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Laboratory Bioassays to Estimate the Lethal and Sublethal Effects of Various Insecticides and Fungicides on *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae)

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ABSTRACT This laboratory bioassay focused on lethal and sublethal effects of five insecticides (chlorantraniliprole, cyantraniliprole, spinetoram, novaluron, and lambda-cyhalothrin) and two fungicide treatments (sulfur and a mixture of copper hydroxide and mancozeb) on the predatory mired bug *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae) using second instars and adult males and females. Formulated pesticides were tested using concentrations that were equivalent to the high label rate (1x) [high rate] and $1/10^{th}$ of that amount (0.1x) [low rate] dissolved in 378.5L of water. Lambda-cyhalothrin was highly toxic to *D. brevis* nymphs and adults at both rates, while both rates of novaluron were highly toxic to nymphs. Cyantraniliprole, chlorantraniliprole and novaluron were less toxic to adults and chlorantraniliprole and spinetoram were less toxic to nymphs. Both rates of spinetoram caused significant mortality to adults. Fecundity of adult females was negatively affected by the high rates of either novaluron or spinetoram while the fertility was affected only by the high rate of novaluron. The high rate of spinetoram reduced survival of nymphs. Adults treated with spinetoram had reduced longevity. Cyantraniliprole caused some mortality to nymphs and affected their survival. Both rates of sulfur were toxic to nymphs and affected emergence to adults. The mixture of copper hydroxide and mancozeb was less toxic to *D. brevis*. Neither adult longevity nor sex ratio was affected by the fungicides. The r values for *D. brevis* treated with lambda-cyhalothrin, novaluron, spinetoram and sulfur were low, indicating these products may have negative impact on population growth.

KEYWORDS biological control, generalist predator, pear psylla, lethal and sublethal effects, reduced-risk insecticides

In the western United States, pest management in apple, pear and walnut orchards is primarily focused on a key pest, codling moth (*Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Beers et al. 1993). Considered as a worldwide pest, the larva of codling moth can cause severe internal feeding damage to apples, pears and walnuts (Hoyt et al. 1983). Codling moth damage reduces the market value of the fruit and makes it unfit for human consumption. Insecticides combined with pheromone-based mating disruption are useful management tactics when used against codling moth (University of California 1991). The type of insecticide used to control codling moth can determine the occurrence of secondary pest outbreaks in tree fruit orchard (University of California 1991).

In the past, organophosphorus (OP) insecticides were commonly used for codling moth control (Hoyt 1969, Hoyt and Burts 1974). Following the implementation of the Food Quality Protection Act of 1996 (FQPA 1996), most were either removed or are in the process of being removed from use (Agnello et al. 2009). Currently, reduced risk insecticides with novel modes of action and OP alternatives are used to control the codling moth (Agnello et al. 2004). However, little is known about how selective these newer insecticide chemistries are to natural enemies. Although most of these newer reduced-risk insecticides are target specific with low mammalian toxicity, there is information that some of these newer insecticides could affect natural enemies that are important for regulating secondary insect and mite pests and thus, integrated pest management (IPM) programs (Brunner et al. 2001, Villanueva and Walgenbach 2005, Kim et al. 2006, Myers et al. 2006, Villanueva and Walgenbach, 2006, Agnello et al. 2009, Crampton et al. 2010). In contrast to neurotoxic OP insecticides, some of the newer reduced risk insecticides have been shown to have chronic reproductive rather than acute effects on natural enemies (Kim et al. 2006). In addition to the reduced risk insecticides, some of the fungicides

71 used in pest management may have insecticidal and miticidal properties that affect natural
72 enemies of secondary insects and mites (Jepson et al. 2007). Thus, additional information is
73 needed to better understand the impacts of pesticides on natural enemies including impacts that
74 may affect population growth (Jones et al. 2009). In this study, we investigated a wide range of
75 pesticide effects on an important predatory Hemipteran *Deraeocoris brevis* (Uhler) (Hemiptera:
76 Miridae).

77 Predatory Hemipterans including some species in family Miridae are important natural
78 enemies in many agricultural systems and are often effective against small, soft-bodied
79 arthropods such as aphids, thrips and mites (Westigard 1973). The mirid bug *D. brevis* (Uhler), a
80 key natural enemy in pear orchards in the Pacific Northwest (Riedl 1991), is widely distributed
81 in apple and pear orchards in western United States and Canada (Kelton 1982, Westigard et al.
82 1968). It is considered a generalist predator that feeds on small arthropod pests such as aphids,
83 leafhoppers, psyllids and mites (McMullen and Jong 1967). *Deraeocoris brevis* is one of the
84 most important predators of pear psylla [*Cacopsylla pyricola* (Forster)] (Hemiptera: Psyllidae)
85 (Westigard et al. 1968). Pesticides used to control pest insects and mites in tree fruit orchards
86 can negatively affect *D. brevis* (Westigard 1973, Kim et al. 2006). These negative effects are
87 either direct: lethal (acute), or indirect: sublethal (chronic) (Kim et al. 2006). Sublethal effects of
88 pesticide exposure can affect the development, reproduction and survival of natural enemies and
89 negatively impact the natural enemy population growth (Kim et al. 2006). In order to predict the
90 total impact of a pesticide on a natural enemy in the field, both sublethal and acute toxicity
91 effects need to be quantified. Hence, investigating sublethal effects should be included in assays
92 to provide a more accurate assessment of a pesticide's impact (Kim et al. 2006). Acute toxicity
93 assays using only topical application may not be predictive of impacts of pesticides in the field

94 (Stark et al. 1995) because beneficial organisms may receive pesticide exposure from multiple
95 sources including direct contact, and oral exposure (Longley and Stark 1996). Therefore,
96 estimating sublethal effects through multiple routes of exposure is necessary to accurately assess
97 insecticides (Banken and Stark 1998).
98 This current study was part of a large, multi-state project conducted in Washington,
99 Oregon and California, with the goal to improve the sustainability of apple, pear and walnut
100 production by enhancing biological control in western USA orchard cropping systems. The
101 overarching theme was to investigate the pesticides used against *C. pomonella* and their
102 secondary impacts on natural enemies found in these three orchard cropping systems. One
103 aspect of this project was to develop new technology and information to allow growers and
104 practitioners to take advantage of natural enemies in fruit and nut orchards. Other studies from
105 this large project evaluated the impact of various pesticides that are key inputs for deciduous tree
106 fruit and nut integrated pest management (IPM) programs. The focus of this current study is to
107 investigate lethal and sub lethal effects of various pesticides in the laboratory against *D. brevis*.
108 We chose to investigate effects of five formulated insecticides containing the following
109 active ingredients: cyantraniliprole, chlorantraniliprole, spinetoram, novaluron and lambda-
110 cyhalothrin and two fungicide treatments, sulfur and a mixture of copper hydroxide plus
111 mancozeb on *D. brevis* nymphs and adults (males and females) in the laboratory using multiple
112 routes of exposure. We then used this information to estimate the impact of these pesticides on
113 the intrinsic rate of population increase (r) for *D. brevis*.
114 Pesticides tested in this study were selected, in part, with input from the grant's
115 Stakeholder Advisory Panel, and also considering whether these products were used in one, two
116 or all three cropping systems targeted in this project. This allowed other labs associated with this

