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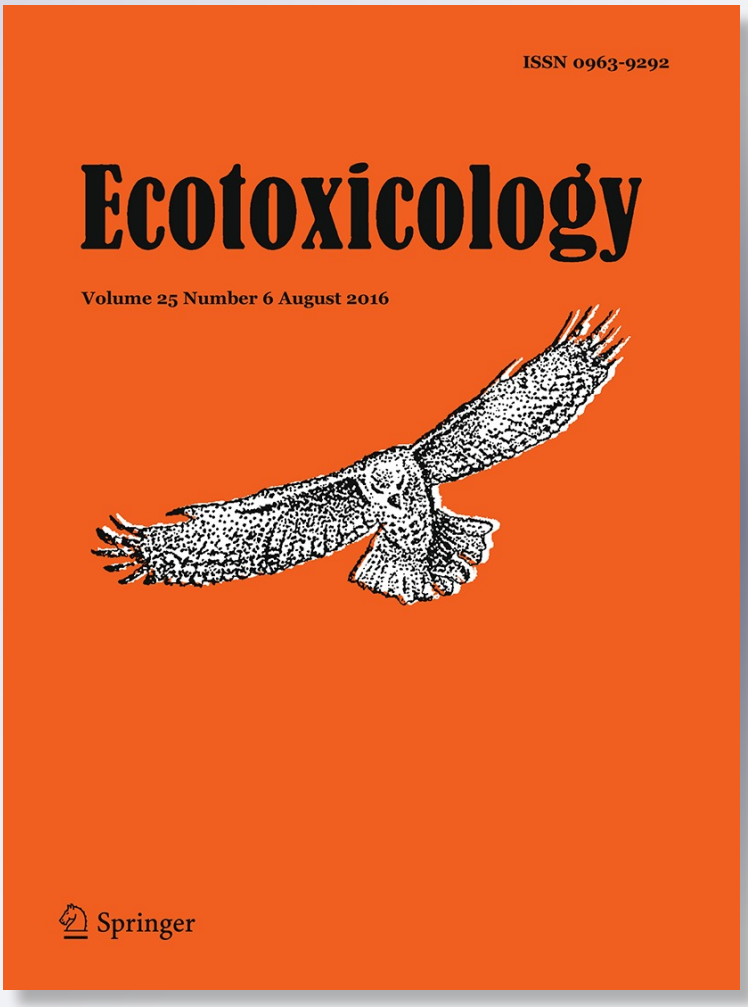

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Unintended effects of the herbicides 2,4-D and dicamba on lady beetles

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Abstract Weed resistance to glyphosate and development of new GM crops tolerant to 2,4-dichlorophenoxyacetic acid (2,4-D) and dicamba is expected to lead to increased use of these herbicides in cropland. The lady beetle, *Coleomegilla maculata* is an important beneficial insect in cropland that is commonly used as an indicator species in safety evaluations of pesticides. Here, we examined the lethal and non-lethal effects of 2,4-D and dicamba active ingredients and commercial formulations to this lady beetle species, and tested for synergistic effects of the herbicides. Second instars of lady beetles were exposed to an experimental treatment, and their mortality, development, weight, sex ratio, fecundity, and mobility was evaluated. Using similar methods, a dose–response study was conducted on 2,4-D with and without dicamba. The commercial formulation of 2,4-D was highly lethal to lady beetle larvae; the LC_{90} of this herbicide was 13 % of the label rate. In this case, the "inactive" ingredients were a key driver of the toxicity. Dicamba active ingredient significantly increased lady beetle mortality and reduced their body weight. The commercial formulations of both herbicides reduced the proportion of males in the lady beetle population. The herbicides when used together did not act synergistically in their toxicity toward lady beetles versus when the chemistries were used independently. Our work shows that herbicide formulations can cause both lethal and sublethal effects on non-target, beneficial insects, and these effects are sometimes driven by the "inactive" ingredients. The field-level implications of shifts in weed management practices on insect management programs should receive further attention.

