12-15-2015

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Recommended Citation

Kaushalya G. Amarasekare, Peter W. Shearer, Nicholas J. Mills, "Testing the selectivity of pesticide effects on natural enemies in laboratory bioassays", Biological Control, Vol. 102, 2016, Pages 7-16, ISSN 1049-9644, https://doi.org/10.1016/j.biocontrol.2015.10.015.

To be submitted for publication in Biological Control

Testing the selectivity of pesticide effects on natural enemies in laboratory bioassays

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Abstract

The toxic effects of older classes of pesticides on natural enemies are typically acute and exposure usually occurs through direct contact with foliar residues. However, older chemistries are being replaced by newer classes of pesticides that can cause sublethal effects in addition to direct mortality. We developed a set of life table response protocols to quantify the effects of multiple routes of exposure to pesticides on individual-level life history parameters of predators and parasitoids. We then integrated the data into population-level endpoint estimates of population growth rates using stage-structured population models. For this study, we evaluated the impacts of five insecticides (cyantraniliprole, chlorantraniliprole, spinetoram, novaluron and lambda-cyhalothrin) and two fungicides (sulfur and a mixture of copper hydroxide and mancozeb) on a generalist insect predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and an aphid parasitoid *Trioxys pallidus* (Haliday) (Hymenoptera: Braconidae). Green lacewings and *T. pallidus* are key members of the natural enemy community in western USA orchards. The results of these laboratory studies demonstrate that both *C. carnea* and *T. pallidus* were negatively affected by cyantraniliprole, spinetoram and lambda-cyhalothrin while only one species was affected by chlorantraniliprole and novaluron (*C. carnea*) or sulfur (*T. pallidus*). The benefits of integrating acute, chronic, and sublethal effects from laboratory bioassays to assess the selectivity of pesticides with respect to natural enemies are discussed.

Key words *Chrysoperla carnea*; *Trioxys pallidus*; reduced risk insecticide; intrinsic rate of increase; sublethal effects; multiple routes of exposure

1. Introduction

From the introduction of organophosphorus (OP) insecticides in the late 1950s until mid-1990s, most integrated pest management (IPM) programs used in the orchards in the western United States relied heavily on them (Jones et al., 2009). Significant findings from previous research that evaluated adverse effects of agricultural pesticides on the environment health (Pimentel, 1995; Pimentel et al., 1993; Pimentel and Levitan, 1988) plus the enactment of the Food Quality Protection Act of 1996 resulted in the removal of many OP insecticides (Jones et al., 2010; US EPA, 1996). This led to the introduction of newer pesticide chemistries with novel modes of action and lower mammalian toxicities (Agnello et al., 2009; Kim et al., 2011; Whalon et al., 1999).

Most of these newer reduced risk insecticides are target specific, but there is evidence that some of these insecticides could affect key natural enemies that regulate secondary insect and mite pests (Agnello et al., 2009; Amarasekare and Shearer, 2013a, 2013b; Brunner et al., 2001; Crampton et al., 2010; Kim et al., 2006; Myers et al., 2006; Villanueva and Walgenbach, 2005, 2006). In contrast to neurotoxic OP insecticides, some of the newer reduced risk insecticides have been shown to have sublethal rather than lethal effects on natural enemies (Amarasekare and Shearer, 2013a, 2013b; Beers and Schmidt, 2014; Desneux et al., 2007; Kim et al., 2006). Many systemic neonicotinoid insecticides have unintended side effects on bees and natural enemies including predators and parasitoids (Cloyd and Bethke, 2011; Cresswell, 2010; He et al., 2012; Laycock et al., 2012; Li et al., 2015; Rahmani and Bandani, 2013; Yao et al., 2015). In addition to reduced risk insecticides, some fungicides used in pest management may have insecticidal and miticidal properties that affect natural enemies (Amarasekare and Shearer,

2013a, 2013b; Hoyt, 1969; Jepsen et al., 2007; Stavrinides and Mills, 2009). Thus, additional information is needed to better understand the impacts of reduced risk pesticides on natural enemies including both lethal and sublethal effects (Jones et al., 2009).

Traditionally, measurement of acute toxicity of pesticides to natural enemies has relied largely on the determination of an acute median lethal dose (LD_{50}) or concentration (LC_{50}) (Desneux et al., 2007). The effects of pesticides on natural enemies were examined further by running selectivity tests (pests/natural enemies) to identify products with the lowest non-target activity. Because of the increasing economic importance of natural enemies in agriculture and the recognition of limitations associated with traditional methods for studying non-target pesticide effects, a growing number of studies have focused on the inclusion of sublethal effects during past several decades (Ahmadi 1983; Banken and Stark, 1998; Desneux et al., 2007; Longley and Stark, 1996; Stark et al., 1995; Stark and Banks, 2003; Stark et al., 2007; Theiling and Croft, 1989). Older classes of pesticides, such as OPs and carbamates, are acutely toxic and thus the analysis of sublethal effects is less straightforward (Wennergren and Stark, 2000). However, newer classes of pesticides are often less lethal to natural enemies than the older classes, and consequently a more comprehensive approach is needed for assessment of their non-target selectivity.

In evaluating a pesticide's potential for compatibility with natural enemies, the International Organization for Biological Control (IOBC) recommends a tiered approach whereby initial pesticide screening is done in the laboratory and depending upon the results obtained, semi-field or field tests may be conducted (Hassan 1992;Vogt et al., 2000). The IOBC classifies pesticides into the following four categories depending on the extent of mortality or reduction in life history performance that they cause to natural enemies: 1 = harmless (<30%), 2

= slightly harmful (30-79%), 3 = moderately harmful (90-98%) and 4 = harmful (>99%) (Hassan 1992; Vogt et al., 2000). Although the tiered approach advocated by the IOBC is admirable, there are limitations to this method for assessment of pesticide side effects (Stark et al., 2004). Laboratory life table response experiments (LTREs) and demographic analyses have proved to be an effective approach to evaluate the combined lethal and sublethal effects of pesticides (Stark and Banks, 2003; Stark et al., 2007; Theiling and Croft, 1989). In contrast to the standardized tests developed by IOBC to study pesticide effects of fresh pesticide residues on natural enemies in the laboratory (Hassan, 1985; Vogt et al., 2000), our objective was to develop a set of bioassays for arthropod predators and parasitoids that were designed as LTREs and that incorporated multiple routes of pesticide exposure including topical, residual and oral (Banken and Stark, 1998; Longley and Stark, 1996; Stark et al., 1995).

The current study was part of a large, multi-state project conducted in apple, pear and walnut orchards in Washington, Oregon and California, respectively (Jones et al., 2015 this issue). A major goal of the study was to enhance the sustainability of biological control in western USA orchard systems. A central theme was to investigate the secondary impacts of pesticides used against codling moth (*Cydia pomonella* (L.), Lepidoptera: Tortricidae), the common key pest found in these three orchard systems. This approach is illustrated here by presenting the methodology, impacts on life history parameters, and population endpoint estimates of the effects of five insecticides (cyantraniliprole, chlorantraniliprole, spinetoram, novaluron and lambda-cyhalothrin) and two fungicides (sulfur and a mixture of copper hydroxide and mancozeb) on a generalist insect predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and an aphid parasitoid *Trioxys pallidus* (Haliday) (Hymenoptera: Braconidae). The fungicide mixture of copper hydroxide and mancozeb is one of the most

important fungicides used in walnuts to control walnut blight but not used in pears and apples. Sulfur is generally used in pears and apples but not in walnuts. We incorporated both fungicides (the mixture of copper hydroxide and mancozeb and sulfur) in our studies for all natural enemies tested across the three cropping systems because most of these natural enemies are commonly found in pear, apple and walnut orchards. In this study we followed Stark and Banks (2003) in using LTREs and population models to explore the demographic effects of pesticides on natural enemy populations.

Green lacewings (Neuroptera: Chrysopidae) are important predators of arthropod pests in many horticultural and agricultural cropping systems, including vegetables, fruits, nuts, fiber and forage crops, ornamentals, greenhouse crops and forests, both in the context of natural biological control as well as in augmentative release programs (Nordlund et al., 2001; Pappas et al., 2011; Ridgway and Kinzer, 1974; Ridgway and Murphy, 1984). *Chrysoperla carnea* is a species native to Eurasia that has been used throughout the world in such programs (Henry et al., 2002). *Trioxys pallidus* is an introduced solitary endoparasitoid of the walnut aphid *Chromaphis juglandicola* (Kaltenbach), a pest of walnuts in the U.S. and many walnut growing areas in the world (Hougardy and Mills, 2009). Thus green lacewings and *T. pallidus* are thus key members of the natural enemy community in western USA orchards and were selected here as examples of two different functional groups of natural enemies to illustrate the approach that we developed for testing the selectivity of pesticides in laboratory bioassays.