grant to test the same pesticides using similar procedures on other natural enemies. Although each cropping system has its uniqueness, majority of the natural enemies existing in these orchards are common for all three systems here in the western USA. Some of the pesticides selected (e.g. chlorantraniliprole and spinetoram) are used in all three crops. Cyantraniliprole is a new unregistered insecticide with effective control of a cross-spectrum of important pests, such as caterpillars, whiteflies, leafminers, thrips and some aphids, in a wide range of crops including pome and stone fruits. In addition to reduced-risk insecticides, we incorporated some OP replacement insecticides including lambda-cyhalothrin (pears and walnuts) and novaluron (pears and apples) as these materials are used in some of the systems to control codling moth which can impact natural enemy balance (EPA 2010 and Kim et al. 2011). The grant's Stakeholder Advisory Panel also suggested that we investigate potential effects from fungicides thus, we included the mixture of copper hydroxide plus mancozeb, primarily used in walnuts for walnut blight control but not in pears or apples, and sulfur, which is mainly used in pears and apples but not in walnuts.

Materials and Methods

***Deraeocoris brevis* Colony Rearing.** A colony of *D. brevis* was maintained at 25°C, 50-60% R. H. and a photoperiod of 16:8 (L: D) h in the laboratory. The initial colony was started in 2007 from *D. brevis* collected from pear and apple orchards in Hood River, OR. In summer 2009, field collected *D. brevis* adults were added to the colony to reduce the risk of inbreeding. *Deraeocoris brevis* are predacious, thus, eggs and nymphal stages were kept separate in ventilated plastic containers (crispers) (30 × 25 × 9 cm). An area of 20 × 15 cm was removed from the lids of these crispers and a piece of insect proof mesh glued for ventilation.

Adults were maintained in a custom-made wooden sleeve cage with a glass top (50×72×54 cm). It had two 18-cm diameter openings in the front panel covered with cloth sleeves for insect handling. Nymphs and adults were fed eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) purchased from a commercial source (Beneficial Insectary, Redding, CA, USA). These eggs were stored in a freezer at -6°C. *Ephestia kuehniella* eggs were sprinkled on a sheet (20.3 ×28cm) of blue paper that was soaked in water for 5 minutes and then drained of excess water. The blue paper (176 gsm [65 lb weight) (216 × 279 mm) (Fireworx (™), Boise Paper Holdings, LLC. Boise ID) provides better visibility when assessing the distribution of eggs. Fresh green beans (organically grown and locally purchased) were provided to supply the moisture needed for development as well as an oviposition substrate for adult females. Before use, beans were soaked in a 0.5% bleach solution for 1 min, rinsed with water and air-dried. Adults and immature *D. brevis* were transferred twice weekly to clean crispers containing fresh beans and *E. kuehniella*. To maintain the colony, bean pods with eggs were collected from the adult cage and placed in clean crispers the eggs hatched. The rearing method was similar to methods used by Alauzet et al. 1992 and Kim and Riedl 2005.

Insects. Second instars (0-1 d old) and adult male and female (1-2 d old) *D. brevis* were used in this study. Newly emerged first instars were collected from the crispers with the *D. brevis* eggs (in bean pods), provisioned with *E. kuehniella* eggs and reared as above until they molted to second instars. Newly emerged adult male and female *D. brevis* were collected from crispers containing fifth instars. Adults were separated by gender using morphology of the female reproductive organs in the family Miridae (Davis 1955) and placed in separate crispers provisioned with *E. kuehniella* eggs and green beans as mentioned in *D. brevis* colony rearing.

Insecticides and Fungicides. The following five insecticides and two fungicides listed with their maximum label rates were tested as formulated material: cyantraniliprole (DuPont Crop Protection, Wilmington, DE) 149.9 g [AI] / ha, chlorantraniliprole (Altacor 35WG, DuPont Crop Protection, Wilmington, DE) 110.4 g [AI] / ha, spinetoram (Delegate 25WG, Dow Agro Sciences LLC, Indianapolis, IN) 122.6 g [AI] /ha, novaluron (Rimon 0.83EC, Chemtura AgroSolutions, Middlebury, CT) 363.4 g [(Active Ingredient) AI] / ha, and lambda-cyhalothrin (Warrior II CS, Syngenta LLC Inc., Greensboro, NC) 46.6 g [AI] / ha, sulfur (Kumulus DF, Micro Flo Company LLC., Memphis, TN) 17.9 kg [AI] / ha, and a mixture of mancozeb (Manzate Pro Stick, DuPont Crop Protection, Wilmington, DE) 1.5 kg [AI] / ha and copper hydroxide (Kocide 3000 WG, DuPont Crop Protection, Wilmington, DE) 2.1 kg [AI] / ha. Distilled water was used as the control treatment. Each pesticide was tested using concentrations that were equivalent to the maximum label rate (1x) and $1/10^{th}$ of that amount (0.1x) dissolved in 378.5L of water.

Bioassay – Lethal Effects. Custom made glass arenas consisting of a glass cylinder (Wheaton Glass Warehouse, Millville, NJ) standing upright on a glass plate (Cincinnati Gasket, Cincinnati, OH) [adult arena: 7.5 cm diameter × 6 cm tall × 3.2 mm thick glass cylinders and 9 × 9 cm and 2.25mm thick glass plates; nymph arena: 4.4 cm diameter × 6 cm tall × 2.3 mm thick glass cylinders and 6 × 6 cm and 2.25 mm thick glass plates] were used in the bioassay. To hold each plate to the cylinder with binder clips, four aluminum strips (1 cm wide × 3 cm long × 1.5 mm thick and bent to 90°angle) were glued to the side of the lower exterior cylinder wall at 90^{o} intervals (corresponding to 4 corners of the plate) with hot glue. To provide multiple routes of

exposure of treatments, *E. kuehniella* eggs (ingestion exposure), beans (ingestion exposure), cheese cloth lids (residual exposure), glass arenas (residual exposure) and insects (contact exposure) were treated as mentioned below (Fig. 1). *Ephestia kuehniella* eggs were used as a food source for both adults and nymphs of *D. brevis* and fresh green beans (organically grown) were provided for moisture. *Ephestia kuehniella* eggs were drenched in 100 ml of a treatment solution and air-dried for 30 min. The green beans were treated by dipping them in 50 ml of a treatment for 5 seconds, suspended from a horizontal wire with a small binder clip and then air-dried. Individual pieces of cheese cloth [10 cm × 10 cm and 15 cm × 15 cm] (#90 [44 ×36], http://www.onlinefabricstore.net) were used as lids to cover the small and large arenas, respectively. The cheese-cloth lids were treated by dipping them in 50 ml of a treatment solution and then air-dried.

