

# Meta-analysis and review of pesticide non-target effects on phytoseiids, key biological control agents

Rebecca A Schmidt-Jeffris,<sup>a\*</sup> Elizabeth H Beers<sup>b</sup> and Chris Sater<sup>b</sup>



## Abstract

Understanding pesticide non-target effects on natural enemies is a key element of successful conservation biological control. Due to their importance in agroecosystems worldwide, the phytoseiid mites are the most well-studied natural enemies in pesticide selectivity research. The wealth of literature associated with this topic allows for a thorough meta-analysis of pesticide non-target effects and may also indicate general trends relevant to many cropping systems. We conducted a meta-analysis using 2386 observations from 154 published papers examining the impact of pesticides on lethal (adult and juvenile mortality) and sublethal (fecundity, egg hatch) effects. Insecticides and herbicides did not statistically differ in toxicity to phytoseiids, but research on herbicide non-target effects is scarce. Specific insecticides, fungicides, and miticides were sorted into least and most harmful categories. Phytoseiid species also differed in sensitivity, with *Galendromus occidentalis* (Nesbitt), *Neoseiulus californicus* (McGregor), and *Typhlodromus pyri* Scheuten among the least sensitive species. Sensitivity variation may be partly due to pesticide resistance; the greatest differences between species were within older mode of action (MOA) groups, where resistance development has been documented. It has been speculated that specialist phytoseiids, which closely associate with *Tetranychus* spp. spider mites, have more opportunities for resistance development due to their necessary proximity to a pest that rapidly develops resistance. Effect sizes were higher for generalist phytoseiid species, supporting this hypothesis. This meta-analysis highlights pesticide types (herbicides) and MOA groups where more research is clearly needed. Our analysis also allows for more robust generalizations regarding which pesticides are harmful or selective to phytoseiids.

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Supporting information may be found in the online version of this article.

**Keywords:** Phytoseiidae; predatory mites; pesticide non-target effects; meta-analysis; biological control; sublethal effects

## 1 INTRODUCTION

Selecting pesticides with minimal non-target effects on pest natural enemies is a critical component of conservation biological control.<sup>1–4</sup> Non-target effects vary between active ingredients (AIs) and species,<sup>1,5</sup> making studies of pesticide effects an essential component of building integrated pest management (IPM) programs. Since the 1950s, pesticide non-target effects research has increased exponentially.<sup>6–8</sup> However, much remains unknown in this field. New pesticides are continually developed, creating a need to examine their effects on key natural enemies to maintain existing IPM programs. Newer materials are often more selective in terms of acute mortality,<sup>1</sup> but this may require examination of their sublethal effects.<sup>9</sup> Natural enemies that have been understudied may be discovered to be important in well-established IPM programs,<sup>10,11</sup> adopted as newly popular augmentation agents,<sup>12</sup> or become key to invasive species control efforts.<sup>13</sup> These developments require continuously expanding research on pesticide non-target effects.

Predatory mites in the family Phytoseiidae are the most important biological control agents of pest mites<sup>14</sup> and more than half of all mite biological control papers include phytoseiids.<sup>15</sup> Additionally, > 60% of the current augmentative biocontrol market is predatory mites.<sup>16</sup> Phytoseiids are predators of tetranychids,

eriophyids, tarsonemids, tenuipalpids, thrips, scales, and whiteflies, and also feed on honeydew, pollen, nectar, fungi, and leaf fluids.<sup>14,17,18</sup> Conserving key phytoseiid species through selective use of pesticides is a critical component of many integrated mite management programs.<sup>19–22</sup>

Phytoseiids are the most studied natural enemies in the field of pesticide non-target effects. In the last quantitative review of the pesticide non-target effects literature, they were the most represented group, making up 19% of the records from the 20 most tested families.<sup>23</sup> Of the 22 most tested species, five were phytoseiids. These five species made up 32% of the records for the top 20 species and the phytoseiid with the most records [*Neoseiulus fallacis* (Garman)] was the second most studied natural

\* Correspondence to: RA Schmidt-Jeffris, Temperate Tree Fruit and Vegetable Crop Research Unit, USDA-ARS, 5230 Konnowac Pass Road, Wapato, WA 98951, USA. E-mail: rebecca.schmidt@usda.gov

a Temperate Tree Fruit and Vegetable Crop Research Unit, USDA-ARS, Wapato, WA, USA

b Tree Fruit Research and Extension Center, Washington State University, Wenatchee, WA, USA

enemy.<sup>23</sup> Phytoseiids also play a large role in studying pesticide resistance development in natural enemies. They comprise the majority of documented resistant natural enemies and they tend to develop higher levels of resistance than other groups.<sup>8,23</sup>

Because of this large body of literature, phytoseiids are an excellent system for examining patterns in pesticide non-target effects. They have a near-worldwide distribution and are found in a wide range of climates,<sup>14</sup> making these predators relevant in many crops. They are key components of conservation,<sup>24,25</sup> augmentation,<sup>16</sup> and classical<sup>26</sup> biological control programs. Phytoseiid diets are highly diverse, making it possible to study both specialists and generalists.<sup>27,28</sup> There are conflicting hypotheses regarding how predator diet specialization influences pesticide resistance development,<sup>8,29</sup> but this has not been formally investigated.

The last quantitative review of the pesticide non-target effects literature (the SELECTV database<sup>5</sup>) primarily contained studies from 1985 and earlier.<sup>30</sup> Since then, many new pesticides have been developed. Of particular significance to phytoseiids, several new miticides with selective modes of action (MOAs) have entered the market<sup>19</sup> and there has been a shift away from broad-spectrum insecticides. Methods for analyzing data from previous studies have also been refined to allow for statistical comparisons between groups (i.e. meta-analysis). There is a clear need to summarize the literature on pesticide non-target effects on phytoseiids to identify existing patterns and determine research priorities.

The purpose of this meta-analysis was to summarize the literature on pesticide non-target effects on phytoseiids to compare effects between pesticide types and phytoseiid species and to identify research needs. Specifically, we examined differences in effect sizes between (i) different life stages/tested parameters (e.g. mortality, fecundity); (ii) pesticide type, MOA, and AI; (iii) phytoseiid species; and (iv) lifestyle type. Our analysis can be used to identify trends in pesticide non-target effects, aiding IPM practitioners in optimizing pesticide selection for conserving phytoseiids within their cropping system. This is especially critical given the prominent role of phytoseiids in augmentative and conservation biological control programs worldwide.

## 2 MATERIALS AND METHODS

### 2.1 Study selection

We examined the existing literature for studies by using combinations of the keywords 'Phytoseiidae', 'non-target', 'pesticide', 'side effect', and 'sublethal' as search terms in Google Scholar. Additional papers were included by reviewing studies referenced in the International Organization for Biocontrol-West Palearctic Regional Section (IOBC-WPRS) Pest Select Database.<sup>31</sup> We also examined the reference lists of papers retrieved in our initial search. Articles in languages other than English were included if some of the content (i.e. abstract, captions) was in English and allowed us to determine relevance prior to requesting translation assistance.

Studies were excluded when measures of variation or sample sizes were not reported. Studies that only reported the IOBC rating<sup>31</sup> instead of the associated data were also excluded. Data were only included for pesticides tested at field rates. We also excluded studies where only median lethal concentration (LC<sub>50</sub>) values were reported, because effect sizes cannot be calculated from these values. When mortality data from multiple time points were collected in one study, we only used data from the time point closest to 48 h after exposure, as this appeared to be the

most common time frame evaluated. If both 24 and 72 h data were provided, 72 h data were used. Entries were excluded if the AI could not be determined or if unformulated pesticides were used. Only studies examining direct contact or fresh residues (as opposed to aged residues) were included. Studies examining other exposure routes, such as ingestion, were very rare; this is an area of research that requires further investigation. Only laboratory trials were included, as we were unable to find enough field studies that included a control, reported variance, and identified phytoseiids to species.

