bugs due to the ability of these insecticides to cross plant membranes and translocate throughout plant tissue where they are readily ingested (Tomazawa & Casida 2005). Neonicotinoids have also been found to be considerably less toxic to *Procambarus* sp. crayfish than pyrethroids or organophosphates when applied to fields managed in the crayfish-rice rotation system common to Louisiana and Texas (Barbee & Stout 2009; Lanka and Stout, unpublished data).

Dinotefuran has been reported to exhibit longer residual effects against rice stink bugs than pyrethroids in studies in Arkansas and Texas (Bernhardt 2009; Way et al. 2009). Our study sought to compare the merits of dinotefuran to those of pyrethroid insecticides. Experiments were conducted to test the hypothesis that dinotefuran has a longer residual effect than  $\lambda$ -cyhalothrin on the mortality of adult rice stink bugs, to compare feeding damage on commercial fields treated with

pyrethroids and neonicotinoids, to determine efficacies and patterns of re-infestation in small plots and commercial fields treated with both insecticides, to compare adult rice stink bug feeding behavior on rice treated with pyrethroids and neonicotinoids, and to develop a baseline  $LC_{50}$  data for pyrethroids on select populations of rice stink bugs in Louisiana and Texas.

## Materials and Methods

### LOCATION AND RICE CULTURE

Small-plot field experiments were conducted at the Louisiana State University Agricultural Center Rice Research Station (RRS) in Crowley, Louisiana in 2011, 2012, and 2013. The soil type at this location was a silt loam (fine, montmoillonitic, thermic, Typic Albaqualf). Plots of rice, 1.5 m  $\times$  5.5 m, were drill-seeded at 67.25 kg/ha and managed following LSU AgCenter recommendations for fertilization and control of weeds and pathogens (Blanche et al. 2009). Rice plots at this location typically become infested by rice stink bugs at levels exceeding current thresholds shortly after heading begins.

### INSECTICIDE EFFICACY TRIALS, 2011-2013

Experiments in 2011 and 2012 compared the effects of pyrethroids, neonicotinoids, and neonicotinoid/pyrethroid combinations on densities of rice stink bugs in small plots. Treatments consisted of an untreated control and 4 insecticides:  $\lambda$ -cyhalothrin,  $\lambda$ -cyhalothrin + thiamethoxam, thiamethoxam, and dinotefuran (Table 1). Treatments were applied to the plots of rice cultivar “Cocodrie”, a widely grown long-grain cultivar. Plots were arranged in a randomized block design with 4 replications. Plots were separated by 1.2 meters on all sides.

In 2013, an experiment similar to the 2011 and 2012 experiments was conducted using the rice cultivar “Cheniere”, another widely grown long grain cultivar. Treatments for the 2013 experiment were the same as those in 2011 and 2012 (Table 1) except for the substitution of a second high rate of  $\lambda$ -cyhalothrin + thiamethoxam (439 mL/ha) in place of thiamethoxam. Treatments were again assigned to plots according to a randomized block design with 4 replications. The arrangement of plots was changed from previous years to improve treatment spacing. The number of rice plots in each block was doubled, but treatments were applied only to every other plot so that treated plots were surrounded on all sides by untreated plots.

Treatments were applied when rice in plots had reached 75 to 100% panicle emergence and stink bug populations exceeded the current action threshold of 3 adults per 10 sweeps. All insecticide solutions were prepared in a laboratory using tap water (pH 7.66) as a carrier and applied between 0730 and 0800. Treatments were applied using a backpack,  $CO_2$ -powered sprayer calibrated to deliver 140.5 L/ha. Plots were sampled at multiple time points after application by making 10 consecutive sweeps across each plot with a 15-inch (38.1 cm) diam sweep net. Numbers of rice stink bug adults and nymphs caught in sweep nets in each plot were recorded in the field separately, but life stages of individual nymphs were not recorded.

**Table 1.** Insecticides and rates used for small plot insecticide trials, 2011-2013. \*Products labeled in Louisiana for use against *O. pugnax* in rice in 2013.

Product	Manufacturer (Location)	Active Ingredient	A.I. Rate
Centric 40 WG	Syngenta (Greensboro, NC)	thiamethoxam	98.1 g/ha
Endigo 2.06 ZC	Syngenta (Greensboro, NC)	$\lambda$ -cyhalothrin + thiamethoxam	90.15 g/ha and 108.18 g/ha
Karate Zeon*	Syngenta (Greensboro, NC)	$\lambda$ -cyhalothrin	44.83 g/ha
Tenchu*	Mitsui Chemicals Agro, Inc. (Tokyo, Japan)	dinotefuran	125.8 g/ha

Repeated measures analyses were conducted in SAS using PROC GLIMMIX to compare the effects of treatments on rice stink bug densities in plots at various time points after application (SAS 2008). The block and treatment x block variables were considered random in the analysis. Means were compared using Protected LSDs. Analyses were conducted separately for nymphs and adults for each year.

## COMPARISON OF RESIDUAL ACTIVITIES

An experiment was conducted in 2011 to test the hypothesis that dinotefuran has longer residual activity than  $\lambda$ -cyhalothrin when both insecticides are applied at label rates. Small plots of rice (cv. 'Cocodrie') were planted and cultured as previously described. At heading, 3 treatments ( $\lambda$ -cyhalothrin, dinotefuran, and an untreated control) were assigned to plots according to a randomized block design with 4 replications. Lambda-cyhalothrin was applied at 44.83 g (AI)/ha and dinotefuran was applied at 126 g (AI)/ha.