The glass arenas and insects were treated with a Potter spray tower (Burkard Scientific, Uxbridge, UK) (103 kPa, intermediate nozzle). Glass plates and cylinders were separately sprayed with 2 ml of solution then removed from the spray tower after a five sec settling period. The treated plates and cylinders were air-dried for 30 min and then assembled and held together with four small (1.9 cm) binder clips at the points where four aluminum strips were glued. Test insects were treated in a 9-cm diameter glass petri dish as a group of four nymphs per replicate or a single pair of adult male and female *D. brevis* per replicate, respectively. Treated insects were then transferred with a soft brush to the assembled glass arenas and then covered with a treated cheese cloth lids (Fig 1). Adult and immature *D. brevis* were provided with treated *E. kuehniella* eggs (approx. 0.2-0.3g) and fresh green beans (one bean pod per replicate). All arenas were placed in an environmental growth chamber (Percival I-36LLVLC8, Percival Scientific Inc., Perry, IA) at 23°C, 60% RH and 16:8 (L:D) h photoperiod. Arenas containing adult or immature

D. brevis were checked daily to assess mortality until 10 d after treatment (DAT). Untreated *E. kuehniella* eggs and fresh green beans were provided to all surviving insects at 72 h after treatment; afterwards, fresh beans and *E. kuehniella* eggs were provided to all surviving insects three times a week. The insecticide and fungicide experiments (insecticides: adults: n=10 [5 replicates], nymphs: n= 20 [5 replicates] and fungicides: adults: n=30 [15 replicates], nymphs: n= 60 [15 replicates]) were conducted independently. The insecticide experiment was repeated twice using the same experimental procedures for a total of 15 replicates.

Bioassay Sublethal Effects: Nymph to Adult Developmental Time, Survival and Sex Ratio. Treated nymphs from the lethal bioassay were reared until they molted to adults and their developmental time and nymph to adult survival (adult emergence) were determined. All surviving nymphs were provided with fresh beans and *E. kuehniella* eggs three times a week. The gender of the emerged adults was determined using the methods described above (Davis 1955). Adult sex ratio was calculated as the percentage of females ([females/ (males + females)] * 100).

Bioassay Sublethal Effects: Adult Longevity, Fecundity, Fertility and Egg Viability. Treated adults were reared until they died. Fresh beans and *E. kuehniella* eggs were provided to all surviving adults three times a week. Green beans from the adult arenas were collected every other day to evaluate the number of eggs each female laid and egg hatch for a period of 20 d (approximately 30% of adult life plus 8 d of preovipositon period). Collected bean pods (egg beans) were checked under a microscope to count the number of eggs laid and then placed individually in a glass petri dish (9 cm diameter) and covered with the lid. A small amount

232 (approx. 0.2-0.3g) of *E. kuehniella* eggs was added to each petri dish as food for emerging
233 nymphs. All petri dishes were placed in an environmental growth chamber set to the conditions
234 above and monitored daily for egg hatch and number of viable nymphs that emerged.
235
236 **Bioassay Sublethal Effects: Intrinsic Rate of Population Increase (r).** An age
237 structured matrix model of *D. brevis* was developed for each insecticide and fungicide using life-
238 history elements of survivorship, developmental rate, fecundity and sex ratio to calculate the
239 intrinsic rate of population increase (r). We used life history stages of eggs (F1 generation), first
240 to second instar (F1 generation), third to fifth instar (treated nymphs), pre-ovipositing females
241 and adult females (treated adults) to obtain the developmental time and survival of each life
242 stage. Daily fecundity was obtained from eggs collected from adult treated females. Sex ratio
243 was calculated from adults that emerged from F1 generation.
244 Newly emerged nymphs from eggs oviposited in beans (eggs from treated adult females)
245 (n=25, 5 replicates per treatment) were collected to study the F1 generation. Five of the nymphs
246 collected from each replicate were placed in a 9-cm diameter petri dish with a fresh green bean
247 and *E. kuehniella* eggs. All petri dishes were placed in an environmental growth chamber and
248 their development and survival were monitored daily till the adult emergence. Gender of the
249 emerged adults was determined.
250 Pop Tools, an add-in for 32 bit PC versions of Microsoft Excel (version 97 and up) was
251 used for the matrix model development and analyses (Hood, 2011).
252
253 **Statistical Analyses.** The experimental design used for both nymph and adult
254 insecticide experiments was randomized complete block design (RCBD). A two-way ANOVA

was performed (PROC MIXED) (SAS Institute 1999) to test for interactions between experiments (blocks) and treatments for mortality, developmental time, survival, sex ratio, fecundity, fertility, egg viability. A three-way ANOVA was performed for adult longevity to test for interactions among experiments (blocks), treatments and gender. Block means were used in mean comparisons.

A completely randomized experimental design (CRD) was used for both nymph and adult fungicide experiments. A one-way ANOVA was performed (PROC MIXED) for mortality, developmental time, survival, sex ratio, fecundity, fertility, egg viability and a two-way ANOVA was performed for adult longevity and gender.

Means were compared at $P \leq 0.05$ significance level for all experiments (LSMEANS) (SAS Institute 1999). Proportion of mortality, survival and sex ratio were arcsine-square root transformed before ANOVA to stabilize variances (Zar 1984).

Insect Identification and Species Verification. Insect identification and species verification (Lot # 1201881) of *D. brevis* was provided by T. J. Henry (Miridae) the Systematic Entomology Laboratory, Agricultural Research Service, US Department of Agriculture, Baltimore, MD.

Voucher Specimens. Voucher specimens of *D. brevis* were deposited in the entomology insect collection at Oregon State University, Mid-Columbia Agricultural Development and Extension Center, Hood River, OR 97031.

