

Susceptibility of *Megachile rotundata* to insecticides used in wild blueberry production in Atlantic Canada

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Abstract The alfalfa leafcutting bee, *Megachile rotundata* (Fabricius), is a valuable wild and managed pollinator of lowbush blueberry (syn. ‘wild blueberry’, *Vaccinium angustifolium* Ait.), in Atlantic Canada. As some insecticides may present a hazard to pollinators, we assessed the susceptibility of *M. rotundata* to insecticides used or projected for future use in lowbush blueberry pest management. In topical direct contact bioassays, adults were susceptible to phosmet, spinosad, spinetoram, and deltamethrin. Based on findings from these laboratory studies, it appears that when used at recommended or projected application rates, each of these compounds poses a hazard to *M. rotundata* by direct contact. In a second experiment, eggs and larvae were collected in the field and their pollen provisions were treated with deltamethrin, flubendiamide, and spinetoram at field relevant concentrations. Larvae treated with deltamethrin and spinetoram in the laboratory either died before spinning a cocoon or, in the case of spinetoram, occasionally pupated without spinning a cocoon. Flubendiamide was not toxic to adult *M. rotundata* by direct contact and had no effect on larval survivorship, or time to complete cocoon spinning. Emergence after overwintering was relatively poor overall, but there was no effect of treatment. Based on these results, flubendiamide

appears safe to use in the presence of *M. rotundata*, whereas the other insecticides we tested may pose a hazard.

Keywords Pollinators · *Megachile rotundata* · *Vaccinium angustifolium* · Insecticide toxicity

Introduction

Many agricultural crops are wholly or partially dependent on bee pollination for proper seed and fruit set (Allen-Wardell et al. 1998; Free 1993; Kevan 1999; Klein et al. 2007), and demand for pollinator-dependant crops is increasing (Winfree 2008; Aizen and Harder 2009). Honey bees (*Apis mellifera* L.) have been considered the most ecologically and economically significant pollinator globally (Allen-Wardell et al. 1998; Delaplane and Mayer 2000; Kevan 1999), but there is increasing awareness of the important role other bees play in pollination. Given the precarious state of the honey bee industry (vanEngelsdorp et al. 2008) and the fact that honey bees are not the most proficient pollinators of all crops, several commercially managed non-*Apis* species are gaining interest in agriculture (Javorek et al. 2002; Kevan and Philips 2001; Tasei 2002).

The alfalfa leafcutting bee, *Megachile rotundata* (Fabricius), is a solitary, cavity-nesting species that is well-recognized for its role in alfalfa seed production in western Canada and other parts of the world (Peterson et al. 1992). Originally from Eurasia, natural populations now occur throughout most of the United States and Canada, following multiple introductions since the 1930s (Bohart 1972; Peterson et al. 1992). *M. rotundata* has been identified as an important managed pollinator of lowbush blueberry (*Vaccinium angustifolium* Ait.) in Atlantic Canada (Javorek et al. 2002). This species nests in almost any

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cavity of appropriate size, including hollow stems, abandoned beetle burrows, holes bored in wood, and various paper, rubber, or metal tubes (Free 1993). Mated *M. rotundata* females build a series of cells constructed from leaf cuttings within the tunnels she has chosen for nesting. Each cell is partially filled with a mixture of pollen and nectar, on to which the adult female lays an egg. The cell is then capped with more leaf cuttings and the larva develops entirely within the cell, nourished by the pollen and nectar provision. As *M. rotundata* nests gregariously and will readily accept artificial nesting blocks, large populations can be easily managed for pollination purposes (Free 1993; McCorquodale and Owen 1997).

Concerns of pollinator declines are well documented (Allen-Wardell et al. 1998; Klein et al. 2007) and have been attributed to multiple factors, including insecticides (Brittain et al. 2010; Kevan and Phillips 2001; Winfree 2010). Pesticides remain an essential pest management tool in most agricultural systems, but exposure to these chemicals may cause numerous negative effects in bees, including mortality, compromised reproduction, or behavioural changes, all of which could result in reduced pollination (Johansen et al. 1983; Johansen and Mayer 1990; Tasei 2002). To mitigate many environmental and ecotoxicological concerns, reduced-risk alternatives to older, broad-spectrum chemistries have been developed. However, depending on the dose and exposure, many of these alternatives can be toxic to bees (Aliouane et al. 2009; Bailey et al. 2005; Gradish et al. 2010; Mommaerts et al. 2009; Morandin and Winston 2003; Morandin et al. 2005). The vast majority of bee toxicity studies have focused on honey bees and data on their susceptibility to a given pesticide are required by many countries prior to registration. Although these data are often extrapolated to predict impacts on all other bee species, there are many important differences in ecology, physiology, and behaviour of honey bees and non-*Apis* taxa (Thompson and Hunt 1999). Indeed, laboratory studies have shown that different bee species vary in their susceptibility to pesticides (Devillers et al. 2003; Johansen 1972; Johansen 1977; Johansen et al. 1983; Scott-Dupree et al. 2009; Zahoor and Johansen 1973), and it is therefore important to generate data on the susceptibility of other bee species to pesticides.