Adult rice stink bugs were confined to rice panicles at two time points following insecticide application and their mortality was assessed 48 h later to compare residual activities of dinotefuran and  $\lambda$ -cyhalothrin. Stink bugs were confined to panicles using tulle net cages measuring 34 cm x 10 cm. Adult rice stink bugs were collected with sweep nets from untreated grassy weeds and rice at the RRS. Collected insects were temporarily held in aluminum cages with fresh rice and grassy weed panicles for approximately 30 minutes. They were then held at 4.5 °C for 15 min to reduce mobility and prevent escape during transfer to nylon sleeve cages. Insects with no visible signs of damage were transferred to cages and confined to the top quarter of the cage with twist ties (Sturdy-Twists, Woodstream Corporation, Lititz, Pennsylvania) for ease of transfer to panicles and to prevent escape during sleeve installation. Cages provided adequate room for insects to feed on all areas of the panicle.

Cages with bugs were transported (<5 min) to the field, placed over individual panicles, and secured at the panicle base using twist ties. Four stink bugs were confined to each cage and 3 cages were placed in each plot at 2 h and 144 h after insecticide applications. Furthermore, to ensure that insecticides had been applied effectively, a single sleeve cage with 3 stink bugs was placed over 1 panicle in each plot before spraying, and mortality was recorded 2 h after spraying. For the cages placed at 2 and 144 h, cages were removed to assess mortality after 48 h. Panicles with cages and insects were detached from the plant below the twist tie and brought back to the lab. Total insects alive and dead were assessed within 30 minutes of removal from the field. Insects were considered dead when they were observed motionless for 15 seconds after being prodded with a sharpened pencil.

In 2014, a different approach was used to test the hypothesis that dinotefuran has longer residual activity than Karate. Twelve small rice plots (cv. 'Cheniere'), arranged in a randomized complete block design, were assigned to three treatments:  $\lambda$ -cyhalothrin, dinotefuran, and an untreated control. Lambda-cyhalothrin was applied at 44.83 g (AI)/ha and dinotefuran was applied at 126 g (AI)/ha; plots were treated with a backpack CO<sub>2</sub> sprayer as described above. Approximately 2 h after insecticide applications, 5 panicles were haphazardly removed from the interior of each of the 12 plots and placed in individual plastic boxes (38 x 20 x 15 cm) labeled with the plot number and lined with moistened paper towels. Five field-collected and apparently healthy adult rice stink bugs were placed in each box and numbers of surviving bugs counted after 48 h. Panicles remained in good condition throughout the 48-hour testing period. Five additional panicles were removed from plots 24 h after insecticide applications, and the entire assay was repeated using fresh plastic boxes and insects.

For the 2011 data, proportions of insects surviving in each cage were calculated and proportions analyzed using PROC MIXED in SAS

(SAS 2008). Comparisons of the effects of treatments on the proportion of insects surviving on each day were done by pair-wise *a priori* contrasts. To estimate appropriate degrees of freedom, Satterthwaite's adjustment of degrees of freedom was used in the model statement. For the 2014 experiment, effects of treatments on numbers of surviving bugs were analyzed using PROC MIXED in SAS. Data from the 2 h and 24 h panicle removals were analyzed separately.

## ACUTE TOXICITY BIOASSAYS

Assays were conducted in 2013 to compare the LC<sub>50</sub>s of  $\lambda$ -cyhalothrin for rice stink bugs from 2 populations with differing histories of pyrethroid use. As an initial step, baseline LC<sub>50</sub> and LC<sub>95</sub> values were established using a population of rice stink bugs collected from the RRS. Serial dilution vial bioassays (0-10 µg/mL) were prepared following Miller et al. (2010a), and assays were conducted on groups of stink bugs collected on 3 Aug, 31 Aug, and 1 Sep (total *n* = 990). For each assay, 1 adult insect was placed into each vial and caps were lightly secured on vials to ensure they were not airtight. Insects were assessed for mortality after 4 h exposure. To assess mortality, insects unable to right themselves in vials were placed on a petri dish and observed for 15 s. If they did not right themselves and remain in an upright position within the allotted time, they were considered dead. Data from the 3 collection dates were pooled, and the LC<sub>50</sub> and LC<sub>95</sub> were determined using SAS PROC PROBIT (SAS 2008). For subsequent assays, vials were prepared using the LC<sub>50</sub> and LC<sub>95</sub> values determined from these initial assays. Analysis of subsequent assays was performed by comparing adjusted percent mortality of each population and treatment level to fiducial limits and confidence intervals in baseline assays. Abbot's Formula was used to correct for control mortality in the RRS population.

Subsequent bioassays compared mortalities at the LC<sub>50</sub> and LC<sub>95</sub> concentrations of rice stink bugs collected from the RRS on 26 Sep and from a site in Wharton County, Texas (N 29° 12.498'; W 96° 29.988') on 9 Oct. The former site was an area of relatively light pyrethroid use, whereas the latter was recently suspected of harboring a resistant population of rice stink bugs (Way 2011). Vials were prepared on 25 Sep for both bioassays.

Insects were collected from rice fields and neighboring areas containing headed barnyard grass and broadleaf signal grass. Insects were transferred to aluminum cages (Bioquip #1450B) and then transported to the lab where assays were initiated. Insects used in Louisiana tests were held for 12 h before assays, while insects for Texas assays were held for approximately 1 h. Special attention was given to ensure caged insects were kept out of direct sunlight and had an adequate water source via moistened cotton balls or paper towels. At the RRS, 40 vials for control (no insecticide), LC<sub>50</sub> (0.297 ppm) and LC<sub>95</sub> (9.772 ppm) concentrations were infested with a single rice stink bug adult. In Wharton County, 60 insects were used for the control and LC<sub>50</sub> treatments, and 59 were used for the LC<sub>95</sub> population. Insects at both locations were assessed for mortality as described above after 4 h exposure.

