

# Effects of Direct and Indirect Exposure of Insecticides to Garden Symphylan (*Symphyla*: *ScutigereLLidae*) in Laboratory Bioassays

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**ABSTRACT** The garden symphylan, *ScutigereLLa immaculata* Newport, is a serious soil pest whose root feeding affects yield and survival of several high valued crops in the California's central coast. Because organophosphate insecticides, widely used for *S. immaculata* control, are rigorously regulated and little is known about the efficacy of alternate insecticides, laboratory bioassays were conducted to determine insecticide efficacy through repellency and lethality. To determine indirect repellency (noncontact) of insecticides, choice assays were conducted where five *S. immaculata* were introduced into the arena to choose between insecticide-treated and untreated wells whereas, in direct repellency (contact) assays, three insecticide-treated 1-cm-diameter discs were pasted into the arena and the number of visits, time spent per visitation, and number of long-duration (>10 s) stays of five *S. immaculata* were quantified. To determine efficacy through direct mortality, number of *S. immaculata* died after 72 h were determined by introducing 10 *S. immaculata* to insecticide-treated soil assays. In indirect exposure bioassays, seven (clothianidin, oxamyl, zeta-cypermethrin, chlorpyrifos, ethoprop, azadirachtin, and a combination of beta-cyfluthrin and imidacloprid) out of 14 insecticides tested elicited repellency to *S. immaculata*. Of six insecticides tested in the direct exposure assays, only tolfenpyrad elicited contact repellency. In soil assays, after 72 h of introduction, bifenthrin, oxamyl, clothianidin, zeta-cypermethrin, and tolfenpyrad caused 100, 95, 80, 44, and 44% *S. immaculata* mortality, respectively, which was significantly greater than distilled water and four other insecticides. The implications of these results on *S. immaculata* management in the California's central coast are discussed.

**KEY WORDS** *ScutigereLLa immaculata*, lettuce, vegetables, broccoli, Salinas valley

The garden symphylan, *ScutigereLLa immaculata* Newport (Family ScutigereLLidae), a white, highly mobile, omnivorous centipede-like, 3- to 6-mm-long soil arthropod (Waterhouse et al. 1969, Waterhouse 1970, Berry and Robinson 1974, Umble et al. 2006), is a serious soil pest of several crops in the United States (Woodworth 1905, Illingworth 1927, Wymore 1931, Michelbacher 1935, Gould and Edwards 1968, Berry and Robinson 1974, Umble et al. 2006). In the central coast of California, high-value crops such as lettuce (*Lactuca sativa* L.), strawberry (*Fragaria cascadenSis* K.E. Hummer), broccoli (*Brassica oleracea* var. *italica* Plenck), cauliflower (*B. oleracea* L. var. *botrytis*), and celery (*Apium graveolens* var. *dulce* Mill.), which are valued at \$1.21, 0.86, 0.43, 0.16, 0.22, and 0.17 billion USD, respectively (Monterey County Crop Report 2013), and several other miscellaneous vegetables are affected by *S. immaculata* feeding. *S. immaculata* feeds on roots of both direct-seeded and transplanted crops alike (Umble et al. 2006), causing severe stunting and plant mortality. Besides feeding on the roots, they also survive feeding on organic matter, and other soil-

dwelling fungi (Waterhouse et al. 1969, Umble et al. 2006). *S. immaculata* use the channels created by other soil organisms such as earthworms for vertical and lateral movement through the soil profile. Their seasonal dynamics in the soil are also influenced by soil moisture (Michelbacher 1939) and temperature (Berry and Robinson 1974). Incidence of *S. immaculata* infestation is mostly reported in heavier or clay soils with higher organic matter content than lighter or sandy soils (Edwards 1958, 1961). Often, *S. immaculata* aggregate in high densities in certain spots in the field and the damage is concentrated in those spots. The spots vary in size but they could be as large as 0.1 ha.

