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Large-plot field studies to assess impacts of newer insecticides on non-target arthropods in Western U.S. orchards

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3 **Large-plot field studies to assess impacts of newer insecticides on non-target arthropods in**

4 **Western U.S. orchards**

5

6

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23

24 *Running head:* P.W. Shearer et al. Testing impacts of reduced risk insecticide in orchards

25

26 **Abstract**

27

28 The non-target impacts of two reduced risk insecticides, chlorantraniliprole and spinetoram, were
29 evaluated for two years in Oregon pear and California walnut orchards. Experiments were
30 conducted in large replicated plots (approximately 0.25-0.4 ha) to assess the impact of these two
31 insecticides on natural enemies of secondary pests when applied against codling moth, *Cydia*
32 *pomonella*. Cumulative insect days (CID) of secondary pests and natural enemies were
33 calculated from leaf samples, plant volatile traps, beat trays or cardboard trunk bands. Ratios of
34 natural enemies and prey were also calculated. Results from these field studies demonstrate that
35 applications of chlorantraniliprole can reduce abundance of predatory Neuroptera and that
36 spinetoram negatively impacts parasitic Hymenoptera. However, these trends did not always
37 occur each year. As a percentage among all trials within a crop, there were more treatment
38 differences for natural enemy/prey ratios (50 and 33% for pears and walnut plots, respectively)
39 than for natural enemy CIDs (25 and 13% for pears and walnut plots, respectively). It is likely
40 that unseasonably cool weather during the two years of this study impacted both pest and natural
41 enemy abundance. The intrinsic value of large-plot field studies is discussed.

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46 Key words biological control; disruption; reduced risk insecticide; integrated pest
47 management; natural enemy; secondary pest

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49 **1. Introduction**

50

51 Integrated pest management (IPM) programs in orchards have evolved rapidly in the past
52 20 years in large part because the Food Quality Protection Act (FQPA) (US EPA, 1996) changed
53 the suite of insecticides available to growers (Agnello et al., 2009; Jones et al, 2009, 2010). Of
54 all the organophosphorus insecticides replaced by FQPA, the loss of azinphosmethyl was of
55 greatest concern to IPM practitioners because it had been used regularly in tree fruit for control
56 of codling moth, *Cydia pomonella* (L.) (Lep.: Tortricidae), since the 1950s (Whalon et al., 1999).

57 To fill the void left after the removal of azinphosmethyl, IPM practitioners began testing a
58 suite of “reduced risk” pesticides as replacements (Viray and Hollingsworth, 2009). These new
59 insecticides were often assumed to be less harmful to natural enemies because of results from
60 previous field studies (Agnello et al., 2009; Atanassov et al., 2002; Roubos et al., 2014).
61 However, laboratory studies have demonstrated detrimental effects of reduced risk insecticides
62 on key natural enemies (Amarasekare and Shearer, 2013a,b; Kim et al., 2006), including studies
63 presented in this issue (Amarasekare et al., this issue; Beers et al., this issue a,b; Mills et al., this
64 issue a). Interestingly, the safety of these products to natural enemies is not a specific criterion
65 used by the United States Environmental Protection Agency (EPA) to define this group of
66 pesticides (National Research Council, 2000). Pesticides can be classified as reduced risk if they
67 meet at least one of nine criteria including, but not limited to, reduced impact on human health,
68 replacement of chemicals that pose health risks to workers, reduced effects on non-target
69 organisms, or presumed compatibility with IPM.

70 While IPM has been practiced for more than 50 years (Stern et al., 1959), the foundation of
71 tree fruit IPM was laid by Hoyt (1969), who developed a program that integrated chemical
72 control of insects (primarily codling moth), with the use of conservation biological control to

73 suppress phytophagous mites. Hoyt demonstrated that proper choice of insecticides, rates, and
74 application methods, allowed *Galendromus* [= *Typhlodromus*] *occidentalis* (Nesbitt) (Acari:
75 Phytoseiidae) to survive and suppress populations of the McDaniel spider mite, *Tetranychus*
76 *mcdanieli* McGregor (Acari.: Tetranychidae), and the European red mite, *Panonychus ulmi*
77 (Koch) (Acari: Tetranychidae) in Washington apple orchards. The methods outlined by Hoyt
78 (1969) have been adapted to IPM programs on tree fruits around the world (Apple and Smith,
79 1976).

80 Orchard IPM programs include the use of pesticides and their selection should be based on
81 efficacy against target pests and safety towards natural enemies. Early examples of successful
82 orchard IPM programs involved applications of pesticides that did not significantly harm natural
83 enemies of phytophagous mite pests in apple (Croft and Soloman, 1981). Most of those early
84 examples were based upon physiological selectivity when selective miticides killed targeted
85 mites but not predatory mites. Later, several predator mite species evolved resistance to key
86 insecticides including azinphosmethyl; a result of its long history of seasonal use against primary
87 apple pests (Croft and Hoyt, 1978). Other natural enemies, including *Pnigalio flavipes*
88 (Ashmead) (Hym.: Eulophidae), a parasitoid of the leafminer, *Phyllonorycter elmaella* (Doganlar
89 and Mutuura) (Lep.: Gracillariidae) (Barrett and Brunner, 1990) and *Trioxys pallidus* (Haliday)
90 (Hym.: Braconidae), a parasitoid of walnut aphid, *Chromaphis juglandicola* (Kaltenbach) (Hem.:
91 Aphididae), have also developed resistance after repeated long-term exposure to azinphosmethyl
92 (Hoy and Cave, 1989). More recently, insecticide resistance management tactics have been
93 incorporated into many new insecticide labels to delay resistance development in primary pests.
94 These refined use patterns and resistance management tactics will likely prevent natural enemy
95 populations from developing natural field resistance (Jones et al., 2009).

