DOI: 10.1002/ps.1058

Lethal and sub-lethal effects of spinosad on bumble bees (*Bombus impatiens* Cresson)

Lora A Morandin,* Mark L Winston, Michelle T Franklin and Virginia A Abbott
Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6

Abstract: Recent developments of new families of pesticides and growing awareness of the importance of wild pollinators for crop pollination have stimulated interest in potential effects of novel pesticides on wild bees. Yet pesticide toxicity studies on wild bees remain rare, and few studies have included long-term monitoring of bumble bee colonies or testing of foraging ability after pesticide exposure. Larval bees feeding on exogenous pollen and exposed to pesticides during development may result in lethal or sub-lethal effects during the adult stage. We tested the effects of a naturally derived biopesticide, spinosad, on bumble bee (*Bombus impatiens* Cresson) colony health, including adult mortality, brood development, weights of emerging bees and foraging efficiency of adults that underwent larval development during exposure to spinosad. We monitored colonies from an early stage, over a 10-week period, and fed spinosad to colonies in pollen at four levels: control, 0.2, 0.8 and 8.0 mg kg^{-1}, during weeks 2 through 5 of the experiment. At concentrations that bees would likely encounter in pollen in the wild (0.2–0.8 mg kg^{-1}) we detected minimal negative effects to bumble bee colonies. Brood and adult mortality was high at 8.0 mg kg^{-1} spinosad, about twice the level that bees would be exposed to in a 'worst case' field scenario, resulting in colony death two to four weeks after initial pesticide exposure. At more realistic concentrations there were potentially important sub-lethal effects. Adult worker bees exposed to spinosad during larval development at 0.8 mg kg^{-1} were slower foragers on artificial complex flower arrays than bees from low or no spinosad treated colonies. Inclusion of similar sub-lethal assays to detect effects of pesticides on pollinators would aid in development of environmentally responsible pest management strategies.
© **2005 Society of Chemical Industry**

Keywords: pesticides; bumble bees; toxicity; spinosad; sub-lethal effects; foraging

1 INTRODUCTION

Managed and wild bumble bees are important pollinators of crop plants and wild flowers.[1] However, few pesticides have been tested on bumble bees or other wild bees prior to commercial release. Wild bees are thought to contribute a substantial amount of pollination service to the approximately 30% of human food that results from bee pollination.[2] North American agriculture relies largely on imported, managed honey bees (*Apis mellifera* L) for crop pollination,[3] and there has been little incentive to investigate the role of native, non-*Apis* pollinators. However, recent declines in feral and managed honey bee colonies due to parasites have led to a growing concern over the state of potentially important unmanaged pollinators.[4,5]

Populations of wild bees also may be declining in agricultural areas,[5] likely due to habitat loss, decreased plant diversity[6–8] and increased pesticide use.[9] Newer generations of pesticides, such as microbial biopesticides, are thought to be less harmful to humans and the environment than older, synthetic organophosphate, carbamate and pyrethroid insecticides.[10] Yet in some of the few studies conducted to date, exposure to these newer, environmentally safer, pesticides has resulted in significant bee mortality in laboratory experiments.[11]

Oral and acute toxicity tests on the domesticated honey bee are now commonly required prior to pesticide registration and commercial use in Canada and the USA. Bees (Apoidea)[12] are a very diverse group, with 20 000–30 000 species from seven families world-wide, and range from solitary to colonial to primitively social species and to the highly social honey bee. There have been few pesticide toxicity studies on any bees other than honey bees, yet bees from different genera and families likely differ widely in their vulnerability to pesticide exposure. Testing at least a few species from genera other than *Apis* would provide some knowledge of the sensitivity of other bees to commonly used insecticides.

Sub-lethal effects of pesticides may have significant impacts on bees and pollination in addition to the more easily observable mortality, disrupting

* Correspondence to: Lora A Morandin, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6
E-mail: lmorandi@sfu.ca
Contract/grant sponsor: Natural Sciences and Engineering Research Council
Contract/grant sponsor: Agriculture–Agri-Food Canada
(*Received 12 October 2004; revised version received 12 February 2005; accepted 16 February 2005*)

© 2005 Society of Chemical Industry. *Pest Manag Sci* 1526–498X/2005/$30.00

foraging and causing decreased pollination and/or bee reproduction. Adult bees perform complex behaviours to collect pollen and nectar and provision their offspring. Exposure in earlier life stages could affect development, resulting in negative impacts that would only be evident if studies were of long enough duration to monitor adult behaviour following larval exposure.

Spinosad (Dow AgroSciences) is a microbial biopesticide made from a mixture of spinosyn A and D, two of the main metabolites formed from fermentation of the actinomycete bacterium, *Saccharopolyspora spinosa* Mertz & Yao. Spinosyns are broad-spectrum insecticides, with activity against Diptera, Lepidoptera, Hymenoptera, Siphonaptera and Thysanoptera,[13,14] yet have little effect on other insects, mammals or other wildlife. Thus spinosad is classified as a reduced-risk pesticide by the US Environment Protection Agency.[15] Spinosad causes activation of the nicotinic acetylcholine receptors and alters the function of GABA-gated chlorine channels.[11,13] Over-activation of the acetylcholine receptors is the primary cause of death, initially resulting in involuntary muscle contractions and tremors and, after long periods of exposure, paralysis.[13] As of 2001, spinosad was registered in 37 countries for use on 150 crops.[15] Application rates of spinosad range from 25 to 150 $g\,AI\,ha^{-1}$ to theoretical 'worst case', high volume sprays up to 540 $g\,AI\,ha^{-1}$.[11]

