ABSTRACT  Effects of treatment of rice seeds with an anthranilic diamide, chlorantraniliprole, and a neonicotinoid, thiamethoxam, on egg laying and first instar survival in rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, were examined under greenhouse conditions. Exposure of adult weevils to rice (6–7 leaf stage) grown from seeds treated with chlorantraniliprole and thiamethoxam resulted in reduction in numbers of eggs and first instars. The low egg numbers by adults exposed to chlorantraniliprole-treated plants was confirmed as a sublethal effect on adults: adult survival was not impacted after 4 d of feeding on foliage from chlorantraniliprole-treated plants but the number of eggs laid by these weevils was reduced when released on untreated plants. Furthermore, a comparison of first instar emergence from chlorantraniliprole-treated plants and from untreated plants infested with weevils previously exposed to this chemical suggested that chlorantraniliprole was also reducing egg or first instar survival. In contrast, adults that fed on foliage from thiamethoxam-treated plants showed increased mortality. Possible sublethal effects of thiamethoxam on the number of eggs laid by adults were investigated by infesting untreated plants with weevils that survived exposure to thiamethoxam via foliar feeding (7 μg active ingredient/seed). Prior exposure to thiamethoxam through adult feeding reduced egg numbers. However, potential larvicidal or ovicidal effects of thiamethoxam seed treatments could not be detected in this study because of low first instar emergence from both thiamethoxam-treated plants and from untreated plants infested with weevils previously exposed to this chemical. These experiments revealed that the two seed treatments accomplish weevil control in different ways.

KEY WORDS  rice water weevil, seed treatment, chlorantraniliprole, thiamethoxam, sublethal effects

The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), is the most destructive early season insect pest of rice in the United States (Way 1990). Over the past 60 yr, this insect has invaded parts of Asia and Europe and thus has now assumed global importance as a pest of rice (Saito et al. 2005). The interaction of the rice water weevil with rice involves all life stages of the insect. Adult weevils feed on leaves of young rice plants causing characteristic feeding scars parallel to the venation of leaves. Oviposition is triggered by the presence of standing water, and females deposit eggs primarily in submerged leaf sheaths (Stout et al. 2002). After an incubation period of 5–9 d, neonates mine through the leaf sheath for an undetermined period then move down to the roots (Grigarick and Beards 1965, Rak-sarart and Tugwell 1975, Zhang et al. 2004). Larvae pass through four instars then pupate in earthen cocoons attached to roots. Thus, adults use rice foliage as a food source, eggs are inserted into leaf sheaths, and larvae feed first on leaf tissue and later on root tissue. Economic losses are caused primarily by feeding by the larval stages on the roots of flooded rice. Root pruning by larvae results in reduced crop stands, reduced tillering and reduced grain weights (Zou et al. 2004a). Small plot research and sampling of commercial fields indicate yield losses would likely exceed 10% in some areas if no control measures were adopted (Zou et al. 2004b).

Two insecticide seed treatments, one containing the anthranilic diamide, chlorantraniliprole (Derma-cor-X-100), and the second containing the neonicotinoid, thiamethoxam (Cruiser Maxx), have been registered over the past several years for use in rice in the southern United States against the rice water weevil. Results of small plot evaluations of these seed treatments have shown that their use reduces densities of rice water weevil larvae by 75–95% (Stout et al. 2011). Neonicotinoid insecticides are agonists of nicotinic acetylcholine receptors and are less toxic to verte-
brates because of their weak affinity toward mammalian receptors (Tomizawa and Casida 2003, 2005). The broad-spectrum insecticidal activity and excellent systemic characteristics of thiamethoxam makes it suitable for use as a seed treatment (Maenisch et al. 2001, Jeschke and Nauen 2008). The anthranilic diamide class of insecticides, which includes chlorantraniliprole, activates ryanodine receptors and stimulates calcium ion release from muscle cells causing paralysis and death in vulnerable species (Lähm et al. 2005, Cordova et al. 2006). This chemical has excellent larvicidal activity against many Lepidoptera and Coleoptera (Lähm et al. 2007, Clark et al. 2008, Koppenhöfer and Fuzy 2008). Chlorantraniliprole is more selective to ryanodine receptors of insects than mammals, which accounts for its low mammalian toxicity (Lähm et al. 2007, 2009).

