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ORIGINAL ARTICLE

Can spinosad be effective for the integrated management of *Anastrepha ludens* (Tephritidae) in soil and fallen fruit, and be compatible with the parasitoid *Diachasmimorpha longicaudata* (Braconidae)?

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Abstract Tephritid fruit flies are susceptible to insecticide treatments when leaving infested fruit to pupate in the soil and when emerging as adults. Laboratory experiments involved placing third instar larvae of the Mexican fruit fly, *Anastrepha ludens* on sand treated with the naturally-derived insecticide spinosad (SpinTor 12SC). Negative correlations were detected between the concentration of spinosad and pupation and adult emergence. Treatment of pupae significantly reduced adult longevity, which could impact pest reproduction as adult flies require approximately two weeks to reach sexual maturity. Brief immersion of naturally infested oranges in 33–66 ppm spinosad solution also significantly reduced adult emergence. Exposure to spinosad-treated sand (33 ppm) did not adversely affect the foraging behavior or mortality of the braconid parasitoid *Diachasmimorpha longicaudata*. We conclude that effective control of *A. ludens* in soil with spinosad is possible but will likely require application of high concentrations of the insecticide, which may not be economically viable under conventional fruit production schemes. In the case of organic orchards surrounded by wild hosts that harbor large fly populations, targeted spinosad soil applications might be desirable as fly numbers could be significantly reduced without harming parasitoids.

Keywords Fruit fly · Pupation · Adult emergence · Sand · *Diachasmimorpha longicaudata* · Parasitoid · Citrus

Introduction

Tephritid fruit flies are major pests of fruit production in many parts of the world (Aluja et al. 2009; Shelly et al. 2014). The Mexican fruit fly, *Anastrepha ludens* Loew is a serious pest of citrus and mango in Mexico and Central America that also threatens fruit production in sub-tropical regions of the United States and elsewhere (Aluja 1994; Birke et al. 2013). It is highly polyphagous and can attack the fruit of many other plant species (Aluja and Mangan 2008; Birke et al. 2015). The presence of this pest limits international trade in fruit produced in areas with high pest populations and often requires that the commodity be subjected to costly post-harvest treatments to minimize the risk of fly larvae surviving in fruit destined for export (Shelly et al. 2014).

Management strategies targeted at this pest include cultural control methods and area-wide population suppression by the sterile insect technique (SIT) (Enkerlin 2005), in combination with spinosad-based bait sprays (Flores et al. 2011) and releases of the braconid larval-pupal endoparasitoid, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), for augmentative biological control of this pest (Montoya et al. 2007). The effectiveness of these measures is determined by area-wide trap monitoring that forms part of a national

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program aimed at the sustainable control of fruit fly pests in Mexico (Williams et al. 2013).

Spinosad is a naturally-derived insecticide that is particularly active against species of Lepidoptera, Diptera, some Coleoptera, Thysanoptera and Hymenoptera, specifically ants (Santos and Pereira 2020). Spinosad is active by ingestion and to a lesser extent by contact. It is a neurotoxin that acts uniquely on a subgroup of the post-synaptic nicotinic-acetylcholine receptors of certain insects (Geng et al. 2013). Due to its favorable ecotoxicological profile, spinosad is classified by the United States Environmental Protection Agency as an environmentally and toxicologically reduced risk material (Thompson et al. 2000). It has been used as the active ingredient in toxic bait sprays against tephritid fruit flies for approximately two decades without serious issues of resistance development in tephritid populations, although laboratory studies have reported selection for resistant strains in some cases (Voudouris et al. 2018; Guillem-Amat et al. 2020).

In the late stages of infestation by *A. ludens*, fruit often fall off the tree and final (third) instar larvae exit the fruit to pupate in the soil, where they remain until adult emergence approximately two weeks later, if environmental conditions are suitable (Hodgson et al. 1998). During this phase, the insects are susceptible to predation and parasitism (Aluja et al. 2005), infection by pathogens (Toledo et al. 2005) and exposure to insecticide treatments (Stark et al. 2014). Thus, there is value in determining whether the soil-dwelling stages of the fly can be controlled through the application of insecticides.

