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PABLO DA COSTA GONTIJO

NON-TARGET EFFECTS OF TWO INSECTICIDAL SUNFLOWER SEED TREATMENTS ON *Orius insidiosus* AND *Chrysoperla carnea*

LAVRAS – MG
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Orientador
Dr. Geraldo Andrade Carvalho

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(EFEITOS NÃO-ALVO DE DOIS TRATAMENTOS INSETICIDAS DE SEMENTES DE GIRASSOL EM *Orius insidiosus* E *Chrysoperla carnea*)

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, para a obtenção do título de Doutor.

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Aos meus pais, João e Selma, meus maiores professores.

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GENERAL ABSTRACT

The use of systemic insecticides as seed treatments is generally considered safer for natural enemies than other modes of application. However, the potential for contamination of plant products, such as extrafloral nectar, has raised concern about the possible impacts of these products on non-target organisms, because many beneficial insects use these products to supplement their diet. Therefore, the aims of the present study were to assess whether either chlorantraniliprole or thiamethoxam cause negative effects on the development and reproduction of (1) *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) or (2) *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) when various life stages of these predators are exposed to sunflower seedlings grown from seed treated with these insecticides. Thiamethoxam caused both lethal and sublethal effects on *O. insidiosus*, whereas chlorantraniliprole was not lethal to any life stage, although sublethal effects were evident. The nymphal stage was most susceptible and insecticidal toxicity diminished with plant development. In *C. carnea*, both chlorantraniliprole and thiamethoxam adversely impacted adults when they fed on extrafloral nectar of seedlings grown from treated seed. These insecticides reduced adult survival and female fecundity, especially thiamethoxam. Offspring of chlorantraniliprole-exposed adults had lower survival than those of controls. Both chlorantraniliprole and thiamethoxam had no effect on any life history parameter of *C. carnea* when larvae were exposed to sunflower seedlings grown from treated seed, but the sex ratio of resulting adults was lower in the thiamethoxam treatment than in the chlorantraniliprole treatment.

Keywords: Chlorantraniliprole. Thiamethoxam. Extrafloral nectar. Systemic insecticides. IPM. Neonicotinoid.

RESUMO GERAL

O uso de inseticidas sistêmicos para tratamento de sementes é geralmente considerado mais seguro para os inimigos naturais do que os outros modos de aplicação. Entretanto, devido ao potencial de contaminação dos produtos vegetais, como o néctar extrafloral, com esses inseticidas, aumentou-se a preocupação sobre os possíveis impactos desses produtos sobre organismos não alvos, visto que muitos insetos benéficos consomem produtos vegetais para complementar sua dieta. Os objetivos do presente estudo foram avaliar se clorantraniliprole e tiametoxam aplicados como tratamento de sementes de girassol causam efeitos negativos no desenvolvimento e reprodução de (1) *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) e (2) *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), quando várias fases de desenvolvimento dos insetos foram expostas a plântulas de girassol cultivadas de sementes tratadas. Tiametoxam causou efeitos letal e subletal em *O. insidiosus*, enquanto clorantraniliprole não foi letal para qualquer fase de desenvolvimento do predador, embora efeitos subletais foram evidentes. A fase de ninfa foi a mais suscetível e a toxicidade dos inseticidas diminuiu com o desenvolvimento da planta. Para *C. carnea*, ambos clorantraniliprole e tiametoxam causaram efeitos negativos sobre adultos quando eles alimentaram-se de néctar extrafloral de plantas cultivadas de sementes tratadas. Estes inseticidas reduziram a sobrevivência dos adultos e sua fecundidade, especialmente tiametoxam. As larvas provenientes dos adultos expostos a clorantraniliprole tiveram sobrevivência menor que o controle. Clorantraniliprole e tiametoxam não causaram qualquer efeito nos parâmetros da história de vida de *C. carnea*, quando larvas foram expostas a plantas de girassol cultivadas a partir de sementes tratadas, exceto que a razão sexual dos adultos resultantes foi menor no tratamento tiametoxam em relação ao clorantraniliprole.

Palavras-chave: Clorantraniliprole. Tiametoxam. Néctar extrafloral. Inseticidas sistêmicos. MIP. Neonicotinoides.

SUMMARY

FIRST PART

GENERAL INTRODUCTION

Sunflower, *Helianthus annuus* L. (Asteraceae), is an important oilseed crop worldwide. World production of confection and oil sunflowers in 2014/15 harvest is estimated at 40.8 and 15.5 million tons, respectively (USDA, 2014). Aside from being an economically important crop on the High Plains of the United States, sunflower plants are an important source of supplementary food for beneficial insects during the hot, dry summers (ROYER; WALGENBACH, 1991). Sunflower plants have abundant extrafloral nectaries along their petioles and leaf veins that secrete nectar from the time the first true leaves expand until the plant senesces. Extrafloral nectar (EFN) is predominantly composed of sucrose, with intermediate concentrations of glucose and fructose and low concentration of amino acids (BAKER; BAKER, 1979; ROGERS, 1985). Due to the abundance of food resources such as EFN, sunflower fields have been associated with increased populations of beneficial insects and improved biological control of pests in neighboring crops such as wheat (BREWER et al., 2008). However, like many other row crops cultivated on the High Plains, the majority of commercial sunflower is now planted with a systemic insecticide seed treatment which poses a potential hazard to natural enemies.

Seed treatments use insecticides with low lipophilicity and an octanol-water partition (log P_{oct}) normally below 4.0 (CLOYD; BETHKE, 2011). These are properties that provide the insecticides with systemic action, facilitating translocation throughout all vascular tissues in the plant (DIVELY; KAMEL, 2012; GOULSON, 2013). Systemic insecticides have been adopted for control of arthropod pests that attack different crops early in the growing season while plants are still small (HODGSON; KEMIS; GEISINGER, 2012; NUYTTENS et al., 2013). They have proven efficacious for pest control in seeds and seedlings of corn, soybeans, sorghum and sunflower (BRADSHAW; RICE; HILL, 2008) and have been promoted as more compatible with biological control than broadcast sprays, primarily because seed treatment involves a smaller amount of pesticide applied in a more selective manner, with consequently reduced impacts on non-target organisms (ALBAJES; LÓPEZ; PONS, 2003; HULL; BEERS, 1985) and lower levels of environmental contamination (TAYLOR; ECKENRODE; STRAUB, 2001). Neonicotinoids such as thiamethoxam are among the insecticides most often used for seed treatment, and more recently diamides such as chlorantraniliprole. Thiamethoxam is a second generation neonicotinoid and acts on nicotinic acetylcholine receptors causing hyperactivity followed by a collapse of the nervous system, which can result in both lethal and sublethal effects (TOMIZAWA; CASIDA, 2005). Chlorantraniliprole acts as a ryanodine receptor modulator that blocks insect muscle contraction. Once ingested, chlorantraniliprole causes Ca^{++} depletion in insect muscle cells leading to feeding cessation, lethargy, muscle paralysis and death (LAHM et al., 2007).

Despite providing benefits in some situations, seed treatments are not always effective in reducing populations of arthropod pests (VERNON et al., 2013) and may pose hazards to non-target organisms (SANCHEZ-BAYO; TENNEKES; GOKA, 2013). Non-target organisms may be exposed to systemic insecticides directly through consumption of pollen, floral and extrafloral nectar, sap and tissues of plants grown from treated seeds, and indirectly by the consumption of contaminated prey or hosts present on these plants (CLOYD; BETHKE, 2011; STONER; EITZER, 2012), with both lethal and sublethal effects (LI et al., 2012). Sublethal effects are often neglected in research, despite their potential for impact on the population dynamics of beneficial organisms (BIONDI et al., 2012; DESNEUX; DECOURTYE; DELPUECH, 2007). Negative effects of systemic insecticides applied to the soil or as seed treatments have been observed in predators such as *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) (TORRES et al., 2010) amd *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) (SMITH; KRISCHIK, 1999) and parasitoids such as *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) (MOSCARDINI et al., 2014).

Many beneficial insects feed on sunflower EFN (e.g. CHARLET; GAVLOSKI, 2011), including flower bugs of the genus *Orius* (Hemiptera: Anthocoridae) (ROYER; WALGENBACH, 1991) and green lacewings, *Chrysoperla* spp. (Neuroptera: Chrysopidae), both of which are cosmopolitan predators in agricultural crops, easily reared in the laboratory, and widely commercialized in biological control programs (ALBUQUERQUE; TAUBER; TAUBER, 1994; PAPPAS; BROUFAS; KOVEOS, 2011.). The most common *Orius* species in the High Plains region is the minute pirate bug, *Orius insidiosus* (Say), an omnivorous generalist predator (LATTIN, 1999; SYMONDSON; SUNDERLAND; GREENSTONE, 2002) that is an important biological control agent of many arthropods pests, including thrips, aphids, mites, whiteflies and the eggs and small larvae of Lepidoptera (RAGSDALE et al., 2011; WEINTRAUD; PIVONIA; STEINBERG, 2011). Both immature and adult stages of *O. insidiosus* utilize plant resources such as pollen, floral and extrafloral nectar, and plant sap (DESNEUX; O'NEIL, 2008; HARWOOD et al., 2007) and thus are at risk of exposure to any systemic insecticides they contain. Lacewings are polyphagous predators that contribute to natural control of many pests of economic importance in agriculture (STELZLA; DEVETAK, 1999). Larvae of *Chrysoperla carnea* (Stephens) feed on a wide range of small, soft-bodied arthropods with a preference for aphids (PRINCIPI; CANARD, 1984). Non-prey foods such as honeydew, floral and extrafloral nectar, and pollen can be important resources for chrysopids in both larval (DOWNES, 1974) and adult (VENZON et al., 2006) stages. Thus, the aims of the present study were to assess whether either chlorantraniliprole or thiamethoxam applied as a sunflower seed treatment can cause negative effects on the development and/or reproduction of *O. insidiosus* or *C. carnea* when various life stages of the insects were exposed to sunflower seedlings grown from treated seed.

