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Sublethal effects of insecticide seed treatments on two nearctic lady beetles (Coleoptera: Coccinellidae)

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Abstract Predatory insects often feed on plants or use plant products to supplement their diet, creating a potential route of exposure to systemic insecticides used as seed treatments. This study examined whether chlorantraniliprole or thiamethoxam might negatively impact *Coleomegilla maculata* and *Hippodamia convergens* when the beetles consumed the extrafloral nectar of sunflowers grown from treated seed. We reared both species on eggs of *Ephestia kuehniella* and then switched adult *H. convergens* to a diet of greenbugs, *Schizaphis graminum*, in order to induce oviposition in this species. Excised sunflower stems, either treated or control and refreshed every 48 h, were provided throughout larval development, or for the first week of adult life. Exposure of *C. maculata* larvae to chlorantraniliprole and thiamethoxam applied as seed treatments delayed adult emergence by prolonging the pupal period. When adults were exposed, thiamethoxam reduced the preoviposition period compared to chlorantraniliprole, whereas the latter treatment cause females to produce fewer clutches during the observation period. Larvae of *C. maculata* did not appear to obtain sufficient hydration from the sunflower stems and their subsequent fecundity and fertility were compromised in comparison to the adult exposure experiment where larvae received supplemental water during development. Exposure of *H. convergens* larvae to thiamethoxam skewed the sex ratio in favor of females; both materials reduced the egg viability of resulting adults and increased the period required for eclosion. Exposure of *H. convergens* adults to chlorantraniliprole reduced egg eclosion times compared to thiamethoxam and exposure to both insecticides reduced pupation times in progeny. The results indicate that both insecticides have negative, sublethal impacts on the biology of these predators when they feed on extrafloral nectar of sunflower plants grown from treated seed.

Keywords Biological control · Chlorantraniliprole · *Coleomegilla maculata* · Extrafloral nectar · *Hippodamia convergens* · Risk assessment · Systemic insecticides · Thiamethoxam

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Introduction

Many beneficial arthropods are omnivorous, consuming both prey and plant material (Coll and Guershon 2002). Natural enemies of insect pests may utilize various plant resources, nibbling tender shoots, sucking sap, or consuming pollen and nectar, both floral and extrafloral (Wackers et al. 2007; Lundgren 2009a; Choate and Lundgren 2013). Although floral and extrafloral nectar (EFN) are both rich in sugar, the latter contains sucrose as the dominant sugar, rather than glucose or fructose (Baker and Baker 1979; Rogers 1985). Sugars can be an essential dietary component for coccinellids, improving their survival and reproductive capabilities, and providing metabolic fuel for flight and other behaviors (Lundgren 2009b; Hodek and Evans 2012). Unlike floral nectar which is available only during flowering, EFN can be available to natural enemies for a much longer period (Pacini et al. 2003; Rose et al. 2006).

On the High Plains of the USA, the EFN secreted by annual sunflowers, *Helianthus annuus* L., (Asteraceae) is an important source of both sugar and hydration for beneficial insects during the hot, dry summers. Although extremely small, the nectaries are highly abundant along petioles and leaf veins and secrete nectar continuously from the time the first true leaves expand until the plants senesce. The production of EFN by plants is associated with attraction of herbivore natural enemies and thus fosters mutualistic protection for the plant (Marazzi et al. 2013). More than 40 species of Coccinellidae are known to utilize EFN in 15 plant families (Pemberton and Vandenberg 1993). A wide variety of insects, beneficial and otherwise, can be directly observed utilizing sunflower EFN as a source of hydration during summer months (Charlet and Gavloski 2011) and sunflower EFN likely accounts for the great diversity of insects associated with this plant (Royer and Walgenbach 1991).