Keywords *Coleomegilla maculata* · GM crop · Herbicide tolerant crop · Pesticide · Risk assessment · Sublethal effects

Introduction

The use of herbicide-tolerant, genetically modified crops has been associated with major shifts in herbicide use throughout the USA. Genetically modified crops were first commercialized in the US in 1996, with the introduction of glyphosate-resistant soybean (Green et al. 2009). Currently nearly all soybeans, cotton, and maize grown in the USA are tolerant to the herbicide glyphosate (Lundgren et al. 2009; NASS 2015). A growing number of weeds have evolved resistance to glyphosate, and in response additional herbicides are now used alone and in mixtures to manage weeds and prolong the remaining effectiveness of glyphosate (Behrens et al. 2007). For example, there have been recent increases in the use of 2,4-dichlorophenoxyacetic acid (2,4-D) and dicamba together alongside glyphosate to sufficiently control weeds (Shaner 2000; Behrens et al. 2007). Moreover, 2,4-D resistant crops were registered by the US-EPA in 2014 (EPA Registration Number 62719-649; Decision Number 457755) and dicamba resistant crops are undergoing registration (Mortensen et al. 2012). If these crops are adopted as widely as glyphosate-resistant crops, these chemistries would

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experience a dramatic increase in use nationally. The realized and potential increases in the uses of 2,4-D and dicamba raise the question of how these chemicals will affect other species in agroecosystems.

One risk posed by pesticides is the potential hazard that their active ingredients may pose to non-target species and the ecosystem services that these species provide. These hazards can be either lethal or sublethal effects (Desneux et al. 2007), and herbicides have been documented as having deleterious effects on animals (Cousin et al. 2013; Lacoume et al. 2009). A number of studies have explored the toxicity of 2,4-D and dicamba on non-target species. For example, honey bee colonies fed 2,4-D experienced reduced brood production at a concentration of 100 ppm, and eggs failed to hatch when the colony was exposed to 1000 ppm (Morton et al. 1972). Dung beetle population declines have been correlated with the use of a 2,4-D commercial formulation (Martinez et al. 2001). Increased earthworm mortality (30–40 % mortality) was observed after a 14 day exposure to 10 mg 2,4-D/kg soil and 100 % mortality was observed at 500 mg/kg of soil (Correia and Moreira 2010), and morphological abnormalities were documented, although the dose and exposure underlying these effects were unclear. These non-target effects have also been observed in vertebrates as well (e.g., amphibians; Aronzon et al. 2011). Dicamba is typically not as toxic to non-target organisms as 2,4-D, but it might have indirect negative effects by reducing plant quality for foliar-dwelling and herbivorous species (Bohnenblust et al. 2013). However, both herbicides adversely affected wild birds and mammals when applied at typical application rates of 0.33 kg/ha of dicamba and 1.11 kg/ha of 2,4-D (Bautista 2007). In sum, toxicological evidence suggests a risk potential for these two herbicides, assuming that exposure levels in the field reach a critical, and hereby undefined, exposure threshold.