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SECOND PART – ARTICLES

ARTICLE 1

NON-TARGET EFFECTS OF TWO SUNFLOWER SEED TREATMENTS ON *Orius insidiosus* (HEMIPTERA: ANTHOCORIDAE)

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Non-target effects of two sunflower seed treatments on *Orius insidiosus* (Hemiptera: Anthocoridae)

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Abstract

BACKGROUND: Systemic insecticides used as seed treatments are generally considered to be safe for natural enemies. However, predatory insects may feed directly on plants or use plant products to supplement their diet. This study examined whether chlorantraniliprole or thiamethoxam might negatively impact *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) when bugs utilize sunflowers grown from treated seed.

RESULTS: When eggs of *O. insidiosus* were laid in the stems of treated sunflower seedlings (two-leaf stage), thiamethoxam reduced egg viability and the longevity of females hatching from these eggs, whereas chlorantraniliprole reduced female survival. Thiamethoxam, but not chlorantraniliprole, reduced female fertility in six-leaf-stage plants. Nymphs exposed to thiamethoxam-treated seedlings had reduced survival, delayed development and reduced fecundity as adults, relative to other treatments, whereas chlorantraniliprole delayed oviposition. Nymphs exposed to six-leaf-stage plants did not differ from controls in either treatment. Adults exposed to treated plants expressed no significant differences among treatments for any parameter evaluated for either plant growth stage.

CONCLUSION: Thiamethoxam treatment on sunflower seeds caused lethal and sublethal effects on *O. insidiosus*, whereas chlorantraniliprole was not lethal to any life stage, although sublethal effects were evident. The nymphal stage was most susceptible, and insecticidal toxicity diminished with plant development.

Keywords: systemic insecticides; neonicotinoid; conservation biological control; ecological selectivity; omnivory; risk assessment

1 INTRODUCTION

The preplant treatment of seeds with systemic insecticides has been widely adopted as a means of early-season pest control in row crops.[1,2] This tactic has proven to be successful in preventing damage from arthropod pests that damage seeds and seedlings of corn, soybean, sorghum and sunflower, among other crops,[3,4] although it is not always effective in reducing pest populations.[5] Compared with broadcast applications of insecticides, the treatment of seeds reduces environmental contamination, decreases the amount of active ingredient used, reduces applicator exposure[6] and generally lowers pesticide impacts on non-target organisms through ecological selectivity.[7,8]

In spite of these advantages, research has revealed lethal and sublethal effects of seed treatments on non-target organisms.[9-12] Beneficial arthropods may be exposed via the direct consumption of pollen and nectar of plants grown from treated seed, by consuming prey or hosts contaminated by feeding on such plants or when they consume the active ingredient via ingestion of plant sap.[13-16] Pollen and nectar, both floral and extrafloral, represent important resources for many natural enemies of insect pests, either in the presence or in the absence of prey.[17-20] Seed treatments also pose a risk to insects with endophytic oviposition, because eggs inserted into plant tissues can absorb plant fluids and, consequently, any insecticides they may contain.[21,22]

The potential risks to beneficial, non-target organisms posed by systemic insecticides used as seed treatments are well recognized.[15,16,23,24] Both thiamethoxam and chlorantraniliprole exhibit systemic activity within plant vascular tissues, which facilitates their use as seed treatments, but also their potential to contaminate pollen and floral and extrafloral nectar.[25-27]

Chlorantraniliprole belongs to the anthranilic diamides, a group of compounds that bind to ryanodine receptors on the sarcoplasmic reticulum of insect muscle cells. Once ingested by an insect, chlorantraniliprole causes Ca ++ depletion in muscle cells, leading to feeding cessation, lethargy, muscle paralysis and death.[28] In contrast, thiamethoxam is a neonicotinoid insecticide that targets nicotinic acetylcholine receptors on the post-synaptic membranes of nerve cell junctions,[29] producing both lethal and sublethal neurological effects. Sublethal effects of neonicotinoids on various natural enemies have included behavioral and reproductive impairments.[9,30] On the High Plains of the United States, sunflower plants are an important resource for beneficial insects during the hot, dry summers owing to abundant extrafloral nectaries along their petioles and leaf veins that secrete nectar from the time the first true leaves expand until the plants senesce. Royer and Walgenbach[31] documented 40 species of foliar and epigeal predators associated with cultivated sunflower in eastern South Dakota. As the majority of commercial sunflowers are now planted with seed treatments to guard against planting-time pests such as wireworms and flea beetles, sunflower fields represent a substantial risk of systemic insecticide exposure for many beneficial insects.

Flower bugs of the genus *Orius* are among the most abundant predators associated with cultivated sunflowers.[31] The most common species in the High Plains region is the minute pirate bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), an omnivorous generalist predator.[32,33] This species is an important biological control agent of many arthropods pests, including thrips, aphids, mites, whiteflies and the eggs and small larvae of Lepidoptera.[34,35] Both immature and adult stages of *O. insidiosus* utilize plant resources such as pollen, floral and extrafloral nectar and plant sap,[36,37] and thus may be exposed to any systemic insecticides they contain. Therefore, the aim of the present study was to assess whether either chlorantraniliprole or thiamethoxam would have any detectable impacts on the survival, development or reproduction of *O. insidiosus* when various life stages of the bug were exposed to sunflower seedlings grown from seed treated with these materials.

2 MATERIALS AND METHODS

2.1 Insects

Adults of *O. insidiosus* were collected from a maize field at the Agricultural Research Center-Hays in Hays, Kansas (38° 51′ 31.14″ N, 99° 20′ 10.86″ W). Approximately 200 bugs were transported to the laboratory and placed in a 1 L glass jar covered with an organdy mesh screen. All bugs were held in a climate-controlled growth chamber set to 24 ± 1 °C, 42 ± 5% RH and a photoperiod of 16:8 h (L:D). The jar was filled with shredded wax paper as harborage, and water was provided on a cotton wick. Stems of greenhouse-grown sunflowers, *Helianthus annuus* L., cv. Triumph Nusun 810CL, were provided as a substrate for oviposition. The insects were fed every 48 h with frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) obtained from a commercial supplier (Beneficial Insectary, Oak Run, CA). Stems containing eggs of the predator were removed every 48 h and transferred to petri dishes (15 cm diameter). The petri dishes were lined with paper towel to provide shelter for hatching nymphs; water was provided on a cotton wick, and frozen eggs of *E. kuehniella* were supplied as food. Once nymphs reached adulthood, they were transferred to a glass jar (as above) for mating and production of the next generation. All bioassays were carried out under the same physical conditions as the stock colony.

2.2 Plants

Seeds of Triumph Nusun cv. 810CL sunflower were obtained with and without thiamethoxam seed treatment, and Pioneer 63N82 sunflower seeds with chlorantraniliprole seed treatment. Ideally, the same sunflower cultivar would have been employed with both seed treatments, but these are commercially applied by different companies on their own proprietary cultivars. Seeds were treated with thiamethoxam at a rate of 50 mg AI 100 kg^{-1} (Cruiser 5FS®; Syngenta Crop Protection, Greensboro, NC) or with chlorantraniliprole at a rate of 1800 mg AI 100 kg^{-1} (DuPont Crop Protection, Wiilmington, DE). All seeds were planted 2.0 cm deep in metal trays (8×51×36 cm) filled with a mixture of soil, peat moss and perlite (1:1:1) and germinated in a greenhouse at 25 ± 2 °C under natural light supplemented during daylight hours with metal halide lamps. Sunflower stalks were harvested beginning at the V2 stage (two true leaves expanded) and at the V6 stage (six true leaves expanded), and every 2 days thereafter for each period of insect exposure. These growth stages corresponded to plants at 14-15 and 20-21 days post-emergence, respectively, at the prevailing temperature. All stem segments were harvested before 9:00 a.m. and constituted the upper portion of the main stem. Before provisioning to insects, the ends of excised stem segments (ca 2 cm long) were dipped in liquid paraffin to seal vascular tissues and maintain turgor, while at the same time preventing exudation of resinous materials that might pose a hazard to the insects.

2.3 Exposure of eggs

For each replicate (*n* = 10 per treatment), three-day-old, mated *O. insidious* females (*n* = 5) were aspirated into a clear plexiglass cylinder (5.0 cm diameter × 10.0 cm height) that was then sealed with a ventilated plastic lid. Each container was supplied with a sunflower stem segment (ca 2 cm long), either treated or control (prepared as described in Section 2.2), for a period of 24 h. The experiment was performed twice, beginning with stems from plants at either the V2 or V6 stage. After a 24 h period for oviposition, stem segments were removed and the number of opercula in each was counted under a stereomicroscope at 40x magnification. Stems containing eggs were then transferred to fresh plastic cylinders containing a moist piece of cotton to prevent desiccation prior to nymphal eclosion. These containers were examined daily for the next week, and all eclosing nymphs were transferred to fresh plastic cylinders with moist cotton (as above), thus preventing any further contact with sunflower stems. Eggs were considered to be unviable if the operculum had not opened by the end of the seventh day. Eclosed nymphs were fed with frozen eggs of *E. kuehniella* every 48 h until all had either died or molted to the adult stage. The live insects were then sexed, and 25 pairs were established from each treatment. Each pair was placed in a petri dish (5.5 cm diameter) containing an untreated sunflower stem segment as an oviposition substrate and a moist cotton wick. Frozen eggs of *E. kuehniella* were provisioned every 48 h for 10 days, and sunflower stems were changed daily.

