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Impacts of orchard pesticides on *Galendromus occidentalis*: Lethal and sublethal effects

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ABSTRACT

Fifteen pesticides were tested in laboratory bioassays on *Galendromus occidentalis* (Nesbitt), the principal phytoseiid mite predator in Washington apple orchards. We developed a rating system for pesticides using lethal and sublethal effects, and applied the rating system to our results. At the 1× dose, only spinetoram and lambda-cyhalothrin caused >75% acute mortality of females. Carbaryl, azinphos methyl, spinosad, spirotetramat, cyantraniliprole, and sulfur had relatively little effect on mortality, but moderate to high effects on fecundity. Egg viability was most affected by carbaryl, spinosad, novaluron, spirotetramat, and sulfur. Lambda-cyhalothrin, spinosad, and sulfur were the most toxic compounds to larvae. Materials such as sulfur and spinetoram had widely divergent toxicity to adults versus larvae. The cumulative impact of these effects was best integrated by the numbers of live larvae of the F_1 generation. Using this measurement, spirotetramat, sulfur, spinetoram, acetamiprid, lambda-cyhalothrin, carbaryl and novaluron caused the greatest percentage reduction compared to the check, yet only spinetoram and lambda-cyhalothrin would have been identified as harmful in acute bioassays. These bioassays provide support for the benefits of measuring a range of sublethal effects and testing multiple life stages to provide an accurate assessment of the harmfulness of reduced-risk pesticides.

1. Introduction

Integrated mite management has historically been one of the most effective and durable biological control programs in Washington's apple pest management. This program, based on the conservation of the predatory mite *G*alendromus *occidentalis* (Nesbitt) was developed and implemented in the 1960s (Hoyt, 1965, 1966, 1969a,b; Hoyt and Caltagirone, 1971), and was still largely effective through the early 2000s. The deterioration of integrated mite management since that time was coincident with shifts in pesticide use patterns that affected large portions of the seasonal spray program.

The most impactful change has been the steady decline in organophosphate use (NASS, 1992, 2008) for a wide variety of pests, including the key lepidopteran pests of apple, viz., codling moth, *Cydia pomonella* L., and obliquebanded leafroller, *Choristoneura rosaceana* (Harris). This change has been driven in part by pesticide resistance of codling moth (Dunley and Welter, 2000; Knight et al., 1994) and leafrollers (Carrière et al., 1996; Dunley et al., 2006; Pree et al., 2002; Waldstein et al., 1999), but primarily by regulatory action. The latter includes the withdrawal of encapsulated methyl parathion, the restriction of chlorpyrifos to the pre-bloom period, and the scheduled phase-out of azinphos methyl after the 2012 growing season. This has necessitated the replacement of the older materials with newer "reduced risk" pesticides, those that have been identified in the registration process as having greater worker safety and fewer environmental impacts. There has been growing evidence, however, that while the newer compounds fit the reduced-risk profile, they are not necessarily safe for predatory mites (Bostanian et al., 2009; Gadino et al., 2011; Lefebvre et al., 2012, 2011; Villanueva and Walgenbach, 2005).

One of the challenges of integrating the new pesticides into an existing integrated pest management (IPM) program has been the evaluation of their nontarget effects (Croft, 1990; Stark and Banks, 2003). For pesticides such as the organophosphates, carbamates, and pyrethroids, nontarget effects could often be adequately described with simple assays of contact mortality; thus, short-term bioassays were reasonable indicators of potential disruption (Croft, 1990). The newer materials have a wide variety of modes of action (IRAC, 2012), often with much more subtle effects than simple mortality. Sublethal effects include any deleterious effect other than mortality, such as reduced prey finding or consumption; lower fecundity or egg viability; reduced longevity or increased

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developmental time; and altered sex ratios (Croft, 1990). Bioassays that address all potential effects of pesticides provide needed information to avoid nontarget effects of pesticides in IPM programs.

The purpose of these experiments was to characterize the lethal and sublethal effects of orchard pesticides on adult western predatory mite, *G. occidentalis*, and to compare contact mortality of adults and larvae. Our goal was to use these nontarget effects to develop a rating system modeled after that of the International Organization for Biological Control for pesticides and natural enemies (Sterk et al., 1999) using fairly rapid and simple screening bioassay.

2. Materials and methods

2.1. Colony maintenance

A *G. occidentalis* colony was started using mites collected from a commercial apple orchard near Bridgeport, WA in September 2008. The orchard was managed conventionally, but used a relatively selective program. The colony was maintained on twospotted spider mite, *Tetranychus urticae* Koch grown on lima bean plants *Phaseolus vulgaris* L. 'Henderson Bush'. A separate colony of *T. urticae* was also grown on lima bean plants; this provided the source of *T. urticae* used in the bioassays, and the prey for *G. occidentalis*. A separate set of uninfested bean plants was used as the source of the bioassay disks.

The materials used in the bioassays are pesticides used in Washington apple production (Table 1). Many are active on Lepidoptera and are used against one or more of Washington's key direct pests. Imidacloprid and spirotetramat are used primarily for hemipteran pests (e.g., aphids). Carbaryl is a broadspectrum insecticide, but its use in apple production is primarily for fruit thinning. Mancozeb and copper hydroxide are fungicide/bactericides, and sulfur is a fungicide with acaricidal properties.

The treatments consisted of three concentrations of each of the candidate pesticides, tested in a separate bioassay with its own distilled water check. Mancozeb and copper hydroxide were tested as a mixture, because they are most commonly applied as a mixture in the field. The doses were based on the maximum label rate applied at 935 l ha^{-1} (Table 1). This was the 1× dose; the other doses were 2× and 0.1×. The 2× dose simulated a more concentrated spray, while the 0.1× dose simulated declining residues. The concentrations were made by mixing the appropriate amount of the formulated pesticide for the 2× dose in 1 L of water, with the 1× and 0.1× doses made by dilution. Pesticides were applied with a Potter Spray Tower (Burkard Mfg, Rickmansworth, England) set at 44.8 kPa using the intermediate nozzle. Each arena with a leaf disk was sprayed with 2 ml (Amarasekare and Shearer, 2013) of the appropriate concentration (17.7 μl solution/cm^2 leaf area); the checks were sprayed with distilled water.