*S. immaculata* are primarily managed using preventative insecticide application (Morrison 1953, Sechrist 1972), although other tactics such as crop rotation (William 1996, Peachey et al. 2002), planting less susceptible crops (Umble and Fisher 2003), flooding the field (Michelbacher 1935), reduced tillage (Morrison 1953, Peachey et al. 2002), and conservation of beneficial organisms (Getzin and Shanks 1964, Swenson 1965, Stimmann 1968; Waterhouse 1969, Berry 1973, Peachey et al. 2002) have been suggested. Success and effectiveness of these nonchemical tactics were constrained by several factors such as lack of fit to the current production practices, susceptible crops being

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grown, varied topography, and enormous population size, although the biology and ecology of *S. immaculata* in California's central coast has not been studied thoroughly. In California, primarily organophosphate insecticides such as chlorpyrifos, diazinon, and mocap were recommended (Natwick 2009) and widely used to manage soil borne pests including *S. immaculata* in the vegetable crops. Until recently, most of these organophosphate insecticides were rigorously regulated because of off-site movement and detection of high residue levels in water bodies posing threats to humans and the environment (Hunt et al. 2003, California Environmental Protection Agency [CEPA] 2013). Thus, growers in the region are switching to other insecticides such as pyrethroids, neonicotinoids, and newer insecticides hitting the market with no or limited research-based knowledge on efficacy to manage *S. immaculata*. Moreover, the incidence and distribution pattern of *S. immaculata* within the field is patchy and unpredictable (Berry and Robinson 1974, Umble et al. 2006), which poses a challenge to carry out successful field efficacy studies against *S. immaculata* (Howitt and Bullock 1955, Howitt et al. 1959; S.V.J., unpublished data). Previous laboratory bioassays against *S. immaculata* tested older insecticides and found that parathion and ammonium cyanamid were effective (Howitt 1959b), but those insecticides are not currently available for use. Clearly, there is a need to establish relative efficacy of current and newer insecticides against *S. immaculata*.

Insecticides as repellents to soil pests have been documented (Herk et al. 2008) but have not been previously studied against *S. immaculata*. *S. immaculata* spend their entire life in the soil and are well adapted to the subterranean habits. They lack eyes but have long antennae and thousands of sensory hairs on the body, which possibly function as mechanoreceptors and chemoreceptors (Eisenbeis 2006). With a pair of long, movable sensory hairs called trichobothria on cerci (Haupt 1970, Kraus and Kraus 1994) *S. immaculata* could perceive stimuli from eight different directions (Haupt 1970). Also, *S. immaculata* is highly mobile with normal speed of 5–20 mm per second and can be as fast as 40 mm per second (Eisenbeis 2006). These features and behaviors possibly aid the foraging *S. immaculata* to sense and evade insecticides in the soil. Thus, the major objectives of this study were to establish relative efficacy of insecticides at commercial field rate against *S. immaculata* through, 1) repellency behavior by indirect exposure (avoidance without contact), 2) direct exposure (avoidance after contact), and 3) *S. immaculata* lethality. The knowledge of relative repellency before and after contact, and lethal effects of insecticides to *S. immaculata* will help to set baseline and later develop IPM strategies for *S. immaculata* management in several economically important crops in California's central coast.

## Materials and Methods

**Arthropod Source.** The garden symphylans were field-collected from cauliflower (*B. oleracea* var.

*botrytis*) and celery (*A. graveolens* var. *dulce*) fields in Moss Landing, CA, and San Jan Bautista, CA, respectively, in 2013 and 2014. Stunted transplant plugs with *S. immaculata* were scooped out from the soil. In addition, potato slices, used as baits to monitor *S. immaculata* in the soil (Umble and Fisher 2003), were placed on the soil surface and covered with plastic bowls for 2 d. Several of these baits were deployed within the *S. immaculata*-infested hot spots in the field. After 2 d, *S. immaculata* on the potato baits were carefully dislodged by tapping into 1.2-liter containers (Rubbermaid, High Point, NC) with moist soil and the containers were transported to the laboratory. In the laboratory, *S. immaculata* were carefully extracted from the transplant plugs and soil using a soft paint brush and maintained in moist soil covered with moist paper towels in 1.2-liter Rubbermaid containers. The containers were kept closed using their lids to reduce desiccation and were maintained in darkness at ~21°C in the drawer of a bench in the laboratory. Extracted *S. immaculata* used in the bioassays were mostly adults with 12 pairs of legs.

