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Comparing efficacy of insecticides against cabbage maggot (Diptera: Anthomyiidae) in the laboratory

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ABSTRACT

The efficacy of 29 insecticides was determined against cabbage maggot, *Delia radicum* (L.) through a laboratory bioassay by exposing field collected *D. radicum* maggots to insecticide-treated soil immediately after application. In an assay, 10 *D. radicum* maggots were exposed to insecticide treated soil and then efficacy of insecticides was determined using three parameters: (1) proportion of maggots on the soil surface after 24 h, (2) proportion of change in weight of turnip bait, and (3) dead maggots after 72 h. Efficacy index (scale of 0–100) was developed based on the three parameters. Efficacy index of 11 insecticides was ≥70 against *D. radicum* and they were zeta-cypermethrin, tolfenpyrad, fenpropathrin, clothianidin, bifenthrin, lambda-cyhalothrin, chlorpyrifos, ethoprop, thiamethoxam + lambda-cyhalothrin, pyrethrins, and oxamyl in the order of highest to lowest efficacy. There was a significant positive correlation ($R^2 > 0.5$) among the three parameters. Furthermore, persistence of efficacy was examined on eight insecticides, where *D. radicum* maggots were exposed to field aged (1, 3, 7, 14, and 30 d) insecticide treated soil. Percentages of *D. radicum* maggots dead and on the soil surface were significantly greater when field aged soil was treated with bifenthrin, tolfenpyrad and clothianidin than other insecticides for most of the field age interval treatments. Efficacy of clothianidin did not change through field age interval treatments. The implications of these results on *D. radicum* management in the central coast of California are discussed.

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1. Introduction

Cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae) is an important insect pest of Brassicaceous crops worldwide (Coaker and Finch, 1971). The pest causes serious economic losses to broccoli (*Brassica oleracea* var. *italica* Plenck), cauliflower (*B. oleracea* L. var. *botrytis*), cabbage (*B. oleracea* L. var. *capitata*), broccoli raab (*Brassica rapa* L. subspecies *rapa*), Brussels sprouts (*B. oleracea* L. var. *gemmifera*), and turnip (*B. rapa* var. *rapa* L.) in the central coast of California, United States of America (USA). The value of Brassicaceous crops was estimated at ~$1 billion USD in 2013 (U.S. Department Agriculture, NASS, 2013). In Monterey County (USA), Brassicaceous crops in California are valued at ~$485 million USD and are grown in >34,390 ha (Monterey County Crop report, 2013).

In California's central coast, *Brassica* crops are grown throughout the year; as a result *D. radicum* problems persist year long (Joseph and Martinez, 2014). In other *brassica* growing regions, *D. radicum* pupae undergo diapause during the winter months, which enabled researchers to determine accurate emergence of adult flies in the spring and subsequent generations (Walgenbach et al., 1993; Jyoti et al., 2003; Dreves et al., 2006). Because typical winters in California's central coast are mild (ave. low temperature: >2.8 °C in the last five years) (US Climate data 2015), *D. radicum* rarely goes into diapause (Johnsen and Gutierrez, 1997); it is presumed that *D. radicum* populations remain active on the roots of *Brassica* crops and weed plants through the winter months (January to March). Similarly, average high temperatures during the summer months in the central coast persist in a cool range (~21 ± 5 °C) (Griffin and White, 1955; US Climate data, 2015). This suggests that *D. radicum* populations are less likely to aestivate in the summer.

D. radicum eggs are primarily laid in the soil around the crown area of the plant. A single female can lay 300 eggs under laboratory conditions (Finch, 1974). The eggs hatch in 2–3 days and the apodous maggots feed on the taproot for up to three weeks and can destroy the root system of the plant. The maggots pupate in the soil surrounding the root system and emerge into flies within 2–4

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weeks (Harris and Svec, 1966). Severe *D. radicum* feeding injury to the roots cause yellowing, stunting even plant death (Natwick, 2009).

Control of *D. radicum* on *Brassica* crops primarily involves the use of soil applied organophosphate insecticides such as chlorpyrifos and diazinon (Natwick, 2009). However, the persistent use of organophosphate insecticides has resulted in high concentrations of the insecticide residues in the water bodies (Hunt et al., 2003) posing risks to non-target organisms and public health through contaminated water. Currently, use of organophosphate insecticides is strictly regulated by California Department of Pesticide Regulation (California Environmental Protection Agency [CEPA], 2013) leaving growers with no clear options to combat *D. radicum* problems in *Brassica*. There is therefore an urgent need to determine the efficacy of alternate insecticides for *D. radicum* control.

Recent research results show that incidence of *D. radicum* infestation in direct seeded broccoli could be severe throughout the growing period except the first 30 d after sowing (Joseph and Martinez, 2014). This suggests that the alternate insecticides applied at sowing should not only be effective against *D. radicum*, but also provide a reasonable level of persistence of efficacy. Research has shown that *D. radicum* infestation can be suppressed by using organophosphate insecticides, particularly chlorpyrifos, for more than a month after planting because product residues persist for an extended period (Getzin, 1985; Chapman and Chapnan, 1986). However, it is not clear if the residues of alternate insecticides could persist and provide extended *D. radicum* control. As a result, growers and pest control advisers are currently using alternate insecticides to combat *D. radicum* without any research based information on their efficacy and level of persistence. Therefore, the objectives of the study were to (1) assess the relative efficacy of some alternate insecticides against *D. radicum* based on lethality, and ability to penetrate the insecticide treated soil and feed on the untreated bait, and (2) assess the persistence of efficacy of selected insecticides through lethality and ability to penetrate the treated field aged soil. Selection of insecticides was based on their efficacy and current usage in the central coast of California.