2.2. Disk arenas

The arena for the adult bioassays consisted of a bean leaf disk cut from an untreated, uninfested bean leaf, and placed with the lower surface facing up in a plastic cup filled with cotton and water. A smaller (2.2 cm diam.) disk was used for single female *G. occidentalis*, and a larger disk (3.5 cm diam.) was used for multiple individuals (*G. occidentalis* larval bioassays). The size of the plastic portion cups for each disk size allowed a ca. 8 mm gap of cotton and water barrier between the disk and the side of the cup. All bioassays were held at 20 ± 2 °C and 16:8 L:D photoperiod until evaluation.

2.3. G. occidentalis female bioassay

This bioassay measured acute mortality, fecundity, prey consumption, egg viability, and survival of the F_1 larvae. Prey was provided in the form of *T. urticae* eggs. Eight to ten *T. urticae* females were transferred to each 2.2 cm disk and allowed to oviposit for 24 h. The *T. urticae* females were removed, the resulting eggs were counted and adjusted to a standard number per disk. Preliminary prey consumption studies indicated 30 eggs per disk would be sufficient; however, the number of eggs per disk was increased to 40 in subsequent bioassays, after a few instances of complete prey consumption occurred in 48 h. Replicates where all eggs were

Table 1
Pesticides tested against *G. occidentalis*.

Common name	Mode of action[a]	Chemical class	Brand name/formulation[b]	Formulation	Use rate (g ai ha^{-1})[c]	mg ai l^{-1} (1x conc)
Carbaryl	1A	Carbamate	Sevin 4F[d]	479 g l^{-1}	3363	3595
Azinphosmethyl	1B	Organophosphate	Guthion 50W[d]	500 g kg^{-1}	1121	1798
Lambda-cyhalothrin	3	Pyrethroid	Warrior II 2.08CS[e]	249 g l^{-1}	47	50
Acetamiprid	4A	Neonicotinyl	Assail 70WP[f]	700 g kg^{-1}	167	179
Thiacloprid	4A	Neonicotinyl	Calypso 4F[d]	479 g l^{-1}	280	300
Imidacloprid	4A	Neonicotinyl	Provado 1.6F[d]	192 g l^{-1}	112	120
Spinosad	5	Spinosyn	Entrust 80W[g]	800 g kg^{-1}	168	180
Spinetoram	5	Spinosyn	Delegate 25WG[g]	250 g kg^{-1}	123	131
Novaluron	15	IGR - benzoyl urea	Rimon 0.83 EC[h]	99 g l^{-1}	233	389
Spirotetramat	23	Tetramic acid	Ultor 1.25L[d]	150 g l^{-1}	99	164
Chlorantraniliprole	28	Anthranilic diamide	Altacor 35WDG[i]	350 g kg^{-1}	110	118
Flubendiamide	28	Anthranilic diamide	Belt 4SC[i]	479 g l^{-1}	175	188
Cyantraniliprole	28	Anthranilic diamide	Exirel 100 g AI/L[i]	100 g l^{-1}	149	160
Copper hydroxide	M1	Inorganic	Kocide 3000[i]	461 g kg^{-1}	4650	4972
Sulfur	M2	Inorganic	Kumulus 80W[j]	800 g kg^{-1}	17,933	19,175
Mancozeb	M3	Dithiocarbamate	Manzate Pro-Stick[i]	750 g kg^{-1}	1513	1618

[a] Mode of action classification taken from Insecticide Resistance Action Committee (IRAC) v 7.0 (URL) or the fungicide Resistance Action Committee (FRAC).
[b] The Registrant listed is from the time the experiments were begun.
[c] The concentrations were based on an application rate of 935 L/ha, or 100 US gallons/acre.
[d] Bayer CropScience, Research Triangle Park, NC.
[e] Syngenta Crop Protection, Inc., Greensboro, NC.
[f] Cerexagri-Nisso LLC, King of Prussia, PA.
[g] Dow Agrosciences LLC, Indianapolis, IN.
[h] Chemtura Corporation, Middlebury, CT.
[i] E.I DuPont de Nemours & Co., Wilmington, DE.
[j] Arysta LifeScience North America, LLC, Cary, NC.

consumed were excluded from analyses, because the true prey consumption could not be determined. Each egg's position was marked by putting a dot next to it with a felt-tip pen. A single *G. occidentalis* female of unknown age was then transferred to each disk. Previous work has indicated that using females of an unknown age does not affect differences found between treatments in pesticide bioassays (Beers et al., 2009). Each pesticide concentration (2×, 1×, 0.1×, check) had 25 replicate disks of a single female *G. occidentalis*.

The treatments were applied by contact to the *G. occidentalis* females and *T. urticae* eggs on the disks. Thus, the females were not only treated topically by the sprays, but were also exposed to residues on the disk surface and contaminated prey (*T. urticae* eggs). At the time the larvae hatched, the residues were five days old.

Numbers of live and dead *G. occidentalis* females were recorded 24 and 48 h after treatment. For the purposes of evaluating mortality, females that were dead or had walked off the disk were counted as dead (Knight et al., 1990), giving a greater weight to materials that were repellent or irritating to mites. After 48 h, the females were removed from the disk arena. *G. occidentalis* eggs and the number of remaining prey items (*T. urticae* eggs) were then counted. The disks were held 3–4 d to allow hatch of the *G. occidentalis* eggs. After eggs had hatched, the numbers of live *G. occidentalis* larvae, and hatched and unhatched eggs on each disk were recorded. The *T. urticae* eggs, some of which hatched by the end of the bioassay, were left on the disk as a food source for the *G. occidentalis* larvae.

2.4. G. occidentalis larval bioassay

Forty *T. urticae* females and 20 *G. occidentalis* females were transferred to each 3.5 cm bean leaf disk and allowed to oviposit for 24 h. The *T. urticae* eggs served as the food source for *G. occidentalis*. After 24 h, females of both species were removed, and the *G. occidentalis* eggs were allowed to hatch (typically 3 d). At this time, any unhatched *G. occidentalis* eggs were removed, and the numbers of larvae per disk were recorded (18–28 or $\bar{x} = 21$). The disks with eggs and larvae were sprayed in a Potter Spray Tower as described previously. Mortality was evaluated after 48 h. Each treatment was replicated five times, with 90–115 ($\bar{x} = 102$) larvae per concentration.