**Insecticides.** The details of the insecticides, formulations, recommended rates, and tested rates for all the experiments are listed in Table 1. Whenever possible, the label recommended rates of insecticides specifically for *S. immaculata* were used to determine the test rate, although most of the tested insecticide products lacked a recommended rate specifically for *S. immaculata*. Thus, a maximum recommended rate of any of the closely related insect pests of vegetable crops was selected for testing. Those novel insecticides which lack current registration for use on vegetables but are on the path of registration against insect pests of vegetables as soil-applied insecticides in other agro-systems were also included in the study. The rates of such new insecticides were determined after consultation with the manufacturer. Because the water volume generally varies between 280.6 and 560.7 liters per ha in the central coast vegetable production when applied using tractor-mounted sprayers; therefore, an intermediate, commonly used rate, 373.98 liters per ha was chosen for the bioassays.

**Indirect Exposure Bioassay.** The bioassay equipment used in Rijal et al. (2014) was used as a model for construction of bioassays. The bioassay arenas were constructed using clear, nonventing 60- by 15-mm polystyrene Petri dishes (Fisher Scientific, Pittsburgh, PA). Two transverse, 10-mm-diameter holes were drilled in the center of opposite quadrants of each bottom dish leaving 15 mm between holes. In the bioassay, to create a barrier and prevent movement of *S. immaculata* back to the dish, 100–1250 µl micropoint pipette tips (Fisher Scientific, Pittsburgh, PA) were cut (upper-end: 6 mm inner diameter, lower-end: 5 mm inner diameter, and ~1.5 cm long) and the upper end of the cut tip was glued into the holes created in the bottom of the Petri dishes so that the tips projected downwards outside the Petri dish. A 10-mm-diameter hole was drilled on the plastic cap that threaded on 6-ml, 19- by 20-mm, clear glass vials (Fisher Scientific, Pittsburgh, PA). The outer side of the vial-cap was inserted through the cut pipette

**Table 1. Insecticides evaluated against *S. immaculata* in laboratory bioassays**

Class	Insecticide	Formulation <sup>a</sup>	Recommended field rate (a.i. per ha) <sup>b</sup>	Tested rate (a.i. per ha)	Tested dose (ppm) <sup>c</sup>
Neonicotinoid	Clothianidin*†‡	EC	168.03–224.05 g	224.05 g	599.78
Pyrethroid	Bifenthrin*†	LFR	44.81–89.61 g	89.61 g	239.88
	Zeta-cypermethrin*†	EC	31.37–55.99 g	55.99 g	149.88
	Lambda-cyhalothrin*	EC	16.97–27.98 g	27.98 g	74.90
Neonicotinoid + Pyrethroid	Imidacloprid + Beta-cyfluthrin*	EC	52.56 g + 25.85 g	105.00 g + 52.51 g	281.08 + 140.56
Organophosphate	Ethoprop*	15% G	n/a <sup>e</sup>	335.99 g	899.44
	Chlorpyrifos*	E in water	1367.12 g	1367.12 g	3659.77
Carbamate	Oxamyl*†† <sup>d</sup>	L	n/a <sup>e</sup>	560.18 g	1499.59
Spinosyn	Spinetoram*†‡	SC	43.76–87.52 g	87.52 g	234.29
Ryanodine receptor activator	Cyantraniliprole*†‡	SC	145.64–197.18 g	197.18 g	527.84
Pyridazinone	Tolfenpyrad*†‡	EC	n/a <sup>e</sup>	237.11 g	634.74
Tetranortriterpenoid	Azadirachtin*† <sup>f</sup>	EC	13.83–27.66 g	27.66 g	74.04
	Essential oils *† <sup>f/g</sup>	–	–	–	–
	Mineral oil * <sup>f</sup>	–	–	–	–

Insecticides included in indirect exposure experiment (\*), direct exposure experiment (†), and mortality experiment (‡).

<sup>a</sup> Abbreviations—EC or E, emulsifiable concentrate; LFR, liquid fertilizer ready; G, granule; L, liquid flowable; SC, suspension concentrate.

<sup>b</sup> Recommended rate for insect pests in vegetable crops but not necessarily for *S. immaculata*.

<sup>c</sup> Dose determined based on 373.98 liters per hectare.

<sup>d</sup> Registered for *S. immaculata* on pepper and celery in the United States.

<sup>e</sup> Not registered on any vegetable crops.

<sup>f</sup> Organic Materials Review Institute (OMRI) certified.