### 2.2 Data extraction

We extracted data for the four most common response variables: adult mortality, juvenile mortality (larvae or nymphs), egg hatch (treated eggs or eggs laid on treated surfaces), and fecundity. Data from graphics were extracted using WebPlotDigitizer.<sup>32</sup> In many cases, only corrected mortality<sup>33</sup> was reported; for these studies, we entered control mortality as 0%. Data regarding mortality and egg hatch was converted to binomial data whenever possible, as weighted effects sizes based on means cannot be calculated when variance is zero, which frequently occurs in mortality data (0% control mortality, 100% mortality for highly toxic pesticides). Pesticides were classified as insecticides, insecticides/miticides, miticides, fungicides, herbicides, or plant growth regulators (e.g. fruit thinners) based on pesticide labels and further categorized by MOA as determined by the Insecticide Resistance Action Committee ([irac-online.org/modes-of-action/](http://irac-online.org/modes-of-action/)), Fungicide Resistance Action Committee ([frac.info/fungicide-resistance-management](http://frac.info/fungicide-resistance-management)), and Weed Science Society of America ([wssa.net/wssa/weed/herbicides](http://wssa.net/wssa/weed/herbicides)), respectively. The species of each phytoseiid tested was updated according to current nomenclature standards by checking the Phytoseiidae Database.<sup>34,35</sup> Phytoseiid diet specialization was classified by lifestyle type (I–IV).<sup>27,34</sup>

### 2.3 Data analysis

Data were analyzed using Open MEE.<sup>36</sup> For means data, the effect size calculated was Hedge's *d* and for binomial data, the log odds ratio was calculated. Effect sizes quantify the difference between groups, which allows for analyses to incorporate data between multiple studies.<sup>37</sup> Log odds ratios were converted to Hedge's *d* to allow for comparison between all data types.<sup>37</sup> Positive values indicated a harmful effect (increase in mortality, decrease in egg hatch or fecundity). The 'Subgroup Meta-analysis' function was used to compare effect sizes of various categorical variables. For each group analyzed, its effect size and 95% confidence interval (CI) were calculated. Effect sizes were considered significantly different from zero or each other when 95% CIs did not overlap<sup>37</sup>; this corresponds to  $\alpha$  ( $P < 0.05$ ). We compared effect sizes for: (i) the type of non-target effect examined (adult mortality, fecundity, etc.), (ii) the type of pesticide (insecticide, fungicide, etc.), (iii) fungicide AI, (iv) insecticide MOA group, (v) miticide AI, (vi) phytoseiid species  $\times$  MOA group, and (vii) phytoseiid lifestyle type.

For most analyses, the effect sizes for adult mortality, juvenile mortality, fecundity, and egg hatch were examined separately. For comparing fungicide AI, the effect size calculated combined the effects of adult and juvenile mortality. For comparing AIs within miticides, we calculated effect sizes for adult mortality, fecundity, and egg hatch, but not juvenile mortality, as relatively few AIs had sufficient observations for analysis. When comparing phytoseiid species, we conducted separate analyses for each of

the most commonly tested MOAs and only examined the effect of adult mortality; this prevented certain species from seeming more or less sensitive if only harmful or harmless pesticides had been tested against them. To compare lifestyle types, we calculated the effect size for adult mortality across all pesticides for each type (I–IV). Only effect sizes based on  $n > 2$  observations were used.

### 3 RESULTS AND DISCUSSION

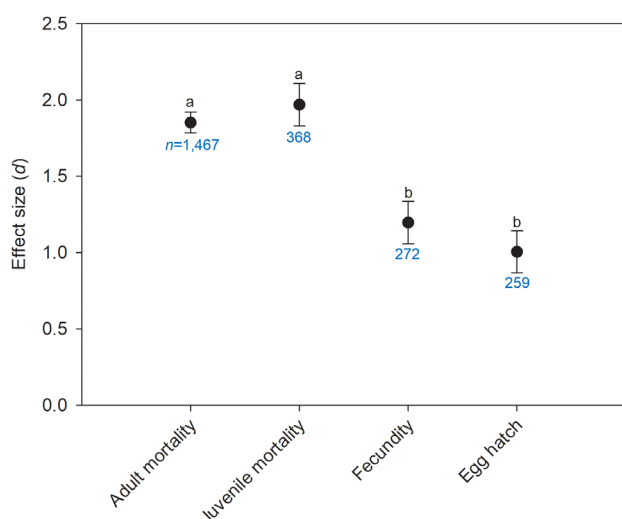
The literature review yielded 2386 observations/records from 154 papers, which were published from 1956 to 2020. The meta-analysis database can be found in Supporting Information Appendix S1.

#### 3.1 Non-target effect type

In our meta-analysis, adult and juvenile mortality did not differ in effect size and were significantly higher than the effects on fecundity and egg hatch (Fig. 1). Previous reviews of pesticide non-target effects indicate that juveniles are more sensitive than adults due to their lower weight, which increases dose per amount body mass, and larger surface to volume ratio, which increases relative surface exposure.<sup>38</sup> Based on this logic, IOBC testing protocols recommend first testing newly hatched phytoseiid larvae, assuming that these are the most susceptible stage.<sup>36,37</sup>

However, if this assumption is incorrect, opportunities to test impacts on reproduction could be missed in assays when only juveniles are tested. In the SELCTV database, phytoseiid adults were the most sensitive group, followed by juveniles, then eggs.<sup>39</sup> Surprisingly little research has been done to directly compare juvenile and adult phytoseiid susceptibility within the same study. One study on *Galendromus occidentalis* (Nesbitt) found that adults were more susceptible to thiacloprid, spinetoram, novaluron, flubendiamide, and mancozeb + copper, whereas larvae were more susceptible to carbaryl, azinphosmethyl, spinosad, and sulfur.<sup>40</sup>

The results of the meta-analysis support prior assumptions that eggs are the least sensitive stage, but also indicate that juveniles



**Figure 1.** Effect sizes ( $d$ ) of all pesticides combined on four types of phytoseiid non-target effects. Error bars indicate 95% bootstrapped confidence interval (CI). Where these intervals do not overlap with  $d = 0$  or each other the effect or difference is significant. Treatments marked with the same letter were not statistically different ( $P > 0.05$ ). Sample size ( $n$  records) is indicated underneath each CI.

and adults do not differ in sensitivity. This indicates that in general, assays could use either life stage. Where resources are limited and only one life stage can be tested, it would be more beneficial to test the adult life stage, as this allows for examination of impacts on fecundity, egg hatch, and reduction of second generation offspring.<sup>37,39</sup> However, for pesticides that specifically target certain life stages (e.g. growth regulators), it is obviously more appropriate to test the stage impacted.

#### 3.2 Pesticide type

Insecticides and insecticide/miticides were the most common types of pesticides tested, making up 61% of records in our meta-analysis (Fig. 2). There were few studies examining plant growth regulators, with only the effect of juvenile mortality containing enough records for analysis. Herbicides were the next least common type, with only 38 records total and an insufficient number to examine effects on fecundity or egg hatch (Fig. 2). In the last review of the literature of non-target effects on all natural enemies, herbicides, fungicides, and miticides made up 1.4%, 9%, and 7% of records, respectively.<sup>23</sup> While research on miticide non-target effects has increased, our meta-analysis and previous work<sup>41,42</sup> highlight that herbicide effects remain poorly described.