2.5. Data summary and analysis

Data from the female bioassays were analyzed using a logistic regression model (PROC GENMOD, SAS 9.3 (SAS Institute, 2013)) using a logit link. The variables mortality, prey consumption, and percentage egg hatch were treated as binomial (live/dead, eaten/uneaten, hatched/unhatched), with the binomial distribution specified in the model statement. Data were corrected for zeros by adding 0.125 to the frequency of all outcomes. Eggs and live larvae produced per female were analyzed with the same procedure, except that the Poisson distribution (non-negative count data) was specified, and 0.25 was added to the all observations to correct for zeros. Concentrations within pesticides were compared when the overall χ^2 was significant using pairwise single degree-of-freedom likelihood ratio contrasts ($P > 0.05$). Statistical letters were assigned manually from the pairwise comparisons, and conflicts in the assignments resolved by using $P > 0.10$ (one case only). The mortality data from the larval contact/residual bioassays were analyzed using a logistic regression model as described for the adult female bioassay.

Means and standard errors presented in Table 2 were taken over all replicates, and thus represent the outcome per initial female. The exception was the percentage egg hatch; if no eggs were laid, division by zero set the replicate to missing. At each subsequent step in the evaluation, any negative effect of the pesticide accumulated, integrating the various lethal and sublethal effects. Larval mortality data were the mean and standard error of the five replicate disks.

Because each bioassay was conducted independently, additional calculations were done to characterize each pesticide on a uniform scale (0–100 or −100) relative to its own check (see Fig. 1). Mortality was corrected for the check mortality using Abbott's formula (Abbott, 1925). An analogous method was used for other variables to calculate percentage reduction from the check, viz., ((E − C)/C) *100, where E is the response in the 1× concentration, and C is the response of the check. Unlike the concentration means (Table 2), these calculations accounted for response of the surviving females at 24 and 48 h rather than the initial number ($n = 25$), partitioning the sublethal effect from simple mortality. A rating scheme of low (<25%), moderate (≥25 and ≤75%), and high (>75%) was used to group corrected percentage mortality and percentage reduction from the check.

3. Results

3.1. G. occidentalis female bioassays

Carbaryl caused a moderate amount of mortality; however, because of the relatively high check mortality, no statistical differences among the three doses and the check were found (Table 2). Prey consumption and fecundity were reduced by all doses, including the 0.1× dose. The percentage hatch was also reduced at the two higher doses. The number of live larvae was greatly decreased by the 2× and 1× doses, and significantly reduced in the 0.1× dose.

Azinphosmethyl was not acutely toxic to *G. occidentalis*, but prey consumption and numbers of live larvae were reduced by all doses relative to the check. Fecundity was reduced by the 2× and 1× doses (Table 2).

Lambda-cyhalothrin caused 96–100% mortality after 48 in the 2× and 1× doses, respectively (Table 2). Even the 0.1× dose was quite toxic (76% mortality after 48 h). All of the eggs laid by *G. occidentalis* hatched, but given the initial low numbers, and a moderate amount of larval mortality post-hatch, the number of live larvae of the subsequent generation was quite low in all three doses.

Mortality caused by direct contact with acetamiprid sprays was moderate, never exceeding 40% (Table 2). All doses caused higher rates of mortality than the check, but were not different from each other. However, this material had a pronounced negative effect on prey consumption and fecundity. The 2× dose of acetamiprid caused a 10-fold reduction in the number of total prey consumed. The reduction in the lowest dose (0.1×) of acetamiprid was nearly as great (5.6-fold), indicating the effect could persist for some time in the field. However, this bioassay does not distinguish between reduced prey consumption due to contaminated prey and a direct effect on the females contacted by the sprays. The females, though still alive, were lethargic and nonresponsive. The reduction in fecundity was similar in magnitude to the reduction in prey consumption at the two higher doses; all had significantly fewer eggs per female than the check. No live larvae were produced in the 2× dose, and numbers were also significantly reduced in the 1× and 0.1× doses, although all eggs hatched.

Thiacloprid caused much higher levels of mortality (68–96%) than did acetamiprid (Table 2). Fecundity and prey consumption were significantly reduced, but egg hatch was not affected by this material. The number of live larvae produced was significantly reduced by all doses of this compound; however, the 0.1× dose

Table 2
Mortality (uncorrected, 48 h), prey consumption, fecundity, egg hatch and larval survival of *G. occidentalis* treated topically as an adult female (all values expressed as mean ± SEM).