## LABORATORY FEEDING ASSAY

Experiments were conducted in 2011 and 2012 to assess potential sub-lethal effects of  $\lambda$ -cyhalothrin and dinotefuran on adult feeding behavior. Whole rice plants, from untreated plots or plots treated with  $\lambda$ -cyhalothrin and dinotefuran at 44.83 g/ha or 126 g/ha, respectively, were uprooted 2-4 hours post treatment and placed individually in plastic 5-gallon buckets (18.9 L). Buckets containing plants were transported inside an air-conditioned truck cab to a greenhouse on the campus of Louisiana State University, where they were held for 72 h before the start of the experiment. Feeding behavior was monitored in



polystyrene petri dishes (14 cm diameter and 2.2 cm depth, Corning™ New York). Petri dish bottoms contained approximately 0.5 cm layer of 2.0% agar to maintain moist conditions. The distal end of panicles was excised, and the 6 cm cut end containing 10-15 spikelets was inserted into the agar bed. Two of these panicle portions were placed in each petri dish: 1 panicle portion from an untreated plant and 1 panicle portion from a plant treated with either dinotefuran or λ-cyhalothrin. Ten petri dishes for both dinotefuran and λ-cyhalothrin treatments were used to compare stink bug feeding behavior on treated and untreated panicles. A third group of Petri dishes contained 2 spikelet portions from untreated panicles.

Feeding assays were initiated by releasing 1 adult rice stink bug in each petri dish. At several time points after experiment initiation, observations on feeding and non-feeding related behaviors were recorded for 1 minute. The extension of stylets to contact with a grain on a panicle was categorized as feeding-related behavior. All other activities, such as antennal brushing, rubbing of legs, and running and walking toward or away from panicle, were categorized as non-feeding behaviors. Observations of behaviors were made at 8 (2011) or 9 (2012) time points. Three observations were made within 1 h of test initiation and successive observations were made at 3 h intervals thereafter.

The total numbers of occurrences of feeding and non-feeding behaviors in petri dishes at each time point were converted into percent time for each of the 2 behavioral categories. Analysis of variance was conducted on untransformed data using percent time as the dependent variable and insecticide treatment as the independent variable. Post hoc comparisons were done by using Tukey comparisons between each category of behavior in λ-cyhalothrin and dinotefuran treatments and in untreated controls.

DEMONSTRATION TRIAL

Demonstration tests were conducted to compare the efficacies of dinotefuran and registered pyrethroids (λ-cyhalothrin or z-cypermethrin) at commercial rates under commercial growing conditions. Nine commercial field sites were selected in 7 Louisiana parishes in the northern, central, and southwestern rice-growing regions of the state (Table 2). Each field site comprised 2 adjacent fields of similar area, all greater than 1 hectare, which could be treated by aircraft. Adjacent fields had been planted with the same variety and were managed in an identical fashion. One field in each pair was assigned to receive an application of a pyrethroid at the label rate, while the other field in each pair was treated with Tenchu (dinotefuran) (Table 2).

Rice stink bug populations were monitored before and after insecticide treatment by sweeping 10 times in 10 locations in each field, and feeding damage was evaluated by analysis of pecky rice in harvest-

ed rough rice. Sweep net sampling was conducted 24 to 48 h before spraying for each site between the growth stages of anthesis and hard dough. Post-treatment sweep net counts were taken at 48 h and 7 days after treatment to determine rice stink bug densities. The pyrethroid-treated field at the Morehouse Parish 2 site remained above threshold at the 48 hour sampling point and was treated with a second application of pyrethroid at 48 h to reduce infestation levels. This field was not included in the statistical analysis for the 7 day sampling period. Samples of rough rice were collected from all 11 sites at harvest and analyzed by a USDA certified inspector at Louisiana Rice Mill in Crowley, Louisiana, to determine percent pecky rice.

Sweep net sampling data were analyzed using the PROC MIXED procedure in SAS (SAS 2008) with field (location) as a random effect. Because there were no untreated fields (controls) in this demonstration, a formal statistical analysis was not possible. However, rice stink bug population densities in pyrethroid- and dinotefuran-treated fields were compared, and, in a second analysis, rice stink bug densities after treatment were compared with pre-treatment densities separately for Tenchu and pyrethroid-treated fields. LSD was used for mean comparisons. The impact of insecticide treatments on percent peck was analyzed by ANOVA with PROC GLIMMIX in SAS (SAS 2008).

Results

INSECTICIDE EFFICACY TRIALS 2011-2013

In 2011, densities of nymphs (nymphs per 10 sweeps) were significantly affected by insecticide treatment ( $F_{5,15} = 18.75$ ;  $P < .0001$ ). Significantly greater nymph densities were found in control plots than in plots of all other treatments at 1 and 2 days after treatment (DAT) (Fig. 1a). Densities in control plots fell considerably between 2 and 5 DAT, and no significant differences were observed among treatments at 5 DAT. Insecticide treatment did not significantly affect adult densities in plots ( $F_{5,18} = 1.96$ ;  $P = 0.1342$ ). However, a significant treatment x day interaction was observed ( $F_{10,36} = 2.61$ ;  $P = 0.0170$ ). Among adult rice stink bug samples, densities in plots treated with thiamethoxam, λ-cyhalothrin, and a mixture of thiamethoxam and λ-cyhalothrin were lower than densities in controls at 1 DAT (Fig. 1b).

Insecticide treatment also affected nymph densities in plots in 2012 (Fig. 2a,  $F_{5,51} = 23.07$ ;  $P < .0001$ ). All insecticides significantly reduced nymph densities at 1, 3 and 6 DAT (Fig. 2a). All treated plots had nymph densities less than half the mean for untreated plots at each time point. Densities of adults in the 2012 experiment were lower than in 2011, and no significant differences in densities of adults were detected among control and treated plots at any time point (Fig. 2b,  $F_{5,18} = 1.08$ ;  $P = 0.4208$ ).

