

Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae)[†]

Angela E Gradish,^a Cynthia D Scott-Dupree,^{a*} Les Shipp,^b C Ron Harris^a and Gillian Ferguson^c

Abstract

BACKGROUND: Bumble bees [*Bombus impatiens* (Cresson)] are widely used for supplemental pollination of greenhouse vegetables and are at risk of pesticide exposure while foraging. The objective of this study was to determine the lethal and sub-lethal effects of four insecticides (imidacloprid, abamectin, metaflumizone and chlorantraniliprole) and three fungicides (myclobutanil, potassium bicarbonate and cyprodinil + fludioxonil) used or with potential for use in Ontario greenhouse vegetable production to *B. impatiens*.

RESULTS: Imidacloprid, abamectin, and metaflumizone were harmful to worker bees following direct contact, while chlorantraniliprole and all fungicides tested were harmless. Worker bees fed imidacloprid-contaminated pollen had shortened life spans and were unable to produce brood. Worker bees consumed less pollen contaminated with abamectin. Metaflumizone, chlorantraniliprole and all fungicides tested caused no sub-lethal effects in bumble bee micro-colonies.

CONCLUSION: We conclude that the new reduced risk insecticides metaflumizone and chlorantraniliprole and the fungicides myclobutanil, potassium bicarbonate and cyprodinil + fludioxonil are safe for greenhouse use in the presence of bumble bees. This information can be used preserve greenhouse pollination programs while maintaining acceptable pest management.

© 2009 Society of Chemical Industry

Keywords: *Bombus impatiens*; pesticide; toxicity; queen-less micro-colonies; greenhouse

1 INTRODUCTION

Bumble bees [*Bombus impatiens* (Cresson)] are important indigenous North American pollinators. Since the early 1990s, they have increasingly been used for pollination in commercial greenhouses and now play an essential role in North American greenhouse vegetable production. Tomato and pepper flowers are self-pollinating, but supplemental bumble bee pollination results in larger, more attractive fruit.^{1,2} Typically, bumble bee colonies are placed in greenhouses for up to 8 weeks and successful pollination depends, in part, on the bees' ability to produce large numbers of offspring to forage during that time.

Greenhouse vegetables are sold for fresh consumption and have a high aesthetic standard required by consumers. Thus, effective pest management is crucial to producing high, marketable yields of greenhouse vegetables, and pesticides remain an important control tactic in integrated pest management programs. Some insect pests occasionally require insecticide applications and fungicides are routinely applied for powdery mildew control.³ These pesticides can negatively affect bumble bees, compromising greenhouse pollination programs. Additionally, regulatory agencies are requiring more data on non-target impacts as part of the pesticide review process. Therefore, as new pesticides are developed it is important to determine their potential impact on bumble bees.

Bumble bees are at risk of pesticide exposure in greenhouses during foraging through direct contact with foliar spray, residues on plants, or by consuming contaminated pollen. The most obvious effect is worker mortality following direct exposure. However, pesticides also may cause significant sub-lethal effects to bees, including shortened life span, behavioral changes, reduction in pollen gathering, reduced fecundity, and abnormal development.⁴ Brood production and vitality can be negatively affected when contaminated pollen is collected and fed to developing larvae. Most studies investigating pesticide impact on

* Correspondence to: Cynthia D Scott-Dupree, Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.
E-mail: cscottdu@uoguelph.ca

[†] Part of this paper was presented at the International Organization of Biological Control Integrated Control in Protected Crops conference in 2008 and appears in the conference bulletin: IOBC/WPRS Bulletin 32: 67–70.

a Department of Environmental Biology, University of Guelph, Guelph, ON, Canada, N1G 2W1

b Agriculture and Agri-Food Canada, Greenhouse and Processing Crops Research Centre, Harrow, ON, Canada, N0R 1G0

c Ontario Ministry of Agriculture, Food and Rural Affairs, Harrow, ON, Canada, N0R 1G0

bees have focused on honey bees (*Apis mellifera* L.); however, there are important physiological and behavioral differences between bumble bees and honey bees that likely result in variation in their susceptibility to pesticides.⁵ Available data suggest that insecticides can have lethal^{6,7} and sub-lethal^{8–11} effects on bumble bees. Currently, there are no studies investigating the effect of fungicides on bumble bees. Therefore, it is essential to generate more toxicity data for bumble bees to accurately assess the potential impacts of pesticide application on greenhouse pollination.