In the present study, we performed a laboratory evaluation of the efficacy of applications of spinosad targeted at *A. ludens* stages in the soil. Previously, soil treatments involving the organophosphate diazinon were used to control pupation of tephritids in soil around fruit trees, but the use of this compound has been largely discontinued for environmental and safety reasons (Stark and Vargas 2009), although it continues to be used in some developing countries (Abdullahi et al. 2020). Specifically, we asked whether control of *A. ludens* in soil could be achieved using lower concentrations of spinosad than were previously tested against pestiferous species of *Ceratitis* and *Bactrocera* (Stark et al. 2013, 2014). Given that spinosad can be toxic to parasitoids (Williams et al. 2003), we also examined the risk that spinosad-treated soil posed to foraging by the parasitoid *D. longicaudata* in a semi-field study.

Materials and methods

Insects and insecticides

Larvae of *A. ludens* were obtained from a laboratory colony maintained in the Instituto de Ecología AC, Xalapa, Mexico. This colony was started using pupae obtained from naturally-infested citrus fruit collected from commercial orchards in Veracruz State, Mexico. The rearing process involved adult flies aged 13–16 days held in acrylic cages (30 × 30 × 60 cm) with continuous access to water and food (3:1 sugar: hydrolyzed protein). Flies oviposited into artificial oviposition devices filled with transparent silicon from which eggs were collected, washed in 0.2% (wt/vol) sodium benzoate solution, rinsed and placed on pieces of polyester cloth on moistened cotton inside Petri dishes, and incubated at 30 ± 1 °C until they hatched. After eggs had hatched, larvae were reared on a standard diet comprising yeast, corn flour, corncob fractions, sugar, citric acid, guar gum, preservatives and water, used for the mass production of *A. ludens* in Mexico (Pascacio-Villafán et al. 2015).

The parasitoid *D. longicaudata* was reared by placing *A. ludens* third instars from the laboratory colony in Petri dish lids containing larval diet and exposing them to oviposition by mated parasitoids, as described previously (Montoya et al. 2000). All insects were maintained at 26 ± 1 °C, 70% RH, 400 lx, and a photoperiod of 12 h:12 h (L:D) at the Instituto de Ecología AC.

Spinosad was obtained as the suspension concentrate formulation SpinTor 12SC (Dow Agrosciences LLC, Indianapolis, USA). Diazinon 25 EC (Anajalsa, Jalisco, Mexico) was obtained as a generic product sold in Mexico for control of numerous pests of field crops.

Effect of spinosad concentration on pupation and adult emergence

The effect of different concentrations of spinosad applied to sand on pupation and emergence of *A. ludens* adults was tested by treating 225 g of washed, dry sand (mean ± SE particle diameter: 1.44 ± 0.05 mm) with 25 ml of spinosad solution containing one of the following concentrations of active ingredient (a.i.): 0.1, 1, 10, 100, 333 or 1000 ppm (ppm, mg a.i./l) of spinosad. This range of concentrations was equivalent to 0.01, 0.11, 1.1, 11, 37, or 111 mg spinosad/kg sand. In all cases, spinosad solutions were prepared with 0.05% (vol/vol)

Tween 80 as wetting agent and thoroughly mixed with sand by stirring and shaking for several minutes. Sand for the control was treated with 0.05% Tween solution alone. Diazinon was included as a reference insecticide treatment at a concentration of 135 ppm, equivalent to 15 mg/kg sand based on the efficacy of this concentration against tephritid pests in previous studies (Stark and Vargas 2009; Stark et al. 2013). The resulting mixture was placed into the base of a plastic Petri dish (30 g sand/dish) and a group of 25 *A. ludens* third instars from the laboratory colony was placed on the sand. The dish was then covered with a piece of paper towel and sealed using a perforated Petri dish lid for ventilation. Experimental dishes were then incubated in a bioclimatic chamber at 27 ± 1 °C, 12 h: 12 h L:D photoperiod. The entire process was performed on twelve occasions (replicates) using different batches of insects.