Neonicotinoids exhibit a variety of lethal and sublethal effects on aspects such as insect feeding, oviposition, and fecundity in Lepidoptera, Coleoptera, and Hemiptera (Nauen 1995, Hu and Prokopy 1998, Biddenger and Hull 1999, Isaacs et al. 1999, Kunkel et al. 2001, Wise et al. 2007, Daniels et al. 2009, Hoffman et al. 2009, Uginé et al. 2011). Fitness impacts on insects after sublethal exposure to chlorantraniliprole, such as disrupted mating behavior and reduced or delayed fecundity, have been previously reported in Lepidoptera (Knight and Flexner 2007, Han et al. 2012) and Diptera (Teixeira et al. 2009). The intimate association of rice water weevils with plants treated as seeds with these insecticides could result in a variety of lethal and sublethal effects on the biology and behavior of rice water weevils that may contribute to weevil suppression in the field. In a previous study, adult rice water weevils experienced increased mortality and reduced feeding on foliage of rice plants treated as seeds with thiamethoxam, while no such impacts were seen in chlorantraniliprole-treated plants (Lanka et al. 2012). The present studies investigated the effects of both insecticide seed treatments on egg-laying and first instar survival of rice water weevils.

Materials and Methods

Insects, Seed Treatments, and Greenhouse Rice Culture. Dermacor-X-100 (active ingredient [AI]: chlorantraniliprole) was supplied by DuPont (Wilmington, DE) and Cruiser 5FS (AI: thiamethoxam) was supplied by Syngenta Corporation (Greensboro, NC). The rice variety CL131, a widely grown long grain variety resistant to the imidazolinone class of herbicides, was used for all experiments. Insecticide formulations were diluted in water containing a small quantity of Brilliant blue dye, and applied by pipette to seeds in plastic bags to attain thiamethoxam treatment rates of 7, 21, and 28 g AI/seed and chlorantraniliprole was applied at 10, 25, and 50 g AI/seed. Seed treated with dye only served as a control (0 g AI/seed). Plants for all experiments were grown by sowing four to six seeds of a single treatment rate in 1.0 liters pots in a greenhouse on the campus of Louisiana State University, Baton Rouge, LA. The potting mixture was composed of two parts of autoclaved silt loam soil with one part each of sand and peat moss. The greenhouse was maintained at 28.0 ± 5°C with ambient lighting. Pots were watered adequately but excessive watering was avoided to prevent leaching of insecticide and disturbance of soil. Plants were thinned to retain two plants/pot ~10 d after sowing. A complex fertilizer (20–14–38 N: P: K) was applied to the soil surface at a rate of 2.5 g/liter after thinning. Plants were grown and experiments were conducted in large wooden basins lined with black plastic pond liner that allowed plants to be flooded.

Adult weevils were collected from unsprayed rice plots at the Louisiana State University Agricultural Center Rice Research Station, Crowley, LA, 1 d before use in experiments and acclimated overnight in large plastic dishes containing rice leaves and water. Weevil mating pairs were separated into plastic cups provided with moisture at the bottom. In all experiments in present studies, mating pairs of weevils were used to ensure a sex ratio of 1:1.