Coleomegilla maculata DeGeer and *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) are two of the most abundant lady beetles in the central USA, the latter species being of particular importance for the biological control of cereal aphids in wheat, sorghum and other grains (Rice and Wilde 1988; Nechols and Harvey 1998; Michaud 2013). Both species consume pollen and nectar, both floral and EFN (Pemberton and Vandenberg 1993; Smith and Krischik 1999). These species breed only during periods of high aphid populations, which are usually limited to several weeks in both spring and fall. Aphids and alternative insect prey are rare during summer months, forcing most species into a reproductive diapause (Michaud and Qureshi 2005). Hydration is critical to survival during this period when the beetles must survive on alternative food sources such as pollen and non-aphid prey that have lower water content than aphids, and sunflower EFN can be a key moisture source (Michaud and Qureshi 2006). Although *C. maculata* can complete development on an exclusive diet of pollen, it has a relatively high water demand when feeding on non-aphid food sources and is sensitive to desiccation stress during development (Michaud and Grant 2005). In contrast, laboratory observations (JPM unpublished) indicate that the daily water consumption of diapausing adult *H. convergens* is only 20–25 % that of diapausing *C. maculata*, and the drought tolerance of the former species is likely key to its success in this arid environment.

Recently, both floral nectar and EFN have been recognized as potential routes of exposure to systemic insecticides applied to soil or seeds. Both lethal and sublethal effects have been observed in honey bees (van der Sluijs et al. 2013), predators such as *C. maculata* (Smith and Krischik 1999), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Seagraves and Lundgren 2012; Gontijo et al. 2014a) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) (Rogers et al. 2007; Gontijo et al. 2014b), and parasitoids such as *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) (Krischik et al. 2007) and *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) (Moscardini et al. 2014). Seed treatment with systemic insecticides has been widely adopted as a means of early-season pest control in row crops (Hodgson et al. 2012; Nuyttens et al. 2013). However, some studies in soybean, corn and canola have questioned the economic benefit of prophylactic seed treatments, aside from the potential non-target hazards they present (Royer et al. 2005; Wilde et al. 2007; Seagraves and Lundgren 2012). Like most other row crops, the majority of commercial sunflowers are now planted with a systemic insecticide seed treatment, usually thiamethoxam.

The focal insecticides in the present study were chlorantraniliprole and thiamethoxam, examples of two very different insecticide groups, diamides and neonicotinoids, respectively. Both insecticides exhibit systemic activity within plant vascular tissues which facilitates their use as seed treatments, and their potential to contaminate plant products, including floral and extrafloral nectar (Maienfisch et al. 2001; Lahm et al. 2009; Li et al. 2012). Chlorantraniliprole acts as a ryanodine receptor modulator to block insect muscle contraction; once ingested by an insect, Ca^{++} depletion in muscle cells leads to feeding cessation, lethargy, muscle paralysis and death (Lahm et al. 2007). In contrast, thiamethoxam targets nicotinic acetylcholine receptors in the central nervous system of insects, producing both lethal and sublethal neurological effects (Tomizawa and Casida 2005). The objective of the present study was to assess the sensitivity of *C. maculata* and *H. convergens* to traces of these materials in sunflower EFN and test whether development or reproduction would be impacted when larvae or adults were exposed to sunflower seedlings grown from treated seed.

Materials and methods

Insect colonies

Adults of *C. maculata* and *H. convergens* were collected from fields of sorghum and corn at the Agricultural Research Center in Hays, Kansas, USA (38°51′31.14″N 99°20′10.86″W). Adults of each species were placed in 1-L glass mason jars (ca. 150 per jar) covered with an organdy mesh screen and filled with shredded wax paper as harborage. Water was provided on a cotton wick and approximately 50 mg of frozen *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs, obtained from a commercial supplier (Beneficial Insectary, Oak Run, CA, USA), were provided daily to each jar. Both species were held in a growth chamber at 24 ± 1 °C, 42 ± 5 % RH, and a photoperiod of 16:8 (L:D). Under these crowded conditions with limited food, the beetles remain in reproductive diapause for many months.