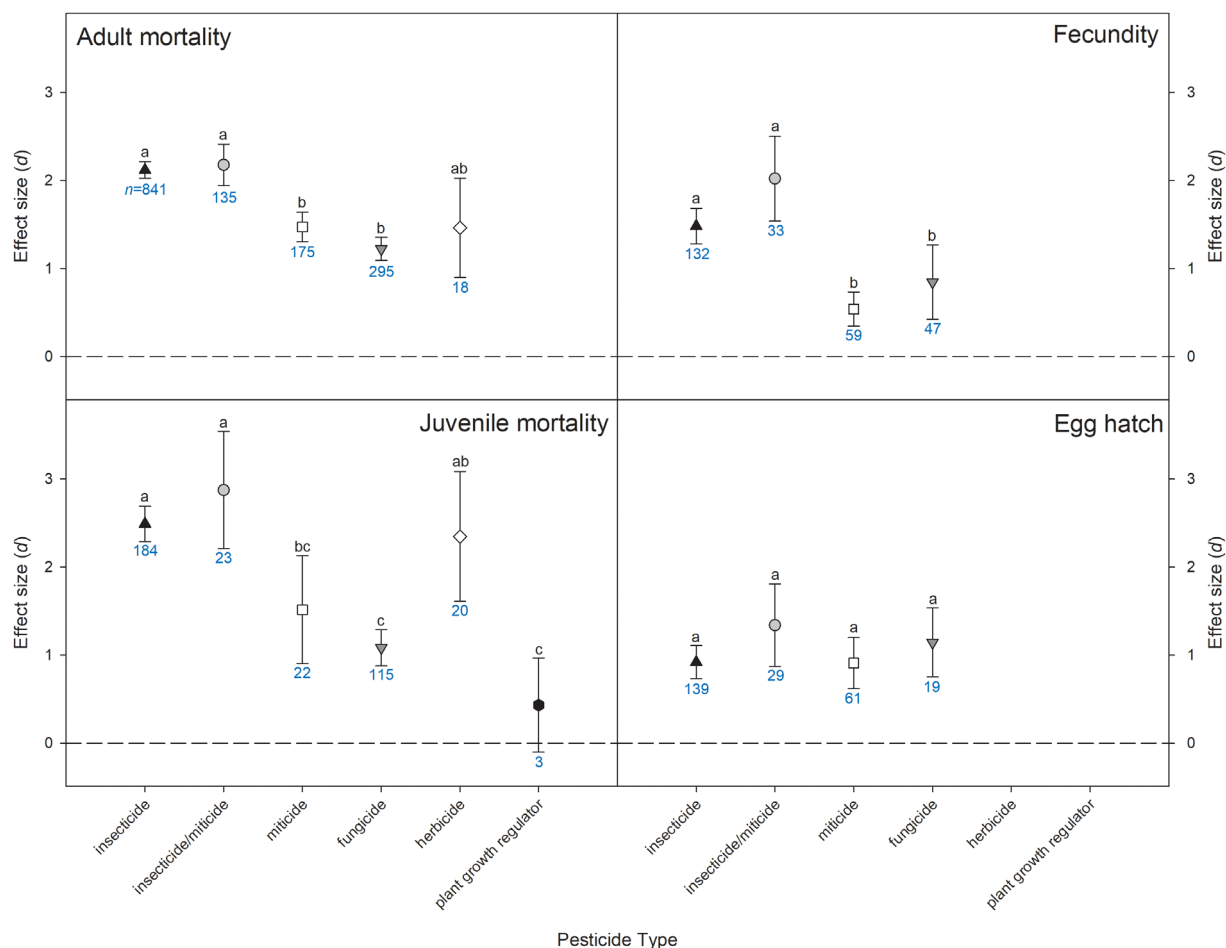
In the meta-analysis, insecticides and insecticide/miticides caused higher phytoseiid adult mortality than miticides or fungicides (Fig. 2). The effect of herbicides was intermediate and differed from none of the other groups. Juvenile mortality was highest for insecticides, insecticide/miticides, and herbicides and lowest for miticides, fungicides, and plant growth regulators (Fig. 2). Insecticides and insecticide/miticides also caused the greatest reduction in fecundity. All groups caused a reduction in egg hatch and did not differ from each other (Fig. 2). While plant growth regulators had no effect on juvenile mortality, all other pesticide types were harmful to phytoseids (effect size  $> 0$ ).

In the SELCTV summary of non-target effects on all natural enemies herbicides were the second most toxic group, after the insecticides.<sup>23</sup> SELCTV indicated that herbicide toxicity varied widely, with herbicides being among both the most and least toxic AIs.<sup>23</sup> In work focusing on *Typhlodromus pyri* Scheuten, 58% of fungicides, 53% of herbicides, and 90% of insecticides reviewed had lethal effects.<sup>43</sup> A review of the literature on pesticide non-target effects on *N. fallacis* found that herbicides were intermediate to insecticides and fungicides in toxicity.<sup>30</sup> Another review indicated that herbicides are generally harmful to phytoseids and tend to be more harmful to phytoseids than to beneficial insects.<sup>38</sup> Because herbicide non-target effects studies were scarce, we were unable to quantitatively compare toxicity between AIs or MOAs. A qualitative review of the herbicide non-target effects on phytoseids found that bromofenoxim, bromoxynil, ioxynil, methabenzthiazuron, glufosinate, and paraquat were the most toxic herbicides.<sup>41</sup>

The current meta-analysis and previous work indicate that herbicides can be moderately to highly toxic to phytoseids. Given the potential of these pesticides to disrupt biological control, the lack of research is striking. This disparity may be due to the large focus on commercially available phytoseids used for augmentation in glasshouses (see Section 3.6 Species identity), where herbicide use is rare. Because phytoseids use weeds for shelter and food resources, this gap in the literature should be addressed.<sup>41,42</sup>

#### 3.3 Fungicides

Unlike the herbicides, several fungicide AIs were sufficiently represented to allow for quantitative comparison between



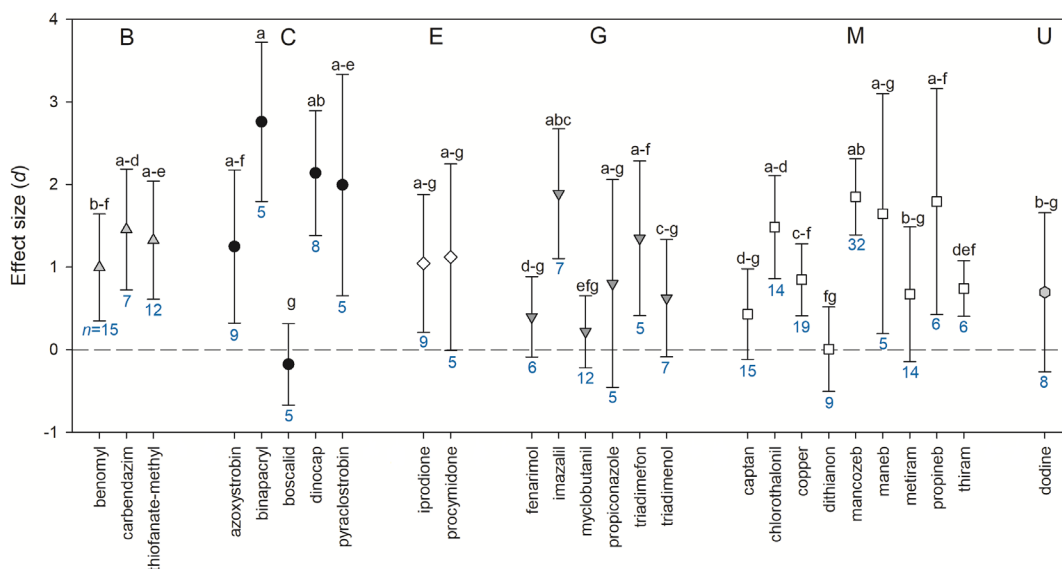
**Figure 2.** Effect sizes ( $d$ ) of various pesticide types on phytoseiid adult mortality, juvenile mortality, fecundity, and egg hatch. Error bars indicate 95% bootstrapped confidence interval (CI). Where these intervals do not overlap with  $d = 0$  or each other the effect or difference is significant. Treatments marked with the same letter were not statistically different ( $P > 0.05$ ). Sample size ( $n$  records) is indicated underneath each CI.