Here, we evaluate the toxicity of several reduced-risk and conventional insecticides to *M. rotundata*, a species that may be more susceptible to insecticides than honey bees and bumblebees (Devillers et al. 2003; Johansen 1977; Johansen et al. 1983; Scott-Dupree et al. 2009). The low-bush blueberry agroecosystem and relevant insecticides that may be used during bloom serve as a framework for these studies. *M. rotundata* is a valued managed pollinator for many blueberry producers, and several insect pests of the crop—e.g. blueberry spanworm, (*Itame argillacearia*

Packard) and blueberry flea beetle (*Altica sylvia* Malloch)—are prominent during bloom. Thus, *M. rotundata* may be at risk of insecticide exposure, either from direct sprays or by consuming potentially contaminated pollen or nectar from treated flowers. We hypothesised that susceptibility to the tested compounds will vary for both *M. rotundata* adults and larvae, and that based on recommended field rates, the insecticides will differ in predicted hazard that they pose to *M. rotundata* in the field.

Materials and methods

Test insects

For all bioassays, *M. rotundata* pre-pupae were purchased from Northstar Seeds (Neepawa, MB) and incubated at 30°C for 20–25 days according to existing emergence protocols. Loose cells were placed in plastic containers (17 × 27 × 7 cm high) with ventilated lids covered in aluminium screening. Each container held a single layer of cells that covered the bottom.

Adult direct contact bioassay

The following formulated insecticides were tested: flubendiamide (Belt™ SC, Bayer CropScience Canada, Calgary AB), phosmet (Imidan® 50 WP, Gowan Company, Yuma, AZ), deltamethrin (Decis® 5 EC, Bayer CropScience Canada, Calgary AB), spinosad (Success® 480 SC, Dow AgroSciences Canada, Calgary, AB), and spinetoram (Delegate® WG, Dow AgroSciences Canada, Calgary, AB). Some of these compounds were also used in larval pollen consumption bioassays (see below).

Insecticides were applied to adult *M. rotundata* using a scaled-down (1/9th size spray tube) version of a Potter spray tower (PST) (Potter 1952). The mini-spray tower operates like a PST, applying an even spray to a fixed area using a mounted air brush sprayer, but allows easier application of formulated product. Stock solutions were prepared by dissolving formulated insecticides in deionized water and desired concentrations were then achieved by serial dilutions. Prior to treatment, bees were randomly selected from rearing containers and placed in 500-ml Mason jars. Each jar was randomly assigned to a treatment. Bees were anaesthetized with CO₂ and placed dorsal side up in a 5 cm diameter glass Petri dish lined with a filter paper. Dishes containing bees were placed in the spray tower and 1 ml of appropriate treatment was applied. Control bees were treated with water only. Following treatment, bees were transferred to post-treatment containers consisting of two 15 cm diameter plastic Petri dish lids, separated by a wire screen insert (45 cm long × 5 cm high). Each dish contained a cotton-plugged

1 ml plastic floral pick (Econoplastik Inc., Saint-Jean-Port-Joli, QE) filled with 50% honey/water solution as a food source. For each insecticide, four to five concentrations were tested, and for each concentration and control, four to five replicates containing 9–12 bees were performed. Post-treatment containers were held in the dark at $25 \pm 1^\circ\text{C}$ and 30–40% relative humidity (RH), and mortality was assessed after 48 h.

For each insecticide, regression lines, LC50 values, χ^2 values, and 95% fiducial limits were calculated using the Probit procedure in SAS 9.2 (SAS Institute 2008). Hazard quotients were calculated by dividing each insecticide's estimated field exposure concentration by its LC50 (Stephenson and Solomon 2007).

Larval pollen consumption bioassay

Deltamethrin, flubendiamide, and spinetoram were tested at 0.1 mg a.i./kg pollen, and half (0.05 mg a.i./kg) and double (0.2 mg a.i./kg) this level. For spinetoram, the 0.1 mg a.i./kg pollen concentration was suggested by Dow AgroSciences and was based on residue studies conducted with spinosad on purple tansy (*Phacelia tanacetifolia*) (J. Routledge, personal communication¹). Pollen residue data were not available for deltamethrin or flubendiamide, but a literature search was performed to determine pollen residue levels for a variety of crops and insecticides (Barker et al. 1980; Chauzat et al. 2006; Kubik et al. 1999; Skerl et al. 2009). These values ranged from 0.001 to 0.9 mg a.i./kg with an average of 0.117 mg a.i./kg, which we used as an estimated realistic residue level likely to be encountered in the field. Due to time and resource constraints, we omitted phosmet and spinosad from this experiment. They are generally less used during bloom than their broad-spectrum and spinosyn insecticide counterparts, deltamethrin and spinetoram.