For each experiment, a series of female beetles (n = 30) of each species were removed from the jar and isolated, *C. maculata* in plastic Petri dishes (5.5 cm diam) and *H. convergens* in ventilated plexiglass cylinders (5.0 cm diam × 10.0 cm ht) under the same physical conditions as the colony. Females of *C. maculata* were fed with frozen *E. kueniella* eggs daily with water provided on a small sponge, whereas females of *H. convergens* were fed with an ad libitum diet of greenbugs, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), because aphids are required to induce oviposition in this species once it has entered diapause (Michaud and Qureshi 2006). The plexiglass cylinders facilitated the provisioning of aphids on excised sorghum seedlings and provided more secure containment of aphids than did the petri dishes. The aphids were obtained from colonies reared on sorghum seedlings in a growth chamber under the same physical conditions as the beetles. Food and water were refreshed daily and eggs, mostly laid on the inner surfaces of the containers, were collected by transferring the beetles to new containers. Upon eclosion, larvae of both species were reared on frozen eggs of *E. kuehniella* in Petri dishes (5.5 cm diam), five per dish, with water provided on a sponge cube, refreshed every 48 h, until they emerged as adults. The first laboratory generation was used for larval exposure experiments and the second generation for adult exposure experiments for each species.

Sunflower plants

Triumph Nusun cv. 810CL sunflower seeds were obtained from Triumph Seed Corp. (Dow Agrosciences, Indianapolis, ID) both with and without treatment with Cruiser 5FS® (thiamethoxam, 50 mg a.i. 100 kg^{-1}, Syngenta Crop Protection, Greensboro, NC); untreated Triumph seed served as the experimental control. Sunflower seeds cv. Pioneer 63N82 were obtained from DuPont Crop Protection (E.I. du Pont de Nemours and Co., Wilmington, DE) treated with chlorantraniliprole (1800 mg a.i. 100 kg^{-1}). All seeds were planted 2.0 cm deep in metal trays (8.0 cm × 51.0 cm × 36.0 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 (L:D = 12:12). Plants were watered daily, but sparingly, to avoid excessive leaching of insecticide. Sunflower stalks were harvested beginning at the V2 stage (14–15 day-old plants with two true leaves expanded) and every 2 days thereafter throughout each period of insect exposure, so that insects were exposed to progressively older plant tissues as material was replaced in the experiments. All stem segments were harvested before 9:00 a.m. and constituted the bulk of the upper portion of the main stem. For provisioning to insects, stem segments (ca. 4.0–5.0 cm in length) were excised from seedlings and the cut ends dipped in liquid paraffin to seal vascular tissues and maintain turgor, while at the same time preventing the exudation of any resinous materials that might pose a hazard to the insects.

Exposure of larvae

Experiments with both species were conducted under the same environmental conditions used for rearing the beetle colonies. Each replicate (n = 8 per treatment) consisted of five first instar larvae held in a Petri dish (5.5 cm diam), their parentage recorded to prevent any subsequent pairing of related beetles. Larvae were fed frozen eggs of *E. kuehniella* ad libitum, refreshed every 48 h. Each Petri dish was supplied with a sunflower stem segment as the only source of hydration, either grown from untreated seed (controls) or from seed treated with one of the two insecticides. The stem segments were replaced every 48 h until larvae pupated.

Data were recorded daily for all insects throughout the experiment. Larval developmental time was tallied as the number of days from the beginning of the experiment until the formation of pupae and pupation time as the number of days from pupal formation to adult emergence. Immature survival was calculated as the percentage of neonate larvae placed in the treatment that successfully emerged as adults. Emergent adults were sexed and, when insects were 7–8 days old, the maximum possible number of pairs were established by confining each female with a male from the same treatment group, checking parentage to prevent the pairing of siblings. After 48 h, males were removed and females were isolated, *C. maculata* in Petri dishes (as above) provisioned with ad libitum frozen eggs of *E. kuehniella* and water on a cube of sponge, *H. convergens* in plexiglass vials (as above) with ad libitum *S. graminum* provided on excised sorghum seedlings. Food and water was refreshed every 48 h.

Eggs were harvested daily by transferring females to new containers and the preoviposition period of each female was calculated as the number of days from adult emergence until first oviposition. A series of ten clutches were collected from each *H. convergens* female and the number of days required to produce them was recorded. Because oviposition by *C. maculata* was much slower, female fecundity was assessed for a 21 day period post-copula, during which period not all females produced ten clutches.

