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The effects of spinosad, a naturally derived insect control agent to the honeybee

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Abstract

Spinosad is a novel insect control agent derived by fermentation of the Actinomycete bacterium, *Saccharopolyspora spinosa.* Spinosad controls many caterpillar pests in vines, pome fruit and vegetables (including tomatoes and peppers), thrips in tomatoes, peppers and ornamental cultivation and dipterous leafminers in vegetables and ornamentals. The effects of spinosad to honeybees have been extensively researched. Testing has been performed under a variety of conditions in a range of countries globally. Studies to determine the acute toxicity of spinosad under laboratory conditions were conducted to generate LD_{50} or LC_{50} values for oral and contact routes of administration. These demonstrated that spinosad was highly toxic to worker honeybees under worst case laboratory conditions. Residue tests conducted under laboratory conditions using worker honeybees exposed to treated foliage, indicated that dry product residues were harmless. Therefore, the effects seen in laboratory acute toxicity tests did not translate to a more realistic exposure scenario. Semi-field cage studies demonstrated that spinosad was safe to bees when applied to flowering crops during periods of low bee activity. At high application rates evidence for repellence was seen and in one study some minor effects were noted on brood survival. In field studies dry residues of spinosad were safe to foraging worker honeybees, with no adverse effects seen on mortality, foraging behaviour, brood or queen. It can be concluded that spinosad, when used according to the approved product label recommendations, would be safe to foraging worker bees, queen and brood and may be safely used in flowering crops if applications are made during periods of low bee activity.

Key Words: spinosad, honeybee, *Apis mellifera*, toxicity.

Introduction

Spinosad is a novel insect control agent derived by fermentation of the Actinomycete bacterium, *Saccharopolyspora spinosa.* The active ingredient is composed of two metabolites, spinosyn A and spinosyn D (Thompson *et al*., 1997). Spinosad controls many caterpillar pests in vines, pome fruit and vegetables (including tomatoes and peppers), thrips in tomatoes, peppers and ornamental cultivation and dipterous leafminers in vegetables and ornamentals. Application rates vary between 25 to 150 g of active ingredient per hectare (g a.i./ha) and 4.8 to 36 g of active ingredient per hectolitre (g a.i./hL) depending on the crop and target pest. High volume sprays in may lead to theoretical worse case application rates of 144, 214 and 540 g a.i./ha.

The mode of action of spinosad is completely novel, making it a useful resistance management tool. A novel mechanism of activity on the nicotinic acetylcholine receptors was identified as the primary cause of death (Salgado, 1997). Spinosad has additional effects on gamma-aminobutyric acid or GABA receptors, although it has not been shown that these effects contribute to insecticidal activity. The action of spinosad on nicotinic receptors is unique in comparison with traditional insecticides and is at a different site from that of nicotine and imidacloprid. Studies so far have found no cross-resistance with a variety of target - site resistance mechanisms, consequently spinosad should have an excellent opportunity in resistance management programs.

It is important that Plant Protection Products (PPP) are authorised for use only in ways that do not pose an unacceptable risk of harm to honeybees and data obtained to enable the safety to be evaluated. This paper summarises the effects of spinosad to the honeybee (*Apis mellifera*). Data comes from a range of Dow AgroSciences reports originating from laboratory, semi-field and field exposure testing. Studies have been taken from a range of locations globally and include Brazil, France, Japan, Netherlands, UK and USA.

Most studies were performed to Good Laboratory Practice (GLP). As in most areas of side-effects evaluation, testing followed a sequential scheme starting with simple laboratory toxicity testing and progressing to higher tier tests such as residue, cage (semi-field) and field studies. As spinosad is not an insect growth regulator (IGR) no studies were carried out specifically on the brood although observations were made on both brood development and viability as part of the semi-field and field trials. Although the protocols and experimental designs for these investigations differ the objective of each was to assess the potential effects of spinosad to honeybees.