<sup>g</sup> Contains rosemary oil and peppermint oil.

tips attached to the Petri dishes, and also glued to the Petri dish. Two glass vials were then screwed to the glued vial-caps attached to each Petri dish creating wells protruding from the base of the dish. The inner bottom surface of the dish was uniformly roughened using sandpaper (3M, St. Paul, MN) to facilitate *S. immaculata* movement.

For the bioassays, “Clear Lake Clay” soil was collected from the fallowed fields in Salinas, CA, and was dried in an oven at > 100°C for 72 h. In a well, 3 g of oven-dried soil was added, then 1 ml of insecticide solution or distilled water was added into the well using a pipette. In the first set of experiments, soil in both the wells was treated only with distilled water. In the later experiments, one well was treated only with distilled water and other with insecticide solution. Fourteen insecticides were tested for indirect exposure, and the details of active ingredients, and the rates used are listed in Table 1 (indicated by symbol \*).

Once the soil was added into the wells, five *S. immaculata* were added to the center of the Petri dish using a soft paint brush. The Petri dish was then covered with the lid and the edges were sealed using Parafilm (Bemis Company Inc., Oshkosh, WI) to reduce desiccation of *S. immaculata*. The bioassays were held in darkness at ~21°C and ~45% relative humidity (room conditions) for 6 h. After 6 h, those five *S. immaculata* were located and their locations inside the arena were documented. For each insecticide treatment, bioassays were replicated 18 times and a total of 90 *S. immaculata* were used for each insecticide product.

**Direct Exposure Bioassay.** A clear, nonventing 60- by 15-mm (5.5-cm-diameter) polystyrene Petri dish (Fisher Scientific, Pittsburgh, PA) was used for the direct exposure bioassay. The bottom surface of the Petri dish was divided and marked into three pie sections. Whatman No. 1 filter paper was dipped for 2 s in

distilled water and placed on the bottom of the dish. For this bioassay, 1-cm diameter filter paper discs were made from 4.7-cm black qualitative filter paper (Ahlstrom filtration LLC, Helsinki, Finland). These black discs were dipped into 45-ml insecticide solution in a container for 5 s then air dried for 20 min in a running fume hood. Three insecticide-treated discs were pasted on to the wet Whatman No. 1 filter paper in the center of each pie using a washable glue stick (Elmer’s product Inc., Columbus, OH). Black colored filter paper was selected for the discs because they would be in contrast to white or off-white *S. immaculata* and help with evaluation.

Five *S. immaculata* were introduced to the center of the Petri dish using a soft paint brush. The movement of five *S. immaculata* was videotaped for 20-min using Dino-Lite scope (BigC, Torrance, CA) mounted on to an adjustable stand. These bioassays were conducted in the laboratory at ~21°C and ~45% relative humidity. Nine insecticides were tested using this bioassay (Table 1, indicated by symbol †) and every insecticide treatment was videotaped and replicated six times per insecticide product. The videos were evaluated for number of times *S. immaculata* visited the black discs, time spent per visit on the black discs, and number of times *S. immaculata* spent > 10 s on the black discs. Stop watch (Apple iPhone 4 s, Cupertino, CA) and tally counter (The Denominator Company, Inc, Woodbury, CT) were used to quantify those parameters. Insecticides belonging to class pyrethroids were not tested in this bioassay because they are more likely to trigger hyperactivity after exposure (Alzogaray and Zerba 2001).

**Soil Bioassay.** The bioassay was developed to determine the efficacy of insecticides against *S. immaculata* through their mortality. Typically, *S. immaculata* forage through the soil profile and it is probable that these foraging *S. immaculata* are exposed to applied insecticides

in soil by contact. In the central coast of California, insecticides targeting *S. immaculata* control were either applied at planting as a narrow band (~12 cm wide) along the seed line for direct-seeded crops or through drip irrigation for transplanted seedlings. Nine insecticides were tested to determine efficacy against *S. immaculata*. The details of active ingredients and tested rate are listed in Table 1 (indicated by symbol ‡).