groups. The fungicide MOAs with AIs represented by enough records for analysis were: cytoskeleton and motor protein (group B), respiration (group C), signal transduction (group E), sterol biosynthesis in membranes (group G), and multi-site activity (group M).

Within fungicide AIs, boscalid, pyrocymidone, fenarimol, myclobutanil, propiconazole, triadimenol, captan, dithianon, metiram, and dodine did not increase phytoseiid mortality (Fig. 3). Group C contained both the most and least harmful AIs, binapacryl and boscalid. Binapacryl is a combination miticide/fungicide, whereas boscalid is a fungicide only. These fungicides are in separate chemical classes; chemical properties of the AI may be more informative as to why some products are more toxic than others, especially in the case of pesticides not intended to target arthropods. For instance, it was speculated that herbicide lipophilicity or volatility may impact phytoseiid toxicity.<sup>41</sup> However, generalizations about MOAs may be useful in making management recommendations. Of the MOA groups represented by more than two AIs, group G had the lowest proportion with  $> 0$  effect size and groups B and C had the highest.

Previous research on natural enemies highlights the role of chemical class, rather than MOA, in fungicide toxicity. For all natural enemies in SELCTV, fungicides that were more toxic were those in chemical classes that also contain insecticides and miticides, such as nitrophenols and carbamates.<sup>23</sup> A literature review

on phytoseiids associated with soft fruit production found that most fungicides were fairly harmless.<sup>42</sup> The effects of mancozeb were highly variable, which the authors noted was likely due to variations in species and life stage tested.<sup>42</sup> Like the present meta-analysis, the review listed boscalid, iprodione, myclobutanil, propiconazole, copper salts, and diiodine as fairly harmless fungicides. However, although pyraclostrobin and azoxystrobin were designated as harmless in the review,<sup>42</sup> these fungicides were grouped with the more harmful AIs in our meta-analysis, potentially due to their large variance (Fig. 3). A review of pesticide effects on *N. fallacis*, which is considered a pesticide-sensitive species,<sup>44</sup> found benomyl and captan to be the most toxic fungicides and propiconazole, dithianon, and metiram to be the least toxic.<sup>30</sup> These results correspond well to those in the meta-analysis, with the exception of captan, which was found to have no effect in our study, but was rated intermediate in the review.<sup>30</sup> Given the higher susceptibility of *N. fallacis*, this result is not surprising and underscores the variability in responses to some AIs between phytoseiid species. While fungicide non-target effects are better understood than those of herbicides, they are still understudied relative to the insecticides and more work is needed to better understand how fungicides impact predatory mites.

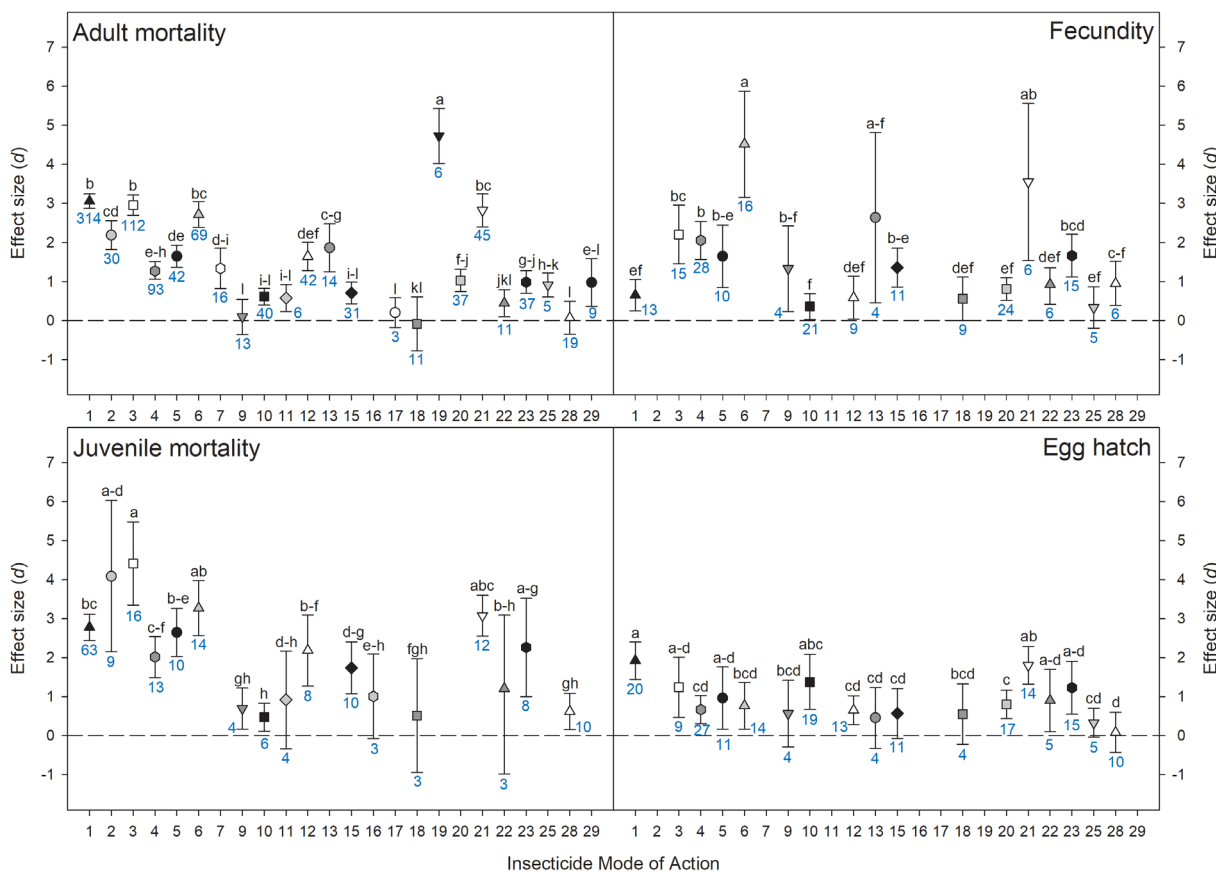


**Figure 3.** Effect sizes (*d*) of different fungicide active ingredients on combined phytoseiid adult and juvenile mortality. Effect sizes are grouped by mode of action, which is indicated at the top of the graph in capital letters, comparisons are made across all modes of action. Error bars indicate 95% bootstrapped confidence interval (CI). Where these intervals do not overlap with *d* = 0 or each other the effect or difference is significant. Treatments marked with the same letter were not statistically different (*P* > 0.05). Sample size (*n* records) is indicated underneath each CI.