A method modified from Abbott et al. (2008) was used to determine insecticidal effects on developing *M. rotundata* larvae. A 160 × 122 × 152 cm high shelter was constructed of plywood and painted with black and white alternating stripes. The shelter faced E on the NW edge (N 43°01.768', W 81°12.826') of a 1 ha plot of alfalfa at the Southern Crop Protection and Food Research Centre, Agriculture and Agri Food Canada, London, ON. Three Styrofoam[®] nests (Northstar Seeds, Neepawa, MB), each containing ca., 3,500 tunnels lined with plastic drinking straws or rolled pieces of craft paper, were mounted inside the shelter. In early June, alfalfa was cut to coordinate crop bloom with adult bee emergence. Starting at early bloom (2 July), bees in their rearing containers were brought to the

field, placed on the ground inside the shelter, and each container was opened to release the bees. Containers were left open for several days to ensure maximum adult emergence. Adults that emerged in the lab prior to transport to the field (i.e. before flowering) were provided a 50% honey/water solution ad lib and were maintained in the dark at 20°C and 50% RH until bloom. Bees were released in this manner in four staggered batches of ca., 5000 adults, spaced 3–4 days apart.

Leaf cell collections were initiated ~1 week after the first release and continued every 3–4 days for 2 weeks. There were four batches of leaf cells collected, each representing an experimental block in time, for a total of 38–42 cells per treatment. Females built their cells almost exclusively in tunnels lined with paper. Paper tubes lined with cells were removed from nesting blocks, brought to the lab, and opened. Individual cells were removed, opened on one end using a scalpel, and placed in wells of 96-well tissue culture plates. Stock solutions were prepared by dissolving insecticides in deionized water and desired concentrations were then prepared by serial dilutions. Pollen provisions of each leaf cell were then injected with 1 µl of the appropriate test solution using an Eppendorf[®] pipette, ensuring that insecticide did not come into direct contact with the egg/larva. Control cells were treated with water only. Only eggs or first instars were used for bioassays to ensure feeding occurred throughout the entire development period. Individually treated cells remained open for ease of observation and the well plates were placed in unused rearing containers for protection and to maintain humidity. Treated cells were maintained in the dark at 30°C and 50–70% RH. Cells were observed daily to determine mortality and/or time taken to complete cocoon spinning. A cocoon was considered completed once the cell opening was entirely closed with silk and the larva inside was no longer visible. Cells that completed cocoons in any treatment were transferred to 24-well tissue culture plates and kept at room temperature for ~1 week. Cells were then held at 6°C and 50–70% RH for overwintering, beginning early-mid August. In May, trays containing pupae were moved to 30°C, 50–70% RH until adult emergence. Upon emergence, adults were sexed and weighed.

Larval mortality data were subjected to an analysis of variance using the Mixed procedure in SAS 9.1 (SAS Institute 2008) with day as a repeated measure. Variance was partitioned into the fixed effects treatment, day and the treatment * day interaction, and the random effect block. The mean number of days to complete spinning a cocoon for each treatment also was subjected to an analysis of variance using the Mixed procedure, with variance partitioned into the fixed effect treatment and the random effect block. In both cases, assumptions of ANOVA were verified

¹ Registration Manager, Dow AgroSciences Canada Inc., Calgary AB, Canada.

by plotting the residuals against the predicted values, block, and treatment. The mean of the residuals was equal to zero and a Shapiro–Wilk test confirmed that the residuals were approximately normally distributed. Differences between means were determined with a Fisher's LSD test (SAS Institute 2008). In spring, emergence success and sex data were analysed with a nominal logistic model, while days to emergence and adult weight data were analysed with a two-way ANOVA with interaction, incorporating factors of treatment and block (SAS Institute 2010). All tests were performed at $\alpha = 0.05$.

Results

Adult direct contact bioassay

Flubendiamide did not cause mortality of adult *M. rotundata* up to 5000 mg a.i./l, the most concentrated solution of the formulated product we were able to prepare. This is ~10-fold the label rate of 526 mg a.i./l, making the compound of low hazard to *M. rotundata* via topical exposure (Table 1). *M. rotundata* was susceptible by topical exposure to all other tested compounds and all of these pose some hazard, although their toxicities differed in terms of both LC50 values and slopes of the probit lines (Table 1). Based on LC50 values, bees were equally susceptible to the insecticides spinosad and spinetoram, and about sixfold less susceptible to the organophosphorus insecticide phosmet. However, the field exposure estimate concentrations calculated using suggested field rates show that spinosad presents over twofold the estimated hazard of spinetoram and phosmet to *M. rotundata* in the field (Table 1). On the other hand, the synthetic pyrethroid deltamethrin was about threefold more toxic to adult bees than the spinosad and spinetoram, but produces a lower hazard quotient when the recommended field rate is considered (Table 1). The slopes of the probit lines varied but were relatively steep in all cases, indicating a homogenous population response within each chemical (Table 1).