2. Materials and methods

2.1. Chrysoperla carnea colony

A colony of *C. carnea* was maintained at 23 °C, 50-60% R. H. and a photoperiod of 16: 8 h L: D in the laboratory using the methods described in Amarasekare and Shearer (2013b). Adults were reared in an open-top glass aquarium (26 × 30 × 50 cm) with a wire mesh screen lid (6 × 6 mm mesh). To facilitate egg laying, the opening at the top of the aquarium was covered with a piece of cheesecloth and secured with a wire mesh top. Artificial diet was prepared in the laboratory and used to feed the adults (Vogt et al., 2000). Adults were provided with new food, water and cheesecloth cover three times a week. The *C. carnea* colony was initiated with commercially purchased lacewing larvae (BioBest, Leamington, Ontario, Canada) and the species identity was confirmed by song analysis (Henry et al., 2002; Henry et al., 2013).

The bioassays were conducted using 0-1 d old second instar larvae and 1-2 d old adult males and females. Identification and feeding of second instar larvae and adult males and females were as described in Amarasekare and Shearer (2013b).

2.2. Trioxys pallidus colony

Colonies of both *C. juglandicola* and *T. pallidus* were initiated from individuals collected from an orchard near Chico, California in June 2008 and were maintained in a glasshouse at 22 ± 1.5 ˚C, 55-65% R. H. and a 16: 8 h L: D photoperiod. Walnut seedlings grown from seed in 1 L pots with standard potting soil (Supersoil: Rod McLellan Company, Marysville, Ohio, USA) were used for maintaining the aphid colony when seedlings were 2-3 months old and had three or more fully expanded leaves. Both aphid and parasitoid colonies were maintained in wooden

sleeve cages (1 × 1 × 1 m) with glass tops and organdy backs. For aphid colony cages, a single fresh seedling was introduced each week to replace the older of two seedlings, such that each plant remained in the cage for a two-week period and the aphids dispersed naturally from the older to the younger seedlings. After two weeks in an aphid colony cage, an infested seedling was either discarded or used for the parasitoid colony. For the parasitoid colony, a new aphid-infested seedling was introduced every two weeks, just after emergence of each new generation of adult parasitoids (generation time ~ two weeks). Aphid-infested seedlings remained in parasitoid colony cages for three weeks to ensure that all adult parasitoids had emerged. Walnut seedlings were watered daily and once a week they were supplemented with Peters Professional® Water Soluble Fertilizer (Scotts-Sierra Horticultural Products, Ohio, Marysville, Ohio, USA) to prevent nutrient depletion in the pots.

Experimental adults of *T. pallidus* consisted of male/female pairs that were 1-2 d old. As it is difficult to be sure whether parasitoid probing always leads to oviposition and successful endoparasitism of aphids, for consistency, we used healthy instead of parasitized aphids in the juvenile bioassays with the implicit assumption that any pesticide that is lethal to the walnut aphid as a host of *T. pallidus* would necessarily kill the larval parasitoid. Unparasitized third instar aphids taken directly from the laboratory colony were used for the juvenile *T. pallidus* bioassays.

2.3. Insecticides and fungicides tested

Pesticides tested in this study were selected, in part, with input from the Advisory Panel for the project (Jones et al., 2015 this issue), and whether these products were used in most or all of the three cropping systems.

The following five reduced risk and/or OP-replacement insecticides and two fungicides were tested as formulated product: insecticides: cyantraniliprole (ryanodine receptor modulator) (reduced risk insecticide) (Exirel® 100SE, DuPont Crop Protection, Wilmington, Delaware, USA) 160.2 mg [AI] / L, chlorantraniliprole (ryanodine receptor modulator) (reduced risk insecticide) (Altacor 35WG, DuPont Crop Protection, Wilmington, Delaware, USA) 117.9 mg [AI] / L, spinetoram (nicotinic acetylcholine receptor allosteric activator) (reduced risk insecticide) (Delegate 25WG, Dow Agro Sciences LLC, Indianapolis, Indiana, USA) 131.1 mg [AI] / L, novaluron (a chitin synthesis inhibitor) (OP-replacement insecticide) (Rimon 0.83EC, Chemtura AgroSolutions, Middlebury, Connecticut, USA) 388.5 mg [AI] / L, and lambda-cyhalothrin (sodium channel modulator) (OP-replacement insecticide) (Warrior II CS, Syngenta LLC Inc., Greensboro, North Carolina, USA) 49.9 mg [AI] / L; fungicides: sulfur (Kumulus DF, Micro Flo Company LLC., Memphis, Tennessee, USA) 19.2g [AI] / L, and a mixture of copper hydroxide (Kocide 3000 WG, DuPont Crop Protection, Wilmington, Delaware, USA) 2.2g [AI] / L and mancozeb (Manzate Pro Stick, DuPont Crop Protection, Wilmington, Delaware, USA) 1.6 g [AI] / L. Distilled water was used as the control treatment. Each pesticide was tested at concentrations that were equivalent to their maximum label rate (100%) and 10% of that amount dissolved in 935 L ha^{-1} of distilled water. Both concentrations of all pesticides were tested against larval and adult life stages of *C. carnea* and for juvenile and adult stages of *T. pallidus* in acute toxicity bioassays. For sublethal bioassays of larval and adult stages of *C. carnea*, both 10% and 100% concentrations were used while the highest concentration of a pesticide that

caused < 75% acute mortality was used for the sublethal bioassays of adult *T. pallidus*. There was no acute toxicity bioassay for the effect of sulfur on juvenile *T. pallidus*.

2.4. Bioassays for C. carnea

Acute and sublethal bioassays for both larvae and adults of *C. carnea* were conducted in arenas consisted of glass cylinders placed on glass plates with cheesecloth lids. Details of their specifications and use were as described in Amarasekare and Shearer (2013b). All individuals in adult and larval bioassays were treated with pesticides through combined topical exposure (experimental insects), residual exposure (arenas and cheesecloth lids) and oral exposure (via *Ephestia kuehniella* Zeller eggs and adult diet). Cheesecloth lids and *E. kuehniella* eggs were treated by drenching them in 100 ml of each treatment for 30 sec and then air-dried. A Potter spray tower (Burkard Scientific, Uxbridge, UK) (103 kPa, intermediate nozzle) was used to treat the glass cylinders and plates, insects and adult diet using 2 ml of solution for each application. Glass cylinders and plates were treated separately. After treatment, they were removed from the spray tower after a 5 sec settling time, air-dried for 30 min and then assembled. The adult food was treated after it was applied as a thin layer to the bottom exterior surface of a 9 cm-dia glass petri dish. Experimental insects were topically exposed as replicate groups of three larvae or a single pair of adults (male and female), and individual larvae and paired adults were transferred to treated bioassay arenas with pesticide residues and placed in an environmental growth chamber at 23 °C, 50-60% R. H. and a photoperiod of 16: 8 h L: D.

For the acute bioassays, larval and adult *C. carnea* were assessed for mortality after 48 h and the proportion dead in each pesticide treatment was Abbott-corrected with respect to mortality that occurred in the control treatments (Abbott, 1925; Hoekstra, 1987).

For the sublethal bioassays, larvae that survived the acute treatments were reared using the same treated arena with pesticide residue until they emerged as adults to determine their survivorship, developmental time and sex ratio of emerged adults (percentage of females).

Similarly, adults that survived the acute treatments were reared using the same treated arena with pesticide residue until they died to provide an estimate of adult longevity. Cheesecloth lids from the adult arenas were collected every other day to evaluate the number of eggs each female had oviposited and their subsequent fertility for 20 d as described in Amarasekare and Shearer (2013b). Egg hatch and daily fertility (the total number of eggs hatched per female per day) were recorded and hatched larvae were removed daily. Larvae and adults were provided with food and water three times a week and untreated *E. kuehniella* eggs and fresh adult food were used after 72 h from initial treatment.