96 Now that azinphosmethyl and several other organophosphorus insecticides have been
97 removed from use and replaced by newer insecticides, growers and pest control advisors need
98 more information on how to use them. Despite their efficacy against primary pests, some of
99 these newer insecticides have been shown in the laboratory to have lethal and sub lethal effects
100 on key natural enemies. In those studies, spinetoram was more toxic to parasitic Hymenoptera
101 than chlorantraniliprole (Amarasekare et al., this issue; Beers et al., this issue a,b; Beers and
102 Schmidt, 2014; Mills et al., this issue a), while chlorantraniliprole had a greater negative impact
103 on predatory Neuroptera than did spinetoram (Amarasekare and Shearer, 2013 b; Amarasekare et
104 al., this issue). While laboratory studies provide useful knowledge about potential impacts of
105 pesticides on natural enemies, field studies are necessary to verify whether similar effects occur
106 under orchard conditions. That is the purpose of this study.

107 This study, in addition to a similar study conducted in apple orchards (Beers et al., this
108 issue), was part of a comprehensive USDA-NIFA Specialty Crops Research Initiative effort to
109 enhance biological control in western orchards (Jones et al., this issue). Our study focused on
110 whether applications of select reduced risk insecticides that were targeted for *C. pomonella*
111 management caused outbreaks of one or more secondary pests by disrupting natural enemies in
112 pear orchards in Oregon and walnut orchards in California.

113

114 **2. Materials and methods**

115

116 *2.1. Site descriptions and experimental treatments*

117

118 Studies to assess the impact of reduced-risk insecticides, applied against *C. pomonella*, on
119 the abundance of key natural enemies and secondary pests found in pear and walnut orchards
120 were conducted during 2010-11. Pear orchards were located in Hood River, OR while the
121 walnut sites were located in Hamilton City, CA.

122 In OR, field trials were set up using a randomized complete block design in both years.
123 The 2010 study was conducted in a 2.4 ha planting of mature ‘D’Anjou’ pear trees interplanted
124 with ‘Bartlett’ pear pollinizers with a tree spacing of 4.6×7.9 m. This pear orchard had for the
125 previous three years been treated with mating disruption for *C. pomonella*, but was not under
126 mating disruption during the course of this study. The experiment was set up with three
127 treatments and three replicate blocks with insecticide treatments applied to plots of
128 approximately 0.27 ha in size in each block. In 2011, a second study was set up in a different
129 orchard. This 2.2 ha planting of mature ‘D’Anjou’ pear trees with ‘Bartlett’ pear pollinizers,
130 with a tree spacing of 3.0×6.0 m, was divided into 4 replicate blocks and each block contained
131 two treatments. Individual plots were approximately 0.28 ha in size. This orchard was in its
132 sixth year of using codling moth mating disruption during our study.

133 Insecticides used in the pear studies were timed for early egg hatch of first-generation *C.*
134 *pomonella* (Brunner et al. 1987) and applied using a Rears Pack Tank research sprayer (Rears
135 Manufacturing, Eugene, OR) delivering 935 L/ha. In 2010, the three experimental treatments
136 (and application rate/ha) consisted of chlorantraniliprole (Altacor[®] 35 WDG, 315 g/ha, DuPont
137 Crop Protection, Wilmington, Delaware, USA), spinetoram (Delegate[®] 25 WG, 490 g/ha, Dow
138 Agro Sciences LLC, Indianapolis, Indiana, USA) and cyantraniliprole (Exirel[®] 100 g [AI] SE,
139 1.242 L/ha, DuPont Crop Protection, Wilmington, Delaware, USA) and were applied 16 Jun
140 (217 DD after biofix) and 15 days later on 1 Jul 2010. One application of methoxyfenozide

141 (Intrepid 2F 877 ml/ha) was applied against second-generation codling moth on 3 Aug (990 DD)
142 across all plots. In 2011, the second study, which was set up in a different 2.2 ha planting of
143 mature 'D'Anjou' pear trees with 'Bartlett' pear pollinizers, was divided into 4 blocks and each
144 block contained two treatments. Individual plots were approximately 0.28 ha. The experimental
145 treatments consisted of chlorantraniliprole (315 g/ha) and spinetoram (490 g/ha) and were
146 applied 17 Jun (231 DD) and 23 days later on 10 Jul 2011. Trees were planted on 3×6 m grid.
147 Both pear orchards received standard disease and insecticide sprays from dormant through
148 shortly after petal-fall.

149 In CA, three field trials were conducted as randomized complete block designs. In 2010, a
150 single field trial was conducted in a 10.12 ha orchard of 'Vina' walnuts, with a tree spacing of
151 6.1 × 6.1m, to compare three experimental treatments; two insecticides targeting codling moth
152 and a no-insecticide control. The three treatments were applied to 0.4 ha plots in each of four
153 replicate blocks in one half of the orchard. In 2011, two trials were conducted, the first in the
154 other half of the same 10.12 ha 'Vina' orchard used in 2010 (orchard A), and the second in part
155 of a 20.23 ha orchard with a 7.6 × 7.6m tree spacing of 'Serr' walnuts (orchard B). In orchard A
156 we compared three treatments (two insecticides plus a control) in 0.4 ha plots in four replicate
157 blocks, and in orchard B we compared four treatments (three insecticide combinations plus a
158 control) in 0.61 ha plots in three replicate blocks. All three orchards used codling moth mating
159 disruption and applications of a combination of mancozeb plus copper hydroxide for control of
160 walnut blight, *Xanthomonas campestris* pv. *juglandis*, early in the season.

161 Insecticide treatments were timed for egg deposition or larval hatch of codling moth in
162 the first two generations and were applied using grower-operated speed sprayers delivering a
163 volume of 935L/ha. In 2010, the two insecticides used were spinetoram (Delegate[®] 25 WG, 448

164 g/ha) applied at 650 DD after biofix of the first generation (1B flight) and 300 DD after biofix
165 for the second generation (2A flight), and chlorantraniliprole (Altacor[®] 35 WDG, 280 g/ha)
166 applied at 500 DD after biofix of the first generation (1B flight) and 150 DD after biofix for the
167 second generation (2A flight). For orchard A in 2011, the two insecticides were
168 chlorantraniliprole (Altacor[®] 35 WDG, 263 g/ha) and chlorantraniliprole combined with lambda-
169 cyhalothrin (Voliam Xpress[®], 876 g/ha, Syngenta Crop Protection, LLC, Greensboro, NC), both
170 applied at 500 DD after biofix of the first generation (1B flight) and 150 DD after biofix for the
171 second generation (2A flight). For orchard B in 2011, four applications were made in total,
172 timed at 200 DD and 550 DD after biofix for both generations. One insecticide combination
173 consisted of half rates of spinetoram applied twice in the first generation and half rates of
174 chlorantraniliprole applied twice in the second generation, and a second insecticide combination
175 consisted of the reverse (chlorantraniliprole applications followed by spinetoram applications).
176 A third insecticide combination, the grower standard, consisted of two applications of lambda-
177 cyhalothrin (Warrior II[®], Syngenta Crop Protection, LLC, Greensboro, NC), 183 ml/ha) in the
178 first generation followed by one application of chlorpyrifos (Warhawk[®], 4.7L/ha, Loveland
179 Products, Greeley, CO) in the second generation (200DD).