2. Materials and methods

2.1. Insect source

Cabbage maggots were collected from field grown broccoli plants in Chaular, CA. Infested broccoli roots were collected in plastic bins and transported to the entomology laboratory (University of California Cooperative Extension, Salinas, CA) where *Delia* spp. maggots were carefully extracted from the roots using forceps and brush. The maggots were randomly sampled and identified as *D. radicum* using Brooks (1951) key. The extracted *D. radicum* maggots were mostly second and third instars and were used in the bioassay. Because the first instar maggots were small sized and could easily be injured during extraction, they were not used in the bioassay. First instar larva has one median hook and a paired plate one either side of the cephalopharyngeal skeleton (Brooks, 1951). On second instar larva, the mouth hooks has two teeth whereas, those hooks are smooth on third instar larva (Coaker and Finch, 1971). Moreover, the first instar larvae are 1 mm or less in length whereas, the second and third instar larvae were more than 2–8 mm in length (Smith, 1927). The collected *D. radicum* maggots were temporarily stored in 60 by 15-mm polystyrene Petri dishes (Fisher Scientific, Pittsburgh, PA) lined with moist paper towel and the edges sealed with Parafilm® (Bemis Company, Inc. Oshkosh, WI) to reduce desiccation.

2.2. Bioassay

The bioassay was developed to determine the efficacy of insecticides against *D. radicum* larvae (second or third instar) because larval stages are the only destructive phase of *D. radicum* and are usually the target of insecticide applications. In addition to larval mortality, the ability of the maggot to penetrate the soil after being exposed to insecticide in the soil and the ability of *D. radicum* maggot to consume untreated *Brassica* root after exposure to insecticide treated soil were evaluated.

Typically, newly emerged *D. radicum* maggots travel through the soil to reach and infest the *brassica* roots. Insecticides targeting *D. radicum* control were therefore either applied at sowing as a narrow band along the seed line or at the base of the seedlings. This is to ensure that the *D. radicum* maggots come in contact with insecticide residues in soil as they attempt to travel through the soil layer before reaching the root system.

The bioassay consisted of translucent a polypropylene cup (6 cm diam. wide and 7.1 cm long), soil and turnip (*B. rapa* var. *rapa* L. variety ‘Tokyo’) bulbs, which were cut into thin 1–1.5 g cuboid slices (~0.3-cm [thickness] × 1-cm × 1-cm) and used as a bait. Untreated bait was placed in the center of the cup before soil was added. The Chualar loam soil (Clay, 44.8%; Sand, 14%; organic matter, 2.5%) was collected from a field in Chualar, CA where *D. radicum* infestation was persistent throughout the growing season. The soil was collected multiple times from the field for the study. Each time, ~1000 g of soil was dried in an oven at >100 °C for 72 h. Several preliminary bioassays were conducted to optimize the soil and water content suitable for *D. radicum*. Once optimized, twenty five grams (25 g) of the oven-dried soil was added to the cup burying the bait in the center-bottom, and 4.5 mL of insecticide solution per cup was uniformly pipetted on to the surface of the soil within the cup. Ten second or third instar maggots were put on the soil surface of each cup (experimental unit) then the cup was later covered with perforated caps to allow air flow. The cups were maintained at ~21 °C and ~45% relative humidity for 72 h before treatment evaluation.

2.3. Insecticide efficacy

Two experiments were conducted. The first experiment was conducted to determine the efficacy of the insecticides whereas, the second experiment was conducted to determine persistence of efficacy. In the first experiment, efficacy of 29 insecticides was tested against *D. radicum* maggots where larvae were introduced immediately after insecticide application (E_0). Distilled water was used as negative control. The details of insecticide, formulation, recommended rate and tested rates are presented in Table 1. Whenever possible, the insecticide recommended rates specifically for *D. radicum* or root maggot were used to determine the test rate. For insecticide products that lacked recommended rates, rates used for closely related insect pests of *Brassica* crops were selected for testing. The novel insecticides whose registration for use on *Brassica* or other crop category in the USA are still in progress or those that demonstrated effectiveness against *D. radicum* as soil applied insecticides in other agricultural systems were also included in the study. The rates of such new insecticides were determined after consultation with the manufacturer. Two insecticides, dinotefuran and tolfenpyrad were tested at maximum recommended rate (1.0×) as well as half rate (0.5×). The active ingredient bifenthrin was tested using two formulations, “Water Soluble Bag” (WSB) and “Liquid Fertilizer Ready” (LFR). Because the water volume generally varies between 280.6 and 560.7 L per ha in the central coast vegetable system when applied using tractor mounted sprayers, an intermediate water volume of 373.9 L per ha was selected for the

Table 1
Insecticides evaluated against *Delia radicum* in laboratory bioassays.

Class	Insecticide	Formulation	Recommended field rate (g of A.I. per ha)[a]	Tested rate (g A.I. per ha)	Tested dose (ppm)[b]
Neonicotinoids	Clothianidin*	EC	168.03–224.05	224.05	599.78
	Dinotefuran	G	245.34–294.64	147.32, 294.64	394.37 (0.5×), 788.74 (1×)
	Acetamiprid	30 SG	42.55–84.02	84.02	224.92
Pyrethroids	Bifenthrin*	LFR	44.81–89.61[c]	89.61	239.88
	Bifenthrin	10% WSB	55.99–112.01[c]	112.01	299.85
	Zeta-cypermethrin*	EC	31.37–55.99	55.99	149.88
	Lambda-cyhalothrin	EC	16.97–27.98	27.98	74.90
	Fenpropathrin	2.4 EC	224.07–336.11	448.14	1199.66
	Pyrethrins[h]	EC	56.01	56.01	149.93
Neonicotinoids + Pyrethroids	Thiamethoxam + Lambda-cyhalothrin	ZC	41.27–45.87 + 30.39–34.47	45.87 + 34.47	122.79 + 92.27
	Imidacloprid + Beta-cyfluthrin	EC	52.56 + 25.85	105.00 + 52.51	281.08 + 140.56
Neonicotinoids + Diamide	Thiamethoxam + Chlorantraniliprole*	EC	146.05–189.60 + 73.02–94.05	189.60 + 94.05	507.55 + 251.77
Organophosphates	Ethoprop	15% G	n/a[e]	335.99	899.44
	Chlorpyrifos	E in water	1367.12[c,d]	1367.12	3659.77
Carbamates	Oxamyl	L	n/a[f]	560.18	1499.59
	Methomyl	LV	503.94–1008.33	672.22	1799.52
Spinosyn	Spinetoram*	SC	43.76–87.52[c]	87.52	234.29
	Spinosad[h]	SC	87.52–175.04[c]	175.04	468.58
Ryanodine receptor activator	Cyantraniliprole*	SC	145.64–197.18[c]	197.18	527.84
	Chlorantraniliprole	SC	50.41–109.79[g]	102.32	273.90
	Cyclaniliprole*	50 SL	n/a	59.89	160.32
Pyridinecarboxamide	Flonicamid	50 SG	69.46–99.71	99.71	266.92
Pyridazinone	Tolfenpyrad*	EC	n/a[f]	118.55–237.11	317.37 (0.5×), 634.74 (1×)
Butenolides	Flupyradifurone	SL 200	n/a[f]	409.2	1095.12
Tetramic acid	Spirotetramat	SC	67.22–89.62	89.62	239.91
s-triazine	Cyromazine	WSP	139.69	139.69	373.94
Benzoylurea	Novaluron	0.83 EC	43.58–87.17	87.17	233.35
Benzamide	Diflubenzuron	2L	35.0–70.02	70.02	187.44
Tetranortriterpenoids	Azadirachtin[h]	EC	13.83–27.66	27.66	74.04