Data were tallied on the number of eggs laid in each replicate, the percentage of eggs hatching, the duration of embryonic and nymphal development, nymphal survival, sex ratio [Σ♀/Σ(♀+♂)], adult survival (♀♀ and ♂♂), preoviposition period and female fecundity. Egg fertility was assessed by harvesting the stems from each replicate on the fifth day of oviposition and counting the number of bugs eclosing from them over the next 7 days.

2.4 Exposure of nymphs

For each replicate (n = 10 per treatment), first instars of *O. insidiosus* (n = 5) were transferred into a petri dish (5.5 cm diameter) containing treated or untreated sunflower stem segments using a fine brush. The experiment was performed twice, beginning with stem segments from plants at either the V2 or V6 stage. Stem segments were prepared as described in Section 2.2 and refreshed every 48 h, along with frozen eggs of *E. kuehniella*, until all nymphs had either died or molted to the adult stage. The bugs were then sexed, and the maximum possible number of pairs from each treatment were established in plastic cylinders. Reproductive performance data were obtained as described in the egg exposure assay.

2.5 Exposure of adults

Male and female *O. insidiosus* (ca 24 h old) were paired in petri dishes (5.5 cm diameter) containing frozen eggs of *E. kuehniella* and sunflower stem segments (n = 25 per treatment), as described in Section 2.2. All stems were replaced after 48 h so that pairs of bugs had 4 days total exposure. After this period, pairs were transferred to fresh petri dishes and provisioned with untreated sunflower stem segments, a moist piece of cotton and frozen eggs of *E. kuehniella*, all refreshed every 48 h. Stem segments were replaced daily for a period of 10 days, and all eggs laid in them were counted. Stems from the fifth day of oviposition were transferred to petri dishes containing a moistened cotton wick and held until eclosion of nymphs. Within 1-2 h of hatching, nymphs were transferred to fresh dishes with moistened cotton and frozen eggs of *E. kuehniella* replaced every 48 h until they reached the adult stage. Adult survival (♀♀ and ♂♂), preoviposition period, fecundity, egg fertility, duration of embryonic and nymphal development, nymphal survival and sex ratio [Σ♀/Σ(♀+♂)] were all tallied.

2.6 Statistical analysis

Data were subjected to Shapiro-Wilk and Bartlett tests ($\alpha = 0.05$) for normality and homoscedasticity, respectively, using PROC UNIVARIATE.[38] Data that passed these tests were subjected to one-way ANOVA, and treatment means were separated using the Bonferroni test ($\alpha = 0.05$). Data that did not meet the assumptions of ANOVA were analyzed using the Kruskal-Wallis test, and treatment means were separated with Dunn's test ($\alpha = 0.05$). The sex ratio was analyzed using the chi-square goodness-of-fit test ($\alpha = 0.05$). Adult survival (♀ and ♂) was submitted to survival analysis using the log-rank test of the non-parametric procedure LIFETEST,[38] and survival curves were fitted using the Kaplan–Meier estimator.

3 RESULTS

3.1 Exposure of eggs

When eggs of *O. insidiosus* were laid in V2 sunflower stems, the thiamethoxam seed treatment reduced egg viability, although other developmental parameters were unaffected (Table 1). The lifespan of adult females hatching from these eggs was reduced in both the thiamethoxam and chlorantraniliprole treatments (Fig. 1), although their reproductive performance appeared to be unaffected (Table 2). When stems from V6 plants were used, no developmental parameters were affected by either treatment (Table 1), but females reared from eggs laid in the thiamethoxam treatment had lower egg fertility (Table 2). There were no differences among treatments in adult survival (Fig. 2).

3.2 Exposure of nymphs

Nymphs of *O. insidiosus* reared with thiamethoxam-treated sunflower stems cut from V2 plants had reduced survival and delayed development (Table 3), whereas chlorantraniliprole-treated sunflower stems produced no effects. Females that matured from these nymphs had extended preoviposition periods in the chlorantraniliprole treatment and reduced fecundity in the thiamethoxam treatment (Table 4). There were no differences among treatments in adult survival (Fig. 1). When stems were cut from V6 sunflower plants, there were no significant effects of treatment on any developmental or reproductive parameter (Tables 3 and 4 respectively) or adult survival (Fig. 2).

3.3 Exposure of adults

Four days of adult exposure to treated sunflower stems produced no significant differences among treatments in survival (Figs 1 and 2) or any parameter of reproductive performance or progeny development (Table 5), whether stems were cut from V2 or V6 plants.

4 DISCUSSION

The use of systemic insecticides as preplant seed treatments is generally viewed as safe for natural enemies because the exposure of beneficial insects is substantially reduced relative to broadcast applications.[13,39] However, exposure can still occur for predatory insects such as *O. insidiosus* that feed directly on plants or use plant products to supplement their diet. The results of this study demonstrate that both chlorantraniliprole and thiamethoxam can negatively impact eggs and nymphs of *O. insidiosus* when they come into contact with sunflower stems grown from treated seeds.

Systemic insecticides are characterized by low lipophilicity, typically exhibiting an octanol-water partition coefficient (log P_{oct}) below 4.0, which ensures high solubility in water and facilitates translocation through plant vascular tissues.[13] Both chlorantraniliprole and thiamethoxam have high systemic activity, with log P_{oct} values of 2.86 and -0.13 respectively. Residues of systemic insecticides can be found in many plant parts following soil application or seed treatment.[23,40] Residues of imidacloprid, a neonicotinoid similar to thiamethoxam, has been found at 13 ppb in sunflower pollen following its application as a seed treatment; residues were 50 times more concentrated in cotyledons than in the first true leaves, and 800 times more concentrated than in the apex of the plant.[41] Concentrations were typically reduced by half in each successive pair of leaves, and this progressive dilution as a function of plant growth likely explains the generally low toxicity of treatments that employed V6 plants.

The consumption of plant resources creates an avenue of natural enemy exposure to systemic insecticides employed as seed treatments. Many insect predators nibble on plant tissues occasionally to supplement their nutrition or simply to obtain moisture, and seedlings are a preferred plant stage because they are succulent and tender.[42] Another consideration is that insect densities are typically at their lowest levels in fields at planting time, which translates into low prey availability for immigrant predators. The risks are especially high for omnivorous predators such as *O. insidiosus*, which rely heavily on plant resources such as pollen and nectar and oviposit into plant tissues.[43-45]

The present results show that seed treatments with thiamethoxam and chlorantraniliprole did not cause *O. insidiosus* females to avoid sunflower plants for oviposition, because treatments did not differ significantly in numbers of eggs laid in the first bioassay. Lankaet al.[46] reported that females of the rice water weevil, *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae), are able to recognize rice plants grown from chlorantraniliprole-treated seeds and avoid laying eggs on them. Three mechanisms were proposed to explain the observations: (1) adult mortality prior to oviposition, due either to contact with contaminated plant tissues or the ingestion of plant sap; (2) sublethal effects on fecundity following contact with treated plants; (3) a repellency effect of the treated plant.[46] As neither lethal nor sublethal effects were observed when *O. insidiosus* were exposed as adults to either seed treatment, the first two mechanisms seem unlikely. However, it is possible that the continuous availability of moth eggs diverted adults away from plant feeding, as toxicity has been attributed specifically to plant feeding in other studies.[13,47,48] It is also known that neonicotinoids can elicit changes in plant chemistry that may impact arthropod physiology indirectly,[49] and it is possible that some such effect contributed to the present results. Furthermore, the exposure of individual life stages was necessary to determine their relative susceptibility, but may have underestimated the natural situation where exposure through successive life stages results in additive or synergistic impact.

Whether adults were less sensitive to the tested materials than juvenile stages, or simply avoided consuming plant tissues, they clearly did not discriminate against such plants for oviposition. Eggs of *O. insidiosus* are inserted deeply in plant tissues, leaving only the operculum exposed.[50] Thus, the eggs are exposed to the plant sap moving through vascular tissues and residues of any insecticide present therein. In the present study, thiamethoxam significantly reduced the viability of eggs laid in treated tissues of V2 plants, and both materials increased the mortality of female survivors.

The toxicity of thiamethoxam to eggs of *O. insidiosus* may derive from their water demand, as these tend to be preferentially deposited in plant tissues with high moisture content.[51] Oviposition by *O. insidiosus* into plant substrates rich in water is thought to be a tactic for avoiding desiccation.[52,53] As *Orius* eggs lack a waxy cuticle that would be impermeable to water, thiamethoxam in aqueous solution would easily penetrate them once they are embedded in plant tissue. However, the exposed operculum does have a wax layer, ostensibly to prevent water loss in the event of desiccating conditions,[54] and this wax might protect eggs externally to some degree, given the low lipophilicity of thiamethoxam.