180

181 2.2. Secondary pest monitoring

182

183 In pear, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae) nymphs were sampled
184 weekly from ‘D’Anjou’ trees by collecting 180 or 240 leaves per treatment (3 leaves [basal,
185 medial and distal] per terminal shoot × 2 terminal shoots per tree × 10 trees per plot × 3 or 4
186 plots/treatment). Leaves were brought back to the lab in coolers and *C. pyricola* nymphs were

187 counted using a stereo-microscope. Sampling started in early-mid June and ended in September
188 both years.

189 In the walnut system, several secondary pests were monitored including walnut aphid, *C.*
190 *juglandicola*, dusky-veined aphid, *Panaphis juglandis* (Goeze) (Hemiptera: Aphididae),
191 twospotted spider mite, *Tetranychus urticae* Koch (Acar.: Tetranychidae) and European red mite
192 *P. ulmi*. These were sampled every two weeks by collecting 45 or 60 leaves per treatment (three
193 compound leaves per tree × 5 trees per plot × 3 or 4 plots/treatment). The two aphid species
194 were first counted directly in the field before the leaves were placed in coolers and returned to
195 the laboratory where they were brushed to give a single count per plot for the two mite species.
196 Collections started in early May and continued until the end of September in both years.

197

198 2.3. Natural enemy monitoring

199

200 Natural enemy abundance was monitored several different ways. Natural enemies were
201 counted and collected in pear orchards during weekly beat tray sampling and collections of
202 corrugated cardboard bands placed around tree trunks. In walnuts, natural enemies were
203 sampled from leaves collected every two weeks. Abundance of natural enemies were also
204 estimated from sticky traps baited with one of several plant volatile lures (PV) deployed in both
205 crops.

206 In pears, *Trechnites* spp. (Hym.: Encyrtidae; a parasitoid of pear psylla), predatory
207 Heteroptera (including adult and immature *Campylomma verbasi* (Meyer), *Deraeocoris brevis*
208 *piceatus* (Knight) (Hem.: Miridae), *Geocoris* spp. (Hem.: Lygaeidae), and spiders (Araneae)

209 were monitored weekly with 15 beat tray samples (Burts and Retan, 1973) per plot (45 trays per
210 treatment in 2010; 60 trays per treatment in 2011).

211 Cardboard bands were deployed in pears to measure the abundance of European earwigs,
212 *Forficula auricularia* Linnaeus (Derm.: Forficulidae). They were made from 7.6 cm wide
213 corrugated wrap (Model S-11450, ULINE, Chicago, IL) cut into 3.8 cm wide strips. These
214 bands, containing one smooth side and one corrugated side, were wrapped around pear tree
215 trunks with the smooth side out and fastened to the bark with 1 cm deep staples. Fifteen
216 cardboard bands per plot ($n = 45$ per treatment) were deployed in 2010 and 10 bands per plot
217 were deployed in 2011 ($n = 40$ per treatment). Bands were replaced at weekly intervals. The
218 cardboard bands were brought back to the laboratory in coolers and the bands from each plot
219 were placed into a large ($33 \times 33 \times 53$ cm) plastic container where they were misted with water
220 from a spray bottle. The water relaxed the glue so the two layers of cardboard could be pulled
221 apart for inspection. The abundance of *F. auricularia* captured in the bands were pooled per plot
222 and recorded.

223 In walnuts, *C. juglandicola* mummies parasitized by *T. pallidus* were counted directly in
224 the field from the leaf samples collected for counts of aphids (see above). Phytoseiid mites were
225 assessed at the same time as the tetranychid mites using the mite brushing machine as previously
226 discussed.

227 Plant volatile (PV) traps were used to assess abundance of adult predatory Neuroptera
228 (primarily *Chrysoperla* spp. and some Hemerobiidae), predatory Syrphidae (primarily
229 Syrphinae) and parasitic Hymenoptera in the various treatments in both crops. Large white
230 plastic delta traps with sticky liners (Suterra, Bend, OR) were used in 2010 and white or yellow
231 sticky cards (Alpha Scents Inc., West Linn, OR) in 2011. The traps were constructed and

232 deployed as described by Jones et al. (this issue a). In 2010, the traps were baited with one of
233 four synthetic plant volatile blends, 1) GMP [geraniol (Sigma-Aldrich Corp. St. Louis, MO), +
234 methyl salicylate (Sigma-Aldrich Corp. St. Louis, MO) + 2-phenylethanol (Sigma-Aldrich Corp.
235 St. Louis, MO)] with components in separate dispensers, 2) acetophenone (Fisher Scientific,
236 Pittsburg PA), 3) squalene (Sigma–Aldrich, St. Louis, MO) or 4) phenylacetaldehyde (Fisher
237 Scientific, Pittsburg PA). In 2011, two trap types were deployed, white sticky cards with the
238 GMP lure and a blank yellow sticky card. In both crops and years, one of each type of trap was
239 deployed in each replicate plot. The sticky cards were replaced weekly in pears and every two
240 weeks in walnuts then covered with clear Saran™ wrap (S. C. Johnson & Son, Inc. Racine, WI)
241 and frozen at -10°C until natural enemy taxa could be identified. Lures were changed monthly
242 and traps were changed and rotated to adjacent trees when serviced. Numbers of captured target
243 insects were pooled across trap types within each replicate plot.