Larval pollen consumption bioassay

Survival of *M. rotundata* larvae was significantly affected by treatment ($F = 200.48$; $df = 9, 237$; $P < 0.0001$), day ($F = 65.3$; $df = 7, 237$; $P < 0.0001$), and the treatment*day interaction ($F = 5.16$; $df = 63, 237$; $P < 0.0001$). At 2 days after treatment (DAT) significant mortality was observed in larvae treated with deltamethrin at 0.1 mg/kg (Fig. 1a) and double this concentration (Fig. 1b), and by 4 DAT high mortality was seen in all three deltamethrin treatments (Fig. 1). Spinetoram was slower acting than deltamethrin, but exposure of larvae to all concentrations of spinetoram-treated pollen eventually resulted in significant mortality. At 6 DAT, spinetoram 0.1 mg a.i./kg (Fig. 1a) and 0.5 mg a.i./kg (Fig. 1c) treatments resulted in significant mortality, although lethal effects were delayed until 8 DAT in larvae exposed to the 2× rate spinetoram treatment (Fig. 1b). By 16 DAT the 0.1 mg ai/kg and 2× rate of spinetoram resulted in mortality equal to that of deltamethrin. Mortality in the 0.5× rate spinetoram treatment levelled off at just over 60% (Fig. 1C). Consumption of flubendiamide-treated pollen at any concentration did not cause significant mortality of *M. rotundata* larvae over the 16 days of the experiment (Fig. 1).

Larvae that consumed pollen treated with deltamethrin and spinetoram did not survive until cocoon completion. Larvae that consumed spinetoram sometimes pupated without spinning a cocoon. For cells treated with flubendiamide, there was no effect of treatment on number of days to complete a cocoon ($F = 0.59$; $df = 3, 123$; $P = 0.62$) (Table 2).

Emergence

Adult emergence was poor overall. Among all treatments, only 20% of larvae that were alive going into overwintering successfully emerged as adults. The nominal logistic model run on bee emergence was not significant ($X^2 = 15.12$; $df = 13$; $P = 0.30$), with no effects of treatment,

Table 1 Direct contact toxicity of formulated insecticides to adult *Megachile rotundata*, 48 h following spray application

Insecticide	<i>n</i>	Slope ± SE	LC50 (mg/l)	95% FL	χ^2	Field rate ^a (mg/l)	Hazard quotient ^b
Flubendiamide	152	—	>5000	—	—	525	<0.11
Phosmet	402	4.73 ± 1.09	288.13	224.98–509.70	6.73	1120	3.9
Spinetoram	336	3.31 ± 0.45	47.01	41.54–54.12	0.73	188	4.0
Spinosad	294	2.52 ± 0.73	46.84	6.41–90.07	8.86	462	9.9
Deltamethrin	313	2.39 ± 0.27	17.15	13.32–21.15	2.21	31	1.8

^a Based application volumes of 200 l/ha (except phosmet at 1000 l/ha). Where a range of rates was presented, the mean rate was used: flubendiamide 105 g a.i./ha; phosmet 1120 g a.i./ha; spinetoram 37.5 g a.i./ha; spinosad 92.4 g a.i./ha; deltamethrin 6.25 g a.i./ha

^b Hazard quotient = estimated field rate concentration divided by its LC50. An insecticide having a hazard quotient of <1 is considered non-hazardous