Formulation is an important consideration when examining the toxicity of a pesticide, with additives to the formulation potentially having important effects on non-target species. "Inactive" ingredients in pesticide formulations are rarely publicized, but typically constitute the majority of a pesticide's volume (Mullin et al. 2015; Tominack 2000). These additives include things like surfactants, solvents, emulsifiers, stabilizers, crystal inhibitors, spreaders, stickers, wetting agents, etc. (Mullin et al. 2015; Tominack 2000). These "inactive" ingredients extend the persistence of a pesticide in the environment (Howe et al. 2004; Krogh et al. 2003; Tominack 2000), affect the spectrum of activity of a pesticide (Stahlman and Phillips 1979), and improve its coverage and effectiveness (Cowles et al. 2000). All told, millions of kilograms of additive ingredients are deployed into the environment annually (Krogh et al. 2003; Mullin et al. 2015). In the U.S., pesticide additives are regulated (Levine 1996), but less so than active ingredients. Moreover, formulations of pesticides have fewer data requirements for registration than active ingredients (Mullin et al. 2015). One challenge is that all of the active ingredients and adjuvants could feasibly combine into tens of thousands of formulations that would each require registrations (Cox 1999). Add to this that the ecological and physiological circumstances affect the relative toxicity of a formulation to a particular organism (Cowles et al. 2000), that the metabolites of the adjuvants can be toxic where the parent molecule is not (Sivey and Roberts 2010), and that pesticides in cocktails can act synergistically (Zhu et al. 2014), and the registration process becomes very complex. Nevertheless, "inactive" ingredients in pesticide formulations affect the toxicity of the active ingredient to a specific organism, and sometimes the adjuvants can be more toxic to non-target species than the active ingredients are (Cox and Surgan 2008; Mann and Bidwell 1999; Mullin et al. 2015; Surgan et al. 2010; Zhu et al. 2014). For example, "inactive" ingredients found in common herbicide formulations used at label rates can be directly toxic to mites (Cowles et al. 2000; Yi et al. 2011), honey bees (Ciarlo et al. 2011; Zhu et al. 2014), aquatic insects (Dunkel and Richards 1998), and soil organisms (Pereira et al. 2009). Indeed, the commercial formulation of 2,4-D can be ten times more toxic to late stage tadpoles of at least one frog species (*Rhinella arenarum*) than the active ingredient (Aronzon et al. 2011), due in large part to the use of poly-ethoxylated tallowamine (POEA) as an adjuvant in aquatic herbicides (Howe et al. 2004; Mann et al. 2009).

Coccinellidae are important biological control agents found in numerous cropping systems throughout the world (Biddinger et al. 2009; Hodek and Honěk 2009; O'Connell et al. 2012; Iperti 1999). They are important predators of aphids and other soft-bodied life stages of insect pests (Evans 2009; Hodek and Honěk 2009; Obrycki et al. 2009). Lady beetles have also been used as an indicator species for testing of non-target effects of pesticides (Stark et al. 2007; Antwi and Peterson 2009; James 2003). For example, toxicity assays focusing on the native lady beetle, *Coleomegilla maculata*, were influential in the safety assessment of genetically modified Bt corn (Duan et al. 2002; Lundgren and Wiedenmann 2002). Recently, laboratory toxicity assays revealed that exposure of *C. maculata* larvae to commercial formulations of 2,4-D at label rates resulted in 25 % mortality and increased development time (Michaud and Vargas 2010). Given the impending use increases of 2,4-D and dicamba, our experimental goal is to explore the toxicity of these herbicides more deeply, examining the dose–response curve for commercial formulations and active ingredients, and examine how combining these herbicides together affects their relative toxicity to this non-target, beneficial insect.

Materials and methods

Insects

The experiments were conducted with eggs obtained from a *C. maculata* DeGeer (Coleoptera: Coccinellidae) colony. Hundreds of adults from the colony were originally collected in untreated, conventional maize fields around USDA-ARS, North Central Agricultural Research Laboratory, Brookings, SD (44.35 and −96.81; latitude and longitude), and annual infusions of new beetles were added to the colony. Neonate larvae were reared until the 2nd stadium on Lundgren's Super C Mac Diet (Lundgren et al. 2011) and a water saturated cotton wick.

Effects of commercial formulations and active ingredients of 2,4-D and dicamba on *C. maculata*