			Prop. prey								
Pesticide	mg AI L^{-1}	*n*	% Mortality	*n*	Consumed	*n*	Eggs laid	*n*	% Egg hatch	*n*	Live larvae
Carbaryl	7190	25	44.00 ± 10.13a	25	0.21 ± 0.05c	25	1.00 ± 0.26b	13	59.62 ± 13.73b	25	0.04 ± 0.04c
Carbaryl	3595	25	32.00 ± 9.52a	25	0.28 ± 0.06b	25	1.48 ± 0.32b	16	79.27 ± 6.22b	25	0.08 ± 0.06c
Carbaryl	360	25	40.00 ± 10.00a	25	0.30 ± 0.05b	25	1.60 ± 0.32b	16	84.38 ± 8.80a	25	0.60 ± 0.21b
Carbaryl	0	25	24.00 ± 8.72a	25	0.51 ± 0.05a	25	3.52 ± 0.42a	21	96.43 ± 2.61a	25	2.40 ± 0.35a
χ^2, *P*			2.61, 0.455		164.02, <0.001		43.97, <0.001		31.60, <0.001		109.84, <0.001
Azinphos methyl	3595	25	8.00 ± 5.54a	25	0.28 ± 0.05b	25	0.88 ± 0.27c	10	100.00 ± 0.00a	25	0.88 ± 0.25b
Azinphos methyl	1798	25	0.00 ± 0.00a	25	0.25 ± 0.04b	25	1.12 ± 0.29bc	12	100.00 ± 0.00a	25	1.16 ± 0.29b
Azinphos methyl	180	25	12.00 ± 6.63a	25	0.28 ± 0.04b	25	1.56 ± 0.39ab	12	100.00 ± 0.00a	25	1.36 ± 0.34b
Azinphos methyl	0	25	0.00 ± 0.00a	25	0.34 ± 0.05a	25	2.20 ± 0.48a	14	100.00 ± 0.00a	25	2.16 ± 0.47a
χ^2, *P*			6.07, 0.108		20.10, <0.001		17.00, <0.001		0.05, 0.997		15.41, 0.002
Lambda-cyhalothrin	100	25	100.00 ± 0.00a	25	0.04 ± 0.01bc	25	0.00 ± 0.00c	0		25	0.00 ± 0.00c
Lambda-cyhalothrin	50	25	96.00 ± 4.00a	25	0.03 ± 0.02c	25	0.16 ± 0.12b	2	100.00 ± 0.00a	25	0.04 ± 0.04bc
Lambda-cyhalothrin	5	25	76.00 ± 8.72b	25	0.06 ± 0.02b	25	0.20 ± 0.16b	3	100.00 ± 0.00a	25	0.16 ± 0.12bc
Lambda-cyhalothrin	0	25	28.00 ± 9.17c	25	0.41 ± 0.05a	25	2.76 ± 0.46a	18	100.00 ± 0.00a	25	2.36 ± 0.39a
χ^2, *P*			45.34, <0.001		561.73, <0.001		144.60, <0.001		1.54, 0.672		133.40, <0.001
Acetamiprid	357	25	36.00 ± 9.80a	25	0.07 ± 0.01c	25	0.44 ± 0.13c	9	100.00 ± 0.00a	25	0.00 ± 0.00c
Acetamiprid	179	25	32.00 ± 9.52a	24	0.09 ± 0.01bc	25	0.60 ± 0.12c	14	100.00 ± 0.00a	25	0.04 ± 0.04c
Acetamiprid	18	25	40.00 ± 10.00a	25	0.11 ± 0.01b	25	1.28 ± 0.14b	22	100.00 ± 0.00a	25	0.40 ± 0.15b
Acetamiprid	0	25	0.00 ± 0.00b	22	0.75 ± 0.03a	25	3.88 ± 0.32a	24	100.00 ± 0.00a	25	3.68 ± 0.34a
χ^2, *P*			17.93, <0.001		926.71, <0.001		108.85, <0.001		0.37, 0.947		204.66, <0.001
Thiacloprid	599	25	96.00 ± 4.00a	25	0.05 ± 0.01c	25	0.88 ± 0.17c	15	100.00 ± 0.00a	25	0.40 ± 0.12c
Thiacloprid	300	25	68.00 ± 9.52b	25	0.21 ± 0.04b	25	1.80 ± 0.25b	22	100.00 ± 0.00a	25	1.52 ± 0.23b
Thiacloprid	30	25	72.00 ± 9.17b	25	0.09 ± 0.01c	25	1.16 ± 0.16bc	19	100.00 ± 0.00a	25	0.64 ± 0.14c
Thiacloprid	0	25	12.00 ± 6.63c	24	0.60 ± 0.04a	25	4.12 ± 0.37a	24	100.00 ± 0.00a	25	3.68 ± 0.33a
χ^2, *P*			44.38, <0.001		756.62, <0.001		73.31, <0.001		0.18, 0.981		99.51, <0.001
Imidacloprid	240	25	80.00 ± 8.16a	25	0.05 ± 0.01c	25	0.68 ± 0.13b	15	100.00 ± 0.00a	25	0.44 ± 0.10c
Imidacloprid	120	25	68.00 ± 9.52a	25	0.02 ± 0.01d	25	0.72 ± 0.14b	15	100.00 ± 0.00a	25	0.48 ± 0.12c
Imidacloprid	12	25	12.00 ± 6.63b	25	0.26 ± 0.03b	25	2.04 ± 0.27a	21	89.68 ± 4.22b	25	1.72 ± 0.24b
Imidacloprid	0	25	8.00 ± 5.54b	25	0.39 ± 0.05a	25	2.88 ± 0.37a	20	100.00 ± 0.00a	25	2.76 ± 0.37a
χ^2, *P*			46.78, <0.001		689.09, <0.001		55.17, <0.001		11.98, 0.008[a]		69.44, <0.001
Spinosad	360	24	58.33 ± 10.28a	24	0.41 ± 0.06a	24	0.67 ± 0.19ab	10	95.00 ± 5.00ab	24	0.63 ± 0.18a
Spinosad	180	25	36.00 ± 9.80ab	25	0.43 ± 0.05a	25	0.36 ± 0.18b	4	75.00 ± 25.00b	25	0.24 ± 0.14b
Spinosad	18	25	24.00 ± 8.72b	25	0.32 ± 0.04b	25	1.00 ± 0.19a	15	100.00 ± 0.00a	25	0.84 ± 0.18a
Spinosad	0	25	16.00 ± 7.48b	25	0.30 ± 0.02b	25	1.12 ± 0.27a	13	100.00 ± 0.00a	25	1.12 ± 0.27a
χ^2, *P*			11.08, 0.011		55.03,<0.001		12.26, 0.007		11.12, 0.011		16.23, 0.001
Spinetoram	262	25	88.00 ± 6.63a	25	0.21 ± 0.03c	25	0.24 ± 0.09c	6	100.00 ± 0.00a	25	0.00 ± 0.00c
Spinetoram	131	25	96.00 ± 4.00a	25	0.21 ± 0.03c	25	0.32 ± 0.10c	8	100.00 ± 0.00a	25	0.00 ± 0.00c
Spinetoram	13	25	60.00 ± 10.00b	25	0.30 ± 0.04b	25	0.72 ± 0.27b	9	88.89 ± 11.11a	25	0.24 ± 0.14b
Spinetoram	0	25	8.00 ± 5.54c	25	0.68 ± 0.05a	25	4.92 ± 0.49a	23	100.00 ± 0.00a	25	4.20 ± 0.41a
χ^2, *P*			56.55, 0.001		484.36, <0.001		207.40, <0.001		3.87, 0.276		255.27, <0.001
Novaluron	777	25	40.00 ± 10.00a	25	0.29 ± 0.04c	25	1.52 ± 0.34b	14	73.10 ± 8.71c	25	0.08 ± 0.06b
Novaluron	389	25	44.00 ± 10.13a	25	0.35 ± 0.06b	25	1.72 ± 0.31b	19	72.46 ± 8.76c	25	0.16 ± 0.07b
Novaluron	39	25	16.00 ± 7.48b	25	0.53 ± 0.05a	25	4.32 ± 0.35a	25	99.00 ± 1.00a	25	2.20 ± 0.35a
Novaluron	0	25	16.00 ± 7.48b	25	0.55 ± 0.05a	25	3.56 ± 0.41a	21	95.24 ± 4.76b	25	2.88 ± 0.38a
χ^2, *P*			8.369, 0.039		158.16, <0.001		51.86, <0.001		39.86, <0.001		136.64, <0.001
Spirotetramat	328	25	52.00 ± 10.20a	25	0.37 ± 0.05b	25	0.08 ± 0.06c	2	0.00 ± 0.00b	25	0.00 ± 0.00b
Spirotetramat	164	24	20.83 ± 8.47b	24	0.53 ± 0.04a	24	0.33 ± 0.10b	9	55.56 ± 17.57b	24	0.00 ± 0.00b
Spirotetramat	16	25	48.00 ± 10.20a	25	0.37 ± 0.05b	25	0.36 ± 0.10b	9	100.00 ± 0.00a	25	0.00 ± 0.00b
Spirotetramat	0	24	12.50 ± 6.90b	22	0.51 ± 0.05a	24	2.88 ± 0.35a	21	100.00 ± 0.00a	24	2.46 ± 0.29a
χ^2, *P*			13.14, 0.004		71.04, <0.001		114.33, <0.001		29.99, <0.001		156.64, <0.001
Chlorantraniliprole	236	25	16.00 ± 7.48a	25	0.65 ± 0.05b	25	4.16 ± 0.51a	20	100.00 ± 0.00a	25	2.28 ± 0.39b
Chlorantraniliprole	118	25	8.00 ± 5.54a	25	0.59 ± 0.05c	25	4.52 ± 0.54a	21	100.00 ± 0.00a	25	3.48 ± 0.46a
Chlorantraniliprole	12	25	8.00 ± 5.54a	25	0.70 ± 0.04a	25	4.52 ± 0.51a	22	100.00 ± 0.00a	25	3.36 ± 0.40a
Chlorantraniliprole	0	25	0.00 ± 0.00a	25	0.71 ± 0.04a	25	5.48 ± 0.47a	23	100.00 ± 0.00a	25	2.80 ± 0.35ab
χ^2, *P*			5.11, 0.164		33.30, <0.001		5.00, 0.172		0.00, 0.999		7.88, 0.049
Flubendiamide	375	25	24.00 ± 8.72a	23	0.38 ± 0.03b	25	2.44 ± 0.28a	21	100.00 ± 0.00a	25	1.72 ± 0.26a
Flubendiamide	188	25	36.00 ± 9.80a	22	0.27 ± 0.05c	25	1.88 ± 0.34a	16	100.00 ± 0.00a	25	1.64 ± 0.30a
Flubendiamide	19	25	28.00 ± 9.17a	25	0.36 ± 0.04b	25	2.76 ± 0.27a	23	100.00 ± 0.00a	25	2.32 ± 0.25a
Flubendiamide	0	25	8.00 ± 5.54a	25	0.42 ± 0.03a	25	2.92 ± 0.32a	22	100.00 ± 0.00a	25	2.48 ± 0.27a
χ^2, *P*			6.27, 0.099		48.04, <0.001		6.57, 0.087		0.01, 0.999		6.53, 0.088
Cyantraniliprole	320	24	41.67 ± 10.28a	24	0.21 ± 0.03c	24	1.13 ± 0.30b	11	100.00 ± 0.00a	24	0.63 ± 0.19a
Cyantraniliprole	160	25	20.00 ± 8.16a	25	0.30 ± 0.04b	25	1.48 ± 0.36b	17	100.00 ± 0.00a	25	0.76 ± 0.19a
Cyantraniliprole	16	24	29.17 ± 9.48a	24	0.23 ± 0.04c	24	0.96 ± 0.24b	11	100.00 ± 0.00a	23	0.61 ± 0.21a
Cyantraniliprole	0	24	16.67 ± 7.77a	24	0.39 ± 0.05a	21	2.76 ± 0.51a	15	86.67 ± 6.89a	24	1.04 ± 0.28a
χ^2, *P*			4.48, 0.214		90.24, <0.001		19.07, <0.001		7.79, 0.051		3.91, 0.272