Direct Effects on Eggs and First Instar Emergence. Initial experiments were conducted to compare weevil egg numbers on, and first instar emergence from, entire plants treated as seeds with chlorantraniliprole and thiamethoxam. Egg counts are a direct measure of oviposition, whereas counts of first instars provide information about both the number of eggs laid and mortality of eggs or first instars resulting from consumption of leaf tissue by first instars. Plants grown to the six to seven leaf-stage from seeds treated with thiamethoxam (0, 21, and 28 g AI/seed) or chlorantraniliprole (0, 10, and 25 g AI/seed) were used for these experiments. Plants were infested with mating pairs of weevils by releasing them on plants in infestation cages. Infestation cages were constructed of cylindrical wire frames (46 cm diameter, 61 cm tall) covered with a mesh fabric screening.

In total, three independent experiments were conducted, two with chlorantraniliprole-treated plants and one with thiamethoxam-treated plants. In all experiments, infestation cages served as experimental units. Two infestation cages were used for each of the three treatment rates in each experiment. Each cage contained plants of a single treatment rate (no choice experiment). Depending on experiment, 9 or 10 pots, each with two plants, were placed in each of the infestation cages. Thus, 18 or 20 plants were placed in each infestation cage. After placing pots in cages and flooding the plants, adult weevils were released in cages at a density of one male:female pair/plant (18–20 pairs released/cage). Weevils were allowed to feed, mate, and oviposit on plants in cages for 4 d. Pots were then removed from cages and any weevils found on plants were removed. One plant from each pot (a total of 9 or 10 plants from each infestation cage) was removed for egg counts. Soil from plants for egg counts was carefully removed from the roots of these plants, and plants were tagged and placed in 75% ethanol until bleached. The numbers of eggs were counted by ex-
Table 1. Foliar and whole plant exposure regimes of *L. oryzophilus* adopted for determining the impact of seed treatments on egg numbers and survival of first instars

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Foliar feeding (µg Al/seed)</th>
<th>Plants infested (µg Al/seed)</th>
<th>Treatment designation*</th>
<th>Regime of exposureb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorantraniliprole</td>
<td>25</td>
<td>0</td>
<td>25–0</td>
<td>Foliar feeding</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0</td>
<td>50–0</td>
<td>Whole plant</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>25</td>
<td>0–25</td>
<td>No exposure</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>50</td>
<td>0–50</td>
<td>No feeding</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0–0*</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>7</td>
<td>0</td>
<td>0–7</td>
<td>No feeding</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>7–0</td>
<td>No exposure</td>
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<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0–0</td>
<td>No exposure</td>
</tr>
</tbody>
</table>

*Prehyphenated term indicates the seed treatment rate used in foliar feeding and posthyphenated term is the seed treatment rate used for infestation.

b Feeding exposure regime consists of weevil feeding on excised foliage from treated plants and subsequent release on untreated plants; whole plant exposure regime had weevil feeding on excised foliage from untreated plants and subsequent release on treated plants.

Weevils were fed on foliage from untreated plants and subsequently released on untreated plants.

Table 1 outlines the regimes of exposure to chlorantraniliprole and thiamethoxam used in this study.

The weevil mating pairs that were exposed to foliage from treated plants (25 or 50 µg Al/seed for chlorantraniliprole or 7 µg Al/seed of thiamethoxam) were subsequently released on untreated plants (0 µg Al/seed) (Table 1). This was done to determine the impact of feeding exposure to insecticides in adults on the number of eggs laid on and resulting first instars. This particular regime of weevil release was termed in this study as ‘Foliar feeding exposure’ and the treatments were designated as 25–0 and 50–0 for chlorantraniliprole and 7–0 for thiamethoxam. The numbers of eggs and first instar weevils resulting from Foliar feeding exposure regime were contrasted with those resulting from another regime of exposure termed ‘whole plant exposure’ (Table 1). This regime involved feeding weevil mating pairs on foliage from untreated plants (0 µg Al/seed) for 4 d, then releasing weevils in infestation cages on plants treated as seeds with chlorantraniliprole (25 or 50 µg Al/seed) or thiamethoxam (7 µg Al/seed). This contrast was done to determine the potential larvicidal/ovicidal effect of seed treatments on first instar-rice water weevils (larvae mine through leaf sheaths to emerge).

