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Incorporating neonicotinoid and anthranilic diamide insecticides into integrated pest management of rice water weevil, Lissorhoptrus oryzophilus

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INCORPORATING NEONICOTINOID AND ANTHRANILIC DIAMIDE INSECTICIDES INTO INTEGRATED PEST MANAGEMENT OF RICE WATER WEEVIL, *LISSORHOPTRUS ORYZOPHILUS*

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ABSTRACT

Seed treatments with chlorantraniliprole and thiamethoxam have been used for the management of rice water weevil, *Lissorhoptrus oryzophilus* in the United States. Both insecticides being plant-systemic could potentially target multiple life stages of *L. oryzophilus*. The rational use of these chemicals as seed treatments in weevil management requires thorough understanding on targeted life stages of *L.oryzophilus* and, the relative persistence and potency of both chemicals. A series of greenhouse and laboratory experiments were conducted on weevils by culturing rice plants treated as seeds with different rates of chlorantraniliprole and thiamethoxam. Adult feeding assays on foliage from treated plants revealed divergent effects: treatments with thiamethoxam but not chlorantraniliprole affected adult mortality and foliar consumption. Dose-mortality relationships for thiamethoxam were determined by combining the estimates of leaf biomass consumed by weevils in conjunction with foliar insecticide residues estimated by LC/MS/MS. The LD_{50} s obtained were the first LD_{50} s in leaf feeding insects feeding on foliage of thiamethoxam-treated plants. Changes in adulticidal activity with plant-growth were related to the residues analyzed by ELISA method.

Adult exposure to treated rice (6-7 leaf stage) led to reduced egg numbers and first instar emergence. The low egg numbers by adults in chlorantraniliprole was confirmed as a sub-lethal effect: survival was not impacted on foliage but the number of eggs by adults 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. Sub-lethal effects of thiamethoxam on weevils that survived feeding exposure were manifested in reduced egg numbers. Finally, differential activities of both seed treatments on

weevil life stages were related to residue patterns in plant portions. In chlorantraniliprole, the greatest reduction occurred in late instars feeding on roots, whereas in thiamethoxam, reduction was largely due to disrupted adult survival and egg-laying. High above ground concentrations of thiamethoxam and high below ground concentrations of chlorantraniliprole in rice plants were consistent with differential activities. The implications of such divergent distribution patterns on *L.oryzophilus* management were discussed.

CHAPTER 1

INTRODUCTION

Rice

Globally, rice is planted on over 159 million hectares (USDA FAS 2012). One fifth of the world population depends on rice cultivation for livelihood and rice farms cover 11% of world's arable area (IRRI 2011). Moreover, rice is the most important staple for a large majority of the population and provides at least half of daily caloric intake (IRRI 2011). So the development of research technologies in rice can have a strong positive impact on world population and will also have significant effect on the environment.

Rice is cultivated largely in more than 50 countries across Asia, Africa, Europe, North America, South America and Australia (USDA 2012a). Although, the US produces less than 2% of the world's rice, it is the major exporter accounting for more than 10% of annual volume of rice trade (USDA ERS 2012). In 2011, the value of US rice harvest was approximately $ 2.63 billion, and in Louisiana, the rice production was worth over $ 360 million (USDA FAS 2012).

Biology of rice water weevil

The rice water weevil is the most destructive early-season insect pest of rice in the United States (Smith 1982, Way 1990). The rice water weevil is found in all rice producing states in the U.S. including California, Arkansas, Louisiana, Missouri, Mississippi and Texas. This pest has recently invaded important rice-producing regions of Asia and Europe (Saito et al. 2005) and thus has assumed global importance as a pest. The insect overwinters as an adult, presumably in a state of diapause (Smith et al. 1983). Overwintering habitats are various and include leaf litter, bunch grasses, grass clumps and plant debris in and around rice fields and riparian habitats (Shang et al. 2004). Emergence of adults from overwintering in spring is influenced by temperature and begins in early April in southern Louisiana (Zou et al. 2004a). Adults are semi-aquatic and upon emergence, they move to rice fields to feed on the leaves of rice and other

aquatic grasses (Tindall and Stout 2003). Feeding injury on leaves is not economically important except under unusually heavy infestations. Females oviposit primarily in the leaf sheaths of flooded plants, beneath the surface of the water (Everett and Trahan 1967, Raksarart and Tugwell 1975, Smith 1983, Way 1990, Stout et al. 2002a). Larval establishment on roots thus begins when standing water is present in a field, a condition usually met only after a permanent flood is applied to a field. The immature stages of this insect are spent in flooded soils, where they feed on or in the roots of their hosts. The insects pass through four instars and a pupal stage in approximately 27-30 days (Zou et al. 2004a). Only a single larval peak occurs in a single field during a growing season (Shang et al. 2004), although populations of the weevil are multivoltine. Feeding by larvae on the roots of rice plants results in reduced tillering and shoot growth in the vegetative phases of development and in reduced panicle densities and grain weights at harvest (Zou et al. 2004b).

Several factors make this insect a particularly severe pest in southern Louisiana. First, weevil populations are typically larger in Louisiana than in other rice growing states, probably because of warm springs and an abundance of native habitat. Second, weevil populations pass through at least two generations and a third partial generation per year. As a result, adults remain in rice fields in significant numbers from April through August and the potential for damaging larval infestations in young rice exists throughout the growing season. Finally, cultural practices like water-seeding and early flooding, commonly used in Louisiana to manage weeds, are conducive for infestations of rice plants at early stages of growth, and thereby increasing yield losses from this pest.

Yield impacts

The economic impact of this pest on rice production in Louisiana is not precisely known. Yield losses attributable to injury by this insect in small plots can exceed 20% and presence of a single weevil larva on a young plant results in a yield loss of approximately 0.5 to 1% (Zou et al. 2004b). Larval densities can easily exceed 25 larvae per plant in southwest Louisiana where populations are large. A recent study at commercial sites not treated with insecticides showed a conservative yield loss of 5% when 12 larvae per core were present (unpublished).

Non chemical tactics in weevil management

Non chemical tactics against the rice water weevil have been investigated but show little potential for reducing weevil populations below damaging levels. (Puissegur 1976, Bunyarat et al. 1977, Smith 1983, Way 1990, N'Guessan and Quisenberry 1994, N'Guessan et al. 1994, Thompson et al. 1994 a, b, Rice 1996, Heinrichs and Quisenberry, 1999, Stout et al. 2001, Stout and Riggio, 2002). Draining and drying fields until the soil cracks during heavy infestations of larvae may be effective in some cases, but is not recommended due to frequent rain and the costs associated with reapplying herbicide and fertilizer (Way, 1990; Thompson et al., 1994a) and pumping of water to reflood.

Thousands of rice lines have been screened for resistance to the rice water weevil, but only a few have shown low or moderate levels of resistance (N'Guessan and Quisenberry, 1994; N'Guessan et al., 1994; Heinrichs and Quisenberry, 1999; Stout et al., 2001; Stout and Riggio, 2002); Levels of resistance in even the most resistant lines identified to date are not sufficient to negate the need for additional control methods.

Early flooding applied at the two to three leaf stage is commonly practiced in southwestern Louisiana. This is especially important where red rice is a severe pest because it

assists in red rice management. In areas where red rice is a less of a problem, floods are delayed until the four to five leaf stage. Nonetheless, the initiation of flooding induces rice water weevil oviposition, with more eggs oviposited in leaf sheaths of flooded rice plants than non-flooded plants. The depth of flood also influences oviposition; floods of 10.2 cm were the most preferred when rice water weevils were provided a choice between multiple flood depths (Stout et al., 2002b). Research has also shown that younger plants are more susceptible to rice water weevil injury (Stout et al 2002a). The time at which permanent floods are applied also affects rice water weevil injury to rice (Rice et al., 1999; Zou et al., 2004). When floods are delayed by two weeks, numbers of rice water weevil larvae on roots were reduced by as much nine times that on roots of early flooded rice. However, delaying floods has not been readily adopted because it compromises red rice management (Dunand, 1988).

Early planting has long been suggested as a management tactic against the RWW (Isley 1934, Bowling 1964, Thompson et al. 1994b, Shang et al. 2004, Espino et al. 2009, Stout et al. 2011). The basis for this suggestion is a potential asynchrony between rice planting, which begins in March in southwest Louisiana, and the emergence of weevils from overwintering sites. Emergence of adults from overwintering habitats in spring is influenced by temperature, beginning in late March to early April in southern rice-growing regions of the United States (Zou et al 2004a) and later in more northern rice-growing regions (Morgan et al. 1984). This emergence is a protracted process that may occur over the course of two or more months (Muda et al. 1981, Shang et al. 2004). Weevil populations are more likely to be low in early planted rice and fields are less likely to suffer yield losses from weevils. However, heavy weevil pressures in certain years necessitate application of insecticides even in early planted rice to control this pest.

Foliar insecticides for rice water weevil management and their demerits

Until recently, the management program for the rice water weevil relied almost entirely on foliar application of insecticides. Six chemical insecticides are registered in Louisiana for aerial application to foliage to target adult weevils: Karate® (lambda-cyhalothrin, Syngenta Crop Protection), Mustang Max® (zeta-cypermethrin, FMC Corporation), Proaxis® (gamma-cyhalothrin, Pytech Chemicals), Prolex® (gamma-cyhalothrin, Pytech Chemicals), Declare® (gamma-cyhalothrin, Cheminova), and Dimilin® (diflubenzuron, Chemtura Corporation). Of these insecticides, Dimilin® has received very little use in Louisiana because it is more expensive than the others. All pyrethroids kill adult rice water weevils but do not cause significant mortality of larvae, eggs, or pupae of the insects (Stout et al. 2000). Current recommendations call for applications of pyrethroids when adult weevils are merely present in flooded fields, with the intent of eliminating adult females before they oviposit. In most cases, the opportunity for effective control of adult rice water weevils is limited to seven days or less after the establishment of the permanent flood. If pyrethroids are not applied within this window of opportunity, significant oviposition occurs and the resulting larval infestations cannot be controlled using foliar insecticides. Most foliar applications take place in April and May, immediately after permanent floods are applied to rice fields. If adult populations are large, fields can be re-infested by weevil adults after initial applications of pyrethroids. In addition, if applications are not timed correctly, efficacy of a single application can be inadequate. A second application of pyrethroid insecticides may be needed in these cases.

In addition to the aforementioned constraints on the use of pyrethroids in weevil management, widespread agricultural and nonagricultural use of this class of insecticides raises concerns about their persistence in soils and non-target effects in aquatic environments due to

their broad-spectrum toxicity (Coats et al. 1989, Weston et al. 2004, Amweg et al. 2006, Lao et al. 2010, Moreira et al. 2010, Palmquist et al. 2011). In particular, pyrethroids used to manage this major early season pest of rice in Louisiana can have non target impact on crawfish (*Procambarus* spp.), a major aquaculture product co-produced with rice in Louisiana. Pyrethroids are extremely toxic to crawfish, with typical LC50's less than 1 μg/L (Jarboe and Romaire 1991, Paul and Simonin 2006, Barbee and Stout 2009). Moreover, the pyrethroids used in rice are formulated as liquids and applied aerially, features that increase the risk of crawfish exposure. Finally, the rice water weevil has a history of developing resistance to insecticides (Bowling 1968). Therefore, there is an urgent need for insecticides that are softer on crawfish, with a mode of action different from that of pyrethroids, and that are more effective against severe infestations.

Seed treatments in weevil management

Over the past several years, two insecticidal seed treatment formulations, DermacorX®100 (active ingredient: chlorantraniliprole) and Cruiser Maxx® (active ingredient thiamethoxam) have been introduced for drill seeded rice in the southern United states against rice water weevil. Chlorantraniliprole belongs to the anthranilic diamide class of insecticides that activate ryanodine receptors, thus stimulating uncontrolled calcium ion release from muscle cells and causing paralysis in insects (Cordova et al., 2006, Lahm et al., 2007). The remarkable safety of chlorantraniliprole on mammals has been attributed to its high selectivity for ryanodine receptors of insects over mammals (Lahm et al., 2007, 2009). This chemical has excellent larvicidal activity against many Lepidoptera and Coleoptera (Lahm et al. 2007, Clark et al. 2008, Koppenhöfer and Fuzy 2008).

Thiamethoxam is a neonicotinoid group of insecticide which exhibits stronger affinity to insect nicotinic acetylcholine receptors than vertebrate receptors (Tomizawa and Casida, 2003, 2005) thus accounting its safety on mammals. It provides excellent control of a broad range of commercially important pests in Hemiptera, Coleoptera as well some Lepidoptera. Thiamethoxam is developed both for foliar/soil applications and as a seed treatment for use in most agricultural crops all over the world (Maienfisch et al. 2001a, b)

A comparative evaluation of the impact of foliar pyrethroids and seed treatments on crawfish survival conducted in simulated rice paddies revealed minimal impact of seed treatments on crawfish. Under the same conditions, pyrethroids caused complete mortality of crawfish (Stout et al. 2011a). In addition lab bioassays on juvenile Procambarid crawfish revealed that thiamethoxam and chlorantraniliprole are at least 2-3 orders of magnitude less acutely toxic (96 h LC(50)) than pyrethroids (Barbee and Stout 2009, Barbee et al. 2010). In addition, being less acutely toxic to crawfish, both seed treatments have been shown to be more effective in suppressing populations of weevil larvae for longer periods than foliar pyrethroids. Thus, chlorantraniliprole and thiamethoxam have the potential to be more compatible with rice-crawfish rotations. Like other neonicotinoids, thiamethoxam exhibits excellent systemic characteristics (Maeinfisch et al. 2001b) while chlorantraniliprole has also been shown to possess some systemic activity (Lahm et al. 2009, Li et al. 2011).Thus these seed treatments possess merits that pyrethroids are lacking.