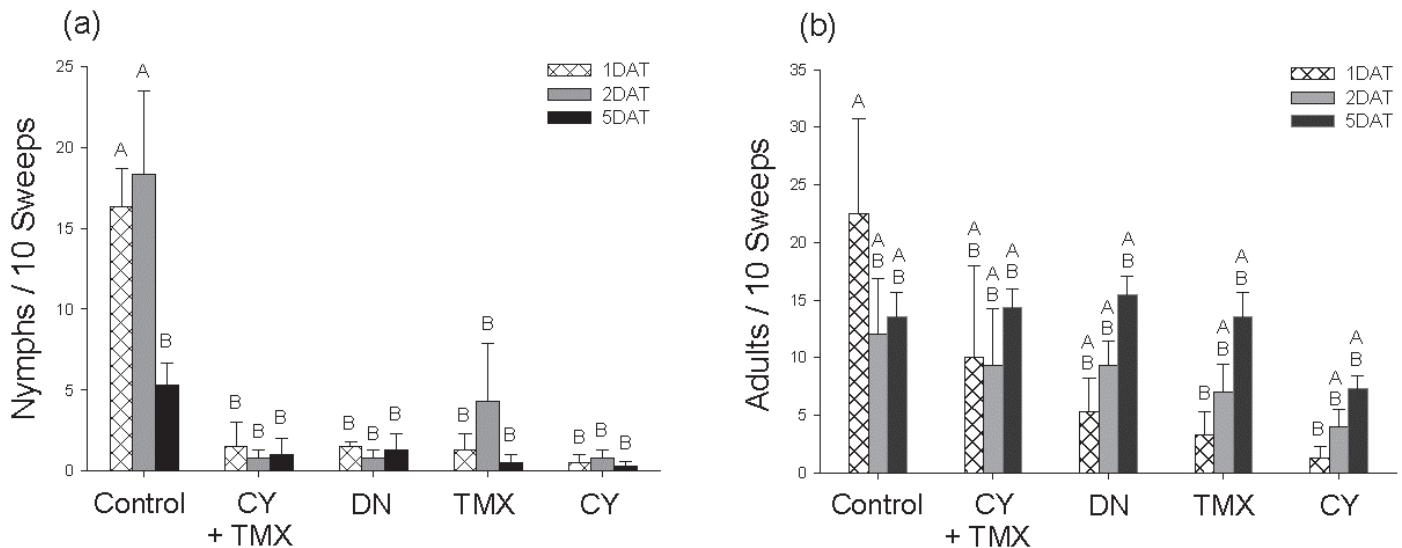
In 2013, a marginally significant difference was observed among treatments for nymph densities (Fig. 3a,  $F_{4,57} = 2.19$ ;  $P = 0.0812$ ). No treatment was significantly different than the control at the  $P = 0.05$  level for any sampling date. At 1 DAT, nymph densities remained below 1 insect per 10 sweeps in all treatments except the untreated control ( $3.00 \pm 1.58$ ) and λ-cyhalothrin ( $1.75 \pm 1.81$ ). Nymph densities remained low at 3 DAT ( $1.00 \pm 0.71$ ). Densities of adults were again low in 2013. As in 2012, no significant differences were observed between treatments in adult densities (Fig. 3b,  $F_{4,12} = 0.55$ ;  $P = 0.6996$ ).

COMPARISON OF RESIDUAL ACTIVITIES

For the 2011 experiment, contrasts showed a significant difference in rice stink bug mortality among treatments immediately after spraying ( $F_{1,7.95} = 9.36$ ;  $P = 0.0157$ ), with approximately 80% mortality in the 2 insecticide treatments but only 10% mortality in controls (Fig. 4). In

Table 2. Demonstration Trial Rice Varieties and Insecticide Rates.

Plot Location	Variety	Tenchu Field Rate
Jeff Davis Parish	XL CL729	112.1 g/ha
Acadia Parish 1	Cheniere	112.1 g/ha
Acadia Parish 2	CL151	112.1 g/ha
Acadia Parish 3	CL151	112.1 g/ha
Acadia Parish 4	CL131	112.1 g/ha
Acadia Parish 5	CL161	112.1 g/ha
Avoyelles Parish	Cheniere	112.1 g/ha
Concordia Parish 1	NA	112.1 g/ha
Concordia Parish 2	NA	112.1 g/ha
Morehouse Parish 1	CL151	112.1 g/ha
Morehouse Parish 2	CL151	112.1 g/ha
Morehouse Parish 3	XL CL729	112.1 g/ha



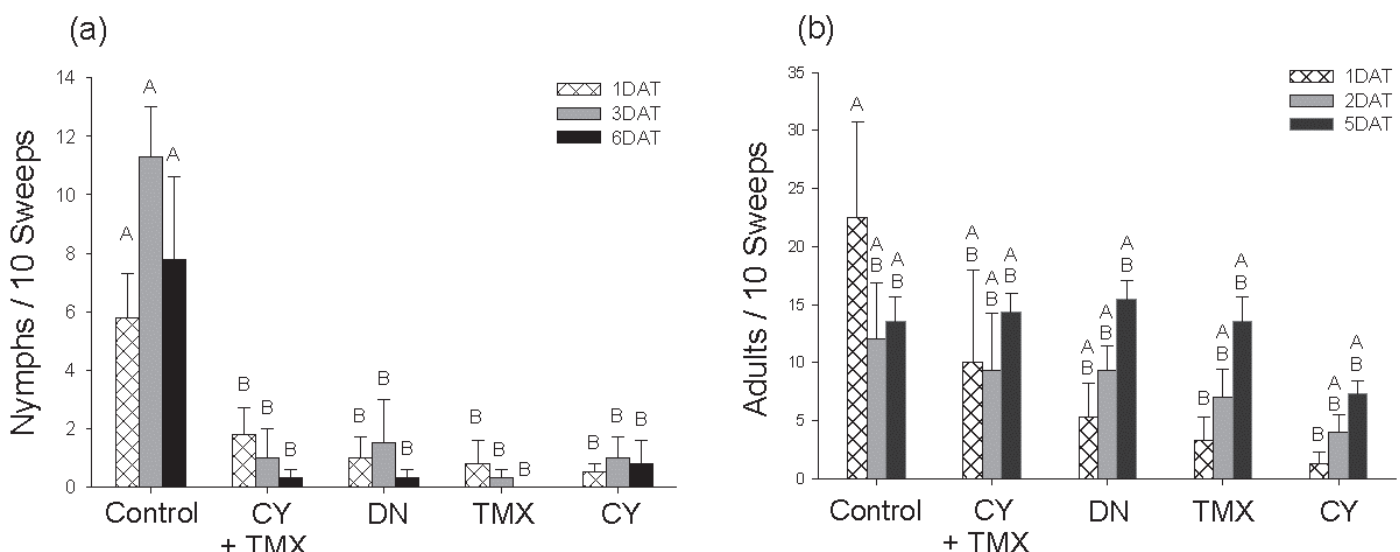
**Fig. 1.** 2011. Mean  $\pm$  SE numbers of rice stink bug nymphs (a) and adults (b) in 10 sweeps in untreated and insecticide-treated rice small-plots. Means accompanied by different letters indicate a significant difference across treatments ( $P < 0.05$ , LSD). (CY =  $\lambda$ -cyhalothrin, TMX = thiamethoxam, DN = dinotefuran).