Insecticide and fungicide studies were conducted independently using the following number of individuals and replicates for acute and sublethal assays for larvae and adults. Insecticide study: acute assay, larvae, n = 12 with 4 replicates and adults, n = 10 with 5 replicates; sublethal assay, larvae, n = 4-12 with 4 replicates and adults, n = 3-5 with 3-5 replicates and fungicides study: acute assay, larvae, n = 30 with10 replicates and adults, n = 30 with15 replicates; sublethal assay, larvae, n = 22-28 with 10 replicates and adults, n = 14-15 with 14 -15 replicates, respectively. The insecticide study was repeated twice using the same experimental procedures and each individual experiment was considered as a separate block (for statistical purposes) for a total of three blocks.

2.5. Bioassays for T. pallidus

Acute bioassays for adult *T. pallidus* were carried out in 3.7 ml glass vials (15 mm dia, 45 mm tall, VWR International, Pasadena, California, USA) with cheesecloth lids and used a combination of residual and oral pesticide exposure. Cheesecloth lids were dipped in pesticide solution for 30 sec before being air dried in a fume hood. Vials were surface coated with pesticide residue by being filled with pesticide solution for 5 min before the solution was poured out and the vials were left to dry overnight in a fume hood. Oral exposure was achieved by placing a narrow streak of 50 % honey solution into a surface-treated vial daily using a single bristle from a paint brush. A 50 % honey solution was prepared by mixing 0.5 ml of the pesticide solution to be tested with 0.5 ml of warm, liquid honey. A replicate consisted of a male and female pair of *T. pallidus* placed into a treated glass vial with a moistened strip of filter paper to increase humidity and closed with a cheesecloth lid.

Acute bioassays for unparasitized third instar walnut aphids were based on residual exposure only and were carried out in 20 mm dia clip cages with cheesecloth screening attached to individual excised walnut leaflets. Pesticide solutions to be tested were diluted in a 0.25 ml Agral 90(Syngenta Canada Inc., Guelph, Ontario, Canada) / 1 L distilled water solution to ensure an even coating of walnut leaflet surfaces and single leaflets were dipped into the pesticide solutions for 3 sec before being laid flat on wax paper until dry. A replicate consisted of a single treated leaflet with 10 aphids transferred into a clip cage on its lower surface. The petiole of each leaflet was placed individually into a cotton-stoppered 3.7 ml glass vial of water and each

of these arenas was enclosed individually in a plastic ventilated sandwich box (15 × 10 × 3 cm) lined with moisten paper towel to maintain humidity.

Acute bioassay arenas were placed in a growth chamber at 22 °C and a 16: 8 h L: D photoperiod. A set of 30 replicates were used for the adult *T. pallidus* bioassays and 8 replicates for the unparasitized aphid bioassays for each pesticide concentration. Acute mortality was recorded after 48 h and Abbott-corrected with respect to mortality that occurred in the control treatments (Abbott, 1925; Hoekstra, 1987).

Bioassays for sublethal effects were conducted for the adult stage of *T. pallidus* only, through combined topical, residual and oral exposure to the pesticides tested. For topical exposure, *T. pallidus* adults of < 24 h old were knocked out by 5 sec exposure to CO_2 at 10 PSI and treated in a Potter spray tower with 1.05 ml of pesticide solution for a mean deposition of 1.5 mg per cm^2. For residual exposure, custom-made glass cylinders (2.5cm dia, 2.8cm tall) were sprayed with 1.95 ml of pesticide solution in a Potter spray tower, turned over, and sprayed with 1.95 ml of solution again, which also resulted in a mean residue of 1.5 mg per cm^2. When dry, these cylinders were used to make clip cages, by attaching a foam base, a hair clip, and a removable organdy lid held in place with a plastic ring. For oral exposure, 10 µl droplets of a 50% honey-water solution were pipetted into a plastic petri dish (10 cm dia) and treated in a Potter spray tower with 1.05 ml of pesticide solution before being used to place two narrow streaks on the inside surface of a treated clip cage using a single bristle from a paint brush.

Female-male pairs were placed in treated clip cages attached to the lower surface of leaflets of potted walnut seedlings together with 35 untreated third instar walnut aphids. After 24 h, the adult *T. pallidus* were transferred to a new treated clip cage containing a fresh set of 35 walnut aphids. The clip cages and aphids was changed daily to avoid a buildup of honeydew that

would cause wasp and aphid mortality. A sequence of three clip cages was used for each replicate, and the aphids from each day of exposure were enclosed in untreated clip cages for a further 20 d to monitor mummy formation, mummy survivorship, timing of adult emergence and sex ratio (% female) of emerged adults. Sublethal bioassays were carried out in a growth chamber at 22 °C and a 16: 8 h L: D photoperiod with 20-25 replicates for each pesticide tested.

2.6. Data analysis

Most of the statistical procedures were carried out in SAS (SAS Institute, 2009), with the exception of the generalized linear models with binomial errors (R Development Core Team, version 2.15.0, 2012) that were used to analyze the Abbott-corrected acute mortality of juvenile and adult *T. pallidus* with pesticide and concentration as fixed factors. Generalized linear models (SAS PROC MIXED) were used to analyze the effects of pesticide and concentration for both interaction and main effects (with block as an additional random effect for the insecticide treatment) on the Abbott-corrected acute mortality after log odds transformation for larval and adult *C. carnea*. When there was a significant interaction between fixed main effects, the effect of pesticide was tested separately for each concentration. In the absence of an interaction, the main effects were tested for significance. Similar generalized linear models were also used to analyze larval survival, developmental time and sex ratio, and adult female longevity, fecundity and fertility as uncorrected responses for both *C. carnea* and *T. pallidus* (SAS, PROC MIXED). Log odds transformation was used for larval survival and sex ratio. For *C. carnea*, separate models were used for the insecticide (pesticide, concentration and block) and fungicide experiments (pesticide and concentration), whereas for *T. pallidus*, all pesticides were analyzed

together with pesticide (single concentration only) as the only factor in the model. In all cases, control treatments were included as a level within pesticide. Means were compared at $P \leq 0.05$ level for all factors that proved to have a significant effect (*C. carnea*, LSMEANS; *T. pallidus*, Tukey HSD).

Following Stark et al. (2007), stage-structured matrix models were developed for both *C. carnea* and *T. pallidus* to extrapolate the effects of pesticide exposure on individual life-history parameters to a single population-level end point measurement. Daily survivorship from acute and/or sublethal bioassays, in addition to developmental rate, daily fecundity and sex ratio from the sublethal bioassays, were used to parameterize the matrix models. The models were analyzed using PopTools (Hood, 2010) to estimate the intrinsic rate of population increase (r) for control treatments and following exposure to each pesticide. To compare the population responses to pesticide exposure for these two natural enemy species that differ considerably in life history traits, the percentage change in potential growth of an initial population of 10 individuals over a single generation ($10*e^{rT}$ where r = intrinsic rate of population increase, T= generation time) was estimated.

3. Results

3.1.1. Acute toxicity bioassays

For corrected acute adult mortality of *C. carnea*, the interaction between insecticide and concentration ($F = 11.15$, $df = 4, 10$, $P = 0.001$) and the main effects (insecticide, $F = 73.14$, $df = 4, 10$, $P = 0.001$; concentration, $F = 132.68$, $df = 1, 10$, $P = 0.001$) were all significant. Hence,

insecticides were tested separately for each concentration and there was a significant insecticide effect for both concentrations (10%, $F = 1859.48$, $df = 4, 8$, $P = 0.001$; 100%, $F = 25.68$, $df = 4, 8$, $P = 0.001$). The significant interaction resulted from the increased mortality for the higher concentration for lambda-cyhalothrin and cyantraniliprole, but a very limited or moderate effect of concentration on mortality for the other pesticides (Fig. 1a). For corrected acute larval mortality of *C. carnea*, the interaction between insecticide and concentration ($F = 1.75$, $df = 4, 10$, $P = 0.22$) and the main effects (insecticide, $F = 1.27$, $df = 4, 10$, $P = 0.34$; concentration, $F = 2.78$, $df = 1, 10$, $P = 0.13$) were not significant. The acute effects of the pesticide treatments were much greater for adults than for larvae of *C. carnea* (Fig. 1a). Corrected acute larval mortalities at 48 h were less than 14% for all insecticide treatments except for the high concentration of lambda-cyhalothrin. In contrast, corrected acute adult mortalities were ≥ 40% for the high concentrations of chlorantraniliprole, cyantraniliprole, spinetoram and lambda-cyhalothrin. All larvae and adult *C. carnea* survived at 48 h and did not exhibit any acute mortality when treated with the two fungicides.