A final regime was maintained in both chlorantraniliprole and thiamethoxam experiments in which weevils were fed for 4 d on foliage from plants containing no insecticide then released on plants not treated with insecticide. This treatment, termed 0–0, served as a control for both insecticide exposure regimes. In total, two experiments were conducted using these exposure regimes with chlorantraniliprole and one experiment was conducted with thiamethoxam.

**Effects on Adult Survivorship, Foliar Consumption, Eggs, and First Instar Emergence.** Subsequent experiments were conducted to determine whether feeding by adults on foliage from chlorantraniliprole- and thiamethoxam-treated plants would reduce subsequent egg numbers on untreated plants. These experiments also allowed monitoring of adult survival and feeding activity on foliage from treated and untreated plants. To accomplish these goals, a two-stage protocol was used. In the first stage, adult weevils were allowed to feed on excised foliage from treated or untreated seeds with chlorantraniliprole or thiamethoxam for 4 d. In the second stage, weevils were released in infestation cages on plants untreated or treated as seeds with chlorantraniliprole and thiamethoxam. Larvae emerging from eggs deposited in these plants eventually move to the roots. Larvae were counted daily after shaking plants to dislodge larvae from roots. After each count, water was replaced and counts were recorded until no larvae were recovered for three consecutive days.

For counts of first instars, the second plant from each pot (9 or 10 plants from each cage) was removed, washed to remove soil from roots, and placed in a test tube filled with water. Test tubes were placed in a controlled environment chamber (Percival Scientific, Boone, IA) maintained at 28°C and a photoperiod of 14:10 (L:D) h. Larvae emerging from eggs deposited in these plants eventually move to the roots. Larvae were counted daily after shaking plants to dislodge larvae from roots. After each count, water was replaced and counts were recorded until no larvae were recovered for three consecutive days.

**Effects on Adult Survivorship, Foliar Consumption.** For exposure of weevils to excised foliage, mating pairs of weevils were placed on excised foliage from treated or untreated plants in petri dishes (22.5 cm diameter; 2.5 cm deep) lined with 1.5% Agar (Fisher Chemical, lab grade). Excised ends of leaf blades were inserted into agar to maintain turgidity. The top two leaves of the rice plants (6–7 leaf stage) closest to the central whorl were used and leaves were replaced daily for 4 d with new leaf material obtained from fresh plants. Mortality of adult weevils was assessed every 24 h of the 96-h feeding period. Mortality was defined as lack of visible movement in weevils in 10 min after being prodded by a camel hair brush. After the daily assessment of mortality, feeding activity was measured according to the methods described previously (Lanka et al. 2012). Data from this previous survey on the impact of seed treatments on egg numbers and survival of first instars of *L. oryzophilus*.
study showed no impact of thiamethoxam on foliar consumption rate/weevil at 7 µg AI/seed).

The number of weevil pairs initiated for feeding on treatments was greater than the number required for subsequent release in infestation cages. This was done to compensate for any adult mortality occurring during feeding on foliage from treated plants. For the first exposure regime experiment with chlorantraniliprole, a total of five petri dishes, each with nine mating pairs of weevils/dish, were used for both chlorantraniliprole treatment rates (45 mating pairs/treatment rate). In total, 15 petri dishes, each with nine mating pairs/dish, were used to provide weevils for the 0–25, 0–50, and 0–0 treatments. In the second experiment with chlorantraniliprole, a total of four petri dishes, each with 12 mating pairs/dish, were used (48 mating pairs/treatment rate). In this experiment, weevil mating pairs were exposed to foliage from untreated plants in a total of 12 petri dishes (12 mating pairs/dish).