Exposure of adults

Pairs of adult beetles (ca. 24 h old) of each species (*C. maculata*, n = 18 per treatment, *H. convergens*, n = 14 per treatment) were established in their respective containers (as above), with parentage checked to prevent the pairing of siblings. Each container contained a sunflower stem segment corresponding to one of the three treatments and was provisioned with ad libitum frozen eggs of *E. kuehniella*. The sunflower stems were refreshed every 48 h for a total exposure period of 10 days, whereupon males were removed and females isolated for oviposition, those of *C. maculata* receiving eggs of *E. kuehniella* and those of *H. convergens* receiving *S. graminum*. Procedures and data collection were thereafter the same as described above for insects exposed as larvae.

A series of ten neonate larvae hatching from the first clutch of each female were isolated in Petri dishes (5.5 cm diam), one per dish and fed ad libitum frozen eggs of *E. kuehniella* with water provided on a sponge cube, both refreshed every 48 h. All insects were observed daily and all developmental data collected until they emerged as adults.

Data analysis

Data were subjected to Kolmogorov–Smirnov and Levine tests ($\alpha = 0.05$) for verification of normality and homoscedasticity, respectively (PROC UNIVARIATE, SAS Institute 2008). For each species and life stage (larvae or adults), data that passed these tests were subjected to one-way ANOVA. When means were significant, these were separated by a Bonferroni test ($\alpha = 0.05$) (PROC GLM; SAS Institute 2008). The preoviposition periods of *C. maculata* in the adult exposure test were transformed to log (x + 1) before being subjected to one-way ANOVA. Untransformed means are presented in all tables. Data that were not normally distributed or failed a Levine test for equality of variances were analyzed using the Kruskal–Wallis test ($\alpha = 0.05$) (PROC NPAR1WAY, SAS Institute 2008). Sex ratio ($\Sigma♀/\Sigma\ (♀ + ♂)$) was analyzed using the Chi square Goodness of Fit test ($\alpha = 0.05$) (PROC FREQ; SAS Institute 2008).

Results

Larvae of *C. maculata* exposed to sunflower stems grown from treated seeds spent about half a day longer in the pupal stage than did controls, but no other treatment effects were significant (Table 1). Survival of pupae was 100 % in all three treatments, and the reproductive performance of the resulting females did not differ among treatments, although egg fertility was below normal values in all three. When *C. maculata* were exposed to sunflower stems as adults, there was no mortality in the three week observation period, but preoviposition periods were reduced by the thiamethoxam treatment relative to chlorantraniliprole, although neither was different from controls. However, females in the chlorantraniliprole treatment laid fewer clutches, although overall fecundity (total number of eggs laid) and egg viability (percentage of eggs hatching) did not vary significantly among treatments (Table 2). There were no significant treatment effects on any parameter of progeny development.

A higher proportion of emergent adults were female when larvae of *H. convergens* were exposed to stems in the thiamethoxam treatment, but no other developmental parameters differed among treatments (Table 3). Pupal survival was 100 % in all three treatments. However, the viability of eggs was significantly reduced for female adults in both the chlorantraniliprole and thiamethoxam treatments and the time required for eclosion of their eggs was slightly increased. Exposure of adults revealed no significant treatment effects on reproductive parameters, but some transgenerational effects were evident in the progeny. Chlorantraniliprole reduced egg eclosion time compared to thiamethoxam, although controls were not different from either, and both treatments reduced pupation time (Table 4). There was no mortality of adults during the period of reproductive observations.

Discussion

Subtle, but significant, negative effects on development and reproductive biology were observed when *C. maculata* and *H. convergens* fed on EFN presumably contaminated with residues of chlorantraniliprole and thiamethoxam. Systemic insecticides become distributed throughout the plant and may contaminate the pollen, floral and extrafloral nectar (Cloyd and Bethke 2011). EFN can be an important food source for many beneficial organisms, especially coccinellids, because it is rich in sugars that are easily digested (Lundgren 2009b). Consumption of EFN may provide energy and increase fitness, especially when prey is scarce. Lundgren and Seagraves (2011) observed that *C. maculata* adult consuming EFN of *Vicia faba* (Fabaceae) in the absence of prey improved their survival, nutrient reserves and reproductive capacity.