Second instars (n = 40 each treatment) were randomly assigned to one of eight treatments: (1) an insecticide application, (2) 2,4-D commercial formulation, (3) 2,4-D active ingredient, (4) dicamba commercial formulation, (5) dicamba active ingredient, (6) 2,4-D and dicamba commercial formulations, (7) water control, and (8) acetone control. The insecticide was esfenvalerate (Asana® XL, DuPont, Wilmington, DE). The commercial product was diluted in water according to label instructions (304 µL/mL). The commercial formulation for 2,4-D was 2,4-D LV4 (Albaugh Inc., Ankeny, IA); active ingredient for this chemical was 2,4-D (Product 31518, Sigma-Aldrich, St-Louis, MO). The commercial product was diluted in water as per label instructions (250 µL/mL) and the active ingredient was diluted in acetone at a rate equivalent to the amount of a.i. in the commercial product (114 mg/mL of a 50:50 acetone:water solvent). Dicamba (3,6-dichloro-2-methoxybenzoic acid) commercial formulation was dicamba DMA (Albaugh Inc.); active ingredient for this chemical was dicamba (Product 45430, Sigma-Aldrich). The commercial product was diluted in water as per label instructions (250 µL/mL) and the active ingredient was diluted in acetone at a rate that equated to the amount of a.i. in the commercial product (120 mg/mL of a 50:50 acetone:water solvent). In the treatment where the commercial products were combined, each was diluted in water according to label instructions (37 µL/mL 2,4-D and 37 µL/mL dicamba); when used in combination, the labels recommend a much lower rate for each of the two herbicides than when they are used alone. Each test substance was administered as a single application of 0.5 µL to the dorsum of the 2nd instar with a micropipette. This quantity of fluid was not sufficient to kill the larvae, but administered a small quantity of toxin to the young larvae, as might happen in a herbicide treated field. Additional work on actual exposure levels in the field would help to hone this assay. Larvae were then transferred to individual 30 mL plastic cups with a water-saturated cotton wick and excess lady beetle diet, which were freshened as needed. Experimental conditions were 27 °C, and a 14:10 h (light: dark; L:D) photoperiod. Larvae were monitored daily through adulthood, and various fitness metrics were recorded. Mortality and the duration of each stadium were recorded daily. Pupal weight and sex ratio of adults were recorded. Larval prey consumption was recorded within 24 h of molting to the 3rd stadium; each larva was provided with an excess (approximately 30 mg) of *Ephestia khueniella* Zeller (Lepidoptera: Pyralidae) eggs. The weight of eggs remaining after 24 h was recorded for each individual. Adult mobility was recorded as in Lundgren and Wiedenmann (2002). Each beetle was placed on its dorsum, and the time that it took to right itself was recorded. Walking speeds of individual beetles were recorded by tracing the distance of the beetle's walking path and timing the duration it required to walk it. Fecundity of each female was also recorded. Within 6 days of eclosion, females were exposed to two randomly selected males from the *C. maculata* colony for 24 h. Eggs laid per day per female were recorded daily for the next 10 days.

Effects of dicamba on the toxicity of 2,4-D

Experimental procedures followed the methods described above, except that a different suite of treatments were applied. Specifically, two series of seven doses of 2,4-D (with or without dicamba) were applied to second instars. These doses were 0, 62, 125, 250, 330, 420, and 500 µL/mL of the commercial 2,4-D formulation. Simultaneously, the same concentrations of 2,4-D were applied with the label rate of the commercial dicamba formulation (37 µL/mL). Larval mortality and development rates were recorded daily, and pupal weight and sex ratio of eclosed adults were recorded for each individual.

Data analysis

In experiment 1, larval development, pupal development and weight, prey consumption, and walking speeds were compared among the treatments using independent ANOVAs. For traits whose treatment effects were significant, differences among treatment pairs were resolved using Fisher's LSD test. Non-parametric Kruskal–Wallis ANOVAs were used to compare the fecundities and flip times among treatments. Sex ratios (females scored 1, males scored 0) in each treatment were compared to a predicted 0.5 proportion (Pilorget et al. 2010) using a one-sample *t* test. A Kaplan–Meier non-parametric model and the

Mantel test statistic were used to compare the longevity of *C. maculata* larvae fed each the eight treatments. Subsequent pair-wise comparisons were conducted to determine which treatments had significantly different survivorship curves.