Exposure to V2 sunflower stems grown from thiamethoxam-treated seeds reduced the survival of nymphs by 40% and increased their developmental duration, but similar exposure of adults had no measurable impact. The difference in toxicity of thiamethoxam to nymphs and adults can be at least partly explained by the higher consumption of plant tissues by immature stages, which are more dependent on plant nutrients for development.[47,48] As mentioned above, the availability of prey (*E. kuehniella* eggs) may also have reduced the consumption of plant tissues by adults, which prefer animal protein. Seagraves and Lundgren[11] found that the addition of prey to thiamethoxam-treated plants increased the survival of adult *O. insidiosus*, indicating that the bugs will alter their diet as prey becomes available. However, prey availability did not affect the survival of nymphs, which were more sensitive to thiamethoxam than adults. Similarly, Prabhaker et al.[10] reported negative effects when *O. insidiosus* and *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae) came into contact with citrus leaves allowed to draw up a thiomethoxam solution. Funderburk et al.[55] observed that soil application of imidacloprid negatively affected populations of *O. insidiosus* in pepper fields for up to 6 weeks after application, and concluded that imidacloprid was not compatible with conservation biological control. These studies consistently indicate that omnivorous hemipteran predators are negatively affected by plants treated with systemic neonicotinoid insecticides, whether these are applied to seed or soil.[56] Other groups of insects shown to be negatively affected by neonicotinoids include the predator *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae)[57] and the parasitoid *Microplitis croceipes* Cresson (Hymenoptera: Braconidae).[58]

Chlorantraniliprole applied as a seed treatment was not lethal to any stages of *O. insidiosus*, but sublethal effects were evident in the form of reduced survivorship of females that hatched from eggs laid in V2 plants and longer preoviposition periods for females exposed as nymphs. Sublethal effects of chlorantraniliprole have also been reported for the pollinator *Bombus terrestris* (L.) (Hymenoptera: Aphidae) when ingested as contaminated pollen.[59] In contrast, Biondi et al.[60] found that chlorantraniliprole was safe for *O. laevigatus* when adults were exposed to residues on tomato leaves. Castro et al.[61] concluded that chlorantraniliprole was selective for *Podisus nigrispinus* Dallas (Heteroptera: Pentatomidae) and *Supputius cincticeps* (Stal) (Heteroptera: Pentatomidae), and Martinou et al.[62] concluded the same for *Macrolophus pygmaeus* (Heteroptera: Miridae). As chlorantraniliprole is still a relatively new class of insecticide, there are little data as yet regarding its impact on natural enemies, and additional work to assess its toxicity to beneficial organisms is warranted.[28,63]

Although seed treatments have proven to be popular with farmers and may have reduced the non-target impacts of pesticides relative to broadcast applications, there is growing evidence that they should be employed judiciously. Their effectiveness does not extend beyond the first few weeks of plant growth, and biological control may remain important for crop protection throught the remainder of the season. Both lethal and sublethal effects have the potential to disrupt the population dynamics of beneficial organisms and jeopardize subsequent biological control in the crop. The fundamental philosophy of integrated pest management (IPM) is to avoid any pesticide application unless there is evidence that (1) the pest is beyond, or will exceed, an economic threshold, and (2) that the costs of any application will be more than recovered in terms of increased yield.[64] Seed treatments employing these materials may, in some contexts, be a useful tactic within an integrated control program. However, the current reliance on seed treatments as a convenience to be applied universally for prophylactic control of pests that may, or may not, be of economic importance is not consistent with sustainable IPM practices. Additional work on the non-target effects of systemic insecticides used as seed treatments in sunflower and other cropping systems would seem to be justified.

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Table 1. Egg exposure. Mean ± SE numbers of *Orius insidiosus* eggs laid in sunflower stems of two developmental stages grown from treated sunflower seeds, their developmental periods and survival. Nymphs were fed *ad libitum* *Ephestia kuehniella* eggs.

Treatment	No. eggs laid	Embryonic period		Nymphal period		Sex ratio
		Egg viability (%)	Duration (days)	Survival (%)	Duration (days)	
V2 plants (two true leaves expanded)						
Control	12.60 ± 2.33	92.22 ± 5.75 a	4.61 ± 0.07	70.00 ± 8.18	11.70 ± 0.17	0.51
Chlorantraniliprole	16.50 ± 2.47	81.74 ± 7.77 ab	4.58 ± 0.09	61.99 ± 8.92	11.87 ± 0.15	0.52
Thiamethoxam	16.33 ± 2.35	73.09 ± 6.84 b	4.82 ± 0.14	49.41 ± 8.07	12.23 ± 0.36	0.51
F, *H* or χ^2	0.85*	6.57**	1.56*	1.53*	1.22*	0.01***
df	2,27	2	2,27	2,27	2,25	2
P	0.439	0.038	0.228	0.235	0.313	0.993
V6 plants (six true leaves expanded)						
Control	22.20 ± 2.09	94.86 ± 2.38	5.07 ± 0.10	79.35 ± 7.24	11.83 ± 0.15	0.55
Chlorantraniliprole	22.60 ± 3.63	83.66 ± 6.06	5.06 ± 0.06	83.49 ± 7.54	12. 03 ± 0.18	0.56
Thiamethoxam	13.80 ± 3.62	97.31 ± 1.45	4.77 ± 0.12	62.25 ± 11.13	11.48 ± 0.13	0.58
F, *H* or χ^2	2.42*	4.81**	3.01*	1.63*	3.20*	0.34***
df	2,27	2	2,27	2,27	2,26	2
P	0.108	0.090	0.066	0.214	0.057	0.845

Analysis by one-way ANOVA (*), Kruskal-Wallis (**), or Chi-square (***).

Means followed by different letters were significantly different within columns (Dunn's test, $\alpha = 0.05$).

Table 2. Egg exposure. Mean ± SE reproductive parameters for *Orius insidiosus* females that eclosed from eggs laid in sunflower stems of two developmental stages grown from treated seeds. Bugs were reared on *ad libitum Ephestia kuehniella* eggs.

Treatment	Preoviposition period (days)	Eggs female^{-1} day^{-1}	Egg fertility (% hatching)
V2 plants (two true leaves expanded)			
Control	3.85 ± 0.34	6.21 ± 0.48	68.73 ± 10.65
Chlorantraniliprole	3.54 ± 0.33	5.35 ± 0.54	86.07 ± 9.94
Thiamethoxam	3.33 ± 0.26	4.75 ± 0.38	75.75 ± 9.57
F	0.76	2.71	0.68
df	2,48	2,48	2,34
P	0.476	0.077	0.513
V6 plants (six true leaves expanded)			
Control	2.74 ± 0.42	6.81 ± 0.34	85.00 ± 4.82 a
Chlorantraniliprole	2.20 ± 0.31	6.47 ± 0.52	63.76 ± 9.00 ab
Thiamethoxam	1.86 ± 0.37	6.71 ± 0.46	54.15 ± 8.47 b
F	1.45	0.16	4.38
df	2,53	2,53	2,46
P	0.243	0.850	0.018

Means followed by different letters were significantly different within columns (Bonferroni, $\alpha = 0.05$).

Table 3. Nymph exposure. Mean ± SE survival, developmental time and sex ratio of *Orius insidiosus* reared on *ad libitum Ephestia kuehniella* eggs and confined with sunflower stems of two different developmental stages grown from treated seeds.

Treatment	Survival (%)	Nymphal period (days)	Sex ratio
V2 plants (two true leaves expanded)			
Control	92.00 ± 3.27 a	12.64 ± 0.19 b	0.43
Chlorantraniliprole	86.00 ± 6.70 a	12.54 ± 0.15 b	0.48
Thiamethoxam	52.00 ± 9.52 b	13.99 ± 0.17 a	0.42
F or χ^2	9.55*	19.84*	0.37**
df	2,27	2,25	2
P	< 0.001	< 0.001	0.830
V6 plants (six true leaves expanded)			
Control	82.00 ± 4.67	11.40 ± 0.21	0.58
Chlorantraniliprole	88.00 ± 4.42	11.26 ± 0.13	0.43
Thiamethoxam	92.00 ± 3.27	11.65 ± 0.21	0.39
F or χ^2	1.46*	1.10*	3.59**
df	2,27	2,27	2
P	0.250	0.348	0.166

Analysis by one-way ANOVA (*) or Chi-square (**).

Means followed by different letters were significantly different within columns (Bonferroni, $\alpha = 0.05$).

Table 4. Nymph exposure. Mean ± SE reproductive parameters of *Orius insidiosus* females reared as nymphs with sunflower stalks of two developmental stages grown from treated seeds and fed *ad libitum Ephestia kuehniella* eggs.

Treatment	Preoviposition period (days)	Eggs female^{-1} day^{-1}	Egg fertility (% hatching)
V2 plants (two true leaves expanded)			
Control	2.79 ± 0.16 b	7.25 ± 0.43 a	68.94 ± 11.48
Chlorantraniliprole	4.36 ± 0.31 a	5.84 ± 0.56 ab	48.37 ± 14.41
Thiamethoxam	2.67 ± 0.21 b	5.45 ± 0.45 b	39.82 ± 18.73
F or *H*	4.48*	6.97**	0.99*
df	2,31	2	2,20
P	0.019	0.031	0.389
V6 plants (six true leaves expanded)			
Control	3.27 ± 0.45	7.18 ± 0.48	60.49 ± 6.12
Chlorantraniliprole	3.36 ± 0.31	6.71 ± 0.61	46.50 ± 9.05
Thiamethoxam	3.64 ± 0.41	5.67 ± 0.68	53.31 ± 13.83
F or *H*	0.23*	3.52**	0.50*
df	2,33	2	2,27
P	0.796	0.172	0.612

Analysis by one-way ANOVA (*) or Kruskal-Wallis (**).

Means followed by different letters were significantly different within columns (Bonferroni or Dunn's test, respectively, α = 0.05).

Table 5. Adult exposure. Mean ± SE reproductive performance of *Orius insidiosus* pairs and developmental parameters of their offspring. Bugs were fed *ad libitum Ephestia kuehniella* eggs and confined with sunflower stems of two developmental stages grown from treated seed.

Treatment	Eggs female^{-1} day^{-1}	Embryonic period		Nymphal period		Sex ratio
		Egg fertility (% hatching)	Duration (days)	Survival (%)	Duration (days)	
V2 plants (two true leaves expanded)						
Control	4.97 ± 0.69	63.09 ± 6.82	4.56 ± 0.12	68.19 ± 9.28	12.12 ± 0.17	0.49
Chlorantraniliprole	4.40 ± 0.66	58.33 ± 7.45	4.67 ± 0.24	63.33 ± 16.79	11.67 ± 0.19	0.54
Thiamethoxam	5.35 ± 0.66	62.49 ± 7.12	4.42 ± 0.13	61.55 ± 8.27	11.73 ± 0.24	0.52
F or χ^2	0.39*	0.08*	0.55*	0.13*	1.37*	0.15***
df	2,42	2,27	2,26	2,26	2,22	2
P	0.679	0.927	0.585	0.878	0.275	0.930
V6 plants (six true leaves expanded)						
Control	7.16 ± 0.60	62.59 ± 6.35	4.68 ± 0.15	54.48 ± 11.16	12.55 ± 0.24	0.56
Chlorantraniliprole	7.53 ± 0.57	66.75 ± 9.29	4.62 ± 0.10	62.53 ± 11.45	12.64 ± 0.31	0.59
Thiamethoxam	7.42 ± 0.52	76.00 ± 7.39	4.49 ± 0.12	75.34 ± 7.70	12.33 ± 0.23	0.48
F or χ^2	0.11*	0.84*	0.60*	1.20*	0.40*	1.55***
df	2,57	2,50	2,46	2,47	2,36	2
P	0.894	0.438	0.553	0.310	0.674	0.462

Analysis by one-way ANOVA (*) or Chi-square (***).

