Lethal and Sublethal Effects of Pesticides Used in Western United States Orchards on *Hippodamia convergens*

by

Lisa Fernandez

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Committee in charge:

Professor Nicholas J. Mills, Chair
Professor Kent M. Daane
Professor George K. Roderick
Professor Steve Selvin

Abstract

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We examined the effects of two fungicides (copper+mancozeb and sulfur) and five reduced-risk insecticides (chlorantraniliprole, cyantraniliprole, lambda-cyhalothrin, novaluron, and spinetoram) on *Hippodamia convergens* (Guerin-Meneville) (Col.: Coccinellidae), an important natural enemy in western United States orchards. Acute toxicity of pesticides was tested via three different exposure routes: oral, residual, and topical. Lambda-cyhalothrin caused significant mortality to adults and larvae via all exposure routes (57-100%). Cyantraniliprole was toxic to adults and larvae when orally exposed, but it was less toxic for other exposure routes. Cyantraniliprole, spinetoram, and novaluron were moderately toxic to larvae when topically exposed (40-63% mortality), but not to adults (<4% mortality). Chlorantraniliprole, copper+mancozeb, and sulfur demonstrated low toxicity (<30% mortality) to adults and larvae, regardless of exposure route. Lethal and sublethal effects were tested by treating insects topically in the inactive stages (egg, pupa) and simultaneously through oral, residual, and topical exposure in the active stages (larva, adult). Larvae were vulnerable to all pesticides except copper+mancozeb, and lambda-cyhalothrin also reduced survivorship for pupae and eggs (3-19%). Exposure to novaluron lowered survivorship for treated eggs (14%) and fertility of treated females (9%). Fecundity was increased by exposure to copper+mancozeb. Corrected acute adult mortality alone was sufficient to detect the harmful effects of chlorantraniliprole and lambda-cyhalothrin, but combined effects of corrected adult mortality and daily fertility were needed to highlight the harmful effect of novaluron. Estimates of the intrinsic rate of increase indicated substantial delays in the population recovery of *H. convergens* following exposure to chlorantraniliprole, cyantraniliprole, novaluron, and sulfur, and local extinction for lambda-cyhalothrin. The effects of a combination pesticide (chlorantraniliprole and lambda-cyhalothrin) were tested on larvae and adults exposed to residues aged 0, 1, 2, 4, 8, 16, and 38 d in a laboratory or field setting. For field-aged residues, both life stages were highly susceptible to 0 and 1d residue (> 80%), but acute mortality declined for residues aged 16 and 38 d adults (< 53%). For laboratory-aged residues, both life stages had high acute mortality rates (> 80%)

regardless of residue age. The rate of decay of the pesticide impact on adults (0.059) was greater than for larvae (0.024) for the field-aged residues, and greater than for adults exposed to laboratory-aged residues (α = 0.004). The results indicate that even reduced-risk insecticides may have harmful effects that vary by pesticide, life stage, and exposure route. Pesticide risk assessments can be improved by using lethal and sublethal data to estimate changes in population growth, and by incorporating both laboratory and field-based bioassays to better understand how pesticides impact natural enemies in the field.

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CHAPTER 1:
Conservation of *Hippodamia convergens*,
a key predator in orchards of the western United States

Biological control is a system of pest control that uses natural enemies to suppress pest populations below levels that damage crops (e.g. below the economic threshold). This can include the use of predators, parasitoids, and/or pathogens to either permanently or temporarily suppress pests. With increasing interest in pesticide use and sustainable agriculture, biological control can provide an economically and environmentally sound means of pest management. Several different approaches exist, including classical biological control, the release of non-native natural enemies to control non-native pests, augmentative biological control, artificially increasing native enemy populations via mass release, and conservation biological control or the manipulation of farming practices to conserve natural enemies.

1. Biological Control

1.1 Classical Biological Control

Classical biological control aims to control an invasive non-native species via the deliberate introduction of its specialized natural enemy. Natural enemy introductions have been used to control over 200 arthropod pests and 40 weeds (Van Driesche et al., 2008). The use of classical biological control became popular after the dramatic success of the control program for cottony cushion scale, *Icerya purchasi* (Hemiptera: Monophlebidae), a major citrus pest, through the introduction of the ladybird beetle, *Rodolia cardinalis* (Coleoptera: Coccinellidae), to California from Australia in 1888 (Caltagirone and Doutt, 1989). More recently, classical biological control continues to be used to suppress increasing numbers of invasive species worldwide. In the United States, it has been estimated that, while invasive insect species are only a small percentage of total arthropod species (2%), they represent approximately 35% of 700 important insect pests (Knutson et al., 1990). Thousands of classical biological control introductions have been made worldwide, and it has been estimated that, in 17% of cases, there has been effective control, to the extent that the targeted invader is no longer considered a pest. In 43% of cases, introductions have led to at least partial control, thereby reducing ecological damage and the need for pesticide use. However, 40% of introductions were found to have negligible impact on the target pest (Van Driesche et al., 2008). Thus, though complete success is possible, it is not characteristic of most classical biological control programs. More commonly, it results in partial control which positively contributes to pest management.

1.2 Augmentative

This method of biological control suppresses pest populations by releasing commercially produced natural enemies. This is particularly useful when natural enemies are not present or are scarce, such as in greenhouses or large monocultures. Within augmentative biological control, there are different methods for releasing insects. For instance, inoculative releases introduce small numbers of natural enemies, allowing them to establish in a crop, reproduce, and provide season-long control. Inundative releases, on the other hand, involve a mass

introduction of natural enemies, with the expectation that they will provide an immediate reduction in pest densities. Yet the effect will be short lived, because they do not reproduce or establish (Van Driesche et al., 2008). For example, mass releases of *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) have proved to be effective for control of two major pests, *Adoxophyes orana* and *Grapholitha molesta* (Lepiodptera: Tortricidae). When released in apple orchards, egg parasitism ranged from 81-99%. In this case, mass reared *T. dendrolimi* were labeled with a radioisotope to distinguish them from naturally occurring populations. The released *T. dendrolimi* were responsible for 74% of the egg parasitism, while 26% of the parasitized eggs were attacked only by wild *T. dendrolimi* (Zhou et al., 2014). This indicates that, not only was augmentation alone significant, but it may compliment scarce populations of natural enemies that are already present in the field.

1.3 Conservation Biological Control

When cropping systems are altered to enhance populations of natural enemies, this is known as conservation biological control. In some cases, this can involve providing natural enemies with a suitable plant surface to forage on, as some crop cultivars can be incompatible with natural enemies. For instance, high densities of trichomes on tomatoes increased entanglement of lacewing larvae (Simmons and Gurr, 2005). Also waxy blooms on cabbage plants can reduce the ability of lacewing larvae to move effectively, reducing predation rates on the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) (Eigenbrode et al., 1999). Providing nutritional supplements, such as floral resources or food sprays, is another way to conserve populations of natural enemies. In New Zealand apple orchards, under-planting apple trees with floral resources increased parasitism rates of *Epiphyas postvittana* (Lepidoptera: Tortricidae) (Irvin et al., 2006). In cotton, reproduction of lacewings was enhanced by spraying a mixture of hydrolyzed protein, water, and sugar (Hagen et al., 1970). While adding food sources and suitable habitat can enhance natural enemies, it is equally important to alter management practices that decrease natural enemies, namely, pesticides. Soon after the introduction of synthetic insecticides in the 1950s, researchers began to link secondary pest outbreaks and pest resurgence to the destruction of natural enemies (DeBach et al., 1976). Since, then it has become abundantly clear that pesticides can affect natural enemies in numerous ways, inducing changes in foraging behavior, predation rates, survivorship, fertility, fecundity, and development rates (Amarasekare and Shearer, 2013b; Beers et al., 2009; Desneux et al., 2007; Desneux et al., 2004b; Martinou et al., 2014; Stark and Banks, 2003).

1.4 Coccinellids in Biological Control

Ladybird beetles, or coccinellids, are important predators of many pest species, including whiteflies, aphids, mealybugs, scales, and mites. In particular, aphidophagous ladybird beetles are prominent generalist predators (Obrycki et al., 2009; Obrycki and Kring, 1998; Weber and Lundgren, 2009). As mentioned above, one of the most well-known examples of the use of coccinellids in classical biological control was the importation of the vedalia beetle, *Rodolia cardinalis* (Coleoptera: Coccinellidae), to control the cottony-cushion scale on citrus in California. Immediately after this success, many classical biological control programs began to focus on Coccinellidae, leading to the “ladybird fantasy” period (Caltagirone and Doutt, 1989).

One coccinellid commonly used in biological control programs in the United States is the generalist predator *Hippodamia convergens* (Coleoptera: Coccinellidae) (Hagen, 1974; Obrycki and Kring, 1998). It is frequently used in augmentative biological control because it is easily collected and stored. In California, they are collected from overwintering sites in the Sierra Nevada and sold commercially, yet still maintain their ability to prey on pests relative those not collected and stored for commercial use (Bjornson, 2008; Hagler, 2009; Lind, 1998). Due to rapid dispersal in a field setting, it is thought that mass releases of commercial *H. convergens* is ineffective (Obrycki and Kring, 1998; Van Driesche et al., 2008). For instance, for aphid control in roses, the effective rate of *H. convergens* release was two orders of magnitude greater than recommended by commercial insectaries, and was no more cost effective than an application of the systemic pesticide imidacloprid (Flint and Dreistadt, 2005). On the other hand, when placed in a more contained environment, such as a greenhouse, *H. convergens* has the ability to reduce pest populations (Dreistadt and Flint, 1996; Raupp et al., 1994).

Additionally, *H. convergens* may be incorporated into conservation biological control programs. In western North America, it is an important natural enemy in high-value tree crops (Gontijo et al., 2012; OSU, 2014; WSU, 2011). However, some reduced-risk pesticides used within this system have been found to be toxic to *H. convergens* (Cloyd and Bethke, 2010; Rodrigues et al., 2013; Roubos et al., 2014). Understanding how pesticides impact populations of natural enemies is key to integrated pest management and the conservation of natural enemies. **A major objective of this dissertation is to test how reduced-risk pesticides commonly used in high-value tree crops affect *H. convergens.***

2. Reduced-Risk Pesticides

For decades, the use of organophosphate pesticides has disrupted the activity of natural enemies. With the passage of the Food Quality Protection Act in the United States in 1996, this class of conventional pesticides became the primary focus of a registration review. With increased restrictions on use of organophosphates, growers began to use alternative pesticides for the control of pest populations. From 1990 to 2007, the use of organophosphates declined by approximately 60%, from 85 million pounds to 33 million pounds, and decreased from 70% of total insecticide use to 36% (Grube et al., 2011).

In 1992, via the Federal Register, the Environmental Protection Agency (EPA) Office of Pesticide Programs called for incentives to promote development and use of reduced-risk pesticides. The following year, the same office announced the Reduced-Risk Pesticide Initiative. The goal of this program was to promote the development and use of pesticide products that would pose a reduced risk to human and environmental health. Additionally, the EPA created incentives to promote registration of these pesticides under the new reduced-risk criteria. These criteria included reducing human health risks by reducing toxicity by a factor of 10-100 times relative to organophosphate insecticides. Of particular concern was reducing health hazards to pesticide applicators and field laborers, which is another reason the EPA actively promoted the replacement of organophosphates. In addition to human health, the criteria specified a reduction in risk to non-target organisms such as birds, beneficial insects, and aquatic

organisms (EPA, 1997). Though the Reduced-Risk Initiative criteria aimed to reduce risks to human and environmental health, reduced risk does not guarantee there are no harmful effects to other non-target organisms, such as natural enemies.

For my dissertation, seven reduced-risk pesticides commonly used in high-value tree crops in western North America were of particular interest, in the context of their impact on *H. convergens* as an important natural enemy. The seven reduced-risk pesticides examined were:

1. chlorantraniliprole (Altacor)
2. cyantraniliprole (Exirel)
3. copper hydroxide (Kocide 3000) and mancozeb (Manzate)
4. lambda-cyhalothrin (Warrior II)
5. novaluron (Rimon 0.83EC)
6. spinetoram (Delegate WG)
7. sulfur (Kumulus)

2.1 Chlorantraniliprole

Chlorantraniliprole belongs to a class of chemicals known as anthranilic diamides. It acts on insect ryanodine receptors, and is most effective when applied to plant material and ingested. One formulation of this chemical is Rynaxypyr, the active ingredient in the pesticide Altacor. Altacor is designed to target various lepidopteran pests, such as leafrollers, crown borers, leafminers, and codling moth. It is approved for use in fruiting shrubs, vines, and tree crops, including banana, citrus, and pome fruit (DuPont, 2014a).

Thus far, chlorantraniliprole appears to have little to no toxicity for most natural enemies. For example, it has shown demonstrated to have no toxic effects on the dogbane beetle *Chrysochus auratus*, (Coleoptera: Chyrsomelidae), a beneficial insect that feeds almost exclusively on problematic weeds in blueberry crops (Crozier and Cutler, 2014). According to the IOBC toxicity classification, chlorantraniliprole was ranked as slightly harmful to *Orius armatus* (Hemiptera: Anthocoridae), which controls western flower thrips in greenhouse grown peppers (Broughton et al., 2014). Similarly, it was found to be compatible with beneficial insects such as the *Bombus impatiens* (Hymenoptera: Apidae), *Copidosoma bakeri* (Hymenoptera: Encyrtidae), an endoparasitoid, the predatory ground beetle *Harpalus pennsylvanicus* (Coleoptera: Carabidae), and the ectoparasitoid, *Tiphia vernalis* (Hymenoptera: Tiphiidae) (Larson et al., 2014). When tested for behavioral effects on the generalist predator *Macrolophus pygmaeus* (Hemiptera: Miridae), exposure to chlorantraniliprole reduced feeding, but had no significant effect on predation rates (Martinou et al., 2014). Furthermore, there were no significant effects on survival, parasitism rates, or emergence of seven different species of parasitic wasps, including two sensitive indicator species, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) and *T. dendrolimi* (Brugger et al., 2010). When treated with chlorantraniliprole, adult and immature *Deraeocoris brevis* (Hemiptera: Miridae) survived at rates comparable to controls (Amarasekare and Shearer, 2013b). Yet, contrary to the aforementioned studies, chlorantraniliprole caused 100% mortality in two species of green lacewing, *Chrysoperla carnea* and *Chrysoperla johnsoni* (Neuroptera: Chrysopidae) (Amarasekare and Shearer, 2013a).

2.2 Cyantraniliprole

Cyantraniliprole is also an anthranilic diamide. Another version of this active ingredient is known as Cyazypyr. Under the trade name Exirel, it is approved for use in several crops, including bushberries, citrus, pome, stone fruit, and tree nuts. With respect to high value tree crops in western North America, it is approved for use in Washington and Oregon, but not in California. This pesticide targets chewing and piercing sucking insects, primarily from the orders Lepidoptera and Hemiptera. Both Altacor and Exirel pose little human health risk. However, unlike Altacor, Exirel has application restrictions due to its risk to insect pollinators (DuPont, 2014b).

Cyantraniliprole has been demonstrated to have no significant impact on the mortality of *Tamarixia triozae* (Hymenoptera: Eulophidae) when it is exposed to residue on either a glass or leaf surface (Liu et al., 2012b). However, 25% of *T. triozae* died 24 h after ingestion of treated honey. For the predator mite, *Galendromus occidentalis* (Acari: Phytoseiidae), cyantraniliprole caused no significant changes in mortality, egg hatch, or larval survivorship, but there were reductions in prey consumption and fecundity (Beers and Schmidt, 2014). The treated larvae of two lacewing species, *C. carnea* and *C. johnsoni*, did not experience significant mortality or differences in sex ratio, yet exposure was highly toxic to adults (Amarasekare and Shearer, 2013a). In nymphs of the predatory mirid, *D. brevis*, 23% mortality was observed when treated with the maximum recommended field rate, but there was no significant impact on mortality for treated adults (Amarasekare and Shearer, 2013b). Additionally, treated nymphs had no significant difference in development time, but, when they emerged as adults, the sex ratio had a significant female bias (73%). When applied as a systemic treatment in peppers , cyantraniliprole was found to have no impact on *Orius insidiosus* (Hemiptera: Anthocoridae) populations, but it also had no impact on populations of the target pest (Funderburk et al., 2013).

2.3 Copper Hydroxide and Mancozeb

Copper hydroxide and mancozeb are two active ingredients that may be mixed in a single pesticide solution and applied as a spray to control plant pathogens. Copper hydroxide is the active ingredient in the pesticide marketed under the trade name Kocide 3000, and mancozeb is the active ingredient in the pesticide Manzate Pro-Stick. They are used to control both bacterial and fungal pathogens in numerous crops, including citrus, conifers, vegetables, tree crops, cereals, and small fruits. In high-value tree crops, they are used in combination to control pathogens such as apple scab, pear scab, fire blight, and powdery mildew (DuPont, 2014c; DuPont, 2014d; OSU, 2014; WSU, 2011).

Fungicide effects on natural enemies are frequently focused on predatory mites. A mixture of copper hydroxide and mancozeb caused significantly high mortality in the predatory mite *G. occidentalis,* but at a concentration that was twice the maximum label rate. The same study found that, even at a diluted concentration, (0.1x the maximum label rate), exposing females to the pesticide mixture did not affect hatch rates, but, once hatched, the larvae had a higher mortality (Beers and Schmidt, 2014). On the other hand, a different predatory mite, *Amblyseius cucumeris* (Acari: Phytoseiidae), appears to be compatible with copper hydroxide, in that

exposure only causes high mortality at levels 10 times the recommended field rate. Copper hydroxide also had no significant effect on mortality or fecundity on the predatory mite *Euseius victoriensis* (Acari: Phytoseiidae), whereas mancozeb significantly lowered mortality as well as fecundity, with no females laying eggs after treatment (Bernard et al., 2010).

With respect to parasitoids, copper hydroxide has shown little to no toxicity on the predator of citrus leafminer, *Ageniaspis citricola* (Hymenoptera: Encyrtidae) (Villanueva-Jimenez and Hoy, 1998) or *Tamarixia radiata* (Hymenoptera: Eulophidae), a parasitoid of the Asian citrus psyllid (Hall and Nguyen, 2010). Mancozeb applications, suspected as the cause of low parasitism rates in Indonesian crops, demonstrated no toxicity to four different parasitoid species: *Hemiptarsenus varicornis* (Hymenoptera: Eulophidae), *Opius* sp., *Gronotoma micromorpha* (Hymenoptera: Eucoilidae) (Lardner 1991), and *Diglyphus isaea* (Hymenoptera: Eulophidae) (Prijono et al., 2004). These results suggest that the effects of copper hydroxide and mancozeb vary for different species, and their compatibility with natural enemies cannot be generalized.

2.4 Lambda-cyhalothrin

Lambda-cyhalothrin is a broad spectrum pyrethroid. One of its trade names is Warrior II, approved for use in a wide variety of crops, such as alfalfa, cereal crops, vegetables, tree nuts, tree fruits, cotton, and tobacco. In high-value tree crops, it is used to control many different pests: apple aphid, walnut aphid, codling moth, pear psylla, leafrollers, leafhoppers, and ants. This active ingredient is highly toxic to bees via direct application or residues on vegetation, and should not be applied when either crops or weeds are in bloom. Lambda-cyhalothrin is also highly toxic to aquatic organisms and wildlife, and areas subject to pesticide drift or runoff are considered hazardous (Syngenta, 2014).

Lambda cyhalothrin has also demonstrated toxic effects on several natural enemies. The predatory mite, *G. occidentalis*, experienced 96% mortality at the full field rate. Even at 0.1x the full field rate, it was highly toxic to *G. occidentalis*, causing 76% mortality (Beers and Schmidt, 2014). In order to test for resistance, one study topically exposed 28 different populations of ladybird beetles to lambda cyhalothrin. However, the majority of populations tested (21 of 28) displayed an LD_{50} below the maximum recommended field rate, including all populations of *Coleomegilla maculata*, *Cycloneda sanguinea*, *Harmonia axyridis*, and 6 of 8 *H. convergens* populations (Rodrigues et al., 2013). Lamba-cyhalothrin is also toxic to both the larvae and adults of two green lacewing species, *C. carnea* and *C. johnsoni* (Amarasekare and Shearer, 2013a). Additionally, it is highly toxic to the parasitoid, *Diadegma insulare* (Hymenoptera: Ichneumonidae), which parasitizes diamondback moth, a major pest of brassica crops. Moreover, *D. insulare* can distinguish between host larvae fed on lambda-cyhalothrin treated plants versus non-treated plants, indicating a change in host preference (Liu et al., 2012a).

2.5 Novaluron

Novaluron is an insect growth regulator, marketed under the trade name Rimon. It is approved for use in crops such as brassica, berries, potatoes, pome fruit, and stone fruit. In pear and apple orchards, it is used to control pear psylla, leafrollers, and codling moth. Its mode of

action is to disrupt cuticle formation and deposition during molting. Although novaluron is not a conventional insecticide, it still may have non-target effects on beneficial insects, such as pollinators. For instance, exposure to novaluron may interfere with the development of honey bee larvae. Therefore, its application may not be compatible with beneficial insects when crops are in bloom. In addition, it has demonstrated toxicity to fish and aquatic organisms (Chemtura, 2014).

Novaluron appears to have variable toxicity on several natural enemies. It demonstrated a low toxicity to *O. insidiosus* and *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), both of which are natural enemies in blueberry production (Roubos et al., 2014). However, when exposed to novaluron residue aged 7 days, nearly 50% of *H. convergens* displayed acute effects, meaning they were either killed or knocked down. For *G. occidentalis*, an important predatory mite in apple orchards, novaluron is moderately toxic. Though it did not significantly reduce fertility, it caused reductions in fecundity as well as fertility (Beers and Schmidt, 2014). It has also demonstrated toxicity to green lacewing larvae, none of which survive to the adult life stage after treatment (Amarasekare and Shearer, 2013a). Moreover, though it did not impact fecundity, it significantly reduced fertility and egg viability. *Podisus maculiventris* (Hemiptera: Pentatomidae), a predator of the potato beetle, is susceptible through both direct contact and residual exposure (Cutler et al., 2006). Additionally, when fed treated prey, 5th instar larvae were unable molt in to adults. Despite its impacts on survivorship and reproduction, novaluron is still seen as a better alternative to pesticides with greater acute toxicity. For instance, when compared to brassica crops treated with broad spectrum pesticides (e.g. pyrethroids or organophosphates), novaluron treated crops had higher yields and higher rates of parasitism in the pestiferous diamondback moth (Ayalew, 2011).