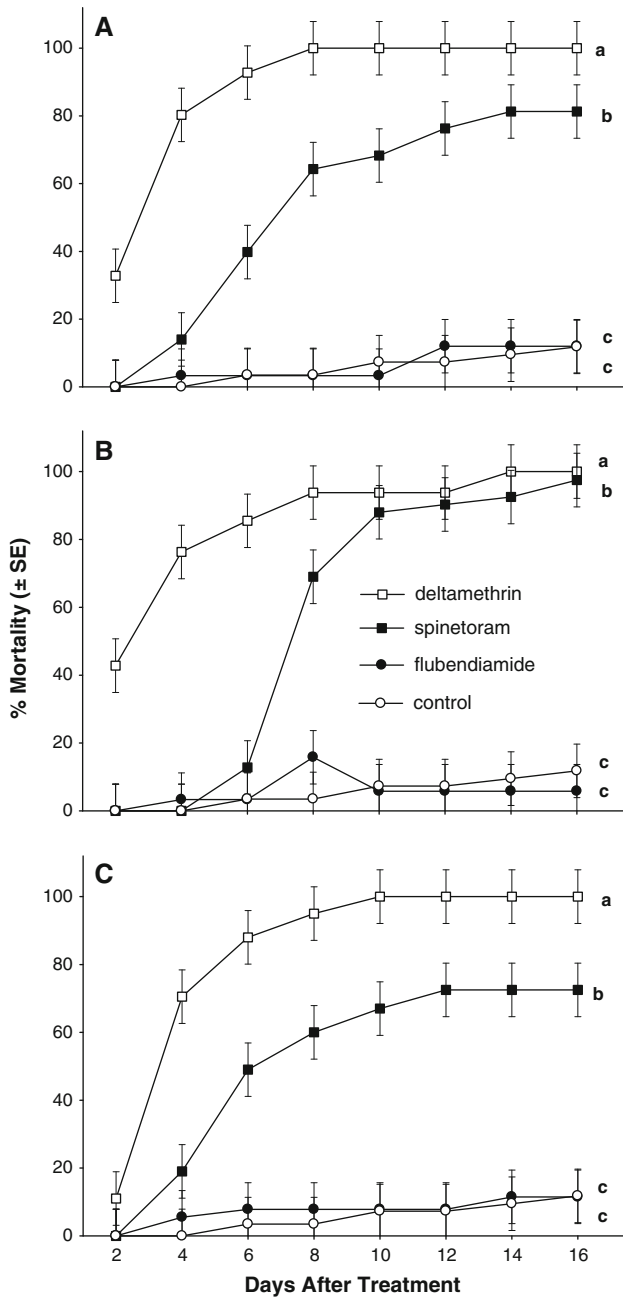


Fig. 1 Mortality of *Megachile rotundata* larvae following ingestion of nectar/pollen provisions treated with insecticides (flubendiamide, deltamethrin, and spinetoram) at a (A) realistic field residue level (0.1 mg a.i./kg pollen), (B) double, and (C) half this concentration. Controls were treated with water. Trend lines followed by the same letter are not significantly different at $\alpha = 0.05$

block or the interaction of these factors. Of bees that did successfully emerge as adults, there was no effect of treatment or blocking (whole model effects) on sex ($X^2 = 8.39$; $df = 9$; $P = 0.50$), days to emergence ($F = 0.16$; $df = 9, 20$; $P = 0.99$), or bee weight ($F = 0.78$; $df = 9, 20$; $P = 0.64$).

Table 2 Number of days for *Megachile rotundata* larvae to complete cocoon spinning following ingestion of nectar/pollen provisions contaminated with formulated flubendiamide at a realistic pollen residue level (0.1 mg a.i./kg pollen), and double and half this concentration

Flubendiamide treatment (mg a.i./kg pollen)	<i>n</i>	Average number of days \pm SE to cocoon completion ^a
0	31	9.8 \pm 0.35 a
0.05	35	9.5 \pm 0.37 a
0.1	33	9.3 \pm 0.45 a
0.2	29	10.1 \pm 0.63 a

^a Values followed by the same letter are not significantly different, $P > 0.05$

Discussion

Pesticide exposure is regarded as a potential contributing factor to global bee declines. To decrease the potential for environmental contamination and health risks to humans and non-target organisms, reduced-risk alternatives to traditional broad-spectrum insecticides have been sought. However, designation of an insecticide as reduced-risk does not assure safety to bees, which will vary depending on the bee species, as well as the compound’s mode of action, intensity of exposure, and probability of exposure. Most lowbush blueberry growers in eastern North America currently rely on honey bees for pollination of their crops, but other managed bees like *M. rotundata* have demonstrated good pollination capability in lowbush blueberry. Numerous wild species of Megachilidae also occur throughout the region.

Our findings that *M. rotundata* adults are susceptible to phosmet, deltamethrin, spinosad and spinetoram, but not flubendiamide, are supported by results from other studies. Significant declines in nesting populations of *M. rotundata* in apple orchards were reported following applications of phosmet (Alston et al. 2007). Scott-Dupree et al. (2009) examined the direct contact toxicity of a number of compounds to *M. rotundata*, including technical grade (unformulated product) deltamethrin and spinosad. Similar to our results, Scott-Dupree et al. (2009) found *M. rotundata* adults to be highly susceptible to both deltamethrin and spinosad, although their LC₅₀ values differed from ours and their 48 h spinosad:deltamethrin LC₅₀ ratio (9.6) was more than triple that found in this study (2.7). This discrepancy is likely due to differences in methodology, e.g. technical versus formulated product, use of a different spray apparatus and different spray volumes. At the highest solution of flubendiamide we were able to achieve, we observed no lethal effects on *M. rotundata*. Hall (2007) similarly reported that flubendiamide was essentially non-toxic to honey bees and bumble bees in both acute contact and acute oral tests. We have observed that flubendiamide is

acutely non-toxic to *B. impatiens* through both direct contact and ingestion (Gradish et al. unpublished data).