Materials and methods

Acute toxicity

Initial laboratory tests were performed on both the active ingredient and a range of formulated products. Studies were performed on technical spinosad (88% active ingredient), a 240 g a.i./L SC formulation (240SC) and a 480 g a.i./L SC formulation (480SC). Tests were conducted to either FIFRA Guideline Series 141-1 (U.S. E.P.A., 1982) or EPPO Guideline No. 170 (EPPO, 1992). Worker honeybees were exposed orally (in sugar water diet) or to topically applied doses. Mortality and sublethal effects were recorded at 24, 48 or 72 hours after treatment. Most studies were designed to generate LD_{50} or LC_{50} values for oral and contact routes of administration.

Laboratory residue tests

These studies were designed to address a more realistic exposure scenario by evaluating the toxicity of spinosad to honeybees exposed to dry residues of spinosad on plant material. Applications were performed to field crops and treated material was harvested (often after different drying times i.e. 2, 3, 8 or 24 hours after application) and placed in laboratory cages. Worker honeybees were exposed to the treated foliage and their survivorship monitored. These studies were conducted in the U.S.A. using field grown alfalfa.

Semi-field (cage) studies

Two semi-field (cage) studies have been conducted according to EPPO Guideline No. 170. Both tests were performed in 4 x 4.5 m cages over a flowering *Phacelia tanacetifolia* Benth. crop. Each cage contained a small nucleus hive (3,000 to 5,000 workers and queen). A toxic reference treatment (dimethoate) and a water treated control were included and each treatment was replicated three times. In the first test spinosad as the 480SC was applied early morning before the onset of bee flight. Two rates of test item were included (144 and 540 g a.i./ha). In the second test a more complicated design was employed to include an assessment of repeated applications. The first treatment (spinosad x4) received four applications at a rate of the 480SC at 216 g a.i./ha at T1, T2, T3 and T4 with spray intervals of 0, 7, 17 and 9 days. The second treatment (spinosad x1) had one application at 216 g a.i./ha on a single occasion at T4. The T4 application was made at midday when bees were active in the crop for both spinosad treatments. In both semi-field studies, bee mortality, foraging behaviour, hive activity was assessed each day and brood and food reserves were examined at the end of the test.

Field studies

A range of field studies have been performed in a number of different crops. Test system details, formulation type and test rates are summarised in table 1. In all tests honeybees were exposed to dry product residues. Studies were performed in alfalfa, almonds, citrus, and avocado. In alfalfa, hives were covered in the field before applications were made and uncovered after 3 hours and bees were then exposed to the treated crop. In almonds and citrus, applications were performed at night.

Results

Acute toxicity

Studies conducted to assess the acute oral toxicity of technical and formulated spinosad are summarised in table 2 and the contact toxicity in table 3. Spinosad was clearly harmful to worker honeybees when administered orally or topically as a technical grade or formulated product. One test item, the 0.2% g a.i./L fruit fly bait was of low toxicity, however this is a highly dilute formulation so bees were exposed to low quantities of spinosad. The findings from these laboratory studies indicated that spinosad had high intrinsic toxicity to honeybees.

Laboratory residue tests

Table 4 summarises the findings from the studies conducted on treated foliage alfalfa. A range of rates were tested from 50 g a.i./ha to 200 g a.i./ha. The 100 g a.i./ha rate was also tested with a range of commercially available wetters. Where bees were allowed to forage on treated alfalfa foliage 2, 3, 8 and 24 hours after application no treatment related mortality was observed. The addition of wetters did not affect the safety of spinosad. The findings from the laboratory residue studies demonstrated that spinosad residues are not acutely toxic to worker honeybees provided that the foliage is allowed to dry before bees are exposed.

Table 1. Field studies conducted to evaluate the effects of spinosad on honeybees (*Apis mellifera*).

Crop	Application rate (g a.i./ha)	Test material	Method
Alfalfa	70, 175	240 SC	Field Exposure to Flowering crop
Almond trees	100	240 SC	Field exposure to flowering trees sprayed at night
Citrus (orange)	157, 210	240 SC	Field exposure to flowering trees sprayed at night
Avocado	96	120 SC	Field exposure to flowering crop

Table 2. The acute oral toxicity of technical and formulated spinosad administered to honeybees under worse case laboratory conditions.