### 3.4 Insecticides

In the meta-analysis, insecticides in MOA groups 9 (chordotonal organ modulators), 17 (dipteran molting disruptors), 18 (ecdysone

receptor agonists), and 28 (ryanodine receptor modulators) did not increase adult phytoseiid mortality; all other groups had an effect size > 0 (Fig. 4). Of these, Group 19 (octopamine receptor



**Figure 4.** Effect sizes (*d*) of different insecticide modes of action on phytoseiid adult mortality, juvenile mortality, fecundity, and egg hatch. Error bars indicate 95% bootstrapped confidence interval (CI). Where these intervals do not overlap with *d* = 0 or each other the effect or difference is significant. Treatments marked with the same letter were not statistically different (*P* > 0.05). Sample size (*n* records) is indicated underneath each CI.



agonists) had the highest effect size. The next most harmful groups were groups 1 (acetylcholinesterase inhibitors), 3 (sodium channel modulators), 6 (glutamate-gated chloride channel allosteric modulators), and 21 (mitochondrial complex I electron transport inhibitors, METI miticides) (Fig. 4). Juvenile mortality results were similar, with groups 2 (GABA-gated chloride channel antagonists), 3, 6, 21, and 23 (inhibitors of acetyl CoA carboxylase) causing the highest mortality (Fig. 4). Only MOA groups 11 (*Bt*), 16 (type I inhibitors of chitin biosynthesis, buprofezin), 18 (ecdysone receptor agonists), and 22 (voltage dependent sodium channel blockers) did not increase juvenile mortality.

The non-target effects of organophosphates (1), carbamates (1), and pyrethroids (3) on phytoseiids are well-documented.<sup>23,38,45</sup> Given the large effect size for octopamine receptor agonists in our meta-analysis, surprisingly little is noted in the literature regarding the toxicity of these insecticides to phytoseiids. Conversely, groups 6 and 21 are well-known for their toxicity to phytoseiids.<sup>44</sup> These pesticides are frequently used for spider mite control, but are more broad-spectrum and also control many sucking pests.<sup>19</sup> Groups 6 and 21 are among the most harmful miticides to phytoseiids (see Section 3.5 Miticides). Our results also highlight the more selective insecticide groups and agreed with Fountain and Medd,<sup>42</sup> who included *Bacillus thuringiensis* (11), diflubenzuron (15), methoxyfenozide (18), and pymetrozine (9) in a list of 'most selective' AIs.

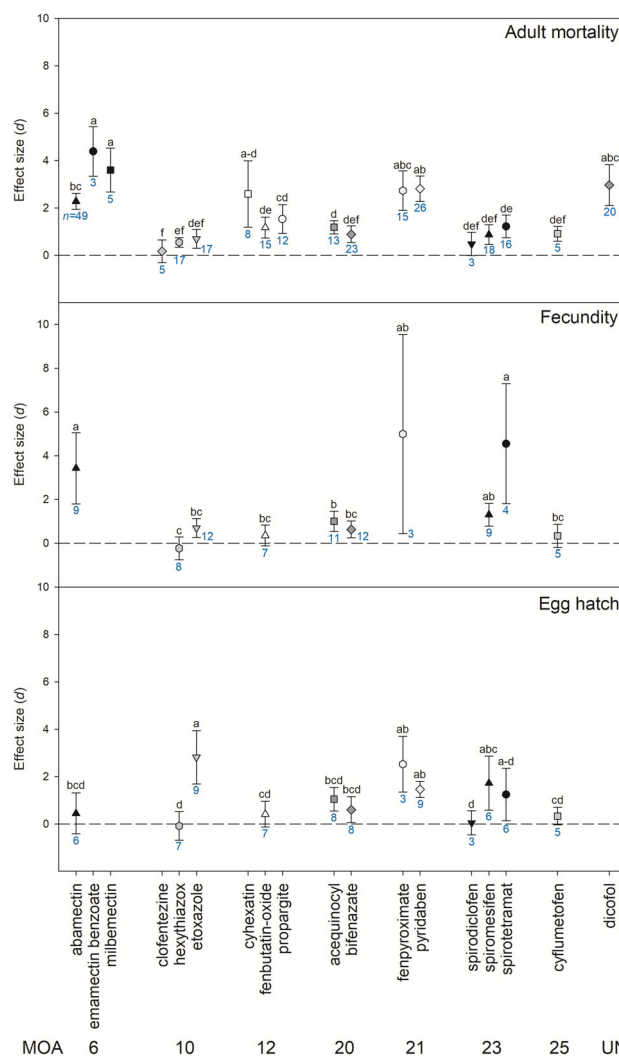
All MOA groups reduced fecundity except groups 18 and 25 (METI II) (Fig. 4). The highest fecundity effects were seen in groups 6, 13 (oxidative phosphorylation uncouplers), and 21. Fecundity effects tended to vary more in highly toxic groups, such as MOA groups 13 and 21 (Fig. 4). This could be because in assays where a field rate is tested, few females survive, resulting in higher variability due to low sample size. In groups where adult mortality is high, fecundity reduction may be because "surviving" females are moribund. Juvenile hormone mimics (6) had the strongest effect on fecundity, which is not surprising, given the role of juvenile hormone in egg production in arthropods. Groups 1, 3, 5, 10, 21, 22, and 23 caused the greatest reduction in egg hatch. Of the harmful groups, only groups 10 and 23 are designed to impact arthropod reproduction or growth. These are both miticidal (see Section 3.5 Miticides).

### 3.5 Miticides

Miticides with the greatest impact on adult mortality were emamectin benzoate, milbemectin, cyhexatin, fenpyroximate, and pyridaben (Fig. 5). Only clofentezine and spiroadiclofen did not increase adult mortality. Including those AIs, the miticides causing the least adult mortality were hexythiazox, etoxazole, bifenazate, spiromesifen, and cyflumetofen. The group 6, 21, and UN MOAs caused higher mortality than the selective miticide groups 10, 12, 20, 23, and 25.

Fenpyroximate, spirotetramat, abamectin, and spiromesifen caused the greatest reduction in fecundity (Fig. 5). Hexythiazox, fenbutatin-oxide, and cyflumetofen did not reduce fecundity. Etoxazole and bifenazate caused a significant, but minimal decrease in fecundity. In general, miticides that caused high mortality also reduced fecundity, but these effect sizes had high variance (Fig. 5). This is likely because few individuals survive field concentrations of these AIs to be later assessed for fecundity.

Etoxazole, fenpyroximate, pyridaben, spiromesifen, and spirotetramat had the greatest effect on egg hatch (Fig. 5). Abamectin, hexythiazox, fenbutatin-oxide, spiroadiclofen, and cyflumetofen did not reduce egg hatch. Within MOAs, acequinocyl and



**Figure 5.** Effect sizes ( $d$ ) of acaricide active ingredients on phytoseiid adult mortality, fecundity, and egg hatch. Effect sizes are grouped by mode of action (MOA), which are indicated at the bottom of the graph. Active ingredients have the same symbol color and shape in all panels. Error bars indicate 95% bootstrapped confidence interval (CI). Where these intervals do not overlap with  $d = 0$  or each other the effect or difference is significant. Treatments marked with the same letter were not statistically different ( $P > 0.05$ ). Sample size ( $n$  records) is indicated underneath each CI.

bifenazate (group 20) were similar, but hexythiazox and etoxazole (group 10) differed dramatically in egg hatch. Spiromesifen was more harmful than spiroadiclofen, with spirotetramat intermediate (group 23). The least harmful miticides across all three effects examined were hexythiazox, bifenazate, and cyflumetofen.