2.6 Spinetoram

Spinetoram is chemical derived from the soil bacterium *Saccharopolyspora spinosa.* It is used as the active ingredient in an insecticide, marketed under the trade name Delegate, which is used to control foliage damaging pests such as leafminers, psyllids, and lepidopterous larvae. It is approved for use in a number of crops, including bananas and plantains, bushberries, hops, citrus, pome fruits, tree fruits, and tree nuts. In pome fruits, it is used to suppress populations of apple maggot, pear psylla, thrips, light brown apple moth and codling moth. In addition to targeting pests, spinetoram is toxic to bees exposed to treated crops, either though foraging on pollen-shedding or nectar producing plant parts. Delegate is recommended for use in integrated pest management programs and is advertised as being compatible with natural enemies, such as ladybird beetles, lacewings, assassin bugs, and spiders (Dow, 2014).

Spinetoram has been demonstrated to be highly toxic to natural enemies and pollinators. For instance, *T. triozae* is a key parasitoid of the tomato psyllid, *Bactericera cockerelli* (Hemiptera: Trizoidae). *T. triozae* experiences 100% percent mortality when exposed to spinetoram via surface residue, even when residue is aged as much as 15 days (Liu et al., 2012b). Moreover, ingestion of spinetoram resulted in 100% mortality within a period of 12 hours. Additionally, spinetoram is highly toxic to eggs and larvae of the predatory mite *G. occidentalis*. It also caused 100% mortality in adult females after 72 h of exposure (Lefebvre et al., 2011).

Spinetoram is also toxic to pollinators via ingestion of treated sugar water, and it may cause mortality through exposure to either dry or wet residue. It is highly toxic to worker bees, and induces 100% mortality in bumble bees when applied at recommended field rates (Biondi et al., 2012a). Moreover, *Neochrysocharis formosa* (Hymenoptera: Eulophidae) and *Ganaspidium nigrimanus* (Hymenoptera: Figitidae) have demonstrated susceptibility to spinetoram (Hernandez et al., 2011). These natural enemies are important parasitoids of *Liriomyza trifolii* (Diptera: Agromyzidae), a major pest of vegetable and ornamental crops worldwide. Furthermore, spinetoram consistently lowers survival of both parasitoid species when exposed by direct application, residue, or ingestion.

2.7 Sulfur

Sulfur is commonly used as both a fungicide and acaricide. Known as dry flowable sulfur or wettable sulfur, it is a dry powder that may be mixed with water and used as a foliar spray. It is the active ingredient in the pesticide marketed under the trade name Kumulus, which is used in an extensive variety of crops: ornamentals, turf, alfalfa, brassica, citrus, berries, cotton, hay, tree fruits, peanuts. In apple and pear orchards, sulfur is used to control plant pathogens, such as powdery mildew and scab, and pests, such as the two spotted mite and the red spider mite (Arysta, 2014). It is also ranked as having variable impacts on natural enemies in tree fruits, ranging from low toxicity to aphid parasitoids to high toxicity on the western predatory mite, *G. occidentalis* (WSU, 2011).

In apple orchards, *G. occidentalis* is a predator of tetranychid mites, such as the two spotted spider mite (*Tetranychus urticae*) and the European red mite (*Panonychus ulmi*) (Acari: Tetranychidae). Sulfur residue demonstrated a low toxicity to *G. occidentalis* adult females, and it had no effect on prey consumption. However, sulfur may have variable toxicity depending on the life stage of natural enemies, because the same study found that sulfur residue was highly toxic to larvae (Beers et al., 2009). It has also been shown to affect populations of another predatory mite, *Iphiseiodes zuluagai* (Acari: Phytoseiidae), which preys on the coffee pest *Oligonychus ilicis* (Acari: Tetranychidae). The recommended field concentration caused approximately 60% mortality of *I. zuluagai* and decreased reproduction in adult females (Teodoro et al., 2005). *Psyllobora vigintimaculata* (Coleoptera: Coccinellidae) is a mycophagous coccinellid that may reduce the severity of the plant pathogen powdery mildew (Erysiphales) fungi. Additionally, sulfur was toxic when applied directly to *P. vigintimaculata,* resulting in 100% mortality within 24 h of exposure (Sutherland et al., 2010). Furthermore, when applied in commercial vineyards, sulfur reduced the density of *P. vigintimaculata*. Sulfur has also shown to affect multiple life stages of the predatory mired bug, *D. brevis*, causing acute toxicity in both nymphs and adults (Amarasekare and Shearer, 2013b). Exposure also decreased survival of nymphs to the adult life stage (approximately 57%), fertility in adult females, and egg viability.

3. Routes of Pesticide Exposure for Natural Enemies

Three main routes of exposure have been addressed in the literature: topical, residual, and oral. It is well known that natural enemy susceptibility to pesticide varies depending on exposure

route (Banken and Stark, 1998; Grafton-Cardwell and Gu, 2003; Longley and Stark, 1996). In a field setting, it is possible for natural enemies to be exposed to pesticide through all three aforementioned routes: topical exposure from direct spraying, residual exposure from a treated plant surface, and/or oral exposure from ingestion of a treated food source (Bernard et al., 2010; Longley and Stark, 1996; Obrycki and Kring, 1998). Therefore, to more accurately assess the effects of pesticide exposure on natural enemies, it is necessary to examine multiple routes of exposure.

3.1 Topical

There are several different methods for assessing the effects of pesticides on natural enemies (Hassan, 1985). One route of exposure for assessing pesticide toxicity is to directly expose organisms via topical application. A common tool for topically applying pesticide is through the use of a Potter Spray Tower, which sprays a specific volume of the pesticide solution over a specified surface area (Potter, 1952). For instance, topical application via a Potter Spray Tower was used to assess the effects of buprofezin, an insect growth regulator that inhibits chitin synthesis, on several general predators, including *H. axyridis*, *Stethorus punctum picipes* (Coleoptera: Coccinellidae), *Orius tristicolor* (Hemiptera: Anthocoridae), *Geocoris pallens*, and *Geocoris punctipes* (Hemiptera: Lygaeidae) (James, 2004). In addition to direct spraying, there are other methods of topical application. For instance, to measure the effects of several conventional and biorational insecticides on the eggs of the generalist predator *Chrysoperla externa* (Neuroptera: Chrysopidae), eggs were directly dipped into maximum field rate solutions for 25-s (Rimoldi et al., 2008). While topical applications are a factor to consider when assessing pesticide risk, in a field setting, natural enemies are more likely to encounter pesticide via a combination of routes, including residual and oral exposure.

3.2 Residual

Another approach is to measure pesticide toxicity via residual exposure, whereby a substrate is treated with a given pesticide, and the organism is then exposed to the treated substrate. For example, several pesticides (e.g. abamectin, deltamethrin, methoxyfenozide, phosmet and trichlorfon) frequently used in peach orchards were tested for their effects on the lacewing predator *C. carnea* using a Potter Spray Tower to apply residues to glass plates, and the predators were then exposed to the dried residue (Giolo et al., 2009). Additionally, a Cornelis spray chamber was used to coat the sides of glass cages, to which the predatory stinkbug *Picromerus bidens* (Heteroptera: Pentatomidae) was then added (Mahdian et al., 2007). Another study used a leaf dip bioassay, in which pesticide residue was formed by dipping green bean leaves into pesticide solution for 5-s. In this manner, reduced-risk pesticides were then tested on multiple natural enemies: *O. insidiosus*, *Amblyseius swirskii* (Acari: Phytoseiidae), and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) (Gradish et al., 2011). While most studies are based on fresh dried residues of pesticides, an equally important, but less commonly studied aspect of residues as a route of exposure for natural enemies is their persistence in the field (Meyerdirk et al., 1979). Several studies have begun to examine how the age of pesticide residue impacts natural enemies (Desneux et al., 2005; Gradish et al., 2011; Van de Veire et al., 2002).

3.3 Oral

A far less common practice is to determine the effects of pesticides via oral exposure, in which the study organism is provided with a pesticide-treated food source. This approach has more frequently been used for testing pesticide toxicity among pollinators. Pesticides are added to a diet of sugar water and fed to pollinators, such as honey bees and bumbles bees (Biondi et al., 2012a). This type of bioassay may be used to determine acute toxicity and/or effects on foraging behavior. With respect to natural enemies, a treated diet may be used to assess oral exposure. For instance, treated honey has been used to assess oral toxicity of several insecticides on parasitoids (Liu et al., 2010; Wang et al., 2008). *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) is a mealybug predator used in integrated pest management programs for citrus. To determine non-target effects of acaricides, *C. montrouzieri* was provided treated prey, the citrus mealy bug *Planococcus citri* (Hemiptera:Pseudococcidae) (Urbaneja et al., 2008). Another key natural enemy in IPM programs is *Coccinella undecimpunctata* (Coleoptera: Coccinellidae), a generalist predator of aphids. In order to evaluate how the neuroactive pesticides pirimicarb and pymetrozine may affect *C. undecimpunctata* consumption of aphids, the predator was provided a diet of treated *Myzus persicae* (Homoptera: Aphididae) (Cabral et al., 2011). As some pesticides are specifically designed for ingestion, oral exposure of natural enemies is an important factor to consider when assessing pesticide impacts.

4. Sublethal Effects

Historically, one of the most common toxicological measurements of pesticide exposure is the median lethal dose (LD_{50}) or the lethal concentration (LC_{50}). Though acute toxicity assays are a good method for beginning to understand the effects of pesticides, they are limited to the measurement of survivorship only. Pesticides can induce a much wider range of sublethal effects (Desneux et al., 2007) that impair physiology (e.g. development, longevity, fecundity, fertility, and sex ratio) or behavior (e.g. mobility, feeding, mating). Studies which consider sublethal effects are viewed as more accurately measuring toxic effects than those which only measure acute effects (Desneux et al., 2007; Stark and Banks, 2003). For instance, when the parasitoid *Aphidius ervi* (Hymenoptera: Aphidiinae) was exposed to lambda-cyhalothrin, the LD_{20} was found to be 50 times lower than the recommended field rate (Desneux et al., 2004a). Additionally, the study found, that even at such low concentrations, exposure to lambda-cyhalothrin decreased the response of *A. ervi* females to its host aphid, *M. persicae*. This same parasitoid species also exhibited a change in response to plant-host odor when exposed to triazamate (Desneux et al., 2004b). Although it is worthwhile to determine lethal doses of pesticides for natural enemies relative to applied field rates, this measurement alone could not have predicted the disruptive behavioral effects on *A. ervi* females.

Studies on sublethal effects can be used determine changes in all aspects of the life history performance of natural enemies that result from pesticide exposure. For instance, pesticides have been shown to significantly affect reproduction in the predatory mirid bugs, *Nesidiocoris tenuis* and *M. pygmaeus* (Arno and Gabarra, 2011). For *N. tenuis,* fresh residues of azadirachtin reduced fertility but those of spinosad did not. Conversely, for *M. pygmaeus*, spinosad reduced

fertility, but azadirachtin did not. Another study found that methoxyfenozide significantly reduced fecundity of *Aphidius colemani* (Hymenoptera: Braconidae), while exposure to pure azadirachtin had no significant effect (Stara et al., 2011). Interestingly, a pesticide may display chronic toxicity, yet have no significant effects on reproduction. For example, exposure to any one of three insect growth regulators (lufenuron, teflubenzuron, or novaluron) caused an 80% reduction in adult emergence of the lacewing *C. externa*, but there was no significant impact on the number of eggs laid per female or hatch rates (Zotti et al., 2013). In this case, solely examining sublethal reproductive effects would have been misleading as a measure of potential demographic effects because the low adult emergence indicates otherwise. Therefore, it is best practice to link several effects of pesticides, lethal and sublethal, to estimate changes in the demography of natural enemy populations.

Demographic toxicology was initially used in 1962 to study the effects of gamma radiation on the intrinsic rate of increase *Daphnia pulex* (Marshall, 1962). Since then, demographic toxicology has been increasingly employed, as it provides a more complete determination of the effects of a toxicant on the population growth rate of an organism rather than simply the acute effects on individuals (Forbes and Calow, 1999; Stark and Banks, 2003). For example, using life table data (e.g. stage duration and survival rates of eggs, larvae, pupae, and longevity and fecundity of adults), critical extinction thresholds were estimated for four economically important parasitoids: *Diachasmimorpha longicaudata*, *Psyttalia fletcheri*, *Fopius arisanus*, and *Diaeretiella rapae* (Banks et al., 2011). Though all species are braconid parasitoids, *D. rapae* was far less vulnerable to pesticide exposure, suggesting that risk assessments cannot be generalized for an entire guild. Another demographic study found that imidacloprid exposure reduced adult emergence of the egg parasitoid *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae), yet mean longevity of emerged females or the mean number of female offspring per female was not significantly different from controls (Saber, 2011).

In order to more fully understand the effects of pesticides, it is useful to measure both lethal and sublethal effects on natural enemies. Acute toxicity studies can be a useful starting point for determining lethal doses relative to recommended field rates. This is especially necessary when maximum field rates cause 100% mortality, making it difficult to study sublethal effects. Moreover, by determining both lethal and sublethal effects, we can begin to measure how these effects translate to changes in population dynamics. The accuracy of these tests can be increased even further by incorporating multiple routes of exposure, thereby predicting what actually may occur to natural enemies in a field setting. Taken together, the aforementioned bioassays yield a more complete assessment of pesticide impacts on natural enemies, ultimately informing more effective integrated pest management strategies.

5. Objectives

The purpose of this dissertation was to assess the effects of reduced-risk pesticides on *Hippodamia convergens.* This coccinellid is an important predator in high-value tree crops in western North America, namely apples, pears, and walnuts. Hence, the pesticides included in this study are those that are commonly used in these cropping systems. In order to more fully

understand pesticide impacts, several different assessment methods were used and are described below. There were three primary objectives, each one addressed in a different chapter.

Chapter 1: Effect of route of exposure of reduced-risk pesticides on acute mortality in *H. convergens*. This chapter discusses how exposure to a set of seven pesticides in laboratory arenas affected survivorship of *H. convergens*. It examines the impacts of exposure of two life stages of *H. convergens* (larvae and adults). Because these are mobile and feeding stages of the life cycle, they can be exposed to pesticides through one of three routes: topical, residual, and oral. Both life stages were exposed to pesticides via each of the three routes, and, after a period of 48 hours, acute effects (i.e. survivorship) were measured. Thus this chapter provides an introductory assessment to the effects of reduced-risk pesticides on the study organism.

Chapter 2: Sublethal effects of reduced-risk pesticides on *H. convergens* via multiple routes of exposure. This chapter examines sublethal effects of reduced risk pesticides on several life stages of *H. convergens* in laboratory bioassays. For egg and pupal life stages, individuals were coated with pesticide, and subsequent stage survival was recorded. First instar larvae and adults were simultaneously treated via three exposure routes (topical, residual, oral), and life table responses were monitored including development time, survivorship and sex ratio for larvae and fecundity, fertility, and longevity for adults. The data were then analyzed to determine the impacts of each pesticide on the population dynamics of *H. convergens*.

Chapter 3: Laboratory and field assessment of the effect of aged residues of a combination insecticide on acute mortality in *H. convergens.* Based on results from the previous two chapters, the pesticide showing the greatest toxicity was a broad spectrum pyrethroid containing the active ingredient lambda-cyhalothrin. This active ingredient is now available as a combination product mixed with chlorantraniliprole and is being increasingly used for pest management in walnuts in California. Using laboratory bioassays, individual larvae and adults were exposed either to field-aged residues of the combination insecticide on leaves or to aged residues in glass vials (residue ages were 0, 1, 2, 4, 8, 16, or 38 days). This provided a comparison between field and laboratory aging of insecticide residues and of the persistence of toxic effects of this insecticide on *H. convergens*.

CHAPTER 2:
Acute toxicity of pesticides used in western U.S. orchards on *Hippodamia convergens*

ABSTRACT This study examined the acute toxicity of a range of seven pesticides (chlorantraniliprole, cyantraniliprole, copper+mancozeb, lambda-cyhalothrin, novaluron, spinetoram, and sulfur) on adults and larvae of *Hippodamia convergens* (Guerin-Meneville) (Col.: Coccinellidae) and determined the difference in acute toxicity through three different exposure routes: oral, residual, and topical. Formulated pesticides were tested using the 100% maximum field rate representing a fresh application, and 10% maximum field rate representing a declining aged residue. Lambda-cyhalothrin and cyantraniliprole were the most acutely toxic pesticides. Lambda-cyhalothrin caused 100% mortality of both life stages of *H. convergens* when topically exposed to the 100% concentration, and mortality of both life stages was >85% when residually exposed at either the 10% or 100% concentration. For oral exposure at either the 10% or 100% concentration, lambda-cyhalothrin caused 100% mortality of larvae and 57% mortality of adults. Cyantraniliprole caused 90% mortality of larvae and 48% mortality of adults at the 100% concentration when orally exposed, but it was less toxic at the 10% concentration and for other exposure routes. Cyantraniliprole, spinetoram, and novaluron were moderately toxic to larvae when topically exposed at the 100% concentration (40-63% mortality), but not to adults (<4% mortality). Chlorantraniliprole, copper+mancozeb, and sulfur demonstrated low toxicity (<30% mortality) to adults and larvae at both the 10% and 100% concentrations, regardless of exposure route. Results from this study indicate that even reduced-risk insecticides may cause substantial acute mortality effects in laboratory bioassays, and that the magnitude of these effects are dependent on pesticide, pesticide concentration, predator life stage and exposure route.

INTRODUCTION

For more than a decade, regulatory limitations have caused a reduction in the use of organophosphates (OPs) in the U.S. as successive products have been restricted or cancelled according to the Food Quality Protection Act (FQPA) (Grube et al., 2011). Additionally, the U.S. EPA's Reduced-Risk Initiative has created a shift away from OPs toward other classes of insecticides (Epstein and Bassein, 2003). The goal of this initiative was to encourage the development and registration of insecticides that, compared to OPs, pose lower human and environmental health risks. This included lower health risks to mammals, aquatic organisms, birds, and other non-target organisms, such as beneficial insects (EPA, 1997). In principle, this initiative would be expected to enhance the potential for biological control of arthropod pests by the natural enemies that had previously been limited by the use of OPs. However, while reduced-risk insecticides may lower human and environmental health risks, they do not necessarily have lower toxicity to natural enemies (Biondi et al., 2012b; Roubos et al., 2014).

It is important to determine the selectivity of insecticide alternatives to OPs, particularly to natural enemies that contribute effectively to pest management (Jones et al., 2009; Letourneau et al., 2009). For instance, several reduced-risk insecticides showed acute toxicity to important natural enemies in high-value tree crops in western North America. Spinetoram and spirotetremat were toxic at recommended field concentrations to the predatory mite *Galendromus occidentalis* (Nesbitt) (Acar.: Phytoseiidae) (Lefebvre et al., 2011). Acetamiprid was toxic to *Deraeocoris brevis* (Uhler) (Hem.: Miridae), a natural enemy of pear psylla, *Cacopsylla pyricola* (Förster) (Hem.: Psyllidae) (Kim et al., 2006). Furthermore, novaluron and lambda-cyhalothrin were highly toxic to larvae of two different green lacewing species, *Chrysoperla carnea* (Stephens) and *Chrysoperla johnsoni* Henry, Wells and Pupedis (Neur.: Chrysopidae) (Amarasekare and Shearer, 2013a).

On the other hand, some reduced-risk insecticides appear to be compatible with certain natural enemy species, demonstrating low toxicity. Chlorantraniliprole had no significant effects on survival, parasitism rates, or emergence of seven different species of parasitoid wasps, including two sensitive indicator species, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hym.: Braconidae) and *Trichogramma dendrolimi* (Matsumura) (Hym.: Trichogrammatidae) (Brugger et al., 2010). In addition, it had no significant impact on the mortality of the predatory ground beetle *Harpalus pennsylvanicus* DeGeer (Col.: Carabidae) when fed treated food (Larson et al., 2014) or on parasitism rates of the ectoparasitoid, *Tiphia vernalis* Rohwer (Hym.: Tiphiidae) when treated directly. Another diamide insecticide, cyantraniliprole, had no significant impact on the mortality of *Tamarixia triozae* (Burks) (Hym.: Eulophidae) exposed to residue on either a glass or leaf surface (Liu et al., 2012b), and caused only 23% mortality of nymphs and no significant mortality of adults of the predatory mirid, *D. brevis* (Amarasekare and Shearer, 2013b).

For many natural enemies, the selectivity of insecticide chemistries is not yet clear. The primary objective of this study was to determine the acute toxicity of a range of pesticides for *Hippodamia convergens* (Guerin-Meneville) (Col.: Coccinellidae), a well-known generalist aphid predator in a wide variety of agricultural systems (Hagen, 1974; Obrycki and Kring, 1998). *H. convergens* is especially prevalent in western North America (Hagen, 1962), where it serves as an indigenous biological control agent for aphid pests in high-value tree crops (Gontijo et al., 2012; OSU, 2014; UC-IPM, 2014; WSU, 2011). As for other natural enemy species, reduced-risk pesticides have also shown acute toxicity to *H. convergens*. In laboratory and greenhouse bioassays, the two neonicotinoid insecticides, acetamiprid and imidacloprid, were toxic to *H. convergens* (Cloyd and Bethke, 2010), and indoxacarb residue was highly toxic even when aged for 14 days (Roubos et al., 2014).