After 24 h incubation, all insects were carefully removed from treated sand and placed in a new Petri dish containing untreated damp sand. Petri dishes were then reincubated in the bioclimatic chamber at 27 ± 1 °C for an additional six days. Larvae and pupae were then removed from sand, counted, and pupae were placed on a filter paper disk inside a new ventilated Petri dish, incubated at 27 ± 1 °C, and checked daily for adult emergence. Adult emergence was calculated based on the number of insects that had pupated in each replicate. The prevalence of pupation and adult emergence were subjected to logit regression by fitting generalized linear models with a quasi-binomial error distribution in GLIM4 (Aitkin et al. 2005). The validity of these models was determined by examination of plots of residual values. The standard error (SE) values of binomially distributed data are asymmetrical and are presented as the range of the SE in the text.

Effect of spinosad treatment of pupae on adult emergence and longevity

Whereas the previous experiment was designed to determine the lowest concentration of spinosad that had significant effects on larvae, this experiment examined the influence of exposure to spinosad-treated sand in the pupal stage, by measuring adult emergence and adult longevity. Based on the results of the previous experiment, we selected 33 ppm (equivalent to 3.7 mg a.i./kg sand) as the lowest concentration of spinosad that was likely to have a marked effect on adult emergence. Consequently, a 25 ml volume of 33 ppm spinosad solution with 0.05% Tween 80 was mixed with 225 g dry sand. Controls consisted of sand mixed with 0.05% Tween 80 solution alone. A group of 25 *A. ludens* pupae from the laboratory colony that had pupated in the previous 36 h period, was placed at the bottom of a 300 ml plastic cup and treated sand was placed over them to a depth of 5 cm, which is typical of the soil depth at which *A. ludens* pupae are found in nature (Hodgson et al. 1998). Cups were sealed with a ventilated acrylic lid and incubated in 30 × 30 × 30 cm acrylic cages in a bioclimatic chamber at 27 ± 1 °C. The procedure was performed on nine occasions using different batches of pupae. After seven days, the lids were removed from experimental cups and adult food (3:1 hydrolyzed protein:sucrose) and water was provided ad libitum. Following emergence, adult flies were transferred to cages with food and water and monitored daily for death for seven days. The numbers of flies that emerged from each cup were tested for normality by Shapiro-Wilk test and compared for control and spinosad-treated groups by t-test. Adult sex ratio was compared by χ^2 test. Differences in adult survival time were examined by log-rank survival analysis using the R-based Jamovi package (Jamovi 2019).

Effect of spinosad treatment of infested fruit on adult emergence

Infested fruit often fall to the ground where larvae emerge and pupate in the soil. To determine the effect of spinosad application to fallen fruit on adult emergence, Valencia oranges (*Citrus aurantium var. sinensis*) known to be infested by *A. ludens* were collected from an untreated orchard in Apazapan, Veracruz, Mexico (19°19′01“ N; 96°42’53” W, altitude 300 m). Each fruit was immersed for 30 s in one of three solutions: (i) 0.5% (vol./vol.) Tween 80 solution as control, (ii) 33 ppm spinosad solution, (iii) 66 ppm spinosad solution. Both spinosad solutions also contained 0.5% Tween 80 as a wetting agent. Fruit were then placed on a paper towel, allowed to dry for 2 h, and then arranged in groups of three on a perforated plastic tray that was placed above a tray containing 1.5 kg of dry sand. Each tray with three fruit was considered as a replicate and a total of 17 replicate trays were prepared for each treatment. Larvae were allowed to emerge from the fruit and pupate in the sand layer for a 15-day period at ambient temperatures (18–23 °C), after which all fruit were removed and discarded. The tray containing sand was sealed with fine nylon mesh and monitored daily for emergence of adult flies. All flies were sexed upon emergence. Numbers of emerged flies were rank transformed to control heteroscedasticity and subjected to analysis of variance (ANOVA) and Tukey test using the Jamovi package.

