The effects of spinosad insecticide to adults of *Apis mellifera, Megachile rotundata* and *Nomia melanderi (Hymenoptera: Apidae)*

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Summary:The toxicity of spinosad to adult female bees tended to be least to the honey bee *(Apis mellifera* L.) (LD_{50} = 0.078 µg/bee), intermediate to the alkali bee *(Nomia melanderi)* (0.065 µg/bee), and greatest to the alfalfa leafcutter bee *(Megachile rotundata* (F.)) (0.058 µg/bee), both in topical drop tests and in tests involving spinosad residues on alfalfa *(Medicago sativa)* foliage. For the calculated LD_{50} µg/g, the honey bee (LD_{50} = 0.612 µg/g) was the most susceptible followed by the alkali bee (0.773 µg/g) and the leafcutter bee (1.908 µg/g). The honey bee oral LD50 was 0.063 µg/bee and the calculated LD_{50} 0.492 µg/g. Adding an adjuvant to spinosad sprays did not change the toxicity of spinosad to bees in residue bioassay studies. Spinosad at as high as 500 ppm in feeders containing a sucrose/honey syrup caused no significant reduction in honey bee visitation or total syrup consumed.

Introduction

Bee poisoning from pesticides is a serious problem worldwide *(Johansen,* 1977; *Crane* and *Walker,* 1983; *Mayer* and *Johansen,* 1983; *Johansen* and *Mayer,* 1990; OEPP/EEPO, 1992; *Barnett* et al., 1996). Major concern exists for the safety of honey bees (*Apis mellifera* L.) as valuable pollinators of many crops and for alfalfa leafcutter bees (*Megachile rotundata* (F.) and alkali bees (*Nomia melanderi* Cockerell) that are used to pollinate alfalfa for seed. Spinosad (a fermentation product of *Saccharopolyspora spinosa*) insecticide, kills as a contact or stomach poison. The registrant, DowAgrosciences, has registered it for insect control on a number of horticultural crops throughout the world at rates of 50–140 gms a.i./hectare.

This study was conducted to evaluate the effects of spinosad on 3 bee species, to classify it as low, moderate or highly hazardous to them and to determine rates and timing for spinosad applications on blooming crops where bees are foraging. *Schoonover* and *Larson* (1995) conducted contact, topical-residual and residue bioassay studies on honey bees with spinosad. In a separate paper we report on the effects of field applications of spinosad on honey bees and alfalfa leafcutter bees. Here we report results of our research involving the effects of spinosad on adult honey bees, alfalfa leafcutter bees and alkali bees.

Material and methods

Topical LD_{50} toxicity of spinosad to three species of bees

Spinosad (technical) was dissolved in acetone to obtain a series of 5 concentrations of active ingredient. Thirty adult bees of each species were treated with each solution as follows. Insecticide solutions were applied with a calibrated Eppendorf microsyringe and disposable tips. For each bee, 2 µl of solution was drawn into the tip and then gently dispensed onto the mesoscutum. For each test, a control group was treated with 2 µl of acetone only. After treatment, bees were kept in screen cages constructed from plastic Petri

dishes (15 cm diameter) with tops and bottoms separated by a wire screen (6.7 meshes/cm) cylinder insert (45 cm long and 5 cm wide) and the cages placed horizontally on shelves.

In preparation for the topical drop application of spinosad, adult bees of the three species were obtained during the summer and treated in the following manner. Worker honey bees (4–5 weeks old) were obtained from the top cover of three colonies and anesthetized with CO_2 to facilitate handling and treatment. Leafcutter bee prepupae in leaf-piece cells were incubated at 30+s.e. 1.2 °C and 50% + 3.0%RH. Female emergence began on day 21 of incubation and was complete on day 23. On day 25, females were allowed to fly in the laboratory, collected off the windows and chilled to facilitate handling and treatment. Alkali bee females (2–3 weeks old) were collected from nesting sites with an insect net and chilled to facilitate handling and treatment. Bees were than treated with spinosad solutions. After treatment, bees were maintained for 24 h mortality counts in cages at 26 to 29 °C and 50% RH. Bees were fed 50% sucrose solution in a cotton wad (5 x 5 cm) placed on the cage bottom.

LD_{50} values (µg/bee) were calculated using Polo-PC Probit and Logit Analysis (*Russell* and *Robertson* 1979).