In this study, seven pesticides were tested that are commonly used in high-value tree crops in western North America (OSU, 2014; UC-IPM, 2014; WSU, 2011). These included five insecticides used for management of key pests such as codling moth, *Cydia pomonella* (L.) (Lep.: Tortricidae), plus two pesticides used for management of bacterial or fungal pathogens in these crops. Each pesticide was tested for acute toxicity using laboratory bioassays for each of three different exposure routes. Natural enemies, such as *H. convergens*, can experience topical

exposure from direct applications of pesticides, residual exposure from a treated plant surface, and/or oral exposure from ingestion of a treated food source (Bernard et al., 2010; Longley and Stark, 1996; Obrycki and Kring, 1998). The objectives of this study were 1) to assess the acute toxicity of selected pesticides on two life stages of *H. convergens* (adults and larvae) and 2) to determine the difference in acute toxicity through three different exposure routes: oral, residual, and topical.

MATERIALS & METHODS

Laboratory rearing

Adult *H. convergens* were obtained from a commercial source (Rincon-Vitova Insectaries, Ventura, California, USA) and stored for up to two months in a screened wooden cage (43 cm x 43 cm x 43 cm) at 6 °C in order to maintain them in a state of overwintering hibernation. To prevent desiccation, several 8.5 cm petri dishes lined with cotton wool were included in the cage and sprayed with distilled water on a weekly basis. Prior to use in the laboratory bioassays, adults were removed from hibernation storage and placed into an incubator maintained at 22 °C, 60-70% RH, and a 16:8 h (L:D) photoperiod. Males and females were identified and approximately 20 adults were placed into 96.1 ml plastic (PETE) cups (Solo Cup Company, Lake Forest, IL). Cups were lined with cotton wool soaked in a 1:1 honey-water solution and covered with a perforated plastic lid to allow for ventilation. After a period of 24 h under these conditions, adults were then used for bioassays.

To obtain larvae of *H. convergens* for the laboratory bioassays, adults were removed from cold storage, sexed, and individual mating pairs were placed into 96.1 ml (PETE) cups, capped with perforated plastic lids for ventilation, and assigned to the same incubator. These adults were reared on a diet of pea aphids, *Acrythosiphum pisum* (Harris) (Hem.: Aphididae), which are known to provide a high quality diet for *H. convergens* (Hinkelman and Tenhumberg, 2013). 20 adult *A. pisum* were provided to each mating pair every day. After 3-4 days, males were removed from the cups and the remaining females were fed approximately 20 adult *A. pisum* per day. Cups were also used as an oviposition substrate and checked daily for the presence of egg clutches. When a female oviposited, it was moved to a new plastic cup, and when eggs hatched, the first instar larvae were fed a diet of *A. pisum*, ad libitum, for a period of 48 h before being used in bioassays.

Pesticides and concentrations tested

Seven pesticides were tested in comparison to a distilled water control: two anthranilic diamides (chlorantraniliprole and cyazypyr), a pyrethroid (lambda-cyhalothrin), an insect growth regulator (novaluron) and a spinosyn (spinetoram) all of which are alternatives to OPs for management of codling moth; a mixture of copper hydroxide and mancozeb used for management of walnut blight and apple scab; and dry flowable sulfur used for management of mildew and apple scab. All pesticides were tested at two different concentrations equivalent to the following label rates: 100% maximum field rate representing a fresh application, and 10%

maximum field rate representing a declining aged residue. The pesticides were tested as formulated materials: chlorantraniliprole, cyantraniliprole and a mixture of copper hydroxide and mancozeb (DuPont, Wilmington, DE); lambda-cyhalothrin (Syngenta, Greensboro, NC); novaluron (Chemtura, Middlebury, CT); spinetoram (Dow AgroSciences, Indianapolis, IN); and dry flowable sulfur (Arysta LifeScience, Cary, NC). The 100% field rates were prepared as 50 ml solutions in distilled water using the following amounts of formulated material: 16.85 mg (118 mg ai/l) for chlorantraniliprole; 80 µl (160 mg ai/l) for cyantraniliprole; 240 mg (2210 mg ai/l) for copper hydroxide and 107 mg (1600 mg ai/l) for mancozeb; 9.9 µL (50 mg ai/l) for lambda-cyhalothrin; 195.2 µl (389 mg ai/l) for novaluron; 26.2 mg (131 mg ai/l) for spinetoram; and 860 mg (13,760 mg ai/l) for dry flowable sulfur. The 10% field rates were made by further dilution.

Bioassay arenas and protocols

For each of the seven pesticides tested (one as a mixture) in comparison to a control, three variables were manipulated 1) life stage (adults or first instar larvae), 2) pesticide concentration (100%, 10%), and 3) exposure route (oral, residual, and topical). For each bioassay, a minimum of 28 replicates was used. In bioassays using adults, the gender ratio was 1:1 (M:F). For all bioassays, test insects were placed individually in 15 x 45 mm glass vial arenas, with cotton wool placed over vial openings to allow for ventilation. Insects were kept at 22 ^{o}C, 60-70% RH, and 16:8 h (L:D) photoperiod. Bioassays lasted for a period of 48 h, after which acute effects were recorded. Acute effects were estimated as the number of live and dead or moribund insects (Martinou et al., 2014; Roubos et al., 2014), where moribund insects were those that were unable to right themselves. For adult bioassays, acute effects were recorded separately for each gender.

To determine acute mortality via oral exposure, *A. pisum* were treated by being individually dipped in a particular pesticide solution. They were removed from the solution with a fine paint brush, and placed on a paper towel in a fume hood until excess pesticide had dripped off and the aphids had dried sufficiently and were able to crawl. 10 topically-treated adult *A. pisum* were placed in a test arena, and a single untreated *H. convergens* (either adult or first instar larva) was added and allowed to feed for a period of 48 h.

To test the effects of residual exposure, individual arenas were treated by adding 4 ml of a given pesticide solution and turning 360 degrees in order to coat the entire inside surface before the solution was poured out. Vials were inverted, suspended on a rack, and allowed to dry in a fume hood for a period of 24 h. Approximately 10 untreated adult *A. pisum* were then added as a food source to the treated arenas. A single untreated *H. convergens* (either adult or first instar larva) was added per arena and allowed to crawl on the treated surface for a period of 48 h.

For topical exposure, individual *H. convergens* were placed in an 8.5 cm petri dish with filter paper lining and treated in a Potter spray tower (Burkard Scientific Limited, Uxbridge, UK) set to 68.9 kPa. For each pesticide solution, the spray volume per application was 1.4 ml, resulting in a spray deposit of 2.50 mg cm^{-2}. This deposit is similar to that used in other studies on non-

target insects (Kim et al., 2006; Martinou et al., 2014) as well as by the IOBC Working Group "Pesticides and Beneficial Organisms" (Hassan et al., 2000). The treated insects were allowed to dry, and were placed individually into an untreated arena with 10 untreated *A. pisum* adults as a food source for a period of 48 h.

Statistical analysis

The statistical program R (R Development Core Team, version 3.1.1) was used for all analyses. Generalized linear models (GLMs) with binomial errors, or quasibinomial errors in cases where there was overdispersion, were used to analyze the effects of pesticide, pesticide concentration (10%, 100%), life stage (larva, adult), and exposure route (oral, residual, topical) on the acute mortality of *H. convergens*. For all GLMs, model reduction and log likelihood ratio tests were used to assess the statistical significance of each factor and their interactions on acute mortality at $\alpha = 0.05$. In a first analysis, the uncorrected acute mortality data were analyzed. Starting with a full model that included all four factors and interactions, the data set was finally subdivided into eight separate models with no significant interaction between factors to compare the relative toxicity of the different pesticides tested. From these models, the pesticides and pesticide concentrations with similar effects on acute mortality were grouped (e.g. factor level reduction), and their toxicity was characterized according to the criteria developed by the International Organisation for Biological Control for classifying pesticide selectivity on beneficial insects in a laboratory bioassay: <30% mortality is harmless, 30-79% mortality is slightly harmful, 80-99% mortality is moderately harmful, and mortality > 99% is harmful (Sterk et al., 1999). Although uncorrected mortality was used in our analysis, the IOBC classification remains applicable due to the fact that control mortalities were low. This classification scheme has been widely adopted for the comparison of differential toxicities of pesticides to natural enemies in a number of laboratory studies (Biondi et al., 2012b; Biondi et al., 2013; Giolo et al., 2009; Martinou et al., 2014; Stara et al., 2011; Zotti et al., 2013).

In a second analysis, all acute mortality data were first corrected to account for variability among control groups (Abbott, 1925), and then used to examine comparative effects of life stage and route of exposure. An initial full model included all four factors, pesticide, pesticide concentration, life stage and exposure route, and their interactions as categorical variables. Due to significant interactions, the corrected data set was then further separated by pesticide and pesticide concentration to examine the effects of life stage and exposure route on acute mortality of *H. convergens*. Additionally, the interaction for effects between pesticide and gender were tested for adults.

RESULTS

Effect of pesticide and pesticide concentration

For the uncorrected acute mortality data, the four-way interaction between pesticide, pesticide concentration, life stage, and exposure route was not significant ($X^2 = 2.50$, df = 14, p = 1.00). However there was a significant three-way interaction between pesticide, pesticide

concentration, and exposure route (X^2 = 27.20, df = 14, p = 0.02). The data was then separated by exposure route and, for oral exposure, the three-way interaction between pesticide, pesticide concentration, and life stage was not significant (X^2 = 2.36, df = 7, p = 0.94), but there was a significant two way interaction between pesticide and pesticide concentration (X^2 = 20.72, df = 7, p = 0.004). The oral exposure data set was further separated by pesticide concentration and there were significant interactions between pesticide and life stage for both pesticide concentrations (10%, X^2 = 47.16, df = 7, p < 0.001; 100%, X^2 = 57.07, df = 7, p < 0.001). Consequently, the data was further separated by life stage for each pesticide concentration, and differences between pesticides were analyzed using four single factor models (Table 1). For residual exposure, the three-way interaction between pesticide, pesticide concentration, and life stage was not significant (X^2 = 3.04, df = 7, p = 0.88). Furthermore, there were no significant two-way interactions (X^2 = 2.63, df = 1, p = 0.10 for removal of all three together) and neither life stage (X^2 = 1.79, df = 1, p = 0.18) nor pesticide concentration (X^2 = 2.06, df = 1, p = 0.15) had a significant effect on acute mortality, resulting in a model with pesticide as the only significant factor (Table 1). For topical exposure, the three-way interaction between pesticide, pesticide concentration, and life stage was not significant (X^2 = 3.93, df = 7, p = 0.79), but there was a significant two-way interaction between pesticide and concentration (X^2 = 31.15, df = 7, p < 0.001). The data set was further separated by pesticide concentration and there was a significant interaction between pesticide and life stage for the 10% concentration (X^2 = 19.58, df = 7, p = 0.007). Thus, the data was further separated by life stage for the 10% concentration and analyzed for the effect of pesticide (Table 1). For the 100% concentration, there was no significant interaction between pesticide and life stage (X^2 = 5.42, df = 7, p = 0.61), but life stage did have a significant effect on acute mortality (X^2 = 50.33, df = 1, p < 0.001).

For oral exposure of adults at the 10% concentration (Table 1, Model 1), there were no significant differences between pesticides, and all were classified as harmless. For oral exposure of larvae at the 10% concentration (Table 1, Model 2), two groups of pesticides caused significantly greater acute mortality than the control treatment, with one classified as harmless and the other moderately harmful (lambda-cyhalothrin). Adults orally exposed to the 100% concentration (Table 1, Model 3) generated three groups of pesticides that differed significantly from the control treatment, one causing less acute mortality than the control (novaluron) and the other two classified as slightly harmful (cyantraniliprole and lambda-cyhalothrin). For larvae orally exposed to the 100% concentration (Table 1, Model 4), there were four groups of pesticides causing significantly greater acute mortality than the control treatment, two classified as harmless, one as moderately harmful (cyantraniliprole), and one as harmful (lambda-cyhalothrin). For residual exposure of adults and larvae at both the 10% and 100% concentrations (Table 1, Model 5), the model yielded two groups of pesticides that caused greater acute mortality than the control treatment, one classified as harmless and the other as moderately harmful (lambda-cyhalothrin). For topical exposure of adults at the 10% concentration (Table 1, Model 6), only one pesticide caused significantly greater acute mortality than the control treatment and was classified as slightly harmful (lambda-cyhalothrin). For topical exposure of larvae at the 10% concentration (Table 1, Model 7), there were two groups of pesticides that differed significantly from the control treatment, one causing less acute mortality, and the other causing greater acute mortality and classified as moderately harmful

(lambda-cyhalothrin). Lastly, at the 100% concentration (Table 1, Model 8), topical exposure of both adults and larvae generated three groups of pesticides causing significantly greater acute mortality than the control treatment, one classified as harmless, one as slightly harmful, and one as harmful (lambda-cyhalothrin).

Effect of exposure route and life stage

For the corrected acute mortality data, there was no interaction between pesticide, pesticide concentration, life stage, and exposure route ($X^2 = 2.17$, df = 12, p = 1.00), but there was a significant three-way interaction between pesticide, pesticide concentration, and exposure route ($X^2 = 20.84$, df = 12, p = 0.05). The data was then separated by pesticide, and, for chlorantraniliprole, there was no interaction between pesticide concentration, exposure route, or life stage ($X^2 = 0.82$, df = 2, p = 0.67), but a significant interaction between pesticide concentration and exposure route ($X^2 = 10.76$, df = 2, p =0.005). The chlorantraniliprole data was further separated by pesticide concentration and there was no interaction between exposure route and life stage for either concentration (10%, $X^2 = 1.75$, df = 2, p = 0.42; 100%, $X^2 = 0.44$, df = 2, p = 0.80). For chlorantraniliprole at the 10% concentration, life stage had no effect ($X^2 = 1.26$, df = 1, p = 0.26), but exposure route had a significant effect on acute mortality ($X^2 = 9.69$, df = 2, p = 0.008, Fig. 1a) and there was no effect of gender on adult mortality ($X^2 = 0.003$, df = 1, p = 0.96). In contrast, for chlorantraniliprole at the 100% concentration, exposure route had no effect ($X^2 = 4.47$, df = 2, p = 0.11), but life stage had a significant effect on acute mortality ($X^2 = 7.99$, df = 1, p = 0.005, Fig. 1b) and there was no effect of gender on adult mortality ($X^2 = 1.47$, df = 1, p = 0.23).

For cyantraniliprole, there was no interaction between pesticide concentration, exposure route, and life stage ($X^2 = 0.57$, df = 2, p = 0.75), but a significant interaction between pesticide concentration and exposure route ($X^2 = 31.45$, df = 2, $p < 0.001$). The cyantraniliprole data was further separated by concentration and there was no interaction between exposure route and life stage for either concentration (10%, $X^2 = 0.48$, df = 2, p = 0.79; 100%, $X^2 = 0.13$, df = 2, p = 0.94). For cyantraniliprole at the 10% concentration, neither life stage ($X^2 = 0.001$, df = 1, p = 0.97) nor exposure route ($X^2 = 4.42$, df = 2, p = 0.11) had an effect on acute mortality (Fig. 1c) and there was no effect of gender on adult mortality ($X^2 = 1.86$, df = 1, p = 0.17). For cyantraniliprole at the 100% concentration, both life stage ($X^2 = 37.15$, df = 1, $p < 0.001$) and exposure route ($X^2 = 59.57$, df = 2, $p < 0.001$) had a significant effect on acute mortality (Fig. 1d), but there was no effect of gender on adult mortality ($X^2 = 0.09$, df = 1, p = 0.77).

For spinetoram, there was no interaction between pesticide concentration, exposure route, and life stage ($X^2 = 0.82$, df = 2, p = 0.66), but there was a significant interaction between life stage and exposure route ($X^2 = 19.83$, df = 2, $p < 0.001$), and concentration had a significant effect on mortality ($X^2 = 14.82$, df = 1, $p < 0.001$). The spinetoram data was further separated by concentration, and, for the 10% concentration, there was no interaction between exposure route and life stage ($X^2 = 5.50$, df = 2, p = 0.06). There were no effects of life stage ($X^2 = 0.53$, df = 1, p = 0.46) or exposure route ($X^2 = 1.42$, df = 2, p = 0.49, Fig. 2a), and gender had no effect on adult mortality ($X^2 = 2.84$, df = 1, p = 0.09). For spinetoram at the 100% concentration, there

was a significant interaction between exposure route and life stage ($X^2 = 13.83$, df = 2, $p < 0.001$). The data was further separated by life stage, and exposure route had no effect on acute mortality of adults ($X^2 = 2.84$, df = 2, $p = 0.24$), but it did have a significant effect for larvae ($X^2 = 53.32$, df = 2, $p < 0.001$, Fig. 2b), and gender had no effect on adult mortality ($X^2 = 0.36$, df = 1, $p = 0.55$).

The lambda-cyhalothrin data had no interaction between pesticide concentration, exposure route, and life stage ($X^2 = 0.59$, df = 2, $p = 0.75$), but there was a significant interaction between exposure route and pesticide concentration ($X^2 = 11.92$, df = 2, $p < 0.003$). The lambda-cyhalothrin data was separated by concentration, and there was a significant interaction between exposure route and life stage for the 10% concentration data ($X^2 = 17.16$, df = 2, $p < 0.001$). The data was further separated by life stage, and exposure route had a significant effect on acute mortality of adults ($X^2 = 55.24$, df = 2, $p < 0.001$), but not for larvae ($X^2 = 1.21$, df = 2, $p = 0.55$, Fig. 2c), and gender had no effect on adult mortality ($X^2 = 0.33$, df = 1, $p = 0.57$). For the 100% concentration data, there was a significant interaction between exposure route and life stage ($X^2 = 8.66$, df = 2, $p = 0.01$). The data was further separated by life stage, and there was a significant effect of exposure route for adults ($X^2 = 27.61$, df = 2, $p < 0.001$), but not for larvae ($X^2 = 4.07$, df = 2, $p = 0.13$, Fig. 2d), and gender had no effect on adult mortality ($X^2 = 0.98$, df = 1, $p = 0.32$).

The novaluron data had no interaction between pesticide concentration, exposure route and life stage ($X^2 < 0.001$, df = 2, $p = 1.00$), nor were there any significant two-way interactions ($X^2 = 2.72$, df = 2, $p = 0.10$ for removal of all three combined). Concentration had a significant effect on acute mortality ($X^2 = 3.75$, df = 1, $p = 0.05$) and so the novaluron data was further separated by concentration. At the 10% concentration, there was no interaction between life stage and exposure route ($X^2 = 2.64$, df = 2, $p = 0.27$), but life stage had a significant effect on mortality ($X^2 = 4.49$, df = 1, $p = 0.03$). The data was further separated by life stage, and exposure route had no effect on mortality for either adults ($X^2 = 1.85$, df = 2, $p = 0.40$) or larvae ($X^2 = 3.35$, df = 2, $p = 0.19$, Fig. 2e), and gender had no effect on adult mortality ($X^2 = 1.41$, df = 1, $p = 0.23$). For the novaluron data at the 100% concentration, there was no interaction between exposure route and life stage ($X^2 < 0.001$, df = 2, $p = 1.00$), but life stage did have a significant effect on mortality ($X^2 = 24.63$, df = 1, $p < 0.001$). Exposure route had a significant effect on mortality for larvae ($X^2 = 23.97$, df = 2, $p < 0.001$), but not for adults ($X^2 < 0.001$, df = 2, $p = 1.00$, Fig. 2f), and gender had no effect on adult mortality ($X^2 < 0.001$, df = 1, $p = 1.00$).

For copper+mancozeb, there was no interaction between pesticide concentration, exposure route, and life stage ($X^2 < 0.001$, df = 2, $p = 1.00$), but there was a significant interaction between life stage and pesticide concentration ($X^2 = 4.86$, df = 1, $p = 0.03$). The copper+mancozeb data was further separated by concentration, and, at the 10% concentration, there was no interaction between life stage and exposure route ($X^2 < 0.001$, df = 2, $p = 1.00$), neither life stage ($X^2 = 2.91$, df = 1, $p = 0.09$) nor exposure route ($X^2 = 4.81$, df = 2, $p = 0.09$) had an effect on acute mortality (Fig. 3a), and there was no effect of gender on adult mortality ($X^2 = 2.91$, df = 1, $p = 0.09$). For copper+mancozeb at the 100% concentration, there was no interaction between life stage and exposure route ($X^2 < 0.001$, df = 2, $p = 1.00$). Exposure route

had a significant effect on mortality (X^2 = 12.09, df = 2, p = 0.002), but life stage did not (X^2 = 2.09, df = 1, p = 0.15, Fig. 3b), and gender had no effect on adult mortality (X^2 = 2.44, df = 1, p = 0.12).

For sulfur, there was no interaction between pesticide concentration, exposure route, and life stage (X^2 < 0.001, df = 2, p = 1.00), nor were there any significant two-way interactions (X^2 = 0.40, df = 2, p = 0.53 for removal of all three combined), but there were significant effects of pesticide concentration on mortality (X^2 = 5.06, df = 1, p = 0.02). The sulfur data was further separated by concentration, and at the 10% concentration, there was no interaction between life stage and exposure route (X^2 < 0.001, df = 2, p = 1.00), and neither life stage (X^2 = 3.12, df = 1, p = 0.08) nor exposure route (X^2 = 1.54, df = 2, p = 0.46) had an effect on mortality (Fig. 3c). Additionally, gender had no effect on adult mortality (X^2 < 0.001, df = 1, p = 1.00). For the sulfur data at the 100% concentration, there was no interaction between life stage and exposure route (X^2 = 1.30, df = 2, p = 0.52), but life stage had a significant effect on mortality (X^2 = 6.82, df = 1, p = 0.009). The data was further separated by life stage, and exposure route had no effect on mortality for either adults (X^2 = 2.43, df = 2, p = 0.30) or larvae (X^2 = 2.16, df = 2, p = 0.34, Fig. 3d), and gender had no effect on adult mortality (X^2 = 1.42, df = 1, p = 0.23).

