# Relative toxicity of three wheat herbicides to two species of Coccinellidae

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**Abstract**   On the High Plains of the USA, herbicides specific for broad-leaf weeds are regularly applied to winter wheat in the early spring, sometimes late enough to coincide with the colonization of fields by cereal aphids and their natural enemies. We tested the toxicity of three such herbicides, Ally (Dupont), Rave (Syngenta) and 2,4-D ester (generic), to neonate larvae of two coccinellid species important in cereal aphid biocontrol, *Coleomegilla maculata* DeGeer and *Hippodamia convergens* Guérin-Méneville. Topical treatment of larvae with 2,4-D resulted in 25% and 60% mortality in the two species, respectively, with surviving *C. maculata* larvae experiencing a 5% increase in developmental time. No significant effects were noted for the other two materials, save for a 2.5% increase in developmental time for *C. maculata* larvae exposed to Rave. No material caused significant mortality in either species when larvae were fed on prey (*Schizaphis graminum* Rondani) treated with herbicide 24 h earlier, although 2,4-D reduced developmental time slightly in *C. maculata*. When herbicide applications are delayed enough in spring to coincide with aphid activity in wheat, farmers can reduce the risk of disrupting biological control by selecting an alternative to 2,4-D.

**Key words**   2,4-D, *Coleomegilla maculata*, dicamba, *Hippodamia convergens*, *Schizaphis graminum*, thifensulfuron methyl

## Introduction

Wheat production in the High Plains region of the US is periodically impacted by several aphid species, primarily the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), the greenbug, *Schizaphis graminum* Rondani and the bird cherry-oat aphid, *Rhopalosiphum padi* L. Fortunately, effective biological control of these species is normally provided by a complex of natural enemies including lady beetles, lacewings, hoverflies and aphid parasitoids (Brewer & Elliott, 2004) such that insecticide applications are rarely necessary. In central and western Kansas, *Hippodamia convergens* Guérin-Méneville is a key predator of cereal aphids (Rice & Wilde, 1988; Nechols & Harvey,

1998; Michaud & Qureshi, 2005) with other species such as *Coleomegilla maculata* DeGeer and *Coccinella septempunctata* L. playing supporting roles, along with assorted chrysopids, syrphids and hymenopterous parasitoids. Aphid populations predictably colonize winter wheat around the time it breaks dormancy in spring and enters a period of rapid growth. Inclement weather conditions or pesticide applications targeting weeds or other insect pests at this time have the potential to impede the activity of key natural enemies and permit aphid populations to reach economic-impact levels.

Considerable research effort has addressed the potential impact of insecticides on coccinellid species (e.g., Croft & Brown, 1975; Kalushkov, 1982; Mizell & Schiffhauer, 1990; Michaud, 2002; Tenczar & Krischik, 2006) and fungicides have also been tested (Edwards & Hodgson, 1973; Michaud, 2001; Babu & Sharma, 2003). However, very few studies have addressed the potential toxicity of herbicides, despite the prevalence of their use on large

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acreages of grain and other crops. In the case of winter wheat, fields that have survived the winter in good condition are often "top-dressed" with an additional dose of fertilizer and herbicide in spring to maximize yield potential, precisely when overwintered coccinellids have emerged from hibernation and begun colonizing wheat fields.

Some 50 years ago, Adams (1960) demonstrated significant toxicity of 2,4-D amine to three coccinellid species that preyed on cereal aphids in grain fields in New Brunswick, Canada: *Hippodamia tredecimpunctata* (L.), *Coccinella transversoguttata* Faldermann, and *Coccinella perplexa* Mulsant. Adams and Drew (1965) suspected that the increasing use of 2,4-D herbicide might be responsible for outbreaks of the aphids *R. padi, Rhopalosiphum maidis* (Fitch) and *Sitobium avenae* (F.) in cereal agroecosystems. Since the springtime application of post-emergent herbicides, including 2,4-D, is widespread in Kansas winter wheat fields, we selected three of the most commonly used materials and tested their toxicity against *H. convergens* and *C. maculata*, the two most abundant species in the region. We subjected first instar larvae of each species to two routes of herbicide exposure considered to be likely under field conditions: direct topical spray application and consumption of treated prey (*S. graminum*). Other possible routes of exposure (not tested) include consumption of herbicides in the pollen or nectar of flowering weeds, or in droplets of dew.