244

245 *2.4. Data analysis*

246

247 The monitoring data from the orchards were summarized each year using cumulative
248 insect-days (CID) to provide a season-long estimate of the potential for secondary pest damage
249 and biological control by natural enemies (Jones and Parrella 1983, Ruppel 1983). CIDs were
250 estimated as the average population density between two consecutive sampling dates multiplied
251 by the number of intervening days and summed over the entire sampling period:

252

$$\text{CID} = \sum 0.5(P_a + P_b)D_{a-b}$$

253 where P_a is the population density (mean arthropods/per unit sampled) at time a , P_b is the
254 population density at time b , and D_{a-b} is the number of days between times a and b . For

255 clarification, seasonal abundance of phytophagous and predatory mites were estimated using the
256 same formula above and referred to as cumulative mite-days (CMD).

257 A season-long estimate of the percent parasitism of walnut aphids was obtained by dividing
258 the cumulative number of *C. juglandicola* mummies by the combined cumulative numbers of *C.*
259 *juglandicola* and mummies. Predator/prey ratios were calculated by dividing the CID (or CMD)
260 of a particular natural enemy by the CID (or CMD) of a particular prey.

261 Cumulative insect-days, CMD, and predator/prey ratios were analyzed using the Statistical
262 Analysis System (SAS 2014). PROC GLIMMIX was used to conduct generalized linear mixed
263 effects models, using insecticide treatments as the fixed effect and replicate blocks as a random
264 effect. CID and CMD data were log transformed to meet the assumptions of normality. Percent
265 parasitism was analyzed similarly, but using a binomial distribution and non-transformed data.
266 Treatment means were separated using pairwise comparisons of least-squares means ($P \leq 0.05$).

267

268 **3. Results**

269

270 *3.1. Secondary pest monitoring*

271

272 Densities of *C. pyricola* nymphs per pear leaf were extremely low in both pear orchards
273 (maximum on any one sample date was < 0.4 and 0.25 nymphs per leaf in 2010 and 2011,
274 respectively) and supplemental summer sprays were not required. There were no statistical
275 differences in the average CID for *C. pyricola* nymphs between insecticide treatments in either
276 year. In 2010, *C. pyricola* nymph CID levels were 17.7 ± 5.0 , 17.6 ± 0.8 , and 13.6 ± 1.7 for
277 chlorantraniliprole, cyantraniliprole and spinetoram, respectively ($F = 0.22$, $df = 2, 4$, $P = 0.82$).

278 Similarly, in 2011 there were no statistical differences in *C. pyricola* nymph CIDs between the
279 chlorantraniliprole and spinetoram treatments, which were 6.3 ± 1.5 and 5.3 ± 2.1 , respectively
280 ($F = 0.98$, $df = 1, 3$, $P = 0.40$).

281 In the 2010 walnut study, *C. juglandicola* CIDs were higher in the spinetoram treatment
282 than in the other two treatments (Table 1). There were no treatment differences in CIDs for *P.*
283 *juglandis*, *T. urticae* or *P. ulmi*. For orchard A in 2011, both the lambda-cyhalothrin plus
284 chlorantraniliprole and control treatments had higher secondary pest CIDs than the
285 chlorantraniliprole treatment. For orchard B in 2011, the plots treated with spinetoram for first
286 generation *C. pomonella* had the highest CID for *C. juglandicola*, compared with other
287 treatments, while the grower standard plots had the lowest CID for *T. urticae*. *Panaphis*
288 *juglandis* was not observed in any of the walnut plots in 2011.

289

290 3.2. Natural enemy monitoring

291

292 Beat tray sampling in the pear orchard in 2010 revealed higher CIDs for *Trechnites* spp. in
293 the chlorantraniliprole and cyantraniliprole treatments than in the spinetoram treatment (Table 2).
294 In 2011, the difference between the chlorantraniliprole and spinetoram treatments was similar to
295 that observed in 2010 for the *Trechnites* spp. CIDs, but was not significant. There were no
296 treatment differences in the beat tray CIDs for predatory Heteroptera or Araneae in either year.
297 In 2010, CIDs for predatory Neuroptera captured on PV traps in pear was highest in plots treated
298 with spinetoram and significantly lower in the plots treated with chlorantraniliprole and
299 cyantraniliprole (Table 2). While the pattern was similar in 2011, the effect was not significant.
300 There were no treatment differences in the CIDs for predatory Syrphidae in either year.

301 In both years, *F. auricularia* was abundant in the cardboard bands in the pear orchards. In
302 2010, CIDs for *F. auricularia* were highest in plots treated with cyantraniliprole, and
303 significantly lower in plots treated with chlorantraniliprole or spinetoram (Table 2). There were
304 no significant treatment differences in *F. auricularia* CIDs in 2011.

305 For the walnut orchards there were no differences in the CIDs for *C. juglandicola*
306 mummies between treatments in either year (Table 3). In 2010, the insecticide treatments had no
307 effect on phytoseiid mite CMDs, but in 2011, the CMDs were highest in the chlorantraniliprole
308 treated plots in orchard A and in both the control plots and those that were treated with
309 chlorantraniliprole first followed by spinetoram second in orchard B. In the walnut orchards,
310 there were no differences between treatments in the CIDs for the natural enemies captured on the
311 PV traps in either year.

312

313 3.3. Natural enemy/prey ratios

314

315 There were some statistical differences in natural enemy/prey ratios between treatments in
316 the pear orchards. In 2010, the ratio of *Trechmites* spp./*C. pyricola* CID's was highest in the
317 chlorantraniliprole treatment and lowest in the spinetoram treatment (Table 4). In contrast, the
318 ratio of predatory Neuroptera/*C. pyricola* CIDs were significantly higher in the spinetoram
319 treatment than the cyantraniliprole treatment in 2011. While the same trends between treatments
320 were observed in the other year of the trials for both of these ratios the effects were not
321 significant. There were no treatment differences in ratios of predatory Heteroptera/*C. pyricola*
322 CIDs in either year.