Effect of spinosad application on parasitoid foraging and survival

To examine the effect of spinosad application to soil on parasitoid foraging and survival, an untreated mango (*Mangifera indica* var. Manila) orchard was selected close to Apazapan, Veracruz, Mexico (19°21′15”N; 96°47′03”W, altitude 423 m). Soil from beneath the canopy of mango trees was sieved to remove stones and other debris and placed in 500 ml plastic cups with a perforated base for drainage. Cups were inserted in the soil up to the lip of the cup at eight randomly-selected points beneath the canopy of four large mango trees (10–12 m diameter canopy). Four cups beneath each canopy were randomly assigned to the control treatment and the remaining four cups were assigned to the spinosad treatment. The control consisted of 100 ml of a 0.5% solution of Tween 80 as wetting agent sprayed on to the soil surface of each cup using a hand-held sprayer, whereas the spinosad treatment consisted of 100 ml of 33 ppm spinosad in 0.5% Tween 80 solution, applied in the same manner. Six hours later, control cup 1 and spinosad treatment cup 1 were removed from the soil under each tree and taken to the laboratory and placed in a 30 × 30 × 30 cm ventilated acrylic cage at 26 ± 1 °C, 12:12 h LD photoperiod. A single *A. ludens* infested orange fruit was placed on the soil surface to imitate a fallen infested fruit. A group of 10 mated 2–3-day-old female *D. longicaudata* was placed in each cage and provided with continuous access to a moist cotton pad and drops of honey placed on a plastic disc. After 24 h in the cage, the cup of soil and fruit were removed and the parasitoids remained with access to food and water. Parasitoid mortality was recorded six days later (seven days after exposure to cups containing soil). The procedure was repeated by collecting cups of soil from under mango trees at 7 days (control and treatment cup 2), 14 days (control and treatment cup 3) and 28 days (control and treatment cup 4) after application of spinosad. The day-night temperature range during this period was 27–16 °C with a total of 66 mm precipitation during the experimental period. For samples taken at 7–28 days post-application, the number of females observed searching over the surface of the fruit and soil in the cup was counted over a 1 min period at 1 h after introducing the parasitoids. The results were subjected to linear regression with spinosad treatment as a factor and sampling time (1–28 days) as a covariable. Numbers of foraging parasitoids at 1 h post-introduction were subjected to ANOVA using the Jamovi package.

Results

Effect of spinosad concentration on pupation and adult emergence

Overall, 97.3% (range of SE: 96.2–98.1%) of control insects pupated and 87.5% (SE: 85.5–89.4%) of those pupae emerged as adults. In contrast, none of the diazinon treated insects pupated or emerged (100% mortality). Pupation of spinosad-treated insects gradually decreased from 96.3% (SE: 91.6–98.4%) in the 0.1 ppm treatment to 20.5% (SE: 14.5–28.2%) in the 1000 ppm treatment ($F_{1,70} = 8.903$; $P < 0.01$, Fig. 1a). The slope (±SE) of the logit regression was −0.4668 ± 0.0691 and the intercept (±SE) was 2.302 ± 0.3432. The emergence response to spinosad treatments differed from that of pupation. Of the insects that pupated, 77.9–70.9% of pupae developed into adults from the 0.1–10 ppm treatments (Fig. 1b). The prevalence of adult emergence was reduced to between 12.6% and 5.3% emergence in the 100, 333 and 1000 ppm treatments ($F_{1,69} = 14.413$, $P < 0.001$). The slope (±SE) of the logit regression for adult emergence was −0.4443 ± 0.0728 and the intercept (±SE) was 0.8772 ± 0.2302.

In terms of the numbers of adult flies that emerged from the total of 300 flies tested at each concentration (25 flies per replicate), the three highest concentrations resulted in the emergence of a total of just 25, 7 and 3 flies in the 100, 333 and 1000 ppm treatments, respectively, compared to 163 adult flies in the 10 ppm treatment, underlining the notable reduction in adult emergence in treatments involving concentrations over 10 ppm.

Effect of spinosad treatment of pupae on adult emergence and longevity

The average (±SE) number of flies that emerged from each experimental cup was 15.7 ± 2.2 in the spinosad treatment and 13.6 ± 1.6 flies/cup in the control (t =