DISCUSSION

Of the seven pesticides tested, only lambda-cyhalothrin and cyantraniliprole were either moderately harmful or harmful, causing greater than 80% mortality. Lambda cyhalothrin was either moderately harmful or harmful to both life stages of *H. convergens* when topically exposed at the 100% concentration or when residually exposed at either concentration. However, for oral exposure at both concentrations, it was moderately harmful or harmful to larvae, but not to adults. Cyantraniliprole was moderately harmful to larvae, but not to adults at the 100% concentration when orally exposed, but it was harmless or slightly harmful at the 10% concentration and for other exposure routes. Chlorantraniliprole, copper+mancozeb, novaluron, spinetoram, and sulfur were either harmless or slightly harmful to both life stages at both concentrations, regardless of exposure route.

The acute toxicities of the seven pesticides to *H. convergens* in this study were classified according to guidelines initially established by the IOBC for laboratory bioassays (Sterk et al., 1999) due to the widespread use of this classification. However, a more recent revision of these guidelines for classifying pesticide selectivity may more accurately describe their potential toxicity to natural enemies in the field (Boller et al., 2005). The revision suggests reducing the number of categories from four to three; the harmless category remaining unchanged at less than 30% acute mortality, pesticides responsible for 30-79% acute mortality being changed from slightly to moderately harmful, and the top two categories of 80-99% and > 99% acute mortality being combined into a single harmful category. Although these revised categories have yet to be widely adopted, they offer a valuable improvement over the original classification as LC_{50} values would no longer be considered only slightly harmful and the artificial separation of > 99% mortality as a distinct category would be dropped.

Under these revised guidelines, lambda-cyhalothrin would be considered harmful rather than moderately harmful to larvae for both concentrations and all three routes of exposure, and to adults at both concentrations for residual exposure and at the 100% concentration for topical exposure. Additionally, lambda-cyhalothrin would change from slightly harmful to moderately harmful for adults orally exposed to the 100% concentration and for adults topically exposed to the 10% concentration. Cyantraniliprole would also be reclassified from slightly harmful to moderately harmful at the 100% concentration for adults orally exposed and for larvae topically exposed, and from moderately harmful to harmful for larvae orally exposed to the 100% concentration. Spinetoram and novaluron would also change from slightly harmful to moderately harmful for topical exposure of larvae at the 100% concentration. Copper+mancozeb and sulfur would remain in the harmless category for all life stages, concentrations, and exposure routes. All further discussion of toxicity will be used in reference to the revised classification system.

For corrected acute mortality, life stage had no significant effect for chlorantraniliprole, cyantraniliprole, spinetoram and sulfur at the 100% concentration or for copper+mancozeb at both concentrations. The most pronounced life stage effects were for cyantraniliprole, lambda-cyhalothrin, novaluron, and spinetoram at the 100% concentration, and with larvae being more susceptible than adults in all cases. Similarly, novaluron has been shown to have a significant acute effect on larvae of *C. carnea*, but not on adults (Amarasekare and Shearer, 2013b). In contrast, while sulfur had limited effects on both adults and larvae of *H. convergens* in this study, for *G. occidentalis*, it had significant effects on larvae while being harmless to adults (Beers et al., 2009). However, immature life stages are not always more susceptible than adults to pesticide exposure. For instance, phosmet and dimethoate were harmless to eggs and pupae of *C. carnea*, but harmful to adults (Giolo et al., 2009). The relative susceptibility of life stages may also vary among pesticides. For instance, spinosad was harmless to both adults and nymphs of the predatory mirid bugs *Macrolophus pygmaeus* (Reuter) and *Nesidiocoris tenuis* (Rambur) (Hem.: Miridae), while indoxacarb was harmless to *M. pygmaeus* nymphs, but moderately harmful to adults (Arno and Gabarra, 2011). Though testing a single life stage can be a good starting point for assessing pesticide toxicity, it is important to identify the most susceptible life stage. For *H. convergens*, larvae were more susceptible to pesticide exposure than adults at the 100% concentrations, but not necessarily for the more dilute 10% concentrations.

A similar pattern was found for exposure route; effects were more frequent at the 100% concentration, for larvae than for adults, and for cyantraniliprole, novaluron, lambda-cyhalothrin and spinetoram rather than the other pesticides tested. For cyantraniliprole at the 100% concentration, oral exposure caused the greatest mortality for both larvae and adults, whereas for the other pesticides, when there were significant effects of exposure route, topical exposure caused the greatest mortality. Lambda-cyhalothrin proved to be an exception to this pattern with residual exposure having the greatest effect on adults at the 10% concentration, residual and topical exposure having a similar effect for adults at the 100% concentration, and all exposure routes having a similar effect for larvae at both concentrations. Other studies have also found that exposure route can affect mortality rates of natural enemies. For instance, the

effects of spinetoram on adult females of the parasitoid *Neochrysocharis formosa* (Westwood) were classified differently for all exposure routes: harmful via oral exposure, harmless via topical exposure, and moderately harmful via residual exposure (Hernandez et al., 2011). Additionally, lambda-cyhalothrin was harmless to *N. formosa* females via oral exposure, but moderately harmful via topical or residual exposure. For spirotetramat, topical exposure was moderately harmful to adults of the parasitoid *Tamarixia radiata* (Waterson) (Hym.: Eulophidae), while residual exposure was harmless (Hall and Nguyen, 2010). At the maximum label rate, novaluron was harmful to nymphs of *Podisus maculiventris* Say (Hem.: Pentatomidae) via residual or oral exposure (Cutler et al., 2006). In contrast, in our study, novaluron was moderately harmful to *H. convergens* larvae via topical exposure, but was harmless via residual or oral exposure. Given the variable effects of exposure route on mortality, bioassays for the effects of reduced risk pesticides on natural enemies should include multiple routes of exposure rather than being restricted to residual exposure alone, the approach that is recommended in the first tier of the IOBC testing guidelines (Sterk et al., 1999).

As testing for all individual exposure routes can be considerably more time consuming, a more practical approach would be to use a combined exposure scenario, where insects are exposed via multiple routes simultaneously rather than each route individually. Another potential benefit to testing multiple exposure routes simultaneously rather than individually is that it would allow for any additive or synergistic effects. (Amarasekare and Shearer, 2013a) assessed the toxicity of reduced-risk insecticides to adults and larvae of two lacewing species, *C. carnea* and *C. johnsoni*, by simultaneously treating insects topically, residually, and orally. From measures of survivorship 10 days after exposure, chlorantraniliprole and cyantraniliprole proved to be harmless to larvae, but harmful to adults. In addition, from the same study, spinetoram and lambda-cyhalothrin were moderately harmful to larvae and harmful to adults. In a similar study of the survivorship of the predatory mite *G. occidentalis* from simultaneous routes of pesticide exposure, (Beers and Schmidt, 2014) showed chlorantraniliprole and cyantraniliprole to be harmless and spinetoram and lambda-cyhalothrin to be harmful to both larvae and adults. While our study with *H. convergens* differed in being focused on acute mortality from individual exposure routes, it does help to explain the variation in toxicity among natural enemy species and life stages observed from these earlier studies on mortality effects from simultaneous routes of exposure. Chlorantraniliprole and cyantraniliprole were harmless to lacewing larvae and to larval and adult stages of *G. occidentalis*, but were harmful to lacewing adults and moderately harmful to harmful for larvae and adults of *H. convergens*. From our study, we found that for *H. convergens* the most important exposure route for these two insecticides was via oral ingestion, suggesting that natural enemy species or life stages with sucking mouthparts (extra-oral digestion by lacewing larvae and predatory mites) may escape direct oral exposure, whereas those with chewing mouthparts (lacewing adults and coccinellids) may experience more toxic effects. In contrast, for spinetoram and lambda-cyhalothrin the most important exposure routes for *H. convergens* were either topical or residual, which helps to explain the more consistent harmful effect of these two insecticides to a broader range of natural enemy species and active life stages.

From our study with *H. convergens*, we have found that even reduced-risk insecticides can, in some cases, cause substantial acute mortality effects in laboratory bioassays. We have also been able to demonstrate that pesticide, pesticide concentration, predator life stage and exposure route can all influence the acute mortality response of *H. convergens* in laboratory bioassays. Of the seven pesticides tested, lambda-cyhalothrin caused the greatest acute mortality for *H. convergens*, and was classified as either moderately harmful or harmful at the full field rate concentration. Both cyantraniliprole and spinetoram were also either moderately harmful or harmful to *H. convergens* larvae at the full field rate concentration. The other pesticides tested caused only low rates of acute mortality in *H. convergens* and were considered harmless. However, in addition to direct effects on mortality, it is also important to test for potential sublethal effects of pesticide exposure, such as effects on development time, sex ratio, fecundity, or fertility, before concluding whether a particular pesticide is selective and compatible with a natural enemy species (Biondi et al., 2012a; Desneux et al., 2004, 2007; Stara et al., 2011; Stark and Banks, 2003; Zotti et al., 2013). Therefore, while our laboratory bioassays based on acute mortality have been effective in identifying potentially disruptive materials for *H. convergens*, they have not been sufficient to identify truly selective and compatible products from among the seven pesticides tested. Further research on potential sublethal effects of these pesticides on *H. convergens* is necessary to expand our understanding of how they might impact this important coccinellid predator under field conditions.

		ORAL		RESIDUAL		TOPICAL	
		AI rate		AI rate		AI rate	
		10%	100%	10%	100%	10%	100%
		MODEL 1 (X^2 = 4.44, p = 0.73)	**MODEL 3** (X^2 = 48.23, p < 0.001)	**MODEL 5** (X^2 = 554.22, p < 0.001)		**MODEL 6** (X^2 = 26.67, p < 0.001)	**MODEL 8** (X^2 = 282.6, p < 0.001)
ADULTS	control	8.3^{a}	8.3^{b}	2.8^{a}	2.8^{a}	3.3^{a}	3.3^{a}
	chlorantraniliprole	6.0^{a}	14.3^{b}	10.0^{b}	5.0^{b}	6.7^{a}	3.3^{a}
	cyantraniliprole	12.5^{a}	47.5^{c}	13.3^{b}	3.3^{b}	6.7^{a}	3.3^{a}
	spinetoram	10.0^{a}	13.3^{b}	3.3^{a}	0.0^{a}	3.3^{a}	10.0^{b}
	lambda-cyhalothrin	6.7^{a}	56.7^{d}	87.5^{c}	95.0^{c}	36.7^{b}	100.0^{d}
	copper+mancozeb	3.3^{a}	16.7^{b}	0.0^{a}	5.0^{a}	6.9^{a}	3.3^{a}
	novaluron	2.5^{a}	0.0^{a}	0.0^{a}	0.0^{a}	6.7^{a}	3.3^{a}
	sulfur	6.7^{a}	6.7^{b}	5.0^{a}	5.0^{a}	0.0^{a}	3.3^{a}
		MODEL 2 (X^2 = 113.32, p < 0.001)	**MODEL 4** (X^2 = 211.99, p < 0.001)			**MODEL 7** (X^2 = 119.62, p < 0.001)	
LARVAE	control	0.0^{a}	0.0^{a}	0.0^{a}	2.9^{a}	3.3^{b}	3.3^{a}
	chlorantraniliprole	0.0^{a}	26.7^{c}	7.1^{b}	17.9^{b}	0.0^{a}	6.7^{a}
	cyantraniliprole	6.7^{b}	90.0^{d}	15.0^{c}	25.0^{c}	6.7^{b}	40.0^{c}
	spinetoram	0.0^{a}	0.0^{a}	2.9^{a}	2.9^{a}	13.3^{b}	63.3^{c}
	lambda-cyhalothrin	80.0^{c}	100.0^{e}	85.7^{c}	94.3^{c}	90.0^{c}	100.0^{d}
	copper+mancozeb	0.0^{a}	0.0^{a}	0.0^{a}	2.9^{a}	0.0^{a}	13.3^{b}
	novaluron	3.3^{b}	3.3^{b}	2.9^{a}	2.9^{a}	13.3^{b}	43.3^{c}
	sulfur	3.3^{b}	10.0^{b}	3.3^{a}	3.3^{a}	0.0^{a}	20.0^{b}

Table 1. Percent acute mortality of *H. convergens* larvae and adults treated with pesticide via oral, residual, or topical exposure with the results of GLM models and means comparisons based on factor level reduction for the effects of pesticide treatments. Each block represents a separate model with no factor interactions. Following the IOBC classification, each pesticide is categorized as either light blue = harmless, dark blue = slightly harmful, pink = moderately harmful, or red = harmful.

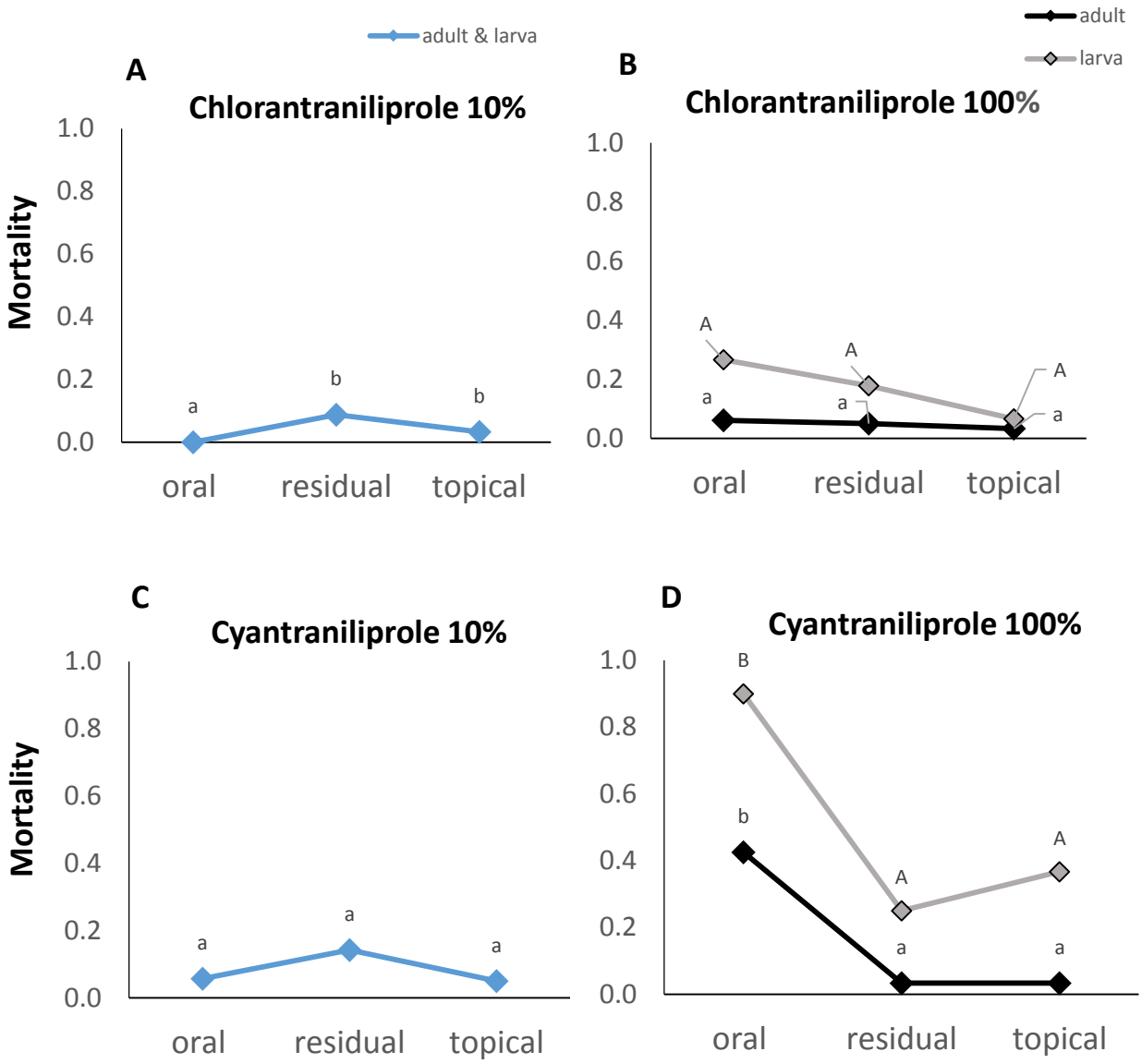

Figure 1. Corrected acute mortality of *H. convergens* treated with dilute (10%) or full field rate (100%) concentrations of anthranilic diamide pesticides in relation to exposure route and life stage. Life stages were pooled in (a) and (c) in the absence of a significant life stage effect, and exposure routes with different letters (upper case for larvae, lower case for adults) are significantly different based on factor level reduction.

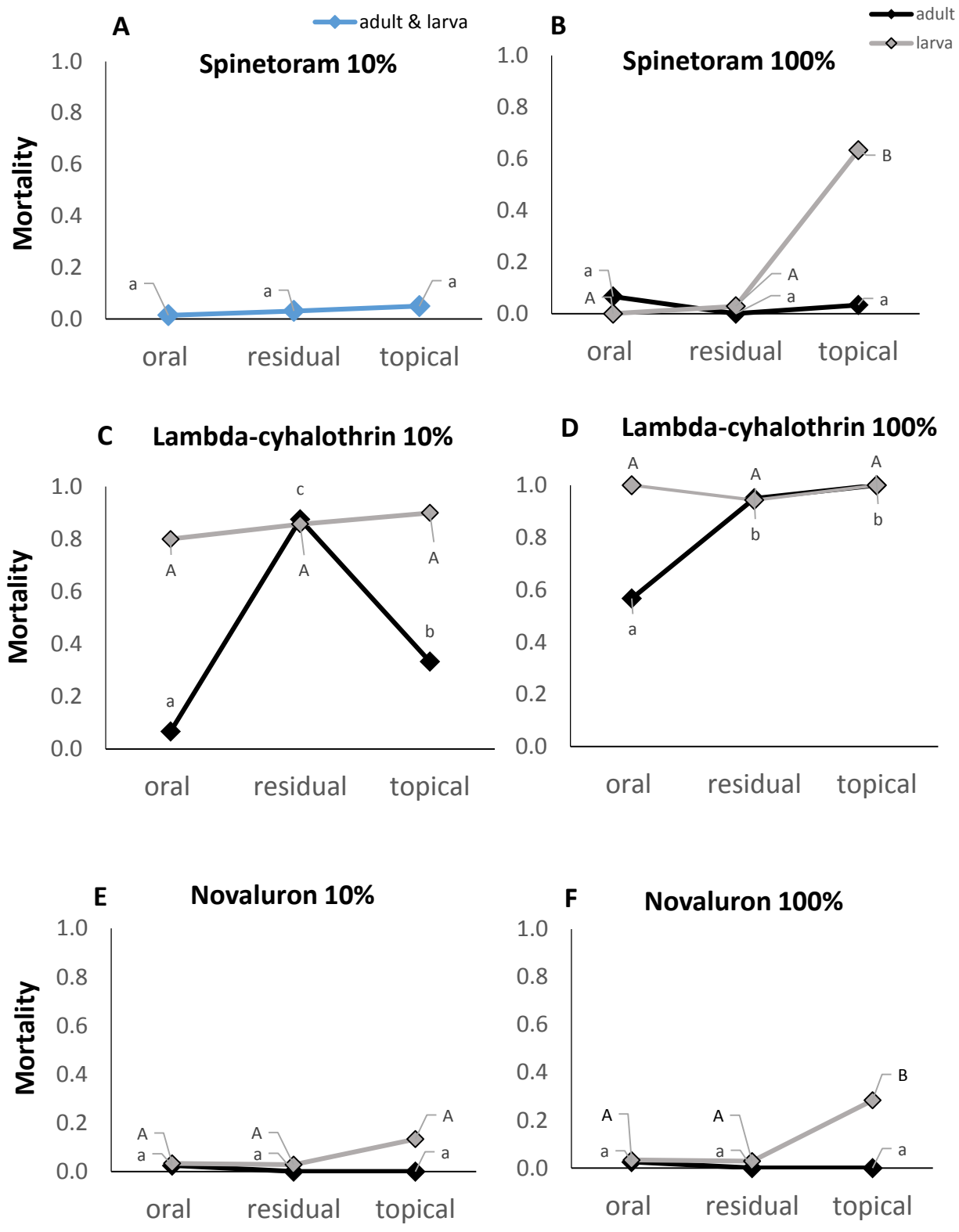

Figure 2. Corrected acute mortality of *H. convergens* treated with dilute (10%) or full field rate (100%) concentrations of two insecticides and an IGR in relation to exposure route and life stage. Life stages were pooled in (a) in the absence of a significant life stage effect.