Negative effects were observed on beetle development. Both seed treatments prolonged *C. maculata* pupation time following larval exposure, possibly because intoxicated larvae had lower mobility and feeding rates, which could have resulted in nutrient limitation and consequent prolongation of the pupal stage or because the materials

Table 1 Mean (±SE) developmental and reproductive parameters of *Coleomegilla maculata* exposed as larvae to sunflower stems grown from treated seeds

Parameter	Seed treatment			*F*, *H* or χ^2	df	*P*
	Untreated	Chlorantraniliprole	Thiamethoxam			
Larval survival (%)	72.5 ± 8.4	70.0 ± 7.6	87.5 ± 6.5	3.06*	2	0.216
Larval development time (days)	11.7 ± 0.2	11.7 ± 0.3	11.9 ± 0.2	0.13	2.21	0.881
Pupation time (days)	3.5 ± 0.1b	3.9 ± 0.1a	3.9 ± 0.1a	8.89	2.21	0.002
No. adults emerged	29	28	35			
Sex ratio[1]	0.55 ± 0.09	0.50 ± 0.09	0.40 ± 0.08	1.54**	2	0.463
No. pairs mated	9	10	12			
Preoviposition period (d)	14.8 ± 2.1	18.1 ± 2.1	16.0 ± 1.6	0.72	2.28	0.494
Fecundity (eggs female^{-1})	102.0 ± 29.1	94.6 ± 31.6	122.1 ± 24.9	0.78*	2	0.677
No. clutches laid in 21 days	8.3 ± 2.1	7.3 ± 1.9	9.5 ± 1.1	0.44	2.28	0.650
Egg viability (% hatching)	50.7 ± 7.1	36.9 ± 6.3	37.5 ± 6.7	1.23	2.28	0.307
Eclosion time (days)	3.0 ± 0.0	3.0 ± 0.0	3.1 ± 0.1	2.55	2.25	0.098

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

Means followed by different letters were significantly different within rows (Bonferroni test, α = 0.05)

[1] Proportion female

Table 2 Mean (±SE) reproductive parameters of *Coleomegilla maculata* adults exposed to sunflower stems grown from treated seed and the developmental parameters of their offspring

Parameter	Seed treatment			*F*, *H* or χ^2	df	*P*
	Untreated	Chlorantraniliprole	Thiamethoxam			
No. pairs established	18	18	18			
Preoviposition period (days)	14.3 ± 1.1ab	16.9 ± 1.6a	12.4 ± 0.6b	3.62	2.51	0.034
Fecundity (eggs female^{-1})	188.1 ± 21.8	152.8 ± 29.6	210.8 ± 26.4	1.25	2.51	0.294
No. clutches laid in 21 days	14.1 ± 1.1a	10.4 ± 1.6b	15.1 ± 1.0a	3.79	2.51	0.029
Egg viability (% hatching)	92.1 ± 2.3	88.9 ± 1.8	83.9 ± 5.5	1.30	2.51	0.283
Eclosion time (days)	3.0 ± 0.0	3.1 ± 0.0	3.0 ± 0.1	0.79*	2	0.673
Larval development time (days)	11.7 ± 0.1	12.0 ± 0.1	11.9 ± 0.1	2.28	2.51	0.113
Pupation time (days)	3.1 ± 0.0	3.1 ± 0.1	3.1 ± 0.1	0.19	2.51	0.828
Immature survival (%)	96.1 ± 1.2	96.7 ± 1.9	94.4 ± 1.8	1.97*	2	0.373
No. adults emerged	173	171	170			
Sex ratio[1]	0.57 ± 0.04	0.53 ± 0.04	0.55 ± 0.04	0.74**	2	0.692

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

Means followed by different letters were significantly different within rows (Bonferroni test, α = 0.05)

[1] Proportion female

impaired neural processes that control pupation. For example, Vargas et al. (2013) showed that *C. maculata* larvae permitted to feed on *E. kuehniella* eggs for only 30 min daily had their total developmental time extended by 10–12 days compared to those permitted ad libitum access to food. Sublethal effects of neonicotinoid insecticides on the foraging behavior and predation rate of beneficial organisms have been previously reported (e.g., Desneux et al. 2007). Smith and Krischik (1999) confined *C. maculata* adults with inflorescences of sunflower plants treated with imidacloprid via soil and observed significantly reduced motor activity. Imidacloprid reduced the functional response *Serangium japonicum* Chapin (Coleoptera: Coccinellidae) to whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) eggs, when applied at a sublethal rate (5 ppm) via egg immersion (He et al. 2012). Thiacloprid applied to tomato at the rate recommended for control of the tomato leaf miner, *Tuta absoluta* (Meyrick)

