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Comparative analysis of pesticide effects on natural enemies in western orchards: a synthesis of
laboratory bioassay data

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Abstract

Pesticides are commonly used for pest management in apple, pear and walnut orchards in the western U.S. and may disrupt biological control of secondary pests in these crops. A comparative analysis was made of results obtained from a series of laboratory bioassays of acute mortality and life table response experiments to estimate lethal and sublethal effects of eight pesticides on seven natural enemy species through use of stage-structured population models. Even though a number of the pesticides tested were reduced-risk products, all of them with the exception of copper plus mancozeb and chlorantraniliprole, caused more than 80% acute mortality of at least one life stage of at least one of the natural enemy species at a full field-rate concentration and could thus be considered moderately harmful according to the International Organization for Biological Control classification for laboratory bioassays. Important sublethal effects included reductions in daily fecundity and egg fertility. From integration of the lethal and sublethal effects in matrix models, the mean of the estimated intrinsic rates of increase for natural enemy species was negative for exposure to cyantraniliprole, lambda-cyhalothrin and spinetoram, but positive and not significantly different from the control for exposure to chlorantraniliprole, copper plus mancozeb, novaluron, and sulfur. For comparisons among pesticides, there appears to be considerable variation in response among natural enemy species that can only be represented effectively from a full life table response experiment and a population-level endpoint, whereas among natural enemy species, their population-level response to the range of pesticides tested could frequently be represented by acute adult mortality alone.

Keywords: *Aphelinus mali*, *Chrysoperla carnea*, *Deraeocoris brevis*, *Galendromus occidentalis*, *Hippodamia convergens*, *Trioxyys pallidus*

1. Introduction

The conservation of natural enemy activity in agricultural crops is one of three key approaches to the biological control of arthropod pests (Mills, 2014). While conservation biological control includes a number of different strategies for manipulating environmental conditions to enhance the abundance and activity of natural enemies (Jonsson et al., 2008), one of the most important is the judicious use of pesticides to avoid disrupting the biological control services provided by natural enemies. Ever since Stern et al. (1959) highlighted the need for a more holistic and integrated approach to pest management, and Carson (1962) popularized the issue of disruptive impacts of insecticides on natural ecosystems, the compatibility of pesticides and natural enemies has been a major concern for conservation biological control.

While the selectivity of pesticides with respect to natural enemies can be tested both in the laboratory and in the field, the majority of studies have been conducted in the laboratory due to the uncertainty of uncontrollable biotic and abiotic influences on field studies (Galvan et al., 2006; Beers et al., 2015). The early classes of synthetic insecticides, such as organochlorines, organophosphates and carbamates, were acutely toxic to a broad range of arthropod natural enemies (Croft, 1990; Sterk et al., 1999) and the focus of laboratory bioassays was on measures of mortality, such as LC50s, as toxicological endpoints (Stark et al., 2007a). However, with the emergence of newer classes of pesticides, such as insect growth regulators, spinosyns, diamides, and strobilurins, effects on natural enemies are less likely to be lethal, but may include sublethal effects on their life history performance and behavior (Stark and Banks, 2003; Desneux et al., 2007). For these newer classes of pesticides it has also been important to use multiple routes of exposure (oral, topical and residual) in laboratory bioassays (Banken and Stark, 1998; Stark and Banks, 2003; Galvan et al., 2006), in contrast to the

standardized methodology of exposing natural enemies to fresh dry residues that had been developed earlier for the older classes of pesticides (Hassan, 1986, 1992; Croft, 1990).

One of the most important challenges in using laboratory bioassays to test for effects of pesticides on different aspects of the life history performance of a natural enemy has been to effectively extrapolate from the multiple life history parameters (development time, sex ratio, fecundity, etc.) obtained from measurement of individuals in the bioassays (individual-level endpoints) to a single index of the response of the natural enemy population to pesticide exposure (population-level endpoint). Two different approaches for integrating combinations of lethal and sublethal effects into single response indices include the total effects or reduction coefficient approach (Overmeer and van Zon, 1982; Urbaneja et al., 2008; Biondi et al., 2012) and the demographic approach (Forbes and Calow, 1999; Stark et al., 2007b; Forbes et al., 2008, 2011; Hanson and Stark, 2011a). The total reduction coefficient is simply the product of the proportional reductions for each individual-level measurement, after correction relative to the control, expressed as an overall percentage reduction. The demographic approach is more complex, but also more inclusive, in that it is based on data from life table response experiments that were specifically designed to estimate population-level responses to environmental factors that are measured as individual-level effects (Caswell, 1989). Life table response experiments have proven to be an effective way to estimate the individual-level effects of exposure to toxicants for organisms with short generation times, such as arthropods (Stark and Banks, 2003; Stark et al., 2007a).