## Materials and methods

### Insects

Adults of *H. convergens* and *C. maculata* were collected from a field of cultivated sunflower, *Helianthus annuus* L., on the Agricultural Research Center in Hays, Kansas on June 12, 2008 by tapping them off plants and into clear plastic cylinders (4.0 cm diameter × 10.0 cm height) that were sealed with ventilated lids. Following transportation to the laboratory, beetles of each species were held separately in 1-L glass Mason jars covered with an organdy mesh screen. The jars were filled with shredded wax paper to serve as harborage and water was provided on a cotton wick. Approximately 150 beetles were placed into each jar and provisioned daily with approximately 50 mg of frozen eggs of *Ephestia kuehniella* Zeller that were obtained from a commercial supplier (Beneficial Insectary, Oak Run, CA, US). *Ephestia* eggs are known to be a suitable food for the development of both *C. maculata* (Michaud & Jyoti, 2007) and *H. convergens* (J.P. Michaud, unpubl. data), yielding survival rates to adulthood that are

not significantly different from 100%. Jars were held in a climate-controlled growth chamber set to 22°C and a photoperiod of 16 : 8 (L : D).

In preparation for experiments, a series of 30 female beetles of each species were removed from the jar and isolated in plastic Petri dishes (5.5 cm diameter) under the same conditions as the stock colony. Females of *C. maculata* were provided with fresh *Ephestia* eggs daily and water on a small cube of sponge, whereas females of *H. convergens* were provided with an *ad libitum* diet of greenbugs, *S. graminum*, a diet superior to *Ephestia* eggs for eliciting oviposition in this species (Michaud & Qureshi, 2006). The aphids were reared in a growth chamber at 20°C under continuous light on seedlings of *Sorghum bicolor* (L.) grown in metal trays in a greenhouse. Eggs were typically laid directly on the Petri dishes and were recovered daily by changing the beetles to new containers. Eggs were held under the same conditions as the adults until they hatched 4–5 days later. All larvae were used in experiments within 12 h of eclosion following natural dispersal from their clusters.

### Herbicides

We selected three herbicides for testing that are commonly used in winter wheat for post-emergent weed control: 2,4-D ester (generic), Ally® Extra (Dupont Corporation, Wilmington, DE, USA, 37.5% thifensulfuron methyl, 18.5% tribenuron methyl, 15.0% metsulfuron methyl, by mass), and Rave® (Syngenta Crop Protection Wilmington, DE, USA, 55.0% dicamba sodium salt, 8.8% triasulfuron, by mass). All three herbicides were tested at concentrations corresponding to recommended field rates: 2,4-D, 8.9 mL/L (1.17 L/ha); Ally, 0.057 gm/L (70 gm/ha); and Rave, 2.3 gm/L (280 gm/ha).

For the topical treatment, equal numbers of newly hatched *H. convergens* larvae from each cluster were assigned to each of the four treatments (3 herbicides + control) and placed in groups of 5–6 in plastic Petri dishes (as above) with a disc of filter paper on the bottom. Dishes containing larvae for each treatment were then each sprayed in turn using an electronic mister (Solo Spraystar 460, Solo Inc., Newport News, VA, USA) to the point where the filter papers appeared visibly damp. Larvae for the control treatment were sprayed with distilled water. Larvae were then immediately transferred to clean, labeled Petri dishes, one per dish, and provisioned with frozen *Ephestia* eggs and water on a sponge cube. All 160 dishes were placed on a tray in a growth chamber under the same conditions as described previously and food and sponges were refreshed every second day until larvae

pupated. Mortality and development stages (pupation, adult emergence) were recorded daily. The experiment was then repeated with larvae of *C. maculata*.

For experiments involving treated prey, *S. graminum* was reared on trays of seedling sorghum as described above. A single, heavily infested tray was sprayed with each material until runoff using an electronic sprayer (as above) and permitted to dry for a period of several hours before use in the experiment. Aphids for the control treatment were sprayed with distilled water. Equal numbers of newly hatched *H. convergens* larvae from each cluster were assigned to each of the four treatments and isolated in labeled plastic cylinders (as above). These provided more room than Petri dishes for the infested plant material and were superior for confining aphids. A single sorghum seedling bearing treated aphids was then introduced into each cylinder ($n = 40$ for each treatment). All 160 cylinders were then placed on a tray in a growth chamber under the same conditions as described above. After 48 h, larvae were each transferred to a labeled Petri dish and provisioned with frozen *Ephestia* eggs (refreshed every second day) with water provided on a sponge cube until they pupated. Two days was considered to be an exposure period sufficient to afford significant consumption of contaminated aphids, and the 2,4-D treatment appeared to cause appreciable aphid mortality which prevented exposure to treated aphids for a longer period. The experiment was repeated with larvae of *C. maculata*.