Results

Bioassay-Lethal Effects: Insecticides. Nymphs treated with either rate of novaluron or lambda-cyhalothrin had significant mortality at 1, 2 and 10 DAT, respectively (1 DAT: F =2.51, df. = 10, 20, P = 0.0380; 2 DAT: F = 47.40, df. =10, 20, P = 0.0001; 10 DAT: F = 276.83, df. = 10, 20, P = 0.0001) (Table 1) (Fig. 2). At 1 and 2 DAT, mortality of immature *D. brevis* treated with either rate of chlorantraniliprole, cyantraniliprole or spinetoram were not statistically different from mortality observed in the control. Approximately 23% mortality was observed for nymphs treated with the high rate of cyantraniliprole at 10 DAT, although the mortality of nymphs treated with chlorantraniliprole or spinetoram was similar to the control mortality. At 10 DAT, both rates of novaluron and lambda-cyhalothrin caused 100% mortality of nymphs.

Adults treated with the high rate of either lambda-cyhalothrin or novaluron had greater mortality than insects in the control and other treatments at 1 DAT (F = 68.13, df. = 10, 20, P = 0.0001) (Table 2). Mortality increased to 50 and 100% in the lambda-cyhalothrin treatments by 2 DAT (F =27.28, df. = 10, 20, P = 0.0001). Both rates of spinetoram caused 46.7% mortality to adult by 10 DAT while insects treated with chlorantraniliprole, cyantraniliprole or novaluron survived at levels that were not statistically different from the control insects (F = 22.03, df. = 10, 20, P = 0.0001) (Fig. 2).

Bioassay-Lethal Effects: Fungicides. Nymphs treated with the high rate of sulfur had significantly more mortality (10%) at 1 DAT than the control insects (F = 1.79, df. = 4, 56, P = 0.0430) (Table 3). At 2 and 10 DAT, significantly higher mortality was observed for insects treated with either rate of sulfur (2 DAT: F = 3.26, df. = 4, 56, P = 0.0180; 10 DAT: F = 5.84, df.

=4, 54, $P = 0.0006$) with ~50% mortality at 10 DAT. The mortality caused by either rate of the mixture of copper hydroxide and mancozeb was not statistically significant at 1, 2 and 10 DAT.

Neither rate of the copper hydroxide and mancozeb mixture caused any statistically significant mortality to adults by 10 DAT (Table 4). Significant mortality was observed for adults treated with the high sulfur rate at 2 DAT compared with the control insects ($F = 1.64$, df. = 4, 56, $P = 0.0438$).

Bioassay-Sublethal Effects: Nymph to Adult Developmental Time, Survival and Sex Ratio - Insecticides. None of the nymphs treated with novaluron or lambda-cyhalothrin survived to adults while 90% survived in the control ($F = 18.97$, df. = 10, 20, $P = 0.0001$) (Table 5). Fewer nymphs treated with either the high rate of cyantraniliprole or spinetoram survived (80 and 73.3%, respectively) when compared with nymph survival in the control (90%) ($F = 18.97$, df. = 10, 20, $P = 0.0001$). There was no difference in nymph developmental time among treatments. Nymph developmental time ranged between 13.5-14.5 d. Nymphs treated with the high rate of cyantraniliprole had significantly higher female biased sex ratio (73.3%) as emerged adults ($F = 1.23$, df. = 6, 12, $P = 0.0376$).

Bioassay-Sublethal Effects: Nymph to Adult Developmental Time, Survival and Sex Ratio - Fungicides. Fewer nymphs survived to adult when treated with either rate of sulfur (~57%) ($F = 3.15$, df. = 4, 54, $P = 0.0213$) compared with levels observed in the control (Table 6). These nymphs also had a longer nymph to adult developmental time (~17 d) ($F = 4.94$, df. = 4, 43, $P = 0.0023$). There was no difference in the sex ratio of emerged adults from treated nymphs. The sex ratio ranged from 36.4-53.3%.

Bioassay-Sublethal Effects: Adult Longevity, Fecundity and Fertility and Egg Viability - Insecticides. Adult longevity was significantly shorter for males treated with the low rate of chlorantraniliprole or either rate of spinetoram or lambda-cyhalothrin when compared with other treatments ($F = 10.73$, df. = 10, 42, $P = 0.0001$) (Table 7). The females treated with the low rate of chlorantraniliprole or cyantraniliprole, either rate of novaluron, spinetoram or lambda-cyhalothrin had shorter longevity compared with the longevity of the females in the control (36.9 d) ($F = 10.73$, df. = 10, 42, $P = 0.0001$).

Females treated with the high rate of novaluron or spinetoram had significantly lower fecundity compared with the fecundity of the females in the control ($F = 3.97$, df. = 8, 16, $P = 0.0091$) (Table 7). Females treated with the high rate of novaluron produced significantly lower numbers of viable eggs ($F = 2.53$, df. = 8, 16, $P = 0.0453$). The lowest level of egg viability (as a percentage of viable eggs to total number of eggs produced) was observed for the eggs laid by females treated with the high rate of novaluron ($F = 1.58$, df. = 8, 16, $P = 0.0283$).

Bioassay-Sublethal Effects: Adult Longevity, Fecundity and Fertility and Egg Viability - Fungicides. Longevity of adult males and females was not affected by the fungicide ($F = 0.58$, df. = 4, 90, $P = 0.6743$) treatments (Table 8) nor were there differences between sexes ($F = 0.58$, df. = 4, 90, $P = 0.6743$). None of the fungicide treatments caused any negative impact on the fecundity of females. Eggs laid by females treated with either rate of sulfur had significantly lower fertility (~9 eggs hatched) compared with the fertility of the eggs in the control (~ 45 eggs hatched) ($F = 1.70$, df. = 4, 33, $P = 0.0445$). Eggs laid by females treated

345 with the high rate of sulfur had significantly lower egg viability compared with eggs laid by
346 females in the control treatment (F = 1.29, df. = 4, 19, P = 0.0387).
347
348 **Bioassay Sublethal Effects: Intrinsic Rate of Population Increase (r) -**
349 **Insecticides.** The intrinsic rates of population increase (r) obtained from the stage structured
350 matrix models were reduced relative to the control for lambda-cyhalothrin (-0.202), novaluron (-
351 0.158) and spinetoram (-0.003). The r values for chlorantraniliprole and cyantraniliprole were
352 0.088 and 0.091, respectively. The r value for the *D. brevis* in the control treatment was 0.085.
353
354 **Bioassay Sublethal Effects: Intrinsic Rate of Population Increase (r) - Fungicides.**
355 The intrinsic rates of population increase (r) for the *D. brevis* treated with the mixture of copper
356 hydroxide and mancozeb or sulfur were 0.071 and 0.029, respectively. The r value for the *D.*
357 *brevis* in the control treatment was 0.094.
358
359 **Discussion**
360 In this study, we discovered negative effects of some of the reduced risk and OP-
361 replacement insecticides and fungicides we tested on *D. brevis*. Effects were either lethal (acute)
362 or sublethal and they hindered the development, survival and reproduction of *D. brevis* and the
363 impact of some of the pesticides tested was gradual.
364 Despite the singular focus of most toxicological studies on mortality/survival estimates,
365 there is an increasing awareness of more subtle toxicant effects that warrants closer attention
366 (Stark and Banks 2003). Sublethal effects of pesticides can be as important as direct toxic
367 effects when evaluating pesticide effects on natural enemies. Decreases in fecundity, fertility,

368 developmental time, longevity and sex ratio are all considered sublethal effects of pesticides
369 (Theiling and Croft 1989, Starks and Banks 2003). Sublethal effects need to be included and
370 quantified to provide a more accurate picture of the total impact of a pesticide on a natural
371 enemy. In contrast to acute toxicity, which is expressed in terms of mortality of a specific stage,
372 the impact of sublethal effects on a natural enemy population is more difficult to assess in the
373 field.