The literature on laboratory bioassays of pesticide effects on natural enemies is extensive and such studies are an integral part of the registration process for pesticides in Europe (Desneux et al., 2007). The majority of studies have been designed to test the effects of a range of different pesticides on one or two species of natural enemy (e.g., Biondi et al., 2012; Liu et al., 2012; Amarasekare and Shearer, 2013; Wang et al., 2013; Beers and Schmidt, 2014). The main objective of these studies is the

ability to rank or to classify the pesticides with respect to their selectivity for the natural enemy species in question. For example, the International Organization for Biological Control (IOBC) uses a standardized classification for the impact of pesticides on natural enemies that consists of four categories: harmless (< 30% effect), slightly harmful (30 - 80% effect), moderately harmful (80 - 99% effect), and harmful (> 99% effect) (Sterk et al. 1999). This is intuitively appealing as it provides an opportunity to consider the use of more or less selective materials with respect to preserving or enhancing the biological control services in cropping systems. However, the predictive ability of such a ranking of pesticide effects will depend on how representative the natural enemy species selected for testing is in terms of its functional role in contributing to the biological control services in different crops and locations. In contrast, other laboratory studies of pesticide effects on natural enemies have tested the effects of a single pesticide on a range of different natural enemy species (e.g., Jansen et al., 2011; Rodriguez et al., 2013). In this case, the objective is to determine how variable the impacts are among individual species within the natural enemy community of a particular crop or within a particular taxonomic group of natural enemies. While this provides valuable data on the variation in selectivity of a particular pesticide, it can seldom be used to guide the choices that are often sought by pest management practitioners in seeking materials that are compatible with biological control. Although a number of laboratory studies fall within the continuum between these two extremes of experimental designs, it is surprisingly difficult to compare different studies due to the wide variation in pesticide concentrations, natural enemy life stages, routes of exposure, and experimental methods and arenas used, and perhaps as a consequence, we know of no meta-analyses of pesticide effects on natural enemies.

Here we focus on a set of laboratory studies designed to determine the selectivity of eight different pesticides (two used only as a mixture) employed for orchard pest management in the western United States with respect to eight different natural enemy species that are well represented in these

tree crops. Each of these studies focused on a single natural enemy species, but all were coordinated to use similar pesticide concentrations, natural enemy life stages, routes of exposure, and experimental methods. This allows us to examine variation in effects of pesticides among natural enemy species and to build a more comparative evaluation of the consequences of pesticide choice on natural enemy communities in western orchards. The objective of this comparative analysis is to address the question of the extent to which laboratory observations from individual species can be generalized to other members of a natural enemy community, and to help guide future laboratory studies of pesticide effects on natural enemies.

2. Natural enemies, pesticides and experimental design

Codling moth, *Cydia pomonella* (L.), (Lep., Tortricidae) is a key pest in apple, pear and walnut orchards throughout the western United States. One of the main objectives of this collaborative study was to assess the risk of pesticides that are used for the management of codling moth, and fungal or bacterial diseases in these crops, to the natural enemies associated with the secondary arthropod pests that occur in these orchards. A set of eight natural enemy species were selected for the laboratory bioassays. Two of the selected natural enemies were parasitoid species, *Aphelinus mali* (Hald.) (Hym., Aphelinidae) a parasitoid of woolly apple aphid *Eriosoma lanigerum* (Hausmann) and *Trioxys pallidus* (Hal.) (Hym., Braconidae) a parasitoid of the walnut aphid *Chromaphis juglandicola* (Kalt.). Three insect predators were selected, *Chrysoperla carnea* (Stephens) (Neur., Chrysopidae) a generalist predator, *Hippodamia convergens* Guérin-Ménéville (Col., Coccinellidae) primarily an aphid predator, and *Deraeocoris brevis* (Uhler) (Hem., Miridae) primarily a psyllid predator. Three arachnid predators were also included, *Galendromus occidentalis* (Nesbitt) (Acar., Phytoseiidae) a predatory mite, *Pelegriana*

aeneola (Curtis) (Aran., Salticidae) a hunting spider, and *Misumenops lepidus* (Thorell) (Aran., Thomisidae) an ambush spider.

The eight pesticides (two as a mixture) used in the bioassays were selected to represent those that are commonly used in apple, pear and walnut orchards in the western United States (Table 1). The five insecticides are all primarily used for management of codling moth in western orchards. The other two pesticides are fungicides/bactericides, with mancozeb plus copper hydroxide being used as a mixture. Three concentrations of each pesticide were used for the laboratory bioassays and 2 ml of each solution was applied with a Potter Spray Tower (Burkard Mfg, Rickmansworth, England) equipped with an intermediate nozzle. The three concentrations consisted of a full field rate (1x), representing the maximum label rate applied at 935 l ha⁻¹ (Table 1), a dilute rate (0.1x), representing 1/10th of that amount to simulate aged residues, and a distilled water control.