Small and large plot field evaluations have shown effective suppression of populations of weevil larvae by both seed treatments. However, reduction in weevil populations is generally greater in chlorantraniliprole-treated plots than in thiamethoxam-treated plots. (Stout et al. 2011b However, based on studies using thiamethoxam as a seed treatment, soil drench and foliar spray,

the systemic activity of thiamethoxam and chlorantraniliprole may decline as plants grow larger and dilute the insecticides (Nault et al. 2004, Castle et al. 2005, McCornack and Ragsdale 2006, Hoffmann et al. 2009, Magalhaeus et al. 2009).

Because these insecticides are systemic, thiamethoxam and chlorantraniliprole seed treatments could affect multiple life stages of the rice water weevil. Adult weevils use rice foliage as food source and insert their eggs into leaf sheaths; larvae mine through the leaf sheaths and later feed on roots. Although field experiments demonstrate the adequacy of chlorantraniprole and thiamethoxam from a crop protection perspective, studies on the activity of seed treatments on life stages of rice water weevil and on the persistence and potency of these insecticides *in planta* will help improve rational use of seed treatments in weevil management.

This dissertation is divided into five chapters. This chapter is a general introduction on rice water weevil and seed treatments in weevil management. The study in the second chapter investigates effects of foliage from plants treated as seeds with thiamethoxam and chlorantraniliprole on adult weevils. Since thiamethoxam seed treatment had adulticidal effects, the relationship between the actual levels of insecticides consumed by weevils (doses) with mortalities was quantified. Plant age-related dilution of thiamethoxam was also studied by comparing toxicities and insecticide residues of plants at distinct ontogenic stages. The study described in the third chapter investigates effects of seed treatments on egg numbers and survival of first instars. The study described in the fourth chapter investigated effects of thiamethoxam and chlorantraniliprole seed treatments on late instars feeding on roots, and differential activity of the two seed treatments on life stages were correlated with based on insecticide concentrations in rice leaves, shoots and roots. The fifth and the last chapter provides summary of all the studies. This study elucidated the biological activity of both insecticidal seed treatments on

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weevils and will hopefully contribute to rational use of seed treatments in weevil management

and will help monitoring insect susceptibility to seed treatments.

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CHAPTER 2

SYSTEMIC EFFECTS OF THIAMETHOXAM AND CHLORANTRANILIPROLE SEED TREATMENTS ON ADULT *LISSORHOPTRUS ORYZOPHILUS* (COLEOPTERA: CURCULIONIDAE) IN RICE[1]

Introduction

The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, is the most destructive insect pest of rice in the United States. This insect is native to the southeastern United States, but has, over the past 60 years, invaded important rice-growing areas in California, Asia, and Europe, and thus poses a global threat to rice production (Saito et al. 2005). All life stages of rice water weevil are intimately associated with its host plant. Adult weevils feed on the leaves of rice plants, leaving narrow feeding scars parallel to the venation of the leaves. Female adult weevils lay eggs primarily in leaf sheaths below the surface of the water in flooded rice fields (Stout et al. 2002). Larval rice water weevils may feed on or in rice leaves or stems for a short period of time, but quickly move down to rice roots and establish feeding sites on or in rice roots. The insects pass through four instars and a pupal stage in approximately 30 days on roots (Zou et al. 2004a) Feeding by larvae on the roots of rice plants results in reduced tillering and shoot growth in the vegetative phases of development, and reduced panicle densities and grain weights at harvest (Zou et al. 2004b). Small plot research and sampling of commercial fields indicate yield losses would likely approach 10% in Louisiana if no insecticides are used (Stout et al. 2011a).

Over the past 15 years, chemical control of the rice water weevil has been accomplished primarily by use of pyrethroid insecticides applied as foliar sprays to eliminate adult weevils in fields. There are several issues with the use of pyrethroids against the rice water weevil. Efficacy of foliar pyrethroid applications is often unsatisfactory when weevil pressure is heavy or sustained (M.J.S, personal observations). Also widespread use of pyrethroids raises concerns about their persistence in soils and non-target effects on invertebrates and fish (Coats et al. 1989, Amweg et al. 2006, Barbee and Stout 2009, Lao et al. 2010, Moriera et al. 2010, Palmquist et al. 2011). Finally, the rice water weevil has a history of developing resistance to insecticides

(Bowling 1968), and therefore, insecticides with alternative modes of action are desirable for resistance management.

Recently, two insecticidal seed treatments, Dermacor-X-100[®], which contains the anthranilic diamide chlorantraniliprole (CAP), and Cruiser [®] Maxx, which contains the neonicotinoid thiamethoxam (TMX), have been registered for use in rice in the southern United States against the rice water weevil. Anthranilic diamides including CAP target ryanodine receptors located on the sarcoplasmic reticulum of muscle cells leading to Ca^{++} depletion, feeding cessation, lethargy, muscle paralysis and death in insects (Cordova et al. 2006, 2007). The neonicotinoid, TMX, and its bioactive metabolite clothianidin (CLO) target nicotinic acetylcholine receptors (Wiesner and Kayser 2000) located at the post synaptic membranes of nerve junctions. Like other neonicotinoids, TMX exhibits excellent systemic characteristics (Maienfisch et al.2001) while CAP has also been shown to possess systemic activity (Lahm et al. 2009, Li et al. 2012). However, based on studies using TMX as seed treatments, soil drenches and foliar sprays, the systemic activity of TMX and CAP may decline as plants grow larger and dilute the insecticides (Nault et al. 2004, Castle et al. 2005, McCornack and Ragsdale 2006, Byrne et al. 2007, Hoffmann et al. 2009, Magalhaes et al. 2009).

Because both TMX and CAP are systemic and because all life stages of the rice water weevil interact with rice, multiple life stages of the insect may be affected by these chemicals when used as seed treatments. One goal of the present study was to compare the effects of TMX and CAP seed treatments on adult rice water weevils, which encounter these insecticides while feeding on the leaves of treated rice plants. Another goal was to quantify the relationship between actual levels of insecticides consumed by weevils (doses) with mortalities determined in acute toxicity assays. Finally, plant age-related dilution of TMX was studied by comparing

toxicities and insecticide residues of plants at seedling and tillering stages. Residues determined from ELISA were used to compare toxicities of the two age classes of rice.

Materials and methods

Insect source and bioassay procedures. Adult *L. oryzophilus* were collected by hand from untreated rice plots at the LSU-Agricultural Center-Rice Research Station (Crowley, LA) one day before use in experiments. Weevils obtained from fields were starved overnight in a large plastic container with water at the bottom. All feeding assays were conducted in polystyrene petri dishes (Corning™, NY 14831 USA) measuring 14.0 cm diameter and 2.5 cm deep. Petri dishes were lined with ca. 1.0 cm layer of 1.5% agar. In all bioassays, the youngest leaves of plants next to the central whorl of the main stem or the top two leaves of rice plants (2-4 leaf stage) were excised using scissors, and the bases of leaves were inserted into agar in a slanting manner to maintain leaf turgidity. Weevils were released into dishes immediately after placement of leaves. The number of weevils released and time of exposure varied in different experiments.

Seed treatment. Seeds of the rice variety CL-131 were used for insecticide treatments during both years of the study. Formulated insecticides were diluted in water containing a small quantity of Brilliant blue dye, and applied by pipette to seeds in Ziploc® bags to attain treatment rates of TMX (0-63 µg AI/seed) or CAP (0-100 µg AI/seed).

Plant rearing and stages tested. Plants for all experiments were grown in a greenhouse on the campus of Louisiana State University, Baton Rouge, LA. The potting mixture was comprised of two parts of autoclaved silt loam soil with one part each of sand and peat moss. The greenhouse was maintained at 28.0 ± 5^0C with ambient lighting conditions. Plants were watered daily but excessive watering was avoided to prevent leaching of insecticide and

disturbance of soil.   Rice plants tested for toxic effects of TMX at 2-4 leaf stage were neither

fertilized nor thinned. For other experiments, a complex fertilizer (19-5-8 N:P:K) was applied on

top of soil in pots at a rate of 2.5 g L^{-1} after plants were thinned to retain four plants pot^{-1}. In

experiments involving plant stages beyond the 6-7 leaf stage, pots with rice plants were placed in

large wooden basins lined with heavy black plastic that allowed plants to be flooded.

Effects of TMX and CAP seed treatments on weevil mortality and feeding. Feeding

assays were conducted to compare adulticidal effects of foliage grown from plants treated as

seeds with TMX or CAP. Plants (4) were grown from seeds (6-8 seeds per 2.0 L pot) treated with

TMX (0-35 µg ai/seed) or CAP (0-50 µg ai/seed). When plants reached the 6-7 leaf stage, the top

two leaves were excised and inserted in petri dishes as described above and 10 weevils were

released per dish and allowed to feed. Mortality was scored at 72 h and defined as lack of any

visible movement in weevils in ten minutes after being prodded by camel hair brush. Leaves

were replaced daily with fresh leaves. Plants were discarded after excision of leaves. Each

experiment was replicated four times (4 petri dishes treatment rate^{-1}) and two experiments were

conducted for both TMX and CAP using different plants and weevils collected on different days.

All experiments were maintained in growth chambers (Percival Scientific, Inc., IA, USA) at 25±

2º C and 14 h light: 10 h dark cycle.

Feeding activity was also estimated daily by measuring areas of feeding scars on leaves

removed after daily assessment of mortality. Leaves were taped to paper and scanned using a

flatbed desk top scanner (HP Scanjet G4050) set at resolution of 200 dpi. A ruler was scanned

next to leaves for scale. The stored images were processed in grey scale and the areas of feeding

scars (mm^2) were obtained by image analysis using Image J (public domain software, National

Institute of Health). The area of leaf surface damaged (mm^2) in a petri dish was divided by mean

number of survivors to estimate daily feeding activity per weevil. Estimates of feeding activity obtained for each petri dish for each day of the feeding assay were averaged to obtain consumption rate in units of $mm^2 day^{-1} weevil^{-1}$.

Statistical analysis. Cumulative (percentage) mortalities and areas of feeding scars were analyzed by analysis of variance with *post hoc* separation of means using Dunnett's test ($\alpha = 0.05$) to compare treatment rate with control. Data from TMX and CAP experiments were analyzed separately. The two separate experiments for each insecticide were treated as blocks for statistical analysis. All analyses were conducted in SAS using PROC MIXED (SAS Institute 2008).

Dose dependence of mortality and reduced feeding following TMX exposure. The goal of these experiments was to characterize the dose dependence of adult weevil mortality following exposure to TMX. To do this, estimates of leaf biomass removed by adult weevils during feeding were used in conjunction with LC/MS/MS analysis of insecticide residues in leaves to estimate oral doses of insecticides. Twenty to twenty –five seedlings were raised in 4.0 L pots as described earlier, and six replicate pots were used for each treatment rate. Two plantings were done: the first planting was assessed at 2-3 leaf stage while the second was assessed at 3-4 leaf stage. Excised leaves (6-8 pairs) were inserted in a petri dish and 40 weevils were released per dish and allowed to feed for one hour, at which time mortality was assessed as described above. The criterion used to score mortality in these experiments was inability of weevils to right themselves in ten minutes after placing them on their backs. For practical reasons (i.e., higher and more rapid mortality following exposure to 2-4 leaf stage compared with 6-7 leaf stage plants), the criterion for mortality used for these one-hour feeding experiments was less conservative compared to experiments described above. Experiments

using 2-3 leaf stage plants and 3-4 leaf stage plants were done at different times using different collections of weevils. For both experiments, assessments were done on three consecutive days using one petri dish/ 40 weevils each day per each treatment rate. Mortalities in treatments were adjusted for control mortality which did not exceed 2.5%.

Leaf samples for analyses of insecticide residues were collected by excising the top two leaves from fresh plants not used for mortality assessments. Two or three replicate samples of 2-5 g were collected. Each replicate sample was obtained by pooling the leaf material from two or three pots. Samples were placed in Ziploc bags on ice, then transported to the lab and stored at -20°C for up to 10 days before analysis.

Concentrations of TMX and CLO in leaves were determined using an LC/MS/MS analytical method. Frozen leaf samples were cut into small pieces and weighed. Cut samples were allowed to sit at room temperature for 30 min after which time 15 ml of MilliQ water were added. Samples were shaken thoroughly for one min, then 15 ml of 1% acetic acid in acetonitrile were added to mixed samples followed by addition of 6g $MgSO_4$ and 1.5g NaOAc. Samples were centrifuged at 3500 rpm for 15 minutes, the organic layers of centrifuged samples were transferred in volumes of 1.0 ml for sample cleanup in dispersive solid phase extraction mini-centrifuge tubes (Restek, Quechers Q 251 150mg $MgSO_4$, 50mg PSA, 50 mg C18) and centrifuged at 6000 rpm for 1 min in a micro-centrifuge. Resulting extracts of each sample were taken in 15ml centrifuge tubes and evaporated to dryness in a nitrogen stream at 35ºC. The dried residues were reconstituted to original volume by the addition of mobile phase (40% 0.01M formic acid/ 60% acetonitrile) and syringe- filtered through 0.2 μm PVDF filter. Thus 10-15 μl of matrix matched standards and filtered extracts of leaf samples from seed treatments were injected for analysis by UPLC/MS/MS (Waters Corp, Milford, MA, USA)) through isocratic

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elution of a reversed phased C_{18} column (2.1 x 50 mm, 1.7 μ particle size) at a flow rate of 0.3ml/min. Triple quadrupole detector was set at positive ESI (Extractor 2.00V RF 0.2 Source Temp 120^0 C Desolvation Temp 400^0 C Desolvation Gas (Nitrogen) 500 L/hr Collision Gas (Argon) 0.18ml/min) to enable specific ion transitions of TMX and CLO, which were filtered through multiple reaction monitoring. Under these conditions expected retention times of TMX and CLO were 0.5 min and 0.52 min and ion ratios of both were 60%. Standard curves were prepared using filtered extract of the control leaf samples mixed with stock solutions of TMX and CLO prepared in acetonitrile to create standards that contain a minimum of 95% matrix. The concentration of each chemical in a sample was estimated based on peak area obtained in the standards.