Acute oral and contact toxicity studies have shown spinosad to be highly toxic to honey bees, bumble bees, alfalfa leafcutter bees and alkali bees.[16] However, dried residues were not harmful to adult honey bees or larvae in laboratory studies, or to adults, brood or foraging rates in field studies. Therefore, recommendations for spinosad application include allowing drying time before bee exposure. Greenhouse studies suggest that development of bumble bee brood may be impaired by spinosad.[16,17]

The purpose of our study was to assess the effects of spinosad on bumble bee (*Bombus impatiens* Cresson) colony health, adult bumble bee mortality and foraging ability of adults exposed during larval development, mimicking realistic levels bees may be exposed to in the field in a controlled laboratory setting. We present a method for testing pesticide effects on bumble bees that could be applied to a wide range of pesticides and, with modifications, on various bee species. We hypothesized that at low doses, bumble bee mortality and brood would not be affected by spinosad, but that larvae developing under exposure to spinosad might exhibit impaired foraging ability as adults. At high doses, we hypothesized that bee mortality would increase, and brood development and foraging ability would be negatively affected.

2 MATERIALS AND METHODS

In the wild, mated bumble bee queens locate a nest site in the spring, collect nectar and pollen to provision the nest, and begin laying eggs. The first brood of eggs usually numbers five to ten, and develops into female worker bees. Once the first brood of worker bees begins foraging, the queen remains in the nest and continues to lay and incubate brood. Eggs generally are laid in or on a mixed mass of pollen and nectar, and, for *B impatiens*, develop for approximately 5 days before they enter the larval or feeding stage.[18] Worker larvae are fed pollen and nectar for approximately 9 days, after which they enter the pupal stage which lasts for approximately 10 days and receive no exogenous food. Adult bees consume little pollen, primarily collecting it to provision their brood.

The experiment was conducted from March to May 2004. A concentrated stock mixture of analytical grade spinosad (90.4%, Dow AgroSciences, Calgary, Alberta, Canada) and pollen from Planet Bee Apiaries, British Columbia, Canada (food grade) was made using the following procedure. Because of the low solubility of spinosad in water, ground pollen and spinosad were mixed with HPLC grade acetone in a round-bottomed flask. The flask was placed on a Rotovap for approximately 30 min to mix the contents and evaporate the acetone. The flask was then dried under vacuum suction at room temperature for approximately 3 h to remove any remaining moisture. All handling of spinosad and mixing procedures involving spinosad were done in the dark or under red light because of the high rate of photodegredation. Complete drying of the pollen and spinosad in the stock mixture would mimic field residues that were dry prior to bee exposure (eg night-time application). Because spinosad residues have been found to be much less toxic to bees if allowed to dry prior to bee exposure,[16] this aspect of our procedure was a 'best case' scenario. It is conceivable that wild bees could be exposed to wet residues if growers apply spinosad during daylight when the crop is in bloom, contrary to label recommendations, or if environmental conditions increase residue drying times.

Three treatment levels of spinosad were made by adding appropriate amounts of stock pollen mixed at $2 + 1$ by weight with 30% sucrose solution to achieve pollen patties containing 0.2, 0.8 and 8 $mg\,kg^{-1}$ spinosad. Treatment control pollen patty was also made to feed to control colonies during times when treatment colonies were fed spinosad-treated pollen. Treatment control pollen was mixed using the highest level of acetone (ie the same that was used for the 8 $mg\,kg^{-1}$ treatment) created by the same methods as above, but with no spinosad. This was to ensure that any effect of possible acetone residues or some other aspect of stock pollen creation, other than spinosad addition, was mimicked in pollen fed to control colonies during treatment weeks.

We are aware of only one residue test examining spinosad levels in pollen after spraying. In sweet corn, with an application rate of 40 $g\,AI\,ha^{-1}$ (Success 480 $g\,litre^{-1}$ SC formulation), residue levels of spinosad were 0.32 $mg\,kg^{-1}$ in pollen.[19] Equivalent spray rates on different crops would likely result in

highly different residue levels in pollen. However, estimates using the available residue data and spray rates[11] result in approximate realistic levels that could be found in pollen of field-treated plants from 0.2 mg kg^{-1} (at 25 g AI ha^{-1}), 1.2 mg kg^{-1} (at 150 g AI ha^{-1}) to 4.3 mg kg^{-1} (at 540 g AI ha^{-1}: theoretical 'worse case' application rate).[11] New maximum use rates as of December 2004 are 216 g AI ha^{-1} (M Miles, Dow AgroSciences, pers comm).

We obtained 28 *B impatiens* colonies from Biobest Canada Ltd (Leamington, Ontario, Canada) at the first brood stage (5–10 workers). Colonies were housed in plastic containers 20 × 28 × 18 cm, surrounded by an outer cardboard casing and equipped with a bag containing a nectar substitute that bees could access freely. The experiment was conducted for 10 weeks, beginning from initial receipt of colonies. Colonies were fed treatment pollen, *ad libidum*, during the second, third, fourth and fifth weeks of the experiment. At all other times colonies were fed untreated pollen and sucrose solution *ad libidum*. At each feeding time, new pollen was weighed and recorded and old pollen was removed and weighed. We fed treated pollen for only four weeks of the colony's life in order to simulate a situation that wild bees could experience in an agricultural setting if foraging on a number of crops that were consecutively treated, and/or a single crop that received consecutive spinosad treatments. With this exposure schedule, we were able to mark and monitor a group of bees that we knew to have developed for their entire larval stage during treated pollen feeding. Weekly, visual estimates were made of the number of egg masses, larval cells, pupae, workers, queens, males and dead bees in each colony. Colonies were monitored daily for newly emerged workers, conspicuous because of their white coloration in the first few hours after emergence. Newly emerged workers were cooled at 4 °C and weighed on an Ohaus Explorer electronic balance (Ohaus Company, Florham Park, New Jersey, USA) to 0.01 g.