Detailed accounts of the experimental methodology used can be found in Amarasekare and Shearer (2013) for *D. brevis*, in Beers and Schmidt (2014) for *G. occidentalis*, and in Amarasekare et al. (2015) for both *C. carnea* and *T. pallidus*. All laboratory bioassays (except those for *G. occidentalis*, which were conducted on leaf disks with prey) were carried out in glass arenas and incorporated multiple routes of pesticide exposure (topical, residual and oral). A two-tiered approach was used, progressing from measurements of acute mortality at 48 h when exposed to all three concentrations of the pesticides to measurements from life table response experiments when exposed to the distilled water control and the higher of the two concentrations that generated < 80% acute mortality. It was considered that meaningful estimates of sublethal effects could only be obtained using pesticide concentrations that allowed for sufficient survivorship of the natural enemies from acute mortality effects. Thus concentrations that resulted in acute mortality that was moderately harmful to harmful according to the IOBC classification were excluded from the life table response experiments. Two life

stages were exposed to the pesticides, young adults and young juveniles (larvae or nymphs for the predators, and unparasitized hosts for the endoparasitoids [see Amarasekare et al., 2015 for further details]), and from 20-30 replicates were used for each pesticide concentration. Measurements from the life table response experiments included longevity, daily fecundity and fertility for the adult bioassays, and survivorship, time to maturity (development time) and sex ratio (percent female) for the juvenile bioassays. Life table measurements were continued throughout juvenile development, and either over the complete lifespan of the adult stage or for a censored period representing at least the first 1/3rd of the lifespan. No life table response experiments were conducted for the two spider species, and acute bioassays were restricted to adult *P. aeneola* and to juvenile *M. lepidus* due to limited availability for the bioassays.

The life history characteristics of the selected natural enemies varied considerably between species and so for comparative purposes each of the measured responses to pesticide exposure were standardized by correction relative to the controls. Correction of mortality has commonly been used in acute bioassays (Abbott, 1925; Hoekstra, 1987) and correction of other vital rates was accomplished in a similar way to provide a reduction coefficient $E(V) = 100 * [(V_C - V_E) / V_C]$, where V_E is the vital rate from the experimental treatment and V_C is the vital rate from the control treatment (Biondi et al., 2012; Hamby et al., 2013; Beers and Schmidt, 2014). The corrected acute mortalities and reduction coefficients were then classified into one of three categories representing a reduced version of the system developed by the International Organization for Biological Control (IOBC) for pesticide risk assessments conducted in the laboratory (Sterk et al., 1999) in which the top two categories are combined; harmless (< 30% effect), slightly harmful (≥ 30 and $\leq 80\%$), and moderately harmful (> 80% effect).

The lethal and sublethal data obtained from these acute mortality and life tables response experiment bioassays were also used to extrapolate from individual-level effects to population-level responses using demographic population models. Stage-structured matrix models, based on PopTools (Hood, 2010), were used to estimate the population growth rate responses of the natural enemy species to exposure to each of the pesticides (with the exception of the spiders for which there was no life table response data). Stage-structured models were used in preference to age-structured models for simplicity, given that results from stage-structured models appear to correlate strongly with those from more complex age-structured models (Levin et al., 1996; Hanson and Stark, 2011b), and due to the censored nature of the data collected during the adult stage for some of the natural enemy species.

3. Synthesis of results from the laboratory bioassays

3.1. Acute mortality

As would be expected, acute mortality of the selected natural enemies varied from 0 to 100% depending on pesticide and natural enemy species (Fig. 1). All of the pesticides tested, with the exception of copper plus mancozeb and chlorantraniliprole, caused more than 80% acute mortality of at least one life stage of at least one of the natural enemy species at the 1x concentration and could thus be considered moderately harmful according to the IOBC classification for laboratory bioassays. At the more dilute 0.1x concentration this was the case for cyantraniliprole, lambda-cyhalothrin and spinetoram only. For the 1x concentration, cyantraniliprole had a much greater acute effect on adult (moderately harmful) than juvenile (harmless) *C. carnea*, and a similar response was found for the effect

of spinetoram on adult and juvenile *G. occidentalis*. Conversely, sulfur had a much greater acute effect on juvenile (moderately harmful) *G. occidentalis* than on adults (harmless), as was also the case for the effect of novaluron on *D. brevis*.

To look for more general differences between the acute mortality responses of adults and juveniles by pesticide or natural enemy species, we used paired t-tests based on mean paired differences and the one-tailed null hypothesis that the adult life stage is more susceptible to pesticides than the juvenile life stage. The acute effects of the pesticides were generally comparable between juvenile and adult stages of the natural enemies (Fig. 1). However, for spinetoram, the acute effects were significantly greater on adults than on juveniles ($t = 2.30$, $df = 5$, $P = 0.04$, Fig. 1A). Similarly, both *A. mali* and *C. carnea* were generally more susceptible to acute effects in the adult rather than juvenile stage. For *A. mali*, this was evident at both the 1x concentration ($t = 2.3$, $df = 6$, $P = 0.03$, Fig. 1B) and the 0.1x concentration ($t = 2.6$, $df = 5$, $P = 0.02$, Fig. 1C), whereas for *C. carnea* a significant difference was evident only at the 1x concentration ($t = 2.3$, $df = 6$, $P = 0.03$, Fig. 1B).