After mortality assessment, damaged leaves were scanned as previously described. However, for these experiments, area of feeding scars was measured using Sigma Scan Pro 5.0 (Systat Software Inc, 2007). Damaged area was determined by the trace measurement mode of operation. Using the values for insecticide residues in leaves and estimates of leaf tissue ingested, the oral dose was estimated. The relationship between the dose and mean weevil mortality was determined using probit analysis by (Finney 1972).

The relationship between area of leaf scars and mass of leaf tissue consumed was estimated by releasing a group of weevils on leaves of untreated plants. Leaves were cut into approximately 2 cm leaf pieces and weights were recorded. Two or three leaf pieces were inserted in each square cell of Integrid plates (BD Falcon™) provided with 1.5% Agar at the bottom and weevils were released in plates for feeding. Damaged leaf pieces were weighed and images were scanned to measure area of feeding scars using Sigma Scan Pro 5.0™. Concurrently, separate leaf pieces were inserted in an agar bottomed cell plate without weevils to

determine mean weight loss in undamaged leaf pieces to allow correction for water loss in weevil-damaged leaf pieces. A simple linear regression was done to predict the mass of leaf consumed based on area of feeding scars (slope: 0.11, $R^2 = 0.72$). Mean estimates of insecticides consumed in feeding assays were used in estimating LD_{50s}.

Statistical analysis: To quantify the toxicity of active substances consumed in these feeding assays PROC PROBIT procedure was used (SAS Institute 2008). Feeding activities of weevils in treatments was expressed as percentage of feeding over control and expressed as percentage relative feeding. Simple linear regression was done to determine the concentration dependence of feeding activity in weevils.

Effects of plant stage on adult mortality due to seed treatments: The effects of plant stage on the toxicity of leaves from plants treated as seeds with TMX were assessed by comparing mortalities of adult weevils fed on leaves from plants at the 5-6 leaf (~ 4 w old) and 4-5 tiller stages (~ 8 w old). Insecticide residues in foliage were analyzed using an ELISA method to quantify toxicity of seed treatments at both stages (see below). Six-eight seeds of various treatments rates were sown in four liter pots, and plants were used for feeding assays as described above. Forty weevils were released on two pairs of leaves collected from rice plants from each seed treatment rate. Assays were terminated at 96 hours and mortalities measured in treatments were adjusted for control mortality. Mortality was defined as complete immobility of weevils in 10 minutes after being placed on their backs. Three determinations were conducted over three consecutive days using foliage from different plants and using separate collections of weevils. The levels of TMX in rice leaves were estimated using a competitive ELISA adapted from that described for the quantification of imidacloprid in avocado leaves (Byrne et al. 2005, 2007) The ELISA kit is available commercially (Thiamethoxam HS plate kit, lot number 1098B;

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Beacon Analytical Systems Inc, 82 Industrial Park Road, Saco, Maine 04072, USA) and has a reported sensitivity range of 0.05 – 2.0 µg TMX L^{-1}. The assay was calibrated before processing samples to test for interference associated with rice leaf tissue homogenates as described previously (Byrne et al. 2005). Preliminary experiments indicated that background interference by leaf material was minimal at 50-fold dilution of leaf extract (0.4 cm^2 leaf disc area). A standard curve was developed by plotting log concentrations of technical grade TMX versus absorbance (slope = -0.482 R^2 = 0.91).

For determination of TMX levels, leaf discs were collected parallel to veins, avoiding mid ribs, using a 3mm diameter hole punch. Leaf discs were punched from the top pair of leaves of plants different from those for bioassays and pooled in 1.5 ml Eppendorf™ vials. Leaf discs were stored at -82^0C until residues were measured. Leaf discs in vials were homogenized in 100% methanol (6 discs/600 µl) by using pellet pestles (Kontes[®]). Homogenates were shaken vigorously for 1 h at room temperature and then centrifuged at 10 000 g for 5 min to pellet the particulate matter. Supernatants were dried completely under nitrogen at 35^0C and 4 psi, resuspended in 600 µl water containing 0.5 g L^{-1}TritonX-100, diluted by 50-fold, and quantified by ELISA. Three duplicate determinations were made from each treatment rate and plant size.

Statistical analysis: Adult weevil mortalities were transformed to arcsine values and analyzed as completely randomized block design with insecticide rate and plant stage as factorial treatment combinations. ANOVA for residues was done for treatment rates in which residues were detected at both growth stages.

Results

Mortality and feeding effects of plants due to seed treatments with TMX or CAP. Feeding

by weevils on leaves from 6-7 leaf stage plants treated as seeds with TMX but not CAP resulted

in significant mortality of weevils after a 72-h feeding period ($F_{5,41} = 9.5$ $P < 0.0001$; Table 2.1;

CAP: $F_{4,34} = 1.8$; $P = 0.15$).

Table 2.1. Comparison of adult rice water weevil mortality ± SE and feeding activity ± SE on
foliage of plants treated as seeds with CAP or TMX [a]

Seed treatment	Rate(μg ai/seed)	% mortality[b]	Leaf consumption rate (mm^2weevil^{-1} day^{-1})
CAP	0	15.0 ± 2.9	8.3 ± 0.7
	10	10.0 ± 5.8	8.3 ± 0.6
	25	20.0 ± 4.1	6.7 ± 0.9
	50	12.5 ± 6.3	7.3 ± 0.9
	100	16.3 ± 4.9	9.4 ± 0.7
TMX	0	3.8 ± 1.8 a	8.3 ± 0.5 a
	7	22.5 ± 4.5 b	6.9 ± 0.3 a
	14	21.3 ± 3.5 a	6.6 ± 0.6 b
	21	28.8 ± 3.0 b	5.5 ± 0.8 b
	28	42.5 ± 6.4 b	5.2 ± 0.3 b
	35	45.0 ± 7.7 b	6.4 ± 0.5 b

[a] Values represent mean (± SE) based on eight replicate petri dishes.
[b] Values followed by same letter are not significantly different from control after
Dunnett mean separation (alpha = 0.05)
[c] Mortality was assessed after 72 hours of feeding and was defined as lack of mobility
for ten minutes after being prodded with a camel hair brush.
All seed treatment rates of TMX increased weevil mortalities significantly compared to

control except for the 14 μg AI/seed. In addition, rates of foliar consumption per

weevil were significantly reduced ($F_{5,41} = 4.5$, $P = 0.002$) in all TMX treatments

except 7 μg AI/seed. In contrast, no significant reduction in consumption was found

due to CAP ($F_{4,34} = 1.7$; $P = 0.17$).

Toxicity of TMX following foliar feeding. Dose dependent weevil mortality was measured during 1 h leaf feeding assays conducted with rice at both the 2-3 and 3-4 leaf stage (Fig. 2.1). The LD_{50} for weevils feeding on 2-3 leaf stage plants was 447 pg TMX+ CLO/weevil
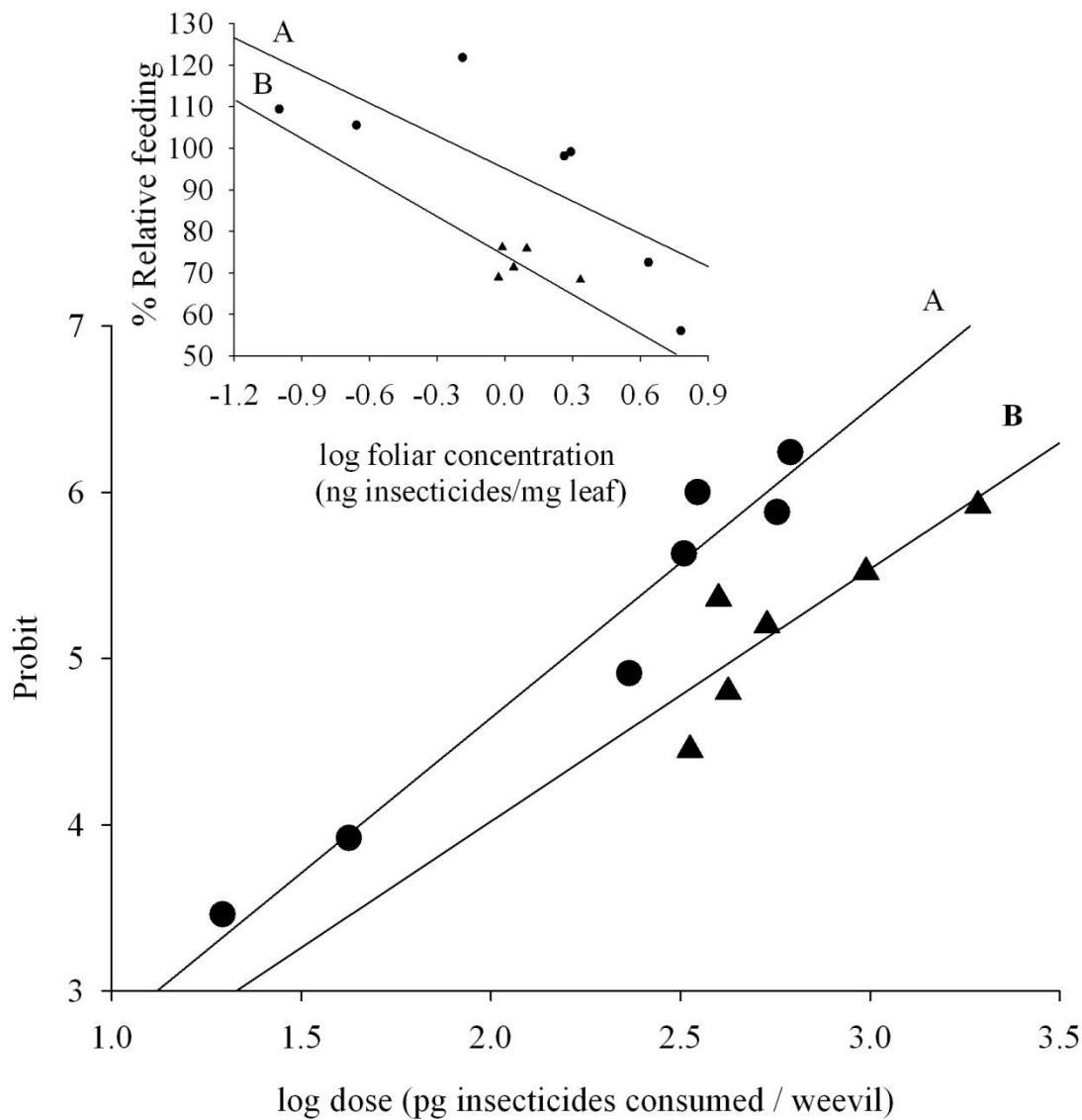
Figure 2.1. Dose responses of mortality and foliar feeding (inset) in rice water weevil after seed treatment with TMX in rice as measured in one-hr feeding assays conducted with plants at 3-4 (A) or 2-3 (B) leaf stage. Dose of TMX + CLN per weevil (pg) was estimated based on LC/MS/MS analysis of residues (ng insecticides / mg leaf) and quantification of leaf mass consumed (mg). Feeding damage was assessed by digital scanning and image analysis and is expressed as percent relative feeding = [(feeding in treatment/feeding on control)*100].

(95% fiducial limits = 25-830 pg/weevil; slope = 1.5 ± 0.5) but was lower (142 pg/weevil; 95%

fiducial limits: 102-180; slope = 1.86 ± 0.23) in experiments with 3-4 leaf stage plants. Leaf

consumption (Fig. 2.1 inset) decreased with increasing foliar concentration of TMX for both 2-3

leaf ($F_{1,4} = 14.9$, $P = 0.02$; slope = -24.3 ± 6.3) and 3-4 leaf stage plants ($F_{1,5} = 7.1$, $P = 0.04$;

slope = -26.2 ± 9.8).

Effects of plant stage on adult mortality: Overall, treatment of seeds with TMX

significantly increased adult mortality (main effect of seed treatment: $F_{5,22} = 44.5$, $P < 0.0001$)
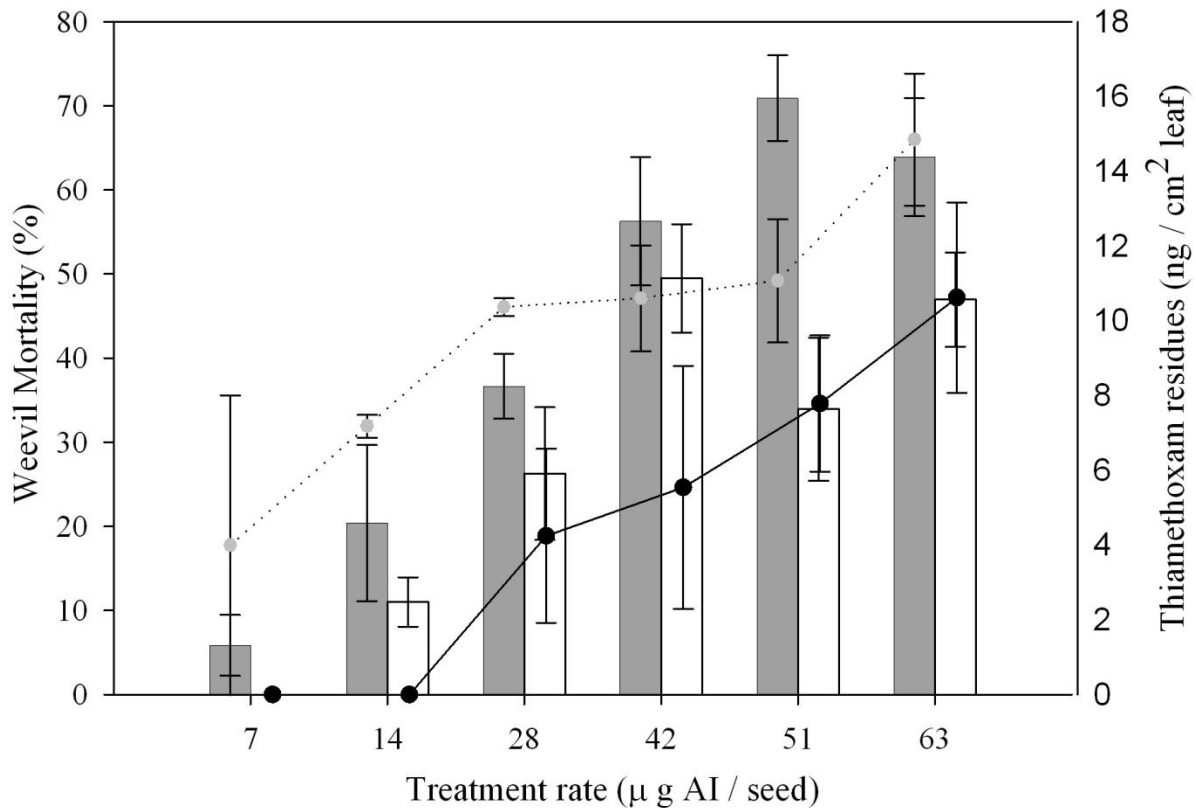
Figure 2.2. Effects of plant stage on adult mortality due to TMX as seed treatment. Mortalities were recorded 96-h after weevil release on excised foliage from plants at 5-6 leaf stage ▨ or tillering stage ▢. Leaves were analyzed for residues of TMX by ELISA. TMX was measured in 5-6 leaf ·●· and tillering stage —●— plants.