In the thiamethoxam experiment, a total of 45 mating pairs were placed in five petri dishes (nine mating pairs/dish) on foliage from seeds treated with 7 µg AI/seed to supply weevils for the 7–0 treatment (Table 1). In total, 10 petri dishes each containing nine mating pairs/dish were used to supply weevils for the 0–7 and 0–0 treatments. At the end of the 4-d exposure period, apparently healthy weevils (i.e., weevils displaying coordinated movement of legs within five min after being placed on their dorsum on a flat surface) were sorted into groups of 18–20 mating pairs for release in infestation cages.

**Effects on Eggs and First Instar Emergence.** To accomplish the goal of contrasting the egg and larval numbers resulting from the foliar feeding exposure and whole plant exposure regimes, mating pairs of weevils were released in infestation cages to achieve the treatment regimens outlined in Table 1. Each cage contained chlorantraniliprole- or thiamethoxam-treated plants of a single treatment rate. Cages were labeled with appropriate designated treatment (i.e., 0–0, 0–25, 0–50, 25–0, or 50–0 for chlorantraniliprole experiments and 0–0, 7–0, or 0–7 for the thiamethoxam experiment). Each treatment was replicated in two cages (18 or 20 plants per cage). After 4 d of infestation, to determine the impact of foliar feeding and whole plant exposure regimes, one plant from each pot was removed for egg counts and the remaining plant was monitored for larval emergence as described previously.

**Statistical Analysis.** For the direct exposure experiments, the impacts of seed treatments on egg and larval numbers were analyzed separately by analysis of variance (ANOVA) using PROC MIXED (SAS Institute 2008). Treatment was a fixed effect whereas cage*treatment was a random term. In analyzing the impact of chlorantraniliprole, experiment was used as an additional random term. Seed treatment rate effects were analyzed by post hoc mean separations using Tukey’s honestly significant difference (HSD). Because of heterogeneity of variances the data were transformed using $\sqrt(X + 0.5)$ before analysis. To estimate appropriate degrees of freedom, the Kenward–Roger adjustment of degrees of freedom was used in model statement.

The effects of chlorantraniliprole seed treatments on adult survival and the rate of foliar consumption were analyzed by mixed-model ANOVA. The impact of seed treatment was analyzed by treating experiment and replicated petri dishes as random terms. Effects of exposure to thiamethoxam on weevil survival were also analyzed by mixed-model ANOVA.

To determine the impact of weevil exposure regimes to chlorantraniliprole and thiamethoxam on egg numbers and first instar emergence, egg, and larval data were analyzed by ANOVA with treatment regime as a fixed effect and cage as a random effect. In the analysis of chlorantraniliprole experiments, experiment was used as an additional random term. Comparisons of effects of exposure regimes were done by pair-wise a priori contrasts of egg-laying by three groups of weevils, that is, whole plant-exposed (chlorantraniliprole: 0–25 and 0–50; TMX: 7–0), foliar-fed (chlorantraniliprole: 25–0 and 50–0; TMX: 7–0), and control (0–0). Data on eggs and first instars were transformed $\sqrt(X + 0.5)$ before analysis because variances were generally proportional to means.

**Results**

**Direct Effects on Eggs and First Instar Emergence.** Both the egg numbers and first instar emergence were reduced on plants treated as seeds with chlorantraniliprole (egg numbers: $F = 5.0; df = 2, 8; P = 0.04$; first instar emergence: $F = 7.5; df = 2, 8; P = 0.01$) (Fig. 1). Egg numbers were reduced by 38 and 50% in the 10 and 25 µg AI/seed treatments, respectively; however, only the difference between control and the 25 µg AI/seed treatment was significant. The first instar emergence between two seed treatment rates did not differ statistically. Larval emergence was reduced by 53% at both seed treatment rates compared with no seed treatment.
Seed treatment with thiamethoxam also significantly impacted egg-laying \((F = 63.4; \text{df} = 2, 57; P < 0.0001)\) and larval emergence \((F = 33.0; \text{df} = 2, 57; P < 0.0001)\) (Fig. 2). Both egg and larval numbers were significantly reduced by >90% at the 21 and 28 \(\mu\)g AI/seed treatments. Both the number of the eggs and first instars did not differ statistically between the two treatments rates.