Mite growth inhibitors (group 10), organotin (group 12), and avermectins (group 6) are among the oldest miticides still in common use,<sup>19,46</sup> with the exception of broad-spectrum insecticides that have activity against mites (e.g. carbamates, pyrethroids). Historically, miticide development and adoption has been driven by spider mite resistance.<sup>46</sup> Unfortunately, this has led to many selective chemistries falling out of use because they are no longer effective on the target pest. For instance, the organotins and related compounds (propargite) are known for their selectivity.<sup>30,47,48</sup> The small effect sizes in our meta-analysis for fenbutatin-oxide and propargite support this (Fig. 5). However, the adult mortality effect size for cyhexatin was

one of the highest. Like other organotins, cyhexatin was considered a selective miticide.<sup>49</sup> Our results suggest it may be less selective than other MOA 12 compounds, although effect size variation was large and cyhexatin did not statistically differ from the other two compounds. Unlike fenbutatin-oxide, cyhexatin is no longer in use due to human health concerns. The use of fenbutatin-oxide and propargite has dramatically declined since their entry into the market due to resistance issues worldwide.<sup>46,48</sup> However, they still comprise a large portion of international miticide sales.<sup>46</sup>

Clofentezine and hexythiazox entered the market in the 1980s and hexythiazox alone dominated the miticide market from 1985 to 1990.<sup>46</sup> Hexythiazox and clofentezine (MOA 10A) are well-known for their selectivity to phytoseiids.<sup>30,44,47,48,50,51</sup> In some cases, hexythiazox even increased phytoseiid oviposition.<sup>44,51</sup> A particular benefit of these acaricides is that they sterilize spider mite eggs, which can still be consumed by phytoseiids, allowing them to remain in treated crops.<sup>47,48</sup> Like the organotins, 10A acaricide use has decreased due to widespread spider mite resistance.<sup>46</sup>

The chloride channel activators (avermectins, milbemectins; MOA 6) were introduced in the 1980s.<sup>46</sup> Prior to registration, abamectin over-use was cautioned against due to its impacts on predatory mites and because its miticidal activity at low concentrations made resistance development more likely.<sup>48</sup> Initially, it was considered selectively favorable because it was less toxic to phytoseiids than pest mites.<sup>48,52</sup> However, in crops where it was used intensively, spider mite abamectin resistance developed in less than 10 years.<sup>53</sup> The MOA 6 compounds can cause substantial phytoseiid adult mortality (Fig. 5), but past work on phytoseiids primarily focused on contact toxicity. Abamectin provides good residual pest control due to translaminar activity, but its surface residues degrade rapidly, which may allow for survival of natural enemies.<sup>54,55</sup> Therefore, field-level effects on phytoseiids may be less severe for abamectin than miticides with long-lasting residues. This is supported by research in apple, where *Typhlodromus caudiglans* (Schuster) populations rebounded more quickly in the abamectin treatment than other miticide treatments (e.g. fenpyroximate, bifentazate), despite initial rapid population decline (Bergeron and Schmidt-Jeffris, unpublished). Because of widely reported abamectin resistance in spider mites<sup>56</sup> and substantial research showing significant harm to phytoseiids, it can no longer be considered selective.

METI acaricides (MOA 21) also became available in the same time period as the MOA 6 acaricides.<sup>46</sup> Like previously popular acaricides, they were heavily relied upon until spider mite resistance developed (~1990–2000), but are still in use.<sup>46</sup> The METIs are also more broad-spectrum and used to control piercing-sucking insects. It is therefore unsurprising that these compounds are also toxic to predatory mites (Fig. 5). Miticides in this group are often found to be the most harmful.<sup>44,57–59</sup>

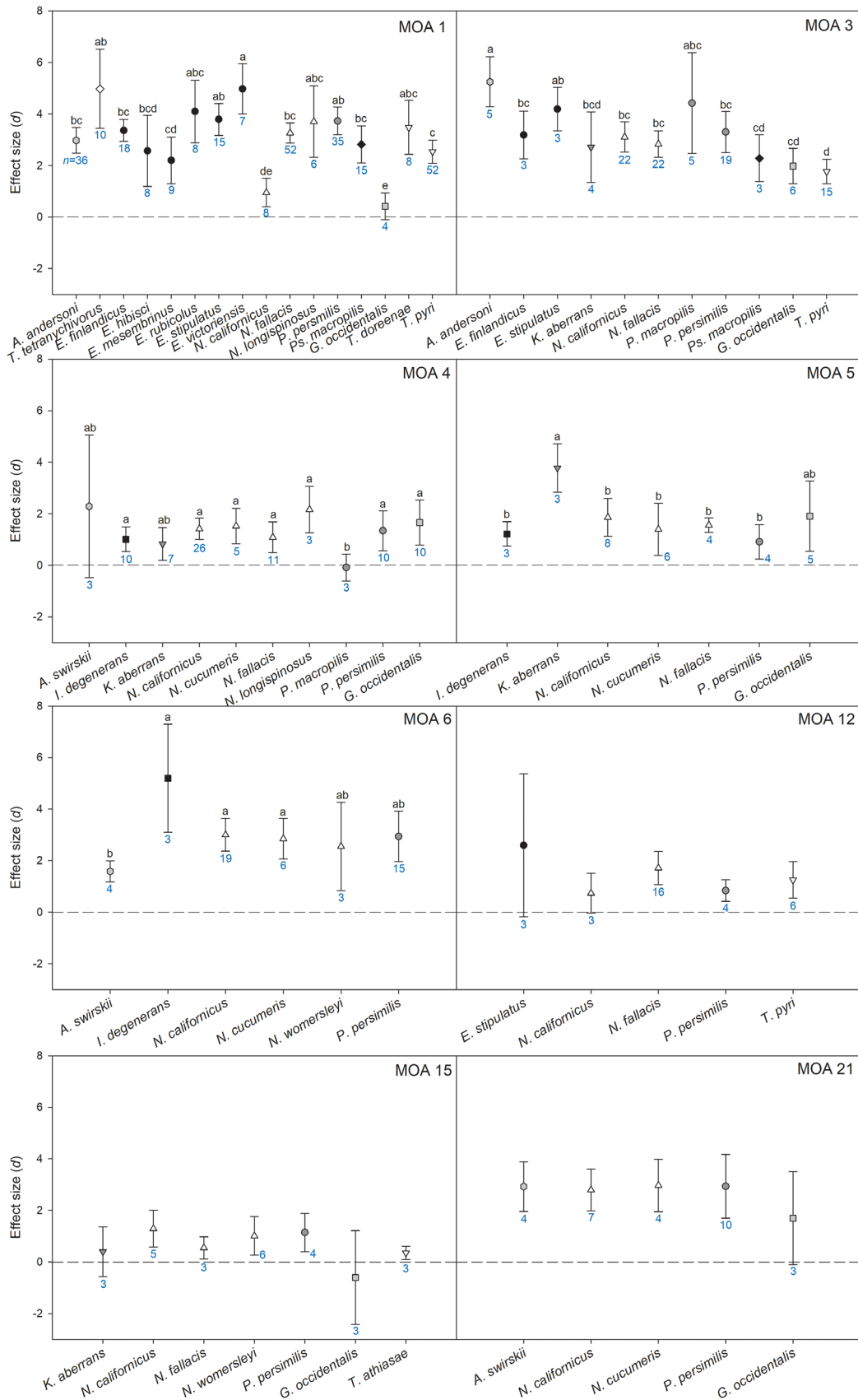
The 10B miticide, etoxazole, entered the market shortly after the MOA 6 and MOA 10A compounds.<sup>46</sup> Unlike 10A miticides, etoxazole (10B) is known for being harmful to phytoseiids, primarily through reduced egg hatch. Our meta-analysis agrees with these observations; etoxazole caused the greatest reduction in egg hatch and was significantly different from hexythiazox (Fig. 5). There is contradicting evidence on whether etoxazole should be grouped with the other mite growth inhibitors, or whether it acts as a chitin synthesis inhibitor and is similar to benzoylureas (MOA 15), in particular triflumeron.<sup>19,60</sup> One study found that triflumeron reduces phytoseiid fecundity,<sup>61</sup> but another study did not.<sup>62</sup> Another benzoylurea, novaluron, has been more thoroughly investigated. Novaluron is known to cause juvenile mortality, reduced fecundity, and reduced egg hatch in