3.2. Life table response experiments

The life table responses, as represented by the corrected reduction coefficients, also varied extensively depending on pesticide and natural enemy species (Table 2). In some cases no life table responses could be measured, such as for spinetoram and the two parasitoid species, for cyantraniliprole and *T. pallidus*, and for lambda-cyhalothrin and both *A. mali* and *H. convergens*, due to very strong acute mortality effects (Fig. 1). In other cases, only partial results could be obtained from the life table response experiments due to extensive reductions in juvenile survivorship, adult longevity,

or adult daily fecundity. Despite this, the response of natural enemy species to exposure to the pesticides could be classified as either moderately harmful or harmful in some cases. The most consistent life table response of the natural enemy species to the pesticides tested was a reduction in either juvenile survivorship or adult longevity. In addition, there were some less consistent, but notable effects on other life history parameters. For example, exposure of adults to novaluron reduced the fertility of all three insect predator species by more than 87% (moderately harmful), whereas the product of the daily fecundity and fertility of *D. brevis* and *G. occidentalis* was reduced by more than 92% (moderately harmful to harmful) following adult exposure to spinetoram. In contrast, however, exposure to some of the insecticides resulted in notable increases in performance among the survivors (represented by negative values in Table 2). For example, an increase in sex ratio (percent females) of 48.9% and 72.5% was observed for *D. brevis* following juvenile exposure to chlorantraniliprole and cyantraniliprole respectively. Similarly, an increase in daily fecundity of 18.3% and 57.4% was observed for *H. convergens* following adult exposure to spinetoram and copper plus mancozeb respectively. While pesticide-induced hormesis is not uncommon among arthropod pests, and is most frequently associated with reproductive traits, it has been less frequently reported for natural enemies (Guedes and Cutler, 2014).

3.3. Demographic population models

Stage-structured matrix models were parameterized using data obtained from the acute mortality bioassays and the life table response experiments. Each of the natural enemy species had very different life history characteristics and consequently population-level parameters (Table 3). In the absence of pesticide exposure, the walnut aphid parasitoid *T. pallidus* was estimated to have the

greatest intrinsic rate of increase ($r = 0.317$) and shortest population doubling time ($t_2 = 2.18$) due to a very short generation time ($T = 10.50$) combined with a moderate net reproductive rate ($R_0 = 27.99$). In contrast, the psylla predator *D. brevis* was estimated to have the lowest intrinsic rate of increase ($r = 0.085$) and longest population doubling time ($t_2 = 8.13$) due to a long generation time ($T = 33.98$) combined with a low net reproductive rate ($R_0 = 18.10$).

By extrapolating the different life table measurements to population-level indices the complete set of effects from exposure to the pesticides are taken into account. As the intrinsic rate of increase can be negative under conditions of exposure to pesticides, there is no equivalent of a corrected reduction coefficient for this population index, and so both r and corrected reduction coefficients for R_0 are presented in Table 4. A negative r indicates that a natural enemy species is unable to sustain a population when exposed to a particular pesticide, whereas a negative $E(R_0)$ indicates that the net reproductive rate was greater when exposed to a particular pesticide than in the control. The instances of a negative r or a greater than 80% reduction in R_0 (classified as moderately harmful to harmful by IOBC) have been highlighted in the table. Moderately harmful to harmful population level effects were detected for each of the seven pesticides tested for at least one of the natural enemy species. In most cases the estimated population-level effects indicated a detrimental effect on the natural enemy species from exposure to the pesticides tested, but enhanced effects occurred for *A. mali* from exposure to the two fungicides/bactericides, for *H. convergens* from exposure to copper plus mancozeb, and for *D. brevis* from exposure to the two anthranilic diamide insecticides.