The soil bioassays were performed using 100-ml translucent polypropylene cups (6-cm diam. wide and 7.1-cm deep) with soil. "Clear Lake Clay" soil was collected from a field in Salinas, CA, where natural *S. immaculata* infestation was previously reported. The soil was dried in an oven at > 100°C for 72 h. Preliminary bioassays were conducted to optimize the soil and water content suitable for this bioassay. Therefore, 25 g of oven-dried soil was added to the cup and 7 ml of insecticide solution or distilled water per cup was uniformly pipetted on to the surface of the soil within the cup. After adding the insecticide solution or distilled water, the soil was stirred five times using a glass rod to facilitate movement of *S. immaculata* within the gaps in soil. Ten *S. immaculata* were introduced into each cup and the cups were covered with perforated caps to allow air flow. Each insecticide treatment was replicated at least 15 times (cups) for a total of 150 *S. immaculata* per insecticide product. The bioassays were maintained in flume hood at ~21°C and ~45% relative humidity for 72 h before evaluation. Seventy-two hours after introduction, the bioassays were evaluated for *S. immaculata* mortality. A needle was used to prod the *S. immaculata* to confirm live, moribund, or dead status. The live *S. immaculata* wiggled easily and moved their legs when poked. The mobility of moribund *S. immaculata* was completely arrested but they sluggishly moved their legs. The dead *S. immaculata* were completely immobile. Because none of the moribund *S. immaculata* reverted to live state, the moribund *S. immaculata* were pooled with dead *S. immaculata* for analysis.

**Statistical Analyses.** For indirect exposure bioassay, the mean number of *S. immaculata* in wells between insecticide or distilled water-treated and distilled water-treated was analyzed (Student's *t*-test) using PROC TTEST procedure in Statistical Analysis System (SAS) software at  $\alpha=0.05$  (SAS Institute 2010). In the direct exposure bioassay, the frequency of *S. immaculata* visiting black discs, total time spent per visit, and number of times *S. immaculata* spent greater than 10 s on the black discs were log-transformed ( $\ln[x + 1]$ ) to establish homogeneity of variance determined using the PROC Univariate procedure in SAS and then analyzed (ANOVA) using PROC GLM procedure in SAS. Similarly, the mortality data from the soil bioassay were analyzed using PROC GLM procedure in SAS at  $\alpha=0.05$  (SAS Institute 2010). Means and standard error for the variables were calculated using PROC MEANS procedure in SAS.