In experiment 2, herbicide dose (log + 1 transformed) was plotted against survival (probit % alive transformed), and linear regressions were fitted to the data when 2,4-D was administered on its own and together with dicamba. From these curves, the LC_{20}, LC_{50} and LC_{90} doses of 2,4-D alone and with dicamba were calculated. Survival was low at doses higher than 250 µL/mL 2,4-D, so mean larval duration and pupal weights were compared among the lower herbicide doses using two-factor ANOVAs; 2,4-D dose and presence or absence of dicamba were the main effects in these analyses. The sex ratio for each of these two doses (with or without the dicamba) were compared to 0.50 using a one-sample *t* test. All statistics were conducted using Systat 13 (SYSTAT Software Inc., Chicago, IL).

Results

Experiment 1: effects of commercial rates of 2,4-D and dicamba

Total larval development rate varied significantly among the treatments ($F_{6,\ 196} = 2.20$, $P = 0.045$) (Table 1). Specifically, the larvae treated with the commercial 2,4-D product had significantly shorter larval durations from all other treatments. Pupal duration was statistically equivalent among the treatments ($F_{6,\ 193} = 0.90$, $P = 0.50$), but pupal weight differed among the treatments ($F_{6,\ 195} = 2.44$, $P = 0.03$) (Table 1). An important point from this analysis is that the larvae treated with dicamba a.i. alone or when both herbicides were combined were significantly smaller than their respective untreated controls. Third instars consumed similar amounts of prey regardless of treatment ($F_{6,\ 202} = 1.19$, $P = 0.32$) (Table 1). Sex ratios were significantly skewed toward a strong female bias in the two treatments fed the commercial herbicides (Table 1). Fecundity (Kruskal–Wallis $\chi^2_6 = 2.59$, $P = 0.86$) and flip times (Kruskal–Wallis $\chi^2_6 = 7.06$, $P = 0.32$) were similar among treatments. Walking speeds were significantly affected by treatment ($F_{6,\ 191} = 3.84$, $P = 0.001$) (Table 1). Specifically, beetles from the acetone control walked faster than all other treatments. Survival analysis showed a significant effect of treatment on *C. maculata* larval longevity (Mantel $\chi^2_7 = 184.63$, $P < 0.001$) (Fig. 1). Most importantly, the larvae treated with dicamba a.i., both herbicides, 2,4-D commercial formulation, or the insecticide died significantly sooner than the water and acetone controls.

Experiment 2

For 2,4-D alone, the LC_{20}, LC_{50} and LC_{90} values were 6.65, 15.18, and 33.26 µL/mL 2,4-D in water (Fig. 2). Adding Dicamba to the 2,4-D did not affect longevity or survival substantially over when 2,4-D alone was applied

Table 1 The effects of herbicide formulations and active ingredients on *C. maculata* life history characteristics

Treatment	Larval duration (days)	Pupal duration (days)	Pupal weight (mg)	Prey consumption (mg)	Proportion male (n)	Fecundity (eggs per female)	Flip time (s)	Mobility (mm/s)
Water control	9.54 ± 0.14 A	3.24 ± 0.07	16.05 ± 0.37 A	3.49 ± 0.13	0.37 (35)	10.00 ± 3.66	29.83 ± 7.42	37.07 ± 1.05 B
Acetone control	9.42 ± 0.12 A	3.26 ± 0.07	15.56 ± 0.29 AB	3.55 ± 0.14	0.42 (38)	11.77 ± 5.00	25.64 ± 7.12	42.44 ± 0.82 A
2,4-D formulation	7.00 ± 1.77 B	3.00 ± 0	16.13 ± 1.03 ABC	4.35 ± 0.36	0 (6) ($P < 0.001$)	7.17 ± 6.77	6.86 ± 1.49	36.17 ± 2.40 B
2,4-D active ingredient	9.53 ± 0.14 A	3.20 ± 0.07	15.57 ± 0.24 AB	3.54 ± 0.14	0.44 (36)	5.80 ± 2.15	16.15 ± 3.06	36.59 ± 1.27 B
Dicamba formulation	9.38 ± 0.14 A	3.15 ± 0.08	15.98 ± 0.34 A	3.70 ± 0.13	0.24 (33) ($P < 0.002$)	8.04 ± 3.25	19.68 ± 4.53	36.47 ± 1.06 B
Dicamba active ingredient	9.38 ± 0.14 A	3.10 ± 0.06	14.63 ± 0.28 C	3.47 ± 0.15	0.48 (29)	27.40 ± 9.77	33.07 ± 8.85	38.54 ± 1.24 B
2,4-D + dicamba	9.71 ± 0.13 A	3.13 ± 0.07	14.94 ± 0.40 BC	3.54 ± 0.17	0.42 (24)	16.00 ± 8.05	17.83 ± 5.73	39.01 ± 1.46 B