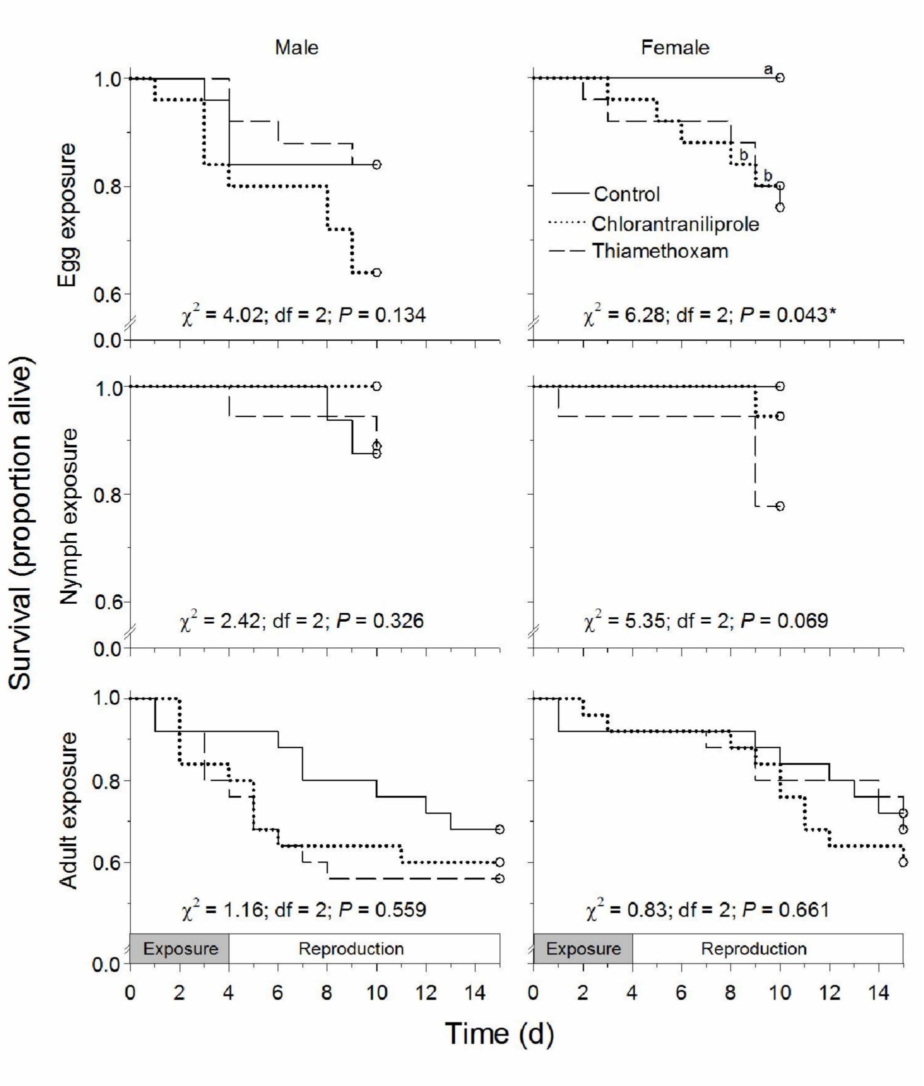

Figure 1. Survival curves for male and female *Orius insidiosus* following exposure as either eggs, nymphs or adults to stems of V2 stage sunflower plants grown from treated seed. Bugs were fed eggs of *Ephestia kuehniella*. Curves bearing different letters were significantly different (Bonferroni, α = 0.05) on the final day of observation.

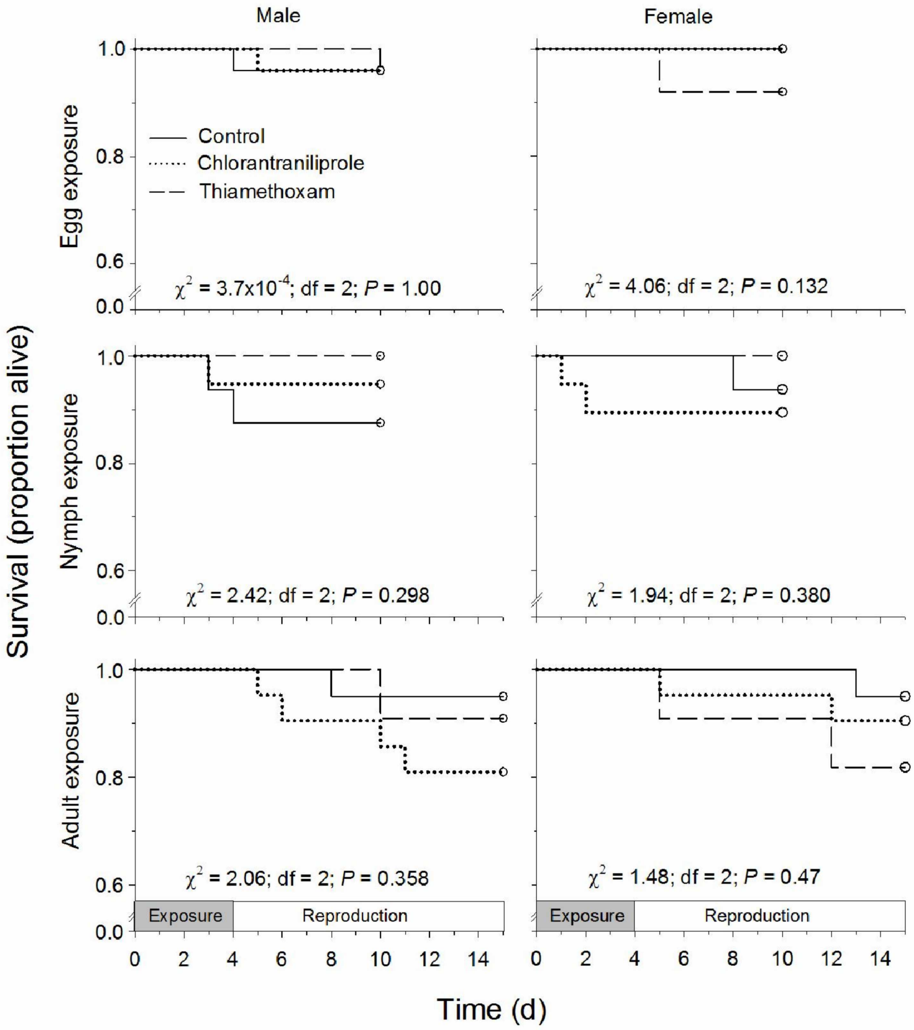

Figure 2. Survival curves for male and female *Orius insidiosus* following exposure as either eggs, nymphs or adults to stems of V6 stage sunflower plants grown from treated seed. Bugs were fed eggs of *Ephestia kuehniella*. There were no significant differences among treatments (Log-rank test, $\alpha = 0.05$) on the final day of observation.

ARTICLE 2

NON-TARGET EFFECTS OF CHLORANTRANILIPROLE AND THIAMETHOXAM ON *Chrysoperla carnea* WHEN EMPLOYED AS SUNFLOWER SEED TREATMENTS

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Non-target effects of chlorantraniliprole and thiamethoxam on *Chrysoperla carnea* when employed as sunflower seed treatments

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Abstract The use of systemic insecticides as seed treatments has raised concern about the possible impacts of these products on natural enemies. This study assessed the effects of sunflower seed treatments with chlorantraniliprole and thiamethoxam on *Chrysoperla carnea* by exposing larvae and adults to sunflower stem segments grown from treated seeds and the nectar secreted by their extrafloral nectaries. Confinement of larvae with stem segments for their entire developmental period had no effect on their survival or any life history parameter, except that the sex ratio of resulting adults was lower in the thiamethoxam treatment than in chlorantraniliprole. However, when adult pairs of *C. carnea* were exposed to treated stem segments during their maturation period, their subsequent survival and fecundity was significantly reduced by both materials, with thiamethoxam reducing median survival (LT_{50}) and fecundity to a greater degree than chlorantraniliprole. Insufficient offspring were obtained from adults exposed to thiamethoxam to permit assessment of their fitness, but the offspring in the chlorantraniliprole-exposed adults had reduced larval survival relative to controls. The greater impact of seed treatments on adult lacewings may be partly attributable to their greater consumption of extrafloral nectar. Our results indicate that seed treatment with systemic insecticides can cause negative effects on beneficial insects, potentially disrupting their population dynamics, and should not be assumed compatible with biological control and IPM simply because this mode of application limits direct exposure.

Keywords Extrafloral nectar Systemic insecticides IPM Green lacewings Environmental risk assessment

Key message

- Sunflower extrafloral nectar is utilized by many beneficial insects and is a potential route of exposure to systemic insecticides applied as seed treatments.
- Both chlorantraniliprole and thiamethoxam adversely impacted *Chrysoperla carnea* adults when they fed on extrafloral nectar of seedlings grown from treated seed.
- Both insecticides reduced adult survival and fecundity, especially thiamethoxam, and offspring of chlorantraniliprole-exposed adults had lower survival than those of controls.