323 In the walnut orchards, there was a significant treatment effect on percent parasitism of *C.*
324 *juglandicola* by *T. pallidus* in 2010, and plots treated with spinetoram had the lowest levels of
325 parasitism (Table 5), but there were no treatment differences for percent parasitism in the two
326 walnut orchards used in 2011. In contrast, there were no treatment differences in predator/prey
327 ratios for phytoseiid and tetranychid mites in 2010, but there were in 2011. In 2011, CMD ratios
328 for phytoseiid mites and either *T. urticae* or *P. ulmi* were higher in the chlorantraniliprole treated
329 plots than in the control or lambda-cyhalothrin plus chlorantraniliprole treated plots for orchard
330 A. However, there were no treatment differences between the CMD ratios for phytoseiid and
331 tetranychid mites in orchard B.

332

333 **4. Discussion**

334

335 Results from these field studies did not show consistent negative effects of
336 chlorantraniliprole or spinetoram on secondary pests and natural enemies when they were
337 applied to manage *C. pomonella* in pear and walnut orchards. Overall, there was a general lack
338 of year-to-year response to the insecticide treatments for most of the sampled taxa making it
339 difficult to demonstrate consistent disruption of natural enemies and increases in secondary pest
340 abundance. The inconsistency in these results is similar to that observed in some of the field
341 studies in apple orchards described by Beers et al. (this issue, a,b). There were two key
342 exceptions. In walnuts, higher levels of *C. juglandicola* abundance were observed in the
343 lambda-cyhalothrin and spinetoram (either applied both generations or applied first generation
344 followed by chlorantraniliprole for second generation) treatments and this was accompanied by
345 lower levels of percent parasitism in the case of the spinetoram treatment in 2010. In pears, the

346 ratio of predatory Neuroptera adults (primarily *Chrysoperla* spp.) to *P. pyricola* nymphs was
347 greater in the spinetoram versus the chlorantraniliprole treatments in both years, although the
348 ratio was significant only in the first year. For all other taxa, if treatment differences occurred in
349 one year, they didn't appear the other year. It is likely that two unseasonably cool summers in
350 the western US was a significant factor that contributed to low levels of secondary pest and
351 natural enemy abundance in both the pear and walnut orchards which may have prevented the
352 detection of more consistent treatment effects.

353 Chlorantraniliprole and spinetoram were the two reduced-risk insecticides that were
354 compared in pear and walnut orchards in both years. In related laboratory bioassays,
355 Amarasekare et al. (this issue) determined that chlorantraniliprole was more detrimental to
356 *Chrysoperla carnea* (Stephens) (Neur.: Chrysopidae) than spinetoram, but that spinetoram was
357 more detrimental to *T. pallidus* than chlorantraniliprole. This was evident from a reduction in
358 the extrapolated intrinsic rates of population increase to negative values in both cases. Although
359 the field study results were not as clear as those from the laboratory bioassays for these two
360 natural enemies, they do provide at least partial verification that strong laboratory effects do
361 translate to the field.

362 However, in general, many of the effects seen from natural enemy exposure to insecticides
363 in laboratory bioassays were not observed in our field studies, and this is thoroughly described
364 elsewhere in this issue (Beers et al., this issue b). For example, both *D. brevis* and *G.*
365 *occidentalis* were shown to be much more susceptible to spinetoram than to chlorantraniliprole
366 in laboratory bioassays (Amarasekare and Shearer, 2013a; Beers and Schmidt, 2014). However,
367 in our field study, there were no significant treatment differences in predatory Heteroptera CIDs
368 collected with beat trays in pear in 2010 and only slightly lower numbers were observed in the

369 spinetoram treated plots the following year. Similarly, phytoseiid mite CIDs were only slightly
370 lower in walnut plots treated with spinetoram during the first generation of codling moth in 2011
371 and not at all in 2010. Overall, as a percentage among all trials within a crop, there were more
372 significant treatment effects for natural enemy/prey ratios (50% for pears, 33% for walnuts) than
373 for natural enemy CIDs alone (25% for pears, 13% for walnut). A difference in natural enemy
374 CIDs alone between insecticide treatments can be more difficult to interpret than a difference in
375 natural enemy/prey ratios as it results from a combination of direct effects on the natural enemies
376 themselves and indirect effects on the availability of prey as a resource for the natural enemies.
377 In contrast, a difference in natural enemy/prey ratios represents a change in natural enemy
378 abundance relative to prey abundance and thus estimates treatment effects on the natural enemy -
379 prey interaction rather than on natural enemies alone. Factoring together the low densities of
380 prey and natural enemies as ratios allowed subtle effects of the insecticide treatments to become
381 more detectable in our field studies.

382 The use of PV-baited traps adds a new dimension to monitoring natural enemies (Jones et
383 al., 2011, this issue a). They can be deployed in and around orchards to capture a wide variety of
384 natural enemies and can be used to measure the impact of IPM programs on natural enemy
385 populations, to measure the diversity of natural enemy communities (Mills et al., this issue b)
386 and to gather information to create natural enemy phenology models (Jones et al., this issue b).
387 One of the main benefits of PV traps is that they capture flying insects for an extended period of
388 time compared with instantaneous collection of insects using beat tray sampling or in-situ visual
389 examinations. PV traps are relatively new tools available for ecologists and IPM practitioners,
390 and in our field studies, we used PV traps baited with several different lures and pooled the
391 catches of natural enemies across lures because several of them have been shown to be cross

392 attractive (Jones et al., this issue a). Despite the high levels of abundance of several natural
393 enemy taxa on these traps relative to the numbers found using other sampling methods, we did
394 not detect differences between insecticide treatments other than for predatory Neuroptera in
395 pears. It is unclear whether the lack of informative results from the PV traps in our field studies
396 reflect the small size of our experimental plots (≤ 0.4 ha) and the strong flight ability of most of
397 the natural enemies captured by the traps including the predatory Syrphidae and Neuroptera and
398 parasitic Hymenoptera. Since the active space of these PV traps has not been determined it is
399 also possible that they may also have attracted natural enemies from outside of the orchards, such
400 as from adjacent refugia, which are known to be important for recruiting natural enemies into
401 orchards (Miliczky and Horton, 2005). It is also possible that natural enemies were captured in
402 the experimental plots as they were transiting through the plots.