(*continued on next page*)

Table 2 (*continued*)

Pesticide	mg AI L^{-1}	*n*	% Mortality	*n*	Prop. prey Consumed	*n*	Eggs laid	*n*	% Egg hatch	*n*	Live larvae
Mancozeb + copper	3236 + 9944	25	68.00 ± 9.52a	25	0.12 ± 0.03c	25	0.24 ± 0.12b	4	100.00 ± 0.00a	25	0.24 ± 0.12c
Mancozeb + copper	1618 + 4972	25	40.00 ± 10.00ab	25	0.20 ± 0.04b	25	0.48 ± 0.14b	9	100.00 ± 0.00a	25	0.40 ± 0.13bc
Mancozeb + copper	162 + 497	24	37.50 ± 10.09b	24	0.34 ± 0.05a	23	0.57 ± 0.18b	9	100.00 ± 0.00a	24	0.63 ± 0.19b
Mancozeb + copper	0	24	16.67 ± 7.77b	24	0.33 ± 0.05a	24	1.38 ± 0.33a	15	100.00 ± 0.00a	24	1.25 ± 0.31a
χ^2, *P*			13.98, 0.003		186.61, <0.001		23.34, <0.001		0.18, 0.980		20.13, <0.001
Sulfur	38,349	25	32.00 ± 9.52a	25	0.25 ± 0.03b	25	1.48 ± 0.23c	18	72.78 ± 9.18b	25	0.12 ± 0.07b
Sulfur	19,175	25	32.00 ± 9.52a	25	0.20 ± 0.04c	25	1.44 ± 0.28c	16	71.88 ± 11.15b	25	0.00 ± 0.00b
Sulfur	1917	25	16.00 ± 7.48a	25	0.28 ± 0.04b	25	2.24 ± 0.28b	24	83.61 ± 6.08b	25	0.08 ± 0.08b
Sulfur	0	25	12.00 ± 6.63a	25	0.41 ± 0.03a	25	3.20 ± 0.29a	25	94.33 ± 2.34a	25	2.60 ± 0.24a
χ^2, *P*			4.75, 0.191		108.55, <0.001		23.45, <0.001		10.48, 0.015		147.43, <0.001

[a] A probability level of $P > 0.10$ was used in order to avoid conflicts in statistical letters assigned manually from the pairwise contrasts.

caused significantly more reduction than the 1× dose in live larvae and prey consumed.