374 Insecticides with little or no acute toxicity may have sublethal effects including effects on
375 reproduction and development that can negatively impact population growth (Stark and Banks
376 2003). Our results show that fecundity and longevity of *D. brevis* was affected by some of the
377 pesticides we used in this study. We found that *D. brevis* treated with lambda-cyhalothrin,
378 novaluron or spinetoram had a negative intrinsic rate of population increase (r) which according
379 to Starks and Banks (2003) is an indication that the population would decline exponentially and
380 head toward extinction. Recent evaluations have indicated that toxicological analyses based on
381 population growth rate provides a more accurate assessment of a pesticide's impacts because the
382 measure of population growth rate combines both lethal and sublethal effects (Stark and Banks
383 2003). Our results support that assessment.

384 Assay methods used to ascertain the toxicity of an insecticide for an insect can have a
385 great effect on the outcome of the test (Banken and Stark 1998). The International Organization
386 for Biological Control (IOBC) has developed standard protocols for the analysis of the impact of
387 pesticides on non target organisms (Hassan 1985). In the laboratory, individual test organisms of
388 uniform age are either exposed to dried residue on treated surfaces or directly sprayed and
389 moved to a clean surface and monitored for mortality or reduction in predation or parasitism
390 (Banken and Stark 1998). These tests are designed to assess the effects of only one route of

391 pesticide exposure, whereas in the field beneficial organisms may be exposed through several
392 routes including direct contact with spray droplets, uptake residues through contact with
393 contaminated surfaces and oral uptake from contaminated food sources (Longley and Stark
394 1996). The influence of natural routes of pesticide exposure may have subsequent effects on
395 development, reproduction and survivorship that would not be detected in the laboratory tests
396 recommended by the IOBC (Longley and Stark 1996). These traditional bioassays can greatly
397 underestimate the impact of a pesticide (Banken and Stark 1998).

398 In contrast to laboratory bioassays, conducting similar studies in the field is difficult
399 because of the cost, labor intensity and in most circumstances unavailability of target insect stage
400 at the right timing. Because of the severity of crop damage due to some of the target pests
401 involved, for most instances it is not feasible to have a control plot in the field to compare the
402 results of pesticide treatments. Although research conducted on how these reduced -risk
403 insecticides react on important natural enemies in the field is scarce there is information that
404 reduced-risk insecticides used in tree fruit orchards are more damaging to the functional ecology
405 of orchards than anticipated. In some situations, the results of laboratory experiments can be
406 very different from the field experiments, studies conducted in commercial orchards to evaluate
407 some of the reduced-risk insecticide tested in this study (chlorantraniliprole, cyantraniliprole and
408 spinetoram) showed that *D. brevis* populations decline drastically after insecticide treatments
409 (unpublished data). Our results on the intrinsic rate of increase of *D. brevis* further support
410 these studies. Because of lack of field studies, it remains unclear whether theses insecticides are
411 reduced-risk enough to complement biological control programs (Gentz et al. 2010).

412 Results from this study demonstrate that some of the newer insecticides that are replacing
413 organophosphorus insecticides in tree fruit IPM programs in the United States are not as

414 selective to natural enemies as initially thought. Natural enemies that survive pesticide
415 exposures may still sustain significant detrimental impact because of sublethal effects (Stark and
416 Banks 2003). The impact of some of our experimental treatments on *D. brevis* varied with
417 chemistry and mode of action from primarily acute toxicity to reproductive or other sublethal
418 effects or combination of both. Studies combining lethal and sublethal effects with population
419 growth measurements provide better estimates for pesticide impacts on natural enemies. The
420 results from this study should be helpful for developing guidelines for using some of these
421 insecticides in order to minimize their impact on *D. brevis* and related natural enemies in tree
422 fruit orchards.

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Table 1. Mortality (%) (mean ± SEM) of *Deraeocoris brevis* treated as second instars with different rates of insecticides or water (control) 1, 2 and 10 d after treatment (DAT)

Treatment	Max. label rate/ha	Rate[2]	Mg AI/liter	Mortality (%)[1] ± SEM 1 DAT	2 DAT	10 DAT
control	-	-	-	3.3 ± 3.3e	3.3 ± 3.3e	3.3 ± 3.3e
chlorantraniliprole	315.2g	0.1x	11.8	6.7 ± 3.3de	6.7 ± 3.3e	13.3 ± 8.8cde
		1.0x	117.9	0.0 ± 0.0e	3.3 ± 3.3e	3.3 ± 3.3e
cyantraniliprole	1.5L	0.1x	16.0	3.3 ± 3.3e	3.3 ± 3.3e	10.0 ± 0.0de
		1.0x	160.2	3.3 ± 3.3e	6.7 ± 3.3e	23.3 ± 8.8bcd
novaluron	3.7L	0.1x	38.9	23.3 ±23.3cd	56.7 ± 8.8d	100.0 ± 0.0a
		1.0x	388.5	33.3 ± 28.5bc	86.7 ± 8.8b	100.0 ± 0.0a
spinetoram	490.4g	0.1x	13.1	0.0 ± 0.0e	0.0 ± 0.0e	6.7 ± 6.7e
		1.0x	131.1	3.3 ± 3.3e	3.3 ± 3.3e	10.0 ± 5.8de
lambda-cyhalothrin	187.1ml	0.1x	5.0	36.7 ± 21.9bc	70.0 ± 5.8cd	100.0 ± 0.0a
		1.0x	49.9	60.0 ± 26.5a	100.0 ± 0.0a	100.0 ± 0.0a

[1]Means within a column followed by the same letters are not significantly different, $P > 0.05$ (Least Square Means (LSMEANS) Test).

[2]Equivalent to the maximum (1x) or 1/10 (0.1x) of maximum labeled rate dissolved in 378.5 L water.