Table 3 Mean (±SE) developmental and reproductive parameters of *Hippodamia convergens* exposed as larvae to sunflower stems grown from treated seeds

Parameter	Seed treatment			F or χ^2	df	P
	Untreated	Chlorantraniliprole	Thiamethoxam			
Larval survival (%)	67.5 ± 6.5	67.5 ± 6.4	57.5 ± 10.9	0.49	2.21	0.620
Larval development time (days)	11.7 ± 0.2	12.0 ± 0.2	11.9 ± 0.3	0.55	2.20	0.587
Pupation time (days)	4.8 ± 0.1	4.6 ± 0.1	4.8 ± 0.1	0.86	2.20	0.448
No. adults emerged	27	26	23			
Sex ratio[1]	0.33 ± 0.09b	0.31 ± 0.09b	0.61 ± 0.10a	6.54*	2	0.043
No. pairs mated	9	7	8			
Preoviposition period (days)	9.6 ± 1.1	10.3 ± 1.3	8.9 ± 1.5	0.27	2.21	0.767
Fecundity (eggs female^{-1})	251.3 ± 19.4	262.1 ± 23.0	254.0 ± 27.7	0.05	2.21	0.947
No. days for 10 clutches	10.6 ± 0.2	11.3 ± 0.5	11.5 ± 0.9	0.76	2.21	0.479
Egg viability (% hatching)	93.2 ± 1.9a	79.9 ± 4.1b	84.1 ± 3.4b	4.87	2.21	0.018
Eclosion time (days)	3.1 ± 0.0b	3.3 ± 0.0a	3.2 ± 0.0a	4.56	2.21	0.023

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

Means followed by different letters were significantly different within rows (Bonferroni test or Chi square, α = 0.05)

[1] Proportion female

Table 4 Mean (±SE) reproductive parameters of *Hippodamia convergens* adults exposed to sunflower stems grown from treated seed and the developmental parameters of their offspring

Parameter	Seed treatment			F, H or χ^2	df	P
	Untreated	Chlorantraniliprole	Thiamethoxam			
No. pairs mated	14	14	14			
Preoviposition period (days)	12.1 ± 0.7	12.4 ± 0.6	13.2 ± 0.3	1.17	2.39	0.332
Fecundity (eggs female^{-1})	247.6 ± 16.7	287.7 ± 18.8	264.1 ± 18.4	1.27	2.39	0.294
No. days for 10 clutches	13.6 ± 0.6	12.1 ± 0.6	12.5 ± 0.4	2.38	2.39	0.106
Egg viability (% hatching)	86.7 ± 2.8	82.8 ± 2.8	79.3 ± 5.7	1.02*	2	0.602
Eclosion time (days)	3.2 ± 0.0ab	3.1 ± 0.0b	3.3 ± 0.0a	3.27	2.39	0.049
Larval development time (days)	11.5 ± 0.1	11.7 ± 0.1	11.7 ± 0.1	1.22	2.38	0.308
Pupation time (days)	4.9 ± 0.1a	4.7 ± 0.1b	4.7 ± 0.1b	4.55	2.38	0.017
Immature survival (%)	86.4 ± 2.9	85.0 ± 4.5	83.8 ± 2.9	0.77*	2	0.682
No. adults emerged	121	119	109			
Sex ratio[1]	0.53 ± 0.05	0.45 ± 0.05	0.46 ± 0.05	2.09**	2	0.353

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

Means followed by different letters were significantly different within rows (Bonferroni test, α = 0.05)

[1] Proportion female

(Lepidoptera: Gelechiidae) was shown to cause reductions in the foraging behavior and predation rate of fifth instar nymphs of *Macrolophus pygmaeus* (Hemiptera: Miridae) (Martinou et al. 2014). Thiamethoxam and clothianidin both caused neurotoxic symptoms (e.g., trembling, paralysis, and loss of coordination) in larvae of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) exposed for six hours to corn plants grown from treated seeds (Moser and Obrycki 2009). Neonicotinoids also cause negative effects on the motor functions of adult worker honeybees, *Apis mellifera* L. (Hymenoptera: Apidae) (Williamson et al. 2014). Lethargic behavior and feeding inhibition are thus sublethal effects often associated with chlorantraniliprole intoxication. Smagghe et al. (2013) found that workers of *Bombus terrestris* (L.) (Hymenoptera: Apidae) showed lethargic behavior and reduced food consumption following chronic oral exposure to chlorantraniliprole via contaminated pollen.