Topical LC_{50} toxicity of spinosad to three species of bees

Spinosad 2SC was dissolved in water to obtain a series of five concentrations of active ingredient. Thirty adult female bees of each species were treated with each solution as follows. Spinosad was applied with a calibrated microsprayer with CO_2 used as the propellent at 20 psi. Just prior to treatment, groups of 30 bees were placed in a 500 cc Sweetheart disposable cup and placed below the nozzle of the microsprayer. Bees were treated with 0.45 ml of solution in 1.5 seconds. One group of control bees was treated with 0.45 ml of water. Bees were obtained as described previously and after treatment maintained in cages as described previously for 24 h mortality counts.

Oral LD_{50} toxicity of spinosad to honey bees

Spinosad 2SC was dissolved in 50% (w/v) sucrose solution in water to obtain a series of five concentrations so that 10 µl of solution contained a known amount of active ingredient. Three groups, each with 10 adult honey bees were treated with each solution as follows. Adult bees were obtained as described previously. Cages as described previously were used except, for this tests, the cotton wad feeder was omitted and a hole was cut in the cage tops for the tube feeders. Ten bees were placed in each cage and empty feeders placed through the hole. The feeder was made from 1.7 volume polypropylene microcentrifuge tubes with closed caps. Near the bottom of the tube was a 3 mm diameter hole for the bees to feed on the solution. After 2 h of starvation, the bee feeder was used to provide each test group of bees with 100 ml of solution for each concentration. One control group of 10 bees (3 replications) was treated with 100 ml of solution without spinosad. After treatment, bees were kept in cages as described previously for 24 h mortality counts. Four h post-treatment the amount of solution consumed by the bees in each cage was determined. In all cages the bees had consumed 95% to 100% of the solution.

The LD_{50} value (u/bee) was calculated using Polo-PC Probit and Logit Analysis (*Russell* and *Robertson*, 1979).

Bioassay of spinosad residues on alfalfa foliage collected from the field

Spinosad 1.6%WP, spinosad 80WDG and spinosad 2SC were applied to 0.004-ha plots of alfalfa with a R&D pressurized sprayer (R&D Sprayers Inc., Opelousas, LA) using 234 l of water/ha. Four samples of alfalfa foliage with field-weathered spinosad residues were collected from each of 12 sites in each treatment at 2 h and 8 h after application. Samples consisting of about 400 cm^2 of vegetative foliage taken from the upper 15-cm portions of plants were placed in cages.

Adult bees were collected and caged as described previously. Residue exposures were replicated 4 times per treatment and time interval each using 4 groups of 30 worker honey bees, 20 female leafcutter bees or 20 female alkali bees caged on a foliage sample. Mortality was assessed after 24 h exposure.

Additionally, the residue bioassay was used to evaluate the bee hazard of spinosad when tank-mixed with different adjuvants using 4 replications per treatment as described above. Growers and fieldmen often add various adjuvants to tank-mixes of insecticides attempting to increase efficacy. Adjuvants tested were (1) Bond (Loveland Industries) (282 ml/ha), (2) Sunspray ultra-fine oil (Clean Crop Company) (2.4 l/ha) and (3) Sylgard 390 (Wilbur-Ellis Co.) 3.5 l/940 l).

Abbott's (1925) formula was used to correct for the natural mortality. The residual degradation time in hours (RT) required to bring bee mortality down to 25% was calculated from the residue bioassay data. It was determined previously that those pesticides causing less than 25% mortality with 8 h residues can be safely applied around bees if applied in the evening when bees are not foraging (*Johansen* and *Mayer*, 1990).

The data were analyzed as a randomized complete block design after transformation by analysis of variance, with Newman-Keuls studentized range test for mean separations (*Lund*, 1989).

Syrup feeding studies – honey bees

Feeding stations were prepared by placing a piece of cotton (9 x 18 cm) in a plastic 15-cm dish; a piece of wood (5 x 5 cm) was placed on top of the cotton to serve as a landing board. A 50% sucrose solution was mixed with honey (3:1 v/v) to prepare a syrup. Six feeders containing 150 ml of syrup with a known concentration of spinosad were established at 1030 h on 4 October 1995 on top of 6 hive boxes set 3 m apart in a line 6 m in front of 10 honey

bee colonies. Free-flying honey bees were allowed to choose between the feeders. Feeders were arranged randomly and rotated after each count. The number of bees per 5-s scan count at each feeder was recorded 4 times at about 0.5 h intervals after feeder placement. After 1.5 h of availability to foragers, the feeders were taken to the laboratory and weighed to determine the amount of syrup consumed. The test was repeated on 10 October.

The untransformed data were analyzed by repeated measures analysis of variance, with Wilks' Lamba Statistics for separations *(Sas*, 1988).