403 Laboratory bioassays can provide meaningful data yet field trials provide the most realistic
404 results (Prasifka et al., 2005). However, there are intrinsic difficulties with conducting on-farm
405 research. Foremost is the difficulty for growers to provide untreated control plots that are used
406 to provide background information about pest and natural enemy abundance. Horticultural crops
407 are expensive to produce and most growers do not want to risk crop loss. In this study, only
408 walnut growers provided plots with unsprayed trees. One solution is to budget for crop loss in
409 grant applications that would compensate growers for losses they might incur.

410 Determining plot size is another aspect of on-farm trials. If investigators were only
411 interested in sedentary or wingless natural enemies, plot size could be small, but larger sized
412 plots are required if natural enemies are able to readily disperse between plots and surrounding
413 habitat. However, large plots can constrain the number of treatment replications that can be

414 included. Larger plot sizes also increase costs in terms of labor, equipment and sampling time
415 (Prasifka et al., 2005).

416 The researchers associated with this current study have participated in large replicated field
417 studies and through experience, have concluded that it is best to conduct replicated studies within
418 an orchard versus blocking the study across multiple orchards. The main reason is that
419 variability is often greater between orchards than between treatments. This limits the ability to
420 determine significant treatment effects. Conducting a replicated study in one orchard minimizes
421 plot-to-plot variability because the orchard unit is relatively uniform which increases the
422 likelihood of treatment differences. Verification of results is then enhanced when the studies are
423 successfully reproduced elsewhere.

424 Another insight into on-farm research is how an investigator decides on which grower and
425 orchard to work with. Orchard management can be classified as a continuum ranging from
426 excellent progressive growers with well-managed orchards to t where growers are considerably
427 less progressive and their pest management is lacking. Researchers, including the authors, tend
428 to work with progressive growers because they are interested in new ideas, having research
429 conducted in their orchards, and are less apt to cause problems such as over-spraying or
430 harvesting the crop before notifying the researchers. The pear growers in these studies were
431 progressive and maintained well managed orchards and they did not need to treat for *C. pyricola*
432 during the summer. They likely conserved their natural enemies by using *C. pomonella* mating
433 disruption instead of insecticides and had extra-orchard habitat that was suitable as natural
434 enemy refugia. Zwick and Fields (1977) showed that the elimination of sprays against *C.*
435 *pomonella* in pears helps to conserve biological control and manage *P. pyricola*. Later, Riedl at
436 al. (2000) also demonstrated that pear growers who successfully implemented an integrated fruit

437 production program that encouraged biological control were able to reduce broad-spectrum
438 insecticide use while maintaining good fruit quality at harvest. More recently, this concept has
439 been expanded in the Hood River, OR pear district. Here growers have substituted *C. pomonella*
440 mating disruption for insecticide sprays. This has allowed significant acreage to avoid
441 treatments for *P. pyricola* during the summer season (Gallardo et al., in this issue; Warner 2012).
442 It is possible that our failure to cause significant disruption of secondary pests in pears in this
443 study was related to several years of low pest abundance in these progressive orchards. A
444 similar situation occurred in the walnut studies that were conducted in well-managed orchards
445 where populations of *C. juglandicola* were low.

446 Again, our studies were conducted during two unseasonably cool summers. Weather also
447 plays a big part where high temperatures can result in more of the population being exposed to
448 the toxicant (because they emerge and develop through more of the sensitive stages before the
449 insecticide residue, which degrades on a calendar date basis, is gone) and cold temperatures have
450 the opposite effect. Thus, year-to-year variation in weather patterns, which are inherent in any
451 field study, contribute some level of serendipity to the success of large-plot field studies of
452 insecticide effects on natural enemies.

453 Given the difficulties associated with conducting large-scale replicated research trials,
454 properly designed laboratory bioassays are often a better alternative to rapidly screen a variety of
455 insecticides. Laboratory bioassays are less expensive and time-consuming to conduct and are
456 likely to yield results that are less variable than field studies. However, field studies conducted
457 on several sites for several years are likely the best way to document the effects of insecticides
458 on secondary pests and natural enemies. As the knowledgebase increases, growers and pest
459 control consultants can then decide which insecticide to use based on efficacy against the target

460 pest and selectivity against natural enemies or they can choose to apply these products at a time
461 when natural enemies are in a less susceptible stage of their life cycle or seasonal phenology
462 (Jones et al., 2009).

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Graphical Abstract



Table 1. Mean (\pm SE) cumulative insect (or mite)-days for secondary pests observed in the walnut orchard trials

| Year | Orchard | Treatment | Cumulative insect (or mite)-days per walnut leaflet | | | |
|----------|-----------|---|---|---------------------|-------------------|-----------------|
| | | | <i>C. juglandicola</i> ¹ | <i>C. juglandis</i> | <i>T. urticae</i> | <i>P. ulmi</i> |
| 2010 | A | Chlorantraniliprole | 55.0 \pm 6.3 b | 32.5 \pm 9.3 | 53.7 \pm 12.8 | 45.6 \pm 10.8 |
| | | Spinetoram | 85.0 \pm 18.9 a | 25.3 \pm 5.7 | 64.4 \pm 30.1 | 71.2 \pm 52.3 |
| | | Control | 57.6 \pm 8.1 b | 34.4 \pm 12.2 | 46.4 \pm 24.6 | 36.4 \pm 13.5 |
| | | <i>F</i> | 6.02 | 0.11 | 1.25 | 0.27 |
| | <i>df</i> | 2, 6 | 2, 6 | 2, 6 | 2, 6 | |
| | <i>P</i> | 0.04 | 0.90 | 0.35 | 0.77 | |
| 2011 | A | Chlorantraniliprole | 4.5 \pm 0.2 b | 0.0 \pm 0.0 | 0.2 \pm 0.1 b | 1.0 \pm 0.4 b |
| | | Lambda-cyhalothrin + chlorantraniliprole | 9.9 \pm 1.0 a | 0.0 \pm 0.0 | 1.4 \pm 0.1 a | 4.8 \pm 1.4 a |
| | | Control | 6.6 \pm 1.5 b | 0.0 \pm 0.0 | 2.0 \pm 0.6 a | 4.7 \pm 0.9 a |
| | | <i>F</i> | 7.63 | | 20.34 | 9.44 |
| | | <i>df</i> | 2, 6 | | 2, 6 | 2, 6 |
| | | <i>P</i> | 0.02 | | 0.002 | 0.01 |
| | B | Chlorantraniliprole/Spinetoram ² | 8.6 \pm 1.3 b | 0.0 \pm 0.0 | 16.5 \pm 5.0 a | 14.6 \pm 3.3 |
| | | Spinetoram/Chlorantraniliprole | 20.8 \pm 4.3 a | 0.0 \pm 0.0 | 10.2 \pm 3.0 a | 13.6 \pm 3.9 |
| | | Grower standard | 6.8 \pm 2.2 b | 0.0 \pm 0.0 | 5.2 \pm 3.6 b | 7.2 \pm 3.3 |
| | | Control | 7.1 \pm 1.2 b | 0.0 \pm 0.0 | 13.9 \pm 2.0 a | 11.2 \pm 3.3 |
| <i>F</i> | | 6.20 | | 6.31 | 1.41 | |
| | | <i>df</i> | 3, 6 | | 3, 6 | 3, 6 |
| | <i>P</i> | 0.03 | | 0.03 | 0.33 | |