These population-level estimates of pesticide effects on natural enemies allow us to address two important comparative questions. The first is how do each of the pesticides rank in terms of their average effects on the set of natural enemy species tested, and the second is whether individual natural enemy species differ in their general susceptibility to the range of pesticides tested. Unfortunately, as

each pairing of pesticide and natural enemy species provided only a single estimate of population growth (r and/or R_0 , Table 4) it was not possible to consider interactions between pesticide and natural enemy species in this analysis. To address the ranking of the pesticides we compared the mean intrinsic rates of population growth (r) for the set of natural enemies with respect to pesticide treatment including the control (Tables 3 and 4). There was significant variation in the ranking of the seven pesticides (ANOVA, $F_{7,40} = 5.14$, $P < 0.001$, Fig. 2A), but only lambda-cyhalothrin had a sufficiently negative effect to be significantly different from those pesticides (copper plus mancozeb, chlorantraniliprole, novaluron, sulfur) that did not reduce the mean r below 0 (Tukey's honest significant difference). It is notable that in addition to lambda-cyhalothrin, both spinetoram and cyantraniliprole also generated negative mean values of r . However, due to the variation in response of different natural enemies to each pesticide, only the more extreme differences in average values of r were significantly different. To address the ranking of the natural enemy species we compared the mean reduction coefficients [$E(R_0)$, Table 4] for the set of pesticides tested (using logit transformation and excluding the controls) with respect to natural enemy species. In this case, we used the reduction coefficient rather than r to avoid the confounding effect of inherent differences in r between natural enemy species (Table 3). There was no evidence for significant variation in the $E(R_0)$ response of the six species of natural enemy tested to the set of pesticides used in the laboratory bioassays (ANOVA, $F_{5,36} = 1.60$, $P = 0.19$, Fig. 2B). *A. mali* had the smallest mean reduction coefficient and *T. pallidus* had the largest, but there was sufficient variation in the response to individual pesticides within natural enemy species to mask any more general effects between natural enemy species.

The population-level effects of the pesticides were often driven by different life history components for each natural enemy species tested. For example, the population-level effects of spinetoram were driven by strong acute adult mortality for the two parasitoids, by acute adult mortality and sublethal effects for *G. occidentalis*, by chronic larval mortality for *C. carnea*, and by reduced adult

fecundity and fertility for *D. brevis*. In contrast, for the two diamides, chlorantraniliprole and cyantraniliprole, the population-level effects were primarily driven by acute or chronic effects on juvenile mortality alone, although chlorantraniliprole also had an important effect on *C. carnea* through reduced adult fecundity. Similarly, the main effects of novaluron and sulfur were through chronic juvenile mortality for the insect and mite predators, but through acute adult mortality and reduced adult fecundity/fertility in the case of *T. pallidus* exposed to sulfur.

The estimated population-level effects also allowed us to ask whether simpler laboratory bioassays, requiring less effort and expense than the acute plus life table response experiments conducted in this study, would be sufficient to capture the same potential disruptive effects of the pesticides on natural enemy populations. Two commonly reported individual-level endpoints for bioassays of pesticide effects on natural enemies are corrected acute adult mortality and corrected reduction coefficients for adult reproduction/fertility (Stark et al., 2007b; Urbaneja et al., 2008; Giolo et al., 2009; Suma et al., 2009; Hanson & Stark, 2011a; Biondi et al., 2012; Hamby et al., 2013). Therefore, we tested the extent to which these two individual-level endpoints or their combination (corrected percent reduction in adult survivorship*daily fecundity*fertility) could account for the variance in intrinsic rate of increase of the natural enemies from our laboratory bioassays using linear regression models with sequential Bonferroni correction for family-wise error rates (Fig. 3). For comparison among pesticides, although corrected acute adult mortality alone accounted for as much as 77% of the variance in r between natural enemy species following exposure to cyantraniliprole, none of the regression models for the seven pesticides were significant after sequential Bonferroni correction. The corrected percent reduction in fecundity/fertility and the corrected percent reduction in acute survivorship and fecundity/fertility combined accounted for significant variation in r for chlorantraniliprole only (Fig. 3A). In contrast, acute adult mortality accounted for a significant amount of the variation in r as a response to the different pesticides for most of the natural enemy species tested (Fig. 3B), with the exception of

D. brevis that showed only a limited acute mortality response to all of the pesticides tested (Fig. 1B). However, corrected percent reduction in fecundity/fertility did not account for any significant variation, and corrected reduction in adult survivorship and fecundity/fertility combined accounted for significant variation for only two of the six natural enemy species tested (Fig. 3B). Thus for comparison among natural enemy species for a single pesticide, there appears to be considerable variation in response that can only be captured effectively from a full life table response experiment and a population-level endpoint, whereas for individual natural enemy species, their population-level response to the range of pesticides tested could frequently be represented by acute adult mortality alone.

4. Discussion

Pesticides continue to be an important component of pest management programs worldwide, but the biological control services provided by natural enemies have become more widely recognized for their role in the development of more comprehensive and sustainable management practices (Jones et al., 2009; Gentz et al., 2010; Hillocks, 2012; Roubos et al., 2014). Moreover, the conservation and enhancement of natural enemy activity in agroecosystems is one of the key elements of sustainable agricultural production (Pretty, 2008; Shennan, 2008; Power, 2010; Ekström and Ekbom, 2011). Effective integration of pesticides and natural enemies can only be achieved if we have sufficient knowledge of the selectivity of pesticide products across a broad range of natural enemy taxa. As newer classes of ‘reduced risk’ pesticides have been replacing the more traditional classes in recent years (Agnello et al., 2009) it is important to understand how selective they are with respect to natural enemies. While new classes of pesticides have different modes of action and may be expected to have improved selectivity, this may not necessarily be the case (Gentz et al., 2010), placing greater emphasis

on the need for laboratory bioassays to take into account multiple routes of exposure and sublethal effects on life history in addition to lethal effects (Stark and Banks, 2003; Desneux et al., 2007).