Introduction

The potential for non-target effects of systemic insecticides when used as seed treatments has recently become a topic of interest and controversy (Sanchez-Bayo et al. 2013). Seed treatments are now widely employed as a prophylactic control measure to protect crops against seed and seedling pests (Bradshaw et al. 2008; Strausbaugh et al. 2010). Their use has, to some extent, reduced the need for broadcast applications of insecticides to ensure stand establishment, thus lowering pesticide exposure for non-target organisms (Hull and Beers 1985; Albajes et al. 2003; Cloyd and Bethke 2011). However, research has shown that seed treatments may also pose a risk to beneficial organisms via less obvious routes of exposure, causing both lethal and sublethal effects (Li et al. 2012). Sublethal effects, in particular, are easily overlooked and yet may have considerable impact on natural enemy population dynamics and the level of ecosystem services contributed by these insects (Desneux et al. 2007; He et al. 2012; Biondi et al 2012a; Biondi et al. 2013; Planes et al. 2013).

Systemic insecticides are characterized by low lipophilicity, typically exhibiting an octanol-water partition coefficient (log P_{oct}) below 4.0 (Cloyd and Bethke 2011) which facilitates their translocation in plant tissues. Residues of such insecticides can be translocated to various plant parts (Dively and Kamel 2012; Goulson 2013). For example, residues of imidacloprid have been found at 13 ppb in sunflower pollen following its application as a seed treatment (Laurent and Rathahao 2003). Chlorantraniliprole and thiamethoxam represent two systemic insecticides used in seed treatments, belonging to two different groups, diamides and neonicotinoids, respectively. Chlorantraniliprole acts as a ryanodine receptor modulator to block insect muscle contraction (Lahm et al. 2007). In contrast, thiamethoxam is a neonicotinoid insecticide that targets nicotinic acetylcholine receptors in the central nervous system of insects, causing overstimulation at low concentrations, and receptor blockage, paralysis and death at higher concentrations (Tomizawa and Casida 2005). Furthermore, thiamethoxam breaks down into clothianidin, a highly active open-chain neonicotinoid with somewhat different properties (Ohkawara et al. 2002; Schwarz et al. 2002) which may contribute to its activity in both plants and insects (Benzidane et al. 2010). Both classes of materials pose potential hazards to non-target organisms because of their ability to contaminate pollen, floral and extrafloral nectar (Lahm et al. 2009).

Pollen and nectar, both floral and extrafloral, are important resources for many natural enemies of insect pests (Wackers et al. 2007; Lundgren 2009; Choate and Lundgren 2013), and thus constitute potential routes of exposure to systemic insecticides for natural enemies and pollinators (Cloyd and Bethke 2011; Stoner and Eitzer 2012; Easton and Goulson 2013). However, floral nectar and pollen are only produced by mature plants in reproductive stages, whereas extrafloral nectar may be produced in much earlier stages of plant development when systemic materials applied as seed treatments are present in plant tissues at much higher concentrations. In sunflower, nectaries occur first on the main stem above the cotyledons, and henceforth on all stems and leaf petioles, so they are present from the time the first pair of true leaves expands, about 2 weeks after germination (JPM, person observation). The vast majority of commercial sunflower seed in the USA is now sold with a factory-applied seed treatment.

Among many beneficial predators in agriculture, green lacewings, *Chrysoperla* spp. (Neuroptera: Chrysopidae), stand out as cosmopolitan species that are easily reared in the laboratory, widely commercialized in augmentation biological control programs, and highly valued for conservation in IPM systems for field crops (Albuquerque et al. 1994; Pappas et al. 2011). Lacewings are polyphagous predators that contribute to natural control of many pests of economic importance in agriculture (New 1975; Principi and Canard 1984; Stelzla and Devetak 1999). The larvae of *Chrysoperla carnea* (Stephens) feed on a wide range of small, soft-bodied arthropods with a preference for aphids (Principi and Canard 1984). Non-prey foods such as honeydew, floral and extrafloral nectar, and pollen can be important resources for chrysopids in both larval (Downes 1974) and adult (Venzon et al. 2006) stages. Limburg and Rosenheim (2001) showed that extrafloral nectar was an important food source for neonate lacewing larvae on cotton and that its consumption increased as the availability of aphid prey declined. Patt et al. (2003) showed that larvae of *C. carnea* used the carbon present in sugar to supplement thier diet and enhance their growth and development. Adults of *C. carnea* are not predaceous, feeding only on extrafloral and floral nectar, pollen, and honeydew (Principi and Canard 1984; Hagen 1986) and provision of a nectar source is known to increase fecundity and extend adult longevity in this species (Gurr et al. 2004). In the central plains of the USA, *C. carnea* adults are among a wide range of beneficial insects that colonize sunflowers during dry summer months (Royer and Walgenbach 1991) and can often be observed exploiting the abundant extrafloral nectar that these plants produce (JPM, personal observation).

Given the potential for contamination of sunflower extrafloral nectar with chlorantraniliprole and thiamethoxam when applied as seed treatments, the objective of the present study was to assess whether either material would affect key life history parameters of *C. carnea* when both larvae and adults were exposed to sunflower seedlings grown from treated seed.

Materials and methods

Insects

Adults of *C. carnea* were collected from a sorghum field at the Agricultural Research Center-Hays in Hays, Kansas, USA (38°51'3114"N 99°20'1086"W). The lacewings were placed in a plastic container (14.0 cm diam x 19.0 cm ht) covered with an organdy mesh screen held in place with a rubber band. The stock colony and all bioassays were held in a climate-controlled growth chamber set to 24.0 ± 1.0 ºC, 42 ± 5% RH and a photoperiod of 16:8 (L:D). Adults in the stock colony were provisoned every 48 h with an artificial diet composed of honey and brewer's yeast (1:1) smeared on two parafin wax strips and water on a piece of sponge, both refreshed every 48 h. Eggs laid on the walls of the container were removed every 48 h by clipping their stalks with scissors. These were then isolated in Petri dishes (5.0 cm diam) to prevent cannibalism by hatching neonates. Larvae were provisioned every 48 h with *ad libitum* frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) obtained from a commercial supplier (Beneficial Insectary, Oak Run, CA, USA), and with water on a piece of sponge until larvae pupated. Emergent adults were transferred to a plastic container (as above) for mating and production of the next generation.

Sunflower plants

Seeds of sunflower were planted 2.0 cm deep in metal trays (8.0 cm x 51.0 cm x 36.0 cm) filled with a mixture of soil, peat moss and perlite (1:1:1) and germinated in a greenhouse at 25.0 ± 2 ºC under natural light supplemented during daylight hours with metal halide lamps (L:D = 12:12). Plants were watered daily, but minimally, to avoid excessive leaching of insecticide from treated seeds. Three treatments consisted of (1) seeds of Pioneer 63N82 factory-treated with chlorantraniliprole (Prevathon®, 1,800 mg ai 100 kg^{-1}, DuPont Crop Protection, Wilmington, DE), (2) seeds of Triumph Nusun cv 810CL sunflower, factory-treated with thiamethoxam (Cruiser 5FS®, 50 mg ai 100 kg^{-1}, Syngenta Crop Protection, Greensboro, NC), and (3) the same Triumph cultivar without any insecticide (control). Ideally, the same sunflower cultivar would have been employed with both seed treatments, but these are commercially applied by different companies on their own proprietary cultivars. Both cultivars had similar agronomic characteristics (oilseed varieties of normal stature and similar maturity group). Sunflower stalks were harvested beginning at the V2 stage (two true leaves expanded) and every 2 days thereafter for each period of insect exposure. The V2 stage corresponded to plants 14-15 days post-emergence in the greenhouse. The extrafloral nectaries of sunflower are very small and distributed widely over the surface of stems, leaf petioles, and even primary leaf veins. Before provisioning to insects, the ends of excised stem segments (ca 4.0 cm long) were dipped in liquid paraffin to seal vascular tissues and maintain turgor, while at the same time preventing the exudation of resinous materials that might pose a hazard to the insects.

Exposure of larvae

In each treatment, first instar larvae of *C. carnea* (neonates within 1-2 h of eclosion) were isolated in Petri dishes (5.0 cm diam) each with a sunflower stem segment (prepared as described above) that was replaced every 48 h until larvae pupated. A total of 40 larvae were placed in each treatment, grouped in eight replicates of five for purposes of assessing mortality. Frozen eggs of *E. kuehniella* were provided *ad libitum* and refreshed every 48 h. Emergent adults were sexed and the maximum number of pairs established in each treatment (control = 16, chlorantraniliprole = 11, thiamethoxam = 12). Adult pairs were isolated in plastic cylinders (5.0 cm diam x 10.0 cm ht), each sealed with a ventilated plastic lid. Artificial diet (as above) and water on a sponge were provisioned and refreshed every 48 h. Developmental time, larval and pupal survival (black pupae and pharate adults were tallied as dead), sex ratio (Σ♀/Σ(♀ + ♂)), adult survival, preoviposition period (time from adult emergence to first oviposition), and female 10-days fecundity (total eggs laid in 10 days of oviposition) were all recorded by examining all replicates daily and harvesting all eggs laid. Egg viability was assessed by harvesting 5-10 eggs per female, per treatment, on the fifth day of oviposition and isolating these in Petri dishes (5.0 cm) until eclosion.