Adult mortalities resulting from feeding on foliage from TMX-treated plants generally increased increased with increasing seed treatment rate, but were consistently higher on foliage from plants at the 5-6 leaf stage than at the tillering stage (main effect of plant stage: $F_{1, 22} = 22.0$ $P = 0.0001$) (Fig 2.2). Mortalities on leaves from 5-6 leaf stage plants ranged from 5.9-64%, depending on seed treatment rate, whereas mortalities on leaves from tillering stage plants ranged from 0-47%. For weevils on leaves from 5-6 leaf stage plants, mortality increased with seed treatment rate between 7 and 51 µg ai/seed, although no significant increase in mortalities were measured at rates > 28 µg ai/seed. On the other hand, for weevils on leaves from tillering plants, no mortality was observed on treatments at 7 µg ai/seed, and mortality on leaves from plants treated at 42, 51 and 63 µg ai/seed differed significantly from that on leaves from the 14 µg ai/seed treatment.

Consistent with differences in weevil mortality at different treatment rates, TMX residues measured by ELISA increased with seed treatment rate and differed between plant stages. Insecticide treatment influenced levels of TMX detected in leaves ($F_{5, 24} = 17.7$, $P = 0.0001$) and foliar TMX residues in the 51 and 63 µg AI/seed treatments were significantly higher than at 7 or 14 µg ai/seed. In addition, foliar residues of TMX were correlated with seed treatment rate in both 5-6 leaf stage ($R^2 = 0.87$ $P = 0.004$) and tillering stage plants ($R^2 = 0.97$, $P < 0.0001$). Finally, adult mortalities were significantly correlated with TMX residues at both the 5-6 leaf stage ($R^2 = 0.73$, $F_{1,4} = 14.5$, $P = 0.019$) and tillering stages ($R^2 = 0.70$, $F_{1,4} = 12.1$, $P = 0.02$). The levels of TMX were significantly higher in rice at the 5-6 leaf stage than at the tillering stage ($F_{1, 24} = 8.2$, $P = 0.0003$). Further no residues were measured at the rates of 7 and 14µg ai/seed in the foliage of tillering stage plants.

Discussion

The rice water weevil, the most important insect pest of rice in the United States, passes through all of its life stages in association with rice plants. Over the past several years, seed treatments with two insecticides, CAP and TMX, have been introduced for control of the rice water weevil in rice. Both provide effective control although reductions in field populations of larvae are generally greater in CAP than TMX-treated plots (Stout et al. 2011). Both insecticides are systemic; thus, use of these chemicals as seed treatments in rice could affect multiple life stages of the rice water weevil. The experiments described here were undertaken to characterize the effects of CAP and TMX seed treatments on adult rice water weevils. In initial experiments contrasting the effects of CAP and TMX on adult mortality and feeding, TMX but not CAP seed treatment was toxic to adults and reduced foliar consumption rate. Further experiments were therefore confined to studies of the effects of TMX on adult weevils. The LD_{50} s for TMX in seed treatments were determined by estimating foliage consumption and determining foliar TMX residues using LC/MS/MS. Also, plant age-related dilution of TMX was demonstrated by combining adult mortality and ELISA-based estimation of residues for two age groups of rice plants.

The fact that seed treatment with TMX but not with CAP affected adult survival and feeding in 6-7 leaf stage plants is consistent with prior studies of these two insecticides. For example, in studies comparing the efficacies of CAP and TMX as soil drenches against black vine weevil, *Otiorynchus sulcatus* (F.) (Coleoptera: Curculionidae), on ornamental *Sedum* spp., TMX but not CAP reduced adult survival and leaf feeding (Reding and Persad 2009, Reding and Ranger 2011). In addition, neonicotinoids at low concentrations were found to reduce feeding in aphids (Nauen 1995, Daniels et al. 2009). The lack of CAP activity on adult mortality in this

study could have been due to limited systemicity of CAP in rice plants or insensitivity of adult weevils to CAP.

The bioassay method described here uses a biologically relevant mode of exposure (adult leaf feeding) to characterize the acute toxicity of TMX to weevils and resulted in, to our knowledge, the first LD_{50} values for leaf feeding insects on foliage of plants treated as seeds with TMX. The LD_{50} measured at 2-3 leaf stage (447 pg insecticides/weevil) was higher than that measured with 3-4 leaf stage plants (142 pg insecticides/weevil), although the overlapping confidence intervals preclude definitive statements about the relative potencies of TMX at 2-3 and 3-4 leaf stages. Some variability in estimates of acute toxicity is not surprising based on factors such as variation in location of adult weevil feeding sites, direct effects of TMX on feeding behavior, and non-uniform distribution of insecticides in leaves. With respect to the latter factor, higher accumulation of TMX in the tips of rice leaves than at basal regions of leaves after foliar treatment has been reported (Maienfisch et al. 2001). Similarly in cotton treated as seeds with imidacloprid, insecticidal concentrations were higher at apical portions of leaf blades than in central portions (El-Hamady et al. 2008).

In the experiment specifically designed to investigate the influence of plant age on adulticidal activity of TMX, adult weevil mortalities following leaf feeding were higher in younger than older rice plants, suggesting a plant age-related decline in adulticidal activity of TMX. Consistent with this finding, TMX residues (as determined by ELISA) were higher in younger than older plants indicating a decrease in insecticide concentrations as plant age. Age-related declines in TMX activity have been found in field monitoring of immature potato leaf hopper, *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae), on snap beans that received TMX as a seed treatment; suppression of *E. fabae* below economic threshold was found before bloom

stage of the crop but not at later stages (Nault et al. 2004). In addition, bioassays on the first and second flushes of leaves on avocado thrips, *Scirtothrips perseae* (Nakahara) (Thysanoptera: Thripidae) following soil drenchings with neonicotinoids revealed excellent activity on thrips during the initial flush, insufficient protection was observed during later flushes (Byrne et al. 2007). Finally, in efficacy trials of TMX seed treatments in soybean, effective suppression of the soybean aphid, *Aphis glycines* (Matsmura) (Hemiptera: Aphididae) was found during early vegetative stages of soybean but not at later stages (McCornack and Ragsdale 2006). In other tests with soybean, foliar residues of TMX declined with time, although no change in efficacy was seen against *A. glycines* (Magalhaes et al. 2009). Age-related declines in TMX residues in rice may help explain why TMX seed treatments show reduced effectiveness against rice water weevil larvae at later stages of rice growth after flooding (Stout et al. 2011b).

In the experiments reported here, two methods were used to determine TMX residues: an ELISA and an LC/MS/MS based analyses. Prior studies from our lab have shown that the two methods give comparable results. For example, in a previous study (Lanka, unpublished data), the foliar concentrations of TMX analyzed by LC/MS/MS showed a decline from the 5-6 leaf stage to tillering stage (4-5 tillers) (0.64 and 0.2 ng/mg, respectively). Similar residue values and similar age-related decline were found by ELISA in the plant age experiment reported here (0.73 and 0.2 ng/mg, at 5-6 and tillering stage plants, respectively). The ELISA method is more sensitive [Limit of Detection (LOD): 0.05 ppb] than LC/MS/MS (LOD: 0.02ppm) and requires less sample clean-up than LC/MS/MS-based methods. However, one major drawback of the ELISA-based assay is the inability to detect CLO, a bioactive product of TMX metabolism that might have contributed to toxicity in weevils. Due to the absence of cross-reactivity to the antibody used in the ELISA kit, CLO could not be detected. Although the levels of TMX found

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were treatment rate dependent, no residues were detectible at 14 µg ai/seed in older plants despite adult mortality being measured. Adult mortality at tillering stage in the absence of detectable residues may be the result of the inability of the TMX kit to detect CLO.

The lack of effect of CAP on adult mortality and feeding in these experiments is interesting in light of field experiments showing more effective suppression of weevil larvae by CAP than TMX treatments (Stout et al. 2011b). There are several possible explanations for the superior field efficacy of CAP. Firstly, greater persistence of CAP than TMX in soil or plant as mentioned above may contribute to greater suppression of weevil larvae. Secondly, the distribution profiles of both insecticides in rice plants are likely to influence the efficacy against rice water weevil. Unlike TMX, which shows high acropetal translocation and strongly protects apical portions of leaves, CAP concentrations are 8- fold higher in roots than in shoots as revealed by residue analysis (Lanka, unpublished), reflecting variation in plant distribution. Finally, CAP may be more inherently toxic than TMX on root feeding stages of rice water weevil. Regardless, the differential effects of CAP and TMX seed treatments on adult weevils demonstrate that the two seed treatments accomplish control of weevil populations in different manner.

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CHAPTER 3

EFFECTS OF CHLORANTRANILIPROLE AND THIAMETHOXAM RICE SEED TREATMENTS ON EGG NUMBERS AND FIRST INSTAR SURVIVAL OF *LISSORHOPTRUS ORYZOPHILUS* (COLEOPTERA: CURCULIONIDAE)

Introduction

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 years, 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 days, neonates mine through the leaf sheath for an undetermined period then move down to the roots (Grigarick and Beards 1965, Raksarart 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 (CAP: Dermacor-X-100[TM]), and the second containing the neonicotinoid, thiamethoxam (TMX: Cruiser Maxx [TM]), 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 vertebrates due to their weak affinity towards

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 (Maienfisch 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 impaired regulation and paralysis in vulnerable

species (Lahm et al. 2005, Cordova et al. 2006). This chemical has excellent larvicidal activity

against many Lepidoptera and Coleoptera (Lahm 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 (Lahm 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, Ugine 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 sub-

lethal 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: chlorantraniliprole) was supplied by Dupont ™ (Wilmington, DE, USA) and Cruiser™ 5FS (active ingredient: thiamethoxam) was supplied by Syngenta Corporation ™ (Greensboro, NC, USA).   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 µg [AI]/seed and chlorantraniliprole was applied at 10, 25 and 50 µg [AI]/seed. Seed treated with dye only served as a control (0 µg [AI]/seed). Plants for all experiments were grown by sowing four to six seeds of a single treatment rate in 1.0 L 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 ± 5^0C 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 approximately 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/l 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 one day 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 μg [AI]/seed) or chlorantraniliprole (0, 10 and 25 μg [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.

A total of 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, nine or ten 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 four days. Pots were then removed from cages and any weevils found on plants were removed. One plant from each pot (a total of nine or ten 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 examining plants under a dissecting microscope (Meiji Techno Co. Ltd, Tokyo, Japan).

For counts of first instars, the second plant from each pot (nine or ten 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, IA, USA) maintained at 28°C and a photoperiod of 14:10 h (L:D). 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, 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 plants treated or untreated as seeds with chlorantraniliprole or thiamethoxam for four days. In the second stage, weevils were released in infestation cages on plants untreated or treated as

seeds with the two insecticides. The rates used for foliar exposure to chlorantraniliprole were 0 (untreated), 25 and 50 µg [AI]/seed (i.e., approximately 1 or 2 times the field rate) and for thiamethoxam were 0 and 7 µg [AI]/seed (approximately 4 times lower than the field rate). These seed treatment rates used were rates shown in a prior study to cause minimal adult mortality in weevils when fed foliage from treated plants (Lanka et al. 2012). Table 3.1 outlines the regimes of exposure to chlorantraniliprole and thiamethoxam used in this study.

Table 3.1. 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.

Chemical	Foliar Feeding (µg AI/seed)	Plants infested (µg AI/seed)	Treatment designation[a]	Regime of Exposure[b]
Chlorantraniliprole	25	0	25-0	Foliar feeding
	50	0	50-0	
	0	25	0-25	Whole plant
	0	50	0-50	
	0	0	0-0[c]	No Exposure
Thiamethoxam	7	0	0-7	Foliar feeding
	0	7	7-0	Whole plant
	0	0	0-0	No exposure[b]

[a]pre-hyphenated term indicates the seed treatment rate used in foliar feeding and post-hyphenated 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.
[c]Weevils were fed on foliage from untreated plants and subsequently released on untreated plants.

The weevil mating pairs that were exposed to foliage from treated plants (25 or 50 µg [AI]/seed for chlorantraniliprole or 7 µg [AI]/seed of thiamethoxam) were subsequently released on untreated plants (0 µg [AI]/seed) (Table 3.1). 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 3.1). This regime involved feeding weevil mating pairs on foliage from untreated plants (0 μg [AI]/seed) for four days, then releasing weevils in infestation cages on plants treated as seeds with chlorantraniliprole (25 or 50 μg [AI]/seed) or thiamethoxam (7 μg [AI]/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 days 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. A total of two experiments were conducted using these exposure regimes with chlorantraniliprole and one experiment was conducted with thiamethoxam.

Effects on adult survivorship and 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 four days 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 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). A total of 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 3.1). A total of 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 four-day 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 3.1. 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 four days 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 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. 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

45

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 i.e., 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. 3.1). 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 to no seed treatment.