Foraging ability of adult worker bees was tested on artificial arrays[20] only if their entire larval stage overlapped with the pesticide feeding period (weeks 2 through 5). Colonies were connected to one of three mesh flight cages (1.2 × 1.2 × 1 m) and allowed to forage on 'simple' artificial flowers made from 1.5-ml clear micro tubes (Sarstedt, Newton, North Carolina, USA) with the caps removed. Tubes were filled with a 30% sucrose solution. On the second morning after colonies had been attached to the foraging cages, we conducted scan surveys, every 15 min for 3 hours, of the number of bees on the array, in flowers on the array, and flying within 30 cm above the array.

Worker bees making regular foraging trips were cooled and marked with a unique paint combination. After 5–10 foragers had been marked from a colony, all bees were returned to the hive, and the simple flower array was replaced with an array of 'complex' flowers designed from the same micro tubes as the simple flowers but with the caps left attached and bent over the opening of the tube, leaving about a 7-mm opening. Sucrose solution (2 µl) was put into each complex flower immediately prior to testing. One marked forager was released into the cage at a time and recorded on a videotape for the duration of at least 30 successful flower visits, defined as the bee completely inside the complex flower and able to access the sucrose solution. We collected data on the amount of time taken until the sucrose in the first complex flower was successfully accessed, handling time (total flower contact time) to access flowers one through 35, and foraging rate for flowers 11 to 20 (experienced forager). Because of time constraints on the life of colonies, we were able to test bees from four to five of the seven colonies from each group.

2.1 Data analysis

All data analyses were done using SAS.[21] Pollen consumption per week was compared among treatments using repeated measures ANOVA with pollen consumption as the response variable and week as the repeated measure. Colony health data were analyzed using repeated measures ANOVA with number of workers, amount of brood and dead bees as the response variables, and week as the repeated measure. In all repeated measures analyses, the interaction between treatment and week was included in the model. Pairwise comparisons of least squares means were conducted among treatments, within weeks, for each response variable. As a measure of brood viability, the number of worker bees emerging ('emergers') was estimated each week, starting in week 2, by the equation:

$$(\mathrm{CS}_t + \mathrm{DB}_t) - \mathrm{CS}_{t-1}$$

where CS = colony size, DB = number of dead bees and t = time in weeks.

The number of worker weights recorded each week was highly variable among colonies and treatments, depending on the number of newly emerged workers that could be found, and we therefore analyzed these data separately from colony health data, using repeated measures ANOVA with treatment as the main effect and week as the repeated unit. Because of the unbalanced design, the test statistics did not follow an exact F distribution, so P values were estimated using an F approximation with fractional degrees of freedom (Satterthwaite approximation).

The number of bees observed in each cage during scan samples was totalled for each colony and divided by the estimated total number of worker bees to obtain a measure of proportional foraging force. Proportional foraging force was arcsine-squareroot transformed and compared among treatments using univariate ANOVA. Flower access times of bees were compared among treatments using repeated measures ANOVA (Satterthwaite approximation) with flower number as the repeated measure and colony as a random factor.

Foraging rates were calculated as the total amount of time for a bee to access flowers 11 to 20, not including time spent in the colony if the bee returned to deposit nectar. Rates were compared among bees in different treatments using a mixed model ANOVA with colony as a random factor. Analyses were followed by comparison of differences of least-squares means among treatments when an overall effect of treatment was found.

3 RESULTS

3.1 Colony health

There was a difference in the amount of pollen consumed among treatments ($F_{4,24} = 137.17$, $P < 0.0001$), with colonies in the $8.0\,\text{mg kg}^{-1}$ treatment consuming the least amount of pollen per week (only significant difference of least-squares means $0.8\,\text{mg kg}^{-1}$ treatment vs $8.0\,\text{mg kg}^{-1}$ treatment; $t_{24} = 2.12$, $P = 0.045$). However, when we controlled for differences in colony size by adding the number of worker bees per colony as a covariate, there was no difference in the amount of pollen consumed per week among treatments ($F_{3,85.9} = 0.13, P = 0.939$). For the amount of brood, number of workers and number of dead bees each week, the $8.0\,\text{mg kg}^{-1}$ treatment was significantly different from the other three groups. All colony health measures began declining in week four or five in the $8.0\,\text{mg kg}^{-1}$ treatment, and by weeks eight and nine, there were virtually no bees or brood left in any of the colonies in this treatment. In four out of seven colonies in the $8.0\,\text{mg kg}^{-1}$ treatment, the queen died before the end of the experiment, which did not happen in any colonies from the other treatments.