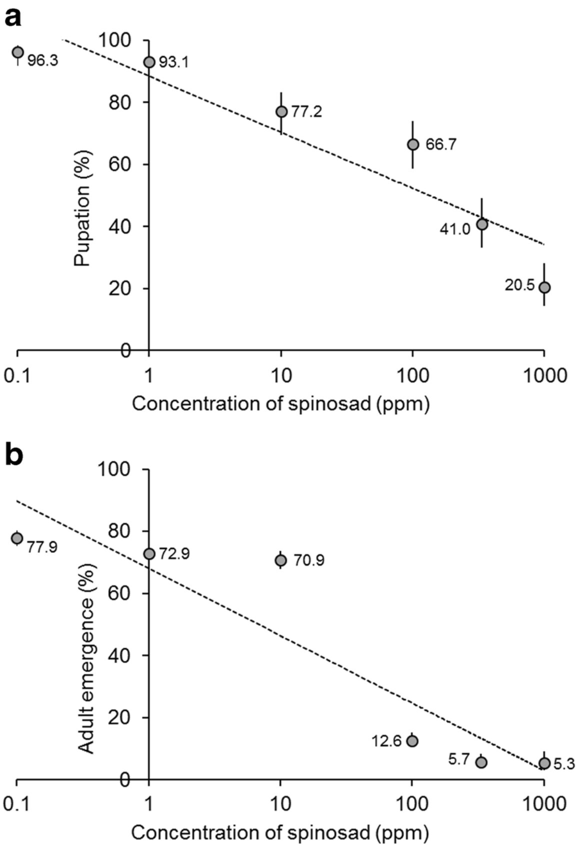

Fig. 1 Relationship between concentration of spinosad applied to sand (logarithmic scale) and (**a**) percentage of pupation observed in groups of 25 larvae of *A. ludens*, or (**b**) percentage of adult emergence based on numbers of insects that pupated in (A). Figures next to data points indicate mean percentage values. Vertical bars indicate asymmetrical SE. Dotted line indicates line of best fit

0.775, d.f. = 16, $P = 0.45$), indicating that the exposure of pupae to spinosad-treated sand did not significantly reduce pupal survival or the fly's ability to emerge through a 5 cm layer of treated sand. The adult sex ratio was similar in the control (44% male) and in the spinosad (40% male) treatment ($\chi^2 = 0.395$, d.f. = 1, $P = 0.530$), and similar numbers of flies emerged in both control and spinosad treatment for males ($t = 0.200$, d.f. = 16, $P = 0.844$) and females ($t = 1.332$, d.f. = 16, $P = 0.201$) (data not shown). The median survival time in *A. ludens* from the control was seven days, at which time 44.5% of flies remained alive (Fig. 2), compared to two days in flies from the spinosad treatment (log-rank test $z = 8.622$, $P < 0.001$). At the end of the 7-day monitoring period, only 4% of flies remained alive in the spinosad treatment (Fig. 2).

Effect of spinosad treatment of infested fruit on adult emergence

Adult emergence from control and treated fruit varied significantly for female ($F_{2,48} = 9.745$, $P < 0.001$) and male flies ($F_{2,48} = 27.316$, $P < 0.001$), and for both sexes together ($F_{2,48} = 18.831$, $P < 0.001$). Compared to the control, emergence of females was reduced by approximately 66–75% in the 33 and 66 ppm spinosad treatments (Fig. 3), whereas emergence of males was reduced by ~75% in the 33 ppm spinosad treatment and by >90% in the 66 ppm treatment. When both sexes were considered together, the emergence of flies was reduced to similar numbers in both spinosad treatments (Fig. 3).

Effect of spinosad application on parasitoid foraging and survival

The mean number of parasitoids observed searching on the fruit and soil surface at 1 h after introduction to the cage varied between 1.0 ± 0.7 and 4.2 ± 1.1 parasitoids/cage, but did not differ significant between control and spinosad treated cups ($F_{1,24} = 0.336$, $P = 0.567$), or according to sample time ($F_{3,24} = 5.948$, $P = 0.079$), indicating that wasps did not avoid contact with spinosad residues. At seven days after exposure to soil in cups, the mortality of parasitoids averaged 2.4 ± 0.4 wasps/cage in the control treatment and 2.7 ± 0.4 wasps/cage in the spinosad treatment across the samples taken at 1, 7, 14 and 28 days (Fig. 4), and did not differ significantly between treatments ($F_{1,29} = 3.026$, $P = 0.093$), or according to sample time ($F_{1,29} = 0.294$, $P = 0.592$).