For the analysis of corrected acute mortality for adult *T. pallidus*, there was a significant interaction between pesticide and concentration ($\chi^2 = 48.64$, $df = 6$, $P < 0.001$), a significant effect of concentration ($\chi^2 = 20.74$, $df = 1$, $P < 0.001$), and a significant influence of pesticide ($\chi^2 = 342.10$, $df = 6$, $P < 0.001$). After separating the two concentrations, there was a significant effect of pesticide for both concentrations (10%, $\chi^2 = 160.19$, $df = 6$, $P < 0.001$; 100%, $\chi^2 = 230.68$, $df = 6$, $P < 0.001$). The significant interaction resulted from a doubling of adult mortality for the higher concentration of lambda-cyhalothrin and sulfur, but a very limited effect of concentration on adult mortality for the other pesticides (Fig. 1b). Although sulfur was not used in the juvenile bioassay, *T. pallidus* adults that were exposed to sulfur experienced 76% and

32% corrected acute mortality for the high and low concentrations, respectively. Similarly, there was a significant interaction between pesticide and concentration (χ^2 = 14.28, *df* = 5, *P* = 0.01), and significant effects of both concentration (χ^2 = 7.86, *df* = 1, *P* =0.01), and pesticide (χ^2 = 593.27, *df* = 5, *P* < 0.001) for the corrected acute mortality of juveniles. After separating the two concentrations, there was a significant effect of pesticides for both concentrations (10%, χ^2 = 310.74, *df* = 5, *P* < 0.001; 100%, χ^2 = 295.13, *df* = 5, *P* < 0.001). The interaction in this case was due to a lack of effect of concentration on larval mortality of chlorantraniliprole, cyantraniliprole and spinetoram compared to the other pesticide tested. The acute effects of the pesticides were more evenly matched between juveniles and adults than was the case for *C. carnea* (Fig. 1b). Novaluron and the copper hydroxide and mancozeb mixture had little effect on either juvenile or adult *T. pallidus*, whereas cyantraniliprole, spinetoram and lambda-cyhalothrin were acutely toxic to both juveniles and adults.

3.2. Sublethal bioassays

For *C. carnea* larval survival to adult in the insecticide experiment, the interaction (*F* = 4.12, *df* = 5, 12, *P* = 0.02) and the main effects (insecticide, *F* = 24.65, *df* = 5, 12, *P* = 0.001; concentration, *F* = 19.44, *df* = 1, 12, *P* = 0.001) were significant. When the insecticide treatment effect was tested for each concentration, there was a significant effect for both concentrations (10%, *F* =8.59, *df* = 5, 10, *P* = 0.002; 100%, *F* = 6.77, *df* = 5, 10, *P* = 0.01). None of the larvae treated with either concentration of novaluron or the high concentration of lambda-cyhalothrin survived to adult emergence and very few of those treated with the high concentration of cyantraniliprole emerged as adults compared with larvae in the control treatment (Table 1).

Most of the larvae treated with the low concentration of spinetoram survived to adult emergences. Larvae treated with the other concentrations and insecticides all had lower larva to adult survival than the control. In the fungicide experiment, the interaction for larva to adult survival was not significant ($F = 0.04$, $df = 2, 35$, $P = 0.96$) and the main effects (fungicide, $F = 1.70$, $df = 2, 35$, $P = 0.20$; concentration, $F = 0.02$, $df = 1, 35$, $P = 0.88$) were not significant (Table 1). More than 76% of the larvae survived to adult emergences for both fungicides.

For those treatments in the insecticide experiment with at least some larvae surviving to adult emergence, there was no significant interaction between insecticide and concentration ($F = 2.37$, $df = 3, 8$, $P = 0.15$) for larva to adult developmental time (Table 1). There was a significant main effect for insecticide ($F = 4.93$, $df = 4, 8$, $P = 0.03$), although concentration was not significant ($F = 3.49$, $df = 1, 8$, $P = 0.10$). The insecticide effect was significant when analyzed with pooled concentrations ($F = 2.84$, $df = 4, 12$, $P = 0.05$). Adults emerged in less than 20 d in all insecticide treatments. The larvae treated with cyantraniliprole had a significantly longer developmental time than the larvae treated with other insecticides (Table 1). In the fungicide experiment, there was no significant interaction for larva to adult developmental time ($F = 0.42$, $df = 2, 35$, $P = 0.66$, Table 1). For the main effects, fungicide was significant ($F = 10.39$, $df = 2, 35$, $P = 0.001$), but concentration was not significant ($F = 0.01$, $df = 1, 35$, $P = 0.93$). The fungicide effect was also significant when analyzed with pooled concentrations ($F = 10.75$, $df = 2, 38$, $P = 0.001$). Adults emerged in less than 21 d in all fungicide treatments. Larvae treated with sulfur had a longer larva to adult developmental time than the larvae treated with the mixture of copper hydroxide and mancozeb or the control. For the sex ratio of emerged adults from treated larvae of *C. carnea*, there was no significant interaction (insecticide, $F = 0.15$, $df = 3, 8$, $P = 0.93$; fungicide, $F = 0.23$, $df = 2, 35$, $P = 0.79$) and the main effects (insecticide, $F =$

2.03, *df* = 4, 8, *P* = 0.18; concentration, *F* = 0.05, *df* = 1, 8, *P* = 0.82 and fungicide, *F* = 0.01, *df* = 2, 35, *P* = 0.99; concentration, *F* = 0.08, *df* = 1, 35, *P* = 0.77) were not significant.

For female longevity, the interaction between insecticide and concentration was not significant (*F* = 2.62, *df* = 5, 12, *P* = 0.08) although both main effects (insecticide, *F* = 11.27, *df* = 5, 12, *P* = 0.001; concentration, *F* = 13.75, *df* = 1, 12, *P* = 0.003) were significant. Adult females treated with either concentration of novaluron or with low concentrations of spinetoram or lambda-cyhalothrin had similar longevities to females in the control treatment. Females treated with the low concentration of chlorantraniliprole had reduced longevity compared with control females (Table 1). For the fungicide experiment, there was no significant interaction between fungicide and concentration for the longevity of females (*F* = 0.05, *df* = 2, 53, *P* = 0.95, Table 1) and the main effects (fungicide, *F* = 0.79, *df* = 2, 53, *P* = 0.46; concentration, *F* = 0.01, *df* = 1, 53, *P* = 0.94) were not significant.

None of the adults treated with either concentration of cyantraniliprole or the high concentration of chlorantraniliprole, spinetoram and lambda-cyhalothrin survived long enough to be able to estimate sublethal effects on fecundity and fertility. For the insecticide experiment, there was no significant interaction for the mean daily fecundity of females (*F* = 0.00, *df* = 1, 4, *P* = 0.97). There was a significant main effect for insecticide (*F* = 9.71, *df* = 4, 4, *P* = 0.02), but the concentration effect was not significant (*F* = 0.15, *df* = 1, 4, *P* = 0.71). The insecticide effect was also significant with pooled concentrations (*F* = 17.5, *df* = 4, 6, *P* = 0.002). Exposure to the low concentration of chlorantraniliprole and lambda-cyhalothrin resulted in a significant reduction in daily fecundity relative to the control and other treatments. Daily fecundities of females treated with the low concentration of spinetoram and either concentration of novaluron were similar to those in the control (Table 1).

For the fungicide experiment, there was no significant interaction between fungicide and concentration for the mean daily fecundity of females ($F = 0.16$, $df = 2, 53$, $P = 0.86$, Table 1). There was a significant main effect for fungicide ($F = 11.69$, $df = 2, 53$, $P = 0.001$) although the concentration effect was not significant ($F = 0.89$, $df = 1, 53$, $P = 0.35$). The fungicide effect was also significant with pooled concentrations ($F = 12.09$, $df = 2, 56$, $P = 0.001$). Females treated with the mixture of copper hydroxide and mancozeb produced a lower number of eggs than in the control.