Imidacloprid was highly toxic on contact to *G. occidentalis* females at the 2× dose (80%) and moderately toxic at the 1× dose (68%); these doses also severely depressed prey consumption and fecundity (Table 2). Percentage egg hatch was slightly reduced in the 0.1× dose. The numbers of live larvae were also suppressed by all three doses, roughly proportional to the numbers of eggs laid.

Mortality levels in the spinosad bioassay were significantly higher than the check only at the 2× dose (Table 2). Surprisingly, there was significantly higher prey consumption in the 2× and 1× doses, with the lowest level occurring in the check. Total fecundity was significantly reduced only in the 1× dose. There was a significant decrease in percentage egg hatch at the 1× dose, which also had a significantly lower number of live larvae.

Mortality levels were relatively high in the spinetoram treatments, with 60–96% mortality overall (Table 2). The 0.1× dose caused lower levels of mortality than the 2× and 1× doses, but was still significantly higher than the check. Most of the mortality was expressed after 24 h (data not shown). Prey consumption was suppressed by all spinetoram treatments. Fecundity was more severely affected than prey consumption; all three doses resulted in significantly lower egg production than the check. Egg hatch was not affected, but no larvae survived in the two higher doses, and larval numbers were significantly reduced in the 0.1× dose.

Novaluron at the 2× and 1× doses caused a moderate (40–44%) amount of mortality (Table 2). Prey consumption was slightly depressed at the two higher doses, while the 0.1× dose was not different than the check. The reduction in fecundity was significant at the 2× and 1× doses. Low fecundity coupled with reduced egg hatch led to significant reductions in numbers of live larvae at the 2× and 1× doses (Table 2).

The 2× and 0.1× doses of spirotetramat caused significantly higher levels of mortality than the check (Table 2). Prey consumption was also reduced in these two doses, while fecundity was reduced dramatically by all three doses. The low egg numbers make the estimates of percentage hatch somewhat unstable in the three treatments; however, the combined low egg numbers, poor hatch and poor larval survival led to a complete absence of live larvae in all three doses.

There were no significant treatment differences in female mortality, fecundity, egg hatch, or live larvae caused by chlorantraniliprole relative to the check (Table 2). However, prey consumption was reduced in the two higher doses.

Flubendiamide was non-toxic in acute mortality measurements, but there was a reduction in prey consumption in all doses

Pesticide	MOA	Larval mortality	Female mortality	Prey Consumption	Fecundity	Egg hatch	Live larvae
Carbaryl	1A	32	11	-36	-52	-18	-97
Azinphosmethyl	1B	40	0	-26	-49	0	-46
Lambda-cyhalothrin	3	87	94	-72	-72	0	-98
Acetamiprid	4A	36	32	-89	-81	0	-99
Thiacloprid	4A	4	64	-59	-36	0	-59
Imidacloprid	4A	51	65	-93	-68	0	-83
Spinosad	5	86	24	-80	-48	-25	-79
Spinetoram	5	15	96	-35	-100	0	-100
Novaluron	15	1	33	-21	-39	-24	-94
Spirotetramat	23	(0)	10	-3	-87	-44	-100
Chlorantraniliprole	28	5	8	-14	-13	0	24
Flubendiamide	28	8	30	-19	-10	0	-34
Cyantraniliprole	28	15	4	-27	-50	15	-27
Mancozeb+Copper	M1/M3	6	28	-38	-64	0	-68
Sulfur	M2	94	23	-42	-51	-24	-100

Fig. 1. Summary of lethal and sublethal effects of 15 pesticides on larvae and adult female *G. occidentalis* (1× dose). Box shading represents the relative strength of the effect, where black is the most harmful (>75%), white is the least harmful (<25%), and gray is intermediate (≥25 and ≤75). Numbers in cells represent either corrected percentage mortality at 48 h (leftmost two columns), or a percentage reduction from the check (all remaining effects). In the latter group, negative values indicate reduction; positive values are shown by a zero in parentheses (0).

(Table 2). Fecundity and egg hatch were not significantly different than the check, corresponding to no significant differences in the numbers of live larvae produced.

No significant differences occurred in mortality, egg hatch, or live larvae in the cyantraniliprole treatments relative to the check (Table 2). There was, however, a significant reduction in prey consumption and fecundity.

Mancozeb + copper hydroxide caused significantly higher mortality than the check at the 2× dose, with corresponding reductions in prey consumption and fecundity (Table 2). The 0.1× dose was intermediate in its effect on these parameters. All three doses had significant fewer live larvae than the check; only egg hatch was unaffected.

Sulfur did not cause an increase in mortality of *G. occidentalis*, even though mortality levels in the 1× and 2× doses were over double that of the check (Table 2). All doses led to decreased prey consumption, fecundity, and egg hatch relative to the check. The numbers of surviving larvae were extremely reduced, even at the 0.1× dose.

3.2. *G. occidentalis* larval bioassay

Sulfur, lambda-cyhalothrin, and spinosad were highly toxic to larvae on contact (Table 3), with all doses (2×, 1× and 0.1×) causing significantly higher mortality than the check. Carbaryl, azinphos methyl, acetamiprid, and imidacloprid were moderately toxic, with one or more doses significantly higher than the check. At the 1× dose, spinetoram, chlorantraniliprole, and cyantraniliprole caused a relatively low level of toxicity (<25%), but one that was significantly higher than the check. Thiacloprid, novaluron, spirotetramat, and flubendiamide were not significantly different from the check at any dose tested.