Topical LC_{50} of spinosad insecticide to three species of bees

The alkali bee (LC_{50} = 510 ppm) was the least susceptible to spinosad followed by the honey bee (311 ppm) and leafcutter bee (251 ppm) *(Table 2)*.

Table 2 Toxicity of spinosad to three species of bees using the microsprayer method

Species	n	Slope	LC50 (95% FL), ppm
A. mellifera	150	2.023	311 (373,249)
M. rotundata	150	1.019	251 (326,199)
N. melanderi	150	1.999	510 (553,468)

Table 1 Toxicity of spinosad to three species of bees using the topical drop method and oral feeding toxicity of spinosad to honey bees

Species	n	Slope	Mean body w, mg	LD_{50} (95% FL), µg/g	LD_{50} (95% FL), µg/bee
A. mellifera	150	2.538	127.4	0.612 (0.912,0.312)	0.078 (0.140, 0.048)
M. rotundata	150	1.356	30.4	1.908 (2.510, .461)	0.058 (0.075, 0.041)
N. melanderi	150	2.122	85.2	0.763 (0.973, 0.553)	0.065 (0.086,0.044)
Oral toxicity					
A. mellifera	150	1.966	128.0	0.0492 (0.532,442)	0.063 (0.098,0.027)

Table 3 Mortality of honey bees (HB), alkali bees (AB), and alfalfa leafcutter bees (LB) exposed to foliage bearing residues of spinosad applied to field plots of alfalfa after correction by Abbott's formula. Bees were confined with the treated foliage for bioassay mortalities and tests done at Touchet and Prosesr, WA. 1995.

		24 h mortalities (%) of bees caged with treated foliage collected 2 or 8 h after spinosad application					
		HB		AB		LB	
Treatment	Kg(AI)/ha	2 h	8 h	2 h	8 h	2 h	8 h
spinosad 1.6% WP	0.05	4a	0a	9a	7a	10a	13a
spinosad 1.6% WP	0.1	0a	3a	10a	2a	12a	5a
spinosad 1.6% WP	0.2	0a	0a	25b	21a	36b	26b
spinosad 80WDG	0.05	0a	0a	8a	8a	18a	5a
spinosad 80WDG	0.1	0a	0a	14a	7a	10a	12a
spinosad 80WDG	0.2	2a	0a	28b	17b	38b	29b
spinosad 2SC	0.05	0a	0a	7a	6a	12a	8a
spinosad 2SC	0.1	3a	0a	16b	3a	12a	12a
spinosad 2SC	0.2	0a	0a	29b	7a	31b	24b
spinosad 2SC + Sylgard	0.1	0a	4a	5a	2a	18ab	10a
spinosad 2SC + Bond	0.1	0a	0a	10a	4a	11a	9a
spinosad 2SC + oil	0.1	1a	2a	14a	3a	10a	11a

Means within a column followed by the same letter are not significantly different at the P = 0.05 level, Newman-Keuls studentized range test

Results

Topical LD_{50} toxicity of spinosad to three species of bees

The honey bee (LD_{50} = 0.078 µg/bee) was the least susceptible to spinosad followed by the alkali bee (0.065 µg/bee) and alfalfa leafcutter bee (0.058 µg/bee) *(Table 1)*. After calculations to obtain the LD_{50} µg/g, the honey bee (LD_{50} = 0.612 µg/g) was the most susceptible to spinosad followed by the alkali bee (0.763 µg/g) and alfalfa leafcutter bee (1.926 µg/g) *(Table 1)*.

Oral LD50 toxicity of spinosad to honey bees

The oral LD_{50} µg/bee was 0.063 and the calculated LD_{50} µg/g 0.492 for honey bees *(Table 1)*.

Bioassay of spinosad residues on alfalfa foliage collected from the field

There was no increase in honey bee mortality as spinosad rates increased, however, there was an increase in alkali and alfalfa leafcutter mortality as rates increased *(Table 3)*. The mortality of bees in 24 h continuous contact with treated foliage was usually reduced when caged with foliage collected 8 h post-application. Using the 8 h residue data for spinosad, the honey bee and alkali bee were more tolerant to

spinosad than the alfalfa leafcutter bee *(Table 3)*. There were no significant differences in bee mortality between the 1.6%WP, the 80WDG and the 2SC formulations of spinosad.

Adding Bond, Sunspray ultra-fine oil or Sylgard to tank-mixes of spinosad had no significant effect on adult honey bee, alkali bee or alfalfa leafcutter bee mortality *(Table 3)*.

Syrup feeding studies – honey bees

Although there were no significant differences in bee visitation to the feeders, adding 50, 100 and 500 ppm of spinosad to the syrup reduced bee visitation for 30 to 60 minutes after placing the feeders *(Table 4)*. There were no significant differences in total bee visitation or observed differences in the percent of syrup consumed *(Table 4)*.