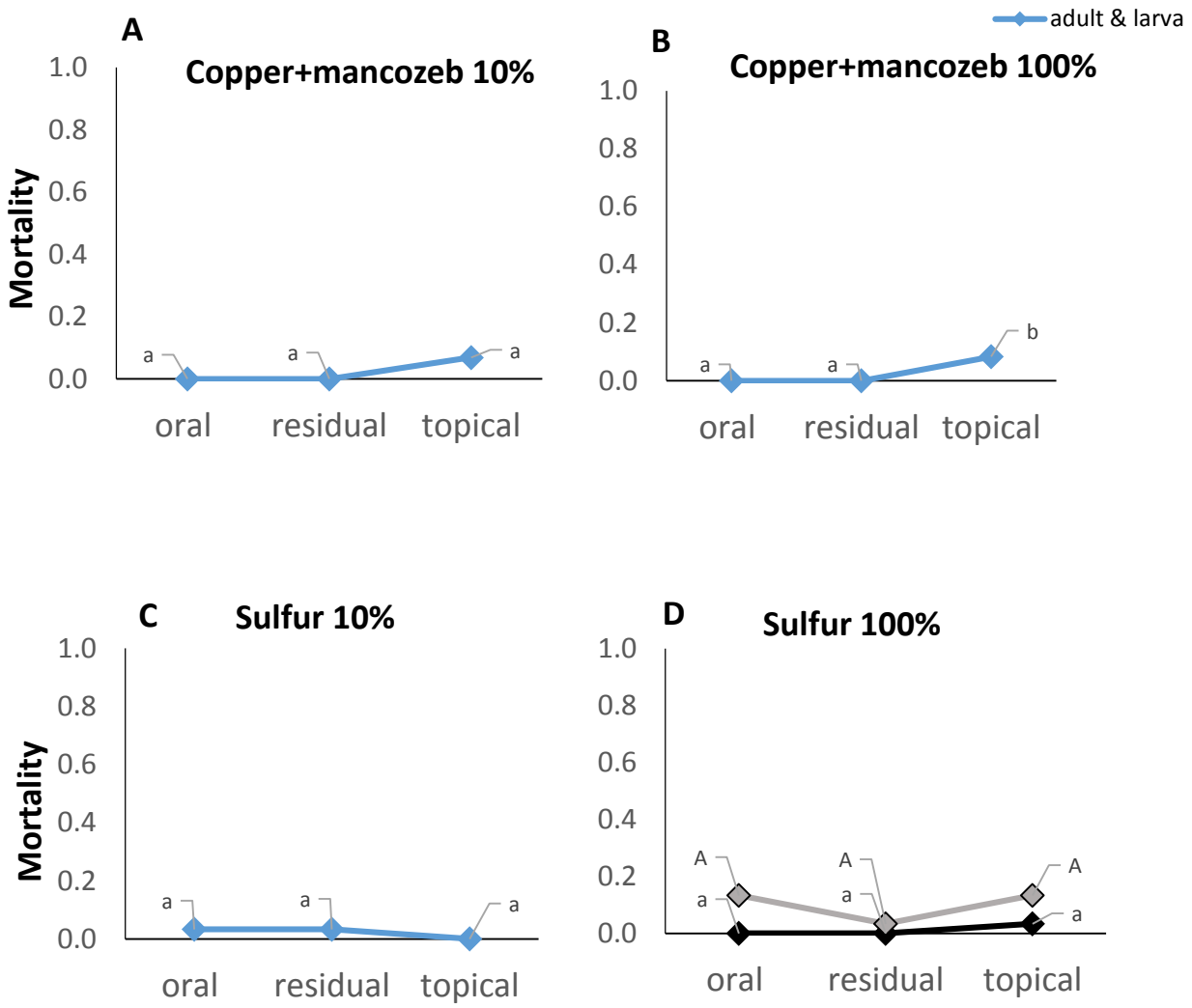

Figure 3. Corrected acute mortality of *H. convergens* treated with dilute (10%) or full field rate (100%) concentrations of two fungicides and an IGR insecticide in relation to exposure route and life stage. Life stages were pooled in (a), (b), and (c) in the absence of a significant life stage effect.

CHAPTER 3:
Lethal and sublethal effects of pesticides used in western U.S. orchards on four life stages of *Hippodamia convergens*

ABSTRACT This study focused on lethal and sublethal of two fungicides (copper+mancozeb and sulfur) and five reduced-risk insecticides (chlorantraniliprole, cyantraniliprole, lambda-cyhalothrin, novaluron, and spinetoram) on egg, larval, pupal, and adult life stages of *Hippodamia convergens* (Guerin-Meneville) (Col.: Coccinellidae). Pesticides were tested using the maximum label rates, and insects were treated topically in the inactive stages (egg, pupa) and simultaneously through oral, residual, and topical exposure in the active stages (larva, adult). Larvae were the most vulnerable life stage, with < 31% survivorship for all pesticides except copper+mancozeb. For adults, lambda-cyhalothrin and chlorantraniliprole were the only pesticides to significantly reduce survivorship (< 40%). Pupae and eggs treated with lambda-cyhalothrin had low survivorship rates (3% and 19%). Novaluron caused low egg survivorship and low egg fertility among treated adult females (14% and 9%). Fecundity was increased by copper+mancozeb, but remained unaffected by other pesticides. Lethal and sublethal effects were used to compare four different measures of the effects of pesticide exposure on *H. convergens*; corrected female mortality, reduction coefficient for the combined effects of corrected adult mortality and daily fertility, and through integration with stage structured matrix models, the intrinsic rate of population increase and delay in population growth. Corrected adult mortality was sufficient to detect the harmful effects of chlorantraniliprole and lambda-cyhalothrin, but a combined reduction coefficient was needed to highlight the harmful effect of novaluron. Based on the intrinsic rate of increase, *H. convergens* populations were estimated not to recover from exposure to lambda-cyhalothrin, while chlorantraniliprole, cyantraniliprole, novaluron, and sulfur were estimated to cause substantial delays in population recovery. These results indicate that different pesticides varied in the strength of their lethal and sublethal effects on *H. convergens* in laboratory bioassays, and that by incorporating life table data into stage-structured models we can better estimate how exposure could affect populations in the field.

INTRODUCTION

There is increasing interest in the effects of pesticides on beneficial insects (Biondi et al., 2012a; Desneux et al., 2007; Hernandez et al., 2011; Liu et al., 2012b). Natural enemies have been a major focus of this research due to their contribution to pest management. Classic methods for establishing the toxicity of pesticides on beneficial insects were to determine the median lethal dose (LD50) and/or lethal concentration (LC50). While acute toxicity assays may be a good starting point for understanding the effects of pesticides, they are limited in that they only measure survivorship and cannot account for sublethal effects. Pesticides are also known to influence physiology, with consequent effects on development, longevity, fecundity, fertility and sex ratio (Amarasekare and Shearer, 2013b; Beers and Schmidt, 2014; Desneux et al., 2007;

Stark and Banks, 2003). Natural enemies exposed to pesticides may also experience changes in behavior, including mobility, feeding, and mating. Therefore, to more accurately assess pesticide toxicity, sublethal effects must be considered.

It is well known that organophosphate pesticides are highly toxic to natural enemies. From 1990 to 2007, the use of organophosphates decreased from 70% of total insecticide use to 36% (Grube et al., 2011). Due to the passage of the Food Quality Protection Act in the United States in 1996, this class of conventional pesticides became increasingly restricted, which initiated the use alternative pesticides for the control of pest populations. One such alterative has been the development of reduced-risk pesticides, promoted by the Environmental Protection Agency Office of Pesticide Programs' (EPA-OPP) Reduced-Risk Pesticide Initiative. In 1992, via the Federal Register, the EPA-OPP called for incentives to promote the development and use of reduced-risk pesticides, and, the following year, announced the Reduced-Risk Pesticide Initiative. The EPA created incentives to promote registration of these pesticides under new reduced-risk criteria that included reducing human health risks and reducing risks to non-target organisms such as birds, beneficial insects, and aquatic organisms (EPA, 1997).

Recently, reduced-risk pesticides have been found to show sublethal effects on several different species of natural enemies. For instance, chlorantraniliprole is an anthranilic diamide designed to target lepidopteran pests, yet when tested for behavioral effects on the generalist predator *Macrolophus pygmaeus* (Hem.: Miridae), exposure to the chemical reduced feeding (Martinou et al., 2014). Similarly, the parasitoid *Diadegma insulare* Cresson (Hym.: Ichneumonidae) was able to distinguish between host larvae fed on plants treated with the pyrethroid, lambda-cyhalothrin, versus non-treated plants, indicating that pesticide exposure can alter host preference (Liu et al., 2012a). Even if a pesticide demonstrates no behavioral effects, it may still induce substantial effects on life history traits. For example, cyantraniliprole, another anthranilic diamide that targets lepidopteran pests, caused a reduction in prey consumption and fecundity for the predator mite, *Galendromus occidentalis* Nesbitt (Acar.: Phytoseiidae) (Beers and Schmidt, 2014). In addition, when *G. occidentalis* females were exposed to copper+mancozeb, there was no effect on egg hatch, but, once hatched, the larvae experienced higher mortality (Beers and Schmidt, 2014). Similarly, while survivorship of female *Chrysoperla carnea* (Stephens) and *Chrysoperla johnsoni* Henry, Wells and Pupedis (Neur.: Chrysopidae) treated with novaluron was unaffected, their subsequent egg viability was reduced to almost zero (Amarasekare and Shearer, 2013a).

When considering sublethal effects, demographic toxicology provides a useful method of assessment for integrating the effects of a toxicant on individual life history parameters into a combined effect on the population growth rate of an organism (Forbes and Calow, 1999; Stark and Banks, 2003). For example, using a demographic approach based on life table data (e.g. stage duration, stage survivorship rates of eggs, and daily fecundity), (Banks et al., 2011) were able to calculate the critical extinction thresholds for four economically important parasitoids: *Diachasmimorpha longicaudata* (Ashmead), *Psyttalia fletcheri* (Silvestri), *Fopius arisanus* (Sonan), and *Diaeretiella rapae* (McIntosh) (Hym.: Braconidae).

Our approach in this study was to examine the lethal and sublethal effects of seven pesticides on the convergent ladybird beetle, *Hippodamia convergens* (Guerin-Meneville) (Col.: Coccinellidae) and their influence on its potential for population growth. The pesticides tested are commonly used in high-value tree crops in western North America, where *H. convergens* is an important natural enemy (OSU, 2014; WSU, 2011). The pesticides included five reduced-risk insecticides that are used for management of codling moth, *Cydia pomonella* (L.), and two fungicides used for the management of bacterial and fungal diseases. The objectives of the study were 1) to assess the lethal effects of these pesticides on egg, larval, pupal, and adult life stages of *H. convergens*, 2) to determine the sublethal effects of exposure of first instar larvae and adults to these pesticides, and 3) to combine the lethal and sublethal effects to determine the impact of each pesticide on the potential population growth rate of *H. convergens*.

MATERIALS & METHODS

Laboratory rearing

Adult *H. convergens* were obtained from a commercial source (Rincon-Vitova Insectaries, Ventura, California, USA). In order to maintain insects in a state of hibernation, adults were stored for up to two months in a screened wooden cage (43 cm x 43 cm x 43 cm) at 6 °C. To prevent desiccation, the colony was sprayed with distilled water on a weekly basis and several 8.5 cm petri dishes lined with cotton wool were included in the cage. Adults were removed from cold storage, sexed, and individual mating pairs were placed into 96.1 ml (PETE) cups (Solo Cup Company, Lake Forest, IL), capped with perforated plastic lids for ventilation, and placed into an incubator maintained at 22 °C, 60-70% RH, and a 16:8 h (L:D) photoperiod. Mating pairs were provided a 1:1 honey-water solution that was replaced every two days and were reared on a diet of *Acrythosiphum pisum* (Harris) (Hem.: Aphididae), which are known to provide a high quality diet for *H. convergens* (Hinkelman and Tenhumberg, 2013). After 3-4 days, males were removed from the cups. Mated females were fed 20 adult aphids per day for a period of 10 days, which allowed time for oviposition to begin. After this period, mated females were then used for bioassays.

A separate group of mated females were used to produce eggs to be used in egg, larval, and pupal bioassays. These females were placed individually to the same plastic cups, fed the same diet and assigned to the same incubator as described above. When an adult female oviposited an egg clutch on the inner surface of a cup, it was transferred to a new cup. Egg clutches aged 1-2 days were used in the egg bioassays. For larval bioassays, newly laid egg clutches were allowed to develop and hatch into first instar larvae (4-5 days). They remained in the same cup and were fed a diet of *A. pisum*, ad libitum, for a period of 48 h. First instar larvae aged 48 h were used in the larval bioassays. For pupal bioassays, egg clutches were allowed to develop into first instar larvae, and individual insects were transferred to 22.1 ml PETE cups with perforated lids to allow for ventilation. On a daily basis, each larva was provided with following number of adult *A. pisum*, first instar larvae were fed 5 aphids, second instar 10 aphids, third instar 15 aphids, and fourth instar 20 aphids. When larvae developed into pupae, they attached themselves to the cup surface. Pupae 1-2 days old were used in the pupal bioassays.

Pesticides and pesticide rates tested

Seven pesticides were tested, including two anthranilic diamides (chlorantraniliprole and cyantraniliprole), a pyrethroid (lambda-cyhalothrin), an insect growth regulator (novaluron) and a spinosyn (spinetoram), all of which are alternatives to OPs for management of codling moth; a mixture of copper hydroxide and mancozeb used for management of walnut blight and apple scab; and dry flowable sulfur used for management of mildew and apple scab. All pesticides were tested at the 100% maximum field rate in comparison to distilled water as a control. The pesticides were tested as formulated materials: chlorantraniliprole, cyantraniliprole and a mixture of copper hydroxide and mancozeb from DuPont, Wilmington, DE; lambda-cyhalothrin from Syngenta, Greensboro, NC; novaluron from Chemtura, Middlebury, CT; spinetoram from Dow AgroSciences, Indianapolis, IN; and dry flowable sulfur from Arysta LifeScience, Cary, NC. The 100% field rate concentrations were prepared as 50 ml solutions in distilled water using the following amounts of formulated material: 16.85 mg (118 mg ai/l) for chlorantraniliprole; 80 µl (160 mg ai/l) for cyantraniliprole; 240 mg (2210 mg ai/l) for copper hydroxide and 107 mg (1600 mg ai/l) for mancozeb; 9.9 µl (50 mg ai/l) for lambda-cyhalothrin; 195.2 µl (389 mg ai/l) for novaluron; 26.2 mg (131 mg ai/l) for spinetoram ; and 860 mg (13,760 mg ai/l) for dry flowable sulfur.

Bioassay arenas and protocols

All bioassays were conducted at 22 °C, 60-70% RH, and a 16:8 h (L:D) photoperiod. Egg clutches and pupae were topically exposed to each of the seven pesticides. Egg clutches and pupae remained in the cups in which they were reared and were coated with 200 µl of a given pesticide dripped from a pipette. Excess pesticide was poured out, and the cups were inverted and allowed to dry in a fume hood for 24 h. Egg hatch was recorded for 6 days after and pupal emergence for 10 days after the exposure event, and stage survivorship was recorded in each case. For eggs, a minimum of 30 replicates (egg clutches) and for pupae a minimum of 29 replicates (individuals) were used.

Mated adult females and first instar larvae were exposed to all pesticides, with the exception of lambda-cyhalothrin. In previous experiments, lambda-cyhalothrin was shown to be acutely toxic to both life stages (86-100% mortality, Chapter 2) which prevented the successful estimation of sublethal effects for this particular pesticide. In a field setting, it is possible for life stages of natural enemies that are actively mobile and feeding to receive topical exposure from direct spraying, residual exposure from a treated plant surface, and/or oral exposure from ingestion of a treated food source (Bernard et al., 2010; Longley and Stark, 1996; Obrycki and Kring, 1998). Consequently, a maximum exposure scenario was assumed, and all three routes were tested simultaneously. 28-30 replicates (individuals) were used for larval bioassays, and 25-30 replicates (individual females) for adult bioassays.

For residual exposure, single 15 x 45 mm glass vials were treated by adding 4 ml of a pesticide solution and turning the vial 360 degrees to coat the entire inside surface before pouring out

the excess solution. Vials were inverted, suspended on a rack, and allowed to dry in a fume hood for a period of 24 h. For oral exposure, *A. pisum* were treated by being individually dipped in a pesticide solution, and then placed on a paper towel in a fume hood until excess pesticide had dripped off the aphids and they were able to crawl. 10 topically-treated adult *A. pisum* were placed in each residually-treated glass vial. For topical exposure, *H. convergens* were placed in an 8.5 cm petri dish with filter paper lining the bottom and sprayed in a Potter spray tower (Burkard Scientific Limited, Uxbridge, UK) set to 68.9 kPa. For each pesticide solution, the spray volume per application was 1.4 ml, resulting in a spray deposit of 2.50 mg cm^{-2}. The deposit is similar to that used in other studies on non-target insects (Kim et al., 2006; Martinou et al., 2014) and to the recommendations of the IOBC Working Group "Pesticides and Beneficial Organisms" (Hassan et al., 2000). The treated insects were allowed to dry and were then placed in treated vials containing treated aphids. Cotton wool stoppers were used for the glass vials to allow for ventilation.

After the initial day of exposure to treated aphids, larvae were fed a diet of untreated aphids on a daily basis. The daily rate of provisioning of *A.pisum* adults for individual larvae was 5 for first instars, 10 for second instars, 15 for third instars, and 20 for fourth instars. Larvae were also transferred to untreated vials two days after the exposure event. They were monitored for 10 days after entering the pupal stage, after which they were considered dead. Larval stage survivorship was recorded, and, for groups with sufficient adult emergence, gender ratio (proportion of females) was recorded. Similarly, after the initial day of exposure to the pesticides, the mated females were transferred to untreated 96.1 ml (PETE) cups and fed approximately 20 untreated adult *A. pisum* per day for a period of 14 days. Throughout this period, survivorship and fecundity were recorded daily. Each day that a female oviposited an egg clutch, she was transferred to a new cup and the egg clutch remained attached to the wall of the initial cups. In order to determined fertility, egg clutches were checked daily for 7 days and hatched larvae were counted, removed, and recorded.

Statistical analysis

Generalized linear models (GLMs) were used to analyze the data with the program R (R Development Core Team, version 3.1.1). Binomial errors and a chi-square test, or quasibinomial errors and an F test in cases where there was overdispersion, were used to analyze the effects of pesticide on the stage-specific survivorship of eggs, larvae, pupae, and adults (truncated to 14 days only for adult females), the fertility of eggs, and gender ratio of emerging adults from the pupal bioassay. Gaussian errors were used to assess the effect of pesticide on larval and pupal development time, and a negative binomial error distribution was used to analyze fecundity, with daily egg production for each female nested as a random effect to account for the repeated daily measurements from the same individuals. For all models, log likelihood ratio tests were used to assess the statistical significance of pesticide at $\alpha = 0.05$. From these models, the pesticides with similar effects on stage survivorship were grouped, and their toxicity was characterized according to the criteria developed by the International Organisation for Biological Control for classifying pesticide selectivity on beneficial insects in a laboratory

bioassay: < 30% impact is harmless, 30-79% impact is slightly harmful, 80-99% impact is moderately harmful, and an impact > 99% is harmful (Sterk et al., 1999).

Additionally, the selectivity of each pesticide with respect to *H. convergens* was expressed as a *Reduction coefficient* E_x which provides a combined estimate of the impact of pesticide exposure based on more than one individual life history response (Biondi et al., 2012b). In this case two life history responses were combined, a lethal effect represented by corrected adult female mortality (E_{mx}), and a sublethal effect represented by corrected reproductive capacity (E_{fx}). The reduction coefficient E_x was estimated from:

$$E_x = 100\left\{1 - \left[\left(1 - \frac{E_{mx}}{100}\right)\left(1 - \frac{E_{fx}}{100}\right)\right]\right\}$$

where

$$E_{mx} = 100\left(1 - \frac{S_T}{S_C}\right) \text{ and } E_{fx} = 100\left(1 - \frac{F_T}{F_C}\right)$$

are Abbott-corrected mortality and loss of daily reproduction respectively, *S* is proportional stage survivorship, *F* is per capita daily reproduction (*daily fecundity * proportional fertility*) and the subscripts *T* and *C* are treatment and control groups.

Lastly, the demographic effects of pesticide exposure were estimated by integrating the individual life history responses of *H. convergens* into a stage-structured matrix model, which generates the intrinsic rate of population increase *r* as a single population level endpoint (Stark et al., 2004). While more complex matrix models have been used to estimate demographic effects of pesticides, (Hanson and Stark, 2011) show that simple stage-structured models perform well in estimating population growth rates and can be readily parameterized using data from life table response experiments. The stage-structured matrix model used in this study included five life stages of *H. convergens* (egg, larvae, pupa, pre-reproductive adults, and reproductive adults):

$$\begin{bmatrix} P_{egg} & 0 & 0 & 0 & F \\ G_{egg} & P_{larva} & 0 & 0 & 0 \\ 0 & G_{larva} & P_{pupa} & 0 & 0 \\ 0 & 0 & G_{pupa} & P_{pre} & 0 \\ 0 & 0 & 0 & G_{pre} & P_{adult} \end{bmatrix}$$

with *P* representing the probability of survival and remaining within the same life stage, *G* the probability of survival and transitioning to the next life stage, and *F* the daily per capita production of female offspring (daily fecundity * proportion of female offspring). These elements of the matrix were estimated as:

$$G = s\left(\frac{1}{t}\right)$$

$$P = s\left[1 - \left(\frac{1}{t}\right)\right]$$
$$F = vf$$

where *s* is the daily survivorship of a stage, *t* is the stage development duration, *f* is the daily per capita fecundity and *v* is the proportion of female offspring. To parameterize the matrix elements for *H. convergens*, individual life history responses from the lethal and sublethal bioassays were used. Corrected stage survivorship from the larval, pupal, and adult lethal bioassays and the greater of two effects on corrected egg survivorship (egg bioassay or fertility measurement from adult bioassay) were first converted to daily survivorship rates for each pesticide treatment. Development times for eggs, larvae, and pupae were taken directly from their respective bioassays, and pre-reproductive and reproductive duration for adults were taken from previously established life spans (Gutierrez et al., 1981). The proportion of female offspring was determined from the pupal bioassay, and daily fecundity from the adult bioassay. When it was not possible to estimate a sublethal effect for a particular pesticide, the corresponding estimate from the control group was used. This was the case for all sublethal effects for lambda-cyhalothrin, and for larval development time for novaluron.

Separate matrix models were developed for the control group of *H. convergens* and for each pesticide treatment group and used to estimate the intrinsic rate of increase (*r*) using the basic matrix analysis tool of PopTools 3.2 (Hood, 2010). After estimating *r* for each pesticide, we also estimated a *population growth time* $T_x = ln(100{,}000/10)/r$, or the time required for a population of *H. convergens* to increase from 10 to 100,000 individuals. The difference between population growth times for control and pesticide treatment groups was used a measure of *delay in population growth* (Stark et al., 2004).