The objective of this study was to determine the lethal and sub-lethal effects on health and reproduction of *B. impatiens* workers of some reduced risk pesticides used or with promise for use in greenhouse vegetable production.

2 MATERIALS AND METHODS

2.1 Test colonies

Class 'A' (capable of pollinating 1400–1850 m²) *B. impatiens* colonies, each containing a queen and ca. 50 workers, were purchased from Biobest Biological Systems Canada (Leamington, ON). A colony consisted of a ventilated plastic nest box contained within a cardboard box. A bottle of Biogluc[®] (Biobest Biological Systems Canada, Leamington, ON), a sugar solution, was included and provided *ad libitum* to the bees as a nectar substitute. Each bumble bee colony received ca. 1 mL of pollen daily. Honey bee-collected mixed floral pollen pellets were purchased from Dutchman's Gold Natural Honey Products (Carlisle, ON), ground to a fine powder and frozen until use.

2.2 Pesticide treatments

Pesticide formulations tested included the insecticides imidacloprid 600 g kg⁻¹ WP (Intercept[®] 60 WP; Bayer CropScience Canada, Toronto, ON), abamectin 19 g L⁻¹ EC (Avid[®] 1.9% EC; Syngenta Crop Protection Canada, Guelph, ON), metaflumizone 240 g L⁻¹ SC (Alverde[™] 240 SC; BASF Canada, Mississauga, ON), and chlorantraniliprole 350 g kg⁻¹ WG (Altacor[®] 35 WG; DuPont Canada, Mississauga, ON), and the fungicides myclobutanil 400 g kg⁻¹ WP (Nova[®] 40 W; Dow Agrosciences Canada, Calgary, AB), potassium bicarbonate (Milstop[®]; Bioworks, Victor, NY), and cyprodinil + fludioxonil 625 g kg⁻¹ WG (Switch[®] 62.5 WG; Syngenta Crop Protection Canada, Guelph, ON). Imidacloprid and abamectin are registered for greenhouse whitefly [*Trialeurodes vaporariorum* (Westwood)] and spider mite (*Tetranychus urticae* Koch) control, respectively. Chlorantraniliprole may be registered for control of cabbage looper (*Trichoplusia ni* Hubner) and other lepidopteran pests. Metaflumizone has been submitted for registration to control cucumber beetle [*Acalymma vittatum* (Fabricius)] and lygus bug [*Lygus hesperus* (Knight)]. Myclobutanil and potassium bicarbonate are currently registered, and cyprodinil + fludioxonil is awaiting registration for greenhouse powdery mildew control.

2.3 Direct contact toxicity

All pesticides tested for direct contact toxicity were technical grade (>95% purity) and included imidacloprid, abamectin, metaflumizone, chlorantraniliprole, myclobutanil, cyprodinil and fludioxonil. A Potter spray tower (PST)¹² was used to apply the pesticides and there was concern that potassium bicarbonate in solution could damage or compromise it. Therefore, potassium bicarbonate was not included. Pesticides were dissolved in acetone + olive oil (19 + 1 by volume) and applied at 0.01, 0.1 and 1 g L⁻¹.

Direct contact toxicity was determined at the Southern Crop Protection and Food Research Centre (SCPFRC), Agriculture and Agri-Food Canada (AAFC) in London, ON. Prior to pesticide application, adult worker bees were aspirated into 1 L Mason jars and each jar was randomly assigned to a treatment. Bumble bees were anesthetized with carbon dioxide for 6–7 s and then were placed dorsal side up in a glass 10 cm Petri dish bottom containing a piece of 9 cm filter paper. Dishes were placed in the PST and 5 mL of the corresponding treatment were applied. Controls were treated with acetone and olive oil only. Four replications of 9–11 bumble bees were performed at each concentration.

Following treatment, the bees were transferred to waxed paper Dixie[®] cups (8.5 × 5 cm) and were covered with a glass Petri dish lid. Two plastic flower picks, one filled with water and the other with 50% sugar solution, were plugged with cotton dental wick and placed in the bottom of each cup. Post-treatment containers were maintained in the dark at 25 ± 1 °C and 35% RH. Mortality was assessed at 72 h for the insecticides and 48 h for the fungicides. Insecticide-treated bees were checked at 72 h as abamectin¹³ and metaflumizone¹⁴ both cause insect paralysis, followed by feeding cessation and eventually death. These insecticides are therefore considered slower acting. Bumble bees that failed to move when probed were considered dead.