bugs placed on panicles 2 h after treatment ( $F_{1,30.7} = 15.56$ ;  $P = 0.0004$ ), survival was significantly lower than controls on dinotefuran treated panicles ( $P = 0.0068$ ) but not on panicles treated with  $\lambda$ -cyhalothrin ( $P = 0.2722$ ). No significant differences in survival were observed among treatments at 144 HAT ( $F_{1,24.6} = 0.25$ ;  $P = 0.6188$ ).

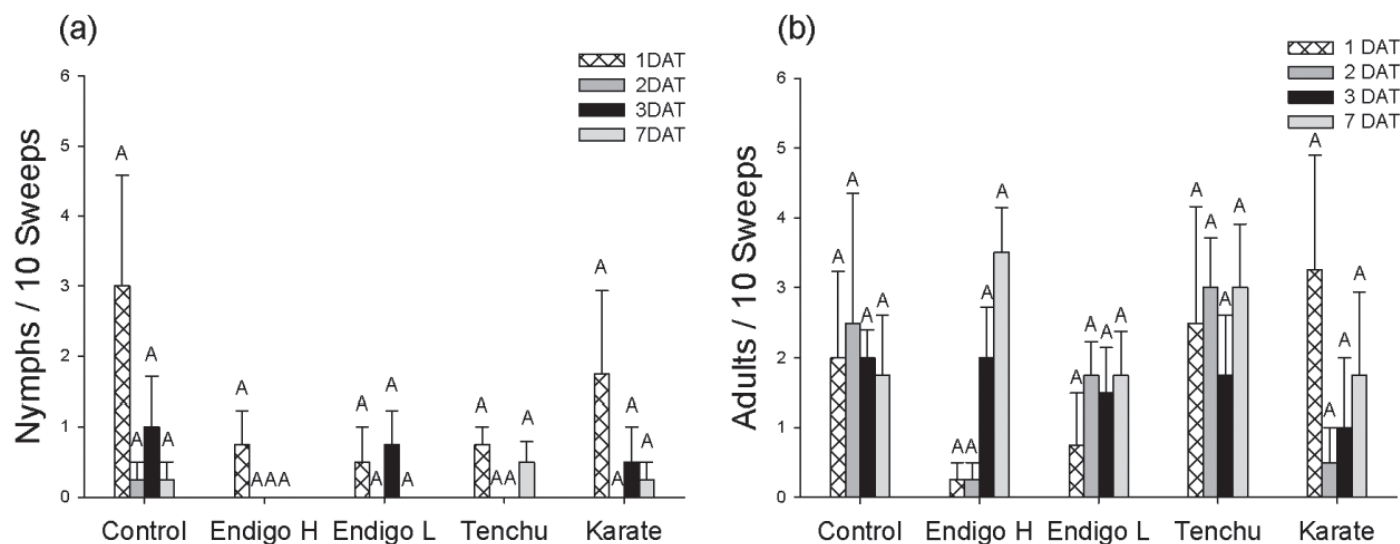
In the 2014 experiment, survival of adult rice stink bugs placed in boxes with panicles collected from plots sprayed with dinotefuran 2 h previously was lower (30%) than survival of bugs placed in boxes with panicles from control plots (95%) ( $F_{2,6} = 8.82$ ;  $P = 0.02$ ). Survival of bugs placed in boxes with panicles from plots treated with  $\lambda$ -cyhalothrin 2 hours previously was intermediate (85%) and did not differ significantly from survival of bugs in the dinotefuran or control treatments. Survival of bugs was uniformly high ( $> 90\%$  on all treatments) when placed in boxes with panicles collected 24 h after treatment and no differences among treatments were found ( $F_{2,6} = 0.3$ ;  $P = 0.75$ ).

#### ACUTE TOXICITY

Serial dilution assays with  $\lambda$ -cyhalothrin to determine baseline  $LC_{50}$  and  $LC_{95}$  values established that mortality of stink bugs collected at the RRS was dose dependent ( $P < 0.001$ ; slope =  $1.941 \pm 0.3376$ ) with an  $LC_{50}$  of 0.2973 ppm (CI: 0.1226-0.6883), an  $LC_{95}$  of 9.7723 ppm (CI: 2.8364-184.2757), and a chi-square value of 33.06 (1.941 df) (Fig. 5). Subsequent comparisons of mortalities of bugs collected from the RRS and Wharton County using vials treated with the  $LC_{50}$  and  $LC_{95}$  concentrations showed a difference between the RRS and Wharton County populations. Mortalities of stink bugs from the RRS population at the  $LC_{50}$  concentration (72%) and the  $LC_{95}$  concentration (100%) were higher than the fiducial limits initially calculated from the baseline assays (35-65% and 85-98% for the  $LC_{50}$  and  $LC_{95}$  concentrations, respectively). In contrast, insects from Wharton County exposed to the same  $LC_{50}$  and  $LC_{95}$  concentrations exhibited only 15% and 66% mortality, respectively,



**Fig. 2.** 2012. Mean  $\pm$  SE numbers of rice stink bug nymphs (a) and adults (b) in 10 sweeps on untreated and insecticide-treated rice small-plots. Means accompanied by different letters indicate a significant difference across treatments ( $P < 0.05$ , LSD). (CY =  $\lambda$ -cyhalothrin, TMX = thiamethoxam, DN = dinotefuran).