Seed treatment with thiamethoxam also significantly impacted egg-laying ($F = 63.4$; df = 2, 57; $P < 0.0001$) and larval emergence ($F = 33.0$; df = 2, 57; $P < 0.0001$) (Fig. 3.2) . Both egg and larval numbers were signficantly reduced by more than 90% at the 21 and 28 µg [AI] /seed treatments. Both the number of the eggs and first instars did not differ statistically between the two treatment rates.

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
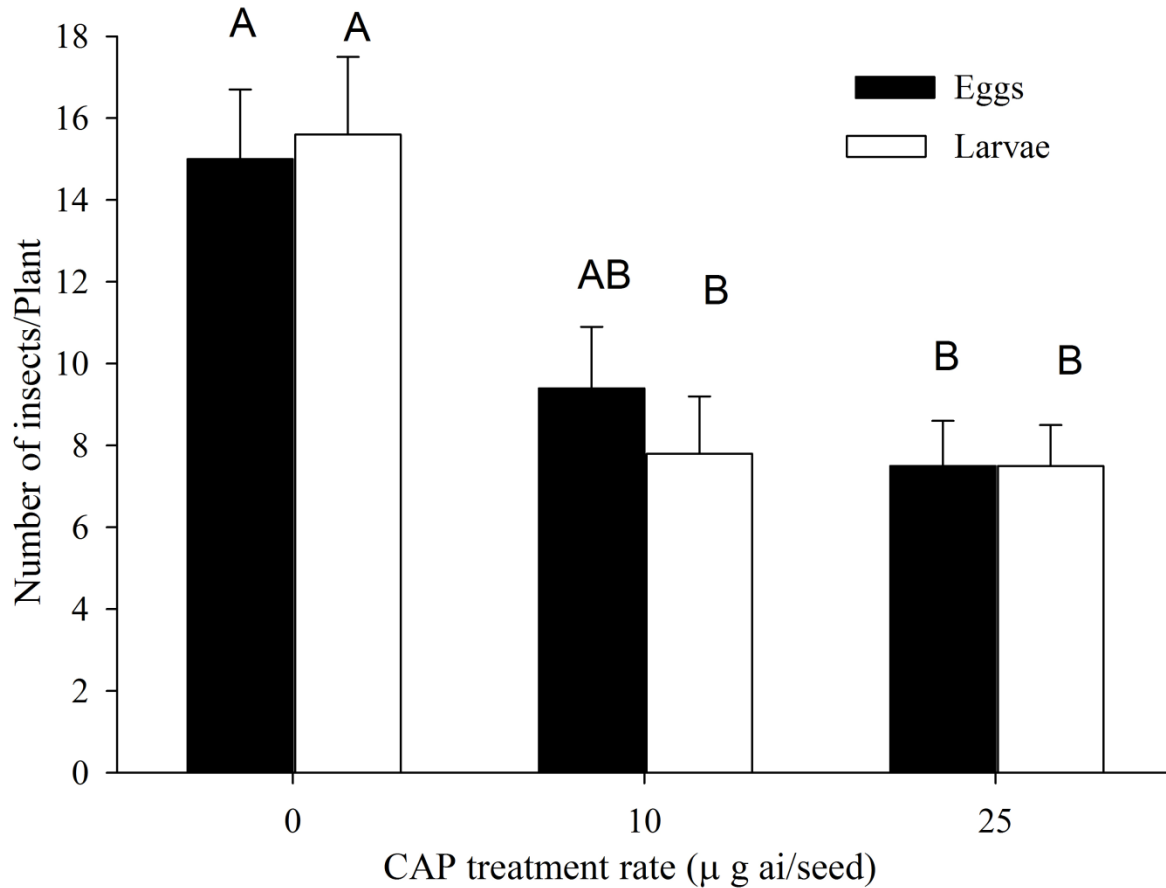
Figure. 3.1. Egg (closed bars; eggs/plant ± SE) and first instar density (open bars; larvae/plant ± SE) by insects exposed to rice plants treated as seeds under no choice with chlorantraniliprole (CAP) placed in cages (n=4; each containing nine or 10 plants for counting egg and larval numbers separately). Bars accompanied by the same letter indicate means not significantly different from one another.

($F = 0.3$; df = 2, 24; $P > 0.34$) or foliar consumption ($F = 0.4$; 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 ($F = 6.0$; df = 1, 12; $P = 0.02$) (data not shown).

Effects on eggs and first instar emergence. Chlorantraniliprole exposure regimes significantly affected subsequent egg numbers ($F = 3.3$; df = 4, 16; $P = 0.04$) and first instar emergence ($F = 13.9$; df = 4, 16; $P = 0.0002$) (Fig. 3.3). 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; $F = 11.5$; 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 ($F = 42.5$; df = 1, 16; $P < 0.0001$).
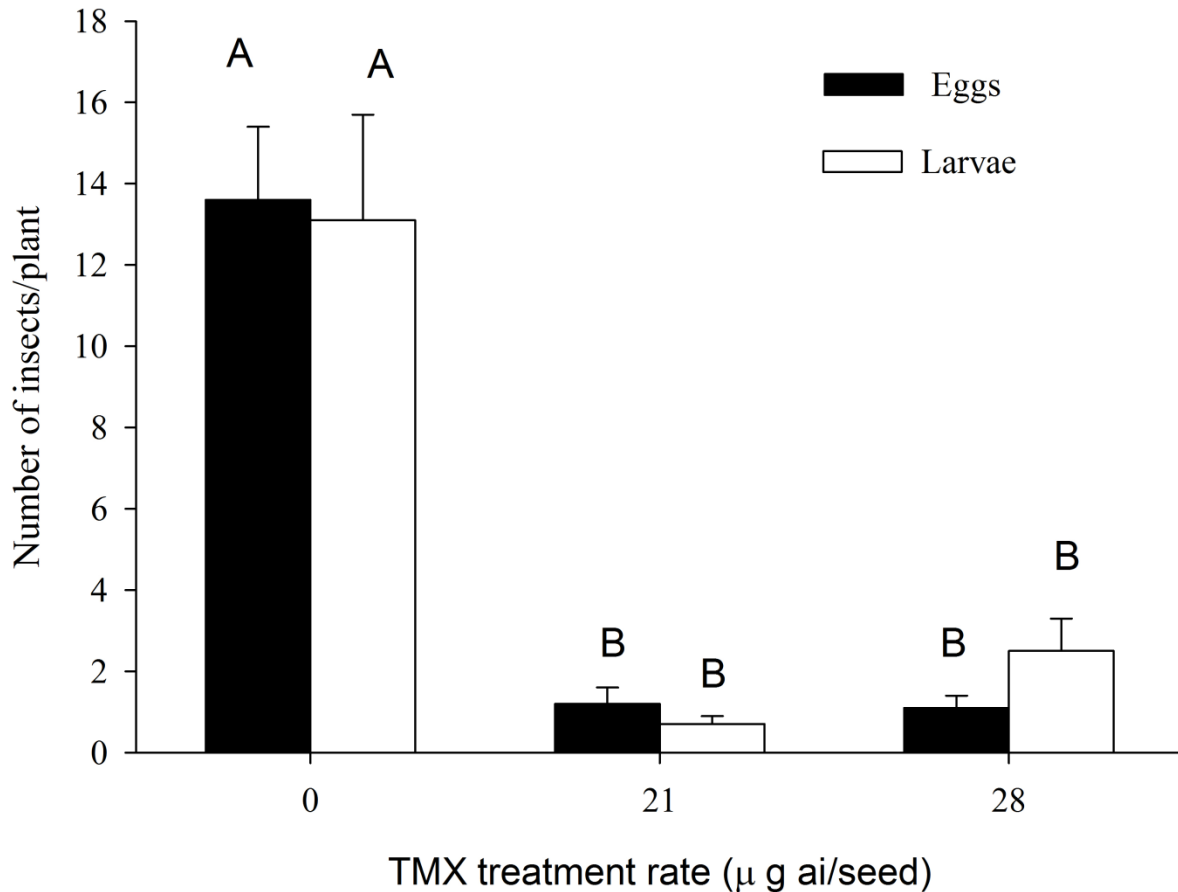
Fig. 3.2. Egg (closed bars; eggs/ plant ± SE) and first instar densities (open bars; larvae/ plant ± SE) by insects exposed to rice plants treated as seeds under no choice with thiamethoxam (TMX) placed in cages (n=2; each containing nine or 10 plants for counting egg and larval numbers separately). Bars accompanied by the same letter indicate means not significantly different from one another.

Furthermore, ingestion of foliage from chlorantraniliprole-treated plants by adults significantly

reduced egg-laying ($F = 8.5$; df = 1, 16; $P = 0.01$) and first instar emergence ($F = 9.9$; df = 1, 16;

$P = 0.007$) on untreated plants when compared to weevils that did not previously ingest

chlorantraniliprole (treatments 25-0 and 50-0 versus treatment 0-0). The magnitude of reduction

Fig. 3.3. The impact of exposure regimes to chlorantraniliprole (CAP) seed treatments on egg and first instar densities of rice water weevils. Weevil adults were fed foliage from plants not treated as seeds (0) and then allowed to access plants either treated as seeds (25 or 50) in WE (Whole plant Exposure; 0-25 and 0-50) or with plants not treated (0-0; control). In FE (Foliar feeding Exposure) weevil adults were fed on foliage from plants treated as seeds (25 or 50 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; larvae/plant ± SE) from plants placed in cages (n=4; each containing nine or 10 plants for egg and larval numbers separately).

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 versus treatments 0-25 and 0-50; $F = 0.33$; 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 versus treatments 25-0 and 50-0; $F = 17.0$; df = 1, 16; $P = 0.001$), suggesting larvacidal activity in chlorantraniliprole-treated plants.