*Insecticides were used for persistence experiment (E_t).
[a] Recommended rate for insect pests in *Brassica* crops but not necessarily for *D. radicum*.
[b] Dose determined based on 373.98 L per hectare.
[c] Registered for root maggot or *D. radicum* in the U.S.
[d] Soil applied rate for *Brassicas* planted in two rows on 101.6 cm wide bed.
[e] Registered only on cabbage.
[f] Not registered on *Brassica* crops.
[g] Registered as transplant water application for *D. radicum* in California.
[h] Organic Materials Review Institute (OMRI) certified.

bioassay. Each insecticide treatment except distilled water treatment (negative control) was replicated 15 times (cups) for a total of 150 maggots per insecticide product. The distilled water was replicated 125 times (1250 maggots). This experiment was conducted in multiple sets where every insecticide was repeated in three sets with five treatment replications at a time. The distilled water treatment (control) was added to every set of the experiment.

In the second experiment, eight insecticides were selected to determine persistence of insecticide efficacy, where *D. radicum* were introduced to field aged assays (E_t) after insecticide application. Eight insecticides (as indicated with asterisks in Table 1) were selected based on superior efficacy in the E_0 experiment, or because they are registered for *D. radicum*. *D. radicum* maggots were introduced at 1 (E_1), 3 (E_3), 7 (E_7), 14 (E_{14}), and 30 (E_{30}) d after application of insecticide solution on the soil. These cups treated with insecticides were field aged by exposing them to natural elements (such as direct sunlight, daily temperature and relative humidity) outside the laboratory. The average minimum and maximum temperatures for each month from May to September 2014 (field aging intervals) were 10–23, 11.4–20.3, 13.6–22.8, 14.4–23.1, and 13.8–23.6 °C. The highest temperature for each month was 36.1, 23.6, 26.6, 28.3, and 28.3 °C (National Oceanic and Atmospheric Administration [NOAA] data in Salinas (Lat, Long, 36.659 N, 121.666 W), respectively. The precipitation for each month during May to September 2014 (field age intervals) was 0.0254, 0, 0.0508, 0, and 0.381 cm (NOAA Salinas), respectively. The insecticide solution (4.5 mL) was added to each experimental unit or cup. Turnip bait was not included in the E_t experiment because turnip would easily rot or dry out if left for long periods of time. After the designated field ageing intervals, 4.5 mL of distilled water was added to each cup prior to introduction of *D. radicum* maggots. Each insecticide treatment except distilled water treatment (negative control) was replicated 10 times (cups) for a total of 100 maggots per insecticide product and the field age interval. The distilled water was replicated 20 times (200 maggots) for each field age interval. The experiment was conducted in multiple sets where every insecticide was repeated in two sets with five treatment replications at a time. The distilled water treatment (control) was added to every set of the experiment.

2.4. Evaluation

In the first experiment, the treatments were evaluated twice (24 h and 72 h) after the introduction of *D. radicum* maggots. After 24 h, the number of maggots (live or dead) on the soil surface of the soil was quantified without disturbing the soil in the cup. After 72 h, the number of dead maggots and pupae in the cup and the change in weight of turnip bait were recorded. In the second experiment (persistence of efficacy of insecticides), turnip bait was not used but the other two parameters were measured. A needle was used to prod the maggots to confirm live, moribund or dead status. The live maggots wiggled when poked and moved their mouthpart plate. The mobility of moribund maggots was completely arrested but they moved their mouthpart plate. Because the moribund maggots neither re-infest the *Brassica* roots nor caused injury after 72 h, they were considered dead for analysis purpose. The dead maggots were completely immobile and their coloration typically changes from off-white to dark brown. Additional follow-up experiments were conducted with zeta-cypermethrin, tolfenpyrad (1.0×), clothianidin, and thiamethoxam + lambda-cyhalothrin to confirm if the moribund maggots reverted and regained mobility (wiggle). These insecticides were selected based on the results from the preliminary efficacy bioassays. Because none of the maggots reverted to live state and did not feed on the untreated turnip bait, the moribund maggots were pooled with dead maggots for analysis. Those maggots successfully pupated with completely developed puparia were considered live, whereas those pupae that had no or partially developed puparia were considered dead. The turnip bait within the cups was weighed (Torbal, Fulcrum, Inc., model: AGZN100, Clifton, NJ) before and after introduction of *D. radicum* maggots to determine if insecticide exposure influenced feeding behavior of the maggots.