Table 2. Mortality (%) (mean± SEM) of adult *Deraeocoris brevis* treated with different rates of insecticides or water (control) 1, 2 and 10 d after treatment (DAT)

Treatment	Max. label rate/ha	Rate[2]	Mg AI/liter	Mortality (%)[1] ± SEM		
				1 DAT	2 DAT	10 DAT
control	-	-	-	0.0 ± 0.0d	0.0±0.0f	13.3±8.9de
chlorantraniliprole	315.2g	0.1x	11.8	0.0 ± 0.0d	0.0±0.0f	20.0±11.5cde
		1.0x	117.9	0.0 ± 0.0d	0.0±0.0f	3.3±3.3e
cyantraniliprole	1.5L	0.1x	16.0	0.0 ± 0.0d	0.0±0.0f	13.3±8.9de
		1.0x	160.2	0.0 ± 0.0d	3.3±3.3ef	10.0±10.0de
novaluron	3.7L	0.1x	38.9	3.3 ± 3.3cd	3.3±3.3ef	23.3±3.3cde
		1.0x	388.5	6.7 ± 3.3bc	6.7±3.3def	20.0±10.0cde
spinetoram	490.4g	0.1x	13.1	3.3 ± 3.3cd	6.7±3.3def	46.7±3.3b
		1.0x	131.1	3.3 ± 3.3cd	10.0±5.8cde	46.7±14.5b
lambda-	187.1ml	0.1x	5.0	3.3 ± 3.3cd	50.0±23.1b	86.7±8.8a
cyhalothrin		1.0x	49.9	66.7 ± 3.3a	100.0±0.0a	100.0±0.0a

[1]Means within a column followed by the same letters are not significantly different, $P > 0.05$ (Least Square Means (LSMEANS) Test).

[2]Equivalent to the maximum (1x) or 1/10 (0.1x) of maximum labeled rate dissolved in 378.5 L water.

Table 3. Mortality (%) (mean± SEM) of *Deraeocoris brevis* treated as second instars with different rates of fungicides or water (control) 1, 2 and 10 d after treatment (DAT)

Treatment	Max. label rate/ha	Rate[2]	Mg AI/liter	Mortality (%)[1] ± SEM		
				1 DAT	2 DAT	10 DAT
control	-	-	-	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
copper hydroxide	4.5 kg,	0.1x	221.0	0.0 ± 0.0c	0.0 ± 0.0c	10.7 ± 5.7bc
+ mancozeb	2.0 kg		161.8			
		1.0x	2209.9	6.7 ± 4.5bc	13.5 ± 5.9bc	20.0 ± 9.5bc
			1617.8			
sulfur	22.4 kg	0.1x	1917.4	3.3 ± 3.3c	16.7 ± 6.3ab	50.0 ± 9.1a
		1.0x	19174.4	10.0 ± 5.3ab	16.7 ± 6.3ab	46.7 ± 10.3a

[1]Means within a column followed by the same letters are not significantly different, $P > 0.05$ (Least Square Means (LSMEANS) Test).

[2]Equivalent to the maximum (1x) or 1/10 (0.1x) of maximum labeled rate dissolved in 378.5 L water.

549 **Table 4. Mortality (%) (mean ± SEM) of adult *Deraeocoris brevis* treated with different rates of fungicides or water**

550 **(control) 1, 2 and 10 d after treatment (DAT)**

Treatment	Max. label rate/ha	Rate[2]	Mg AI/liter	Mortality (%)[1] 1 DAT	2 DAT	10 DAT
control	-	-	-	0.0±0.0a	0.0±0.0c	7.1±4.9a
copper hydroxide +	4.5kg,	0.1x	221.0	0.0±0.0a	0.0±0.0c	13.3±7.7a
mancozeb	2.0kg		161. 8			
		1.0x	2209.9	3.3±3.3a	3.3±3.3bc	20.0±8.2a
			1617.8			
sulfur	22.4kg	0.1x	1917.4	0.0±0.0a	3.3±3.3bc	16.7±7.9a
		1.0x	19174.4	6.7±4.5a	10.0±5.3ab	20.0±6.5a

551 [1]Means within a column followed by the same letters are not significantly different, $P > 0.05$ (Least Square Means (LSMEANS) Test).

552 [2]Equivalent to the maximum (1x) or 1/10 (0.1x) of maximum labeled rate dissolved in 378.5 L water.

553 **Table 5. Nymph to adult survival (%), nymph to adult developmental time (d) and adult sex ratio (adults emerged from treated nymphs) (%)**

554 **(mean ± SEM) of *Deraeocoris brevis* treated as second instar with different rates of insecticides or water (control)**

Treatment	Max. label rate/ha	Rate [2]	Mg AI/liter	Survival (%)[1, 4] nymph to adult	Developmental time (d) [1, 4] nymph to adult	Adult sex ratio (%)[1,3,4]
control	-	-	-	90.0±5.8abc (60)	13.8±0.9a (56)	42.5±3.8d (54)
chlorantraniliprole	315.2g	0.1x	11.8	76.7±6.7cde (60)	13.5±0.3a (46)	50.0±0.0cd (46)
		1.0x	117.9	86.7±8.8abcd (60)	13.7±0.5a (52)	63.3±6.7cd (52)
cyantraniliprole	1.5L	0.1x	16.0	80.0±5.8bcde (60)	13.9±0.8a (50)	56.7±12.0cd (48)
		1.0x	160.2	66.7±8.8e (56)	13.7±0.8a (34)	73.3±14.5abc (36)
novaluron	3.7L	0.1x	38.9	0.0±0.0f (60)	-	-
		1.0x	388.5	0.0±0.0f (60)	-	-
spinetoram	490.4g	0.1x	13.1	80.0±5.8bcde (60)	14.1±0.9a (50)	70.0±5.8bcd (48)
		1.0x	131.1	73.3±6.7de (60)	14.5±0.8a (52)	46.7±16.7cd (44)
lambda-cyhalothrin	187.1ml	0.1x	5.0	0.0±0.0f (60)	-	-
		1.0x	49.9	0.0±0.0f (60)	-	-

555 [1]Means within a column followed by the same letters are not significantly different, $P > 0.05$ (Least Square Means (LSMEANS) Test).

556 [2]Equivalent to the maximum (1x) or 1/10 (0.1x) of maximum labeled rate dissolved in 378.5 L water.

557 [3] Adult sex ratio calculated as the percentage of females = ([females/ (males + females)] * 100).

558 [4] Total number of nymphs used in each treatment is stated in the parenthesis.