There was no significant interaction between fungicide and concentration for the mean daily fertility of *C. carnea* females in the insecticide experiment ($F = 0.03$, $df = 1, 4$, $P = 0.86$, Table 1). There was a significant main effect for insecticide ($F = 41.86$, $df = 4, 4$, $P = 0.001$) although the concentration effect was not significant ($F = 0.01$, $df = 1, 4$, $P = 0.94$). The insecticide treatment effect was also significant with pooled concentrations ($F = 62.35$, $df = 4, 6$, $P = 0.001$). Mean daily fertilities of females treated with either concentration of novaluron were very low (≤ 0.4), although their mean daily fecundities were the same as for the controls (≥ 26 eggs). Fertility of females treated with either the low concentration of chlorantraniliprole or lambda-cyhalothrin was also much lower than in the control while females treated with the low concentration of spinetoram was not different than the control (Table 1). There was no significant interaction for the mean daily fertility of *C. carnea* females ($F = 0.19$, $df = 2, 53$, $P = 0.82$, Table 1) in the fungicide experiment. There was a significant main effect for fungicide ($F = 13.66$, $df = 2, 53$, $P = 0.001$) although the concentration effect was not significant ($F = 1.11$, $df = 1, 53$, $P = 0.30$). The fungicide effect was also significant with pooled concentrations ($F = 14.12$, $df = 2, 56$, $P = 0.001$). A slight, but significant, reduction from the control was observed for the females treated with sulfur and the mixture of copper hydroxide and mancozeb.

Adult female longevity of *T. pallidus* was significantly influenced by pesticide (F = 35.76, df = 5,115, $P < 0.001$), and this was due to the reduced longevity of females exposed to sulfur relative to the control and other pesticide treatments (Table 2). Daily fecundity of females was also significantly influenced by pesticide (F = 21.11, df = 5,115, $P < 0.001$) and in this case exposure to both lambda-cyhalothrin and sulfur resulted in a significant reduction in daily fecundity relative to the control and other pesticide treatments (Table 2). Although juvenile development time from egg to adult emergence did not vary greatly between pesticide treatments, there was significant variation (F = 8.85, df = 5,102, $P < 0.001$). In this case, *T. pallidus* adults exposed to novaluron produced juveniles with a significantly shorter development time, and those exposed to the mixture of copper hydroxide and mancozeb produced juveniles with a significantly longer development time than for other pesticide treatments (Table 2). There was no detectable influence of any of the pesticides on either mummy survivorship (F = 0.82, df = 5,102, P = 0.54) or juvenile sex ratio (F = 0.15, df = 5, 56, P = 0.98) for *T. pallidus* although sample sizes for sex ratio were considerably reduced due to effects of the pesticides on daily fecundity (Table 2).

3. 3. Intrinsic rate of population increase (r) and percentage change in population growth over a single generation of C. carnea and T. pallidus

The intrinsic rates of population increase (r) were negative for *C. carnea* treated with chlorantraniliprole, cyantraniliprole and lambda-cyhalothrin with more than a 99% reduction in population growth over a single generation (Table 3). For *T. pallidus*, cyantraniliprole and lambda-cyhalothrin also generated negative values for r, as did spinetoram and sulfur, but

corresponding reductions in population growth over a single generation ranged from 83-91%. *Chrysoperla carnea* exposed to novaluron and spinetoram had positive, but low, *r* values, and were estimated to experience a 94-96% reduction in population growth over a single generation. When *C. carnea* was treated with fungicides there was little effect on *r* or on the percentage reduction in population growth over a single generation. Similarly, for *T. pallidus* treated with chlorantraniliprole, novaluron or copper hydroxide plus manzate the estimated values of *r* remained positive with from 10-61% reduction in the population growth over a single generation.

4. Discussion

We demonstrated, using bioassays with multiple routes of exposure and life table response experiments, that some of the pesticides (chlorantraniliprole, cyantraniliprole, novaluron, spinetoram, lambda-cyhalothrin and sulfur) evaluated in this laboratory study could negatively affect the population growth of *C. carnea* and/or *T. pallidus*. Cyantraniliprole and lambda-cyhalothrin had direct lethal effects and caused significant mortality to adult and/or immature *C. carnea* and *T. pallidus*. Cyantraniliprole and lambda-cyhalothrin also caused lower survivorship and/or negative sublethal effects on fecundity of *C. carnea* and *T. pallidus*. Novaluron and sulfur were differentially selective for the two natural enemy species tested in this study. Novaluron caused both lethal and sublethal effects on *C. carnea* but had no significant negative effects on *T. pallidus* while the opposite was true for sulfur. The combined acute and sublethal responses to exposure of novaluron and sulfur by *C. carnea* and *T. pallidus* translated into distinctly different effects on their intrinsic rates of natural increase and potential population growth over a single generation. The potential impact of chlorantraniliprole, cyantraniliprole,

novaluron, spinetoram, lambda-cyhalothrin and sulfur on the stability of orchard IPM is an important concern as the management of primary tree-fruit pests transitions from traditional broad spectrum OP insecticides to newer reduced risk and OP-replacement insecticides.

Despite the singular focus of most toxicological studies on mortality/survival estimates, there is increasing awareness of more subtle toxicant effects that warrant closer attention (Stark and Banks, 2003). Sublethal effects of pesticides can be as important as acute mortality when evaluating pesticide effects on natural enemies and include effects on fecundity, fertility, developmental time and sex ratio as life table responses (Ahmadi 1983; Blumel et al., 2000; Calow and Forbes, 2003; Forbes and Calow, 2002; Forbes and Calow, 2012; Hansen et al, 1999; Stark and Banks, 2003; Theiling and Croft, 1989). Sublethal effects should be studied and quantified to provide a more accurate picture of potential impacts of a pesticide on a natural enemy. In this study, we integrated acute, chronic, and sublethal effects of new pesticide chemistries into laboratory bioassays to understand the selectivity of a range of pesticides for two key natural enemies.

We compared lethal and sublethal effects of these insecticides on a predator, *C. carnea*, and a parasitoid, *T. pallidus*, both of which are important natural enemies in orchard cropping systems. Hymenopteran aphid parasitoids are short-lived natural enemies (Weisser et al., 1994). The longevity of *T. pallidus* adults is approximately 7 d (N. J. Mills, unpublished observation; Talebi et al., 2002), in contrast to the more than 30 d longevity of *C. carnea* and other orchard predators, such as *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae), which provides for greater periods of pesticide exposure (Amarasekare and Shearer, 2013a, 2013c).

With a lower intrinsic rate natural increase, the population-level impacts of pesticide exposure on *C. carnea* tended to be greater than for *T. pallidus*. This difference in pesticide

impacts between the two model natural enemy species was reduced, however, for lambda-cyhalothrin, the pesticide with the highest toxicity, and even reversed for sulfur, suggesting that *C. carnea* may have developed some tolerance to this product. The greater longevity of *C. carnea* also permitted study of the chronic effects of pesticides on *C. carnea* that was not possible for the shorter-lived *T. pallidus* (Amarasekare and Shearer, 2013b). Studying the lethal and sublethal effects of pesticides using similar treatments and methods on natural enemy species with varied feeding habits, biological properties, and life history characteristics, can provides a broader perspective and a greater level of insight into the selectivity of pesticides to important natural enemies (Mills et al., 2015, this issue).

In this study, we conducted laboratory bioassays using detailed protocols for two different functional groups of natural enemies, a general predator and an aphid parasitoid and found some important differences. As a general predator, both *C. carnea* larvae and adults could readily be topically exposed to pesticides in this study, whereas for the aphid parasitoid, only adult *T. pallidus* could be topically exposed. Juvenile stages of endoparasitoids, such as *T. pallidus*, need live hosts (the walnut aphid) for their development (Hougardy and Mills, 2009; Rakhshani et al., 2004) and thus cannot be subject to the same routes of pesticide exposure that can readily be applied to juvenile predators. This is why the majority of studies on pesticide effects for endoparasitoids are restricted to either exposed cocoon (Biondi et al., 2013; Longley, 1999) or adult stages (Biondi et al., 2013; Brunner et al., 2001; Longley and Stark, 1996) of the life cycle. However, if pesticide effects during the juvenile stages of endoparasitoids with externally-feeding hosts are ignored, then effective extrapolation of individual-level effects to population responses will inevitably be limited and conservative. This would be a critical factor when using traditional method for estimating side effects of pesticides on natural enemies that

are based on LD_{50} and/or LC_{50} values (Desneux et al., 2007). In this study, we used similar bioassay methods to expose unparasitized walnut aphid hosts as well as adult *T. pallidus* to the same set of pesticides with the implicit assumption that mortality/survival of unparasitized aphids would approximate the mortality/survival of juvenile *T. pallidus* developing inside the pesticide-exposed walnut aphids. It could be argued that it would have been better to use parasitized aphids than unparasitized aphids for the juvenile parasitoid bioassays, but it is notoriously difficult to know when probed aphids have actually been parasitized, and thus for consistency we elected to use unparasitized aphids rather than a mixture of parasitized and unparasitized aphids.