## Results

**Indirect Exposure Bioassay.** Of the 1,338 *S. immaculata* used for this experiment, 90.5%

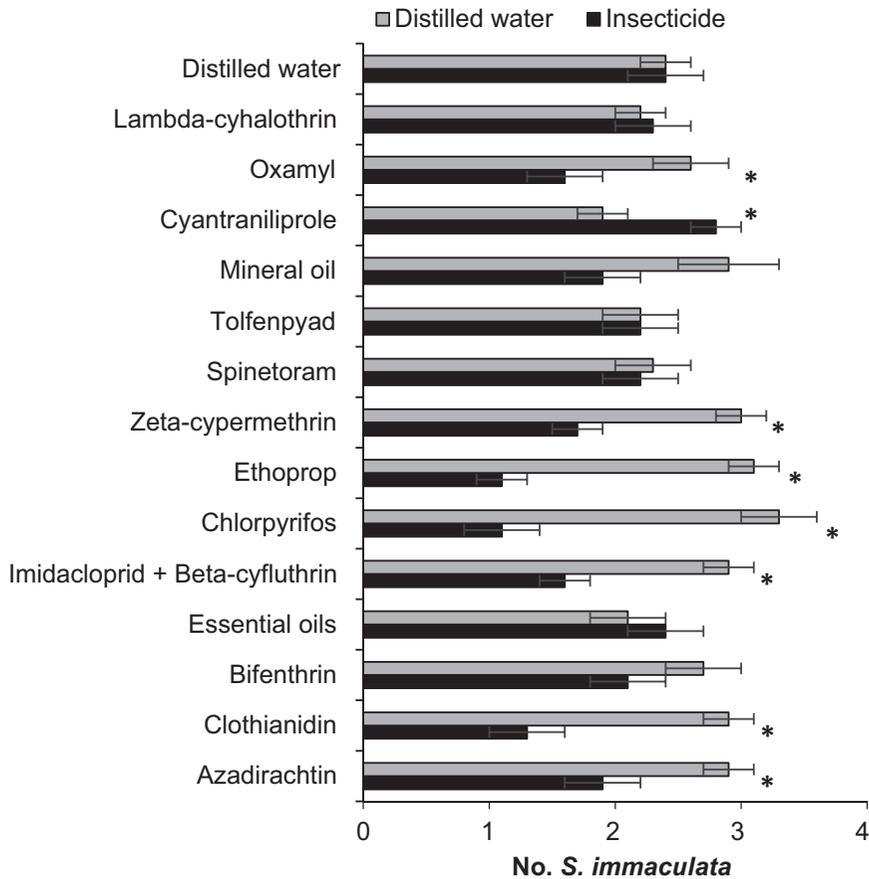
entered either of the wells in 6 h. *S. immaculata* were equally distributed between wells treated with distilled water (Fig. 1). A significantly greater number of *S. immaculata* was found in the well with distilled water when the other well was treated with clothianidin ( $t=-3.2$ ;  $df=32$ ;  $P=0.004$ ), chlorpyrifos ( $t=-6.1$ ;  $df=34$ ;  $P<0.001$ ), ethoprop ( $t=-6.4$ ;  $df=34$ ;  $P<0.001$ ), zeta-cypermethrin ( $t=-2.3$ ;  $df=34$ ;  $P<0.001$ ), oxamyl ( $t=-6.1$ ;  $df=34$ ;  $P=0.027$ ), and imidacloprid + beta-cyfluthrin ( $t=-3.9$ ;  $df=34$ ;  $P<0.001$ ). On the other hand, significantly more *S. immaculata* were found in the well with cyantraniliprole than in the well with distilled water ( $t=3.3$ ;  $df=34$ ;  $P=0.002$ ). Among the organically certified insecticides, significantly more *S. immaculata* was recovered in the wells containing soil treated with distilled water than azadirachtin ( $t=-3.2$ ;  $df=32$ ;  $P=0.004$ ), although these numbers were not significantly different between wells containing essential oil product (rosemary and peppermint oils) ( $t=-1.7$ ;  $df=34$ ;  $P=0.399$ ) or mineral oil ( $t=-1.9$ ;  $df=34$ ;  $P=0.058$ ) and distilled water. *S. immaculata* were equally distributed between wells when one well was treated with bifenthrin ( $t=-1.7$ ;  $df=32$ ;  $P=0.105$ ), lambda-cyhalothrin ( $t=0.3$ ;  $df=34$ ;  $P=0.771$ ), tolfenpyrad ( $t=0.0$ ;  $df=34$ ;  $P=1.00$ ), or spinetoram ( $t=-0.3$ ;  $df=34$ ;  $P=0.795$ ) and distilled water in other.

**Direct Exposure Bioassay.** A significantly fewer *S. immaculata* visits were observed when they were in direct contact with tolfenpyrad-treated surface than spinetoram, cyantraniliprole or distilled water, although there was no significant difference in *S. immaculata* visits among tolfenpyrad, clothianidin, oxamyl, or essential oils (Table 2). Total time spent per visit was significantly lower on tolfenpyrad-treated discs than oxamyl or distilled water. Similarly, number of times *S. immaculata* spent >10 s on distilled water discs was significantly higher than that on tolfenpyrad-treated discs.

**Soil Bioassay.** Soil treated with insecticides oxamyl, bifenthrin, tolfenpyrad, clothianidin, and zeta-cypermethrin caused significantly greater *S. immaculata* mortality than distilled water-treated soil ( $F=78.8$ ;  $df=9$ ;  $146$ ;  $P<0.001$ ; Fig. 2). Mortality of *S. immaculata* was 100% and was significantly greater with tolfenpyrad-treated soil than with all other insecticide treatments except bifenthrin. Significantly more *S. immaculata* died from oxamyl-treated soil than from clothianidin- or zeta-cypermethrin-treated soil.

## Discussion

Indirect and direct repellency and lethality effects of insecticide against *S. immaculata* were examined through a series of laboratory bioassays. Because the entire body of *S. immaculata* is covered with sensory hairs, the assumption was that they could recognize and differentiate the volatile or tactile cues, or both, to interact with their environment (Eisenbeis 2006). In the indirect exposure assays, *S. immaculata* were offered a choice of commercial dose of an insecticide product in one well and only distilled water in the