In addition exposure regimes to thiamethoxam significantly affected subsequent egg numbers ($F = 28.1$; df = 2, 3; $P = 0.01$) and first instar emergence ($F = 21.7$; df = 2, 3; $P = 0.02$) (Fig. 3.4). 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 versus 0-0; $F = 8.5$; 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 without prior exposure to thiamethoxam ($F = 9.9$; df = 1, 3; $P = 0.007$). Ingestion of thiamethoxam by adults during the pre-exposure period significantly reduced egg-laying ($F = 28.1$; df= 1, 3; $P = 0.01$) and first instar emergence ($F = 1.7$; df = 1, 3; $P = 0.02$) on untreated plants when compared to weevils not exposed to thiamethoxam (treatment 7-0 versus 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 versus 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
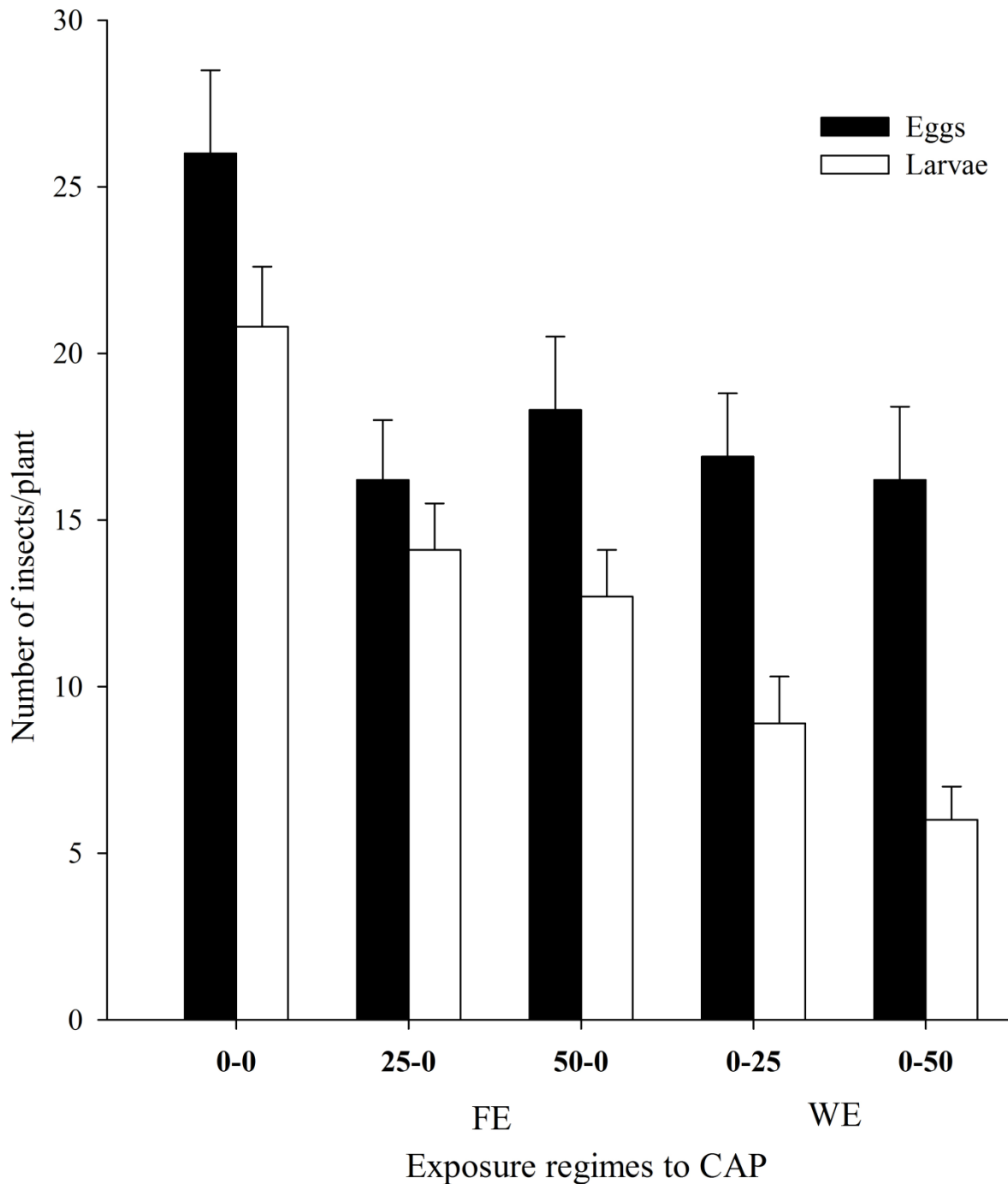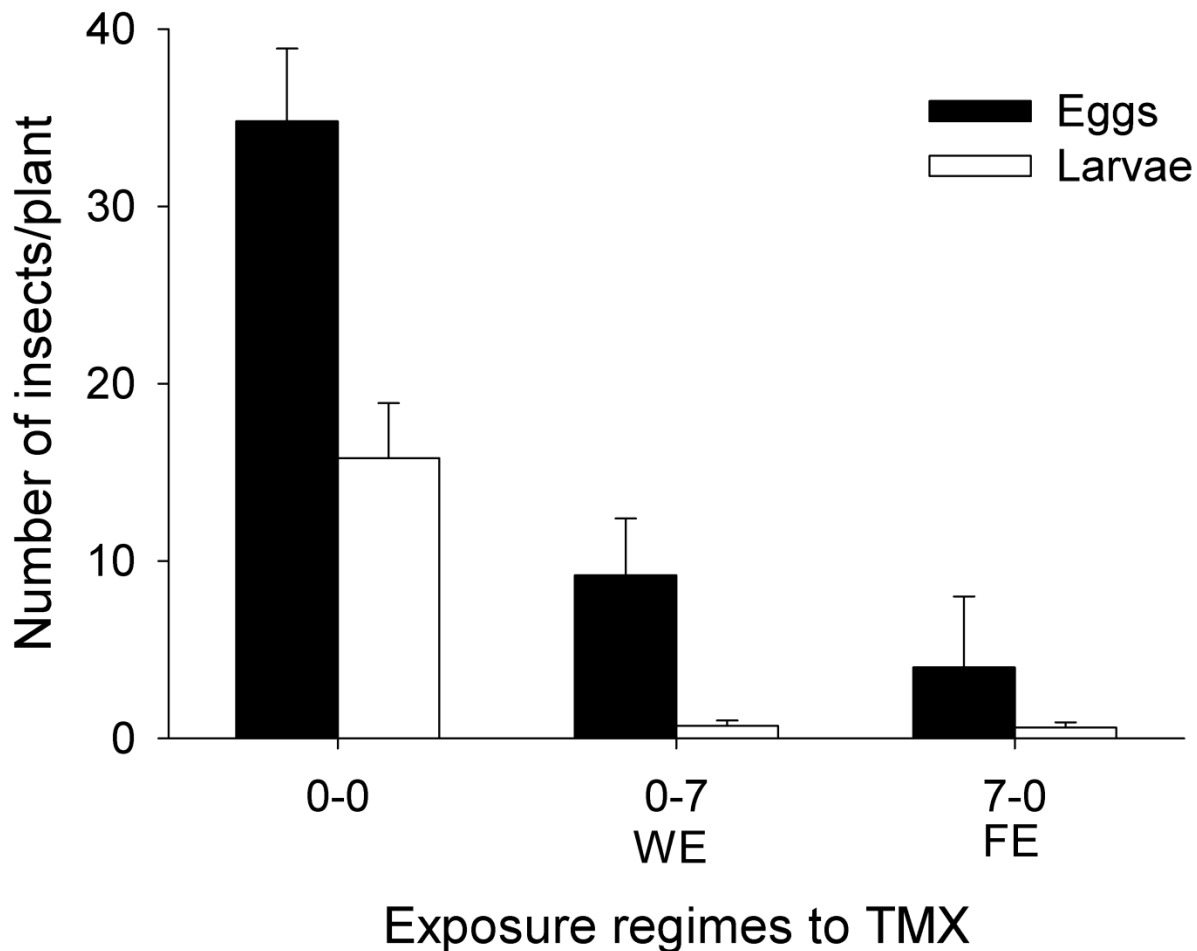
Figure 3.4. The impact of exposure regimes to thiamethoxam (TMX) seed treatment on egg and first instar densities of rice water weevils. Weevil adults were fed foliage from plants not treated as seeds (0) and then allowed to access plants either treated as seeds (7) in WE (Whole plant Exposure; 0-7) or with plants not treated (0-0; control). In FE (Foliar feeding Exposure) weevil adults were fed on foliage from plants treated as seeds (7µg [AI]/seed) and then provided with plants not treated as seeds (0). Egg (closed bars; eggs/plant ± SE) and first instar densities (open bars; larvae/plant ± SE) from plants placed in cages (n=2; each containing nine or ten plants for egg and larval numbers separately).

and by74% 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 weevillarval 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 present 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 present 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 adulticidal activity is low. Two other mechanisms for reduction in egg numbers could involve oviposition deterrence due to the presence of insecticides in above-ground tissues and 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 due to 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 present 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 following 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), as well as reductions in fertility of offspring in beet armyworms, *Spodoptera exigua* (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 sub-lethal concentrations. The reduction in egg numbers by weevils that survived exposure to thiamethoxam treatment in the present study is similar to

effects recorded on fecundity of the black vine weevil, *Otiorynchus sulcatus* (Coleoptera: Curculionidae) (Son 2004). Weevils that were prior fed for seven days 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 six weeks 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 following 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 whole plant exposure regime were lower than 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 insect larvae in chlorantraniliprole treated plants is scarce. Effective control of annual bluegrass weevil, *Listronotus maculicollis* Kirby (Coleoptera: 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 i.e., no difference was found in larval numbers between whole plant and foliar feeding regime. Due to 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 sub-lethal 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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CHAPTER 4

ACTIVITY OF CHLORANTRANILIPROLE AND THIAMETHOXAM SEED TREATMENTS ON LIFE STAGES OF RICE WATER WEEVIL AS AFFECTED BY DISTRIBUTION OF INSECTICIDES IN RICE PLANTS

Introduction

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 years, this insect has invaded into other important rice producing regions of world including Asia and Europe (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 (Everett and Trahan 1967, Muda et al. 1981, Smith 1983, Stout et al. 2002). After the incubation period of eggs is 5-9 days (Raksarart and Tugwell 1975), neonate larvae may feed on or in rice leaves or stems for a short period of time, but quickly move down to rice roots and establish feeding sites on or in rice roots (Grigarick and Beards 1965, Zhang et al. 2004). The insect passes through four instars and pupate in 27-30 days (Zou et al. 2004a). Severe root pruning can result in poor crop stand, reduced tillering, panicle size and grain weight thus causing economic losses to crop (Zou et al. 2004b). This pest has a potential to cause economic losses in excess of 10% under heavy weevil pressures (Stout et al. 2011).

Over the past several years, two insecticidal seed treatment labels i.e., Dermacor-X-100[TM] (active ingredient: chlorantraniliprole; CAP) and Cruiser Maxx [TM] (active ingredient thiamethoxam; TMX;) have being used in drill seeded rice in the southern United states against rice water weevil. The anthranilic diamide class of insecticides, which includes CAP, activates ryanodine receptors thus stimulating uncontrolled calcium ion release from muscle cells causing paralysis in insects (Cordova et al. 2006, Lahm et al. 2007). The high selectivity of CAP on insects has been attributed to its ~ 300 - >2000-fold selectivity for ryanodine receptors of insects

over mammals (Cordova et al. 2006, Lahm et al. 2007, 2009). As with anthranilic diamides, neonicotinoid class of insecticides, which includes thiamethoxam, exhibits stronger affinity to insect nicotinic acetylcholine receptors than mammalian receptors thus interfering with transmission of stimuli in insect nervous system (Tomizawa and Casida 2003, 2005).

Small and large plot field evaluations have shown effective suppression of populations of weevil larvae by both seed treatments, However, reduction in weevil populations is generally greater in CAP-treated plots than in TMX-treated plots (Stout et al. 2011). Although such field trials demonstrate the adequacy of CAP and TMX from a crop protection perspective, studies on the differential activity of seed treatments on life stages of rice water weevil and on the persistence and potency of these insecticides *in planta* will help improve rational use of seed treatments in weevil management. Prior studies on TMX and CAP rice seed treatments showed divergent effects on weevil life stages (Lanka et al. 2012). The activity of TMX was adulticidal and it suppressed oviposition by weevils, but did not impact emergence of first instars. In contrast, CAP showed no activity as an adulticide but reduced both the number of eggs and survival of first instars. However, these prior studies did not examine the effects of TMX and CAP seed treatments on late instars feeding on roots, nor did they attempt to correlate differential activity of the two seed treatments on life stages with differences in the distribution of these two insecticides in rice tissues.

In the present study, differential activities of CAP and TMX on weevil life stages were determined by monitoring densities of different life stages of the rice water weevil (adults, eggs, first instars, and late instars) after infestation of plants with adult rice water weevils. The residues of CAP and TMX were also determined in leaves, stems and roots with the objective of

explaining the differential activity on life stages of weevils in the context of distribution of insecticides in different tissues.

Materials and methods

Seed treatments, plant rearing and insect material: Dermacor™-X-100 (active ingredient: CAP) was supplied by DuPont ™ (Denver, CO, USA) and Cruiser™ 5FS was supplied by Syngenta Corporation ™ (Greensboro, NC, USA). A widely grown, long grained variety of rice (CL 131) 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 desired rates of TMX (0-50 μg AI/seed and CAP (0, 5 and 50 μg AI/seed). (Field rates of CAP and TMX were 25 and 28 μg AI/seed, respectively).Plants were grown in 2.0 L pots by sowing nine seeds of each treatment rate 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 ± 5^0C with ambient lighting. Pots were watered daily but excessive watering was avoided to prevent leaching of insecticide and disturbance of soil. A complex fertilizer (20-14-38 N: P: K) was applied to soil surface at a rate of 2.5 g l^{-1}after thinning to retain six plants per pot 10 days after germination. Experiments were conducted in large wooden basins lined with black plastic pond liner that allowed plants to be flooded. All experiments were conducted when rice plants reached the 6-7 leaf stage.

Weevils were collected from untreated rice plots at the Louisiana State University-Agricultural Center-Rice Research Station, Crowley, LA one day 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 trials involving infestation of plants, mating pairs were used to ensure a sex ratio of 1:1.

Impact of seed treatments on life stages of rice water weevil: Relative impacts of seed treatments on different life stages of rice weevil were measured with plants infested for four days with mating pairs of adults, then monitored for numbers of eggs laid in leaf sheaths, first instars emerging from plants, and densities of late instars on roots.

The weevils were confined to plants by inserting cages over each pot. Cages measured 10 cm diameter by 30 cm height with one end of each cage forced into the soil and top end closed with a mesh screen lid. These cylindrical cages also had two mesh-lined holes to allow air and water circulation. Pots were placed in basins in the greenhouse, basins were flooded to depth of approximately 20 cm. Weevils were allowed to feed, mate and oviposit on plants for a period of four days, then cages were removed to assess adult weevil mortality, which was defined as the inability of weevils to swim within two minutes after being placed them in plastic cups with water. The number of live adults was counted and all weevils were removed from plants. Numbers of weevils at three succeeding life stages (i.e., eggs, first instars and late instars) were obtained by destructive sampling of two plants from each pot at appropriate time points. Out of the 6 plants in each pot, four were removed immediately after assessment of adult mortality, two plants were used for counting eggs and two for counting emergence of first instars. For egg counts, soil was carefully removed from the roots, and plants were labeled and placed in 75% ethanol until bleached. The eggs were counted by examining plants under a dissecting microscope (Meiji Techno Co. Ltd, Tokyo, Japan). For first instars, soil was removed from roots of the second pair of plants and placed in test tubes that were filled with water, then placed in a controlled environmental chamber (Percival Scientific, IA, USA) maintained at 28°C

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at photoperiod of 14h L: 10h D. Larvae emerging from eggs deposited in these plants eventually move to the roots from which they were dislodged by shaking, and counted daily. After each count, water was replaced and counts were recorded until no larvae were recovered for three consecutive days. Pots containing the last pair of plants were placed back in the flooded basin for determination of late instar densities three weeks later. Soil from pots along with roots was placed in sieve buckets and soil was washed from roots. Buckets were placed into basins of salt water and larvae were counted as they floated to the surface of the solution (N'Guessan and Quisenberry 1994). Two experiments, each on CAP and TMX, were conducted. In each of these experiments, there were 6 or 12 pots of TMX-treated or CAP-treated plants, respectively.

Analysis of CAP and TMX residues in plants: In order to relate survival of different life stages of rice water weevil to insecticidal concentrations in plant tissues, residues were quantified in plants treated as seeds at 5 and 50 µg AI/seed for both the chemicals. Because rice water weevil interacts with different parts of rice plants as described earlier, insecticide concentrations were determined in leaves, roots and shoots (including leaf sheaths) of treated rice plants.

The groups of plants used for residue analyses were separate from those used in weevil experiments but were grown under identical conditions. The plant material from each pot represented a replicate of each treatment rate. Plant material from each pot was separated into three portions foliage (comprised of leaf blades), shoots (leaf sheaths were retained with shoots) and roots. Foliage was collected by excising leaf blades while retaining leaf sheaths on shoots. After the excision of foliage, shoots were collected by cutting the plants at the collar region. Finally, pots were washed in sieve bucket and soil was washed-off to separate root material. A total of 6 or 5 replicate pots were used for chemical analysis of CAP and TMX residues,

respectively. Approximately 2-5 g of each tissue type per pot were placed in Ziploc bags on ice, and then transported to the lab and stored at -20°C for up to 10 days before analysis.