There was a significant treatment by week interaction in the number of worker bees per colony ($F_{27,207} = 6.00$, $P < 0.0001$). Overall, there were fewer workers in colonies from the $8.0\,\text{mg kg}^{-1}$ treatment than in the other treatments (control vs $8.0\,\text{mg kg}^{-1}$ $t_{23} = 5.26$, $P < 0.0001$; $0.2\,\text{mg kg}^{-1}$ vs $8.0\,\text{mg kg}^{-1}$ $t_{23} = 3.80$, $P = 0.0009$; $0.8\,\text{mg kg}^{-1}$ vs $8.0\,\text{mg kg}^{-1}$ $t_{23} = 4.67$, $P < 0.0001$; Fig 1). The number of workers was not different among treatments until week 5, after which the number of workers declined significantly in the $8.0\,\text{mg kg}^{-1}$ treatment and was different in weeks 5–10 from all other treatments. There was no significant difference in the number of workers at any time between colonies in the control, $0.2\,\text{mg kg}^{-1}$ and $0.8\,\text{mg kg}^{-1}$ treatments.

There was a greater proportion of dead workers (dead workers week t/colony size week $t-1$) in colonies from the $8.0\,\text{mg kg}^{-1}$ treatment than in colonies from the other treatment groups (control vs $8.0\,\text{mg kg}^{-1}$ $t_{23} = -5.79$, $P < 0.0001$; $0.2\,\text{mg kg}^{-1}$ vs $8.0\,\text{mg kg}^{-1}$ $t_{23} = -4.76$, $P < 0.0001$; $0.8\,\text{mg kg}^{-1}$ vs $8.0\,\text{mg kg}^{-1}$ $t_{23} = -4.68$, $P < 0.0001$; Fig 2). There were proportionally more dead workers in the $8.0\,\text{mg kg}^{-1}$ treatment in weeks five, six, seven, eight and nine than in the other three treatments. We did not compare number of dead bees from the $8.0\,\text{mg kg}^{-1}$ treatment with the other treatments in week 10 because only one $8.0\,\text{mg kg}^{-1}$ colony had more than three workers, and most had no bees.

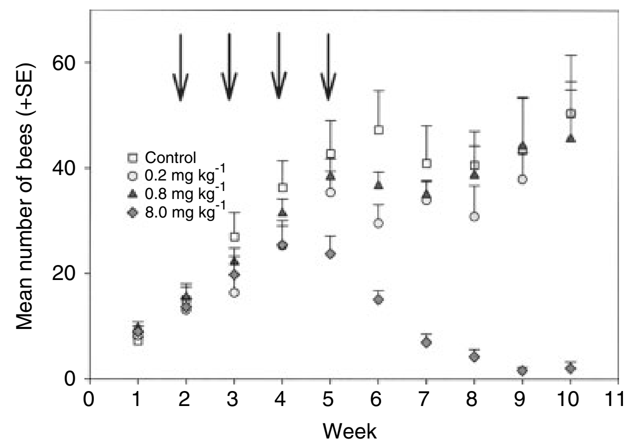

Figure 1. Mean number of worker bumble bees (+SE) in 28 colonies (seven per treatment) from one of four treatments: control, $0.2\,\text{mg kg}^{-1}$ spinosad, $0.8\,\text{mg kg}^{-1}$ spinosad and $8.0\,\text{mg kg}^{-1}$ spinosad. Treated pollen was fed to colonies *ad libitum* during weeks two to five of the experiment, indicated on the graph by arrows.

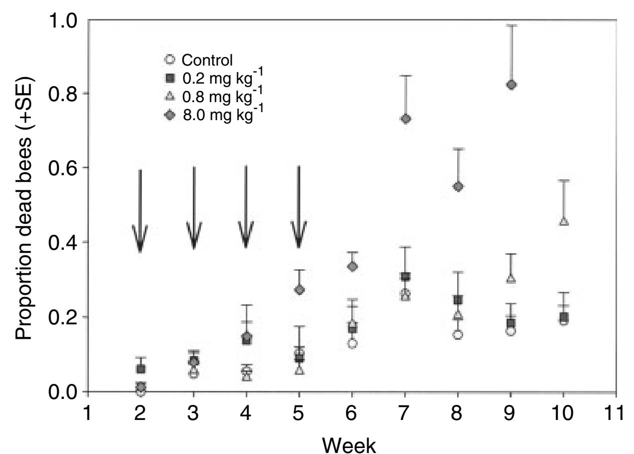

Figure 2. Mean proportion of dead bumble bees (+SE) found in colonies from one of four treatments: control, $0.2\,\text{mg kg}^{-1}$ spinosad, $0.8\,\text{mg kg}^{-1}$ spinosad and $8.0\,\text{mg kg}^{-1}$ spinosad. Arrows indicate weeks where treated pollen was given to colonies.

Worker weights declined over the first four weeks of the experiment and then increased in week five in all treatments except in the $8.0\,\text{mg kg}^{-1}$ treatment (Fig 3). Because of the low number of new bees after week four in the $8.0\,\text{mg kg}^{-1}$ treatment, we were only able to obtain newly emerged worker weights for the first four weeks. Worker bee weights in the $8.0\,\text{mg kg}^{-1}$ treatment were comparable to weights of worker bees from the other treatments, and because of the missing values after week four, we removed the $8.0\,\text{mg kg}^{-1}$ treatment from analyses of worker weights. Worker bees that had been fed treated pollen during their entire larval development began emerging as adults in week five and continued through week 8 of the experiment. Worker weights (±SE) in the control, $0.2\,\text{mg kg}^{-1}$ and $0.8\,\text{mg kg}^{-1}$ treatments during these four weeks were 0.147 (±0.007), 0.143 (±0.005)