In this study we used a shared set of experimental protocols to conduct laboratory bioassays on the effects of the same set of pesticides commonly used in western U.S. orchards on a range of natural enemy species that contribute to the biological control of secondary pests in these orchards. This allowed us to develop a comparative dataset from which the generality of natural enemy responses to particular pesticides, and the generality of the response of particular natural enemy species to a range of pesticides, can be addressed. In addition, by using both acute and life table response bioassays on the natural enemies, we provide the first comparative data that can be used to address how well some of the more commonly used individual-level toxicological responses of natural enemies in laboratory bioassays might reflect their population-level responses. The wide range of pesticide concentrations and experimental protocols more generally used in laboratory bioassays of pesticide effects on natural enemies has made it difficult to conduct detailed comparative studies. The Western Palearctic Regional Section of IOBC has played a lead role in testing the side effects of pesticides since 1980, and has developed a database of selectivity data for more than 140 pesticide products and 20 beneficial arthropod species (Sterk et al., 1999; IOBC-WPRS, 2014). However, most bioassays are focused on lethal effects, although some include adult reproduction, and the resultant database is currently only available to its membership (IOBC-WPRS, 2014). While the range of pesticides and natural enemies included in our comparative dataset was inevitably small in comparison to the IOBC-WPRS database, due to the time and effort required to conduct these types of bioassays, it includes a more comprehensive set of bioassay experiments.

One of the most important outcomes of this analysis for orchard pest management in the U.S. is that even the reduced-risk insecticides had significant impacts on some of the natural enemy species

tested and that many of the observed population-level effects of the pesticides occurred through chronic effects on survivorship or effects on other life history components rather than through acute mortality. An important question associated with laboratory bioassays is whether the observed disruptive effects of pesticides on natural enemies can be extrapolated to similar effects in the field. The experience from the IOBC-WPRS program has been that an effect of greater than 80% in laboratory bioassays is needed before any such effects are likely to be seen in the field (Sterk et al. 1999). From use of the same classification system in this study, our findings suggest a number of potential disruptive effects would be expected on natural enemies in western U.S. orchards. However, there are some important caveats and limitations that need to be considered in extrapolating from laboratory bioassays to the field, including substrate choice for pesticide residues, persistence of effects, natural enemy behavior, and recolonization by natural enemies.

Although the majority of laboratory bioassays, including our own, have used glass substrates for the application of pesticide residues, this may overestimate the toxicological impacts and represent a worst-case scenario that could be avoided through use of leaf surfaces as application substrates (Desneux et al., 2006; Biondi et al., 2012). Similarly, under field conditions the impact of pesticide applications on natural enemy life history parameters will change over time due to effects of aging and persistence of pesticide residues. Although stage-structured demographic models effectively capture the initial effects of pesticides on natural enemy populations, they do not allow for a reduction in effects on life history over time (Banks et al., 2008a); inclusion of a temporal reduction in toxicological effects requires the use of more complex differential equation models (Banks et al. 2008b). In addition, while life table response experiments provide an effective way to estimate the direct impact of pesticides on life history parameters of natural enemies, they do not incorporate indirect impacts of pesticides on behavior (Desneux et al., 2007; Delpuech and Leger, 2011). As pesticides can have significant impacts on foraging, learning, feeding, mating and oviposition behaviors, these indirect effects can further

compromise natural enemy populations beyond the direct effects measured in laboratory table response experiments. Finally, while both direct and indirect effects of pesticides can have localized effects on natural enemy populations, little is known about the speed of recovery of biological control services in agroecosystems through natural enemy recolonization. Clearly the availability of natural enemies for recolonization from local refuges and from the general landscape pool (Roubos et al., 2014) and the degree of persistence of pesticide residues are likely to influence the speed of recovery; this aspect of extrapolation deserves greater attention in the future.