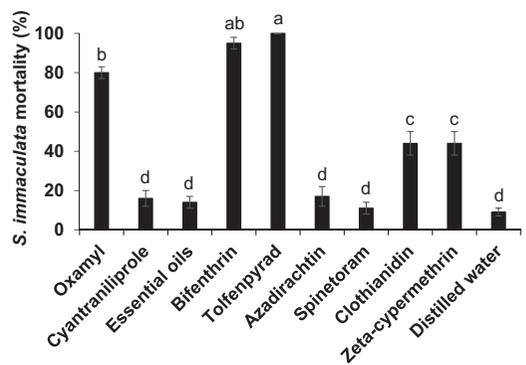
**Fig. 1.** Mean ( $\pm$ SE) number of *S. immaculata* distributed in indirect exposure choice bioassay arenas with insecticide or distilled water in one well and distilled water in the other well. Pairs of bars with asterisks (\*) are significantly different at  $\alpha = 0.05$  (Student's *t*-test).

**Table 2.** Mean ( $\pm$ SE) *S. immaculata* response to direct exposure of insecticides

Insecticide	<i>S. immaculata</i> on treated black discs		
	No. of visits	Time spent (s)	>10-s spent
Clothianidin	67.8 $\pm$ 9.1ab	7.8 $\pm$ 1.3ab	5.7 $\pm$ 1.5abc
Spinetoram	118.2 $\pm$ 26.5a	7.4 $\pm$ 1.5ab	3.3 $\pm$ 0.8bc
Cytraniliprole	126.2 $\pm$ 32.9a	7.1 $\pm$ 1.4ab	4.1 $\pm$ 2.5bc
Oxamyl	100.3 $\pm$ 19.5ab	8.3 $\pm$ 0.8a	7.2 $\pm$ 1.2ab
Essential oils <sup>a</sup>	80.5 $\pm$ 20.0ab	7.3 $\pm$ 1.8ab	6.2 $\pm$ 1.6ab
Tolfenpyrad	49.7 $\pm$ 13.3b	3.1 $\pm$ 0.9b	0.9 $\pm$ 0.4b
Distilled water	104.1 $\pm$ 8.1a	10.1 $\pm$ 1.8a	12.4 $\pm$ 1.4a
<i>F</i> (d1, d2)	2.5 (6, 31)	3.2 (6, 31)	6.4 (6, 31)
<i>P</i>	0.045	0.016	<0.001

<sup>a</sup> Contains rosemary oil and peppermint oil. Same letters within each column are not significantly different at  $\alpha = 0.05$  (Tukey's HSD test).

other. Once an individual *S. immaculata* selects and drops into a well, it cannot reverse the decision or leave the well. *S. immaculata* are susceptible to desiccation and possibly lose about 80% of water from their body every hour in a completely dry environment (Waterhouse 1968, Eisenbeis 2006); therefore, the assays were not ventilated. It is presumed that the well that



**Fig. 2.** Mean ( $\pm$ SE) percentage *S. immaculata* mortality when exposed to insecticides in the soil bioassay for 72h. Bars with same letters are not significantly different at  $\alpha = 0.05$  (Tukey's HSD test).

received insecticide emitted volatiles and created a concentration gradient within the assay guiding *S. immaculata* to respond to the stimuli by avoidance, attraction or being neutral without any physical contact. Of 14 insecticides tested, seven insecticides showed

signs of repellency to *S. immaculata* because a greater number of *S. immaculata* settled in the well with only distilled water when the other well received clothianidin, oxamyl, zeta-cypermethrin, chlorpyrifos, ethoprop, azadirachtin, or combination of beta-cyfluthrin and imidacloprid. When both the wells received distilled water, *S. immaculata* were equally distributed in both the wells. This suggests that *S. immaculata* could perceive the presence of certain insecticides and tend to avoid them before contact.