Most pesticide toxicity studies focus on the lethality of insecticides to insects. However, pesticides also can affect bees through changes to life span, development, and behaviour (Abbott et al. 2008; Gradish et al. 2010; Morandin et al. 2005; Tasei et al. 1994; Tasei et al. 2000; Torchino 1983), and immature stages are generally more sensitive than adults. *M. rotundata* adults are effective blueberry pollen foragers (Javorek et al. 2002) and when in blueberry fields they would be expected to collect blueberry pollen to feed their offspring. In our experiment with *M. rotundata* larvae, flubendiamide had no impact on larval vitality or development. Hall (2007) also reported no adverse effects of flubendiamide on honey bee and bumble bee brood development, and in another study, we have found no effects of flubendiamide on *B. impatiens* colony development (Gradish et al. unpublished data). This is encouraging for wild blueberry growers given that flubendiamide is efficacious against lepidopteran pests like blueberry spanworm, which frequently infests the crop during bloom (Ramanaidu et al. 2011). Conversely, *M. rotundata* larvae exposed to deltamethrin and spinetoram treated pollen had poor development and survival, suggesting that larval feeding on pollen and/or nectar contaminated with these insecticides could result in a reduced pollinator force if fewer individuals are able to successfully reach adulthood and emerge the following spring.

Of larvae that were still alive after 14 days and allowed to overwinter, there were no effects of treatment on adult bee emergence, or the sex and weight of emerged bees. However, our emergence success was low overall at approximately only 20%. We are not certain of the cause of this low emergence, but it is possible that our cutting into cells to add treatments (pesticide or water) compromised development. In studies specifically examining factors that limit or optimise emergence, success can be very high under suitable conditions but can decrease significantly with prolonged storage, low humidity, or variation in temperature regimes at various stages of development (Johansen and Eves 1973; Kemp and Bosch 2000; Pitts-Singer and James 2009), but our rearing conditions and treatment techniques were similar to those used by others with good emergence success (e.g., Abbott et al. 2008).

Additional field and residue studies are needed to fully evaluate the risks that deltamethrin and spinetoram pose to *M. rotundata* larvae in wild blueberry fields since plant architecture may impact the potential residue levels of those insecticides in pollen and nectar. We based our exposure concentrations on a limited amount of field pollen residue data from other cropping systems and we are not certain of the true residue levels of these compounds on blueberry pollen following sprays. Given that most flowers

on a *V. angustifolium* stem tend to hang down and that anthers are poricidal, concentrations of insecticide on blueberry pollen may be relatively low. There are also differences in suggested application rates of tested products. The registered use rate for spinetoram is 25–50 g a.i./ha. Flubendiamide is not yet registered in Canada but we anticipate its use rate will be similar to spinetoram. This brackets the 36 g a.i./ha rate of spinosad applied in the purple tansy study that yielded a residue of 0.09 mg/kg pollen (J. Routledge, personal communication). The registered use rate of deltamethrin, however, is only 6.25 g a.i./ha, which could result in lower pollen residue levels for this insecticide. Field degradation rates of these compounds differ as well and exposure concentrations for bees therefore will vary over time. Nonetheless, the pollen pesticide residue data that are available in other studies suggest our test concentrations of 0.05, 0.1, and 0.2 mg a.i./kg pollen are appropriate to assess relative toxicities of these active ingredients to *M. rotundata* larvae.

Bees differ in their susceptibility to pesticides and experimental methods for toxicity testing are quite variable among researchers. However, our results indicate that toxicities of compounds to worker honey bees—the surrogate used for most toxicology studies with bees—may be useful to predict relative toxicities to *M. rotundata*. For example, reported topical LD50 values indicate that deltamethrin (0.05 µg/bee; Inglesfield 1989), spinosad (0.053 µg/bee; Miles 2003) and spinetoram (0.024 µg/bee; PMRA 2008) are all similar in their toxicities to honey bees, while phosmet is relatively moderately toxic (1.06 µg/bee; USEPA 2011) and flubendiamide is non-toxic (>200 µg/bee; Hall 2007). We noticed the same trend of relative toxicities in our direct contact bioassays with *M. rotundata* adults and oral toxicity experiments with larvae. Thus, although precise extrapolations of toxicity values and predictions of hazard in the field may not be possible, relative estimates of bee susceptibility to different products should be achievable in some cases.

The types of toxicity tests done here can be used to select pesticides that are less toxic to bees so that pest management will not compromise yields by unintended side-effects of pollination loss. Based on our results, deltamethrin, spinosad, spinetoram, and phosmet are potentially hazardous to *M. rotundata* adults by direct contact at label rates for use in wild blueberries. To lessen the hazard posed by insecticides to honey bees, it is often recommended that hives be removed or closed during application and returned once residues have dried (Johansen and Mayer 1990; Mayes et al. 2003). These recommendations are not practical for managed *M. rotundata*, as their nests cannot be easily moved or protected (Tasei 2002). Thus, if it is necessary to spray one of these insecticides, applications should take place when there is low probability of adult

direct contact exposure, such as before sunrise and after sunset. *M. rotundata* exposure to spinosad and spinetoram would certainly be of concern to growers, but risks to bees will be greatly reduced if adults are not exposed by direct contact, since dried residues of spinosyns are practically non-toxic (Miles 2003). Exposure of larvae to contaminated pollen collected by adult females potentially presents an additional risk to *M. rotundata*. Flubendiamide, however, was non-toxic to both life stages and is predicted to pose little hazard in the field. Given that insecticide toxicity may vary greatly between the laboratory and field (Stark et al. 1995), and that susceptibility in an agricultural setting depends on a multitude of factors including application rates, timing, and degradation, additional higher tier semi-field or field studies are advisable to clarify the risks different insecticides pose to *M. rotundata* in blueberries.