phytoseiids.<sup>40,63,64</sup> In orchards, novaluron applications can reduce phytoseiid populations and disrupt spider mite biological control.<sup>65,66</sup> Therefore, our current knowledge of how etoxazole and benzoylureas impact phytoseiids also suggests they may have similar activity.

Bifenazate and acequinocyl are among the newer acaricides, are METI III pesticides (MOA 20),<sup>19</sup> and became available shortly after etoxazole. Both AIs are generally considered selective for phytoseiids;<sup>46</sup> the label for bifenazate mentions its selectivity and lists the specific natural enemy species it does not disrupt in the field. Bifenazate and acequinocyl were among the least harmful AIs examined in our meta-analysis (Fig. 5). Bifenazate has been successfully used with augmentative releases of phytoseiids in field tomatoes<sup>67</sup> and strawberries<sup>68</sup> and has been found to not disrupt resident phytoseiids in hops.<sup>69</sup> However, laboratory results testing impacts of bifenazate or acequinocyl on various phytoseiid species have been mixed.<sup>44</sup> There is also evidence that bifenazate can significantly decrease *N. fallacis* populations in watermelon<sup>70</sup> and *T. caudiglans* populations in apple.<sup>10</sup> Therefore, even selective acaricides may be harmful, especially to more sensitive phytoseiid species.<sup>44,71</sup>

Tetronic/tetramic acid derivatives (MOA 23) make up the largest share of the worldwide miticide market.<sup>46</sup> These miticides inhibit fatty acid synthesis, resulting in toxicity to immature phytophagous mites and reduction in fecundity and egg hatch in treated females.<sup>19</sup> Spirodiclofen is somewhat more selective than spiromesifen and spirotetramat; the former is primarily used for controlling phytophagous mites, whereas the latter two compounds are also used for piercing-sucking insects.<sup>46</sup> Our meta-analysis also found spirodiclofen to be somewhat more selective to phytoseiids than the other MOA 23 acaricides; it was one of the only miticides that did not cause an increase in adult or egg mortality, based on overlap of its 95% CI with zero (Fig. 5). However, spirodiclofen did not significantly differ from the other tetronic/tetramic acid derivatives in adult mortality effect size and did not differ from spirotetramat in impacts on egg hatch (Fig. 5). All three miticides had minor impacts on adult mortality, whereas spiromesifen and spirotetramat were among the most harmful to fecundity and egg hatch (Fig. 5). Given that their primary activity is against immature stages, more studies should be conducted examining these effects for multiple phytoseiid species, as results seem to be variable.<sup>44,59,72</sup> Both spirotetramat<sup>73</sup> and spiromesifen<sup>44</sup> have an unfavorable selectivity ratio when comparing phytoseiid and spider mite toxicity in laboratory assays. While spirotetramat did not harm released *Amblyseius swirskii* Athias-Henriot in pepper,<sup>74</sup> it was associated with reduced *N. fallacis* populations in watermelon.<sup>70</sup> We are unaware of additional field studies examining impacts of MOA 23 acaricides on phytoseiid populations. More research is needed to determine if impacts on immature phytoseiids are substantial enough to disrupt biological control.

Cyflumetofen (MOA 25) is one of the newest miticides<sup>46</sup> and is marketed as selective. There are few studies on its impacts on predatory mites, but all prior work indicates that it is one of the most selective miticides available, even in worst-case-scenario, laboratory exposures.<sup>44,75,76</sup> In our meta-analysis, it was always among the least harmful miticides and had no impact on fecundity or egg hatch (Fig. 5). Because the role of phytoseiids in preventing pest mite outbreaks is well-understood, it is also established that new miticides should be both effective against their target pest and not harmful to phytoseiids.<sup>19</sup> Croft<sup>77</sup> noted that 'Selectivity to predatory mites has become ... a prerequisite for a new acaricide' and that selectivity had become a key point



**Figure 6.** Effect sizes ( $d$ ) of the eight insecticide modes of action (MOAs) most represented in the dataset on phytoseiid adult mortality by species. Species within the same genus have the same symbol shape and color. Error bars indicate 95% bootstrapped confidence interval (CI). Where these intervals do not overlap with  $d = 0$  or each other the effect or difference is significant. Treatments marked with the same letter were not statistically different ( $P > 0.05$ ). Sample size ( $n$  records) is indicated underneath each CI. *Ps.* = *Phytoseius*, *P.* = *Phytoseiulus*.



in advertising new chemicals, which was due to the demonstrated economic benefits: conservation of predatory mites saves growers money.<sup>77</sup> As new miticides are developed, it will likely be with an eye towards further increasing selectivity.

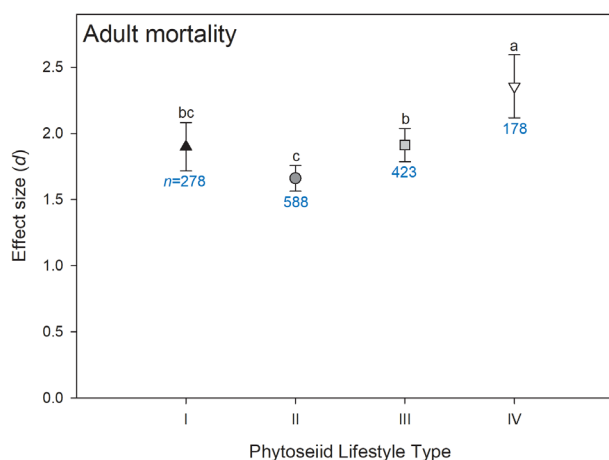
### 3.6 Species identity

This analysis examined differences between species in adult mortality for the eight most tested MOA groups. Older MOAs (groups 1 and 3) had a greater variety of species tested than new MOAs (Fig. 6). Within the entire meta-analysis database (all MOA groups, effect types), the most common species tested were *Phytoseiulus persimilis* Athias-Henriot, *N. californicus*, *N. fallacis*, *G. occidentalis*, and *T. pyri*. This is likely due to their importance in augmentation in glasshouses (*P. persimilis*, *N. californicus*, *N. fallacis*) or their dominance in perennial crops that rely on selective pesticide applications to maintain biological control (*N. fallacis*, *G. occidentalis*, *T. pyri*). There were no differences between species in adult mortality for MOA 12, 15, or 21 (Fig. 6), potentially indicating that current knowledge of these non-target effects may generalize across many species.