Exposure of adults

Male and female *C. carnea* (ca 24 h old) were paired in plastic cylinders (5.0 cm diam x 10.0 cm ht) sealed with a ventilated plastic lid (*n* = 20 pairs per treatment) for the duration of the entire experiment. Each container had a piece of parafilm with artificial diet and a sunflower stem segment (as above). The insects were exposed to sunflower stems for a period of 8 days, and all stems were replaced every 48 h. After this period, pairs were provisioned with water on a sponge and artificial diet refreshed every 48 h. The eggs were counted and removed daily by clipping their pedicels with scissors. To assess the egg viability and offspring fitness, the third and fifth clutches (oviposition days) of each female were collected and the eggs isolated in Petri dishes (5.0 cm diam). Adult survival, preoviposition period, fecundity and egg viability were all scored (as above). After eclosion, 10 larvae from each female were reared and monitored until they emerged as adults. Development time, larval and pupal survival (black pupae and pharate adults were tallied as dead) and sex ratio (Σ♀/Σ(♀ + ♂)) were all recorded.

Statistical analysis

Developmental data from the larval bioassay and reproductive data from the adult bioassay were subjected to one-way ANOVA followed by a Bonferroni test ($\alpha = 0.05$) to separate means (PROC GLM; SAS Institute 2008). Data obtained from the offspring of exposed adults were analyzed using an independent *t* test ($\alpha = 0.05$) (PROC TTEST, SAS Institute 2008). Sex ratio was analyzed using the Chi-square Goodness of Fit test ($\alpha = 0.05$) (PROC FREQ; SAS Institute 2008). Adult survival data was submitted to survival analysis using the non-parametric procedure LIFETEST (SAS Institute 2008) which produces survival curves from Kaplan-Meier estimators. Data from pairs that survived beyond the tenth day of oviposition were censored. Median survival times (LT_{50}) were estimated for treatments in which adult mortality was higher than 50% and their respective 95% confidence intervals determined. Fecundity and egg viability data violated ANOVA assumptions of normality and homoscedasticity (PROC UNIVARIATE; SAS Institute 2008) and were $\sqrt{x}$ and arcsine $\sqrt{x}$ transformed, respectively; prior to analysis; untransformed means are presented in the tables.

Results

Larval exposure

There were no significant differences among treatments in larval survival ($F_{2,21} = 0.42$; $P = 0.662$), duration of larval development ($F_{2,21} = 2.15$; $P = 0.141$), pupal survival ($F_{2,21} = 0.53$; $P = 0.597$) or duration of pupal development ($F_{2,21} = 0.12$; $P = 0.884$) (Table 1). The sex ratio (proportion female) was reduced by thiamethoxam relative to chlorantriniliprole ($\chi^2 = 8.40$; $df = 2$; $P = 0.015$); although neither treatment was significantly different from controls, the power of the test was likely limited by sample sizes. The survival of the resulting adults was unaffected by any treatment (log-rank test, $\chi^2 = 2.39$; $df = 2$; $P = 0.303$) (Fig. 1a); neither was the preoviposition period ($F_{2,35} = 0.50$; $P = 0.608$), fecundity ($F_{2,35} = 0.55$; $P = 0.582$) or egg viability ($F_{2,31} = 0.02$; $P = 0.983$) (Table 1). No treatment caused mortality of adults higher than 50%, so LT_{50} values were not computed.

Adult exposure

Adults of *C. carnea* exposed to sunflower stems grown from treated seeds had significantly lower survival in both chlorantraniliprole and thiamethoxam treatments than in controls (log-rank test, $\chi^2 = 11.35$; $df = 2$; $P = 0.003$) (Fig. 1b) and median survival times (LT_{50}) were lower in the thiamethoxam treatment than in the chlorantraniliprole treatment (Fig. 2). LT_{50} was not computed for control insects because mortality was <50 %. Only 11, 10 and 7 females out of 20 oviposited in the control, chlorantraniliprole and thiamethoxam treatments, respectively, and the fecundity of these females was reduced by both insecticide treatments relative to controls ($F_{2,25} = 7.05$; $P = 0.004$; Table 2). Too few eggs were obtained in the thiamethoxam treatment to monitor development of offspring. The chorantraniliprole treatment significantly reduced the survival of offspring produced by exposed adults ($t = 2.59$; $df = 8$; $P = 0.031$), but other development parameters of offspring remained unaffected; egg viability ($t = 0.47$; $df = 10$; $P = 0.650$), duration of larval development ($t = -1.02$; $df = 8$; $P = 0.337$), pupal survival (100%), pupal duration ($t = 0.86$; $df = 8$; $P = 0.415$) and sex ratio ($\chi^2 = 0.39$; $df = 1$; $P = 0.533$) (Table 2).

Discussion

The present study confirms that diamides and neonicotinoids, when used as seed treatments, can adversely affect certain non-target organisms that come into contact with seedling plants. Both chlorantraniliprole and thiamethoxam caused lethal and sublethal effects to *C. carnea* adults exposed to sunflower stems grown from treated seeds. However, thiamethoxam demonstrated higher toxicity than chlorantraniliprole and greater negative impacts on survival, fecundity and LT_{50}. The most likely explanation for these results is the contamination of extrafloral nectar with residues of the insecticides, since these insects lack chewing mouthparts and are unable to consume leaf or stem tissues, but were often directly observed consuming nectar. Both adults and larvae of *C. carnea* are known to consume extrafloral nectar to supplement their diet (Limburg and Rosenheim 2001; Rogers et al. 2007). The effects on reproductive parameters observed in the present work suggest that populations of *C. carnea* might be reduced in sunflower fields when seed is treated with chlorantraniliprole or thiamethoxam. This is a significant agricultural finding because sunflower extrafloral nectar is an important source of hydration and nutrition for a wide range of beneficial insects during the hot, dry summers on the Great Plains when little free moisture is available (Michaud and Qureshi 2006). For example, the presence of sunflowers has been shown to improve the area-wide survival of natural enemy species important in the biological control of key pests in neighboring crops such as wheat, i.e. *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) (Brewer et al. 2008).

Although not quantified in these experiments, we observed that the water demand of adults appeared to be much greater than that of larvae. This becomes visually evident if the water supply is not maintained. Whereas larvae do not require a supplementary water source to develop successfully, adults quickly desiccate in the absence of moisture, their abdomens becoming visibly shrivelled. This was evident to some degree in all treatments of adults after the exposure period, during which the sunflower stem segments were the only, and likely insufficient, source of hydration; adult hydration recovery, characterized by visible re-inflation of the abdomen, was clearly evident within 24 h of provision of free water. We hypothesize that dehydration during the exposure period is the likely cause of greater female infertility, longer pre-oviposition periods and lower fecundities following adult exposure compared to larval exposure (Table 1 vs Table 2). Therefore, the high water demand of adults likely drove greater consumption of extrafloral nectar relative to larvae, which likely consume it primarily for its sugar content.

Because extrafloral nectar lacks the protein needed for growth and development, its consumption by lacewing larvae tends to decrease with increasing availability of prey (Limburg and Rosenheim 2001). Thus, the availability of ad libitum prey (eggs of *E. kuehniella*) during larval exposure in these experiments may have somewhat reduced the consumption of extrafloral nectar by larvae. If so, our results may underestimate the potential impact on larvae of extrafloral nectar contaminated with these materials when prey is scarce. Also, it could be argued that some effects observed in the thiamethoxam treatment may be attributable to the Pioneer 63N82 cultivar itself, since the controls were Triumph 810CL, but this is unlikely in our view.

Cloyd and Bethke (2011) reviewed the various potential routes of exposure to neonicotinoid insecticides for beneficial insects in interior plantscapes, including floral nectar, and emphasized the importance of plant species and age as factors influencing concentrations. Residues of neonicotinoids used in seed treatments have been detected in the nectar and pollen of rapeseed, corn and sunflower at concentrations from 20 to 39 ppb in pollen (Cutler and Scott-Dupree 2007; Krupke et al. 2012) and from 22 ppb to 30 ppb in nectar (Bonmatin et al. 2005; Cutler and Scott-Dupree 2007). However, floral nectar and pollen are produced by mature plants in late stages of reproduction and, given the role of plant age in diluting the concentration of insecticide in plant tissues, concentrations are likely much greater in the extrafloral nectar of younger plants, such as those provided in this study.

Seagraves and Lundgren (2012) found reduced numbers of adult natural enemies, e.g., *Chrysoperla* sp and *Nabis americoferus* Carayon (Hemiptera: Nabidae), in soybean fields grown from neonicotinoid-treated seed. When applied as a soil drench, imidacloprid reduced the survival of adults of *C. carnea* (Rogers et al. 2007) and the parasitoid *Anagyrus pseudococci* (Girault)

(Hymenoptera: Encyrtidae) that fed on flowers of *Fagopyrum esculentum* (Polygonaceae) (Krischik et al. 2007).

Effects of chlorantraniliprole on lacewings have been recorded in other studies. Amarasekare and Shearer (2013) found that chlorantraniliprole was highly toxic to adults and larvae of *C. carnea* and *C. johnsoni* when the predator ingested contaminated diet or prey. Topical application of chlorantraniliprole on larvae of *C. externa* did not cause negative effects (Zotti et al. 2013), as this material must be ingested to reach its active sites. For example, Smagghe et al. (2013) found negative effects on reproduction when *Bombus terrestris* (L) (Hymenoptera: Apidae) fed on pollen contaminated with chlorantraniliprole. However, chlorantraniliprole was considered safe for *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) when exposed to the insecticide via direct, residual and oral routes (Martinou et al. 2014). Similarly, no significant contact toxicity of chlorantraniliprole, either in terms of lethal or sublethal effects, was recorded on the anthocorid predator *Orius laevigatus* (Fieber) (Biondi et al. 2012b). Thus, the toxicity of chlorantraniliprole for beneficial insects may depend on both the species tested and the route of exposure.