Table 4 Effect of spinosad on honey bee (HB) visitation to honey sugar syrup. Numbers given are HB/dish/5 seconds at 4 different times, percent reduction of HB's from untreated check, and percent syrup consumed. Tests done at Prosser, WA, 1995.

Spinosad (ppm	Time				% reduction in	% syrup	
(in syrup)	1100 h*	1130 h	1200 h	1230 h	Total	total bee visits	consumed
0	56	75	81	100	312	—	77.5
2	22	86	87	100	295	5	75.9
10	37	83	80	100	300	4	77.5
50	3	79	90	100	272	13	76.5
100	0	21	83	100	204	35	75.9
500	0	22	87	100	209	33	75.4

* syrup stations set out at 1030 h

** $P \leq 0.01$

Means within a column followed by the same letter are not significantly different at the P = 0.05 level, Newman-Keuls studentized range test.

Discussions

According to the classification of Johansen and Mayer (1990), pesticides with an LD_{50} of < 2.0 µg/bee, are considered highly toxic to bees. Spinosad fits this category and would probably cause 100% mortality of foraging bees if applied to flowering crops or weeds, or if it drifts onto adjacent bloom where bees are foraging. There was a little, though not unusual, difference in the LD_{50} µg/bee between the three bee species. Helson et al. (1994) also found little differences in the LD_{50} µg/bee among four species of bees with some insecticides. As expected, because of differential weights between the bee species, the toxicity based on µg/g changed the position of susceptibility of the species. This is not unexpected because of the differential weights of the three bee species. Schoonover and Larson (1995) reported a contact LC_{50} of 11.5 and a topical residue LC_{50} of 27.0 for honey bees treated with spinosad 480SC. However, the methodology reported is unclear and is difficult to understand the reference for the LC_{50}s reported.

For many pesticides evaluated in reside bioassay studies, the honey bee is the more tolerant, followed by the alkali bee and leafcutter bee (Johansen and Mayer, 1990) as we found with spinosad. For most pesticides, the leafcutter bee is more susceptible, likely a function of size or surface to volume ratio, which is related to chance adherence of residues to the body of a foraging bee (Johansen et al., 1983). Schoonover and Larson (1995) treated apple branches with two rates of spinosad 480SC for honey bee residue bioassay studies and reported 5 to 20% higher mortality than we report. However, the mortality they reported did not exceed 20%.

Mayer et al. (1987) conducted a series of tests using different adjuvants and pesticides including Bond and another series using Sylgard *(Mayer* et al., 1994). In those tests, the adjuvant had no effect, or increased or decreased mortality, with the change depending on the bee species, pesticide and adjuvant. In this study neither Bond, Sunspray ultra-fine oil or Sylgard effected bee mortality.

Adding spinosad had no effect on the total number of bees feeding at the syrup feeders or the amount of syrup consumed. Apparently, there was not enough spinosad in the syrup in the feeders for the bees to detect, or if they could detect the spinosad, it did not prevent them from feeding. Solomon and *Hooker* (1989) and *Mayer* et al. (1990) conducted syrup feeding studies with different pesticides, some of which, at varying rates, repelled bees.

These studies show that spinosad is toxic to bees to varying degrees, depending on dosage and bee species. These data for spinosad can be used in risk assessment schemes *(Stark* et al., 1995; *Mayer* et al., 1999). In our studies with spinosad, the residual degradation time in hours (RT) required to bring bee mortality down to 25% in cage-test exposures to field-weathered spray deposits was less than 2 h for honey bees for all rates tested. For alkali bees and leafcutter bees it was less than 2 h for the two lower rates and greater than 2 h, but less than 8 h for the high rate. Materials with a RT25 of 2 hr or less can be used safely if applied during the early morning, night or evening *(Johansen* and *Mayer*, 1990). Materials with a RT25 greater than 2 h but less than 8 h can be used safely if applied only during late evening. Spinosad at rates of 0.05, 0.1 and 0.2 kg (ai)/ha can be considered non-hazardous to honey bees when applied to flowering crops where bees are foraging only during late evening, night or early morning. Spinosad at rates of 0.5 and 0.1 kg (ai)/ha can be considered non-hazardous to alkali and leafcutter bees when applied to flowering crops where bees are foraging only during late evening, night or early morning. Spinosad at the 0.02 kg (ai)/ha can be considered non-hazardous to alkali and leafcutter bees when applied to flowering crops where bees are foraging only during late evening.

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