Table 6. Nymph to adult survival (%), nymph to adult developmental time (d) and adult sex ratio (adults emerged from treated nymphs) (%) (mean ± SEM) of *Deraeocoris brevis* treated as second instar with different rates of fungicides or water (control)

Treatment	Max. label rate/ha	Rate[2]	Mg AI/liter	Survival (%)[1,4] nymph to adult	Developmental time (d) [1,4] nymph to adult	Adult sex ratio (%)[1,3,4]
control	-	-	-	90.0±5.3ab (52)	15.7±0.2d (46)	53.3±11.4a (46)
copper hydroxide	4.5kg,	0.1x	221.0	78.6±6.9bc (56)	15.5±0.3d (44)	53.6±12.3a (44)
+ mancozeb	2.0kg		161.8			
		1.0x	2209.9	73.3±9.6bc (54)	16.1±0.2cd (38)	46.2±13.2a (38)
			1617.8			
sulfur	22.4kg	0.1x	1917.4	57.1±10.3c (50)	16.8±0.3bc(26)	36.4±15.2a (26)
		1.0x	19174.4	56.7±8.3c (58)	17.3±0.6ab (24)	50.0±16.7a (24)

[1]Means within a column followed by the same letters are not significantly different, $P > 0.05$ (Least Square Means (LSMEANS) Test).

[2]Equivalent to the maximum (1x) or 1/10 (0.1x) of maximum labeled rate dissolved in 378.5 L water.

[3] Adult sex ratio calculated as the percentage of females = ([females/ (males + females)] * 100).

[4] Total number of nymphs used in each treatment is stated in the parenthesis.

Table 7. Adult longevity (d), fecundity, fertility and egg viability (%) (mean ± SEM) of *Deraeocoris brevis* treated as adults with different rates of insecticides or water (control)

Treatment	Max. label rate/ha	Rate[3]	Mg AI/liter	Adult Longevity (d) [1,2,7]		Fecundity[1,4,7]	Fertility[1,5,7]	Egg Viability (%)[1,6,7]
				Male	Female			
control	-	-	-	33.0±2.3abc (13)	36.9±7.0a (13)	92.2±23.8abcd (12)	36.3±11.0abcd (12)	33.3±5.0ab (12)
chlorantraniliprole	315.2g	0.1x	11.8	21.4±4.7de (14)	8.2±5.8bcd (15)	56.4±0.5de (13)	18.5±5.8cde (13)	21.5±5.5b (13)
		1.0x	117.9	36.9±6.5ab (10)	39.4±2.8a (14)	71.0±6.1cd (13)	27.1±7.3bcd (13)	35.3±6.4ab (13)
cyantraniliprole	1.5L	0.1x	16.0	32.2±2.6abc (12)	27.3±9.5bcd (13)	66.7±6.2cde (11)	29.3±9.1bcd (11)	33.4±13.2ab (11)
		1.0x	160.2	24.6±4.1cde (14)	35.4±8.3ab (13)	80.8±24.4bcd (15)	29.9±11.7bcd (15)	30.8±10.1ab (15)
novaluron	3.7L	0.1x	38.9	33.2±5.7abc (12)	27.6±4.8cd (13)	70.2±22.8cd (12)	37.4±16.1abcd (12)	33.8±13.0ab (12)
		1.0x	388.5	31.9±10.1abcd (12)	17.7±3.0ef (14)	20.3±13.2e (12)	3.3±3.3e (12)	4.4±4.4c (12)
spinetoram	490.4g	0.1x	13.1	18.4±4.7ef (14)	22.9±6.5cde (13)	90.0±21.2abcd (5)	30.5±14.0bcd (5)	31.6±6.7ab (5)
		1.0x	131.1	11.6±4.4fg (13)	21.1±9.0de (14)	20.8±7.2e (7)	10.2±8.8de (7)	25.4±19.3b (7)
lambda-cyhalothrin	187.1ml	0.1x	5.0	4.9 ± 1.9g (15)	7.3 ± 2.2fg (14)	-	-	-
		1.0x	49.9	1.7 ± 0.3g (15)	1.9 ± 0.1g (15)	-	-	-

[1]Means within a column followed by the same letters are not significantly different, $P > 0.05$ (Least Square Means (LSMEANS) Test).

[2]Means between males and females followed by the same letters are not significantly different, $P > 0.05$ (Least Square Means (LSMEANS) Test).

[3]Equivalent to the maximum (1x) or 1/10 (0.1x) of maximum labeled rate dissolved in 378.5 L water.

[4]Fecundity = total number of eggs laid per 20 d period.

[5]Fertility = total number of eggs hatched.

[6]Egg viability (%) = [(Fertility/Fecundity)*100].

[7]Total number of individuals used in each treatment is stated in the parenthesis.

575 **Table 8. Adult male and female longevity (d), fecundity, fertility and egg viability (%) (mean ± SEM) of *Deraeocoris brevis* treated as adults with**

576 **different rates of fungicides or water (control)**

Treatment	Max. label rate/ha	Rate[3]	Mg AI/liter	Adult Longevity (d) [1, 2,7]		Fecundity[1,4,7]	Fertility[1,5,7]	Egg viability (%)[1,6,7]
				Male	Female			
control	-	-	-	32.6±6.4ab (9)	34.7±5.1ab (9)	97.5±40.3a (4)	44.8±22.1ab (4)	51.3±13.3ab (4)
copper hydroxide	4.5kg,	0.1x	221.0,	30.3±4.7ab (12)	31.1±4.4ab (14)	79.0±22.4a (14)	26.1±9.8bc (14)	29.8±4.9bc (14)
+ mancozeb	2.0kg		161.8					
		1.0x	2209.9,	36.9±3.6a (10)	30.7±4.8ab (13)	73.5±26.0a (11)	25.9±10.0bc (11)	35.7±9.7bc (11)
			1617.8					
sulfur	22.4kg	0.1x	1917.4	31.2±3.5ab (12)	30.5±4.1ab (11)	39.9±13.9a (12)	9.3±5.2c (12)	29.8±11.4bc (12)
		1.0x	19174.4	22.7±2.9b (12)	29.0±2.6ab (12)	39.4±16.4a (11)	9.1±6.1c (11)	12.6±8.9c (11)

577 [1]Means within a column followed by the same letters are not significantly different, $P > 0.05$ (Least Square Means (LSMEANS) Test).

578 [2]Means between males and females followed by the same letters are not significantly different, $P > 0.05$ (Least Square Means (LSMEANS) Test).

579 [3]Equivalent to the maximum (1x) or 1/10 (0.1x) of maximum labeled rate dissolved in 378.5 L water.