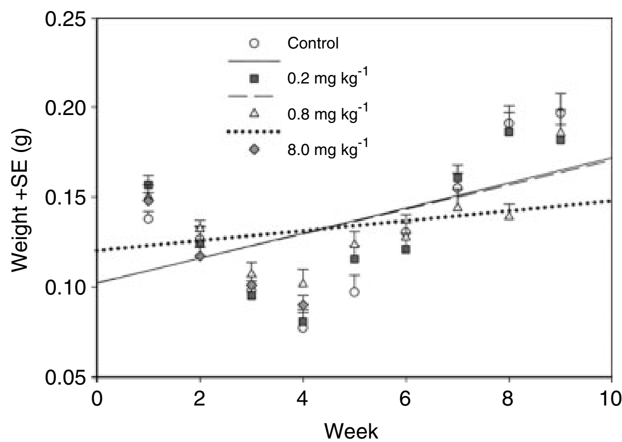

Figure 3. Mean weights of newly emerged worker bees (+SE) in four treatment groups: control, 0.2 mg kg^{-1} spinosad, 0.8 mg kg^{-1} spinosad and 8.0 mg kg^{-1} spinosad. Spinosad treated pollen was fed to bees from weeks two through five of the experiment and bees emerging in weeks five through eight were exposed to spinosad treatment during their entire larval development. Lines were calculated by least-squares regression from weeks one to ten of the experiment.

and 0.134 (±0.004) g, respectively. There was an interaction between worker weights and treatment ($F_{6,232} = 5.44$, $P < 0.0001$). From week five to eight, the slope of the relationship between weight and week was lower in the 0.8 mg kg^{-1} treatment than in the control and 0.2 mg kg^{-1} treatments.

The total amount of brood increased in all treatments from weeks one to three but then decreased in all treatments in week four (Fig 4). Between weeks four and six, the amount of brood generally stayed the same or slightly decreased in all treatments, and then from weeks seven to ten the amount of brood in the 8.0 mg kg^{-1} treatment continued to decline while the amount of brood in colonies from the control, 0.2 mg kg^{-1} and 0.8 mg kg^{-1} treatments increased. From weeks one to six there was no difference

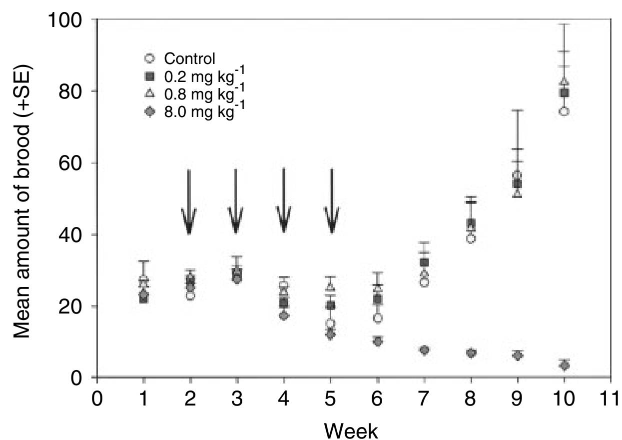

Figure 4. Mean amount of brood (+SE) in bumble bee colonies from one of four treatments: control, 0.2 mg kg^{-1} spinosad, 0.8 mg kg^{-1} spinosad and 8.0 mg kg^{-1} spinosad. The brood number is the estimated sum of egg masses, larval masses, larval cells and pupae in the colonies. Arrows indicate weeks where treated pollen was given to colonies.

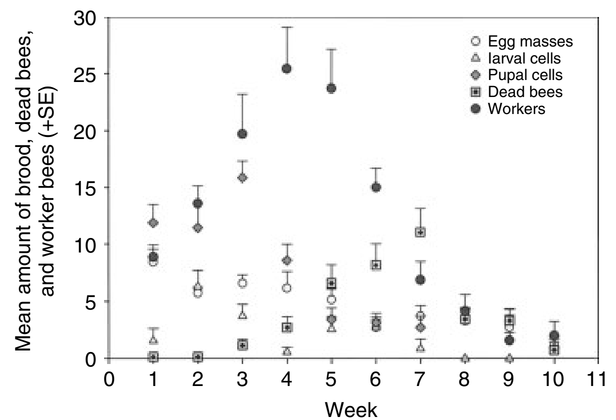

Figure 5. The mean (+SE) number of eggs, larval cells, pupae, worker bees and dead worker bees each week from seven bumble bee colonies fed pollen with 8.0 mg kg^{-1} spinosad during weeks two to five.

among treatments in the amount of brood (all pairwise comparisons of least-squares means >0.05). From weeks seven to ten there was a significant difference in the amount of brood between the 8.0 mg kg^{-1} treatment and the control, 0.2 mg kg^{-1} and 0.8 mg kg^{-1} treatments (pairwise comparisons of least-squares means <0.05). There were no differences among the control, 0.2 mg kg^{-1} and 0.8 mg kg^{-1} treatments in the amount of brood at any time.

Closer examination of the number of eggs, larval masses, distinct larval cells, pupal cells, worker bees and dead workers found each week in colonies from the 8.0 mg kg^{-1} treatment can provide some indication at which stage the bumble bee life cycle was affected by exposure to 8.0 mg kg^{-1} spinosad (Fig 5). In the 8.0 mg kg^{-1} treatment, the number of egg masses declined slightly over the course of the experiment; however, the mean number of egg masses remained similar to that in other treatment groups until week eight when the control, 0.2 mg kg^{-1} and 0.8 mg kg^{-1} treatments began to increase. Egg masses did not appear to develop into larval cells after week two. The mean number of larval cells declined in week three, only one week into spinosad feeding, and never went above approximately three per colony. The mean number of pupal cells declined sharply between weeks three and four. Most of these pupal cells must have developed into adult bees as is evident from the mean increase in colony size and number of dead adult worker bees found. Between weeks three and four, few larval cells developed into new pupal cells. These data taken together indicate that the queen bees in the 8.0 mg kg^{-1} treatment were continuing to lay eggs for three to four weeks after spinosad feeding began. However, few eggs developed into larvae. After one week of exposure at levels of 8.0 mg kg^{-1} spinosad in pollen, larval cells were not developing into pupal cells.