The results of our comparative laboratory bioassays suggest that while individual natural enemy species did not differ in their mean population-level response to the range of pesticide tested, many of them did exhibit a close relationship among the pesticides tested between acute adult mortality and intrinsic rate of population increase. This was surprising, as it was anticipated that the sublethal effects of the pesticides would have been equally as strong as the lethal effects in impacting the natural enemies in the laboratory bioassays. While a clear relationship between acute and population effects is not unique (Kuhn et al. 2000), it may be uncommon (Stark, 2005), and in our study may have been related to the wide variation in acute toxicity effects among the pesticides tested. In contrast, there was almost never a clear link between the intrinsic rate of population increase and the reduction coefficient for loss of fecundity and/or fertility and seldom a relationship with the reduction coefficient for combined acute survivorship and fecundity/fertility. Thus despite the increasing use of combination reduction coefficients to represent the broader life history effects of pesticides (Urbaneja et al., 2008; Biondi et al., 2012; Hamby et al., 2013), the results from our study suggest that such indices may not predict population-level impacts. Nonetheless, whether comparative population effects of pesticides for individual natural enemy species can more generally be represented by acute adult toxicity responses alone is intriguing and deserves closer attention, as the bioassays would be clearly be much quicker and easier to conduct and would enhance the value of previously published acute mortality studies.

In conclusion, our comparative laboratory bioassays suggest that under field conditions disruptive effects on natural enemies would be more commonly expected from applications of lambda-cyhalothrin and spinetoram. In contrast, copper plus mancozeb and chlorantraniliprole would not be expected to cause disruptive effects on natural enemies, although more specific impacts could occur for predatory mites and green lacewings respectively. In addition, novaluron appeared more likely to disrupt predator than parasitoid populations, while other products had less predictable effects among natural enemy species. While it is tempting to search for general patterns of pesticide effects among functional groups of natural enemies or among classes of pesticides, as attempted here, in reality our dataset is far too small to be able to provide robust predictions. In this context, Banks et al. (2011) caution against extrapolation of pesticide effects from individual species to other members of the same functional group due to extensive variation in life history traits which affects the resilience of individual species to disruption by pesticides at the population level. While our comparative study has provided a step in the right direction, a more extensive comparative database of population-level effects of pesticides on natural enemies will be needed to improve our ability to predict disruptive effects and to provide robust recommendations to growers.

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Figure legends

Fig. 1. Comparison of acute mortality responses of adult and juvenile natural enemy species to the pesticides tested in acute bioassays arranged firstly by pesticide for the different natural enemy species at the 1x concentration (A), and secondly by natural enemy species for the different pesticides for both the 1x concentration (B) and the 0.1x concentration (B). The smaller white square delimits pesticide effects that are harmless, the larger grey square indicates effects that are slightly harmful, and the area above and to the right side of the grey square represents effects that are moderately harmful to harmful according to the IOBC classification system for laboratory bioassays. Data points above the diagonal line and positive mean paired differences (across natural enemy species in A and across pesticides in B and C) show a greater response in the adult stage, while those below the line and negative mean paired differences show a greater response in the juvenile stage. Asterisks indicate significant differences from zero (one-tailed, paired t -test, $P < 0.05$).

Fig. 2. A comparison of (A) the mean population-level effects of the pesticides tested, and (B) the mean population-level susceptibility of the natural enemy species tested, as represented by the estimated intrinsic rate of increase when exposed to the pesticides. Bars followed by different letters are significantly different at $P < 0.05$ (Tukey's honest significant difference).

Fig. 3. A comparison two individual-level endpoints (corrected acute adult mortality and corrected percent reduction in daily fecundity*fertility) or their combination (corrected percent reduction in adult survivorship*fecundity*fertility) as predictors of the effect of pesticide exposure on the intrinsic rate of natural increase (r) of natural enemy populations. The coefficient of determination represents the proportion of the total variance in r explained (i.e., the goodness of fit of the linear regression model) by each of the individual-level endpoints in relation to (A) pesticide and (B) natural enemy species. Bars

with asterisks represent regression models that were significant at $P < 0.05$ after sequential Bonferroni correction for family-wise error.

Table 1

Chemical name	Chemical class	Brand name /formulation	Use rate (g ai ha ⁻¹)	Assay concentration (mg ai l ⁻¹) (1x conc)
Lambda-cyhalothrin	Pyrethroid	Warrior II 2.08CS	47	50
Spinetoram	Spinosyn	Delegate 25WG	123	131
Novaluron	IGR - benzoyl urea	Rimon 0.83EC	363	389
Chlorantraniliprole	Anthranilic diamide	Altacor 35WDG	110	118
Cyantraniliprole	Anthranilic diamide	Exirel 0.83SE	149	159
Copper hydroxide	Inorganic	Kocide 3000WG	2067	2210
Mancozeb	Dithiocarbamate	Manzate Pro-Stick 75W	1,513	1,618
Sulfur	Inorganic	Kumulus 80W	17,933	19,174