RESULTS

Lethal and sublethal bioassays

Pesticide treatment had a significant effect on adult female survivorship of *H. convergens* over a period of 14 days ($F_{6,194}$ = 2.77 (quasibinomial), p = 0.01) and factor level reduction generated two groups of pesticides (Fig. 1A). According to the IOBC toxicity classification, one group was not significantly different from the control and was classified as harmless, while the second group, comprised of chlorantraniliprole only, was slightly harmful. For pupae, pesticide treatment had a significant effect on survivorship (X^2 = 135.5, df = 7, p < 0.001, Fig. 1B), generating two groups, one harmless group with a similar level of survivorship to the control and a second group, containing lambda-cyhalothrin only, that caused a significant reduction and was classified as slightly harmful. Both larval survivorship to the pupal stage (X^2 = 111.59, df = 6, p < 0.001, Fig. 1C) and larval survivorship to the adult stage were significantly affected by pesticide (X^2 = 110.86, df = 6, p < 0.001, Fig. 1D), with three groups, one harmless group not significantly different from the control, and two groups with reduced survivorship. From these two groups, spinetoram was slightly harmful, chlorantraniliprole, cyantraniliprole were moderately harmful, novaluron was harmful, and sulfur was either moderately harmful (larval

survivorship) or harmful (larval + pupal survivorship). Egg survivorship was significantly affected by pesticide ($F_{7,359}$ = 32.64 (quasibinomial), $p < 0.001$, Fig. 1E), generating three significantly different groups of pesticides, one that included the control and was classified as harmless, one with slightly lower survivorship than controls but still classified as harmless, and a third group classified as moderately harmful (lambda-cyhalothrin and novaluron).

Based on life history parameters other than survivorship, the bioassays showed that some of the pesticides also had sublethal effects on *H. convergens*. For daily fecundity of females from the adult bioassays, there was a significant effect of pesticide treatment (X^2 = 32.03, df = 6, $p < 0.001$, Fig. 2A), where only copper+mancozeb differed from the control with a greater daily fecundity. For fertility there was also a significant effect of pesticide ($F_{6,151}$ = 26.98 (quasibinomial), $p < 0.001$), generating three significantly different groups, one no different from the control, a second group (spinetoram) with slightly reduced fertility and third group (novaluron) with greatly reduced fertility (Fig. 2B). Of the individuals that survived to pupation in the larval bioassays, pesticide had no effect on larval development time (X_2 = 52.53, df= 5, p = 0.12, Fig. 2C). The pupal development times of the same individuals from the larval bioassays were not affected by pesticide (X_2 = 5.20, df= 5, p = 0.42, Fig. 2D). In contrast, for insects exposed as pupae, pesticide did have a significant effect on development time (X_2 = 123.08, df= 6, $p < 0.001$, Fig. 2E), generating three groups, one having a slightly greater development time (sulfur), a second similar to controls, and a third with a slightly reduced development time (spinetoram). Of the individuals that were exposed either as larvae or as pupae and survived to adult emergence, pesticide had no effect on gender ratio (larvae, $F_{5,59}$ = 0.93 (quasibinomial), p = 0.47; pupae, $F_{7,183}$ = 0.61 (quasibinomial), p = 0.75).

Corrected mortality, reduction coefficient and population growth rate

Based on corrected adult female mortality (14 day period) of *H. convergens* alone, chlorantraniliprole would be categorized as slightly harmful and lambda-cyhalothrin as harmful, while all other pesticides would be categorized as harmless (Table 1). By adding sublethal effects on fecundity and fertility, the reduction coefficients generated some notably different results in comparison to corrected adult mortality. Novaluron was categorized as moderately harmful rather than harmless due primarily to the strong sublethal effects on fertility. Cyantraniliprole and chlorantraniliprole were categorized as slightly harmful rather than harmless due to a combination of effects on adult survivorship and daily fecundity. Copper+mancozeb also had a much larger negative reduction coefficient due to the fact that treated adults had higher daily fecundity as well as survivorship than controls. The intrinsic rate of increase was negative for lambda-cyhalothrin, suggesting that populations exposed to this insecticide would decline to local extinction. It was also reduced for chlorantraniliprole, cyantraniliprole, novaluron, and sulfur and estimated to cause significant delays in the recovery of *H. convergens* populations following exposure, ranging from 5 d to over a year. The estimates of the intrinsic rate of increase and delay in population growth showed a similar pattern of effects from pesticide exposure as seen for the reduction coefficients. The main difference observed was that although sulfur produced a negative reduction coefficient, it produced a reduced rate of increase and consequently a delay in population growth due to low

larval survivorship. In addition, exposure to copper+mancozeb and spinetoram lead to improved performance for a number of the individual life history parameters measured for *H. convergens*, and this was reflected in all four of the endpoint measurements (Table 1).

DISCUSSION

Effects of life stage on survivorship

The effects of pesticide exposure were highly variable among each of the four life stages of *H. convergens*. Adults and larvae had lower rates of survivorship than the pupal and egg life stages, and larvae were more affected than adults. This is most likely due to the fact that as non-mobile and non-feeding life stages, eggs and pupae were only treated topically, whereas adults and larvae were treated via all three routes of exposure. Adult survivorship was reduced by just two of the insecticides tested; lambda-cyhalothrin showed acute toxicity (Chapter 2) and exposure to chlorantraniliprole reduced survivorship by 45%. This effect of chlorantraniliprole was unexpected given that numerous studies have demonstrated it to have little impact on beneficial insects through a variety of exposure routes (Broughton et al., 2014; Brugger et al., 2010; Crozier and Cutler, 2014; Larson et al., 2014; Lefebvre et al., 2011; Martinou et al., 2014). For example, it was harmless via oral, residual, and topical exposure to seven species of parasitoid wasps, including the two indicator species *Aphidius rhopalosiphi* (DeStephani-Perez) (Hym.: Aphidiinae) and *Trichogramma dendrolimi* (Hym.: Trichogrammatidae) (Brugger et al., 2010). Moreover, the weed control agent, *Chrysochus auratus* Fabricius (Coleoptera: Chrysomelidae), was not affected by chlorantraniliprole via topical exposure or ingestion of treated foliage (Crozier and Cutler, 2014). Contrary to this trend, however, both chlorantraniliprole and cyantraniliprole were toxic to immature and adult *C. carnea* and *C. johnsoni* (Amarasekare and Shearer, 2013a). In contrast to adult survivorship, survivorship of *H. convergens* larvae was greatly reduced by all of the pesticides tested, with the exception of copper+mancozeb. Copper+mancozeb has also been demonstrated to be harmless for nymphs and adults of *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae) (Amarasekare and Shearer, 2013b), and copper hydroxide alone was harmless for the predatory mite, *Euseius victoriensis* (Acari: Phytoseiidae) (Bernard et al., 2010).

Egg and pupal stages of *H. convergens* were more resilient to topical pesticide exposure for most of the pesticides tested. Similar observations of greater resilience of egg and pupal stages to pesticide exposure have been demonstrated for *C. carnea* (Giolo et al., 2009), and for eggs of *Stethorus punctum picipes* Casey (Col.: Coccinellidae) and *Geocoris pallens* Stål (Hem.: Geocoridae) and pupae of *Harmonia axyridis* (Col.: Coccinellidae) (James, 2004). However, survivorship of both eggs and pupae of *H. convergens* were significantly reduced when exposed to lambda-cyhalothrin, and egg survivorship was reduced by exposure to novaluron. Although 82% of *H. convergens* pupae treated with novaluron were able to emerge as adults, nearly one third of the emerged adults could not be identified as male or female. Moreover, even where gender could be identified, the elytra were noticeably malformed compared to the control group. Thus, it appears that novaluron, an insect growth regulator, affected the success of development during pupation even though it did not prevent adult emergence. Similarly, few

larvae hatched successfully from eggs of *H. convergens* that were treated with novaluron and lambda-cyhalothrin, and it is likely that the hatched larvae would not have survived if they had been monitored through the larval stage. Such larvae were frequently unable to crawl and/or could not right themselves, conditions that have been categorized as moribund and excluded from survivorship in some other studies (Martinou et al., 2014; Roubos et al., 2014). For instance, when eggs of the predator *Podisus maculiventris* (Say) (Het.: Pentatomidae) were treated with novaluron, there was no significant effect on emergence, but the larvae that emerged from treated eggs were unable to molt (Cutler et al., 2006). In addition, although egg hatch of *H. convergens* was only slightly reduced from controls following exposure to cyantraniliprole, the hatching larvae were often lethargic and may not have developed successfully to the pupal stage. As cyantraniliprole has a greater impact from oral exposure (Chapter 2) it is possible that larvae experienced a brief oral exposure when hatching from treated eggs. These observations highlight the importance of following the potential for carry forward effects of pesticide exposure on survivorship between life stages of longer lived natural enemies.

In view of these observations, egg hatch and adult emergence may not be the best measures of the effects of pesticide exposure on egg and pupal survivorship because they do not take longer term viability into consideration. For instance, when eggs of *Chrysoperla externa* (Hagen) (Neur.: Chrysopidae) were treated with several conventional and biorational pesticides, egg hatch was not affected, but larval survivorship declined significantly when allowed to develop for 48 h after hatch (Rimoldi et al., 2008). Similarly, when *Aphidius ervi* Haliday (Hym.: Braconidae) mummies were exposed to deltamethrin there was no significant effect on adult emergence or mortality 48 h after emergence, but longevity was significantly reduced (Desneux et al., 2006). In addition, the effects of pesticide exposure on egg hatch is frequently measured as a sublethal effect of treated adult females (Biondi et al., 2012b; Broughton et al., 2014; Liu and Stansly, 2004; Mahdian et al., 2007; Wang et al., 2008), but as for acute bioassays, hatch rates may not reflect the subsequent viability of larvae. For example, hatch rates for eggs laid by *G. occidentalis* females exposed to copper+mancozeb were normal, but the larvae that did hatch experienced significantly greater mortality (Beers and Schmidt, 2014). Thus, endpoint measurements for the effects of pesticide exposure on survivorship of non-mobile life stages in laboratory bioassays could be improved by monitoring juveniles a minimum of 48 h after egg hatch and monitoring adults for several days after emergence from pupae.

Sublethal effects

Effects of pesticide exposure on development time and gender ratio of *H. convergens* were minimal, but there were significant effects on fecundity and fertility. Daily fecundity was not affected by pesticide exposure with the exception of copper+mancozeb, which produced a 57% increase in daily fecundity compared to the control group, but had no effect on fertility. This observation for *H. convergens* differs from that for lacewings, which when exposed to copper+mancozeb, had reduced daily fecundity and fertility by 24% and 18.9%, respectively (Amarasekare and Shearer, 2013b). However, pesticide-induced hormesis is not uncommon among arthropods (Guedes and Cutler, 2014), and while they have been observed in laboratory

studies with natural enemies, including the coccinellid *Coleomegilla maculata* de Geer (Atallah and Newson, 1966), it has been argued that beneficial effects of pesticides on natural enemies are less likely to occur under field conditions (Morse, 1998). From a similar laboratory study, when treated with diazinon, *Daphnia pulex* (Leydig) (Clad.: Daphniidae) experienced a 41% increase in fertility compared to the control group (Stark and Vargas, 2003). In contrast, novaluron caused substantial reductions in fertility of *H. convergens* and thus has both lethal and sublethal effects on egg viability, via treated eggs or treated adult females, respectively. Given that novaluron inhibits cuticle formation, its effect on egg viability is to be expected. For instance, novaluron exposure also reduced fertility in other natural enemies, such as *C. carnea*, *C. johnsoni*, and *D. brevis* (Amarasekare and Shearer, 2013a; Amarasekare and Shearer, 2013b).

Comparison of individual and integrated endpoint measurements

For most of the pesticides tested, corrected acute adult mortality and reduction coefficients, that also included sublethal effects on reproduction, gave a similar ranking of pesticide effects on *H. convergens*. The greatest discrepancy was for novaluron, which changed from harmless for corrected acute adult mortality to moderately harmful for the reduction coefficient due to low fertility rates. Similarly, in testing the effects of six pesticides on *C. carnea* using laboratory bioassays, both acute mortality and reductions coefficients that also incorporated reproductive effects yielded very similar results as endpoint measurements (Giolo et al., 2009). However, spinosad and spinetoram were considered harmless or slightly harmful from acute mortality effects on *Orius armatus* (Gross) (Hem.: Anthocoridae) under semi-field conditions, but when reproductive effects were incorporated, the reduction coefficients were classified as moderately harmful (Broughton et al., 2014). The importance of including reproductive effects into reduction coefficients for a particular pesticide may also vary between natural enemy species. For example, the reduction coefficient for *H. convergens* exposed to chlorantraniliprole was 65%, but was only 23% for *Orius laevigatus* (Fieber) (Hem.: Anthocoridae) (Biondi et al., 2012b). Moreover, the reduction coefficients for *H. convergens* from exposure to sulfur and spinetoram were negative, but for *O. laevigatus* were nearly 70% for sulfur and over 80% for spinosyns. When life table data was used to estimate the intrinsic rate of increase and expected delay in population growth of *H. convergens* following exposure to the pesticides, the results were largely consistent with the reduction coefficients, with the exception of sulfur. Sulfur generated improved performance for corrected adult mortality and the reduction coefficient, but caused a slight delay in population growth, due to the inclusion of the low survivorship of treated larvae. Similarly, for the parasitoid *B. nigricans*, while the reduction coefficient for adults exposed to abamectin was classified as slightly harmful, the intrinsic rate of increase indicated that the population would become extinct (Biondi et al., 2013).

Conclusions

From this study, we found that fungicides and reduced-risk insecticides can have both lethal and sublethal effects on *H. convergens* in laboratory bioassays. We demonstrated that, in some cases, pesticides can cause a broad range of sublethal effects, including changes in fertility,

fecundity, and pupal development time. Moreover, the apparent effects of pesticide exposure can vary depending on the endpoint measurement chosen. Survivorship rates may be a valuable starting point for assessing the effects of pesticides on natural enemies, but are not sufficient to understand how natural enemy populations will be impacted under field conditions (see Chapter 2). For a slightly more inclusive perspective, reduction coefficients can be used to combine effects of multiple individual-level life table parameters into a single index. While this allows for some integration of lethal and sublethal effects, such as adult survivorship and reproduction, it can still fail to incorporate other important life table responses of a natural enemy species, as was the case for the effects of sulfur on *H. convergens*. To estimate the potential disruptive effect of a pesticide on a natural enemy species, the most rigorous endpoint measurement comes from the use of life table response experiments to parameterize a demographic model that estimates effects on population growth rate (Forbes and Calow, 1999; Stark and Banks, 2003). Such models can be used to incorporate a complete set of lethal and sublethal effects on individual-level responses from all life stages. Of the pesticides tested, exposure to lambda-cyhalothrin was estimated to cause local extinction of *H. convergens*, while chlorantraniliprole, cyantraniliprole, novaluron, and sulfur were estimated to cause significant delays in the recovery of *H. convergens* populations following exposure. In contrast, both copper+mancozeb and spinetoram seemed to be compatible with *H. convergens*. Incorporating life table data into a matrix model allowed us to estimate the demographic effects of exposure to pesticides without conducting a full life cycle assessment that would be impractical for *H. convergens* and for many other natural enemies that have longer life cycles and adult life spans. We therefore recommend that laboratory bioassays measure both lethal and sublethal effects, be conducted with multiple life stages of the test species, and utilize population-level endpoints in order to best inform our understanding of how pesticides could impact natural enemies under field conditions.

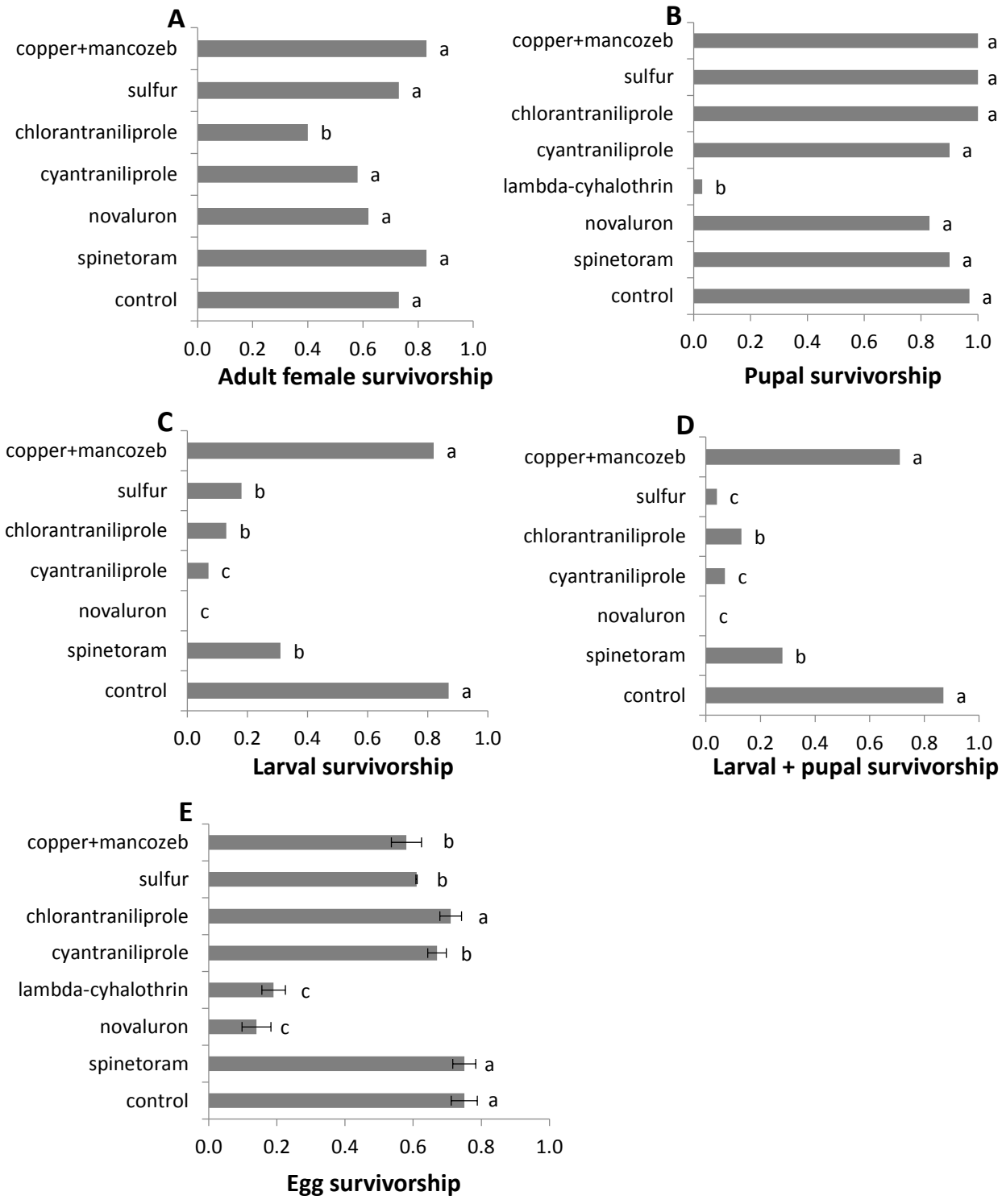

Figure 1. Proportional survivorship of *H. convergens* exposed to pesticides in lab bioassays for (A) adult females for a period of 14 days after treatment, (B) the pupal stage for treated pupae, (C) the larval stage for treated larvae, (D) the larval plus pupal stages for treated larvae, and (E) the egg stage for treated eggs.

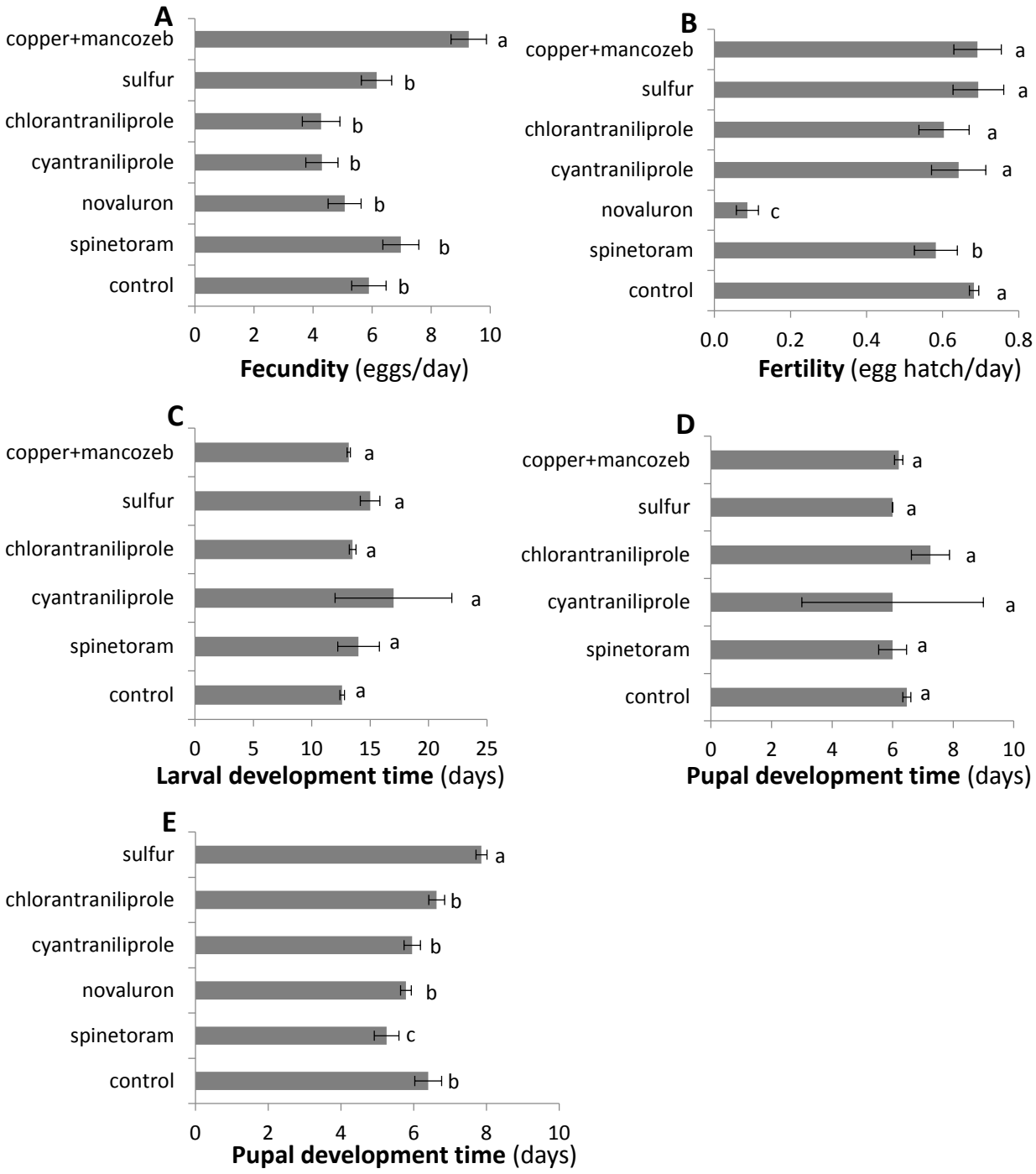

Figure 2. Sublethal effects of pesticide exposure for *H. convergens* in lab bioassays: (A) mean daily fecundity of treated adult females, (B) mean proportion of eggs hatched for treated adult females, (C) mean larval development time for treated larvae, (D) mean pupal development time for treated larvae, and (E) mean pupal development time for treated pupae.