**Effects on Adult Survivorship, Foliar Consumption, Eggs, and First Instar Emergence.**

**Effects on Adult Survivorship and Foliar Consumption.** In the pre-exposure period, adult feeding on leaf material from chlorantraniliprole-treated plants did not impact survivorship \((F = 0.3; \text{df} = 2, 24; P > 0.34)\) or foliar consumption \((F = 0.4; \text{df} = 2, 24; P > 0.7)\) (data not shown). In contrast, exposure of adult weevils to foliage from thiamethoxam-treated plants reduced survival of adults \((F = 6.0; \text{df} = 1, 12; P = 0.02)\) (data not shown).

**Effects on Eggs and First Instar Emergence.** Chlorantraniliprole exposure regimes significantly affected subsequent egg numbers \((F = 3.3; \text{df} = 4, 16; P = 0.04)\) and first instar emergence \((F = 13.9; \text{df} = 4, 16; P = 0.0002)\) (Fig. 3). As in the direct exposure experiments described above, adult weevils not previously exposed to chlorantraniliprole laid significantly fewer eggs on chlorantraniliprole-treated plants than on untreated plants (treatments 0–25 and 0–50 vs. treatment 0–0; \(F = 11.5; \text{df} = 1, 16; P = 0.004)\). Also as seen previously, fewer first instars emerged from chlorantraniliprole-treated plants than from untreated plants when infested with weevils that had not been previously exposed to chlorantraniliprole \((F = 42.5; \text{df} = 1, 16; P < 0.0001)\). Furthermore, ingestion of foliage from chlorantraniliprole-treated plants by adults significantly reduced egg-laying \((F = 8.5; \text{df} = 1, 16; P = 0.001)\) and first instar emergence \((F = 9.9; \text{df} = 1, 16; P = 0.007)\) on untreated plants when compared with weevils that did not previously ingest chlorantraniliprole (treatments 25–0 and 50–0 vs. treatment 0–0). The magnitude of reduction in egg numbers in these latter treatments was similar to the reduction observed in weevils exposed directly to chlorantraniliprole-treated plants (treatments 25–0 and 50–0 vs. treatments 0–25 and 0–50; \(F = 0.33; \text{df} = 1, 16; P > 0.5)\). However, significantly fewer larvae emerged from plants when weevils were directly exposed to chlorantraniliprole-treated plants without prior exposure to chlorantraniliprole than when weevils were pre-exposed to chlorantraniliprole then exposed to untreated plants (treatments 0–25 and 0–50 vs. treatments 25–0 and 50–0; \(F = 17.0; \text{df} = 1, 16; P = 0.001)\), suggesting larvicald activity in chlorantraniliprole-treated plants.

In addition, exposure regimes to thiamethoxam significantly affected subsequent egg numbers \((F = 28.1; \text{df} = 2, 3; P = 0.01)\) and first instar emergence \((F = 21.7; \text{df} = 2, 3; P = 0.02)\) (Fig. 4). As shown previously in the direct exposure experiments, adult weevils without prior exposure to thiamethoxam laid fewer eggs on thiamethoxam-treated plants than on untreated plants (treatment 0–7 vs. 0–0; \(F = 8.5; \text{df} = 1, 3; P = 0.01)\). Also as seen previously, significantly fewer first instars emerged from thiamethoxam-treated plants than from untreated plants when infested with weevils that had not previously exposed to thiamethoxam \((F = 9.9; \text{df} = 1, 3; P = 0.007)\). Ingestion of thiamethoxam by adults during the pre-exposure period significantly reduced egg-laying \((F = 28.1; \text{df} = 1, 3; P = 0.01)\) and first instar emergence \((F = 1.7; \text{df} =
placed in cages (instar densities (open bars; larvae/plant treated as seeds (0)) and then allowed to access plants either treated as seeds (7) in WE (Whole plant Exposure; 0–7) or with plants not treated (0–0; control). In FE (Foliar feeding Exposure) weevil adults were fed on foliage from plants treated as seeds (7 μg AI/seed) and then provided with plants not treated as seeds (0). Egg (closed bars; eggs/plant ± SE) and first instar densities (open bars; larva/plant ± SE) were determined on treated plants placed in cages (n = 2, each containing 9 or 10 plants for egg and larval numbers separately).