¹Means in a column for each year and orchard followed by different letters are significantly different ($P \leq 0.05$). Data natural log (X+1) transformed, actual means reported.

²Order of treatments refer to sprays applied to first then second generation codling moth.

Table 2. Mean (\pm SE) cumulative insect-days for natural enemies observed in the pear orchard trials

| Year | Orchard | Treatment | Cumulative insect-days per beat tray | | | Cumulative insect-days per trap | | Cumulative insect-days per band |
|------|-----------|---------------------|--------------------------------------|--------------------------|----------------|--------------------------------------|------------------------|---------------------------------|
| | | | <i>Trechnites</i> spp. ¹ | Predatory Heteroptera | Araneae | Predatory Neuroptera ¹ | Predatory Syrphidae | <i>Forficula auricularia</i> |
| 2010 | A | Chlorantraniliprole | 11.9 \pm 3.7 a | 63.8 \pm 8.4 | 17.9 \pm 3.2 | 75.4 \pm 12.1 b | 576.9 \pm 46.4 | 593.9 \pm 233 b |
| | | Cyantraniliprole | 9.6 \pm 3.2 a | 76.1 \pm 10.1 | 17.6 \pm 0.7 | 46.7 \pm 7.5 c | 449.8 \pm 5.7 | 910.2 \pm 265 a |
| | | Spinetoram | 1.9 \pm 0.9 b | 63.2 \pm 2.8 | 16.9 \pm 3.2 | 131.2 \pm 11.6 a | 577.3 \pm 20.1 | 262.5 \pm 133 b |
| | | <i>F</i> | 27.05 | 2.13 | 0.04 | 12.67 | 6.28 | 9.73 |
| | <i>df</i> | 2, 4 | 2, 4 | 2, 4 | 2, 4 | 2, 4 | 2, 4 | |
| | <i>P</i> | 0.005 | 0.23 | 0.96 | 0.02 | 0.06 | 0.03 | |
| 2011 | B | Chlorantraniliprole | 8.8 \pm 1.9 | 2.7 \pm 0.4 | 3.9 \pm 0.5 | 69.8 \pm 35.3 | 32.8 \pm 12.6 | 252.2 \pm 96 |
| | | Spinetoram | 4.1 \pm 2.7 | 1.3 \pm 0.3 | 3.9 \pm 0.6 | 216.9 \pm 33.0 | 70.1 \pm 24.2 | 156.6 \pm 43.4 |
| | | <i>F</i> | 6.71 | 7.46 | 0.02 | 6.5 | 4.3 | 1.37 |
| | | <i>df</i> | 1, 3 | 1, 3 | 1, 3 | 1, 3 | 1, 3 | 1, 3 |
| | <i>P</i> | 0.08 | 0.07 | 0.91 | 0.08 | 0.15 | 0.326 | |

¹Means in a column for each year and orchard followed by different letters are significantly different ($P \leq 0.05$). Data natural log (X+1)

transformed, actual means reported.

Table 3. Mean (\pm SE) cumulative insect (or mite)-days for natural enemies observed in the walnut orchard trials

| Year | Orchard | Treatment | Cumulative insect (or mite)- days per walnut leaflet | | Cumulative insect-days per trap | | |
|------|-----------|---|---|---------------------|---------------------------------|------------------------|--------------------------|
| | | | <i>Trioxys pallidus</i> mummies ¹ | Phytoseiid mites | Predatory Neuroptera | Predatory Syrphidae | Parasitic Hymenoptera |
| 2010 | A | Chlorantraniliprole | 26.8 \pm 5.3 | 17.2 \pm 5.8 | 827.0 \pm 85.8 | 293.1 \pm 12.5 | 948.0 \pm 255.9 |
| | | Spinetoram | 24.7 \pm 4.7 | 35.2 \pm 27.3 | 825.9 \pm 89.1 | 515.1 \pm 128.7 | 782.9 \pm 178.1 |
| | | Control | 34.1 \pm 4.0 | 17.4 \pm 2.6 | 753.1 \pm 141.0 | 400.1 \pm 60.5 | 709.1 \pm 96.7 |
| | | <i>F</i> | 1.97 | 11.32 | 0.26 | 1.36 | 0.29 |
| | <i>df</i> | 2, 6 | 2, 6 | 2, 6 | 2, 6 | 2, 6 | |
| | <i>P</i> | 0.22 | 0.95 | 0.78 | 0.33 | 0.76 | |
| 2011 | A | Chlorantraniliprole | 4.2 \pm 0.5 | 28.4 \pm 2.1 a | 48.4 \pm 24.1 | 171.8 \pm 42.2 | 3258.4 \pm 232.8 |
| | | Lambda-cyhalothrin + chlorantraniliprole | 5.4 \pm 0.4 | 19.7 \pm 1.8 b | 59.6 \pm 17.6 | 121.9 \pm 33.3 | 2983.9 \pm 346.3 |
| | | Control | 4.7 \pm 1.0 | 19.9 \pm 0.2 b | 38.0 \pm 14.2 | 155.6 \pm 88.0 | 3738.8 \pm 940.8 |
| | | <i>F</i> | 1.12 | 11.32 | 0.47 | 0.31 | 0.19 |
| | <i>df</i> | 2, 6 | 2, 6 | 2, 6 | 2, 6 | 2, 6 | |
| | <i>P</i> | 0.39 | 0.01 | 0.65 | 0.75 | 0.84 | |
| | B | Chlorantraniliprole/Spinetoram ² | 5.3 \pm 0.6 | 20.8 \pm 1.0 a | 74.0 \pm 5.0 | 984.3 \pm 0.6 | 1836.7 \pm 433.3 |
| | | Spinetoram/Chlorantraniliprole | 8.4 \pm 1.1 | 15.1 \pm 1.2 b | 86.2 \pm 24.7 | 959.0 \pm 1.5 | 2011.8 \pm 306.8 |
| | | Grower standard | 6.1 \pm 1.5 | 14.3 \pm 1.0 b | 101.7 \pm 31.0 | 1065.7 \pm 1.3 | 2352.8 \pm 150.7 |
| | | Control | 7.7 \pm 1.3 | 19.1 \pm 1.0 a | 115.8 \pm 9.3 | 459.5 \pm 1.1 | 1167.8 \pm 220.2 |
| | <i>F</i> | 1.41 | 8.78 | 0.62 | 0.75 | 3.15 | |
| | <i>df</i> | 3, 6 | 3, 6 | 3, 6 | 3, 6 | 3, 6 | |
| | <i>P</i> | 0.33 | 0.01 | 0.63 | 0.56 | 0.11 | |