Discussion

In this laboratory-based study we attempted to determine whether application of spinosad (SpinTor 12SC) to sand, at concentrations lower than those tested previously (Stark et al. 2013, 2014), would result in high mortality in *A. ludens*. Application of spinosad solutions between 0.1 and 1000 ppm (equivalent to 0.01–111 mg a.i./kg sand) resulted in a gradual decrease in the percentage of pupation in *A. ludens* third instars. In contrast, a marked drop in the prevalence of adult emergence was observed when sand was treated with spinosad solutions between 10 and 100 ppm (Fig. 1b). The period of exposure of larvae in the laboratory was

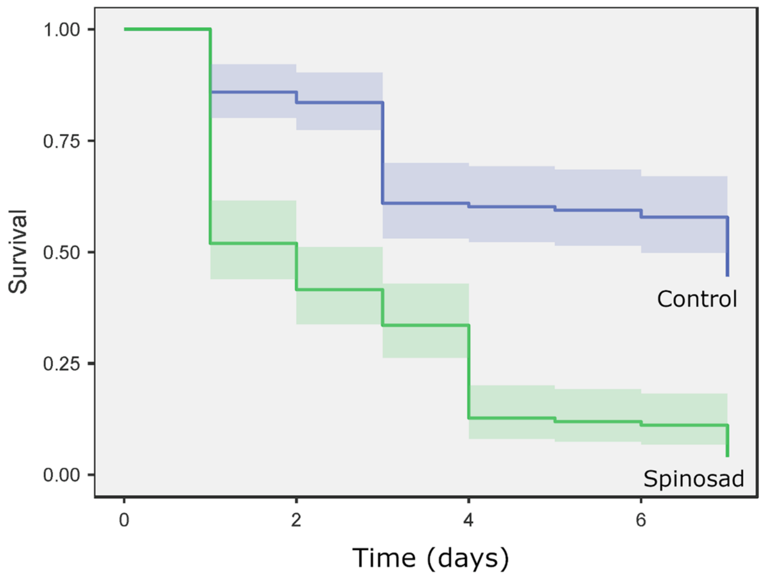

Fig. 2 Kaplan-Meier survival curve for *A. ludens* adults that emerged through sand treated with spinosad (33 ppm, 3.7 mg a.i./kg) and control insects. Shaded area indicates 95% confidence interval. The experiment was terminated at seven days post-emergence

precisely controlled at 24 h, whereas under field conditions larval exposure times are likely to be influenced by biotic and abiotic factors, including temperature, vegetation cover and soil composition, among others. In general, the dose of spinosad acquired by larvae in soil is likely to increase with exposure time.

In terms of the numbers of adult flies that developed and emerged from larvae in spinosad-treated sand, a marked decrease in emergence occurred between 10 and 100 ppm treatments. This led us to select 33 and 66 ppm as suitable concentrations for subsequent testing, which would be less costly that treatments involving markedly higher concentrations of spinosad, given the large quantities of soil requiring treatment beneath tree canopies in the field.

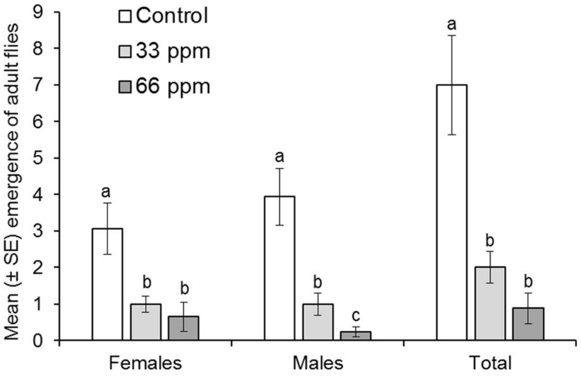

Fig. 3 Mean (±SE) numbers of *A. ludens* adults (females, males and total files) that emerged following treatment of naturally-infested oranges with spinosad solutions (33 or 66 ppm) compared to control fruit. Columns headed by identical letters did not differ significantly for comparisons of treatments within each sex (ANOVA, Tukey test $P > 0.05$, non-transformed values shown)

When *A. ludens* pupae were placed in spinosad-treated sand (33 ppm) adult emergence was not adversely affected whereas adult longevity was markedly reduced compared to that of control insects. Pupae are usually less susceptible to insecticides than larval stages (Croft 1990), although in the case of spinosad, a previous study reported a > 90% decrease in adult emergence when pupae of *Ceratitis capitata* or *Bactrocera* spp. were exposed to a high concentration of spinosad (300 mg a.i./kg) in sand (Stark et al. 2013).