1, 3; P = 0.02) on untreated plants when compared with weevils not exposed to thiamethoxam (treatment 7–0 vs. treatment 0–0). There was no significant difference in egg deposition between unexposed weevils placed directly on thiamethoxam-treated plants and weevils pre-exposed to thiamethoxam placed on untreated plants (treatment 0–7 vs. treatment 7–0; F = 1.7; df = 1, 3; P = 0.4). Also first instar density did not differ between these two groups of weevils (F = 0.4; df = 1, 3; P = 0.9). Egg deposition was reduced by 89% in weevils pre-exposed to thiamethoxam and by 74% in un-exposed weevils placed on treated plants. Finally, first instar larval emergence was reduced to levels by 96% in both regimes of exposure.

Discussion

Over the past several years, insecticide formulations containing chlorantraniliprole and thiamethoxam have been introduced in rice as seed treatments for management of the rice water weevil in the southern United States. Both seed treatments provide effective control of weevil larval populations, although control provided by chlorantraniliprole is superior in most cases to that provided by thiamethoxam (Stout et al. 2011). Because of the intimate association of rice water weevils with rice plants, plants treated as seeds with systemic insecticides could reduce weevil populations via effects on several different life stages of the rice water weevil. The current study investigated possible influences of these seed treatments on egg numbers and mortality of eggs or first instars. The results suggest that reduction in egg numbers may contribute to suppression of weevil larvae for both seed treatments. In addition, egg and/or early instar mortality are additional mechanisms of the control of weevil larvae in chlorantraniliprole treatments. Furthermore, the divergent effects of these insecticides on adult weevils found in the current study are consistent with prior studies that showed thiamethoxam but not chlorantraniliprole seed treatments reduced adult rice water weevil survival and foliar consumption (Lanka et al. 2012).

Reduction in the number of eggs on treated plants could be the consequence of three mechanisms. Firstly, reduction in egg numbers could have resulted from mortality of adults before they oviposited. This mechanism likely provides a partial explanation for reduced egg number on thiamethoxam-treated but not on chlorantraniliprole-treated plants, where adult toxicidal activity is low. Two other mechanisms for reduction in egg numbers could be the oviposition deterrence because of the presence of insecticides in above-ground tissues and the toxicant-induced malaise resulting from sublethal exposure through ingestion because these insecticides were reported to induce oviposition dysfunction in insects (Wise et al. 2006, Teixiera et al. 2009). To more directly test the toxicant-induced malaise, the egg numbers by weevils exposed to chlorantraniliprole and thiamethoxam via foliar feeding and by weevils not exposed to these insecticides were compared. The reduction in egg numbers observed in weevils that fed on foliage from chlorantraniliprole- and thiamethoxam-treated plants was comparable to that observed in weevils directly exposed to treated plants for oviposition. The fact that exposure of weevils to insecticides through foliar feeding resulted in reduced egg numbers is consistent with toxicant-induced malaise. However, this result does not exclude the possibility of direct deterrence of weevil oviposition on treated plants because of the presence of insecticides in leaf tissue. Further studies on feeding behavior and other behaviors on whole plants are required to disentangle the relative roles of oviposition deterrence and toxicant-induced malaise.