Insecticides such as oxamyl, chlorpyrifos, and ethoprop have been used for soil pest management for several decades (Berry 1973, EPA 2007, Natwick 2009). The results show that these insecticides elicited repellency to *S. immaculata* before contact. Although not registered on all vegetables, oxamyl is used in important crops such as pepper (*Capsicum* spp.), celery (*A. graveolens* var. *dulce*), and garlic (*Allium sativum* L.) in California. Chlorpyrifos is now strictly regulated in vegetable fields in California's central coast and its usage has dramatically reduced in the past five years. Alternatively, the pyrethroid and novel insecticides are considered and used to combat *S. immaculata*. Pyrethroid insecticides were known to elicit repellency to several pests (Ebeling et al. 1966, Knight and Rust 1990, Herk et al. 2008, Reeves et al. 2010, Mehlhorn et al. 2011). For example, tefluthrin has showed repellency against wireworms, *Agriotes obscurus* L. and *Limonius canus* LeConte when placed on wheat seeds (Herk et al. 2008). The data suggest that zeta-cypermethrin and a combination of beta-cyfluthrin and imidacloprid elicited noncontact repellency to *S. immaculata*. Azadirachtin is the only organically approved insecticide that elicited noncontact repellency as other researchers also shown repellent activity of azadirachtin on several other arthropod pests (Mikami and Ventura 2008, Ikeura et al. 2013). Reduced-risk insecticide products, spinetoram, cyantraniliprole, essential oil product (rosemary and peppermint oils), and mineral oil did not show any evidence of noncontact repellency in this study.

In the direct contact assay *S. immaculata* moved freely on the surface of the discs treated with distilled water. However, the data suggest that their free movement was affected on tolfenpyrad-treated discs as evidenced by fewer visits, lesser time spent per visitation, and lower number of long-duration (>10s) stays than on distilled water-treated discs. Tolfenpyrad is not yet registered on vegetable crops for pest management but shows a greater potential as a repellent. According to Insecticide Resistance Action Committee (IRAC), tolfenpyrad alters the function of mitochondria by affecting the complex I of the respiratory electron transport chain (IRAC 2014). Currently used insecticides such as clothianidin, spinetoram, and cyantraniliprole did not elicit any repellency by contact. The pyrethroid insecticides were not tested in direct contact exposure bioassays because they could possibly incite hyperactive response in *S. immaculata* (Alzogary and Zerba 2001). Gammon et al. (1981) showed that pyrethroid insecticides increase the motor activity of cockroach (*Periplaneta Americana* L.) and would excite rapid movement

once in contact. This hyperactive behavior would confound the result and may not help determine the contact repellency behavior of *S. immaculata*.

Mortality of *S. immaculata* was assessed using bioassays that allowed free movement in the insecticide-treated soil. Among currently used insecticides, bifenthrin, oxamyl, clothianidin, and zeta-cypermethrin caused 44 to 95% *S. immaculata* mortality. Mortality of *S. immaculata* was 100% on tolfenpyrad-treated soil after 72h. Previous studies showed that parathion, dyfonate, and diazinon provided sufficient suppression of *S. immaculata* (Berry and Robinson 1974). Similarly, soil fumigants, Vapam, Vidden-D, Telone, and Vorlex provided satisfactory efficacy (Berry and Robinson 1974). Conversely, Howitt (1959a) found that Vapam, chloropicrin, and ethylene dibromide were less effective than methyl bromide and 1,3-dichloropropene against *S. immaculata*. These soil insecticides and fumigants are either phased-out, or regulated in the vegetable production.

In conclusion, this study established relative efficacy of insecticides against *S. immaculata* by determining relative repellency, and lethality of insecticides through laboratory bioassays. The data suggest that certain insecticides, but not all, are repellent to *S. immaculata* before contact. Only tolfenpyrad elicited repellency after contact. Reduced-risk insecticides included in the assays were neither repellent nor lethal to foraging *S. immaculata*. Because *S. immaculata* appear in the upper soil profile starting almost immediately after planting and are able to persistently feed on the young roots for several weeks after planting (Berry and Robinson 1974), it is important to determine the length of residual activity of these insecticides as repellent or lethal to the foraging *S. immaculata*. Another challenge in *S. immaculata* management is insecticide delivery or placement where *S. immaculata* feeding damage occurs for few reasons: 1) the crop damage from *S. immaculata* feeding appear in hot spots within the field, 2) these spots shift from season to season, and 3) their incidence relative to the growing crop is not completely clear in the central coast of California. In addition, the properties of soil types, insecticides, and cultural practices vary from field to field in the central coast vegetable production complicating how the applied insecticides interact and provide satisfactory *S. immaculata* control. Currently, insecticides are applied along the seed line as narrow bands for direct seeded crops, whereas, insecticides are delivered through sprinkler or drip irrigation system for transplanted crops. The future studies will evaluate the efficacy of insecticides when delivered using various methods encompassing the properties of insecticide and soil, and the behavior and ecology of *S. immaculata* in vegetable production.

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