2.4 Sub-lethal toxicity

Formulated pesticides were mixed at the recommended rate (RR) for greenhouse use and included imidacloprid, abamectin, metaflumizone, chlorantraniliprole, myclobutanil, potassium bicarbonate and cyprodinil + fludioxonil. If a range of rates was presented on the product label, the middle rate was tested as RR. The concentration (mg L⁻¹) of each pesticide in spray solution at RR was determined as in the following example: at a standard spray volume of 1000 L ha⁻¹, the concentration of abamectin in spray solutions at the RR of 5.7 g ha⁻¹ is: 5.7 g ha⁻¹ × 1 ha 1000 L⁻¹ × 1 L 1000 mL⁻¹ × 1 L 1000 g⁻¹ × 1 000 000 mg L⁻¹ × 19 g Al L⁻¹ = 0.108 mg L⁻¹.¹⁵ A standard spray volume of 1000 L ha⁻¹ was used for all calculations. The RR of each product and calculated concentration of pesticide in the spray solution at RR were: imidacloprid 267 g ha⁻¹ or 160 mg L⁻¹; abamectin 5.7 g ha⁻¹ or 0.108 mg L⁻¹; metaflumizone 288 g ha⁻¹ or 69 mg L⁻¹; chlorantraniliprole 25 g ha⁻¹ or 9 mg L⁻¹; myclobutanil 340 g ha⁻¹ or 136 mg L⁻¹; potassium bicarbonate 560 g ha⁻¹ or 476 mg L⁻¹; cyprodinil + fludioxonil 833 g ha⁻¹ or 521 mg L⁻¹. Pesticides were dissolved in water to create stock dispersions of 1000 mg L⁻¹; dilutions were subsequently made to obtain the desired concentrations.

A paste was created by mixing pollen, honey and pesticide dispersion together in a ratio of 5 : 1 : 1. Concentrations (mg Al g⁻¹ pollen) of each pesticide were: imidacloprid 0.0192, abamectin 3.8 × 10⁻⁶, metaflumizone 3.32 × 10⁻³, chlorantraniliprole 6.15 × 10⁻⁴, myclobutanil 0.011, potassium bicarbonate 0.081, and cyprodinil + fludioxonil 0.065. Pollen for control colonies was mixed with honey and water in a ratio of 5 : 1 : 1. Balls were formed from this paste and coated with melted beeswax to maintain their integrity. Each micro-colony was initially provided with a 2 g ball contaminated with one of the eight treatments on which they started their brood. This ball remained in each colony for the duration of the experiment. Two days later the colony received a supplemental 1 g pollen ball mixed with the same pesticide or control treatment as the larger ball. This ball was weighed and replaced with a fresh ball twice weekly for the entire experiment. Treated pollen was provided for 30 d; colonies were then maintained on untreated pollen balls for an additional 30 d.

Each micro-colony was housed in a 473 mL clear plastic container (11 × 8 cm; Plastipak Packaging, La Prairie, Quebec (QC), Canada) with the bottom cut out and replaced with a piece of craft netting (35 × 35 cm) secured with a rubber band. A beeswax-coated plastic dish to hold the pollen balls and a small piece of small animal nesting material (Riga Pet Supplies, Toronto, ON) were placed in the bottom of each micro-colony. The plastic containers were then placed into 946 mL waxed paper cups (11 × 15 cm; Solo® Canada; Toronto, Ontario (ON), Canada). A feeder containing cotton dental wick soaked in a 60% honey (University of Guelph Apiaries, Guelph, ON) 40% water solution was placed in the bottom of each paper cup. The wick sat just beneath the mesh and provided the bees with honey solution *ad libitum*. Three callow workers were randomly selected from one of six commercial colonies, marked with a different coloured paint dot on the thorax (Elmer's Painters® Medium Opaque Paint Marker; Elmer's Products Inc, Columbus, OH), weighed, and placed in the micro-colony. All remaining workers in the commercial colony were marked with a white paint dot to distinguish newly emerged bees. Once isolated, one worker became dominant and began ovipositing; the other two assisted in rearing the brood.

Paper cups and feeders were replaced three times per week. The bees, their brood, and pollen were transferred to a new plastic container when fecal contamination occurred, *ca.* every 10 d. Micro-colonies were maintained at $25 \pm 1^\circ\text{C}$, 50–70% RH, and 16:8 h light: dark photoperiod.