Test material	Test results	Toxicity classification
Technical (88% a.i.)	48 hr LC_{50} 0.053 μg a.i. /bee	Highly toxic
240 SC	24 hr LC_{50} 0.063 μg a.i. /bee	Highly toxic
480 SC	48 hr LC_{50} 0.053 μg a.i. /bee	Highly toxic

Table 3. The acute contact toxicity of technical and formulated spinosad administered to honeybees under worse case laboratory conditions.

Test material	Test results	Toxicity classification
Technical (88% a.i.)	48 hr LD_{50} 0.0025 μg a.i./ bee	Highly toxic
Technical (88% a.i.)	48 hr LD_{50} 0.04 μg a.i./ bee	Highly toxic
Technical (88% a.i.)	24 hr LD_{50} 0.078 μg a.i./bee	Highly toxic
480 SC	48 hr LD_{50} 0.058 μg a.i./bee	Highly toxic
480 SC	24 hr LD_{50} 0.88 μg a.i./bee	Moderately toxic
0.2 g a.i./ L Fruit Fly Bait	48 hr LD_{50} > 100 μg formulation/ bee	Non-toxic

Table 4. Laboratory studies to determine the effects of dry residues of spinosad to foraging worker honeybees. All products were field applied and treated foliage harvested and taken to the laboratory for bioassay.

Rates tested (g a.i./ha)	Crop	Product	Results	Classification
50, 100, 200	Alfalfa	1.6%WP, 80WG, 240SC	2 and 8 hour residues non-toxic to bees	Harmless
100 + wetters*	Alfalfa	1.6%WP, 80WG, 240SC	2 and 8 hour residues non-toxic to bees	Harmless
42	Alfalfa	240SC	3, 8 and 24 hour residues non-toxic to bees	Harmless
180	Alfalfa	240SC	3, 8 and 24 hour residues non-toxic to bees	Harmless

*The 100g a.i./ha rate was tested with three commercial wetters used in alfalfa Sylgard, Bond or oil.

Semi-field (cage) studies

The mortality of the bees exposed to spinosad applied in the morning before the onset of bee flight is presented in figure 1. The number of dead bees found in dead bee traps and pathways was counted twice per day. Before application bee mortality was similar in all treatments. After application in the dimethoate treatment, exposure to dry product residues caused clear harmful effects. The lower rate of spinosad (144 g a.i./ha) was harmless in terms of worker honeybee mortality. At the higher rate of spinosad (540 g a.i./ha) a slight increase in mortality was seen up to one day after application, at all other observations there was no difference from the control. Following application there was no apparent change in hive activity in any of the treatments. Foraging activity was assessed four times a day and prior to application activity was similar in all treatments (figure 2). After treatment the number of bee foraging in the dimethoate treatment was reduced dramatically. The number of bees recorded foraging in the 540 g a.i./ha rate of spinosad was reduced relative to the control on the day of application and at most assessments 2, 3, 5 and 7 days after application. There was a small, but much less marked reduction in foraging activity in the 144 g a.i./ha rate of spinosad lasting on for only 1 to 2 days. Since there was no marked increase in mortality in the 540 g a.i./ha spinosad rate the observations suggest that the product residues may be repellent to the bees. Nine days after treatment the amount of brood and food reserves (as pollen and nectar) was recorded for each hive (figure 3). Both spinosad treatments and the dimethoate treatment showed a greater reduction in brood compared to the control. At the end of the test an indication of minor effects on the survival of the brood was noted in 2 out of the 3 hives in the 540 g a.i./ha spinosad treatment. Overall these observations suggested some minor treatment related effects at the higher rate of spinosad.