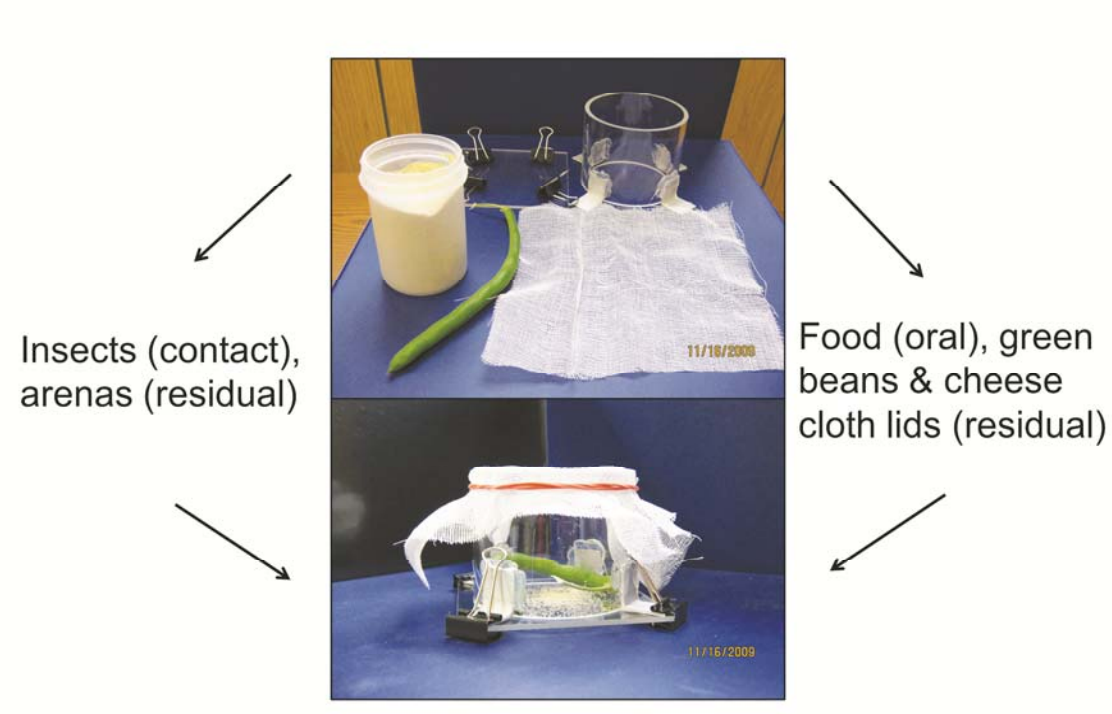
580 [4]Fecundity = total number of eggs laid per 20 d period.

581 [5]Fertility = total number of eggs hatched.

582 [6]Egg viability (%) = [(Fertility/Fecundity)*100].

583 [7]Total number of individuals used in each treatment is stated in the parenthesis.

584 **Fig 1.** Multiple routes of exposure bioassay arena for *D. brevis*.


Insects (contact), arenas (residual)
Food (oral), green beans & cheese cloth lids (residual)
11/16/2009
11/16/2009

585
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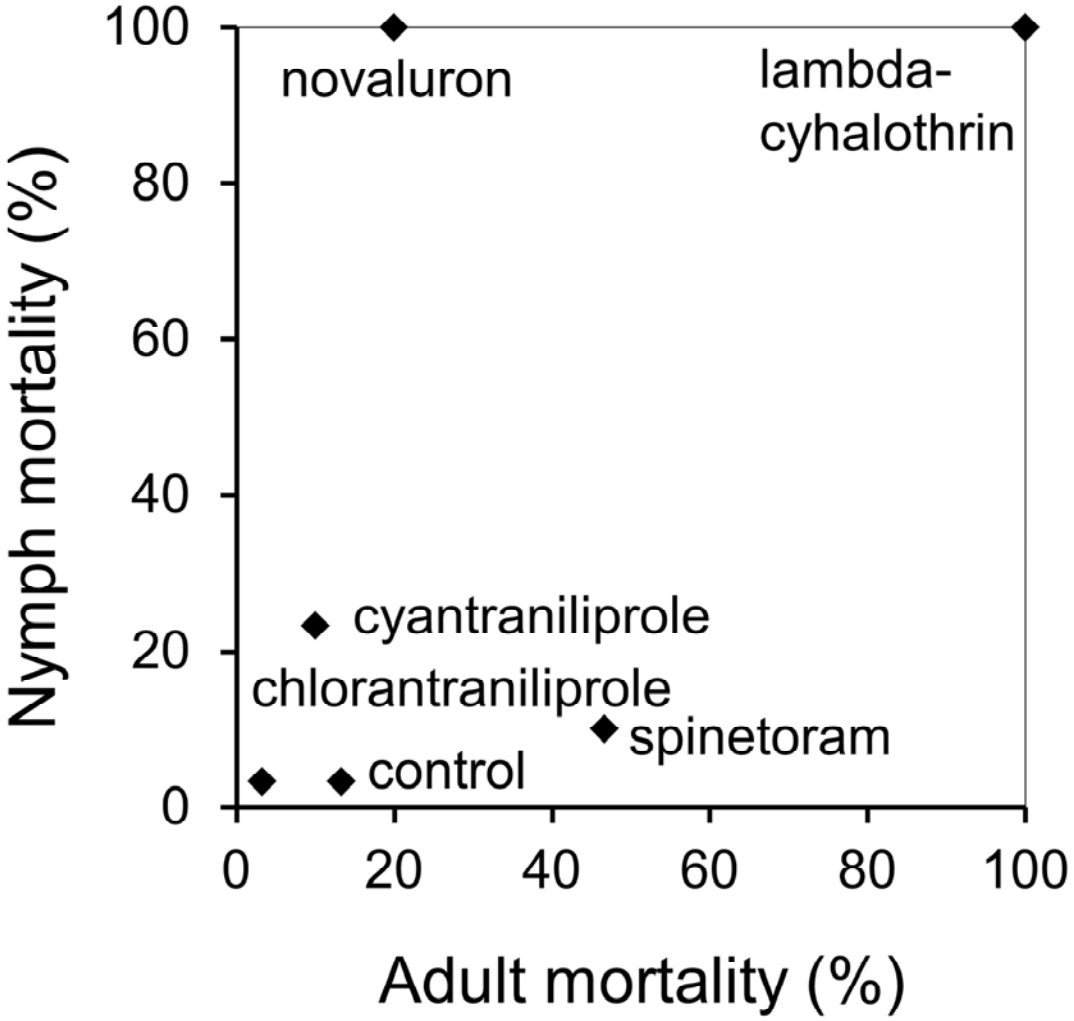
587 **Fig. 2.** Effects of insecticides on *D. brevis* at 10 DAT in relation to percentage nymph and adult
588 mortality at high label rate.


100
80
60
40
20
0
Nymph mortality (%)
novaluron
lambda-
cyhalothrin
cyantraniliprole
chlorantraniliprole
spinetoram
control
0
20
40
60
80
100
Adult mortality (%)

589