Bees were checked daily and dates of first oviposition were recorded. Dead workers were removed and their final weight and date of death were recorded. Ejected larvae were counted and removed. To determine the amount of supplemental pollen consumed, pollen balls were weighed before and after being placed in the micro-colony. At the end of the experiment, any remaining pollen from the initial ball was weighed; if none remained, the entire 2 g was considered to have been consumed.

Initially, 10 micro-colonies were established for each pesticide treatment and 20 for the controls. In some cases, entire micro-colonies perished before pollen could be consumed, micro-colonies failed to initiate oviposition and thus did not produce larvae, or workers escaped. These colonies or workers were not included in analysis. Therefore, for pesticide treatments sample size varied between 7 and 10 for pollen consumption, date of first oviposition and larval ejection, and between 26 and 30 for individual worker lifespan. For control colonies, sample size ranged from 19 to 20 for pollen consumption, date of first oviposition, and larval ejection and equaled 60 for individual worker lifespan.

2.5 Data analysis

2.5.1 Direct contact toxicity

Control mortality did not exceed 10% and corrections for natural mortality were made using Abbott's formula.¹⁶ Insecticide data were subjected to an analysis of variance using PROC GLM in SAS v. 9.1¹⁷ and means were separated using Tukey's multiple means comparison. Prior to analysis, data were arcsine transformed to better meet the assumptions of variance. Pesticides were classified as harmless (<30% mortality), slightly harmful (30–79%), moderately harmful (80–99%), or harmful (>99%) according to standards of the International Organization of Biological Control for laboratory studies.¹⁸ Fungicide data were almost entirely null and did not conform to the assumptions of any statistical test, thus data were not subjected to analyses. Tests were performed at a significance level of $\alpha = 0.05$.

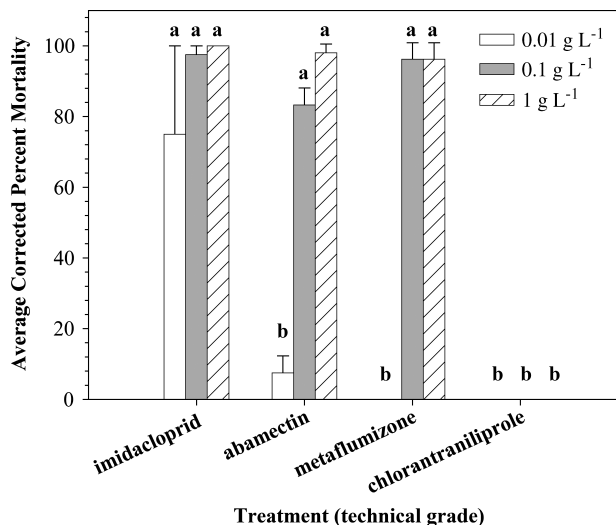


Figure 1. Average corrected percentage mortality of *Bombus impatiens* workers 72 h following exposure to technical grade imidacloprid, abamectin, metaflumizone or chlorantraniliprole using a Potter spray tower. Data were arcsine square root transformed prior to analysis; back transformed means are shown. Columns with the same letter are not significantly different (Tukey's test, $\alpha = 0.05$).

2.5.2 Sub-lethal toxicity

Data were log transformed prior to analysis and number of days to first oviposition, number of ejected larvae and total pollen consumption data were subjected to an analysis of variance in PROC MIXED.¹⁷ Worker lifespan data failed to meet the assumptions of a parametric test and therefore a non-parametric Kruskal–Wallis test was performed using PROC NPAR1WAY to determine differences between means. Tests were performed at a significance level of $\alpha = 0.05$.

3 RESULTS

3.1 Direct contact toxicity

Following direct application, technical grade imidacloprid was moderately harmful to harmful at all concentrations, causing up to 100% mortality after 72 h ($F = 25.94$; $df = 3, 19$; $P < 0.001$; Fig. 1). Abamectin ($P < 0.001$) and metaflumizone ($P < 0.001$) were moderately harmful at 0.1 and 1 g L^{-1} , whereas chlorantraniliprole was harmless at all concentrations (Fig. 1). The three technical grade fungicides myclobutanil, cyprodinil and fludioxonil were harmless (<5% mortality) at all concentrations.