Chlorantraniliprole has been reported to reduce larval feeding in herbivores such as *Plutella xylostella* L. (Lepidoptera: Plutellidae), *Trichoplusia ni* (Hubner), *Spodoptera exigua* (Hubner) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Hannig et al. 2009). Oral exposure of neonate *S. exigua* larvae to a sublethal concentration (LC_{30}) of chlorantraniliprole prolonged larval development and increased the appearance of supernumerary instars (Lai and Su 2011). Notwithstanding, exposure to surface residues of chlorantraniliprole does not seem to affect the foraging behavior of predatory bugs, including *Amphiareus constrictus* (Stal), *Blaptostethus pallescens* Poppius, *Orius tristicolor* (White) (Hemiptera: Anthocoridae) (Pereira et al. 2014), *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stal) (Heteroptera: Pentatomidae) (Castro et al. 2013). Sunflower EFN contaminated with chlorantraniliprole and thiamethoxam caused no lethal effects when consumed by *L. testaceipes* adults, but female foraging behavior was impaired and fewer greenbug nymphs were attacked and parasitized in each bout of foraging (Moscardini et al. 2014). Imidacloprid and chlorantraniliprole also impeded the parasitism of *Nilaparvata lugens* (Stal) (Heteroptera: Delphacidae) by *Anagrus nilaparvatae* (Pang and Wang) (Hymenoptera: Mymaridae) when the wasps consumed honey contaminated with these insecticides (Liu et al. 2010, 2012). Parasitism of aphids by *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae) was reduced when the wasps host-fed on aphids that, in turn, fed on imidacloprid- and thiamethoxam-treated soybean plants (Frewin et al. 2014).

Negative impacts were also observed on the reproductive biology of both beetle species. Effects of chlorantraniliprole on reproduction, similar to those on *C. maculata* in this study, have been reported in other insect groups. For example, Gontijo et al. (2014a) found that female *O. insidiosus* exposed as nymphs to chlorantraniliprole via sunflower EFN suffered extended preoviposition periods. When newly enclosed workers of *B. terrestris* consumed sugar water supplemented with chlorantraniliprole at 40 mg L^{-1}, or pollen sprayed with the insecticide, fewer drones were produced per nest (Smagghe et al. 2013). Negative effects of both insecticides on *H. convergens* biology were delayed and only observed later in life history (reduced egg viability and longer embryonic development when larvae were exposed) or in the next generation (faster pupation time in progeny of exposed adults). In the case of thiamethoxam, increased toxicity is associated with its metabolism into clothianidin, a process which can occur within both plant (Cloyd and Bethke 2011) and insect (Nauen et al. 2003) tissues, and might account for some delayed impact. Benzidane et al. (2010) reported low toxicity of thiamethoxam to *Periplaneta americana* (L.) (Blattodea: Blattidae) adults was associated with a lack of breakdown into clothianidin within 24 h after its ingestion. In contrast, the primary metabolites of chlorantraniliprole are thought to have low toxicity (FAO 2008).

Sublethal concentrations of insecticides have the potential to distort sex ratios in the progeny of exposed insects. Exposure of *H. convergens* larvae to thiamethoxam resulted in a sex ratio skewed toward females when compared to the chlorantraniliprole and control treatments. In contrast, Gontijo et al. (2014b) observed a reduced sex ratio in *C. carnea* when larvae were exposed to sunflower stems grown from seed treated with thiamethoxam as compared to chlorantraniliprole. Thus, patterns of gender-specific larval susceptibility to this material appear to vary among insect groups.