The water control was used as a reference for the formulated herbicides, and the acetone control was used as a reference for the active ingredients. Sample sizes for these metrics are provided in the "proportion male" column. Within a column, values followed by different letters are significantly different from one another ($\alpha = 0.05$)

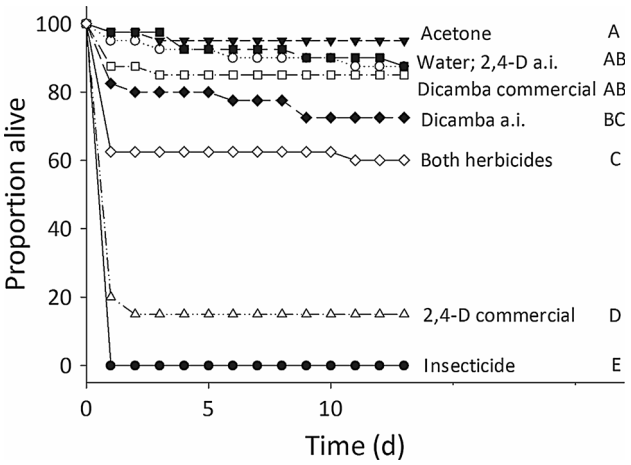

Fig. 1 Survival of *C. maculata* larvae exposed to 2,4-D and dicamba herbicides. Treatments were administered to larvae at the beginning of the 2nd stadium. Treatments followed by *different letters* in the *right column* of the figure had mortalities that were significantly different from one another ($\alpha = 0.05$)

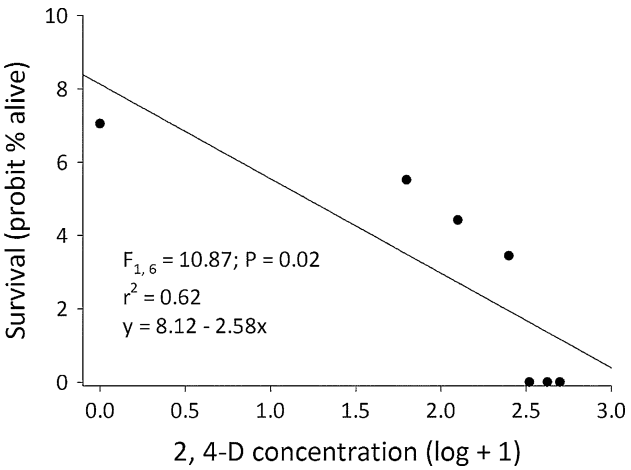

Fig. 2 Dose–response of *C. maculata* larvae to the herbicide 2,4-D. Larvae were treated with the commercial 2,4-D formulation at the beginning of the 2nd stadium