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References

- Abbott VA, Nadeau JL, Higo HA, Winston ML (2008) Lethal and sublethal effects of imidacloprid and clothianidin on *Osmia lignaria* and *Megachile rotundata* (Hymenoptera: Megachilidae). *J Econ Entomol* 101:784–796
- Aizen MA, Harder LD (2009) The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Current Biol* 19:915–918
- Aliouane Y, El Hassani AK, Gary V, Armengaud C, Lambin M, Gauthier M (2009) Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior. *Environ Toxicol Chem* 28:113–122
- Allen-Wardell G, Bernhardt P, Bitner R, Burquez A, Buchmann S, Cane J, Cox PA, Dalton V, Feinsinger P, Ingram M, Inouye D, Jones CE, Kennedy K, Kevan P, Koopowitz H, Medellin R, Medellin-Morales S, Nabhan GP (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conserv Biol* 12:8–17
- Alston DG, Tepedino VJ, Bradley BA, Toler TR, Griswold TL, Messinger SM (2007) Effects of the insecticide phosmet on solitary bee foraging and nesting in orchards of capitol reef national park, Utah. *Environ Entomol* 36:811–816
- Bailey J, Scott-Dupree C, Harris R, Tolman J, Harris B (2005) Contact and oral toxicity to honey bees (*Apis mellifera*) of agents registered for use for sweet corn insect control in Ontario, Canada. *Apidologie* 36:1–11
- Barker RJ, Lehner Y, Kunzmann MR (1980) Pesticides and honey bees: nectar and pollen contamination in alfalfa treated with dimethoate. *Arch Environ Contam Toxicol* 9:125–133
- Bohart GE (1972) Management of wild bees for the pollination of crops. *Annu Rev Entomol* 17:287–312
- Brittain CA, Vighi M, Bommarco R, Settele J, Potts SG (2010) Impacts of a pesticide on pollinator species richness at different spatial scales. *Basic Appl Ecol* 11:106–115
- Chauzat MP, Faucon JP, Martel AC, Lachaize J, Cougoule N, Aubert M (2006) A survey of pesticide residues in pollen loads collected by honey bees in France. *J Econ Entomol* 99:253–262
- Delaplane KS, Mayer DF (2000) Crop pollination by bees. CABI Publishing, New York
- Devillers J, Decourtye A, Budzinski H, Pham-Delègue MH, Cluzeau S, Maurin G (2003) Comparative toxicity and hazards of pesticides to *Apis* and non-*Apis* bees. A chemometrical study. *SAR QSAR Environ Res* 14:389–403
- Free JB (1993) Insect pollination of crops, 2nd edn. Academic Press, San Diego
- Gradish AE, Scott-Dupree CD, Shipp L, Harris CR, Ferguson G (2010) Effect of reduced-risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae). *Pest Manag Sci* 66:142–146
- Hall T (2007) Ecological effects assessment of flubendiamide. *Pflanzenschutz-Nachrichten Bayer* 60:167–182
- Inglesfield C (1989) Pyrethroids and terrestrial nontarget organisms. *Pestic Sci* 27:387–428
- Javorek SK, Mackenzie KE, Vander Kloet SP (2002) Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). *Ann Entomol Soc Am* 95:345–351
- Johansen C (1972) Toxicity of field-weathered insecticide residues to four kinds of bees. *J Econ Entomol* 1:393–394
- Johansen CA (1977) Pesticides and pollinators. *Annu Rev Entomol* 22:177–192
- Johansen CA, Eves JD (1973) Effects of chilling, humidity and seasonal conditions on emergence of the alfalfa leafcutting bee. *Environ Entomol* 2:23–26
- Johansen CA, Mayer DF (1990) Pollinator protection: a bee and pesticide handbook. Wicwas Press, Cheshire
- Johansen CA, Mayer DF, Eves J, Kiou CW (1983) Pesticides and bees. *Environ Entomol* 12:1513–1518
- Kemp WP, Bosch J (2000) Development and emergence of the alfalfa pollinator *Megachile rotundata* (Hymenoptera: Megachilidae). *Ann Entomol Soc Am* 93:904–911
- Kevan PG (1999) Pollinators as bioindicators of the state of the environment: species, activity and diversity. *Agric Ecosyst Environ* 74:373–393
- Kevan P, Phillips TP (2001) The economic impacts of pollinator declines: an approach to assessing the consequences. *Conserv Ecol* 5:8
- Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. *Proc R Soc B Biol Sci* 274:303–313
- Kubik M, Nowacki J, Pidek A, Warakomska Z, Michalczyk L, Goszczynski W (1999) Pesticide residues in bee products collected from cherry trees protected during bloom period with contact and systemic fungicides. *Apidologie* 30:521–532
- Mayer MA, Thompson GD, Husband B, Miles MM (2003) Spinosad toxicity to pollinators and associated risk. *Rev Environ Contam Toxicol* 179:37–71
- McCorquodale DB, Owen RE (1997) Allozyme variation, relatedness among progeny in a nest, and sex ratio in the leafcutter cell, *Megachile rotundata* (Fabricius) (Hymenoptera: Megachilidae). *Can Entomol* 129:211–219
- Miles M (2003) The effects of spinosad, a naturally derived insect control agent to the honeybee. *Bull Insectol* 56:119–124
- Mommaerts V, Sterk G, Hoffmann L, Smagge G (2009) A laboratory evaluation to determine the compatibility of