Upon emergence, adult beetles from all experiments were each transferred to a labeled 18.5 mL glass vial with the cap screwed on loosely and then dried in an oven at $60°C$ for 3 days before weighing on an analytical balance. Mortality in the herbicide treatments was corrected for control mortality using Abbott's formula (Abbott, 1925) and a Chi-square, goodness-of-fit test was used to test

for significance. Data for developmental time (days to formation of pupa, duration of pupal period) and adult weight were compared among treatments in each experiment by one-way analysis of variance (ANOVA) followed by Tukey's test to separate means.

## Results

### Topical application

The corrected mortality of first instar *C. maculata* larvae exposed to Ally at 24 h of age was zero, compared to 7.5% mortality for Rave ($\chi^2 = 2.25$, ns) and 25.0% mortality for 2,4-D ($\chi^2 = 25.0$, $P < 0.001$). This compared to zero, 10.5% ($\chi^2 = 0.64$, ns), and 57.9% ($\chi^2 = 19.36$, $P < 0.001$) mortality, respectively, for the same materials applied to *H. convergens* larvae. There were significant effects of treatment on the developmental time of *C. maculata*, but not on that of *H. convergens*, whereas adult dry weight was not affected by treatment in either species (Table 1).

### Consumption of treated prey

The corrected mortality of first instar *C. maculata* larvae fed *S. graminum* exposed to topical sprays of herbicide 24 h earlier was zero for Ally and 2.4% for both Rave and 2,4-D ($\chi^2 = 0.21$, ns). For *H. convergens* larvae, the corrected mortalities were zero, 2.0% ($\chi^2 = 0.04$, ns) and 2.3%, ($\chi^2 = 0.05$, ns). The 2,4-D treatment had a significant effect on the developmental time of *C. maculata*, but not *H. convergens*, and there were no effects of any treatment on adult weight in either species (Table 2).

**Table 1** Mean ($\pm$ SE) developmental times (eclosion to adult emergence, in days) of two coccinellid species treated topically as first instar larvae on their first day of life with three herbicides at field rate concentrations.

| Variable | *Coleomegilla maculata* | | *Hippodamia convergens* | |
| --- | --- | --- | --- | --- |
| | Developmental time (d) | Adult dry weight (mg) | Developmental time (d) | Adult dry weight (mg) |
| Control | 19.9 ± 0.12 a | 3.46 ± 0.07 a | 22.3 ± 0.17 a | 3.45 ± 0.12 a |
| Ally | 20.1 ± 0.07 ab | 3.63 ± 0.08 a | 22.5 ± 0.46 a | 3.16 ± 0.13 a |
| Rave | 20.4 ± 0.15 bc | 3.57 ± 0.08 a | 22.7 ± 0.24 a | 3.37 ± 0.12 a |
| 2,4-D | 20.8 ± 0.17 c | 3.48 ± 0.10 a | 22.4 ± 0.47 a | 3.03 ± 0.16 a |
| *F* | 7.95 | 1.11 | 0.27 | 1.73 |
| df | 3,134 | 3,134 | 3,83 | 3,83 |
| *P* | < 0.001 | 0.348 | 0.845 | 0.168 |

Values bearing the same letter were not significantly different among treatments within columns (Tukey's test, $\alpha = 0.05$).