Table 2

Natural enemy species tested	Life table response	Chlorantran-iliprole	Cyantran-iliprole	Spinetoram	Lambda-cyhalothrin	Novaluron	Sulfur	Copper+ mancozeb
<i>Aphelinus mali</i>	Sex ratio	7.8	7.7			8.3	1.4	3.1
	Longevity	8.2	21.1			10.3	9.8	2.0
	Fecundity/fertility	5.1	55.9			11.5	0.3	0.8
<i>Trioxys pallidus</i>	Sex ratio	25.1			25.5	22.7	14.5	0.5
	Longevity	0.0			9.7	5.2	57.7	4.5
	Fecundity/fertility	25.2			84.5	14.8	64.4	21.4
<i>Chrysoperla carnea</i>	Survivorship	80.9	97.1	69.5	72.3	100.0	17.9	14.2
	Time to maturity			0.0	-9.0		-12.4	-0.5
	Sex ratio			11.5	-27.0		10.2	-10.0
	Longevity	62.3	85.0	1.5	4.1	-14.8	-18.3	-14.7
	Daily fecundity	78.8		3.4	70.1	1.5	11.5	31.9
	Fertility	4.1		12.2	61.5	95.4	13.5	10.3
	Fecundity/fertility	79.6		15.2	88.5	95.5	23.4	38.9
<i>Deraeocoris brevis</i>	Survivorship	3.7	25.9	18.6	100.0	100.0	37.0	18.6
	Time to maturity	0.7	0.7	-5.1			-10.2	-2.5
	Sex ratio	-48.9	-72.5	-9.9			6.2	13.3
	Longevity	-6.8	4.1	42.8	80.2	52.0	16.4	11.5
	Daily fecundity	22.4	11.8	77.6		77.6	56.8	24.7
	Fertility	13.1	8.9	62.9		90.2	78.2	36.4
	Fecundity/fertility	32.5	22.3	92.5		97.9	91.5	53.7
<i>Hippodamia convergens</i>	Survivorship	85.1	92.0	64.4		100.0	79.3	5.7
	Time to maturity			-11.1			-19.0	-4.8
	Sex ratio			-20.0			0.0	0.0
	Longevity	45.2	20.5	-13.7		15.1	0.0	-13.7

	Daily fecundity	27.5	27.0	-18.3		13.9	-4.4	-57.4
	Fertility	11.7	5.9	14.7		86.8	-1.5	-1.5
	Fecundity/fertility	36.0	31.3	-0.9		88.6	-5.9	-59.7
<i>Galendromus occidentalis</i>	Survivorship	24.0	27.0	100.0	98.0	94.0	100.0	68.0
	Daily fecundity	12.6	50.0	100.0	72.1	38.9	51.2	63.6
	Fertility	0.0	15.0		0.0	23.9	23.8	0.0
	Fecundity/fertility	12.6	57.5	100.0	72.1	53.5	62.8	63.6

Table 3

Functional group	Natural enemy species	r	R_0	T	t_2
Insect parasitoid	<i>Aphelinus mali</i>	0.131	7.64	15.56	5.30
	<i>Trioxys pallidus</i>	0.317	27.99	10.50	2.18
Insect predator	<i>Chrysoperla carnea</i>	0.177	146.09	28.08	3.91
	<i>Deraeocoris brevis</i>	0.085	18.10	33.98	8.13
	<i>Hippodamia convergens</i>	0.112	39.07	32.63	6.19
Mite predator	<i>Galendromus occidentalis</i>	0.157	11.53	15.57	4.41

Table 4

Natural enemy species	Index	Chlorantran -iliprole	Cyantran- iliprole	Spinetoram	Lambda- cyhalothrin	Novaluron	Sulfur	Copper + mancozeb
<i>Aphelinus mali</i>	<i>r</i>	0.125	0.057	0.020	0.036	0.096	0.136	0.147
	$E(R_0)$	32.3	69.4	82.3	79.2	48.6	-19.9	-80.2
<i>Trioxys pallidus</i>	<i>r</i>	0.134	-0.106	-0.033	-0.149	0.296	-0.065	0.263
	$E(R_0)$	86.5	99.0	97.4	99.7	30.9	98.3	45.1
<i>Chrysoperla carnea</i>	<i>r</i>	-0.107	-0.254	0.020	-0.174	0.042	0.176	0.166
	$E(R_0)$	99.9	100.0	99.0	100.0	97.4	12.1	38.3
<i>Deraeocoris brevis</i>	<i>r</i>	0.088	0.091	-0.003	-0.202	-0.158	0.029	0.071
	$E(R_0)$	-6.0	-0.3	95.0	100.0	100.0	89.1	55.6
<i>Hippodamia convergens</i>	<i>r</i>	0.056	0.056	0.106	-0.546	0.018	0.080	0.122
	$E(R_0)$	88.3	86.1	26.5	100.0	95.5	62.9	-56.9
<i>Galendromus occidentalis</i>	<i>r</i>	0.142	0.106	-0.261	-0.320	0.005	-0.111	-0.009
	$E(R_0)$	12.1	56.6	100.0	99.9	90.6	98.6	92.5

Figure 1

Figure 2

A

B

Figure 3