The reduction in adult longevity (Fig. 2) likely reflects the mode of action of spinosad, in which

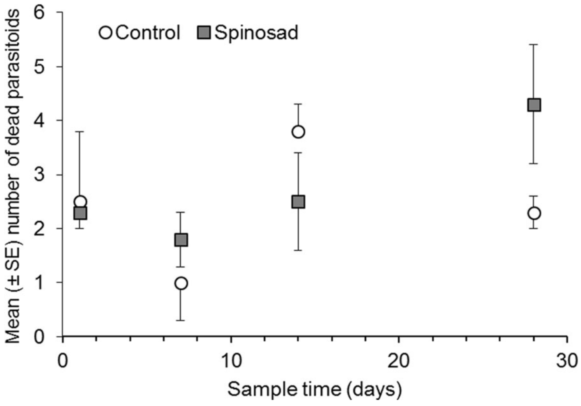

Fig. 4 Mean (±SE) number of deaths at seven days post-exposure in groups of 10 *D. longicaudata* females exposed to spinosad-treated soil (33 ppm) and control soil in samples taken from the field at 1, 7, 14 and 28 days after treatment

cumulative mortality can increase for several days following exposure to spinosad (Yee and Alston 2006; Wang et al. 2013; Vélez et al. 2017). Low doses of spinosad can also result in a range of sublethal effects on growth, reproduction, and longevity observed in insects from various orders (Williams et al. 2003; Yin et al. 2008; Wang et al. 2009; Fernandes et al. 2019), and in other invertebrates (Stark and Vargas 2003; Duchet et al. 2010). Reduced longevity could also impact the reproduction of this pest, as females require approximately 12–15 days to mature, mate and begin oviposition (Aluja 1994).

Treatment of fruit by brief immersion in spinosad solutions resulted in a ~ 70% reduction in adult emergence at 33 ppm and a 90% reduction at 66 ppm (Fig. 3). These insects presumably acquired a lethal dose of spinosad in the final larval stage, while chewing through the treated outer flavedo or exocarp layer when leaving the fruit to pupate. Additional acquisition of spinosad residues on the fruit may have occurred as larvae crawled over the surface when leaving the fruit. Nonetheless, we were surprised at the high mortality observed in insects that exited spinosad-treated oranges. As such, spot applications of spinosad sprays on to fallen fruit may be more efficient and more cost effective than soil drenches in orchard settings, although this notion requires validation under field conditions.

The effectiveness of soil treatments of diazinon against other tephritid pests, such as *C. capitata*, *Bactrocera dorsalis* and *B. cucurbitae*, was established several decades ago (Saul et al. 1983; Purcell and Schroeder 1996; Stark and Vargas 2009), and continues to be evaluated (Abdullahi et al. 2020). This was confirmed in our study with 100% mortality observed in the treatment involving 135 ppm diazinon solution (equivalent to 15 mg a.i./kg sand). However, although still commonly used in Mexico in a wide range of field crops, the use of diazinon is increasingly restricted in the United States and is prohibited in the European Union. This has stimulated the search for effective biorational alternatives, such as described in the present study. Alternative management strategies include the collection, bagging and burial of tephritid-infested fruit at depths (>50 cm) that prevent adult emergence through the soil (Dhillon et al. 2005; Klungness et al. 2005), but growers may be reluctant to employ field sanitation techniques due to the high labor costs of such practices.

Although previously studies clearly demonstrated the effectiveness of spinosad based products such as Entrust and Entrust SC applied at concentrations equivalent to 30 mg a.i./kg sand or 300 mg a.i./kg soil (Stark et al. 2013, 2014), here we considered markedly lower concentrations due to the high cost of spinosad-based products. For example, 1 l of SpinTor 12SC costs approximately US$125 in Mexico, which would be sufficient to treat just 40 kg of soil if applied at a concentration of 300 ppm a.i. used by Stark et al. (2014). This motivated our desire to evaluate this product at significantly lower concentrations.