2.5. Efficacy index

Efficacy index (EI) was developed to determine the relative efficacy of insecticides against *D. radicum* based on the three parameters. The percentages of live or dead *D. radicum* on the soil surface, dead maggots and amount of unfed turnip by weight were converted into a scale (0–9) denoted as 0, ≤9.9%; 1, 10–19.9%; 2, 20–29.9%; 3, 30–39.9%; 4, 40–49.9%; 5, 50–59.9%; 6, 60–69.9%; 7, 70–79.9%; 8, 80–89.9%; and 9, ≥90%. The sum of individual scores for three parameters was calculated for each insecticide and was divided by 27 (sum of maximum scale values of the three parameters).

$$\text{Efficacy index(EI)} = \frac{(\text{Scale : maggot on surface}) + (\text{Scale : maggots dead}) + (\text{Scale : unfed bait})}{27} \times 100 \quad (1)$$

2.6. Statistical analyses

The number of *D. radicum* maggots and pupae recovered after 24 and 72 h was converted to percentages. Mean percentages of *D. radicum* on the soil surface and dead per insecticide were expressed as high (≥70%), moderate (40–69.9%) and low (≤39.9%). Similarly, mean percentages of unfed turnip per insecticide after 72 h was expressed as high (≥90%), moderate (80–89.9%), and low (≤79.9%). The efficacy index data of insecticides were arcsine square root transformed and subjected to analysis of variance (ANOVA) using the PROC GLM procedure of SAS (SAS Institute, 2010) and means were separated using the Tukey's HSD method ($\alpha = 0.05$). Efficacy index values of insecticides were classified as high (EI ≥ 70), moderate (EI = 40–69.9), and low (≤39.9). To determine the relationship among *D. radicum* maggots on the soil surface, dead and unfed bait, the mean percentages data of each insecticide were analyzed (linear regression) using PROC REG procedure of SAS. For the field aged bioassays, both proportions for *D. radicum* maggots on soil surface after 24 h and dead maggots after 72 h of introduction were arcsine square root transformed then analyzed (ANOVA) using PRO GLM procedure of SAS by field age interval and by each insecticide to determine differences within insecticides at each field age interval and efficacy across field age intervals. Means and standard error for the variables were calculated using PROC MEANS procedure in SAS.

3. Results

3.1. Insecticide efficacy (E_0)

Based on the efficacy index (EI), overall 11 insecticides were ranked "high" (EI ≥ 70) among 29 insecticides tested against *D. radicum* maggot ($F = 69.4$; df = 31, 463; $P < 0.001$) (Table 2). Similarly, 14 insecticides were ranked "moderate" with EI between 40 and 69.9. The insecticides with "high" EI were pyrethrins, pyrethroids [zeta-cypermethrin, fenpropathrin, bifenthrin (LFR), lambda-cyhalothrin], organophosphates (chlorpyrifos and ethoprop), a carbamate (oxamyl), neonicotinoid (clothianidin), pyridazinone (tolfenpyrad), and thiamethoxam + lambda-cyhalothrin. Among those insecticides that were ranked "high EI", six insecticides affected the penetration of 70% or greater of the *D. radicum* larvae after 24 h of exposure (Table 2). Similarly, greater than 70% of *D. radicum* were dead when exposed to seven insecticides that were ranked "high EI". *D. radicum* ate 10% or less of the turnip bait when exposed to 10 "high EI" ranked insecticides (Table 2).

There was a positive significant relationship between *D. radicum* that did not penetrate the soil after 24 h and those dead after 72 h ($R^2 = 0.63$; $P < 0.001$; $y = 15.03 + 0.81x$; Fig. 1). More than 70% of *D. radicum* found on the soil corresponded to more than 80% of dead maggots when the soil was treated with zeta-cypermethrin, tolfenpyrad (1.0×), fenpropathrin, and bifenthrin (LFR). Similarly, when the soil was treated with organophosphates (chlorpyrifos and ethoprop), about half of the 90% dead *D. radicum* were found on the soil surface.

The weight of the unfed turnip bait significantly related with dead *D. radicum* after 72 h ($R^2 = 0.53$; $P < 0.001$; $y = -34.92 + 1.06x$; Fig. 2). More than 75% of unfed turnip bait corresponded to more than 80% of dead maggots when the soil was treated with zeta-cypermethrin, tolfenpyrad (1.0×, 0.5×), fenpropathrin, clothianidin, bifenthrin (LFR), chlorpyrifos, and ethoprop. Similarly, when the soil was treated with bifenthrin (WSB), lambda-cyhalothrin, pyrethrins, acetamiprid, denotefuran (1.0×, 0.5×), thiamethoxam + lambda-cyhalothrin, thiamethoxam + chloran traniliprole, spinosad, cyclaniliprole, methomyl, and oxamyl more than 80% of the unfed turnip bait corresponded to 30–80% of dead maggots (Fig. 2). When the soil was treated with the rest of the insecticides, *D. radicum* showed a greater degree of variation in their feeding (34–73% of turnip bait unfed) while they caused less than 30% maggot mortality.

The relationship between weight of the unfed turnip bait and those *D. radicum* that did not penetrate the soil was significantly correlated ($R^2 = 0.55$; $P < 0.001$; $y = -42.37 + 1.06x$; Fig. 3). When the soil was treated with zeta-cypermethrin, tolfenpyrad (1.0×),

Table 2
Efficacy index for each insecticide.