**Fig. 3.** 2013. Mean  $\pm$  SE rice stink bug nymphs (a) and adults (b) in 10 sweeps on untreated and insecticide treated rice small-plots. Means accompanied by different letters indicate a significant difference across treatments ( $P < 0.05$ ).

values below the fiducial limits from the initial baseline assay. Thus, the population of rice stink bugs from Wharton County was more tolerant of  $\lambda$ -cyhalothrin than the population of stink bugs from the RRS.

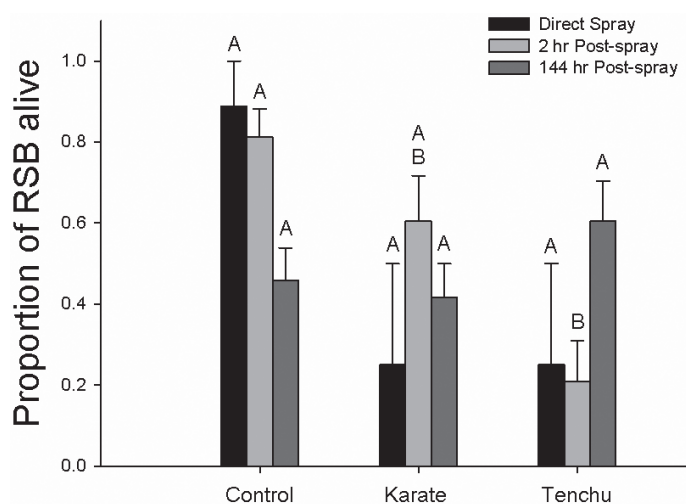
#### LABORATORY FEEDING ASSAYS

No insect mortality was observed in the feeding assays. The percent time spent feeding by rice stink bugs differed with insecticide treatment in both the 2011 ( $F_{2,27} = 5.3$ ;  $P = 0.01$ ) and 2012 ( $F_{2,27} = 6.0$ ;  $P = 0.007$ ) assays (Fig. 6). In 2011, the proportion of time spent feeding by rice stink bugs in the control treatment (in which both panicles in dishes were untreated) was significantly higher ( $P = 0.01$ ) than in the dinotefuran treatment (1 untreated panicle, 1 dinotefuran panicle) but was not significantly higher than the feeding time in the  $\lambda$ -cyhalothrin treatment (1 untreated panicle, 1  $\lambda$ -cyhalothrin panicle)

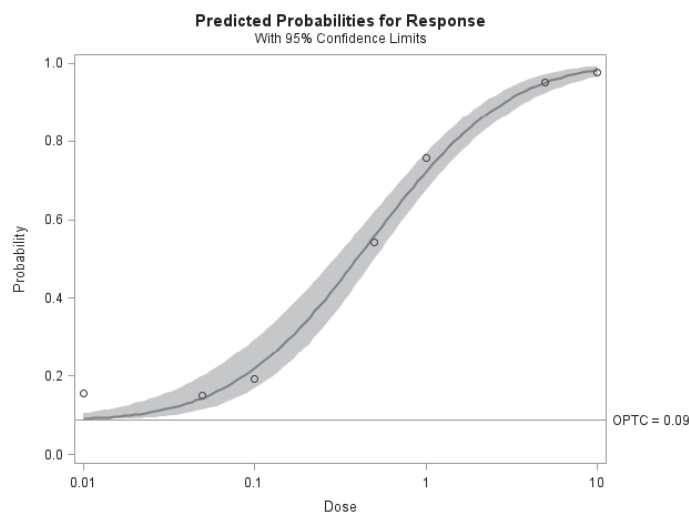
( $P = 0.1$ ). No significant difference was found between  $\lambda$ -cyhalothrin and dinotefuran treatments. Consistent with results from 2011, stink bugs in control dishes (2 untreated panicles) in the 2012 experiment spent a significantly greater proportion of time feeding than did bugs in the dinotefuran ( $P = 0.009$ ) or  $\lambda$ -cyhalothrin ( $P = 0.03$ ) treatments. Once again, no significant difference was found between  $\lambda$ -cyhalothrin and dinotefuran treatments in 2012.