3.2 Foraging experiment

There were not enough worker bees in the colonies in the 8 mg kg^{-1} treatment to be included in this part

of the study. Scan samples of the number of bees on or above flower arrays showed no difference in the foraging force among colonies from the three remaining treatments included in the experiments below ($F_{2,11} = 0.16$, $P = 0.852$).

Mean handling times (±SE) for each treatment, for flowers 1 to 35, was 2.8 (±0.6), 2.9 (±0.5) and 5.5 (±0.5) for control, 0.2 mg kg^{-1} and 0.8 mg kg^{-1}, respectively. Repeated measures ANOVA on flower handling time, with flower number as the repeated measure, showed no interaction between the treatment and the flower number ($F_{68,1044} = 1.14$, $P = 0.206$). There was a treatment effect on handling times ($F_{2,31} = 7.84$, $P = 0.0018$) with bees in the 0.8 mg kg^{-1} treatment having longer handling times than bees from the control and 0.2 mg kg^{-1} treatments (differences of least-squares means control vs 0.2 mg kg^{-1} $t_{31} = -0.11$, $P = 0.914$, 0.2 mg kg^{-1} vs 0.8 mg kg^{-1} $t_{31} = -3.29$, $P = 0.002$, control vs 0.8 mg kg^{-1} $t_{31} = -3.50$, $P = 0.0014$; Fig 6). Handling times (±SE) during the flower 'learning phase' (flowers 1 to 10) were 3.6 (±0.9) s, 4.3 (±0.7) s and 7.3 (±0.8) s for the control, 0.2 mg kg^{-1} and 0.8 mg kg^{-1} treatments, respectively. There was no treatment by flower number interaction ($F_{18,279} = 0.79$, $P = 0.716$) but there was an effect of treatment ($F_{2,31} = 5.85$, $P = 0.007$). Bees from the control and 0.2 mg kg^{-1} treatments did not differ (difference of least-squares means; $t_{31} = -0.59$, $P = 0.561$) and were both faster than bees from the 0.8 mg kg^{-1} treatment (control vs 0.8 mg kg^{-1} $t_{31} = -3.08$, $P = 0.004$, 0.2 mg kg^{-1} vs 0.8 mg kg^{-1} $t_{31} = -2.76$, $P = 0.010$). Separate analysis of flowers 11 to 35 ('experienced foragers') showed an interaction between flower number and spinosad treatment ($F_{48,734} = 1.46$, $P = 0.025$). Mean handling times (±SE) were 2.5 (±0.2) s, 2.4 (±0.1) s and 4.7 (±0.2) s for bees in the control, 0.2 mg kg^{-1} and 0.8 mg kg^{-1} treatments, respectively.

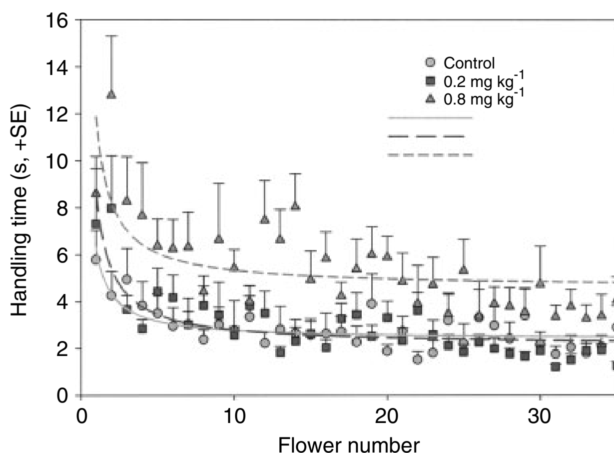

Figure 6. Handling time(s) (+SE) of bumble bees on arrays of artificial complex flowers in three treatment groups: control, 0.2 mg kg^{-1} spinosad and 0.8 mg kg^{-1} spinosad. Treated pollen was fed to bees during weeks two to five of the experiment and bee handling times are from adult worker bees whose entire larval stage overlapped with the treated pollen feeding period. Handling times were calculated as the total time that bees touched artificial flowers until they successfully accessed the sucrose solution in a flower. Bees were videotaped individually on the foraging arrays for approximately 35 flowers.

Foraging rates were calculated as the total time taken to access 10 flowers (flowers 11 to 20 for every bee) and were longer for bees in the 0.8 mg kg^{-1} treatment [5.2 (±0.7) min; overall $F_{2,32} = 4.49$, $P = 0.019$] than for bees from the control treatment [3.0 (±0.3) min; $t_{32} = -2.54$, $P = 0.016$] and 0.2 mg kg^{-1} treatment [3.35 (±0.5) min; $t_{32} = -2.57$, $P = 0.015$] colonies. Foraging rates were not different between the control and 0.2 mg kg^{-1} treatments ($t_{32} = -0.21$, $P = 0.835$). On a per flower basis, the foraging rates convert to approximately 18, 20 and 31 s for bees in the control, 0.2 mg kg^{-1} and 0.8 mg kg^{-1} treatments, respectively. Therefore on a typical foraging excursion in which a bee may successfully access 40 complex flowers, bees exposed to no or 0.2 mg kg^{-1} spinosad are estimated to require 12–13 min, whereas bees exposed to 0.8 mg kg^{-1} spinosad would require about 21 min to access the same number of flowers (note that these estimates only involve a bee foraging on one flower type in one patch and do not include other activities such as flying to and from the nest site, and between patches).