The high toxicity of thiamethoxam to *C. carnea* in the present study compared to chlorantraniliprole may be related to its high mobility in phloem elements and its non-acidic nature (Nauen et al. 1999; Buchholz and Nauen 2002). Lanka et al. (2014) studied rice plants grown from treated seed and found concentrations of thiamethoxam four-fold greater in above-ground tissues than in the roots. In contrast, chlorantraniliprole concentrations in rice roots were up to ten times higher than those in above-ground parts. This demonstrates the high mobility of thiamethoxam in plant tissues, which can result in contamination of pollen, floral and extrafloral nectar. Furthermore, the conversion of thiamethoxam into highly toxic metabolites such as clothianidin (Casida 2011) may contribute to increased toxicity to insects, including natural enemies. Nauen et al. (2003) demonstrated that clothianidin is one of the primary metabolites of thiamethoxam in true leaves of cotton plants treated via soil drench. These authors inferred that clothianidin, not thiamethoxam, was responsible for toxicity of thiamethoxam-treated leaf disks to *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) larvae. However, its greater lipophilicity and lower solubility in water make clothianidin less likely than thiamethoxam to contaminate nectar.

In summary, both chlorantraniliprole and thiamethoxam caused negative effects on the survival and reproduction *C. carnea* adults and the survival of their progeny when the adults were exposed to seedlings grown from seeds treated with these insecticides. In companion studies on the same pesticides identically applied as sunflower seed treatments, no direct mortality was observed on adults of the braconid wasp *Lysiphlebus testaceipes* (Cresson), although various sublethal effects on its behavior and biological performance were recorded (Moscardini et al. 2014). Moreover, both lethal and sublethal effects were caused by thiamethoxam and only sublethal effets by chlorantraniliprole on nymphs and adults of the anthocorid predator *Orius insidiosus* (Say) (Gontijo et al. 2014). The treatment of seeds with systemic insecticides is convenient for farmers and profitable for seed companies. However, seed treatment as an 'insurance policy' without identification of a target pest runs counter to the basic principles of IPM.

The results of the present study and others cited above are converging toward a conclusion that systemic insecticides used as seed treatments can cause negative effects on beneficial organisms, potentially disrupting their population dynamics, and should not be assumed compatible with biological control and IPM simply because their mode of application limits direct exposure of non-target insects and reduces overall environmental exposure to the material compared to broadcast applications. More research is warranted to assess the toxicity of systemic insecticides applied as seed treatments in commercial sunflower seed and other crops. These studies should evaluate the concentration of insecticide in different developmental stages of the plant and in different plant parts, taking into account the distinct biology and behavior of different beneficial species in different life stages, both in the presence and absence of prey. The use of systemic insecticides in the treatment of sunflower seeds may be justified in specific contexts, but further investigation is required to determine the long-term compatibility of this approach with IPM programs that rely on conservation biological control of significant pests.

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Table 1 Mean (± SE) survival, developmental time and sex ratio (proportion female) of *Chrysoperla carnea* larvae when exposed to sunflower stems grown from treated seeds, and their subsequent reproductive parameters

Treatment	Larval period[1]		Pupal period[1]		Sex ratio[2] (n)	Reproductive parameters[1] (*n*)		
	Survival[a] (%)	Duration (days)	Survival[b] (%)	Duration (days)		Preoviposition period (days)	10-d fecundity (eggs female^{-1})	Egg viability (% eclosing)
Control	97.5 ± 2.50	8.9 ± 0.13	85.0 ± 5.00	9.1 ± 0.03	0.46 ab (33)	6.7 ± 0.51 (16)	105.4 ± 10.62	93.1 ± 3.22
Chlorantraniliprole	95.0 ± 3.27	9.1 ± 0.14	85.0 ± 7.32	9.0 ± 0.03	0.65 a (32)	6.1 ± 0.29 (11)	107.5 ± 8.41	92.7 ± 5.06
Thiamethoxam	92.5 ± 5.26	8.7 ± 0.10	92.5 ± 5.26	9.0 ± 0.05	0.36 b (33)	6.3 ± 0.38 (12)	93.3 ± 8.38	92.2 ± 6.62

Means followed by different letters were significantly different within columns (Chi-square, $\alpha = 0.05$).

Analysis by one-way ANOVA (1) or Chi-square (2).

[a] Percentage of neonate larvae (*n* = 40/treatment) yielding pupae.

[b] Percentage of pupae yielding viable adults.

Table 2 Mean (± SE) reproductive parameters of *Chrysoperla carnea* adult pairs exposed to sunflower stems grown from treated seed and the developmental parameters of their offspring.

Treatment	Reproductive parameters[1,2]			Larval period[2]		Pupal period[2]		Sex ratio[3] (n)
	Preoviposition period (days)	10-days fecundity (eggs female^{-1})	Egg viability (% eclosing)	Survival[a] (%)	Duration (days)	Survival[b] (%)	Duration (days)	
Control	13.9 ± 0.75	73.3 ± 11.77 a	83.8 ± 12.23	97.1 ± 1.84 a	9.3 ± 0.15	100	9.2 ± 0.09	0.61 (66)
Chlorantraniliprole	15.5 ± 1.03	46.2 ± 14.76 b	72.5 ± 24.28	73.3 ± 10.53 b	9.7 ± 0.44	100	9.1 ± 0.04	0.54 (22)
Thiamethoxam	16.4 ± 1.48	15.1 ± 9.41 c	-	-	-	-	-	-

Means followed by different letters were significantly different within columns (Bonferroni or *t* test, $\alpha = 0.05$).

Fecundity in the thiamethoxam treatment was too low to permit analysis of offspring development - dashes indicate no data.

Analysis by one-way ANOVA (1); *t* test (2) or Chi-square (3).

[a] Percentage of neonate larvae producing pupae.

[b] Percentage of pupae producing viable adults.

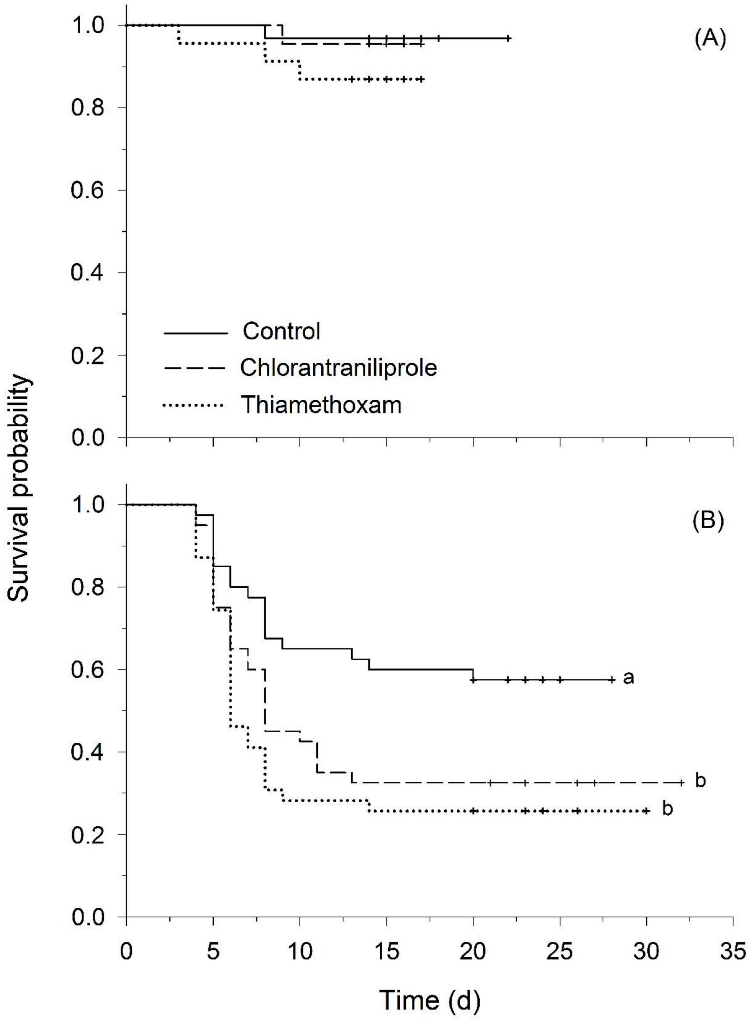

Fig. 1 Survival plots for adult *Chrysoperla carnea* exposed as larvae (a) and adults (b) to stems of sunflower plants grown from seed treated with either chlorantraniliprole or thiamethoxam. All data were considered (+ censored data). Curves bearing different letters were significantly different (log-rank test, $\alpha = 0.05$).

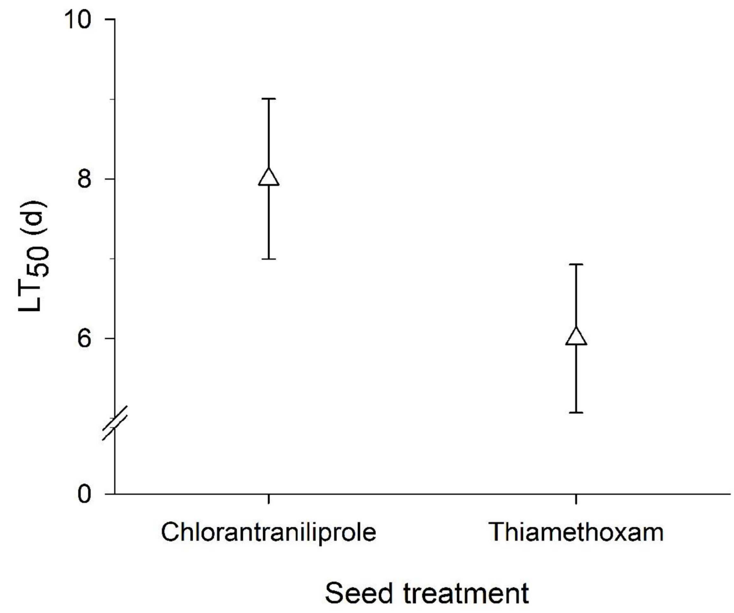

Fig. 2 Median (± 95% CIs) survival times (LT_{50}) for adult *Chrysoperla carnea* exposed to stems of sunflower plants grown from seed treated with either chlorantraniliprole or thiamethoxam.