to the beetles; the probit distribution significantly described the relationship between dose and proportion alive ($F_{1,\ 6} = 9.07$, $P = 0.03$; $r^2 = 0.64$; $y = 7.68-2.17x$). The LC_{20}, LC_{50} and LC_{90} for 2,4-D when Dicamba was included at the label rate were 6.05, 16.18 and 65.82 µL/mL. Neither treatment nor adding Dicamba to the herbicide affected larval duration (treatment: $F_{2,\ 175} = 2.84$, $P = 0.06$; Dicamba: $F_{1,\ 175} = 1.25$, $P = 0.27$; interaction: $F_{2,\ 175} = 0.16$, $P = 0.85$), nor pupal weight (treatment: $F_{2,\ 175} = 0.18$, $P = 0.84$; dicamba: $F_{1,\ 175} = 2.80$, $P = 0.10$; interaction: $F_{2,\ 175} = 0.37$, $P = 0.69$) (Table 2). Sex ratios varied from 50 % male in only one of the treatments: namely 125 mL 2,4-D/mL solution ($t_{12} = 3.32$, $P = 0.006$). Larvae exposed to 62 mL 2,4-D/mL solution ($t_{32} = -0.17$, $P = 0.87$), water control ($t_{45} = 1.81$, $P = 0.07$) produced an even sex ratio. Adding dicamba to the 2,4-D had no effect on the sex ratio of *C. maculata* for 125 mL 2,4-D/mL solution + dicamba ($t_{13} = 1.08$, $P = 0.30$), 62 mL 2,4-D/mL solution + dicamba ($t_{28} = 0.55$, $P = 0.59$), nor dicamba control ($t_{43} = 0.90$, $P = 0.37$).

Discussion

Several treatments significantly reduced the longevity and survival of *C. maculata* larvae. By far, the most toxic herbicide was the 2,4-D formulated product, which reduced survival by 80 %, a rate nearly as high as the insecticide-treated controls (Fig. 1). This same trend was not observed when the larvae were treated with the 2,4-D active ingredient, leading us to conclude that either the "inactive" ingredients of 2,4-D were toxic to *C. maculata* larvae, or these inactive ingredients increased the toxicity of the active ingredient to this non-target organism. Indeed, the LC_{90} for the commercial 2,4-D formulation was 33 µL/mL (Fig. 2), approximately 13 % of the label rate applied for weed control. The active ingredient of dicamba also significantly reduced longevity and survival of larvae relative to the acetone control, but the commercial formulation of dicamba did not (Fig. 1). Studies have found that survival of other arthropod species were not reduced by commercial formulations of dicamba (Egan et al. 2014; Michaud and Vargas 2010; Morton et al. 1972). Michaud and Vargas (2010) evaluated the effect of commercial formulations of 2,4-D and dicamba on *C. maculata* larvae and found a much lower level of mortality compared to our study (25 and 7.5 % for 2,4-D and dicamba, respectively). The formulations in their study varied from ours, as did the timing and method of application (they treated 1st instars with a mist). In all cases in the current experiment, most of the lethality of the herbicides occurred within a few days of administering the product to the larvae (Fig. 1). Those surviving the exposure experienced sublethal effects of both herbicides.

In addition to the lethal effects, both active ingredients and commercial herbicides had deleterious non-lethal effects on the fitness and performance of lady beetles. Lady beetles administered dicamba active ingredient were significantly smaller than the acetone-treated control, and the larvae administered the combined herbicides produced significantly smaller pupae than the water-treated control. Pupal size was reduced by 7 % in Experiment 1, and although we found similar weight reductions in the same treatments of Experiment 2, these were not statistically significant. The active ingredient 2,4-D did not reduce body size, but larval development was significantly shorter

Table 2 Sublethal effects of 2,4-D on *C. maculata* when offered alone or with the label rate of dicamba

2,4-D dose (μL/mL)	Dicamba dose (μL/mL)	Larval duration (days)	Pupal weight (mg)	Proportion male (n)
0 (water control)	0	9.37 ± 0.08	15.27 ± 0.23	0.37 (46)
62	0	9.64 ± 0.14	15.01 ± 0.32	0.52 (33)
125	0	9.62 ± 0.21	15.53 ± 0.50	0.15 (13)*
0 (dicamba control)	37	9.54 ± 0.12	14.88 ± 0.30	0.43 (44)
62	37	9.86 ± 0.14	14.78 ± 0.29	0.45 (29)
125	37	9.64 ± 0.27	14.57 ± 0.71	0.36 (14)