Frozen samples were cut into small pieces and weighed. After 30 minutes at room temperature, 15 ml of MilliQ water were added to each cut sample. Samples were shaken thoroughly for one min, then 15 ml of 1% acetic acid in acetonitrile were added followed by addition of 6g MgSO4 and 1.5g NaOAc. Samples were centrifuged at 3500 rpm for 15 minutes, the organic layers of centrifuged samples were transferred in volumes of 1.0 ml for sample cleanup in dispersive solid phase extraction mini-centrifuge tubes (Restek, Quechers Q 251 150mg MgSO4, 50mg PSA, 50 mg C18) and centrifuged at 6000 rpm for 1 min in a micro-centrifuge. Resulting extracts of each sample were transferred into 15ml centrifuge tubes and evaporated to dryness in a nitrogen stream at 35ºC. The dried residues were reconstituted to original volume by the addition of mobile phase (40% 0.01M formic acid/ 60% acetonitrile) and syringe- filtered through 0.2 µm PVDF filter. Thus 10-15 µl of matrix matched standards and filtered extracts of leaf samples from seed treatments were injected for analysis by UPLC/MS/MS (Waters Corp, Milford, MA, USA) through isocratic elution of a reversed phased C$_{18}$ column (2.1 x 50 mm, 1.7 µ particle size) at a flow rate of 0.3ml/min. Triple quadrupole detector was set at positive ESI (Extractor 2.00V RF 0.2 Source Temp 120^{0}C Desolvation Temp 400^{0}C Desolvation Gas (Nitrogen) 500 L/ hr Collision Gas (Argon) 0.18ml/min) to enable specific ion transitions which were filtered through multiple reaction monitoring[(CAP: 484.2 $>$ 453, 453$>$286;TMX:292$>$ 211, 211$>$189.1)]. Under these conditions expected retention times (CAP and TMX: 0.79 and 0.52 min, respectively) and ion ratios (CAP: 90%; TMX: 60%) were the bases for detection of insecticides. Standard curves were prepared using filtered extract of the control leaf samples mixed with stock solutions of CAP prepared in acetonitrile to create

standards that contain a minimum of 95% matrix. The concentration the compound in a sample was estimated based on peak area obtained in the standards.

Statistical analysis: Two experiments were conducted each on CAP and TMX. In each of thse experiments, there were 6 or 12 pots of TMX-treated or CAP-treated plants, respectively. The effect of CAP or TMX on each life stage was analyzed separately using generalized linear mixed models with seed treatment rate as a fixed effect and experiment as a random effect. Data were analyzed in SAS using PROC GLIMMIX (SAS Institute, 2008). Densities of eggs, first and late instars (number/plant) were all modeled using the Poisson distribution for residuals. Percent adult survival was modeled using the Gaussian distribution after being converted to arcsines before analysis. Differences among treatment rates were compared using Tukey's mean separation. Mean numbers of eggs, first instars and late instars obtained from plants were used to estimate percent reduction in weevil survival (relative to untreated controls) as insects progressed from egg through late instar stages.

The influence of CAP and TMX application rate and plant tissue on the concentrations of insecticides was analyzed with a two-way ANOVA by using PROC GLIMMIX (SAS Institute 2008). The seed treatment rate and the type of plant tissue were used as fixed effects.

Results

Impact on life stages: Seed treatment with CAP had no effect on adult survival ($F_{2, 68} = 1.0$, $P = 0.4$) (data not shown) whereas, number of eggs by weevils on CAP-treated plants was significantly reduced ($F_{2, 68} = 41.2$, $P < 0.0001$) (Fig. 4.1). The egg-laying on plants treated at 5 and 50 µg AI/seed was reduced by 24 and 50%, respectively. Although the impact on egg-laying was significant at both treatment rates, plants treated at 50 µg AI/seed had significantly fewer

Figure 4.1. Impact of CAP seed treatment on life stages of rice water weevil. Densities of egg (dark bars; eggs per plant ± SE), first instar (open bars; larvae per plant ± SE) and late instar (gray bars; larvae per plant ± SE) following infestation of whole plants in a greenhouse (n=24). For a given life stage bars accompanied by the same letter are not significantly different from each other. Inset: Impact on life stages expressed as percent reduction relative to densities on treated plants. Percent reductions represent averages of two experiments.

eggs than those treated at 5 µg AI/seed. The emergence of first instars from CAP-treated plants

was also significantly reduced ($F_{2, 68} = 66.4$, $P < 0.0001$). Significantly fewer first instars

emerged from plants treated at 50 µg AI/seed than from untreated or treated plants at the low

rate. The reduction in first instar emergence was 61% from plants treated at 50 µg AI/seed.

Densities of late instars were reduced by 90% relative to controls at both low and high

application rates ($F_{2, 68} = 50.3$, $P < 0.0001$).

Treatment of seeds with TMX significantly reduced adult survival ($F_{2, 32} = 113$, P <

0.0001). Adult survival on plants treated at both rates was significantly lower than on untreated

plants, and lower on plants treated at 28 µg AI/seed than 14 µg AI/seed (data not shown). The

number of eggs laid by weevils was also reduced due to TMX treatments ($F_{2, 32} = 13.9$, P

<0.0001). This reduction was 52 and 67% at treatment rates of 14 and 28 µg AI/seed,

respectively. The difference in egg-laying between treatment rates was not significant (Fig. 4.2).
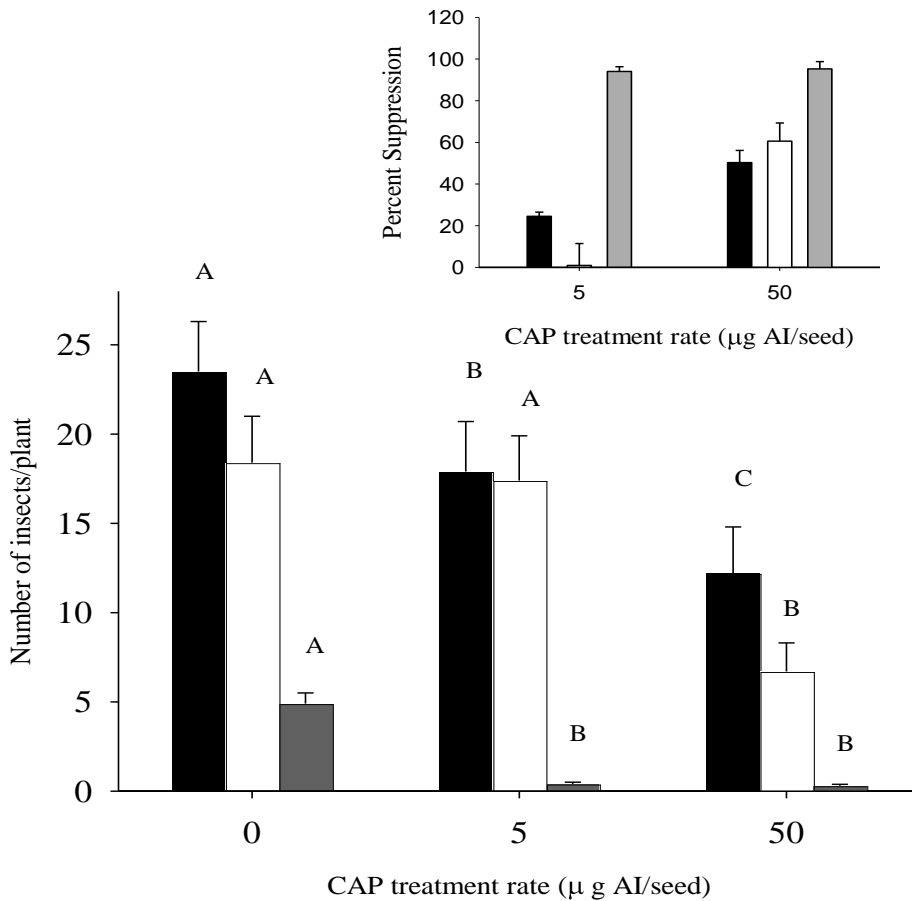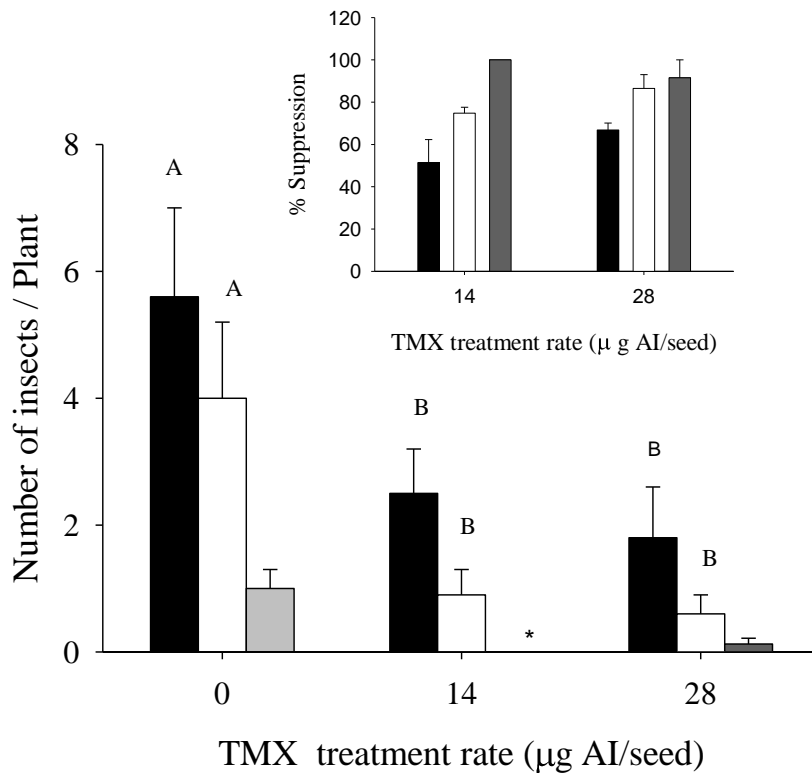

Figure. 4.2. Impact of TMX seed treatment on life stages of rice water weevil. Densities of egg (dark bars; eggs per plant ± SE), first instar (open bars; larvae per plant ± SE) and late instar (gray bars; larvae per plant ± SE) following infestation of whole plants in a greenhouse (n=12). For a given life stage bars accompanied by the same letter are not significantly different from each other. *No late instars were recovered. Inset: Impact on life stages expressed as percent reduction relative to densities on treated plants. Percent reductions represent averages of two experiments.

In addition, first instar emergence from plants was adversely impacted due to treatment with TMX ($F_{2, 32} = 18.5$, $P < 0.0001$) with reductions of 75 and 80% at 14 and 28 µg AI/seed, respectively. Finally, the mean number of late instars recovered from untreated plants was low (1.0 ± 0.3) and only three larvae from 28 µg AI/seed and none from 14 µg AI/seed were recovered.

Residues of CAP and TMX in plants: The concentration of CAP in treated rice plants was significantly influenced by the type of tissue ($F_{2, 30} = 5.9$, $P = 0.007$) (Table 4.1). Higher levels of CAP were found in roots than in leaves ($P= 0.02$) or shoots ($P= 0.01$). Although residues in foliage tended to be higher than in shoots, the difference was not significant. Also, residues found in plants were significantly impacted by seed treatment rate ($F_{1, 30} = 6.9$, $P = 0.01$). The interaction between seed treatment rate and tissue type was significant ($F_{2,30} = 5.9$, $P = 0.007$). In plants treated at 5µg AI/seed, residues levels in tissues did not differ significantly from each other. The concentration of CAP in roots of 50 µg AI/seed treatment was highest compared to shoots or leaves at 5 or 50 µg AI/seed.

Table 4.1. Effect of plant tissue on residues in rice plant treated as seeds with CAP

Treatment rate (µg AI/seed)	Tissue		
	Foliage	Shoots	Roots
5	73.0 ± 13.0	7.0 ± 0.9	43.0 ± 27.0
50	161.0 ± 17.0	24.0 ± 2.0	1868 ± 730
rate	$F = 6.9$	df = 1, 30	$P = 0.01$
tissue	$F = 5.9$	df = 2, 30	$P = 0.007$
rate*tissue	$F = 5.8$	df = 2, 30	$P = 0.006$

Limit of Detection in ppb (ng/g): Shoots: 1.0; Roots: 20; and Leaf: 2.0

The concentration of TMX residues in treated rice plants was significantly influenced by the type of tissue ($F_{2, 24} = 9.5$, $P = 0.0009$) (Table 2). Higher levels of TMX were found in foliage than in roots ($P= 0.009$) or shoots ($P= 0.001$). Also, residues found in plants were

significantly influenced by seed treatment rate ($F_{1, 24} = 30.6$, $P < 0.0001$). The foliar TMX concentration at 50 µg AI/seed treatment was highest compared to concentrations of TMX in roots or shoots at 5 or 50 µg AI/seed. However, in 5µg AI/seed, residues levels in tissues did not differ significantly from each other. The interaction between seed treatment rate and tissue type was significant ($F_{2,24} = 4.2$, $P = 0.03$).

Table 4.2. Effect of plant tissue on residues in rice plant treated as seeds with TMX

Treatment rate (µg AI/seed)	Tissue		
	Foliage	Shoots	Roots
5	116.0 ± 20.0	64.0 ± 11.0	52.0 ± 14.9
50	478.0 ± 99.0	228.0 ± 30.0	156.0 ± 38.0
rate	$F = 30.6$	df = 1, 24	$P < 0.0001$
tissue	$F = 9.5$	df = 2, 24	$P = 0.0009$
rate*tissue	$F = 5.9$	df = 2, 24	$P = 0.007$

LOD (ng/g) : 20 ng/g (foliage, shoots and roots)

Discussion

Seed treatments have been used for the management of *L.oryzophilus* in rice in the southern United States for the past several years. The systemic nature of the active ingredients and the complexity of rice water weevil interactions with rice plants suggest that several life stages of this pest could be affected by seed treatments. Previous studies revealed divergent activity of seed treatments on life stages of rice water weevil: TMX but not CAP impacted adult survival (Lanka et al. 2012) both TMX and CAP suppressed egg-laying in adults; CAP but not TMX affected egg or first instar survival (Lanka et al. 2012 in press). However, effects on late instar weevil larvae were not assessed in these previous studies. The emphasis of the present study was to investigate the differential activity of these seed treatments on all weevil life stages, including late instars, and to relate the differential biological activity of CAP and TMX to patterns of distribution of active ingredients in plants. This study revealed the greatest reduction

in weevil densities occurs in late instars feeding on roots in CAP-treated plants while patterns of effects on other life stages were similar to those observed in prior studies. Similarly, TMX impacted adult survival and egg-laying while effects on first instar were unclear due to poor oviposition by weevils in present study. The analyses of plant residues were consistent with biological activities: High above ground concentrations of TMX in plants were consistent with high biological activity of TMX on adult weevils, and, high CAP residues found in roots was consistent with strong suppression of root feeding larvae.