Rank[a]	Insecticide	*D. radicum* on soil surface after 24 h[b]	*D. radicum* Mortality after 72 h[b]	Unfed turnip after 72 h[c]	Efficacy index[d]	Overall efficacy[e]
1	Zeta-cypermethrin	High	High	High	94.8 ± 2.2 a	High
2	Tolfenpyrad (1.0×)	High	High	High	92.7 ± 1.4 ab	High
3	Fenpropathrin	High	High	High	92.1 ± 1.9 ab	High
4	Clothianidin	Moderate	High	High	89.5 ± 2.5 ab	High
5	Bifenthrin (LFR)	Moderate	High	High	88.9 ± 2.1 ab	High
6	Lambda-cyhalothrin	High	Moderate	High	85.9 ± 2.1 bc	High
7	Chlorpyrifos	Moderate	High	High	83.8 ± 2.0 bc	High
8	Ethoprop	Moderate	High	Moderate	83.4 ± 2.3 b-d	High
9	Thiamethoxam + Lambda-cyhalothrin	High	Moderate	High	82.6 ± 2.8 b-d	High
10	Pyrethrins	High	Moderate	Moderate	79.2 ± 3.3 b-e	High
11	Tolfenpyrad (0.5×)	Moderate	High	Low	78.4 ± 4.1 b-e	High
12	Oxamyl	Moderate	Moderate	High	71.9 ± 3.1 c-f	High
13	Thiamethoxam + Chlorantraniliprole	Moderate	Low	High	66.1 ± 2.7 d-g	Moderate
14	Methomyl	Moderate	Moderate	Moderate	64.1 ± 2.3 e-h	Moderate
15	Bifenthrin (WSB)	Moderate	Low	Moderate	63.7 ± 4.7 e-h	Moderate
16	Dinotefuran (1.0×)	Moderate	Low	Moderate	62.7 ± 2.5 e-i	Moderate
17	Spinosad	Low	Moderate	Low	54.7 ± 3.1 f-j	Moderate
18	Cyclaniliprole	Low	Low	Low	54.3 ± 2.9 f-j	Moderate
19	Dinotefuran (0.5×)	Low	Low	High	53.6 ± 2.8 f-j	Moderate
20	Flupyradifurone	Low	Moderate	Low	52.3 ± 3.1 f-j	Moderate
21	Acetamiprid	Low	Low	High	46.8 ± 2.9 g-k	Moderate
22	Imidacloprid + beta-cyfluthrin	Low	Low	Moderate	45.1 ± 4.8 g-k	Moderate
23	Cyantraniliprole	Low	Moderate	Moderate	43.1 ± 3.7 h-l	Moderate
24	Spinetoram	Low	Low	Low	42.2 ± 3.8 i-m	Moderate
25	Diflubenzuron	Low	Low	Low	34.5 ± 3.3 j-n	Low
26	Novaluron	Low	Low	Low	31.2 ± 4.9 k-o	Low
27	Spirotetramat	Low	Low	Low	26.8 ± 3.3 k-o	Low
28	Chlorantraniliprole	Low	Low	Low	25.2 ± 5.2 n-o	Low
29	Distilled water	Low	Low	Low	24.9 ± 1.4 l-o	Low
30	Cyromazine	Low	Low	Low	24.7 ± 4.2 m-o	Low
31	Flonicamid	Low	Low	Low	23.9 ± 4.2 m-o	Low
32	Azadirachtin	Low	Low	Low	15.8 ± 2.9 o	Low

[a] Based on the mean value of the efficacy index.
[b] Based on mean percentages per insecticide, High, ≥70%; Moderate, 40–69.9%; Low, ≤39.9%.
[c] Based on mean percentages per insecticide, High, ≥90%; Moderate, 80–89.9%; Low, ≤79.9%.
[d] Calculated using Formula [1].
[e] Based on efficacy index, High, ≥70; Moderate, 40–69.9; Low, ≤39.9.

fenpropathrin, clothianidin, bifenthrin (LFR), lambda-cyhalothrin, thiamethoxam + lambda-cyhalothrin, and pyrethrins, those 68–87% of the maggots were found on the soil surface corresponded to 88–100% of the unfed turnip baits.

3.2. Insecticide efficacy (E_t)

3.2.1. D. radicum on soil surface

At E_1 (field aged for a day), a significantly greater percentage of

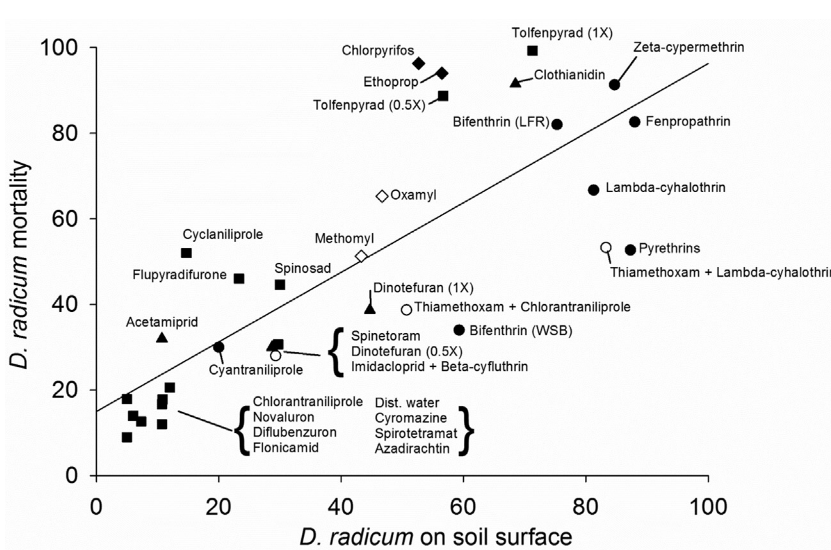

Fig. 1. The relationship between *D. radicum* mortality at 72 h and mortality on the soil surface after 24 h. The symbols: ● = pyrethroid; ▲ = neonicotinoid; ◆ = organophosphate; ◊ = carbamate; ■ = reduced-risk insecticides; and ○ = combination products.

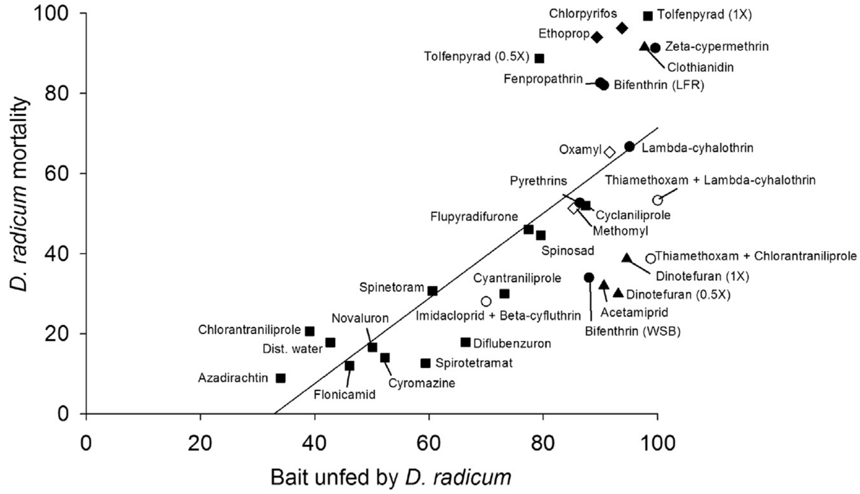

Fig. 2. The relationship between *D. radicum* mortality and turnip bait not fed by *D. radicum* after 72 h. The symbols: ● = pyrethroid; ▲ = neonicotinoid; ◆ = organophosphate; ◊ = carbamate; ■ = reduced-risk insecticides; and ○ = combination products.