Doses >125 μL 2,4-D/mL had low survival (<3 individuals) and were omitted from these analyses. The * in the proportion male column indicates a significant deviation from the expected 0.5 proportion ($\alpha = 0.05$)

for the treatment administered the 2,4-D commercial product. Most of this treatment died (Fig. 1), and it is possible that this herbicide formulation killed weaker larvae that develop more slowly first, which may have skewed the experimental results. Smaller lady beetles have lower fecundity (Honěk 1993), consume fewer prey (Agarwala et al. 2001), and likely have lower dispersal capacity (Kazmer and Luck 1995; Ness et al. 2004). While this is a noteworthy result, it remains unclear whether the size reductions observed in our study are biologically meaningful.

The sex ratios were skewed as a result of exposing the larvae to the commercial herbicide formulations. Specifically, males were selectively killed by the inert ingredients of both herbicides. Previous work has shown that herbicides can produce abnormalities in male genitalia of other non-target taxa (namely, vertebrates) (Hayes et al. 2002) as a direct result of exposure to herbicide formulations. We did not examine the genitalia of the lady beetles in this study, but the results would be consistent with these studies showing sex-specific effects of herbicide formulations. At least one other study has described that 2,4-D formulations have sex specific effects on the parasitoid wasp, *Tiphia vernalis* (Hymenoptera: Tiphiidae) (Oliver et al. 2006). Another interpretation of our data is that female *C. maculata* are larger than males, and thus may have been better able to tolerate the herbicides.

The exact mechanisms for the observed effects of the herbicide active ingredients and their formulations on animal taxa are seldom well understood. Due to their known target and chemical structures, mechanisms underlying non-target effects of pesticide active ingredients should be more tractable than "inactive" components of formulations. Nevertheless, mechanisms underlying lethal and sublethal effects of pesticides on non-target organisms are seldom explored, even in regulatory documentation on safety testing. As part of the phenoxy class of herbicides, 2,4-D and dicamba mimic the action of auxins, a group of plant hormones. To our knowledge, exact mechanisms whereby either of these herbicides affect insects have not been identified. Arguably, these mechanisms also remain unstudied in mammals, although mechanisms of high acute doses of 2,4-D have been identified as disrupting cellular membranes, metabolic pathways involving acetyl-CoA, and the herbicide can uncouple oxidative phosphorylation in cells (Bradberry et al. 2000). "Inactive" ingredients are regarded as proprietary information, and so scientists have little background on these components of the formulation; this poses a clear challenge in predicting the effects of formulated herbicides on insects.

Synergistic, deleterious effects of multiple chemistries can complicate risk assessments of individual compounds for non-target organisms (Meled et al. 1998; Pilling and Jepson 1993; Zhu et al. 2014). Our study did not reveal any synergistic effects of the two herbicides when dicamba was combined with 2,4-D; in other words, dicamba did not increase the lethality of 2,4-D to lady beetle larvae. Furthermore, when the two commercial products were administered together, an intermediate survival rate was observed. The reason for this intermediate mortality is that tank mixing these herbicides substantially reduces the recommended amount of herbicide applied. Thus, mixing herbicide chemistries not only improves the resilience of weed management programs (Mortensen et al. 2012), but it may be a way to reduce the non-target effects of particular chemistries if the reduced quantity of formulated product is as effective as when used individually. An important area of future research should focus on how herbicides, insecticides, and fungicides (along with their "inactive" ingredients associated with each) interact to form a more ecologically realistic picture of pesticide exposure and effects on non-target organisms.

Acknowledgments This research was supported by institutional contributions by the authors.

Compliance with ethical standards

Conflict of interest J.G.L. has sold predatory beetles to Monsanto for safety research, and received honoraria for speaking at Beyond

Pesticides and various Conservation Agriculture and Pollinator-related groups. L.F. has no conflicts of interest to report.

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