#### DEMONSTRATION TRIAL

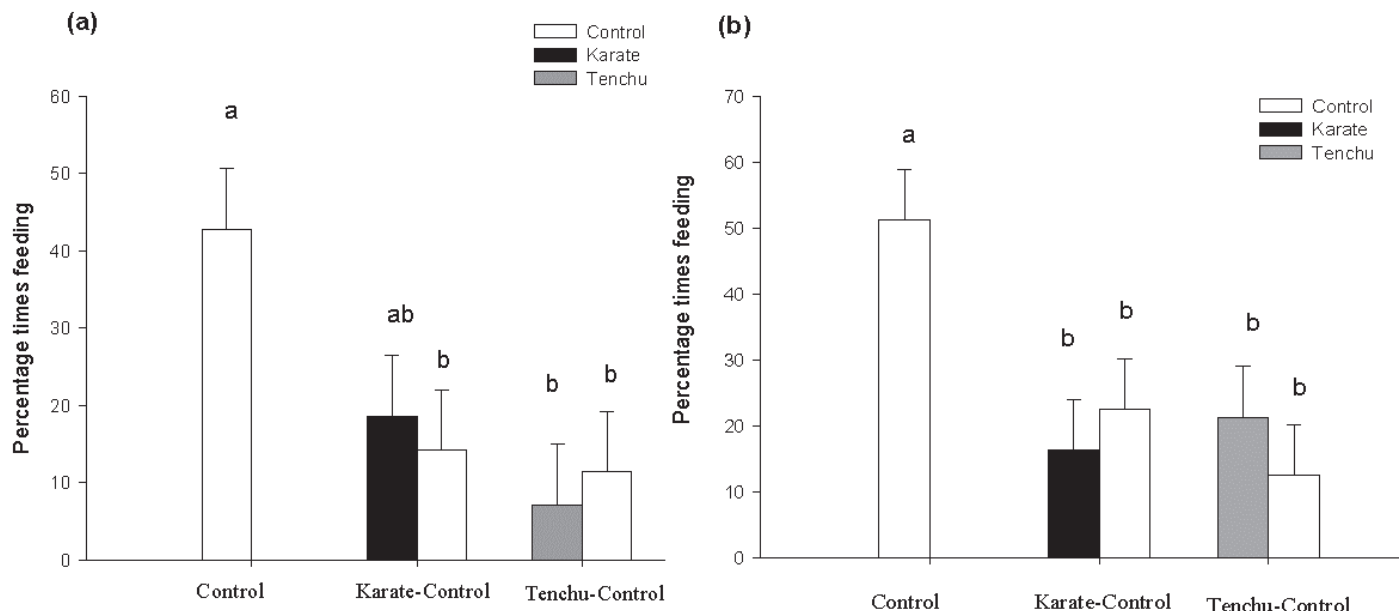
Pre-treatment population densities of rice stink bugs varied greatly, from 1.8 to 48 bugs per 10 sweeps among the nine field sites in the demonstration trial. Post-spray bug densities in fields treated with dinotefuran were lower than pre-treatment densities at 148 but not 72 h after application (Fig. 7; overall effect of Tenchu treatment,  $F_{2,16} = 3.03$ ,



**Fig. 4.** Mean  $\pm$  SE proportion of rice stink bugs surviving after confinement for 48 h to sleeve cages at 3 time points after insecticide applications in 2011. Means accompanied by asterisks indicate a significant difference from controls for the respective sampling date (*a priori* contrasts,  $P < 0.05$ ). (CY =  $\lambda$ -cyhalothrin, DN = dinotefuran).



**Fig. 5.** Probit graph for probability of rice stink bug mortality at given doses of  $\lambda$ -cyhalothrin. Insects were collected at the Rice Research Station in Rayne, LA. Graph was created in SAS 9.3 using PROC PROBIT. Circles represent average mortality for vials treated with serial concentrations of technical grade  $\lambda$ -cyhalothrin: 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 ppm.



**Fig. 6.** Comparison of percent time engaged in feeding activities by rice stink bug adults on treated and untreated rice panicles when given a choice between panicles treated with Karate or untreated and when given a choice between panicles treated with Tenchu or untreated. Control arenas contained two untreated panicles. Experiments were conducted in 2011 (a) and 2012 (b). Bars accompanied by same letter indicate that means on treated and untreated panicles in choice conditions are not significantly different from means on untreated panicles under no choice (control) conditions.

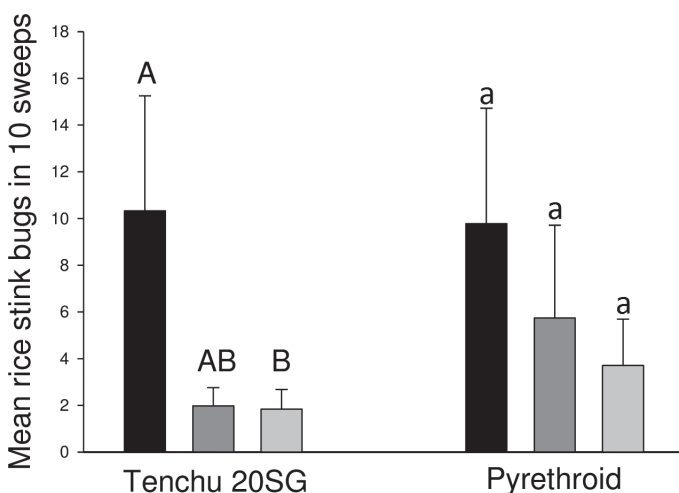
$P = 0.08$ ). Post-treatment densities in pyrethroid-treated fields were not significantly lower than pre-treatment densities (Fig. 7; overall effect of treatment,  $F_{2,15} = 0.69$ ,  $P = 0.52$ ). Population densities of rice stink bugs did not differ significantly among pyrethroid- and dinotefuran-treated fields ( $F_{1,43} = 0.84$ ,  $P = 0.3647$ ), although only in dinotefuran-treated fields did post-spray bug densities remain lower than the current action threshold (3-5 bugs per 10 sweeps) over the 7 d sampling period. A reapplication of pyrethroid was required to reduce *O. pugnax* populations below threshold at 1 of the sites, but no reapplication was necessary for the adjacent field treated with dinotefuran. Fields treated with dinotefuran had a marginally lower ( $P = 0.08$ ) mean percentage

of pecky rice in milled samples (0.4,  $n = 9$ ) than  $\lambda$ -cyhalothrin treated fields (0.5,  $n = 9$ ).