It was clear that spinosad applied to sand at 33 ppm was less effective that markedly higher concentrations of spinosad or the organophosphate diazinon. Insecticides based on other active ingredients have also proved effective against tephritids in soil when applied at relatively high concentrations (300 mg a.i./kg), particularly products based on pyrethroids such as lambda-cyhalothrin, permethrin and tefluthrin and to a lesser degree the spinosad derivative spinetoram (Stark et al. 2013, 2014). Also, high concentrations of these products seem to be necessary for effective control when the soil has a high content of organic matter, whereas equivalent mortality can be achieved at lower concentrations in relatively inert substrates, such as sand.

The impact of spinosad applications on the parasitoid *D. longicaudata* was an issue of concern as this product is toxic to a considerable number of endo- and ectoparasitoids from different families (Williams et al. 2003; Biondi et al. 2013; Kim et al. 2018). In the United States and Mexico, spinosad is normally applied against tephritids in bait formulation (GF-120) which is not consumed by parasitoids (Stark et al. 2004; Wang et al. 2005). Contact with GF-120 residues on foliage can reduce the survival of parasitoids such as *D. longicaudata* (Wang et al. 2005; Ruiz et al. 2008), whereas other formulations have few if any sublethal effects on this parasitoid (Bernardi et al. 2019). In the present study, exposure of *D. longicaudata* to spinosad residues applied in the field did not affect numbers of wasps foraging or the survival of females, which is likely a result of the modest concentration of active ingredient present (33 ppm), and the fact that residues in the soil are not contact-repellent and are not consumed by female wasps when searching for hosts.

An additional issue related to soil treatment with insecticides is the potential for adverse effects on the natural enemies of tephritids that inhabit the soil. Insecticide effects on soil-dwelling pathogens, such as bacteria, nematodes and fungi, vary widely depending on pathogen type and insecticidal compound (Morris 1977; Rovesti and Deseö 1990; De Nardo and Grewal 2003; Mochi et al. 2006; Laznik and Trdan 2014), whereas predatory insects, such as carabid or staphylinid beetles (Potter 1994; Larson et al. 2012) and ants (McCoy et al. 2001; Schläppi et al. 2020), are usually sensitive to broad-spectrum insecticides. Ants are common in fruit orchards in many parts of the world and are often the most abundant predators of tephritid pupae (Aluja et al. 2005; Cao et al. 2012; Urbaneja et al. 2006). Any benefits from tephritid mortality following insecticide applications to soil must therefore be weighed against potential reductions in natural predation by soil populations of predatory insects. Laboratory and field studies indicate that carabids and staphylinids are not highly sensitive to spinosad, whereas this product is toxic to ants, which may experience reductions in their populations in spinosad-treated soil (Williams et al. 2003; Lefkaditis et al. 2017).

Our results could be of help to organic mango and citrus growers in areas where environmental manipulation schemes have been implemented (Aluja and Rull 2009), as spinosad is an acceptable insecticide in organic fruit production (NOSB 2020). For example, mango orchards in Mexico are often surrounded by hedges or patches of tropical plum (*Spondias purpurea* L.), a highly preferred host of the West Indian fruit fly, *A. obliqua* (Macquart). As large fly populations build up in areas of natural vegetation that then move to the adjacent mango orchards (Aluja and Birke 1993), targeted soil applications of spinosad may be warranted and economically-feasible as the number of pupae in the soil can be enormous. Given that pupal populations in soil also harbor large numbers of parasitoids (López et al. 1999), our results are encouraging as *D. longicaudata* adults were not harmed by spinosad treatments. Targeted spinosad treatments under the canopies of *S. purpurea* trees may effectively reduce *A. obliqua* populations while sparing parasitoid populations. A similar approach could be followed in the case of *Citrus aurantium* L. a highly preferred host of *A. ludens*, that is often present in the vicinity of organic grapefruit orchards. These ideas require field testing in future studies.

We conclude that application of spinosad to sand significantly reduced pupation and adult emergence of *A. ludens* at concentrations above 10 ppm. Adults that emerged from spinosad-treated sand experienced reduced longevity, which could reduce or prevent the reproduction of this pest. Direct application of spinosad to infested orange fruits was also effective at reducing adult emergence in both sexes of *A. ludens*. At the concentration tested, soil application of spinosad is unlikely to represent a risk to *D. longicaudata* when searching for tephritid larvae in fallen fruit, but its effects on wasps emerging from fly puparia in treated soil need to be examined.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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