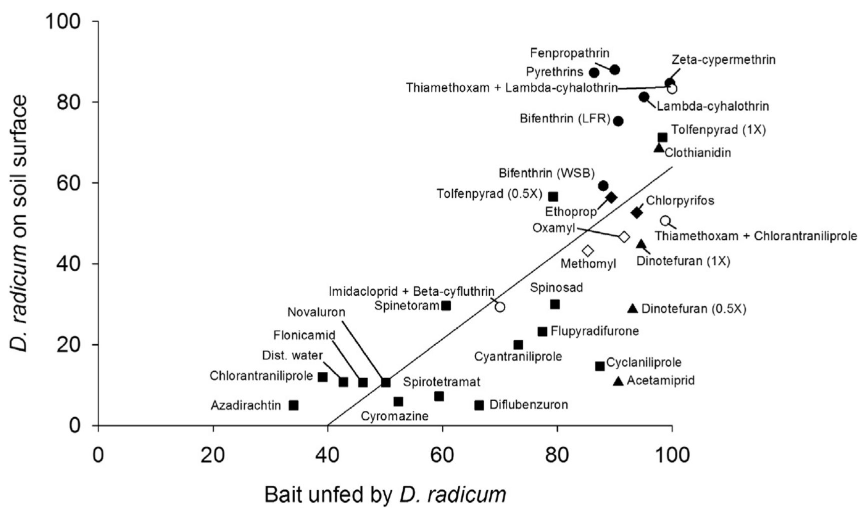

Fig. 3. The relationship between *D. radicum* on the soil surface after 24 h and turnip bait not fed by *D. radicum* after 72 h. The symbols: ● = pyrethroid; ▲ = neonicotinoid; ◆ = organophosphate; ◊ = carbamate; ■ = reduced-risk insecticides; and ○ = combination products.

D. radicum was on clothianidin treated soil surface (more than 7 maggots) than thiamethoxam + chlorantraniliprole, cyclaniliprole, spinetoram, tolfenpyrad, cyantraniliprole, and distilled water, but not with bifenthrin and zeta-cypermethrin treated soil surface (Table 3). At E_3, E_7, E_{14}, and E_{30}, percentage of *D. radicum* maggots was significantly greater on clothianidin, bifenthrin, zeta-cypermethrin, and thiamethoxam + chlorantraniliprole-treated soil surface than on other insecticides treated soil.

3.2.2. *D. radicum mortality*

At E_1, percentage mortality of *D. radicum* was significantly greater on tolfenpyrad treated soil (>9 maggots) followed by clothianidin, bifenthrin, and spinetoram (>8 maggots) than on cyclaniliprole, thiamethoxam + chlorantraniliprole, cyantraniliprole, and distilled water treated soil (Table 3). At E_3, mortality was significantly greater on tolfenpyrad treated soil (>9 maggots) than on clothianidin, cyclaniliprole, spinetoram, and cyantraniliprole treated soil. At E_7, both tolfenpyrad and clothianidin treated soil caused significantly greater *D. radicum* mortality than on cyclaniliprole, spinetoram, cyantraniliprole and distilled water treated soil. Mortality of *D. radicum* was 100% and significantly greater with clothianidin treated soil than other insecticide-treated soil at E_{14}, and there was no significant difference in mortality between tolfenpyrad and spinetoram treated soil. At E_{30}, mortality of *D. radicum* was significantly greater when soil was treated with tolfenpyrad and clothianidin than bifenthrin, thiamethoxam + chlorantraniliprole, zeta-cypermethrin and distilled water. The mortality of *D. radicum* was similar between soil treated with clothianidin and spinetoram.

3.3. *Persistence of insecticide efficacy*

3.3.1. *D. radicum on soil surface*

When treated with clothianidin, cyclaniliprole, spinetoram and distilled water, *D. radicum* on the surface of soil did not significantly differ among field age intervals (Table 3). Soil treated with bifenthrin and thiamethoxam + chlorantraniliprole had significantly greater percentage of maggots on soil surface at 3, 14, and 30 d than 1 d field age intervals. In the zeta-cypermethrin treated soil, percentage *D. radicum* was significantly greater on surface at 7, 14, and 30 d than 1 d field age intervals. When treated with tolfenpyrad, percentage *D. radicum* on the soil surface was significantly greater

Table 3
Insecticide efficacy based on *D. radicum* (Mean ± SE) on soil surface and mortality when exposed to field aged insecticide treated soil.