4 DISCUSSION

Spinosad at a level of 8.0 mg kg^{-1} in pollen was clearly detrimental to bumble bee colony health. The impact was evident first in the proportion of dead adult worker bees, which was greater than in the other treatments by week four, two weeks after treatment feeding began. Egg laying by queens did not appear to be directly affected by 8.0 mg kg^{-1} spinosad, but larval development was quickly disrupted to the extent that very few (less than four per colony) pupal cells formed after week three of the experiment. However, bees in the wild are unlikely to be exposed to levels of spinosad in pollen and nectar as high as 8 mg kg^{-1} at current recommended application rates; such a high rate of exposure would only occur if recommended spraying rates or times were not followed.

The only colony health measure suggesting that levels of spinosad within likely concentrations following normal field applications may affect bumble bee colonies was the lower worker weight of bees exposed to 0.8 mg kg^{-1} spinosad during larval development. Our results suggest that levels of spinosad in pollen of 0.8 mg kg^{-1} spinosad will have minimal immediate ill effects on bumble bee colony health. These results agree with other studies on honey bee and bumble bee colonies that have found minimal to no effects on colony health of spinosad applied at low or medium application rates.[16] However, one study found that bumble bee colonies put into greenhouses 0–9 days following spinosad application to tomatoes at 120 g AI ha^{-1} (comparable with 0.8 mg kg^{-1} spinosad treatment in our experiment) showed some detrimental effects to bumble bee brood, and it was concluded that there may be a transient effect on bumble bee

colonies in greenhouses sprayed with spinosad.[17] Our procedure of drying the spinosad pollen mixture prior to re-hydration and feeding may have resulted in reduced effects compared with fresh, wet residues that are believed to have greater toxicity to bees.[16] Thus, situations in which spinosad residues do not dry prior to bee exposure may cause greater toxicity than our results indicate.

There was no avoidance of treated pollen, as indicated by equal pollen consumption among treatments, although bees in our experiment did not have a choice between pollen with or without spinosad. In the wild, where there are multiple pollen sources, bees may avoid pollen containing spinosad. In a study on honey bees foraging in cages on *Phacelia tanacetifolia* Benth treated with 144 and 540 g AI ha^{-1} spinosad, fewer bees were observed foraging on treated crops than on controls.[11] There was no increase in bee mortality in the treated crops and it was suggested that spinosad residues may be repellent to honey bees.

Although colonies exposed to 0.2 and 0.8 mg kg^{-1} showed only minimal effects on colony health measures, bees that were exposed to 0.8 mg kg^{-1} spinosad during development showed impaired foraging ability on artificial flowers. They took longer to access complex flowers, resulting in longer handling times and slower foraging rates. Studies on the mode of action of spinosad indicate that exposed insects experience hyper-excitation of the nervous system, followed by inhibition of neural firing.[13] This process results in initial involuntary muscle tremors followed by paralysis and death. These effects may result from disruption of nicotinic receptors and GABA-gated chloride currents.[22] The mode of action appears to be similar to that of insecticides in the chloronicotinoid family, such as imidacloprid, that bind to acetylcholine receptors, but spinosad works through a mechanism that is different from other known insecticides.[16] At high doses, imidacloprid causes foraging impairment in both honey bees[23] and bumble bees.[20] We noted similar trembling in bees when they were foraging on arrays from the 0.8 mg kg^{-1} spinosad treatment as was observed in bumble bees exposed to 30 mg kg^{-1} imidacloprid foraging on arrays.[20] Trembling behaviour, most likely caused by excitation of the central nervous system,[13] appeared to impair the bees' ability to land and enter the flower tube. In the 0.8 mg kg^{-1} spinosad treatment, we observed that the bees often would land on the lip of an artificial flower, tremble slightly and fall back, and then proceed to enter the flower tube. This behaviour was not observed in bees from the control or 0.2 mg kg^{-1} treatments.

The importance of the decrease in foraging ability that we observed in bumble bees from the 0.8 mg kg^{-1} treatment to colonies in the wild is difficult to assess. Resource availability may play an important role in determining if impaired foraging would be important to colony health and rearing of the reproductive caste. In areas of low resource availability in which colonies are only marginally meeting nutritional requirements, any decline in foraging efficiency of workers may result in lower reproductive output and consequently lower representation in subsequent years. Conversely, if resources are abundant, a decline in foraging efficiency may not have as significant an impact on colony survival or production of reproductive bees. Decreased foraging rates could lead to pollination limitation and lower seed set.[24]

In summary, we found that spinosad at levels estimated to be twice the likely worst-case exposure to bees in the wild resulted in complete colony death within seven weeks after commencement of a four-week exposure period. Colonies exposed to more realistic field levels of spinosad in pollen did not show any lethal effects and only minimal immediate colony health effects. However, bees that had developed during their larval stage with 0.8 mg kg^{-1} spinosad treated pollen demonstrated impaired foraging on an artificial complex flower foraging array. Bees need to not only survive exposure to pesticides, but also forage effectively. Our results suggest that testing of novel pesticides should include measurement of sub-lethal foraging effects on adult bees that have come in contact with the pesticide in their adult and larval stage. As we showed in this study, adult bees that have been exposed to a pesticide during larval development may display symptoms of poisoning that are not detected with current tests required by regulatory agencies. Pesticide exposure levels that have previously been thought to be safe for pollinators may prove harmful if larval-exposed adults are screened for sub-lethal foraging effects.