**Table 2** Mean (± SE) developmental times (eclosion to adult emergence, in days) of two coccinellid species fed for their first 2 days of life with prey (*Schizaphis graminum*) treated with one of three herbicides at field rate concentrations.

| Variable | *Coleomegilla maculata* | | *Hippodamia convergens* | |
| --- | --- | --- | --- | --- |
| | Developmental time (d) | Adult dry weight (mg) | Developmental time (d) | Adult dry weight (mg) |
| Control | 20.3 ± 0.13 b | 3.50 ± 0.05 a | 19.7 ± 0.11 a | 3.89 ± 0.09 a |
| Ally | 20.5 ± 0.14 b | 3.65 ± 0.14 a | 19.7 ± 0.20 a | 3.50 ± 0.15 a |
| Rave | 20.5 ± 0.12 b | 3.64 ± 0.07 a | 19.9 ± 0.15 a | 3.68 ± 0.10 a |
| 2,4-D | 19.9 ± 0.10 a | 3.53 ± 0.06 a | 19.6 ± 0.47 a | 3.62 ± 0.09 a |
| *F* | 6.07 | 1.02 | 0.60 | 2.24 |
| df | 3,123 | 3,123 | 3,118 | 3,118 |
| *P* | = 0.001 | 0.345 | 0.615 | 0.087 |

Values bearing the same letter were not significantly different among treatments within columns (Tukey's test, $\alpha = 0.05$).

## Discussion

Whereas non-target effects of insecticides on beneficial coccinellid species have received substantial attention, very little effort has been made to examine possible impacts of herbicides, despite the fact that the latter are far more regularly and widely applied to agricultural crops. This is particularly true for low-value field crops such as wheat in which insecticide applications are often difficult to justify, while herbicides are applied routinely on a calendar basis, contingent only on the crop being in a reasonably good condition.

The results of the present study indicate that Ally and Rave, two herbicides widely applied to winter wheat in early spring, present no measurable hazards to the two coccinellid species tested, whether they come into direct contact with spray, or consume residues on prey. In contrast, 2,4-D ester represents a significant hazard for young larvae of both species, and caused 25% and 60% mortality for *C. maculata* and *H. convergens*, respectively, when applied as a topical spray at the recommended application rate. Consumption of prey contaminated with these herbicides did not have measurable impact beyond a slight acceleration of development in the former species, although we suspect residues on aphids may have been minimal due to the cuticle being largely repellent to these aqueous formulations. In contrast to the report of Adams and Drew (1965), we did notice aphid mortality following treatment with 2,4-D, suggesting that many aphids receiving higher doses of herbicide may have died and been unavailable for larvae to consume. *Coleomegilla maculata* larvae that survived topical treatments of 2,4-D and Rave also experienced a change in developmental time, although in these cases it was slightly extended. Thus, *C. maculata* development seemed more sensitive than that of *H. convergens*, although the inconsistent responses to 2,4-D between the two forms of exposure are anomalous and defy meaningful interpretation.

Although these findings are consistent with those of Adams (1960) for other coccinellid species, it bears comment that our trials used an ester of 2,4-D, whereas the former trials employed an amine formulation. Presumably, the ester formulation would have greater capacity to penetrate the waxy surface of an insect cuticle, but the proportional mortality observed was remarkably similar to that reported by Adams (1960). Additional research has demonstrated a higher toxicity of 2,4-D to birds and mammals relative to other herbicides (Bautista, 2007). Adams and Drew (1965) linked the use of 2,4-D to aphid outbreaks in oat fields in New Brunswick, Canada, and demonstrated that aphid species did not share the negative impacts experienced by coccinellid larvae. In contrast, we observed, but did not quantify, aphid mortality as a consequence of the 2,4-D treatment, possibly as a result of the ester formulation. Biological aphid control in annual cereal crops usually relies on the timely recruitment of an entire guild of aphid natural enemies – lacewings, syrphids and aphidiid wasps in addition to coccinellids. Although these insect groups can be expected to vary in susceptibility to particular agrochemicals, the development of their immature stages could be impacted by 2,4-D in a manner similar to coccinellids. Thus, the disruptions of aphid biocontrol observed by Adams and Drew (1965) should not be assumed to result solely from toxicity to coccinellids, but may have involved impacts on other aphid natural enemies, even though their presence and potential effects of herbicides on them was not examined in this study.

It should be noted that 2,4-D will not likely disrupt biological control of aphids in Kansas wheat fields if the application is made early in spring before aphid populations build and overwintered coccinellids enter the fields to begin oviposition. This is also desirable for herbicide

efficacy, which will be optimal when both the crop and the weeds are still small in stature. Nevertheless, wet conditions may persist for long enough in spring to force a delay in herbicide application past the point where noticeable insect activity has commenced. Under these conditions, farmers should be advised to select an alternative to 2,4-D in the interest of conserving natural biological aphid control.

## Acknowledgments

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