In the second semi-field test the effects of applications made during bee flight was investigated. Dimethoate caused a short term increase in mortality lasting up to 2 days after application (figure 4). A slight increase in mortality was observed for both the x1 and x4 spinosad treatments up to one day after application. At all other observations up to 5 days after application no treatment was significantly different from the control ($p<0.05$). However the timing of this trial coincided with some cooler weather and lower bee activity. This may have lead to a higher level of variation than would be expected in this type of study contributing to the lack of statistically significant differences in mortality . In this second trial the rate of dimethoate was half that used in the first which may have also lead to less effect of the harmful reference. Following application there was no apparent change in hive activity in any of the treatments. Foraging activity was assessed four times a day and before application activity was similar in all treatments (figure 5). After application a reduction was seen in all treatments, including the untreated, which was probably due to a change to cooler weather. Dimethoate caused a clear reduction in foraging compared with the control. Both spinosad treatments also caused reduction in foraging with the greater reduction noted in the x4 treatment. The reduction in the x1 spinosad treatment only lasted for 1 to 2 days, for the x4 treatment the reduction was seen up to 5 days. No effect was noted on brood or food reserves for any treatment compared with the control (figure 6).

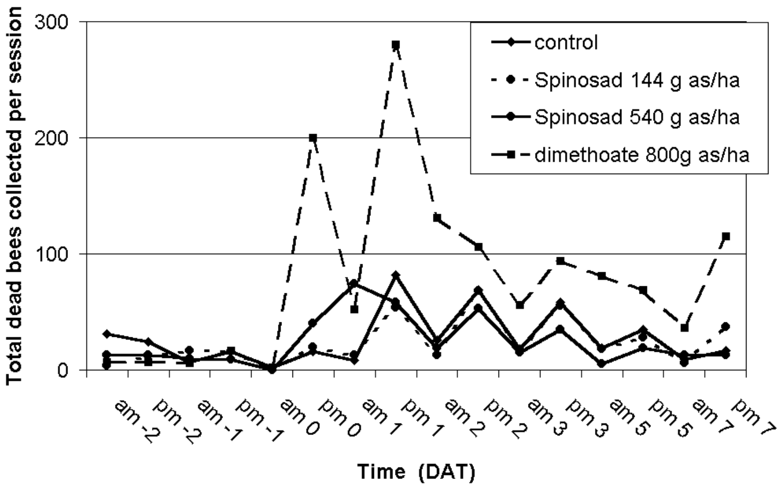

Figure 1. Effect of exposure to dry residues of spinosad on the mortality of worker honeybees in a semi-field (cage) trial.

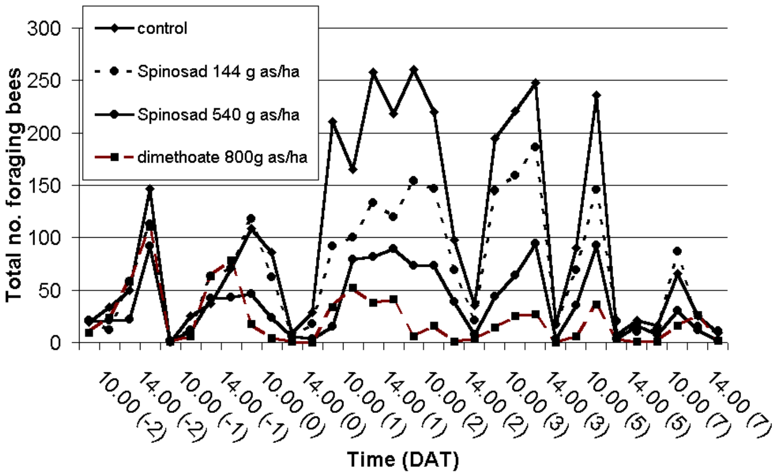

Figure 2. Effect of exposure to dry residues of spinosad on the foraging rate of worker honeybees in a semi-field (cage) trial.