¹Means in a column for each year and orchard followed by different letters are significantly different ($P \leq 0.05$). Data natural log (X+1)

transformed, actual means reported.

²Order of treatments refer to sprays applied to first then second generation codling moth.

Table 4. Mean (\pm SE) natural enemy/prey ratios based on cumulative insect days in the pear orchard trials

| Year | Orchard | Treatment | Predator CID / prey CID ratios ¹ | | |
|------|---------|---------------------|--|---|--|
| | | | <i>Trechmites</i> spp. / <i>C. pyricola</i> | Predatory Heteroptera / <i>C. pyricola</i> | Predatory Neuroptera / <i>C. pyricola</i> |
| 2010 | A | Chlorantraniliprole | 0.7 \pm 0.1 a | 4.1 \pm 0.9 | 4.8 \pm 0.9 ab |
| | | Cyantraniliprole | 0.5 \pm 0.2 b | 4.3 \pm 0.5 | 2.7 \pm 0.4 b |
| | | Spinetoram | 0.1 \pm 0.1 c | 4.7 \pm 0.6 | 9.7 \pm 0.2 a |
| | | <i>F</i> | 85.22 | 0.05 | 7.93 |
| | | df | 2, 4 | 2, 4 | 2, 4 |
| | | <i>P</i> | 0.001 | 0.95 | 0.04 |
| 2011 | B | Chlorantraniliprole | 1.5 \pm 0.4 | 0.5 \pm 0.1 | 9.8 \pm 3.2 b |
| | | Spinetoram | 1.3 \pm 0.4 | 0.6 \pm 0.4 | 49.6 \pm 10.8 a |
| | | | <i>F</i> | 1.01 | 0.03 |
| | | df | 1, 3 | 1, 3 | 1, 3 |
| | | <i>P</i> | 0.39 | 0.87 | 0.03 |

¹Means in a column for each year and orchard followed by different letters are significantly different ($P \leq 0.05$). Data natural log (X+1)

transformed, actual means reported.

Table 5. Mean (\pm SE) percent parasitism and predator/prey ratios (based on cumulative mite-days) in the walnut orchard trials

| Year | Orchard | Treatment | Percent parasitism <i>C. juglandicola</i> | Predator CMD / prey CMD ratios ¹ | |
|------|-----------|---|---|---|-------------------------------------|
| | | | | Phytoseiid mite / Twospotted mite | Phytoseiid mite / European red mite |
| 2010 | A | Chlorantraniliprole | 32.8 \pm 3.8 a | 0.32 \pm 0.1 | 0.30 \pm 0.0 |
| | | Spinetoram | 23.8 \pm 3.2 b | 0.41 \pm 0.2 | 0.44 \pm 0.2 |
| | | Control | 38.6 \pm 5.1 a | 0.69 \pm 0.2 | 0.48 \pm 0.1 |
| | | <i>F</i> | 7.71 | 1.35 | 1.22 |
| | | <i>df</i> | 2, 6 | 2, 6 | 2, 6 |
| | | <i>P</i> | 0.02 | 0.33 | 0.36 |
| 2011 | A | Chlorantraniliprole | 46.8 \pm 3.6 | 312.3 \pm 109.8 a | 205.3 \pm 179.6 a |
| | | Lambda-cyhalothrin + chlorantraniliprole | 42.9 \pm 8.6 | 14.1 \pm 7.0 b | 5.2 \pm 2.6 b |
| | | Control | 33.7 \pm 2.3 | 12.0 \pm 2.5 b | 4.5 \pm 0.6 b |
| | | <i>F</i> | 2.35 | 34.63 | 6.82 |
| | | <i>df</i> | 2, 6 | 2, 6 | 2, 6 |
| | | <i>P</i> | 0.18 | 0.001 | 0.02 |
| | B | Chlorantraniliprole/Spinetoram ² | 39.2 \pm 1.8 | 1.50 \pm 0.4 | 1.55 \pm 0.3 |
| | | Spinetoram/Chlorantraniliprole | 29.8 \pm 4.4 | 1.98 \pm 0.9 | 1.50 \pm 0.7 |
| | | Grower standard | 48.3 \pm 2.1 | 9.10 \pm 10.5 | 2.98 \pm 2.1 |
| | | Control | 51.2 \pm 3.4 | 1.45 \pm 0.5 | 2.03 \pm 1.0 |
| | <i>F</i> | 1.33 | 3.38 | 0.92 | |
| | <i>df</i> | 2, 6 | 3, 6 | 3, 6 | |
| | <i>P</i> | 0.35 | 0.10 | 0.48 | |

¹Means in a column for each year and orchard followed by the different letters are significantly different ($P \leq 0.05$). Data natural log ($X+1$) transformed, actual means reported.

²Order of treatments refer to sprays applied to first then second generation codling moth.