For MOA 1, the most sensitive species were *Transeius tetranychivorus* (Gupta), *Euseius rubicolus* (van der Merwe & Ryke), *E. stipulatus* (Athias-Henriot), *E. victoriensis* (Womersley), *Neoseiulus longispinosus* (Evans), *P. persimilis*, and *Typhlodromus doreanae* Schicha. *Galendromus occidentalis* and *N. californicus* were the least sensitive; the effect size CI for *G. occidentalis* included zero (Fig. 6). There was less variability between species in phytoseiid response to pyrethroids (MOA 3). The most sensitive species were *Amblyseius andersoni* (Chant), *E. stipulatus*, and *Phytoseiulus macropilis* (Banks) (Fig. 6). The least sensitive species were *Typhlodromus pyri*, *G. occidentalis*, *Phytoseiulus macropilis* (Banks), and *Kampimodromus aberrans* (Oudemans). For the remaining MOA groups (4, 5 and 6), differences between species were relatively minor (Fig. 6). *Phytoseiulus macropilis* was less sensitive to neonicotinoids (MOA 4) than most other species tested, but the sample size was relatively small and all records used represent one population. *Kampimodromus aberrans* was more sensitive to spinosyns (MOA 5) than most other phytoseiids and *A. swirskii* was less sensitive to MOA 6 insecticides (Fig. 6).

Prior work has compared pesticide sensitivity between small groups of phytoseiid species. Several previous studies have found *P. persimilis* to be the least sensitive when compared to other phytoseiids,<sup>23,44,78</sup> but a study comparing citrus phytoseiids found *P. persimilis* to be the least pesticide tolerant (versus *E. stipulatus* or *N. californicus*).<sup>72</sup> Our meta-analysis suggests *P. persimilis* is moderately sensitive to pesticides (Fig. 6), but this varies with the MOA group tested. Prior literature has ranked *N. fallacis*<sup>44</sup> and *A. andersoni*<sup>79</sup> among the more sensitive species, which is somewhat consistent with the meta-analysis results (Fig. 6). Like the meta-analysis (Fig. 6), prior work has regularly found *G. occidentalis* to be less pesticide sensitive than other phytoseiids, particularly in regards to MOA 1 insecticides.<sup>10,71,80</sup>

Lack of pesticide sensitivity in *G. occidentalis* is likely due to resistance development; field-selected resistance to azinphosmethyl, carbaryl, and a variety of pyrethroids has been well-documented in this species.<sup>81,82</sup> Phytoseiids appeared to be more similar in their response to newer MOAs compared to older MOAs (Fig. 6). This may indicate that many observed differences between species are due to resistance development, which is more likely to have occurred in older pesticides. However, the pesticide exposure history of the population used in each study was not always described, so it is impossible to be certain if differences between



**Figure 7.** Effect sizes ( $d$ ) for all pesticide effects on adult mortality for the four phytoseiid lifestyle types. Error bars indicate 95% bootstrapped confidence interval (CI). Where these intervals do not overlap with  $d = 0$  or each other the effect or difference is significant. Treatments marked with the same letter were not statistically different ( $P > 0.05$ ). Sample size ( $n$  records) is indicated underneath each CI.

species are due to differences in innate susceptibility or resistance. It is also difficult to determine which factors have the greatest influence on phytoseiid resistance development. For instance, phytoseiid species from perennial crops may be more likely to develop resistance than those from annual crops because their more stable habitat results in yearly exposure of the same populations; phytoseiid mites on tree fruit were the first insecticide-resistant natural enemies discovered.<sup>83,84</sup> Conversely, species of phytoseiids commonly used in augmentation may appear more sensitive if insectary-reared populations that are pesticide-naïve are tested.

### 3.7 Lifestyle type

Diet specialization may also affect both innate susceptibility and resistance development to pesticides. Phytoseiids have been classified into four categories based on diet specialization (Types I–IV).<sup>27,28</sup> Type I species are the most specialized, thriving primarily on *Tetranychus* spp. spider mites, Type II prefer *Tetranychus*, but will consume other species of tetranychids and other mite prey, Type III tend to prefer non-web-spinning spider mites or are insect predators and can reproduce on pollen diets, and Type IV species are specialized pollen feeders and generalist predators.<sup>27,28</sup> In our meta-analysis of adult mortality, generalists tended to be more sensitive than specialists; Type II phytoseiids were less sensitive than Type IIIs, which were less sensitive than Type IVs (Fig. 7). Type I phytoseiids were intermediate in pesticide sensitivity to Types II and III.

Prior reviews have reached opposite conclusions regarding the propensity of resistance development in specialist versus generalist phytoseiids. Generalists are more likely to feed on plant products and therefore more likely to encounter plant toxins, potentially preadapting them to pesticides.<sup>38</sup> It has also been argued that specialist predators are less likely to develop resistance because they cannot survive without the presence of prey, which would die after a pesticide application.<sup>38</sup> However, this fails to account for the ability of *Tetranychus* spp. (particularly *Tetranychus urticae* Koch) to rapidly develop resistance to pesticides. Provided that the prey develop resistance first, specialist predators then also can develop resistance. Therefore, close association with

*Tetranychus* could increase the likelihood that specialist phyto-seiids develop resistance.<sup>8</sup> Evidence for this has been found in perennial agroecosystems that are minimally sprayed, where phyto-seiid communities are dominated by generalists and spider mites are not abundant.<sup>18,85</sup> The results from our meta-analysis support that diet specialization on *Tetranychus* spp. is associated with either low innate pesticide sensitivity or higher incidence of resistance development (Fig. 7). Future work should directly compare phyto-seiid species from similar systems for pesticide resistance to determine the extent to which this occurs. Studies could also compare mechanisms of pesticide detoxification in related phyto-seiid species with different diet specializations.

## 4 CONCLUSIONS

As new pesticides are adopted and as new phyto-seiid species are reared for augmentation or determined to be important for conservation biocontrol, agricultural researchers and biological control practitioners must constantly renew the pesticide non-target effects literature.<sup>42</sup> Our meta-analysis shows that general recommendations about specific pesticides or pesticide groups can be made, but also highlights that the unique biology of some species may contradict overall trends. Additional research on pesticide non-target effects on phyto-seiids should focus on herbicides, sublethal effects, and the newer insecticide/miticide MOAs, which are understudied. Prior work has also heavily focused on a short list of phyto-seiid species, the majority of which are *Tetranychus* spp. specialists. Research on additional species, especially Type IV phyto-seiids, will be needed as the role of generalist phyto-seiids in agroecosystems becomes better understood.

In focusing on the most common and more standardized type of non-target effects testing, direct sprays or fresh residues, our meta-analysis results are limited in that they do not account for residue duration; some pesticides degrade more rapidly in the field than others. Additionally, these assays do not test imperfect pesticide coverage, which creates unsprayed refuges that can interact with phyto-seiid behavior via repellency to potentially alter outcomes of field applications.<sup>86</sup> Furthermore, in specialist phyto-seiids, effective miticides may eliminate prey, resulting in local predator extinction, despite no harmful effects in assays. These complicating factors make it difficult to predict how pesticides will impact natural enemies and secondary pest outbreaks.<sup>87</sup> This further emphasizes the need for ongoing summaries of pesticide non-target effects trends, but also highlights the importance of field studies testing specific key natural enemy and pest combinations in many agroecosystems.

## ACKNOWLEDGEMENTS

The authors thank J. Nelson-Ichido, M. Nelson-Ichido, and M. Seo for providing language translations. We also thank D. Ludwick for creating the spider mite illustration used in the graphical abstract. Graphical abstract created with BioRender.com. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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\*Indicates a study used in the meta-analysis.

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