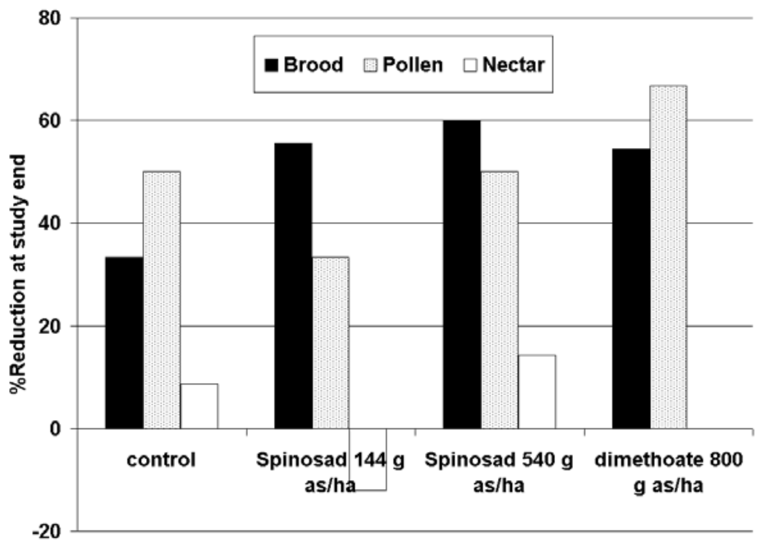

Figure 3. Effect of exposure to dry residues of spinosad on the brood and food stores of honeybee hives in a semi-field (cage) trial.

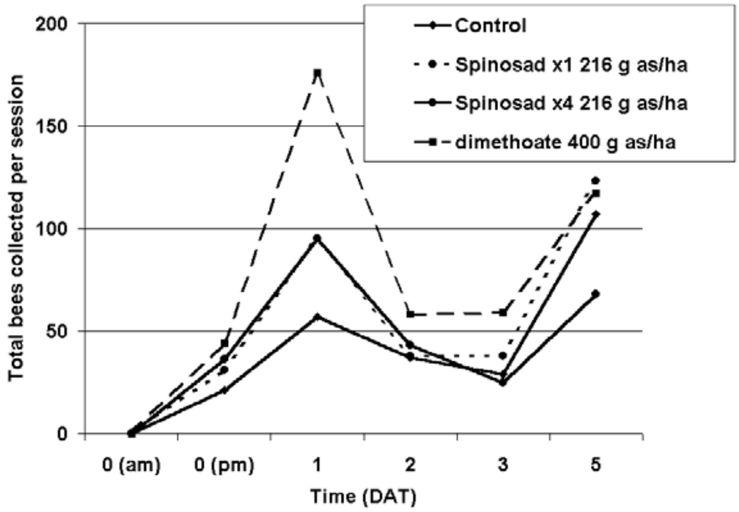

Figure 4. Effect of exposure to spinosad on the mortality of worker honeybees in a semi-field (cage) trial. Applications were made during bee flight. See text for explanation of rates.

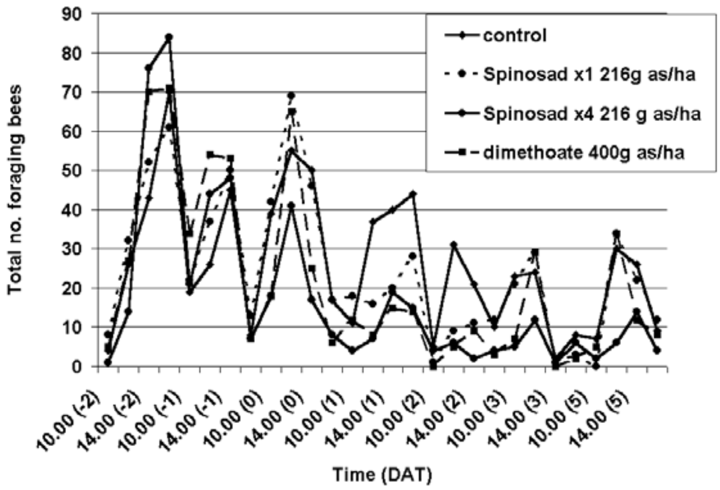

Figure 5. Effect of exposure to spinosad on the foraging rate of worker honeybees in a semi-field (cage) trial. Applications were made during bee flight. See text for explanation of rates.