Sunflower stalks alone did not appear to supply provide sufficient hydration for larvae of *C. maculata*. Both larvae and adults of *C. maculata* have a high water demand and require supplementary water when feeding on non-aphid foods (Michaud and Grant 2005). The viability of *C. maculata* eggs was abnormally low in the larval exposure experiment, regardless of treatment, where the only moisture available was EFN from the exised stalks, but normal in the adult exposure experiment where larvae were reared with access to water. Thus, we infer that reproduction by *C. maculata* was compromised in this particular trial (reduced fecundity and egg viability), even though larval survival was relatively good. It is also possible that treatment effects on egg viability, such as those evident in the analogous *H. convergens* experiment, were obscured in these drought-stressed larvae. In *H. convergens*, preoviposition periods averaged two days longer for beetles exposed as adults compared to those exposed as larvae, likely due to the fact the latter received aphid prey two to three days earlier in adult life. Interestingly, both insecticides altered egg eclosion times in *H. convergens* whether the beetles were exposed as larvae or adults, but only the progeny of *H. convergens* exposed as adults to either insecticide had faster pupation times, with no such effect evident in larvae exposed directly. Thus, the transgenerational effects of these materials do not necessarily mimic the effects of direct exposure.

Transgenerational effects of neonicotinoids have been reported in some coccinellid species. Yu et al. (2014) observed that exposure of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) larvae to imidacloprid in laboratory microcosms reduced fecundity and egg viability in the next generation. Similar results were reported for topical exposure of *Eriopis connexa* (Gemar) (Coleoptera: Coccinellidae) larvae to acetamiprid that reduced subsequent egg viability (Fogel et al. 2013). Transgenerational effects of systemic insecticides have also been reported for other insect groups, for example thiamethoxam in *Bemisia* *tabaci* Gennadius (biotype B) and *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) (Liang et al. 2012) and chlorantraniliprole in *P. xylostella* (Guo et al. 2013).

Overall, treatment of sunflower seeds with chlorantraniliprole and thiamethoxam caused few negative effects in *C. maculata* and *H. convergens* compared to other beneficial species that have been similarly examined (Gontijo et al. 2014a, b; Moscardini et al. 2014). Because most commercial crops on the High Plains (e.g., sunflower, sorghum, soybeans and corn) are all planted with seed treatments, the beetles colonizing these crops may be regularly exposed to sublethal doses of these insecticides, especially thiamethoxam which has been widely used for more than 10 years. Increasing insecticide tolerance in natural enemies, including lady beetles, has been reported as a function of chronic insecticide exposure (Head et al. 1977; Ruberson et al. 2007; Rodrigues et al. 2013a). Rodrigues et al. (2013b) investigated lambda-cyhalothrin susceptibility in 31 populations of lady beetles (Coleoptera: Coccinellidae), focusing on seven species common in cotton fields, and found significant variation between species and among populations of a given species which they inferred to reflect historical field exposure of the beetles to this insecticide. Thus, the relatively robust responses of both coccinellid species in these experiments may not be representative of other geographic populations that may have different insecticide exposure histories.

In summary, our results suggest that both chlorantraniliprole and thiamethoxam have subtle negative effects on the developmental and reproductive biology of these important predators with the potential for cumulative impacts on population dynamics. Other authors have suggested that the widespread use of systemic insecticides poses a risk to biodiversity and ecosystem services (Biondi et al. 2012; Chagnon et al. 2014; van der Sluijs et al. 2015). The integrated approach to pest management with insecticides is predicated on economic justification prior to application, rather than prophylactic use. Although these seed treatments have been shown effective in controlling particular pests, for example rice water weevil on rice, *Lissorhoptrus oryzophilus* Kuschel (Hummel et al. 2014), and various thrips species on soybean seedlings (Reisig et al. 2012), many studies have now failed to identify economic benefits of prophylactic seed treatments in wheat (Royer et al. 2005) corn (Wilde et al. 2007) or soybeans (Seagraves and Lundgren 2012) and others have found them to be ineffective in reducing pests populations (Vernon et al. 2011). Field studies examining a range of nontarget arthropods, conducted over longer time frames, are therefore warranted to determine whether or not these materials are truly compatible with IPM in field crops.

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