4. Discussion

The results of these experiments bear some similarities to other studies of nontarget effects of pesticides on phytoseiids, but with a number of differences. The development of resistance to azinphos methyl was documented in many apple-growing regions of North America (Croft and Jeppson, 1970; Hislop and Prokopy, 1981; Rock and Yeargan, 1971; Watve and Lienk, 1975); this resistance enabled integrated mite control programs in Washington apple systems to be successful while still providing control of direct pests (Hoyt, 1969a). Despite 40+ years of exposure, some sublethal effects remain, even though adult acute toxicity is low. Carbamates in general are among the more toxic materials to phytoseiids (Croft, 1990). The toxic effect of carbaryl has been mainly attributed to acute toxicity in the past (Croft, 1990; Croft and Nelson, 1972; Hoyt, 1969a; Swift, 1968; Thistlewood and Elfving, 1992; Watve and Lienk, 1975). Despite its toxicity, use of carbaryl as a fruit thinner is important to fruit production, therefore its use has been retained with appropriate cautions integrated into grower recommendations (Bush et al., 2009). Like azinphos methyl, acute toxicity has been reduced over time (Babcock and Tanigoshi, 1988), but sublethal effects remain. The acute toxicity of pyrethroids to phytoseiids, and their incompatibility in integrated mite control programs, is likewise well documented (Bostanian et al., 1985; Croft, 1990; Croft and Whalon, 1982; Hoyt et al., 1978; Zwick and Fields, 1978); these materials have seldom been recommended (Bush et al., 2009) or used (NASS, 2008) for control of key pests in Washington for this reason.

Among the newer classes of pesticides, the neonicotinyls were found to have a relatively high deleterious effect as a group in our studies; this class of compounds has had similar effects reported in other laboratory studies (Bostanian et al., 2009; James, 2003; Stavrinides and Mills, 2008). Although hormoligosis (elevated levels of reproduction due to pesticide stress) has been studied in relation to these compounds in both predatory (James, 1997) and phytophagous mites (Ako et al., 2004; James, 2003), we found no evidence for this effect. In fact, the reverse was true in that moderate to severe reductions in fecundity were found for *G. occidentalis*. These reductions may have resulted in part from reduced prey consumption, which has also been documented for these compounds (Poletti et al., 2007; Villanueva and Walgenbach, 2005).

Both of the spinosyn materials had a high level of negative impact on *G. occidentalis*, with subtle differences between the two compounds. Other authors have also noted the detrimental effects of these compounds on various life stages of phytoseiids (Hull et al., 2007; Lefebvre et al., 2012; Villanueva and Walgenbach, 2005). Conversely, Bostanian et al. (2009) and Kim et al. (2005) found spinosad to be relatively harmless, although repellent to *G. occidentalis*. Lefebvre et al. (2011) found spinetoram to be highly toxic to both adults and larvae, whereas larvae suffered little mortality in the present study.

Novaluron, as an insect growth regulator targeting primarily Lepidoptera, would be predicted to have little effect on the Acari. However, the association with mite outbreaks was first noted in

Table 3
Mean percentage mortality of *G. occidentalis* larvae following contact/residual exposure to 15 pesticides.

Pesticide	χ^2	*P*	% Mortality (±SEM)			
			2×	1×	0.1×	Check
Carbaryl	95.05	<0.001	54.00 ± 4.85a	33.70 ± 9.78b	12.08 ± 3.76c	2.77 ± 1.96d
Azinphos methyl	82.09	<0.001	44.33 ± 13.29a	42.75 ± 6.14a	7.07 ± 1.79b	4.14 ± 2.54b
Lambda-cyhalothrin	239.94	<0.001	97.00 ± 1.22a	89.00 ± 4.00b	94.00 ± 2.92ab	14.00 ± 5.34c
Acetamiprid	77.37	<0.001	45.56 ± 2.72a	37.78 ± 6.43a	6.67 ± 2.72b	3.33 ± 1.36b
Thiacloprid	3.92	0.270	17.00 ± 4.06a	12.00 ± 2.00a	11.00 ± 1.87a	8.00 ± 2.00a
Imidacloprid	68.97	<0.001	62.00 ± 7.00a	60.00 ± 4.18a	23.00 ± 7.18b	19.00 ± 6.20b
Spinosad	167.36	<0.001	89.00 ± 4.30a	88.00 ± 4.06a	55.00 ± 2.24b	15.00 ± 4.74c
Spinetoram	41.49	<0.001	37.78 ± 7.06a	20.90 ± 3.84b	6.84 ± 2.45c	7.33 ± 3.03c
Novaluron	1.24	0.743	18.26 ± 1.63a	15.65 ± 1.06a	13.04 ± 1.37a	14.78 ± 1.06a
Spirotetramat	3.46	0.327	3.00 ± 1.22a	7.00 ± 1.22a	6.00 ± 2.45a	9.00 ± 1.00a
Chlorantraniliprole	8.89	0.031	1.05 ± 1.05b	9.55 ± 4.10a	5.46 ± 2.01b	5.00 ± 2.24b
Flubendiamide	3.36	0.339	7.82 ± 5.82a	11.63 ± 2.31a	6.81 ± 2.54a	4.24 ± 4.24a
Cyantraniliprole	20.06	<0.001	27.54 ± 5.39a	21.67 ± 3.91a	5.45 ± 2.67b	7.88 ± 2.31b
Mancozeb + copper	8.90	0.031	32.00 ± 2.55a	20.00 ± 6.52ab	20.00 ± 3.16ab	15.00 ± 2.24b
Sulfur	191.76	<0.001	97.80 ± 1.40a	95.00 ± 2.11a	79.00 ± 6.40b	12.02 ± 3.20c

Means within a pesticide (rows) followed by the same letter are not significantly different ($P > 0.05$), likelihood ratio for type 3 χ^2 (df = 3 for all bioassays). Doses (2×, 1×, 0.1×) are relative to the maximum label rate; see Table 2 for actual concentrations.

field studies (Martinez-Rocha et al., 2008). The sublethal effects on reproduction found in the present study help shed light on this observation. Interestingly, other studies rated this material as non-toxic regarding fecundity, but with marginal acute toxicity to both larvae and females (Lefebvre et al., 2012, 2011).