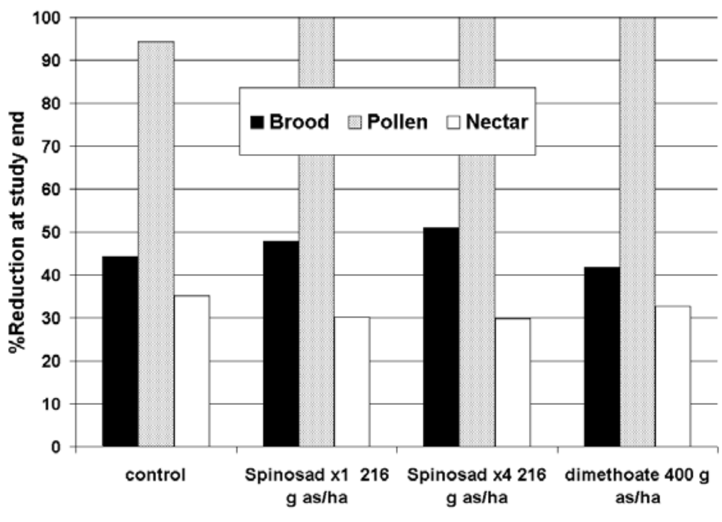

Figure 6. Effect of exposure to spinosad on the brood and food stores of honeybee hives in a semi-field (cage) trial. Applications were made during bee flight. See text for explanation of rates.

Table 5. Effects of spinosad on the honeybee (*Apis mellifera*) under field conditions in alfalfa. Hives were covered at the time of application and uncovered after 3 hours drying time.

Treatment	Rate g a.i./ha	Mortality (total dead bees)	Brood (number / hive)	
			Pre treatment	Post treatment
Spinosad	70	833a	12.2a	12.8a
Spinosad	175	813a	9.4a	10.4a
Carbaryl	1000	2620b	11.2a	10.2a
Control	----	721a	11.2a	11.8a

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

Field studies

The results from the field trial in flowering alfalfa are presented in table 5. Over twice as many bees were found dead in the 1000 g a.i./ha carbaryl treatment indicating clear harmful effects. Numerically more bees were found dead in the spinosad treatment group compared to the control. However statistically ($p<0.05$) there were no treatment related effects of spinosad on either bee mortality, foraging behaviour or on brood. In the field tests in almonds and citrus no effects were seen on mortality, foraging or brood (data not shown). Brood effects only were measured in the trial in avocado where there was no difference between spinosad and the untreated hives. There was no significant difference between the treated and untreated orchards. Brood viability was 69% and 63% for spinosad and untreated orchards respectively. Post application both treatments underwent a 7% decrease in average viability. The average increase in brood area for the untreated and spinosad treatment was 75 cm^2 and 20 cm^2 respectively. This increase was not statistically different ($p = 0.885$ at 95% confidence).

Discussion and conclusions

From the wide range of studies conducted much has been learned about the potential effects of spinosad to honeybees. Tests were performed in a wide range of countries, crops and conditions. Initial laboratory studies clearly indicated risk to bees. Consequently, higher tier tests were performed. Exposure of worker honeybees to spinosad treated foliage under laboratory conditions did not result in increased mortality, indicating that the intrinsic toxicity of spinosad observed in acute toxicity tests was not seen under conditions of more realistic exposure. In semi-field tests dry product residues did not adversely affect bee mortality or brood however some reduction in foraging was noted. A small risk was seen to bees present at the time of application. In the field, spinosad was safely used on flowering crops without causing undue risk to bees, this was especially true where the bees were exposed to dry product residues. The safety of dry residues of spinosad to honeybees has been reported by other workers (Mayer, *et al* 2001).

Since the commercial launch of spinosad in 1998 million of hectares of crops have been treated. Although largely anecdotal evidence, no adverse effects on pollinators have been reported.

It was concluded that spinosad can be safely applied to crops in flower during periods of low or no honeybee activity.

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