Insecticide	Field age interval (days)					*F*	Df	*P*
	1	3	7	14	30			
D. radicum on soil surface								
Clothianidin	79.0 ± 5.0 aA	78.0 ± 3.2 aA	72.0 ± 8.2 aA	80.0 ± 5.9 abA	92.6 ± 2.4 aA	2.5	4, 36	0.056
Bifenthrin (LFR)	61.0 ± 6.5 abB	89.0 ± 5.0 aA	83.0 ± 6.3 aAB	92.0 ± 3.2 aA	92.0 ± 5.1 aA	6.3	4, 36	<0.001
Thiamethoxam + Chlorantraniliprole	42.0 ± 4.6 bcB	73.0 ± 4.2 aA	80.0 ± 4.7 aA	82.0 ± 3.8 abA	87.0 ± 3.3 aA	11.9	4, 36	<0.001
Cyclaniliprole	12.0 ± 3.8 dA	13.0 ± 7.6 cdA	24.0 ± 5.4 bcA	10.0 ± 2.9 eA	14.0 ± 4.0 cdA	1.2	4, 36	0.332
Zeta-cypermethrin	66.0 ± 8.4 abB	82.0 ± 4.2 aAB	89.0 ± 5.4 aA	87.0 ± 4.9 aA	93.0 ± 2.1 aA	2.8	4, 36	0.042
Spinetoram	27.0 ± 4.2 cdA	30.0 ± 4.4 bcA	38.0 ± 5.1 bA	37.0 ± 5.1 cdA	32.0 ± 4.1 cA	0.9	4, 36	0.491
Tolfenpyrad	42.0 ± 7.6 bcAB	40.0 ± 5.1 bAB	39.0 ± 6.7 bB	63.0 ± 5.9 bcAB	64.0 ± 6.7 bA	6.3	4, 36	<0.001
Cyantraniliprole	17.0 ± 4.5 cdAB	10.0 ± 2.5 cdB	32.0 ± 4.4 bA	22.0 ± 3.5 deAB	37.3 ± 6.3 cA	6.4	4, 36	<0.001
Dist. water	11.5 ± 2.6 dA	4.0 ± 1.7 dA	6.5 ± 2.7 cA	6.5 ± 1.3 eA	11.2 ± 3.3 dA	2.0	4, 86	0.073
F (df1, df2)	17.9 (8, 82)	51.4 (8, 82)	31.7 (8, 82)	50.1 (8, 82)	55.8 (8, 102)			
P	<0.001	<0.001	<0.001	<0.001	<0.001			
D. radicum mortality								
Clothianidin	82.0 ± 4.4 bCD	68.0 ± 7.1 bcC	84.0 ± 7.0 aB	100.0 ± 0.0 aA	92.6 ± 3.1 aB	8.6	4, 36	<0.001
Bifenthrin (LFR)	80.0 ± 5.2 bA	83.0 ± 5.5 abA	82.0 ± 5.3 abA	84.0 ± 4.0 bcA	58.0 ± 9.7 bB	4.1	4, 36	<0.001
Thiamethoxam + Chlorantraniliprole	42.0 ± 6.2 cdB	77.0 ± 5.3 abcA	74.0 ± 4.9 abA	47.0 ± 7.3 deB	54.0 ± 5.8 bAB	0.1	4, 36	<0.001
Cyclaniliprole	18.0 ± 5.1 deB	44.0 ± 7.4 cdA	30.0 ± 6.3 cdAB	16.0 ± 3.3 fB	17.0 ± 4.2 cB	4.5	4, 36	0.005
Zeta-cypermethrin	67.0 ± 8.0 bcA	75.0 ± 6.8 abcA	67.0 ± 9.3 abA	68.0 ± 6.7 cdA	59.0 ± 6.4 bA	1.0	4, 36	0.421
Spinetoram	82.0 ± 3.5 bAB	56.0 ± 8.8 bcB	59.0 ± 7.9 bcB	94.0 ± 2.2 abA	68.0 ± 7.2 abAB	5.2	4, 36	<0.001
Tolfenpyrad	99.0 ± 1.0 aA	96.0 ± 1.6 aAB	91.0 ± 3.1 aB	92.0 ± 3.2 abB	91.3 ± 2.3 aB	2.9	4, 36	0.037
Cyantraniliprole	24.0 ± 5.2 deAB	24.0 ± 9.3 deB	21.0 ± 7.6 dB	28.0 ± 6.2 efAB	59.3 ± 7.1 bA	8.8	4, 36	<0.001
Dist. water	13.5 ± 2.6 eA	8.0 ± 2.3 eA	12.0 ± 3.0 cA	17.5 ± 4.1 fA	17.2 ± 4.8 cA	1.3	4, 86	0.294
F (df1, df2)	36.4 (8, 82)	25.6 (8, 82)	24.8 (8, 82)	49.8 (8, 82)	21.9 (8, 102)			
P	<0.001	<0.001	<0.001	<0.001	<0.001			

Lower case letters indicate comparisons of insecticides within each field aged interval, while upper case letters indicate comparisons of field age intervals by insecticide. Letters with similar case (upper or lower) are not significantly different (Tukey's HSD Test, $\alpha = 0.05$).

at 30 d than at 7 d field age interval. Similarly, in the cyantraniliprole treated soil, percentage of *D. radicum* on soil was significantly greater at 7, and 30 d than 3 d field age intervals and there was no significant difference between 14 and 30 or 1 and 3 d field age intervals on percentage of *D. radicum* on the soil.

3.3.2. D. radicum mortality

D. radicum mortality in clothianidin treated soil was significantly greater at 14 d field age interval than at 7 and 30 d, which were significantly greater than at 1 and 3 d field age intervals. With bifenthrin treated soil, a significantly greater percentage of mortality was noted at 1, 3, 7, and 14 d than at 30 d field age intervals. When thiamethoxam + chlorantraniliprole insecticide was applied to soil, a significantly greater percentage of *D. radicum* were dead at 3 and 7 d than at 1 and 14 d field age intervals. On cyclaniliprole treated soil, percentage mortality of maggots was significantly greater at 3 d field age interval than at other field age intervals. When soil was treated with spinetoram, mortality of *D. radicum* larvae was significantly greater at 14 d field age interval than at 1, 3, and 7 d field age intervals but was similar between 14 and 30 d field age intervals, and among 1, 3, 7 and 30 d field age intervals. In the tolfenpyrad treated soil, mortality of *D. radicum* was significantly greater at 1 d field age interval than at other field age intervals. Conversely, when treated with cyantraniliprole, mortality of *D. radicum* was significantly greater at 30 d field age interval than at 1, 3, and 7 d field age intervals but was not different from the 14 d field age interval. When treated with zeta-cypermethrin and distilled water, the mortality of *D. radicum* did not significantly differ among field age intervals.

4. Discussion

This research assessed the efficacy of insecticides against *D. radicum* through laboratory bioassays. In the field, the emerged first instar larvae at the crown area of the plant penetrate the soil and feed on the tap root of the plant. Thus, the insecticide efficacy was measured by quantifying dead larvae and reduction in behavioral events such as penetration into the soil and ingestion of the turnip bait placed within the assay after exposure to insecticides. Results show that when the assay was treated with distilled water, *D. radicum* larvae freely penetrated into the soil in the cup and fed on the bait. An efficacy index was developed to determine relative efficacy of tested insecticides using these three parameters, mortality, larvae on soil surface and unfed turnip bait.

In this study, the second and third instar *D. radicum* larvae were used in the bioassay. It is assumed that if the second and third instar larvae are susceptible to certain insecticides, then the first instar larvae would also be susceptible to those insecticides. For *D. radicum* control in the central coast of California, insecticides are mostly applied immediately after sowing along the seed lines as granules or narrow band spray which likely target first instar larvae. Also, delayed insecticide applications are administered to the crown area of the plants once plant roots are infested with *D. radicum* larvae or when noticeable adult flight activity occurs, where all larval stages including second and third instar larvae are targeted.