Destructive sampling of subsets of infested plants at appropriate time points in this study allowed characterization of natural attrition in weevil populations on plants as well as differential attrition in weevil populations on CAP-treated and TMX-treated plants as insects progressed through different life stages. The decline in adult weevil densities on untreated plants over the four-day infestation period was 8.4% (91.6% survivorship) in experiments with CAP, while mortality in adult weevils on untreated plants was approximately 35.0% in experiments with TMX. In both the CAP and TMX experiments, the decline in weevil densities on untreated plants from the egg stage to first instar and from first to late instars were at ~22% and ~74%, respectively. In contrast to this natural attrition, weevils underwent progressive declines of 2.8 and 98% from egg to first instar and from first instar to late instars on plants treated with CAP at 5 µg AI/seed. The corresponding declines on plants treated at 50 µg AI/seed of CAP were 62 and 96%. In TMX-treated plants, in contrast, reductions in adult weevil densities during the four-day infestation period were higher than on untreated plants (57 and 72% at low and high rate of TMX, respectively). The decline in weevils from egg stage to first instar and first to late instars on TMX-treated plants was about 65% and 100%, respectively. Thus, the reduction in weevil population in CAP-treated plants was characterized by pronounced activity on larval weevils

feeding on roots. On the other hand, TMX accomplished weevil control primarily by reducing adult survival and egg-laying.

The insensitivity of adult survivorship to CAP treatments (in contrast to high activity of TMX against adults) could, in theory, be attributable to limited systemicity of CAP or to poor adulticidal activity (potency) of CAP. Consistent with the former hypothesis foliar concentrations of CAP were three-fold lower than foliar concentrations of TMX at a seed treatment rate of 50 µg AI/seed. The poorer adulticidal potency of CAP relative to TMX is supported by prior studies that revealed mortality of weevils when weevils were fed on foliage containing the concentrations of TMX lower than the foliar CAP at high treatment rate in present studies (prior studies showed 15% mortality in adult weevils when corresponding foliar TMX concentrations were 100 ng/g leaf). Thus the low adulticidal activity of CAP may also be attributable to limited inherent potency of CAP on adult weevils. Further studies are required to test the hypothesis that CAP has lower potency than TMX against adults. Lack of adult mortality was reported earlier in adult black vine weevil *Otiorynchus sulcatus* (Coleoptera: Curculionidae), when weevils were fed on foliage or exposed to potted ornamental *Sedum* sps treated with CAP as surface drenches to soilless medium (Reding and Persad 2009). These authors also speculated that the lack of adult mortality to insensitivity in insects or limited systemicity of CAP.

Although foliar CAP concentrations in rice plants were apparently insufficient to cause adult mortality, they were sufficient to reduce egg-laying. The reduced egg-laying in the present study was 24 and 48% at 5 and 50 µg AI/seed. A previous study showed 38 and 50% reduction in number of eggs at 10 and 25µg AI/seed, respectively (Lanka et al. 2012 in press). This previous study also showed that adult consumption of foliage containing CAP suppressed

oviposition when adults were subsequently presented non-treated plants as oviposition substrates. Similarly, the reduced egg-laying on TMX-treated plants in present study was also found in a previous study, although the reduction in the previous study was somewhat higher (>90% reduction in egg-laying on plants treated at 21 and 28 µg AI/seed). In contrast to CAP, the reduction in egg-laying on TMX-treated plants could be attributed partly to reduced adult survival.

The reduction in the emergence of first instars from CAP-treated plants, also seen in a previous study, suggests ovicidal or larvicidal activity of CAP. The higher percent reduction in weevil densities from egg to first instar stages in plants treated with 50 (45%) than in plants treated with 5 µg AI/seed (2.8%) was consistent with higher CAP residues in shoots at the higher treatment rate relative to the lower rate. The apparently small effect on first instars on plants at the 5 µg AI/seed treatment rate contrasts with the fact that egg-laying was reduced by 24% compared to untreated plants at this rate. It may be possible that egg-laying in weevils is more sensitive to CAP than are first instars, or that CAP in shoots ingested by first instars were too low to cause mortality. Shoot exposure bioassays using first instar larvae on treated plants are needed to elucidate the threshold concentrations for larvicidal impact of CAP on first instars. Such studies are also needed to verify the impact of TMX-treated plants on first instars because data in the present study could not reveal such an impact due to low oviposition on plants.

The greatest reduction in weevil densities on CAP-treated plants occurred in larvae as they as feed on roots. Despite the difference in the effects of CAP at low and high rates of seed treatments on first instars, both rates were equally efficacious in reducing the density of later instars on roots. This high biological activity on root feeding forms was consistent with residue data, which revealed a tendency of CAP to accumulate in roots. Other studies reported higher

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efficacy of CAP on early than on late instars in root feeding June beetles (Coleoptera: Scarabaeidae) following granular application of CAP to soil (Koppenhöfer and Fuzy 2008).

Overall, the high concentrations of TMX in above ground portions of rice plants was consistent with patterns of biological activity characterized by high adult mortality and significant reductions in oviposition. Such biological partitioning of the activity of TMX has interesting consequences for field control of rice water weevil. The inconsistent control of rice water weevil in TMX-treated plots observed in some experiments (Stout et al. 2011) may be explained by dilution of adulticidal effect due to abiotic (such as UV light, transpiration, temperature, root to soil ratio) and biotic factors (such as metabolism in soil, plant, and plant growth- related dilution). A previous greenhouse study on adulticidal effects of TMX-treated rice conducted at distinct ontogenic stages of rice (5-6 leaf and tillering stages) revealed attrition in adulticidal activity manifested by decreased mortality and reduction in foliar concentrations at the tillering stage relative to the 5-6 leaf stage (Lanka et al. 2012) Such dilution in insecticidal activity due to plant growth and metabolism could conceivably occur at much accelerated rates under field conditions as in imidacloprid-treated plants (Sur and Stork 2003).

In contrast, the superior field efficacy of CAP seed treatments, which provide persistent control of larval populations of weevils for up to 6 weeks after permanent flooding, is partially explained by the tendency of CAP to accumulate in roots. Concentrations of CAP in roots were approximately 8-fold higher than concentrations in shoots and leaves in some cases. The tendency of CAP to accumulate in roots may allow reduced seed treatment rates to be used effectively. A recent small-plot field study showed excellent control of larvae at application rates as low as 7 g AI/acre. Further field studies are required to optimize the commercial rates of this highly potent larvicide. The affinity of this insecticide with roots may be explained by its

slightly lipophilic nature. This insecticide is expected to accumulate in soils if used continuously (USEPA 2008).

The contrasting patterns of distribution of CAP and TMX in plants are explained by reference to the different physical and chemical properties of both chemicals such as solubility, biphasic and dissociation constants. Despite these differences, both insecticides provided good control of weevils under greenhouse conditions, albeit by different mechanisms: TMX primarily on adult weevils and egg-laying while CAP on egg-laying and larval control. However, the different physico-chemical properties of CAP and TMX apparently result in different levels of persistence and ultimately efficacy.

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CHAPTER 5

Summary and Conclusions

Seed treatments with chlorantraniliprole and thiamethoxam have been used in rice water weevil management in the past several years. Weevil life stages are intimately associated with rice plants. Adult weevils feed on leaves causing longitudinal leaf feeding scars. Upon flooding, females lay eggs largely in basal half of leaf sheaths. Upon hatching, larvae mine through the leaf sheaths and quickly move down to roots and start pruning roots. Thus adults use rice foliage as food source and leaf sheaths for laying eggs.Llarvae first feed on leafsheaths and on roots. Thus seed treatments with systemic insecticides can impact different stages of rice water weevil.

Systemic effects of seed treatments on adult weevils were investigated for both thiamethoxam and chlorantraniliprole by using foliage from plants treated at different rates (thiamethoxam: 0-35 μg AI/seed; chlorantraniliprole: 0-50 μg AI/seed). Feeding for 96 h on foliage from plants treated as seeds with thiamethoxam (6-7 leaf stage) increased adult mortality and decreased foliar consumption rate while chlorantraniliprole did not cause such effects. The LD-50 of systemic foliar thiamethoxam was determined by combining leaf area lost due to feeding and insecticide residues analyzed by LC/MS/MS. The loss in leaf area was converted to mass by using simple linear regression. The 1 h LD50 for weevils feeding on foliage from plants at 2-3 leaf stage was 447pg insecticides/weevil (95% CL: 25-830 pg/weevil) but was lower (142pg/weevil; 95% CL: 102-180 pg/weevil) in experiments with 3-4 leaf stage plants. Plant age-related decline in adulticidal activity of thiamethoxam was also investigated by comparing adult mortalities on foliage from plants at two different ontogenic stages i.e., 5-6 and tillering (1 or 2 tillers) stages. In consistent with observed high mortalities on foliage young plants, a competitive ELISA for thiamethoxam detected higher insecticide residues in plants at 5-6 leaf stage than at tillering stage. This study provided a baseline for future monitoring adult weevil response.

The effects of rice seed treatments on the weevil egg laying and first instar survival were investigated. In preliminary experiments, greenhouse grown plants from seeds treated with chlorantraniliprole at 10 and 25 µg AI/seed and thiamethoxam at 21 and 28 µg AI/seed (6-7 leaf stage) were placed in infestation cages (pots of single treatment rate were placed), infested with weevil mating pairs for four days, allowed to mate and oviposit on plants. Such exposure of weevils to treated plants led to reduced egg numbers in and reduced first instars emergence from plants (in each cage half of the infested plants were counted for egg numbers and the other half of infested plants were incubated for larval emergence). Both the egg-laying and first instar emergence were suppressed in both chemicals (at both rates). On chlorantraniliprole-treated plants, suppression was 50% , but on thiamethoxam treatments, suppression was 90%. 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 plants treated or untreated as seeds with chlorantraniliprole or thiamethoxam for four days. In the second stage, weevils were released in infestation cages on plants untreated or treated as seeds with the two insecticides.

The low egg numbers by adults exposed to chlorantraniliprole-treated plants was confirmed as a sub-lethal effect on adults: adult survival was not impacted after four days 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 sub-lethal 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 (7µg [AI]/seed). Prior exposure to thiamethoxam through adult feeding reduced egg numbers. However, potential larvacidal 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.

Relative impacts of seed treatments with chlorantraniliprole (5 and 50 µg Ai/seed) and thiamethoxam (14 and 28 µg AI/seed) on different life stages of rice weevil were measured on plants infested for four days with mating pairs of adults, then monitored for numbers of eggs laid in leaf sheaths, first instars emerging from plants, and densities of late instars on roots. Impacts on life stages were related to insecticide residues in different portions of rice plants. Although chlorantraniliprole did not affect adult survival, decreased the number of eggs on treated plants and reduced survival of the first and late instars. Greatest magnitude reduction in weevil population occurred at late instar feeding on roots. Weevil exposure to thiamethoxam-treated plants reduced both the adult survival and egg numbers. The high biological activity of chlorantraniliprole on root feeding stages was consistent with tendency of chlorantraniliprole to accumulate in roots, whereas, in thiamethoxam-treated plants, high activity on adults correlated with high foliar thiamethoxam concentrations. Concentrations of chlorantraniliprole in roots were approximately 8-fold higher than concentrations in shoots and leaves in some cases. The

tendency of this chemical to accumulate in roots may allow reduced seed treatment rates to be used effectively. A recent small-plot field study showed excellent control of larvae at application rates as low as 7 g AI/acre. Further field studies are required to optimize the commercial rates of this highly potent larvicide. High foliar concentrations of thiamethoxam in leaves and shoots could lead to accelerated loss in the activity of seed treatment.

APPENDIX

LETTER OF PERMISSION

Request for a reprint permission to Pest Management Science

Lanka, Srinivas K. Sent: Sunday, October 30, 2012

Editor

Pest Management Science

Subject: Request for reprint permission

Dear Editor,

The following article
"Srinivas K Lanka,James A Ottea,Jeffrey A Davis,Amy B Hernandez,Michael J Stout. 2012. Systemic effects of thiamethoxam and chlorantraniliprole seed treatments on adult *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae) in rice" has been published in the Pest Management Science DOI: 10.1002/ps.3382.The data and information from this article will also be published as a chapter of my dissertation for Louisiana State University. This dissertation will be submitted to the graduate school this fall. Could I request a letter or email stating that Journal of Entomological Science grants me the permission to reprint the materials from this article for my dissertation?
Thank you,
Srinivas

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Title: Systemic effects of thiamethoxam and chlorantraniliprole seed treatments on adult *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae) in rice

Author: Srinivas K Lanka,James A Ottea,Jeffrey A Davis,Amy B Hernandez,Michael J Stout

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VITA

Srinivas K Lanka was born and raised in Vijayawada town in the state of Andhra Pradesh, India. After he had high school education in the same town, he obtained his undergraduate degree in agricultural science from Acharya N G Ranga Agricultural University, Agricultural College of Bapatla campus. He then continued his education for Master's degree in Entomology from Acharya N G Ranga Agricultural University, Rajendranagar campus. After receiving his degree in 1997, he worked as an associate in research laboratory in Directorate of Oilseeds Research and International Crops Research Institute for Semi-Arid Tropics, India. Later, he took up his job as Agricultural officer in Karimnagar serving agricultural extension in the Department of Agriculture, Andhra Pradesh. During this time he got an opportunity to study local cropping systems and took up field crop advisory on agronomic and plant protection issues of major crops in the region. Suddenly, his life took a turn when he decided to pursue a doctorate degree from the United States. Mr.Lanka left his job and moved to the United States in June 2007 and enrolled into a doctorate program under the supervision of Dr. Michael Stout in the Department of Entomology at Louisiana State University and Agricultural and Mechanical College.