Spirotetramat is a relatively new compound and while it primarily targets hemipterans, it is also used as an acaricide, and thus may reasonably be expected to affect predatory mites. The severe effects seen in this study were derived primarily from reproductive effects, rather than mortality. Lefebvre et al. (2011) found much higher levels of mortality of both larvae and females, however, their assessments were taken at 120 h versus 48 h in the present study. They found a reduction in fecundity after 24 h, which grew more severe over time.

The anthranilic diamides are a newer group of compounds primarily targeting Lepidoptera (Lahm et al., 2005). These materials appear to be the least toxic of the materials tested to *G. occidentalis,* and we conclude that they are a good fit with integrated mite control programs. Lefebvre et al. (2011) likewise found chlorantraniliprole and flubendiamide to have little or no toxicity, although the former was recommended for further (field) evaluation. Nontarget effects on other natural enemies have been reported (Amarasekare and Shearer, 2013; Jones et al., 2011), thus further field and lab testing is needed to determine the overall fit with IPM programs.

Surprisingly, the fungicides tested had moderate to high impact on *G. occidentalis*. The deleterious effects of sulfur on phytoseiids have been well documented in both laboratory studies and field experiments (Beers et al., 2009; Costello, 2007; Martinez-Rocha et al., 2008; Prischmann et al., 2005). Despite their toxic effects on phytophagous mites, they are still known to cause pest mite outbreaks. Because the other two fungicides were tested as a mixture, it is not possible to determine the relative contribution of the two compounds, however, deleterious effects have been reported for mancozeb for many phytoseiid species (Angeli and Ioriatti, 1994; Baynon and Penman, 1987; Bernard et al., 2004; Blumel et al., 2000; Bostanian et al., 1998; Gadino et al., 2011; Hassan, 1987; Ioriatti et al., 1992; James and Rayner, 1995; Park et al., 1996; Pozzebon et al., 2002; Zacharda and Hluchy, 1991). These effects, however, vary widely with the species, exposure method, and stage tested. Copper fungicides have been rated as highly toxic in some cases (van de Vrie, 1962), but for the most part are considered more selective than mancozeb (Pozzebon et al., 2002; Smith and Papacek, 1991).

Insights into the inherent toxicity of the compounds can be seen in the differences among the three doses. In general, the most toxic materials were toxic at all three doses, indicating little potential for using reduced doses as a strategy for reducing impact. However, novaluron, imidacloprid, and spinosad had much lower impact at the 0.1× dose. While this provides an opportunity for the use of ecological selectivity (Hull and Beers, 1985), usage dose in the field is likely to be dictated more by efficacy against the target pest. Another factor that could greatly influence the overall impact is the length of time the residues remain toxic. Even highly toxic materials may have less of an impact if their residues fall below a critical threshold for a given natural enemy in a short amount of time.

In a number of cases, there was a surprising lack of correspondence between toxicity to larvae and toxicity to the adult females (Fig. 1). The larvae were substantially more susceptible to carbaryl, azinphos methyl, spinosad, and sulfur than the adult females. Conversely, the adult stage was more susceptible to thiacloprid, spinetoram, novaluron, flubendiamide, and mancozeb + copper hydroxide. Toxicity to the two stages was similar for the highly toxic lambda-cyhalothrin, and moderately toxic acetamiprid and imidacloprid. While these results cannot be compared statistically because they are from different bioassays, it is clear that each compound must be evaluated completely, given the unpredictability of the outcomes.

While the results presented in Table 2 represent the cumulative effect of the pesticide at various life stages, some insight can be gained by examining the patterns of contributing mechanisms (Fig. 1). The impact of lambda-cyhalothrin was clearly due to acute toxicity to motile stages, although near-lethal levels of intoxication affected the sublethal parameters. For the neonicotinyl insecticides, the contribution of mortality was variable in its effect on the overall impact, but effects on prey consumption and fecundity were also a factor. Spinetoram and spinosad were differentially toxic to adults and larvae (Villanueva and Walgenbach, 2005); the life stage effect was reversed depending on material, although the net effect was similar. Spirotetramat was unique in that the effects appear to be almost exclusively on fecundity and fertility, with little contribution from acute toxicity. However, this compound was just as devastating to net reproduction as those with extreme acute toxicity. With sulfur, the highest impact was caused by larval toxicity (Beers et al., 2009), although sublethal effects also contributed.

The percentage reduction in live larvae produced per treated female in relation to the check can be used as an overall measure to compare pesticides across different bioassays, and classify materials as to their potential risk for field effects (Fig. 1). In this measurement, certain patterns become apparent. The anthranilic diamide insecticides (chlorantraniliprole, flubendiamide, cyantraniliprole) tended to have the least impact on *G. occidentalis*, with all materials having <35% reduction in live larvae from the check. Azinphosmethyl, thiacloprid, and mancozeb + copper hydroxide had a moderate negative effect. All the other materials tested had >75% reduction at the 1× dose.

In conclusion, there has been increasing emphasis on sublethal effects and use of a demographic approach to assess the effects of pesticides (Stark and Banks, 2003; Stark and Wennergren, 1995; Stavrinides and Mills, 2008). While testing multiple life stages, using longer evaluation periods, and more rigorously standardized individuals provides much greater consistency, detail and resolution to toxic effects (Angeli and Ioriatti, 1994; Lefebvre et al., 2011), it also tends to be more laborious and expensive (Stark and Banks, 2003), potentially limiting the number of compounds screened. The method of the adult bioassay in the current study used elements of a demographic approach, but was greatly simplified, with compromises between the greater precision obtainable with more rigorous standardization or higher numbers of replicates. The cumulative nature of the effects on larvae produced from the initial females (integrating the preceding effects of female mortality, reductions in fecundity and egg viability, or mortality of larvae upon exposure to residues) allows both a summary of effects, and some information as to which effects are dominant. Because of the short generation time of phytoseiids, this bioassay can be performed in eight days (20 °C) with minimal materials. Further simplification (assessing only adult mortality and F_1 larvae) would decrease the amount of information obtained regarding the effects on various life stages and parameters, but could also decrease the time required for testing while still retaining the summary of population-level effects.

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