Table 1. Comparative analysis of different endpoint measurements for pesticide effects on *Hippodamia convergens* in laboratory bioassays. Endpoint measurements include corrected mortality of adult females (over 14 days), reduction coefficient (E_x) incorporating effects on both adult survivorship and fecundity/fertility for adult females, intrinsic rate of increase (r), and delay in population growth. For the control treatment, the baseline r was 0.112 and population growth time was 82.0 d for estimation of the delay.

Pesticide	Corrected mortality (%)	Reduction coefficient E_x	Rate of increase r	Population growth delay (d)
copper+mancozeb	-13.4	-80.8	0.122	-6.3
sulfur	-0.4	-6.8	0.080	33.4
chlorantraniliprole	45.2	64.9	0.056	83.9
cyantraniliprole	21.0	45.7	0.056	83.1
lambda-cyhalothrin	100.0*	100.0	-0.546	NA
novaluron	15.7	90.8	0.018	428.9
spinetoram	-13.4	-14.5	0.106	5.3

* Data from the acute mortality study for topical exposure in Chapter 2

CHAPTER 4:
Acute toxicity of aged chlorantraniliprole and lambda-cyhalothrin residues on *Hippodamia convergens*

ABSTRACT Laboratory tests of acute toxicity of pesticide residues are a commonly used method for risk assessment to natural enemies, but it is also important to consider how these effects may change over time in a field setting. This study focused on the acute toxicity of a combination insecticide (chlorantraniliprole and lambda-cyhalothrin) on larval and adult stages of *Hippodamia convergens*. Insects were exposed to insecticide residues on either a walnut leaf aged in a field setting or a glass vial in a laboratory setting. Residues were aged 0, 1, 2, 4, 8, 16, and 38 d. For field-aged residues, larvae were highly susceptible, with mortality rates $\geq$ 78% for 0-8 d residues, while for adults mortality was > 80% for only 0 and 1d residues. After 16 days, field-aged residues were harmless to adults (< 20%) and slightly harmful to larvae ($\leq$ 53%). For laboratory-aged residues, both adults and larvae had high mortality rates for 0-38 d residues (> 80%). Based on corrected acute mortality, the rate of decay of the insecticide toxicity to *H. convergens* for adults was greater for field-aged residues (a = 0.059) than for laboratory-aged residues (a = 0.004), and the same was true for larvae (0.024 versus 0.00). For field and laboratory-aged residues, the residue age at which adult mortality was reduced by half was 11.8 d and 173.3 d, while that for larval mortality was 29.0 d and non-measureable, respectively. These results indicate that the chlorantraniliprole and lambda-cyhalothrin combination insecticide is highly toxic to *H. convergens* adults and larvae, and can still impact larval mortality as long as 38 d after application. Although traditional laboratory bioassays can yield accurate estimates of acute toxicity, field-based bioassays are needed to more accurately estimate insecticide persistence. Thus it is important to incorporate both toxicity and persistence into risk assessments to understand how insecticides impact natural enemies under field conditions.

INTRODUCTION

Natural enemies can be influenced by pesticides through various routes of exposure (Banken and Stark, 1998; Grafton-Cardwell and Gu, 2003; Longley and Stark, 1996). Under field conditions, it is possible for active life stages of natural enemies to be exposed to pesticides through topical exposure from direct spraying, residual exposure via treated surfaces, and/or oral exposure from contaminated prey, water droplets, or honey dew (Bernard et al., 2010; Longley and Stark, 1996; Obrycki and Kring, 1998; Wang et al., 2008). Measuring toxicity from residual exposure is a common approach for testing the selectivity of pesticides for natural enemies, based on applications of pesticide residues to a variety of substrates from glass slides to plastic petri-dishes and plant foliage (Biondi et al., 2012b; Giolo et al., 2009; Gradish, 2011; Mahdian, 2007; Roubos et al., 2014).

While most studies have been based on fresh dried residues of pesticides, an equally important, but less commonly studied aspect of residues as a route of exposure for natural enemies is their persistence in the field (Mahdian, 2007; Meyerdirk et al., 1979). For example, *Anagrus nilaparvatae* (Pang et Wang) (Hym.: Mymanidae) had low mortality when exposed to 7 d aged residue of methamidophos, chlorpyrifos, or avermectin, whereas imidacloprid had a much longer residual toxicity, causing 80% mortality from 7 d aged residue (Wang et al., 2008). On the other hand, deltamethrin residues aged ranging from 1-14 d had no effect on the mortality of the parasitoid *Diaeretiella rapae* (M'Intosh) (Hym.: Braconidae), suggesting this pesticide may be compatible with biological control efforts (Desneux et al., 2005). Similarly, abamectin was harmless to *Eretmocerus eremicus* Rose and Zolnerowich (Hym.: Aphelinidae) regardless of residue age, but metaflumizon was slightly harmful for aged residues of 1-8 d and was not harmless until 15 d (Gradish, 2011). Increasingly, pesticides that have a reduced risk to nontarget organisms are being developed and used. Laboratory bioassays indicate that reduced risk pesticides can, in some cases, still be toxic to natural enemies (Chapter 3, Amarasekare and Shearer 2013a; Kim et al. 2006; Lefebvre et al. 2011) and thus an assessment of the toxicity of field aged residues of these products is key to understanding how well laboratory bioassays translate to a field setting.

To encourage a reduction of organophosphate use, the Environmental Protection Agency Office of Pesticide Programs' called for incentives to promote the development and use of alternative reduced-risk pesticides. Subsequently, the EPA announced the Reduced-Risk Pesticide Initiative, which created incentives to promote registration of pesticides under the following reduced-risk criteria: reducing human health risks and reducing risks to non-target organisms such as birds, beneficial insects, and aquatic organisms (EPA, 1997). One such insecticide developed under the new criteria is chlorantraniliprole, which has frequently been shown to have little to no effect on several natural enemy traits, including survivorship, predation rates, parasitoid emergence, or parasitism rates (Amarasekare and Shearer, 2013b; Broughton, 2014; Brugger, 2010; Crozier, 2014; Martinou, 2014). For example, it was found to be compatible with beneficial insects such as *Copidosoma bakeri* Howard (Hym.: Encyrtidae) via direct contact, *Harpalus pennsylvanicus* DeGeer (Col.: Carabidae) via treated food, and *Tiphia vernalis* Rohwer (Hym.: Tiphiidae) and *Bombus impatiens* Cresson (Hym.: Apidae) via residual exposure (Larson et al., 2014). However, for two species of green lacewing, *Chrysoperla carnea* (Stephens) and *C. johnsoni* Henry, Wells and Pupedis (Neur.: Chrysopidae), chlorantraniliprole caused 100% mortality (Amarasekare and Shearer, 2013a).

Lambda-cyhalothrin is another reduced-risk insecticide, but, unlike chlorantraniliprole, it has demonstrated lethal effects on several natural enemies. Lambda-cyhalothrin is lethal to larvae of *C. carnea* and *C. johnsoni* (Amarasekare and Shearer, 2013a). Additionally, it is highly toxic to the parasitoid, *Diadegma insulare* (Hym.: Ichneumonidae), and resulted in a change in host preference (Liu et al., 2012a). At the full field rate and, it caused high mortality in adults of the predatory mite, *Galendromus occidentalis* (Nesbitt) (Acar.: Phytoseiidae) and for larvae that hatched from the eggs of treated females (Beers and Schmidt, 2014). Coccinellid populations topically exposed to lambda-cyhalothrin had significant decreases in suvivorship, including populations of *Coleomegilla maculata* (DeGeer), *Cycloneda sanguinea* (Linnaeus), *Harmonia*

axyridis (Pallas), and *Hippodamia convergens* (Guerin-Meneville) (Col.: Coccinellidae) (Rodrigues et al., 2013).

Recently, both lambda-cyhalothrin and chlorantraniliprole were formulated into a new combination foliar insecticide (Syngenta, 2012b). This combination was developed to provide broad spectrum control of both sucking and chewing insects, including lepidopteran, coleopteran, and hemipteran pests. Additionally, because the compound coats and penetrates the leaf tissue, it affects insects via multiple routes of exposure (e.g. chlorantraniliprole via ingestion and lambda-cyhalothrin via contact). Hence, the combination may target feeding and mobile life stages (e.g. adults and larvae) as wells as eggs. The advantages of a combination insecticide are purported to be multiple modes of action that reduce insect resistance (Syngenta, 2012a). A range of other combination insecticides have been developed for use in agricultural crops, however, there is no evidence that they preclude resistance (UC-IPM, 2014; Wise et al., 2009).

The toxicity of insecticides to *H. convergens* is important for biological control efforts throughout the western United States, where it is a well-known generalist aphid predator (Hagen, 1974; Obrycki and Kring, 1998). It is a particularly important natural enemy in high-value tree crops, where both chlorantraniliprole and lambda cyhalothrin are used to control codling moth (Gontijo et al., 2012; OSU, 2014; WSU, 2011). In this study, we evaluated the acute toxicity to *H. convergens* of aged residues of a combination insecticide, chlorantraniliprole plus lambda-cyhalothrin. The objectives of this study were to 1) assess acute toxicity of the combination insecticide residues on two life stages of *H. convergens* (larvae and adults), 2) determine how acute toxicity of residues is influenced by age, and 3) evaluate the differences in acute toxicity between residues aged in glass arenas in the laboratory bioassay versus residues aged on foliage in the field.

METHODS

Laboratory rearing

Adult *H. convergens* were obtained from a commercial insectary (Rincon-Vitova Insectaries, Ventura, California, USA) and stored in a screened wooden cage (43 cm x 43 cm x 43 cm) for up to two months at 6 °C in order to maintain them in a state of overwintering hibernation. Several 8.5 cm petri dishes lined with cotton wool were included in the cage and sprayed with distilled water on a weekly basis to prevent desiccation. Prior to use in the laboratory bioassays, adults were removed from hibernation storage and placed into an incubator maintained at 22 °C, 60-70% RH, and a 16:8 h (L:D) photoperiod. Males and females were identified, separated, and approximately 20 adults of each gender were placed into 96.1 ml plastic (PETE) cups (Solo Cup Company, Lake Forest, IL). Cups were lined with cotton wool soaked in a 1:1 honey-water solution and covered with a perforated plastic lid to allow for ventilation. After a period of 24 h in these conditions, adults were used for bioassays.

To obtain *H. convergens* larvae for the laboratory bioassays, adults were removed from cold storage, sexed, and individual mating pairs were placed into 96.1 ml (PETE) cups, capped with perforated plastic lids for ventilation, and assigned to the same incubator. Mating pairs were reared on a diet of pea aphids, *Acrythosiphum pisum* (Harris) (Hem.: Aphididae), which provided a high quality diet for *H. convergens* (Hinkelman and Tenhumberg, 2013). 20 adult *A. pisum* were provided to each mating pair every day. After 3-4 days, males were removed from the cups and the remaining females were fed approximately 20 adult *A. pisum* per day. Cups also served as an oviposition substrate and were checked for the presence of egg clutches on a daily basis. When a female oviposited, it was moved to a new plastic cup. When eggs hatched, the first instar larvae were fed a diet of *A. pisum*, ad libitum, for a period of 48 h before being used in bioassays.

Laboratory bioassays

Aged residues of the combination insecticide, chlorantraniliprole plus lambda-cyhalothrin (Voliam Xpress, Syngenta, Greensboro, NC), were tested in comparison to a distilled water control. The combination insecticide was formulated as 0.835 lb of chlorantraniliprole and 0.417 lb of lambda-cyhalothrin per gallon (Syngenta, 2012b). The solutions used for both laboratory and field aged residues were the equivalent of 0.73 l per ha (31.3 g ai/l chlorantraniliprole and 15.6 g ai/l lambda cyhalothrin). For the laboratory aged residues, the insecticide solution was prepared as 16.89 mg of formulated product in 50 ml of distilled water, and, for the field aged residues, the solution was prepared as 35.8 g of formulated product per 106 l of water.

Four variables were manipulated 1) treatment (insecticide versus control), 2) life stage of *H. convergens* (adults or first instar larvae), 3) residue age, and 4) setting (glass vials in the laboratory versus foliage in a walnut orchard in Tracy, CA). A minimum of 28 replicates was used in all bioassays. For all bioassays, test insects were placed individually in 15 x 45 mm glass vial arenas, with cotton wool placed over vial openings to allow for ventilation. Insects were kept at 22 ^{o}C, 60-70% RH, and 16:8 h (L:D) photoperiod. Bioassays lasted for a period of 48 h, after which acute toxicity was recorded. Acute toxicity was estimated from the number of live and dead or moribund insects (Martinou et al., 2014; Roubos et al., 2014), where moribund insects were those that were unable to right themselves. For adult bioassays, equal numbers of each gender were used and acute toxicity was recorded separately for each gender.

The effects of laboratory and field aging were evaluated for residues 0, 1, 2, 4, 8, 16, and 38 d old. To test the toxicity of laboratory-aged residues, individual glass vials were treated by adding 4 ml of insecticide solution and turning 360 degrees in order to coat the entire inside surface before the solution was poured out. For residues aged 0 d, vials were inverted on a rack and allowed to dry for 4 h until they were used in the bioassays. For all other residue ages, vials were inverted, suspended on a rack, allowed to dry in a fume hood for a period of 24 h, and then placed in an incubator maintained at 22 ^{o}C, 60-70% RH, and 16:8 h (L:D) photoperiod under fluorescent lights until used for the bioassays. For field-aged residues, individual walnut trees (cultivar Chandler) were sprayed in a commercial walnut orchard in Tracy, CA. Eight

walnut trees were treated with the insecticide and 7 trees were treated with water on June 19, 2013. Foliar sprays were applied with an orchard sprayer (HD Hudson 800 PSI Max GES High Pressure Handgun, Chicago, IL) operating at 250 psi with a finished spray volume of approximately 2338.5 l per ha. For each sample date (residue age) after spray application four leaves were collected from the lower canopy of each tree in each cardinal direction. In the laboratory, 4 cm x 5 cm sections were cut from each of the leaves and arranged as liners in individual glass vials such that the inside surface of each vial was covered by the lower surface of the leaf sections. Cotton wool plugs were placed at the bottom of each vial to secure the leaf sections in place. For both the laboratory and field aged residues, approximately 10 untreated adult *A. pisum* were then added to each glass vial as a food source, and a single untreated *H. convergens* (either adult or first instar larva) was introduced and allowed to crawl on the treated surface for a period of 48 h.

Statistical analysis

The statistical program R (R Development Core Team, version 3.1.1) was used for the analysis. Generalized linear models (GLMs) with binomial errors, or quasibinomial errors in cases where there was overdispersion, were used to analyze the effects of treatment (insecticide versus control), life stage (adult versus larva), residue age (0 – 38 d), and setting (laboratory versus field) on the acute mortality of *H. convergens*. For all GLMs, model reduction and log likelihood ratio tests were used to assess the statistical significance of each factor and their interactions on acute mortality at $\alpha = 0.05$. The uncorrected acute mortality data were analyzed, starting with a full model that included all four factors and interactions. The data set was finally subdivided into four separate models with no significant interaction between factors to compare the relative acute toxicity of treatment and age: 1) adults exposed to field-aged residues, 2) larvae exposed to field-aged residues, 3) adults exposed to laboratory-aged residues, and 4) larvae exposed to laboratory-aged residues. Additionally, we tested the effects of gender on adult mortality.

In a separate analysis, the data was corrected to account for variability among control groups (Abbott, 1925), and then used to examine comparative effects of life stage and setting. Due to significant interactions, the corrected data set was subdivided when necessary. Additionally, the decay in insecticide impact over time was fitted to a first-order degradation curve $M_t = M_0 e^{-at}$ where M_t is the corrected acute mortality of *H. convergens* exposed to residues of age t (days), M_0 is the corrected acute mortality of *H. convergens* exposed to residues of age 0, and a is a rate constant (Wang and Hoffmann, 1991). A linearized version of the degradation curve, a plot of $\ln (M_t / M_0)$ on residue age, was used to fit a linear regression model and to estimate the rate constant a from the slope. From this we estimated the residue age that represents a 50% loss of the acute mortality response of *H. convergens* to the combination pesticide from its initial effect on day 0 (ET50) from $t_{1/2} = (\ln 2)/a$.

RESULTS

Effect of insecticide and residue age

For the acute mortality data set from the bioassays conducted with *H. convergens*, there was no four-way interaction between treatment, life stage, residue age, and setting ($X^2 = 0.00$, df = 1, $p = 1.00$), nor any three-way interactions ($X^2 = 2.08$, df = 1, $p = 0.15$ for removal of all four together). However there was a significant two-way interaction between treatment and setting ($X^2 = 138.89$, df = 1, $p < 0.001$). The data set was then separated by setting, and, for field-aged residues, there was no three-way interaction between treatment, life stage, and residue age ($X^2 = 1.64$, df = 1, $p = 0.20$), but there was a significant two way interaction between treatment and life stage ($X^2 = 6.01$, df = 1, $p = 0.01$). The field-aged residue data set was further separated by life stage and, for adults, there was no interaction between treatment and residue age ($X^2 = 3.29$, df = 1, $p = 0.07$). Acute adult mortality was significantly affected by treatment ($X^2 = 85.97$, df = 1, $p < 0.001$) and residue age ($X^2 = 42.34$, df = 1, $p < 0.001$), but not by gender ($X^2 = 0.85$, df = 1, $p = 0.36$). For larvae exposed to field-aged residues, there was no interaction between treatment and residue age ($X^2 = 0.01$, df = 1, $p = 0.92$), but there were significant effects on acute larval mortality for both treatment ($X^2 = 170.99$, df = 1, $p <0.001$) and residue age ($X^2 = 29.13$, df = 1, $p < 0.001$).

For the laboratory-aged residue data set, there was no three-way interaction between treatment, life stage, and residue age ($X^2 = 0.00$, df = 1, $p = 1.00$), but there was a significant two way interaction between treatment and life stage ($X^2 = 4.18$, df = 1, $p = 0.04$). Thus, the data set was further separated by life stage, and, for adults, there was no interaction between treatment and residue age ($X^2 = 0.02$, df = 1, $p = 0.88$). Treatment had a significant effect on acute adult mortality ($X^2 = 413.31$, df = 1, $p < 0.001$), but there were no effects of residue age ($X^2 = 0.85$, df = 1, $p = 0.36$) or gender ($X^2 = 1.57$, df = 1, $p = 0.46$). For the larvae exposed to laboratory-aged residues, there was no interaction between treatment and residue age ($X^2 = 0.00$, df = 1, $p = 1.00$), treatment had a significant effect on acute larval mortality ($X^2 = 518.84$, df = 1, $p < 0.001$), but there was no effect of residue age ($X^2 = 0.50$, df = 1, $p = 0.48$).

Effect of life stage and setting

For the corrected acute mortality data set from the bioassays conducted with *H. convergens* there was a significant two-way interaction between residue age and setting for corrected adult mortality ($X^2 = 14.72$, df = 1, $p < 0.001$), but not for corrected larval mortality ($X^2 = 0.00$, df = 1, $p = 1.00$). Corrected larval mortality was affected independently by residue age ($X^2 = 23.45$, df = 1, $p < 0.001$) and setting ($X^2 = 98.47$, df = 1, $p < 0.001$), due to the slower rate of degradation of the toxicity to larvae under field conditions. Based on the degradation models, the rate of decay was greater for adults exposed to field-aged residues ($a = 0.059$) than to laboratory-aged residues ($a = 0.004$). The corresponding residue age that represented a 50% loss of initial insecticide effect on corrected acute mortality of *H. convergens* (ET50) was 11.8 d for exposure to field-aged residues and 173.3 d for exposure to laboratory-aged residues. The rate of decay was also greater for larvae exposed to field-aged residues ($a = 0.024$) than to laboratory-aged

residues (a = 0.000). Consequently, the half-life (ET50) was 29.0 d for larvae exposed to field-aged residue, but could not be estimated for the laboratory-aged residues due to the lack of any decay over the duration of the experiment.

DISCUSSION

There were substantial differences in the acute mortality responses of *H. convergens* to aging of field residues of the combination insecticide (chlorantraniliprole and lambda-cyhalothrin), but not of laboratory residues. Based on the decay rate of the responses over time, we were able to estimate the effects of differently aged residues on *H. convergens* mortality, and to categorize the residue ages according to the criteria used for toxicity classification by the International Organization for Biological Control (Sterk et al., 1999). Field-aged residues were moderately harmful to adults (> 80%) for up to 1 day, but residues were harmless after 18 days from the initial application. Similarly, field-aged residues were estimated to be moderately harmful to larvae for 9 days, and were still slightly harmful after 51 days. In contrast, laboratory-aged residues were estimated to be moderately harmful to adults (> 80% mortality) for a period of 29 days, and harmful to larvae (100% mortality) regardless of residue age. Laboratory-aged residues produced higher mortality rate responses than field-aged residues, and the responses of larvae were greater than those of adults.