To our knowledge, the current study is the first to show an impact of adult exposure to chlorantraniliprole and thiamethoxam seed treatments on egg numbers in Coleoptera. Disruption of mating behavior and reduced fertility in Lepidoptera and Diptera after exposure to chlorantraniliprole has been previously reported. Disruption in the response of males to pheromones and significant decrease in proportion of mated female codling moths, Cydia pomonella L. (Lepidoptera: Tortricidae) were reported when virgin moths contacted dried residues on containers or on treated foliage (Knight and Flexner 2007). In addition, effects on fertility in other Lepidoptera and Diptera were detected after ingestion of sublethal doses of chlorantraniliprole. For example, delayed oviposition was reported in apple maggot flies, Rhagoletis pomonella Walsh (Diptera: Tephritidae) after prior exposure to chlorantraniliprole (Teixiera et al. 2009), and as reductions in fertility of offspring in beet armyworms, Spodoptera exigua Hübner (Lepidoptera: Noctuidae) (Lai and Su 2011) and diamondback moths, Plutella xylostella L. (Lepidoptera: Plutellidae) (Han et al. 2012) were reported after larval feeding on
diets incorporated with insecticide at sublethal concentrations. The reduction in egg numbers by weevils that survived exposure to thiamethoxam treatment in the current study is similar to effects recorded on fecundity of the black vine weevil, Otiorynchronus sulcatus (Coleoptera: Curculionidae) (Son 2004). Weevils that were prior fed for 7 d on foliage from Astilbe thunbergi sprayed with sublethal concentration of thiamethoxam laid fewer eggs when released on untreated foliage than weevils fed on untreated leaves. However, this reduction was temporary and fecundity returned to normal after 6 wk of feeding on untreated leaves of A. thunbergi. Evidence of sublethal influences of neonicotinoids is mostly confined to sucking pests (Nauen et al. 1998, Isaacs et al. 1999, Daniels 2009).

In addition to demonstrating reduction in egg numbers after ingestion of thiamethoxam and chlorantraniliprole by adults, the exposure regime experiment provides evidence for egg and/or early instar mortality of rice water weevils in chlorantraniliprole-treated plants. This evidence comes from the fact that the larval counts in the whole plant exposure regimes were lower than in the foliar feeding exposure regime, although both exposure regimes resulted in equivalent reductions in egg numbers. This particular pattern of additional reduction in larval numbers from whole plant exposure regimes suggested mortality in eggs or larvae. Published evidence for systemic control of non-lepidopteran larvae in chlorantraniliprole treated plants is scarce. Effective control of annual bluegrass weevil, Lepidoptera: Curculionidae) is thought to depend on systemic effects of insecticide on young larvae as they begin to chew their way into the stem of annual bluegrass plant, Poa annua L. treated with Acelepryn (chlorantraniliprole: 20%) (Anonymous 2012).

In contrast, exposure regimes to thiamethoxam, did not reveal additional reduction in larval numbers, that is, no difference was found in larval numbers between whole plant and foliar feeding regime. Because of poor larval numbers in this study, evidence for the absence of ovicidal or larvicidal effects of thiamethoxam was equivocal.

The results of this and a previous study (Lanka et al. 2012) demonstrate that chlorantraniliprole and thiamethoxam seed treatments have lethal and sublethal effects on multiple life stages of the rice water weevil. Seed treatments with chlorantraniliprole had no effect on adult feeding or mortality but reduced egg numbers and killed eggs and/or first instars. In contrast, seed treatments with thiamethoxam reduced adult survivorship and egg numbers. The effects of thiamethoxam were more pronounced than the effects of chlorantraniliprole in these experiments. This is somewhat surprising in light of field experiments showing more effective suppression of weevil larvae by chlorantraniliprole than thiamethoxam (Stout et al. 2011). One possible explanation for the superior field efficacy of chlorantraniliprole is greater persistence in the soil or plant. Another explanation relates to the distribution profiles of both insecticides in rice plants. Investigations are under way to relate the differential activities of both the seed treatments on weevil life stages with the levels of insecticide residues in various plant parts.

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