The reduced risk insecticides (cyantraniliprole, chlorantraniliprole and spinetoram) and OP-replacement (novaluron and lambda-cyhalothrin) used in this study are considered more environmentally desirable than OP or carbamate insecticides. Many of these newer insecticides have a much lower "knock down" effect on natural enemies, and hence, attain much of their damaging effects through reduction in reproduction, development rate and survival. Consequently, many of the insecticides and some of the fungicides tested in these bioassays had strong population impacts on either *C. carnea* or *T. pallidus* or both. The non-selectivity of cyantraniliprole, lambda-cyhalothrin and spinetoram led to particularly strong population effects for both *C. carnea* and *T. pallidus*. In contrast, chlorantraniliprole and novaluron had a greater impact on *C. carnea* than on *T. pallidus*, while the reverse was true for sulfur. Pesticide exposure can also induce subtle behavioral changes that influence the sexual competitiveness, navigation ability, feeding and oviposition potential and even learning success of natural enemies (Stark et al., 2004; Desneux et al., 2007).

Bioassay methods used to ascertain the impact of an insecticide on an insect population can have a considerable effect on the assessment of apparent toxicity (Banken and Stark, 1998). The International Organization for Biological Control (IOBC) has developed standard protocols for the analysis of the impact of pesticides on non-target organisms (Hassan, 1985). In the laboratory, individual test organisms of uniform age are either exposed to dried residue on treated surfaces or topically sprayed and moved to a clean surface and monitored for mortality or reduction in predation or parasitism (Banken and Stark, 1998; Hassan, 1985). These tests are designed to assess the effects of residual or topical exposure alone, whereas in the field natural enemies may be exposed through several routes including topical exposure to spray droplets and oral uptake from contaminated food sources (Longley and Stark, 1996). By excluding oral exposure and sublethal effects, the standardized laboratory tests recommended by the IOBC in its tiered approach could fail to detect several important effects of exposure to a toxicant, such as shortened life-span, reduced number of offspring, altered time to first reproduction, reduced growth and mutations in offspring (Stark et al., 2004). Thus these traditional bioassays can greatly underestimate the impact of a pesticide on the population growth of natural enemies (Banken and Stark, 1998).

Results from our laboratory bioassays demonstrate that some of the newer insecticides that are replacing OPs in tree fruit IPM programs in the U.S. are not as selective to natural enemies as initially promoted. It is likely that natural enemies that survive pesticide exposure may still acquire significant detrimental impacts from sublethal effects (Stark and Banks, 2003). The impact of our laboratory bioassays on *C. carnea* and *T. pallidus* varied with pesticide chemistry and mode of action, and ranged from acute toxicity to loss of reproduction and other sublethal effects. Thus our study contributes to the growing awareness that laboratory bioassays

which incorporate multiple routes of exposure, LTREs and extrapolation through population models provide a more holistic and accurate approach to estimating pesticide impacts on natural enemies compared with using traditional LD_{50} and/or LC_{50} values. The results from this study should also provide helpful guidelines for managers in choosing between pesticides to minimize their impact on *C. carnea* and *T. pallidus* and other natural enemies in orchard systems.

Acknowledgements

This research was supported in part by grant 2008-04854 from the United States Department of Agriculture, National Institute of Food and Agriculture (USDA-NIFA) Specialty Crop Research Initiative (SCRI) program. Matching funds were provided by Oregon State University, Washington State University, University of California Berkeley, the Washington Tree Fruit Research Commission, California Walnut Board and growers in Hood River County, OR, USA. We thank DuPont Crop Protection, Dow Agro Sciences LLC, Chemtura AgroSolutions, Syngenta LLC and Micro Flo Company LLC for providing the pesticides used for this study. Thanks to Elizabeth H. Beers (Department of Entomology, Tree Fruit Research and Extension Center, Washington State University, Wenatchee, WA) and Thomas R. Unruh (USDA- Agriculture Research Service (ARS), Yakima Agricultural Research Lab, Wapato, WA) for assisting with the design of these experiments. We thank C. S. Henry of University of Connecticut for the identification of *C. carnea* and Amanda Borel, Nicole Allum, Anne Iskra, Katie Calkins and Erin Hart of Oregon State University and Kevi Mace-Hill of the University of California Berkeley for their assistance with these experiments.

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Table 1

Mean (± SEM) survival (%), developmental time (d) and adult sex ratio (% female) from treated larvae and longevity, daily fecundity and fertility from treated adults of *Chrysoperla carnea* with pesticide concentrations equivalent to the maximum (100%) and reduced (10%) label rate or water (control).

Table 2

Effects of the highest pesticide concentration that did not cause extensive acute mortality on mean (± SE) female longevity and daily fecundity, and on juvenile development time from egg to adult, mummy survivorship and adult sex ratio (% female) of the progeny produced by *Trioxys pallidus* treated as adults.

Table 3

Estimated intrinsic rate of increase (*r*) and percent reduction in population growth rate over a single generation of *Chrysoperla carnea* and *Trioxys pallidus* when exposed to pesticides.

Tables

Table 1

Mean (± SEM) survival (%), developmental time (d) and adult sex ratio (% female) from treated larvae and longevity, daily fecundity and fertility from treated adults of *Chrysoperla carnea* with pesticide concentrations equivalent to the maximum (100%) and reduced (10%) label rate or water (control).

Treatment	Concentration (%)	Larva			Adult		
		Survival (%)[1]	Developmental time (d) [1]	Adult sex ratio (%)[1]	Female longevity (d) [1]	Daily fecundity[1]	Daily fertility[1]
Insecticides							
control*	10	87.5 ± 2.5ab	16.6 ± 1.0c	47.2 ± 0.1	40.1 ± 5.9b	25.6 ± 2.8a	18.6 ± 0.9ab
	100	87.5 ± 2.5A	16.9 ± 0.9c	55.7 ± 0.7?	38.9 ± 10.3B	26.8 ± 1.8a	18.2 ± 3.5ab
chlorantraniliprole	10	35.8 ± 10.6cde	16.8 ± 0.8c	39.8 ± 13.5	14.8 ± 3.4cd	5.6 ± 2.2b	3.4 ± 1.4cd
	100	16.7 ± 13.1CD	16.7 ± 0.8c	43.1 ± 17.8	4.7 ± 1.0C	-	-
cyantraniliprole	10	30.8 ± 15.6de	17.1 ± 0.7ab	62.4 ± 10.5	5.9 ± 1.3d	-	-
	100	2.5 ± 2.5DE	19.6 ± 1.6ab	63.0 ± 13.0	2.1 ± 0.7C	-	-
novaluron	10	0.0 ± 0.0f	-	-	49.5 ± 9.6ab	24.6 ± 2.9a	0.2 ± 0.1d
	100	0.0 ± 0.0E	-	-	45.1 ± 12.8AB	26.0 ± 6.8a	0.4 ± 0.1d
spinetoram	10	66.7 ± 15.2bcd	16.5 ± 0.8c	49.9 ± 15.8	38.7 ± 5.3b	25.5 ± 6.3a	16.0 ± 5.6b
	100	26.7 ± 15.6BC	16.7 ± 1.2c	45.5 ± 10.6	3.8 ± 0.4C	-	-
lambda-cyhalothrin	10	24.2 ± 9.2e	18.2 ± 1.3bc	65.3 ± 1.4	37.7 ± 6.6b	7.9 ± 4.2b	3.1 ± 1.6cd
	100	0.0 ± 0.0E	-	-	1.0 ± 0.0C	-	-
Fungicides							
control	10	93.4 ± 4.4	18.4 ± 0.8b	50.0 ± 5.1	36.1 ± 3.4	34.1 ± 3.3ab	31.1 ± 3.8a
	100	93.4 ± 4.4	18.5 ± 0.8b	50.0 ± 5.1	36.0 ± 4.3	33.9 ± 2.1ab	31.2 ± 1.9a
copper hydroxide	10	83.5 ± 5.5	19.2 ± 0.6b	41.6 ± 12.0	40.4 ± 4.8	23.9 ± 1.2c	20.2 ± 1.2c
+ mancozeb	100	80.1 ± 7.4	18.6 ± 0.7b	55.0 ± 9.7	41.4 ± 5.7	21.3 ± 1.8c	17.7 ± 1.6c
sulfur	10	80.2 ± 5.4	20.7 ± 0.4a	46.6 ± 11.1	44.7 ± 4.0	30.8 ± 2.7b	25.4 ± 2.6b
	100	76.7 ± 8.7	20.8 ± 0.2a	44.9 ± 11.1	42.7 ± 6.7	27.7 ± 2.7b	22.1 ± 2.3b

[1]Means within a column followed by the same lower or uppercase letters are not significantly different at $P \geq 0.05$ (LSMEANS) for each pesticide group.