High mortality rates from residual exposure to the combination insecticide were most likely due to the lambda-cyhalothrin component, which has been shown to be toxic to several natural enemies (Devotto et al., 2007; Tillman and Mulrooney, 2000). For example, *Neochrysocharis formosa* (Westwood) and *Ganaspidium nigrimanus* (Kieffer) (Hym.: Eulophidae) experienced high mortality rates when exposed to lambda-cyhalothrin residues (Hernandez et al., 2011). *C. carnea* and *C. johnsoni* adults and larvae experienced 100% mortality when treated with the maximum label rate (Amarasekare and Shearer, 2013a). Likewise, lambda-cyhalothrin exposure resulted in 100% mortality for *D. brevis* adults and nymphs (Amarasekare and Shearer, 2013b). When exposed to a treated leaf surface, the parasitoid *D. insulare* and the predator *C. maculata* each had mortality rates > 80% (Liu et al., 2012a). Exposure of *H. convergens* to fresh lambda-cyhalothrin residues at the maximum field rate resulted in mortality rates > 90% for adults and larvae (Chapter 2), and topically exposed pupae and eggs had mortality rates > 80% (Chapter 3). Although the concentration of lambda-cyhalothrin in the maximum field rate dosage used in Chapter 2 (50 mg ai/l) was much greater than its concentration in the combination insecticide (15.6 g ai/l), the mortality rate response of *H. convergens* to the combination insecticide remained high for 0-1 d of the field-aged residues and for all ages of the laboratory residues.

In contrast to the lambda-cyhalothrin component, it is unlikely that the chlorantraniliprole component of the combination insecticide accounted for the high mortality rates of *H. convergens*. Chlorantraniliprole was harmless to *H. convergens* adults and larvae via residual, oral, and topical exposure (Chapter 2). Moreover, its primary mode of action is via ingestion, and it is unlikely that adults or larvae of *H. convergens* received oral exposure in our bioassays with either field-aged or laboratory-aged residues. Chlorantraniliprole has also been demonstrated to be compatible with several natural enemies (Crozier, 2014; Larson et al.,

2014). For example, residual exposure was harmless to *Orius armatus* (Gross) nymphs and adults (Broughton, 2014). It was also harmless to multiple parasitoid species, including *Aphelinus mali* Haldeman (Hym.: Eulophidae), *Diadegma semiclausum* Hellen (Hym.: Ichneumonidae), and *Dolichogenidea tasmanica* Cameron (Hym.: Braconidae) (Brugger, 2010). Even in a maximum exposure scenario, where the predator *Macrolophus pygmaeus* (Hem.: Miridae) was treated orally, residually, and topically, chlorantraniliprole had no effect on mortality (Martinou, 2014). In a similar maximum exposure treatment, it had no effect on the mortality of *G. occidentalis* adults, and an LD_{50} could not be established due to its low toxicity (Lefebvre et al., 2011).
Given the low acute toxicity of chlorantraniliprole, the half-life response of *H. convergens* to the combination insecticide was probably unaffected by its rate of degradation under field conditions or by the difference in substrates used for laboratory and field-aged residues. Chlorantraniliprole was harmless to *H. convergens* adults and larvae exposed to residues on glass (Chapter 2), and to *Trichogramma pretiosum* Riley (Hym.: Trichogrammatidae) as residues on filter paper or cotton leaves (Brugger, 2010). In a laboratory setting, chlorantraniliprole was harmless to *Orius laevigatus* (Fieber) (Hem.: Anthocoridae) for residues aged 1-14 d, in contrast to abamectin, which resulted in nearly 100% mortality up to 14 d (Biondi et al., 2012b). In a field setting, the half-life of chlorantraniliprole on rice straw was 3.5 d (Zhang et al., 2012), and in sugarcane, was 8 d at both 1x and 2x the recommended field rate (Sharma et al., 2014). Hence, the relatively short half-life of chlorantraniliprole compared to that of lambda-cyhalothrin, further suggests that it had minimal effect on the acute mortality of *H. convergens* mortality in our study, which was still substantial by day 8 (> 50% for adults and > 80% for larvae).

The persistence of the toxicity effects of pyrethroid residues in the field appears to vary considerably among natural enemy species and active ingredients. For example, field-aged residues of esfenvalerate remained highly toxic to adult *Trichogramma platneri* Nagarkatti (Hym.: Trichogrammatidae) after 21 days, but were harmless after 7 days for *Colpoclypeus florus* (Walker) (Hym.: Eulophidae) (Brunner et al., 2001). In addition, residues of permethrin and esfenvalerate on almond twigs were sufficient to cause 50% acute mortality of *G. occidentalis* even after 7 months of aging in the field (Zalom et al., 2001). Nonetheless, pyrethroids are subject to photodegradation under field conditions. When exposed to UV light, lambda-cyhalothrin can degrade to 5% of the initial amount applied after 20 min (Fernandez-Alvarez et al., 2007). Similarly, when applied to a rice paddy, the half-lives of both components of the combination insecticide (lambda-cyhalothrin and thiamethoxam) were determined to be 5 days (Barik et al., 2010). Thus rates of degradation of lambda-cyhalothrin under field conditions may vary with formulation, UV exposure within the canopy of the crop, and with residue substrate. Despite the potential for degradation of lambda-cyhalothrin under field conditions, the high level of sensitivity of the voltage-gated sodium channels of insects to disruption by pyrethroids (Soderlund, 2012) resulted in the persistence of acute effects on *H. convergens* larvae in a walnut orchard after 38 days.

From this study we have been able to show that acute mortality response of *H. convergens* to the insecticide combination of chlorantraniliprole and lambda-cyhalothrin was affected by

residue age, setting, and life stage. Adults and larvae both experienced high levels mortality when exposed to fresh residues of the combination insecticide. Chlorantraniliprole is frequently not toxic, whereas lambda-cyhalothrin is highly toxic, to a number of natural enemy species, and therefore the latter active ingredient was likely responsible for the high mortality of *H. convergens*. Larvae were more susceptible to the insecticide residues than adults, and aging had a greater effect for field-aged residues that were exposed to UV from natural daylight than for laboratory-aged residues that were exposed to fluorescent lighting. Thus, traditional laboratory bioassays can yield greater estimates of the persistence of acute mortality responses among natural enemies than bioassays based on field-aged residues. However, the acute mortality responses of *H. convergens* to fresh residues of the combination insecticide were similar for insects exposed to field-aged or laboratory-aged residues. This indicates that traditional laboratory bioassays (i.e. using insecticide residues on a glass substrate) remain a useful tool for assessing acute toxicity for fresh residues, while bioassays with field-aged residues are needed to accurately assess the persistence of toxic effects on natural enemy mortality.

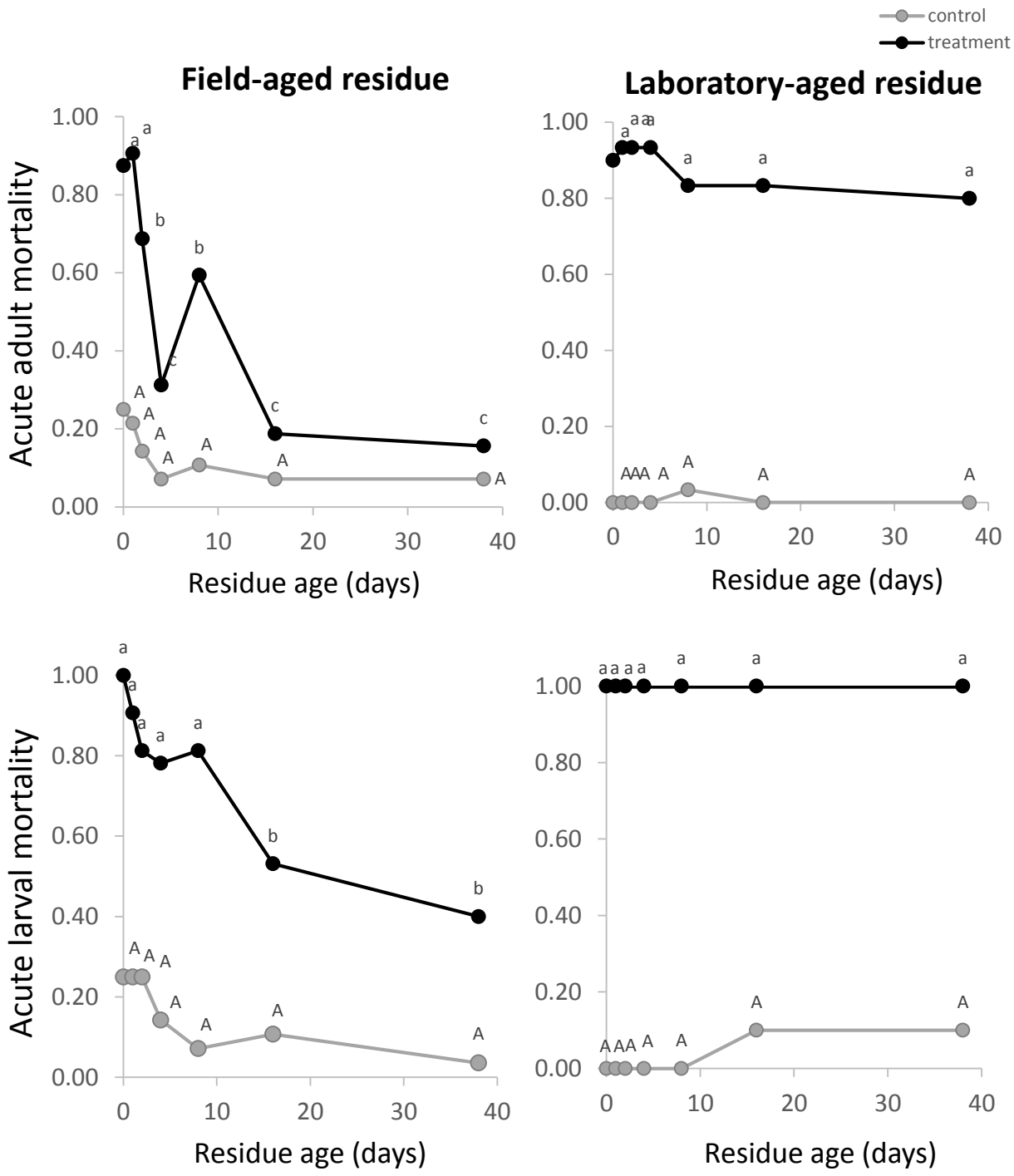

Figure 1 Acute mortality of *H. convergens* treated with a combination insecticide (chlorantraniliprole plus lambda-cyhalothrin) in comparison to control groups. Residue ages with different letters (upper case for the control group, lower case for insecticide group) are significantly different.

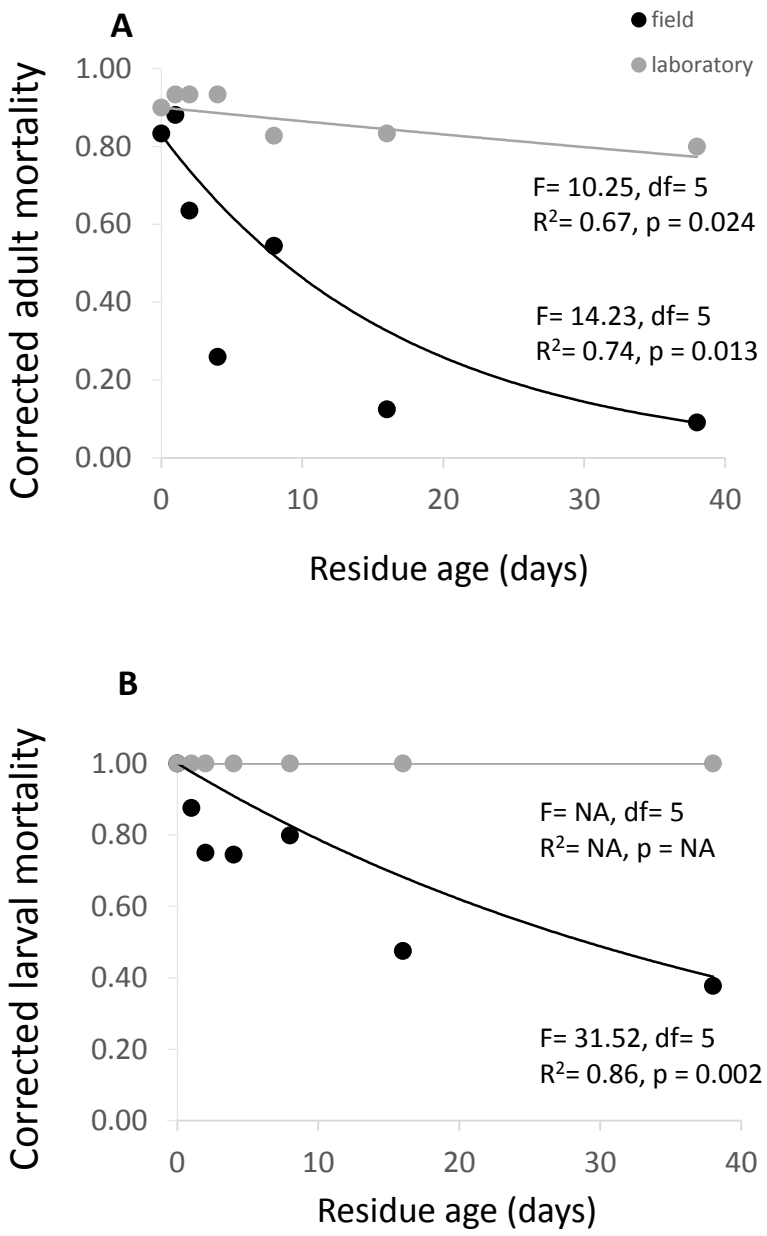

Figure 2 Corrected acute mortality of A) adult and B) larval *H. convergens* treated with residues of a combination insecticide (chlorantraniliprole plus lambda-cyhalothrin) in relation to setting (field versus laboratory).

CONCLUSION

This dissertation assessed the effects of reduced-risk pesticides on *Hippodamia convergens*, a key natural enemy in high-value tree crops in western North America, namely apples, pears, and walnuts. The seven pesticides tested were those that are commonly used in these cropping systems: two fungicides (copper+mancozeb and sulfur) and five reduced-risk insecticides (chlorantraniliprole, cyantraniliprole, lambda-cyhalothrin, novaluron, and spinetoram). As these pesticides are increasingly used to replace the more acutely toxic organophosphates, it is important to determine their impacts on natural enemies. Furthermore, pesticide exposure has been shown to negatively impact natural enemies in a number of ways, including changes in foraging behavior, predation rates, survivorship, fertility, fecundity, and development rates. Thus, to gain a more comprehensive understanding of pesticide effects, we used a range of assessment methods to address three primary objectives.

The first objective was to determine the effect of exposure route on acute mortality in *H. convergens*. Chapter 2 discussed how exposure to either the full field rate (100%) or to a dilute rate (10%) of the seven pesticides in laboratory arenas affected survivorship of *H. convergens* adults and larvae. These are mobile and feeding stages of the life cycle and can therefore be exposed to pesticides via three routes: topical, residual, and oral. Both life stages were exposed to each pesticide via one of the three routes, and acute effects on survivorship were measured. We found that lambda-cyhalothrin and cyantraniliprole had the greatest effects on survivorship. Lambda-cyhalothrin significantly lowered survivorship via all three exposure routes. When topically or residually exposed to the maximum field concentration it caused > 80% mortality to both life stages, while oral exposure greatly lowered survivorship of larvae, but not adults. Cyantraniliprole was moderately harmful to larvae via oral exposure, but it was harmless or slightly harmful at the 10% concentration and for other exposure routes. Chlorantraniliprole, copper+mancozeb, novaluron, spinetoram, and sulfur had little to no impact on both life stages at both concentrations, regardless of exposure route.

With respect to life stage effects, larvae were more susceptible to pesticide exposure than adults at the 100% concentrations, but not necessarily for the more dilute 10% concentrations. The greatest life stage effects were for cyantraniliprole, lambda-cyhalothrin, novaluron, and spinetoram at the 100% concentration, with larvae having lower survivorship than adults. Oral exposure caused the greatest mortality for both life stages for cyantraniliprole, while, when there were significant effects of exposure route, topical exposure caused the greatest mortality for the other pesticides. For lambda-cyhalothrin residual and topical exposure had a similar effect for adults at the 100% concentration, and all exposure routes having a similar effect for larvae at both concentrations. From this introductory assessment of the effects of exposure route on acute mortality, we found that even reduced-risk insecticides can cause substantial acute mortality effects in laboratory bioassays, depending on the pesticide, pesticide concentration, predator life stage, and exposure route.

Although many of the pesticides tested showed no acute effects on *H. convergens*, this did not exclude the possibility for other life table responses, and hence the second objective addressed

sublethal effects of pesticide exposure. Chapter 3 examined sublethal effects of reduced risk pesticides on several life stages of *H. convergens* in laboratory bioassays. The egg and pupal life stages were topically exposed to pesticide, and subsequent stage survival was recorded. First instar larvae and adults were simultaneously treated via three exposure routes (topical, residual, and oral), and life table responses were recorded: development time, survivorship and sex ratio for larvae and fecundity, fertility, and longevity for adults. We found that larvae were more susceptible to exposure than adults, and that egg and pupal stages are the more resilient life stages. Exposure to lambda-cyhalothrin significantly reduced survivorship of all life stages. Adult survivorship was significantly reduced by exposure to chlorantraniliprole, larval survivorship was reduced by exposure to all of the pesticides tested, with the exception of copper+mancozeb, and egg survivorship was reduced by exposure to novaluron.

There were significant sublethal effects on the fecundity and fertility of *H. convergens*; fecundity increased from exposure to copper+mancozeb and fertility greatly decreased from exposure to novaluron. However, development time and gender ratio of *H. convergens* were minimally affected by pesticide exposure. When several toxicity endpoint measurements were compared, corrected acute adult mortality and reduction coefficients (combined effects of corrected adult mortality and daily fertility) gave a similar ranking of pesticide effects on *H. convergens*, except for novaluron, which changed from harmless for corrected acute adult mortality to moderately harmful for the reduction coefficient due to reduced fertility. Additionally, we incorporated life table response data into stage-structured matrix models to estimate the intrinsic rate of increase of *H. convergens* following exposure to each pesticide. Lambda-cyhalothrin was estimated to cause local extinction, while chlorantraniliprole, cyantraniliprole, novaluron, and sulfur were estimated to cause significant delays in the recovery of *H. convergens* populations following exposure. In contrast, both copper+mancozeb and spinetoram seemed to be compatible with *H. convergens*. These findings illustrate the importance of the design of laboratory bioassays for estimating pesticide effects on natural enemies, and the differences that can arise in interpretation of potential harmful effects depending on the endpoint measurement chosen.

Lastly, the third objective was to conduct a laboratory and field assessment of the effect of aged residues of a combination insecticide on acute mortality for *H. convergens.* Based on the survivorship data from Chapters 1 and 2, lambda-cyhalothrin had the greatest toxicity. Recently, this broad spectrum pyrethroid was made available as a combination product mixed with chlorantraniliprole, and is being increasingly used for pest management in walnuts in California. In laboratory bioassays, individual larvae and adults were exposed to the combination pesticide with either field-aged residues on leaves or laboratory-aged residues in glass vials. Through a comparison between field- and laboratory-aged residues, we demonstrated that larvae were more susceptible to field-aged residues than adults, while there was no difference between life stages for laboratory-aged residues. Moreover, laboratory-aged residues caused greater mortality and were more persistent than field-aged residues. For adults, the rate of decay of the pesticide toxicity to *H. convergens* was an order of magnitude greater for field-aged residues compared to laboratory-aged residues, and was greater for adults than for larvae. These results indicate that the chlorantraniliprole and lambda-

cyhalothrin combination pesticide is highly toxic to *H. convergens* adults and larvae, and can impact mortality 38 d after application.

In conclusion, based on the results from these studies, the following recommendations can be provided for growers who are interested in conserving populations of *H. convergens* as an important natural enemy in their orchards:

1) Avoid applications of lambda-cyhalothrin altogether, even at low concentrations. Exposure to lambda-cyhalothrin causes high mortality rates to all life stages of *H. convergens*, and is likely to result in extinction of local populations.

2) Avoid applications of novaluron for codling moth management. Though adults of *H. convergens* may survive exposure and provide temporary pest control, they will not produce viable offspring. Local populations are expected to decline and will not recover quickly enough to provide further biological control within a growing season.

3) Chlorantraniliprole and cyantraniliprole are best used to control codling moth early in the growing season, before *H. convergens* larvae are present. Otherwise, applications of either pesticide could cause a decline in *H. convergens* populations, and require approximately 3 months for populations to recover.

4) Copper+mancozeb is compatible with *H. convergens* conservation, and is preferable to sulfur as a fungicide. Sulfur may be compatible with *H. convergens* when there are no larvae present, but would otherwise be likely to cause an estimated one month delay in population recovery.

5) Spinetoram is the most selective insecticide and compatible with *H. convergens* conservation. It could be used at any time during the growing season with no expected negative impacts on *H. convergens*.

In addition, the results from our laboratory bioassays suggest that the following methods could be used to further improve laboratory assessments of pesticide risk to natural enemies:

1) As an initial step, test the effect of combined routes of pesticide exposure on acute mortality of immature and adult life stages. Determine if there are harmless levels of a given pesticide by testing the maximum field rate and diluted concentrations. Strong acute mortality responses (> 80%) are sufficient to indicate that a pesticide is not selective.

2) For pesticides that do not elicit strong acute mortality responses, measure sublethal effects of combined routes of pesticide exposure on multiple life stages. Collect life table response data, such as changes in development time, longevity, fecundity, fertility, and sex ratio. Determine population effects of pesticide exposure by incorporating life table response data into stage-structured matrix models to estimate the intrinsic rate of increase. A population-level endpoint for toxicity studies, such as the intrinsic rate of increase, provides the best estimate of the probable selectivity of a pesticide under field conditions.

3) For long-lived natural enemies, such as *H. convergens*, life table response data can be further improved by extending the time period of measurements to observe the potential for carry forward effects of pesticide exposure on survivorship between life stages.

4) Determine the persistence of the toxicity effect of a pesticide on a natural enemy by testing the effect of field-aged pesticide residues on acute mortality of immature and adult life stages. A long persistence period would indicate the incompatibility of a pesticide, whereas a short persistence period would indicate that even a non-selective pesticide could be more compatible if timed to avoid the most susceptible life stages of a natural enemy.

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