Of 11 insecticides that showed high efficacy, five of them were pyrethrins plus pyrethroid insecticides (zeta-cypermethrin, fenpropathrin, bifenthrin, and lambda-cyhalothrin) and they had superior or similar efficacy to chlorpyrifos which also showed high efficacy against *D. radicum* in the laboratory bioassay (Table 2). Most of the dead *D. radicum* larvae exposed to pyrethroids were on the soil surface suggesting that these insecticides quickly knocked the larvae down and the larvae did not feed on the bait provided in the assay. Clothianidin was the only neonicotinoid insecticide that showed evidence of high efficacy in the study. Besides causing severe *D. radicum* mortality, clothianidin also reduced the ability of larvae to feed on the bait. This suggests that, unlike pyrethroid insecticides, the larvae exposed to clothianidin were not entirely knocked down but likely died during the transit in the soil without successfully reaching or feeding on the bait. Clothianidin is registered for use on *Brassica* against *D. radicum* in California and would be a handy tool for management of *D. radicum* if its efficacy is consistent in the field conditions. Another insecticide that showed high efficacy in the bioassay was tolfenpyrad. Both the full and half recommended rate of tolfenpyrad showed high efficacy. Currently, tolfenpyrad is not registered in Brassicaceous crops against *D. radicum* in the U.S. and showed promising efficacy against *D. radicum* in this study. Previous field studies showed that dimethoate and thiamethoxam were effective against *D. radicum* maggots when applied as tray drench and foliar spray (Bažok et al., 2012). In this study, although thiamethoxam was not tested alone, thiamethoxam was tested through two combination products. Based on the efficacy result of thiamethoxam plus chlorantraniliprole, and chlorantraniliprole tested alone, thiamethoxam showed a moderate efficacy against *D. radicum*. There are several thiamethoxam insecticide products available in the market place and its concentration vary within or between sole and combination products. The products with higher thiamethoxam concentration may have a better efficacy against *D. radicum* but not tested in this study.

The mode of exposure of insecticides in this study was entirely by contact (through epidermis) and other modes of exposure such as ingestion (oral exposure) or through spiracles (respiratory exposure) were not investigated. Some of the insecticides tested in the study were insect growth regulators (IGRs) (diflubenzuron, novaluron, cyromazine, and azadirachtin), which interfere with the growth and development of the insect and showed a low efficacy. Spinosad was effective against other *Delia* spp. (Ester et al., 2003; Nault et al., 2006; Wilson et al., 2015) and insects are likely exposed to spinosad by ingestion and contact (Hertlein et al., 2011). In this study, spinosad showed a moderate efficacy possibly because the primary mode of exposure to spinosad is by ingestion. The turnip baits used in the study were not directly treated with any insecticide prior to exposing the *D. radicum* larvae and they may not have ingested spinosad while feeding on the turnip bait. The results show that diamides have a moderate efficacy. The diamide insecticides (flonicamid, cyclaniliprole, chlorantraniliprole and cyantraniliprole) have systemic activity as they move within the plant and likely away from the site of application. It is possible that the soil applied diamide insecticides are absorbed by the roots and translocated to the above ground plant parts with little effect on the feeding larvae in the tap roots.

The bioassays were conducted under controlled conditions in the laboratory and the results may not be consistent in field conditions. Several biotic and abiotic factors influence the efficacy of insecticides in the field such as precipitation (Bažok et al., 2012), temperature (Harris and Kinoshita, 1977), soil organic matter and insecticide-soil interaction (Gevao et al., 2000; Arias-Estévez et al., 2008). Precipitation can increase leaching of applied insecticides (Arias-Estévez et al., 2008; Bažok et al., 2012) and drastically reduce the concentration at the time of actual infestation. The *Brassica* fields in the California's central coast are profusely sprinkler irrigated up to three weeks after sowing to ensure uniform germination and proper establishment of plants. It is likely that applied insecticides are partially or completely leached out of the root zone area without providing anticipated maggot control. In this study, insecticides were drenched into the cup and none of the applied insecticide solution leached out. Therefore, it is likely that the insecticides were more effective in the laboratory assay than they would be in the field. Certain insecticides such as pyrethroids tend to bind to the soil organic matter (Harris et al., 1981). The organic matter in the California's central coast soils can be as high as 4% (USDA Soil conservation service, 1978), which could reduce the availability of soil applied pyrethroid insecticide to the root zone where *D. radicum* larvae typically colonize. In situations with poor insecticide spray coverage, invading *D. radicum* larvae are possibly exposed to no or sub-lethal doses of the soil applied insecticide and may be able to penetrate the soil and infest the roots. The air temperature in the field at the time of insecticide application may influence the efficacy of the applied insecticide. Harris and Kinoshita (1977) showed that efficacy of pyrethrins decreased as the temperature increased against onion maggot, *Delia antiqua* (Meigen). This suggests that application of pyrethroid insecticides should be avoided during warmer periods of the day.

Other field conditions that influence efficacy of insecticides are *D. radicum* phenology and frequency of invading *D. radicum* flies on *Brassica* crop in the central coast of California. Joseph and Martinez (2014) showed that earliest peak of *D. radicum* infestation occur a month after sowing broccoli seeds and infestations can be continuous until harvest. Also, insecticides applied at sowing as a banded spray on the seed lines did not provide adequate *D. radicum* control based on the insecticide efficacy trials conducted in commercial broccoli fields (Joseph, 2014). These findings suggest that delaying the insecticide application by 2–3 weeks after sowing is more likely to maximize maggot control. Because the *D. radicum* infestation can last several weeks, insecticides with extended persistence of efficacy would increase the value for *D. radicum* control. Overall, results show that bifenthrin, clothianidin and tolfenpyrad which performed effectively against *D. radicum* in the first experiment were also persistent for a month after application. This indicates that insecticides used before the first peak of infestation may protect the younger stages of the *Brassica* plants allowing them to establish and tolerate milder *D. radicum* infestations thereafter.

In conclusion, this study evaluated the efficacy of 29 insecticides against *D. radicum* maggots in the laboratory and identified 11 insecticides with high efficacy index for future investigation in the field. Also, this study indicated that efficacy of certain insecticides persisted for up to a month in the laboratory. Furthermore, future studies will focus on determining the effects of application timing and delivery methods compatible with *D. radicum* phenology in both directly sown and transplanted *Brassica* crops in the central coast of California.

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