## Discussion

Insecticide applications remain the primary management tactic for reducing rice stink bug populations in all affected rice growing states, and pyrethroids (including  $\lambda$ -cyhalothrin) are the most widely used insecticides (Blackman et al. 2014). Recently, the neonicotinoid insecticide dinotefuran has been registered for rice stink bug management in the southern U.S. In the small-plot experiments, densities of *O. pugnax* adults were significantly reduced by applications of  $\lambda$ -cyhalothrin but not dinotefuran at a few time points, and densities tended to be lowest in plots treated with  $\lambda$ -cyhalothrin at most time points. Densities of *O. pugnax* nymphs in the small-plot experiments, on the other hand, differed significantly among control and treated plots for all insecticides and time points in 2011 and 2012, and both  $\lambda$ -cyhalothrin and dinotefuran were effective at maintaining average nymph populations at approximately 1 per 10 sweeps. In the commercial demonstration trials, post-spray densities of rice stink bugs remained below the action threshold (3-5 bugs per 10 sweeps) only in dinotefuran-treated fields, and dinotefuran provided a marginal advantage ( $P = 0.08$ ) over  $\lambda$ -cyhalothrin at reducing percent pecky rice in milled samples. Overall, the results of these insecticide trials suggest that the efficacies of dinotefuran and  $\lambda$ -cyhalothrin in reducing *O. pugnax* population are comparable.

The differences among the results of small-plot experiments and commercial trials and among the results for nymphs and adults in the small plot experiments point to the important influence of adult movement on the results of these experiments. Movement of large numbers of adult rice stink bugs into commercial fields after insecticide treatments was far less likely than was migration of adults into treated plots in the small-plot experiments, where treated plots were in close proximity to large areas of untreated rice. Similarly, migration of large numbers of wingless nymphs into treated small plots was prob-



**Fig. 7.** Average numbers of rice stink bugs caught in 10 sweeps in commercial fields before insecticide application (black bars), 48 hours after insecticide applications (dark gray bars), or 7 d after insecticide applications (light gray bars). Means accompanied by different letters indicate a significant difference across treatments ( $P < 0.05$ , LSD).



ably minimal, because nymphs remain aggregated within fields until adulthood (Reay-Jones 2010) and because wingless nymphs would be expected to have difficulty traversing open areas between flooded plots. Thus, the results of both the commercial demonstration trials and nymph sampling in small plots provided insights into the efficacies of insecticides not provided by only monitoring densities of adult stink bugs, the standard practice.

In the 2011 residual cage experiment, rice stink bug adults confined to panicles in sleeve cages and directly exposed to  $\lambda$ -cyhalothrin and dinotefuran experienced high levels of mortality compared to controls. More importantly, bugs confined at 2 HAT to dinotefuran-treated panicles showed higher levels of mortality than controls, but bugs confined at 2 HAT to  $\lambda$ -cyhalothrin-treated panicles did not. Likewise, in the 2014 residual experiment, mortalities of bugs exposed to panicles from dinotefuran-treated plots but not from  $\lambda$ -cyhalothrin-treated plots at 2 HAT differed significantly from control mortality. Results at 144 HAT in the 2011 experiment were obscured by high levels of mortality in control cages; the reasons for this high mortality are unknown but are probably related to adverse environmental conditions at the time of the experiment. In the 2014 experiment, mortality of bugs on insecticide-treated panicles collected 24 HAT did not differ from control mortality. The results of these residual experiments are similar to those reported by Way et al. (2009), who found significantly higher mortality of rice stink bug adults feeding on rice panicles treated with dinotefuran than on panicles treated with  $\lambda$ -cyhalothrin. Thus, dinotefuran may possess longer residual activity than  $\lambda$ -cyhalothrin; this possibility must be explored further to better characterize the duration of residual activity.

Rice producers in Southeast Texas often spray more pyrethroid applications to maintain rice stink bug populations below economic thresholds than farmers in surrounding states (Smith 2010; Way 2011). Results of vial bioassays in this study were consistent with the suggestion that repeated exposure of rice stink bugs to pyrethroids like  $\lambda$ -cyhalothrin is contributing to resistance development in populations of Southeast Texas. More selective insecticides like dinotefuran must be introduced into IPM programs to provide alternatives to pyrethroids and limit further resistance caused by continued insecticide applications that act on a single target site in the rice stink bug.

The feeding assay in which bugs were given a choice of treated and untreated panicles allowed the detection of previously undocumented effects of insecticides on the behavior of this insect. Rice stink bugs spent a lower percentage of their time feeding when placed in petri dishes with dinotefuran treated panicles (2011 and 2012) and  $\lambda$ -cyhalothrin treated panicles (2012), even though untreated panicles were available. The results of this choice assay are consistent with observations made of rice stink bugs feeding on dinotefuran-treated rice. Adult *O. pugnax* feeding on dinotefuran treated panicles in the lab, experimental small plots, and commercial fields sometimes appeared extremely lethargic. These insects were observed grasping onto panicles, but they were unresponsive to prodding with a fingertip. This behavior was observed at later sampling dates, suggesting that dinotefuran affects insect feeding behaviors differently than  $\lambda$ -cyhalothrin after the initial application. Experiments need to be designed and conducted using confined insects feeding solely on treated panicles to further document these behaviors and the effects they may have on fecundity and development of rice stink bugs.

Collectively, these experiments show that neonicotinoids, specifically dinotefuran, provide effective control of rice stink bugs when compared with currently labeled products. From the standpoint of residual activity, reductions in population densities, and feeding deterrence of rice stink bugs, dinotefuran appears to be equivalent if not slightly more effective than  $\lambda$ -cyhalothrin. Safer and effective insecticides with

varying modes of action targeting rice stink bug are needed to relieve the selection for resistance resulting from the widespread application of pyrethroids for rice stink bug control throughout the rice-growing region of the southern USA. Dinotefuran exhibits a low mammalian toxicity ( $LD_{50} = 1,000\text{--}3,000$  mg/kg) (EPA 2004), while  $\lambda$ -cyhalothrin is considered moderately toxic to mammals (56 mg/kg)(EPA 1988). Dinotefuran also differs from  $\lambda$ -cyhalothrin in that it acts at a different target site on the rice stink bug than pyrethroids like  $\lambda$ -cyhalothrin. Widespread adoption of dinotefuran among rice IPM programs across the rice-producing southern states will benefit producers and consumers by reducing total insecticide applications and subsequent costs for *O. pugnax* control, as well as delaying resistance development in *O. pugnax* populations.

## Endnote

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