*Half of the control data are used with each concentration 10% and 100% , respectively, to facilitate the data analysis.

Table 2

Effects of the highest pesticide concentration that did not cause extensive acute mortality on mean (± SE) female longevity and daily fecundity, and on juvenile development time from egg to adult, mummy survivorship and adult sex ratio (% female) of the progeny produced by *Trioxys pallidus* treated as adults.

Treatment	Concentration (%)[2]	Female longevity[1] (d)	Daily fecundity[1]	Developmental time[1] (d)	Mummy survival[1] (%)	Adult sex ratio[1] (%)
control		2.9 ± 0.0a	14.7 ± 1.6a	14.8 ± 0.2b	83.0 ± 1.5	45.3 ± 6.2
chlorantraniliprole	100	3.0 ± 0.0a	12.0 ± 1.2a	14.4 ± 0.1bc	79.5 ± 2.7	51.3 ± 9.4
novaluron	100	2.9 ± 0.1a	14.6 ± 1.7a	13.9 ± 0.1c	84.1 ± 1.9	53.7 ± 6.5
lambda-cyhalothrin	10	2.6 ± 0.2a	1.7 ± 0.5b	14.3 ± 0.1bc	85.4 ± 7.2	49.8 ± 15.8
copper hydroxide + mancozeb	100	2.8 ± 0.1a	12.3 ± 1.2a	15.2 ± 0.2a	82.0 ± 2.3	48.3 ± 8.8
sulfur	10	1.5 ± 0.1b	4.2 ± 1.0b	14.4 ± 0.2abc	79.2 ± 6.5	44.4 ± 3.9

[1]Means within a column followed by the same lowercase letters are not significantly different at $P \geq 0.05$ (LSMEANS).

Table 3

Estimated intrinsic rate of increase (*r*) and percent reduction in population growth rate over a single generation of *Chrysoperla carnea* and *Trioxys pallidus* when exposed to pesticides.

Treatment	Intrinsic rate of increase *r*		Percent reduction in population growth rate [1]	
	C. carnea	*T. pallidus*	*C. carnea*	*T. pallidus*
Insecticides				
control	0.177	0.317	0.0	0.0
chlorantraniliprole	-0.107	0.134	99.8	60.7
cyantraniliprole	-0.254	-0.106	100.0	88.5
novaluron	0.042	0.296	94.3	10.1
spinetoram	0.020	-0.033	96.4	83.1
lambda-cyhalothrin	-0.174	-0.149	99.9	90.7
Fungicides				
control	0.191	-	0.0	-
copper hydroxide + mancozeb	0.166	0.263	40.0	23.8
sulfur	0.176	-0.065	26.4	85.7

[1] Percent reduction in population growth rate in a single generation = $[\{10*e^{rT}(\text{control}) - 10*e^{rT}(\text{treatment})\}/ 10*e^{rT}(\text{control})]*100$ where *r* = intrinsic rate of population increase and T = generation time.

Figure captions

Fig. 1: Abbott corrected acute toxicity (%) of pesticides to (a) larval and adult *Chrysoperla carnea* and (b) juvenile and adult *Trioxys pallidus* at concentrations equivalent to the maximum (100%) and reduced (10%) label rate.

Figure 1a

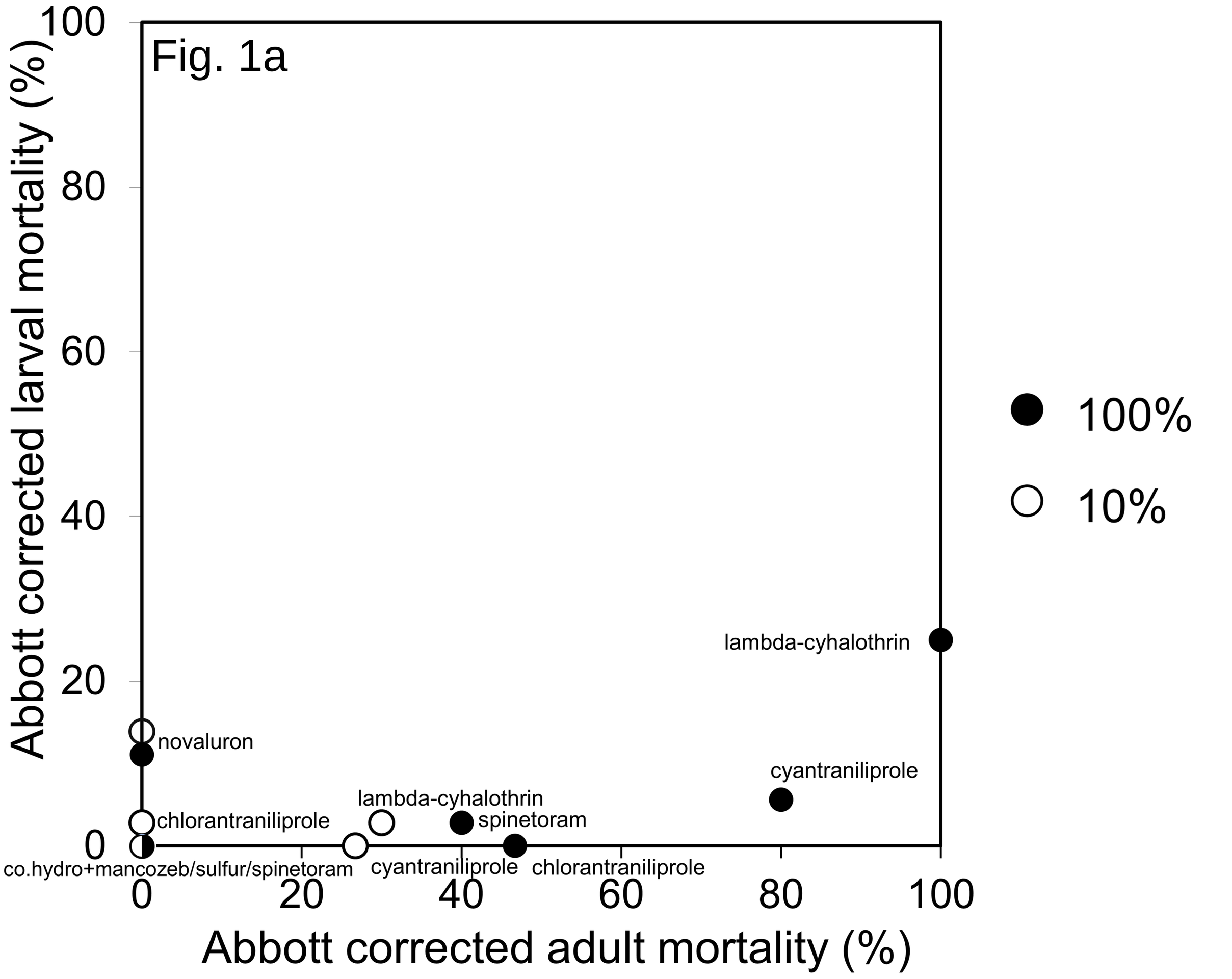


Fig. 1a
Abbott corrected larval mortality (%)
100
80
60
40
20
0
novaluron
chlorantraniliprole
lambda-cyhalothrin
spinetoram
cyantraniliprole
co.hydro+mancozeb/sulfur/spinetoram
cyantraniliprole
chlorantraniliprole
lambda-cyhalothrin
cyantraniliprole
100%
10%
0
20
40
60
80
100
Abbott corrected adult mortality (%)