ACKNOWLEDGEMENTS

We thank Dow Agri-Science for providing spinosad and Cynthia Scott-Dupree for assistance with the data collection. Elizabeth Elle, Zamir Punja and two anonymous reviewers provided helpful comments on the manuscript. We greatly appreciate advice from James Inkster on spinosad dose preparation. Funding was provided by a Natural Sciences and Engineering Research Council/Agriculture-Agri-Food Canada Research Partnership Agreement and an NSERC Discovery Grant to MLW, as well as an NSERC postgraduate scholarship to LAM.

REFERENCES

1 Corbet SA, Williams IH and Osborne JL, Bees and the pollination of crops and wild flowers in the European community. *Bee World* **72**:47–59 (1991).
2 O'Toole C, Diversity of native bees and agroecosystems, in *Hymenoptera and biodiversity*, ed by La Salle J and Gould ID, CAB International, London, pp 169–196 (1993).
3 Kearns CA and Inouye DW, Pollinators, flowering plants, and conservation biology. *BioScience* **47**:297–307 (1997).
4 Watanabe ME, Pollination worries rise as honey bees decline. *Science (Washington)* **265**:1170 (1994).
5 Allen-Wardell G, Bernhardt P, Bitner F, Burquez A, Buchmann S, Cane J, Cox PA, Dalton V, Feinsinger P, Ingram M,

Inouye D, Jones EC, Kennedy K, Kevan P, Koopowitz H, Medellin R, Medellin SM and Nabhan GP, The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conserv Biol* **12**:8–17 (1998).
6 Williams PH, Environmental change and the distribution of British bumblebees (*Bombus* Latr). *Bee World* **67**:50–60 (1986).
7 Banaszak J, Ecological bases of conservation of wild bees, in *The conservation of bees,* ed by Matheson A, Buchmann SL, O'Toole C, Westrich P and Williams IH, Academic Press, London, pp 55–62 (1996).
8 Cane JH and Tepedino VJ, Causes and extent of declines among native North American invertebrate pollinators: detection, evidence, and consequences. *Conserv Ecol* **5**:[online] URL (2001).
9 Johansen CA and Mayer DF, *Pollinator protection: A bee and pesticide handbook*, Wicwas Press, Cheshire, CT (1990).
10 Koul O and Dhaliwal GS (eds), *Microbial biopesticides: an introduction*, Taylor and Francis, London, pp 1–12 (2002).
11 Miles M, The effects of spinosad, a naturally derived insect control agent to the honeybee. *Bull Insectol* **56**:119–124 (2003).
12 Michener CD, McGinley RJ and Danforth BN, *The bee genera of North and Central America (Hymenoptera: Apoidea)*, Smithsonian Institution, Washington, DC (1994).
13 Salgado VL, Studies on the mode of action of spinosad: symptoms and physiological correlates. *Pestic Biochem Phys* **60**:91–102 (1998).
14 Sparks TC, Thompson GD, Kirst HA, Hertlein MB, Larson LL, Worden TV and Thibault ST, Biological activity of the spinosyns, new fermentation derived insect control agents, on tobacco budworm (Lepidoptera: Noctuidae) larvae. *J Econ Entomol* **91**:1277–1283 (1998).
15 Cleveland CB, Mayes MA and Cryer SA, An ecological risk assessment for spinosad use on cotton. *Pest Manag Sci* **58**:70–84 (2001).
16 Mayes MA, Thompson GD, Husband B and Miles MM, Spinosad toxicity to pollinators and associated risk. *Rev Environ Contam Toxicol* **179**:37–71 (2003).
17 Kaneshi K, Toxicity and side effects of spinosad to bumble bees, *Bombus terrestris*, in lab and greenhouse conditions. Report GHF-P-2265. Dow AgroSciences, Indianapolis, IN (2000).
18 Cnaani J, Schmidt-Hempel R and Schmidt JO, Colony development, larval development and worker reproduction in *Bombus impatiens* Cresson. *Insect Soc* **49**:164–170 (2002).
19 Bailey JC, Efficacy of alternative sweet corn pest control agents and their impacts on honey bees, *MSc Thesis*, Department of Environmental Biology, University of Guelph, Guelph, ON (2004).
20 Morandin LA and Winston ML, Effects of novel pesticides on bumble bee (Hymenoptera: Apidae) colony health and foraging ability. *Environ Entomol* **32**:555–563 (2003).
21 SAS, SAS Systems for Windows, SAS Institute Inc, Cary, NC (1999).
22 Watson GB, Actions of insecticidal spinosyns on gamma-aminobutyric acid responses from small-diameter cockroach neurons. *Pestic Biochem Physiol* **71**:20–28 (2001).
23 Schmuck R, No causal relationship between Gaucho seed dressing in sunflowers and the French bee syndrome. *Pflanzenschutz Nachrichten Bayer* **52**:257–299 (1999).
24 Kwon YJ and Saeed S, Effect of temperature on the foraging activity of *Bombus terrestris* L (Hymenoptera: Apidae) on greenhouse hot pepper (*Capsicum annuum* L). *Appl Entomol Zool* **38**:275–289 (2003).