- microbiological control agents with the pollinator *Bombus terrestris*. *Pest Manag Sci* 65:949–955
- Morandin LA, Winston ML (2003) Effects of novel pesticides on bumble bee (Hymenoptera: Apidae) colony health and foraging ability. *Environ Entomol* 32:555–563
- Morandin LA, Winston ML, Franklin MT, Abbott VA (2005) Lethal and sub-lethal effects of spinosad on bumble bees (*Bombus impatiens* Cresson). *Pest Manag Sci* 61:619–626
- Peterson SS, Baird CR, Bitner RM (1992) Current status of the alfalfa leafcutting bee, *Megachile rotundata*, as a pollinator of alfalfa seed. *BeeScience* 1:230–236
- Pitts-Singer TL, James RR (2009) Prewinter management affects *Megachile rotundata* (Hymenoptera: Megachilidae) prepupal physiology and adult emergence and survival. *J Econ Entomol* 102:1407–1416
- PMRA (2008) Evaluation report ERC 2008-01—Spinetoram (XDE-175). Health Canada Pest Management Regulatory Agency, p 107
- Potter C (1952) An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomized spray fluids. *Ann Appl Biol* 39:1–28
- Ramanaidu K, Hardman JM, Percival DC, Cutler GC (2011) Laboratory and field susceptibility of blueberry spanworm (Lepidoptera: Geometridae) to conventional and reduced-risk insecticides. *Crop Prot* 30:1643–1648
- SAS Institute (2008) PROC user manual, version 9.2. SAS Institute, Cary
- SAS Institute (2010) JMP 9.0.2. SAS Institute, Cary
- Scott-Dupree CD, Conroy L, Harris CR (2009) Impact of currently used or potentially useful insecticides for canola agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymenoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae). *J Econ Entomol* 102:177–182
- Skerl MIS, Velikonja Bolta S, Basa Cesnik H, Gregorc A (2009) Residues of pesticides in honey bee (*Apis mellifera carnica*) bee bread and pollen loads from treated apple orchards. *Bull Environ Contam Toxicol* 83:374–377
- Stark JD, Jepson PC, Mayer DF (1995) Limitations to use of topical toxicity data for predictions of pesticide side effects in the field. *J Econ Entomol* 88:1081–1088
- Stephenson GR, Solomon KR (2007) Pesticides and the environment. Canadian Network of Toxicology Centres Press, Guelph
- Tasei JN (2002) Impacts of agrochemicals on non-*Apis* bees. In: Devillers J, Pham-Delegue MH (eds) Honey bees: estimating the environmental impact of chemicals. Taylor and Francis, New York, pp 101–131
- Tasei JN, Sabik H, Pirastru L, Langiu E, Blanche JM, Fournier J, Taglioni JP (1994) Effects of sublethal doses of deltamethrin (DecisCe) on *Bombus terrestris*. *J Apic Res* 33:129–135
- Tasei JN, Lerin J, Ripault G (2000) Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. *Pest Manag Sci* 56:784–788
- Thompson HM, Hunt LV (1999) Extrapolating from honeybees to bumblebees in pesticide risk assessment. *Ecotoxicology* 8:147–166
- Torchino PF (1983) The effects of field applications of naled and trichlorfon on the alfalfa leafcutting bee, *Megachile rotundata* (Fabricius). *J Kansas Entomol Soc* 56:62–68
- USEPA (2011) Office of pesticide programs. Pesticide ecotoxicity database 2000 on S-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)methyl]O,O-dimethyl ester, phosphorodithioic acid (732-11-6). http://cfpub.epa.gov/ecotox/quick_query.htm
- vanEngelsdorp D, Hayes J, Underwood RM, Pettis J (2008) A survey of honey bee colony losses in the US, fall 2007 to spring 2008. *Plos One* 3:e4071
- Winfree R (2008) Pollinator-dependent crops: an increasingly risky business. *Curr Biol* 18:R968–R969
- Winfree R (2010) The conservation and restoration of wild bees. *Ann N Y Acad Sci* 1195:169–197
- Zahoor A, Johansen C (1973) Selective toxicity of carbophenothion and trichlorfon to the honey bee and alfalfa leafcutting bee. *J Econ Entomol* 2:27–30