Figure 1b

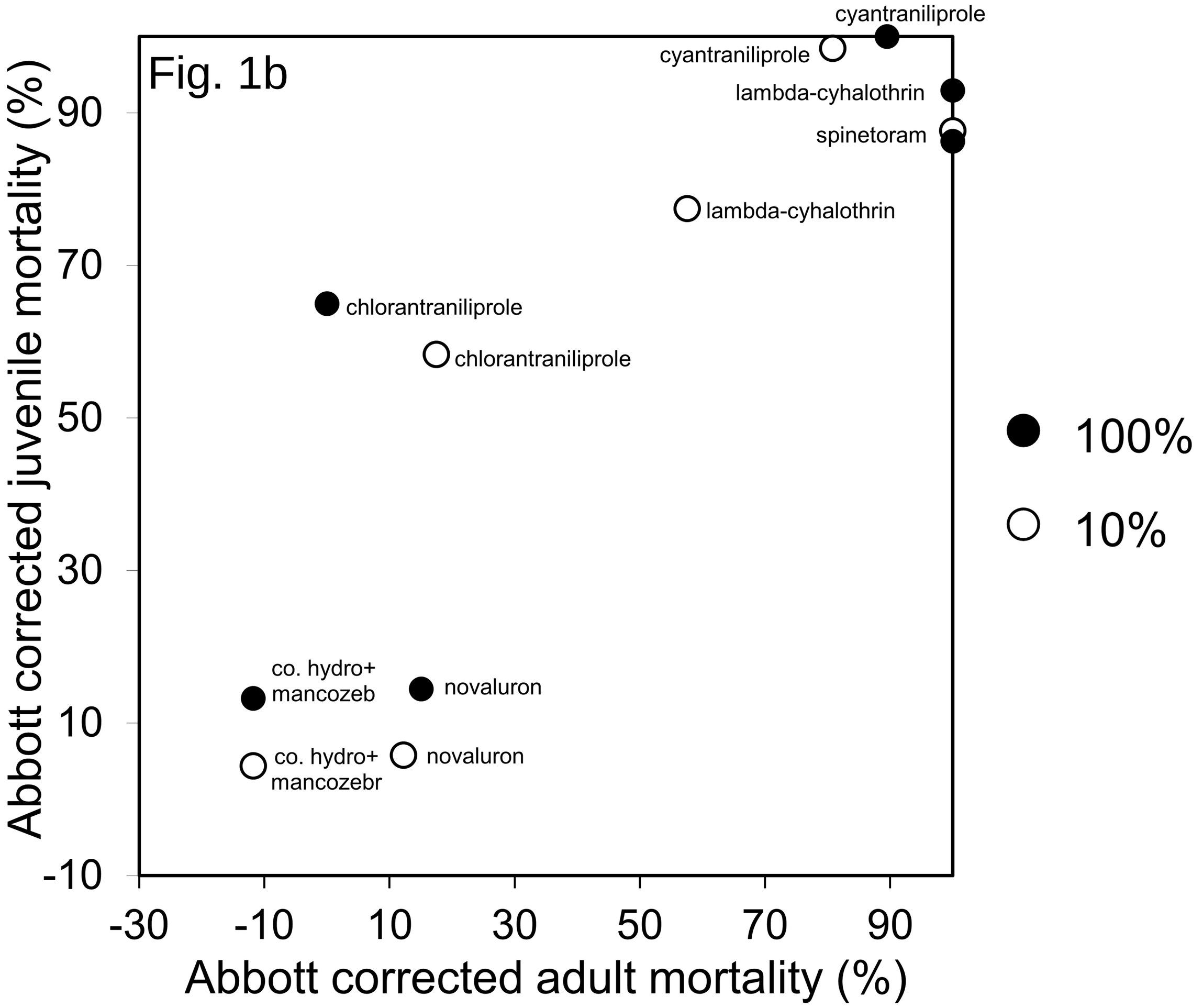
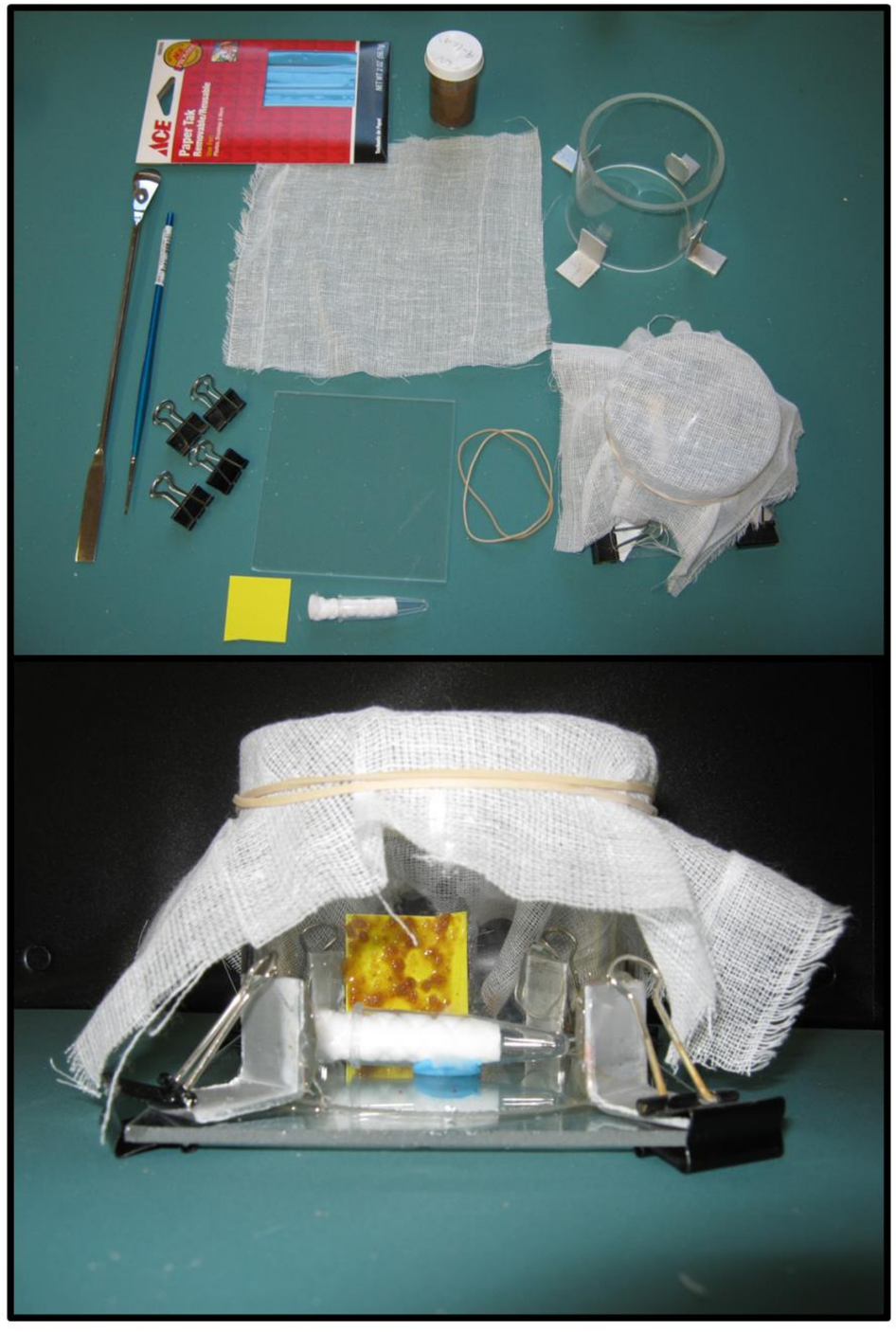


Fig. 1b
cyantraniliprole
cyantraniliprole
lambda-cyhalothrin
spinetoram
lambda-cyhalothrin
chlorantraniliprole
chlorantraniliprole
co. hydro+ mancozeb
novaluron
co. hydro+ mancozebr
novaluron
100%
10%
Abbott corrected juvenile mortality (%)
90
70
50
30
10
-10
-30
-10
10
30
50
70
90
Abbott corrected adult mortality (%)

Graphical Abstract

Multiple routes of exposure bioassay arena for *C. carnea*

Insects (contact), arenas (residual)



Food (oral), cheesecloth lids (residual)