3.2 Sub-lethal toxicity

Worker bees provided with imidacloprid-contaminated pollen had significantly shorter life spans than all other treatments ($P^2 = 146.89$; $df = 7$; $P < 0.001$; Fig. 2) and consumed significantly less pollen ($F = 8.05$; $df = 7, 59$; $P < 0.001$; Fig. 2). Those provided with abamectin-contaminated pollen had significantly shorter lifespans than colonies treated with metaflumizone ($P = 0.0402$) and cyprodinil + fludioxonil ($P = 0.0402$; Fig. 2) and consumed significantly less pollen than untreated micro-colonies ($P = 0.011$) and metaflumizone ($P = 0.0148$) and myclobutanil-treated ($P = 0.0016$) colonies (Fig. 2). Worker bees provided with chlorantraniliprole-contaminated pollen consumed significantly less pollen than worker bees provided with myclobutanil-contaminated pollen ($P = 0.0238$).

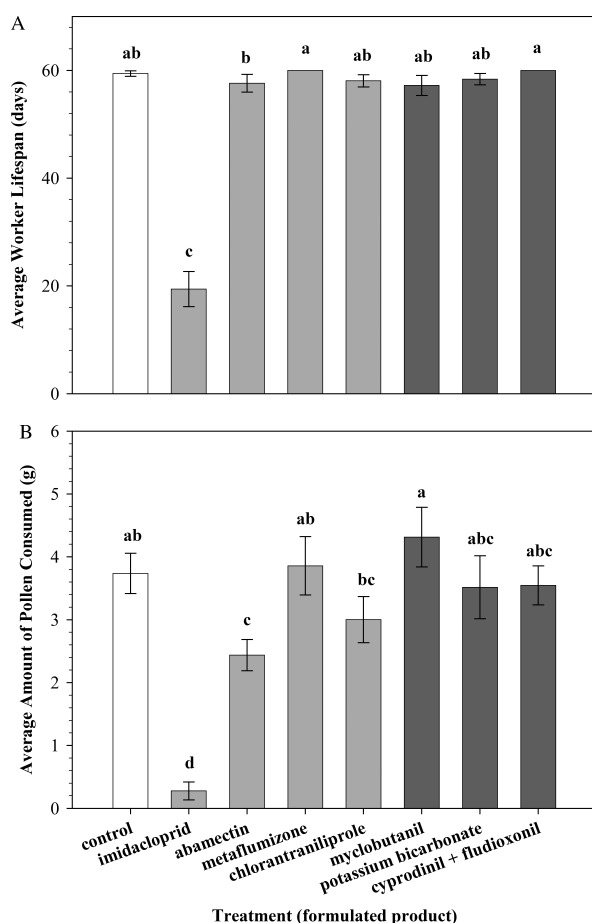


Figure 2. (A) Average lifespan (d) of and (B) average total amount (g) of pollen consumed by *Bombus impatiens* micro-colony workers. Queen-less micro-colonies received pollen contaminated with formulated imidacloprid, abamectin, metaflumizone, chlorantraniliprole, myclobutanil, potassium bicarbonate or cyprodinil + fludioxonil. Control colonies were provided with pollen mixed with water and honey. Columns with the same letter are not significantly different ($\alpha = 0.05$).

(Fig. 2). Micro-colonies provided with imidacloprid-contaminated pollen did not initiate oviposition and therefore did not produce any larvae. Worker bees given abamectin-contaminated pollen initiated oviposition significantly later than worker bees provided with untreated pollen ($P = 0.0095$) or pollen contaminated with metaflumizone ($P = 0.0073$), potassium bicarbonate ($P = 0.0186$), or cyprodinil + fludioxonil ($P = 0.0257$; Fig. 3). There were no significant differences in the number of days to first oviposition among all other treatments ($F = 2.05$; $df = 6, 51$; $P = 0.0759$; Fig. 3). Micro-colonies treated with myclobutanil ejected significantly more larvae than the controls ($P = 0.0244$) and those contaminated with abamectin ($P = 0.0379$) and metaflumizone ($P = 0.015$; Fig. 3). Micro-colonies treated with potassium bicarbonate ejected significantly more larvae than colonies treated with metaflumizone ($P = 0.044$). There were no significant differences in numbers of ejected larvae among all other treatments ($F = 1.87$; $df = 6, 53$; $P = 0.1037$; Fig. 3).

4 DISCUSSION

The toxicity of many pesticides depends on their route of exposure.¹⁹ The queen-less micro-colony experimental design

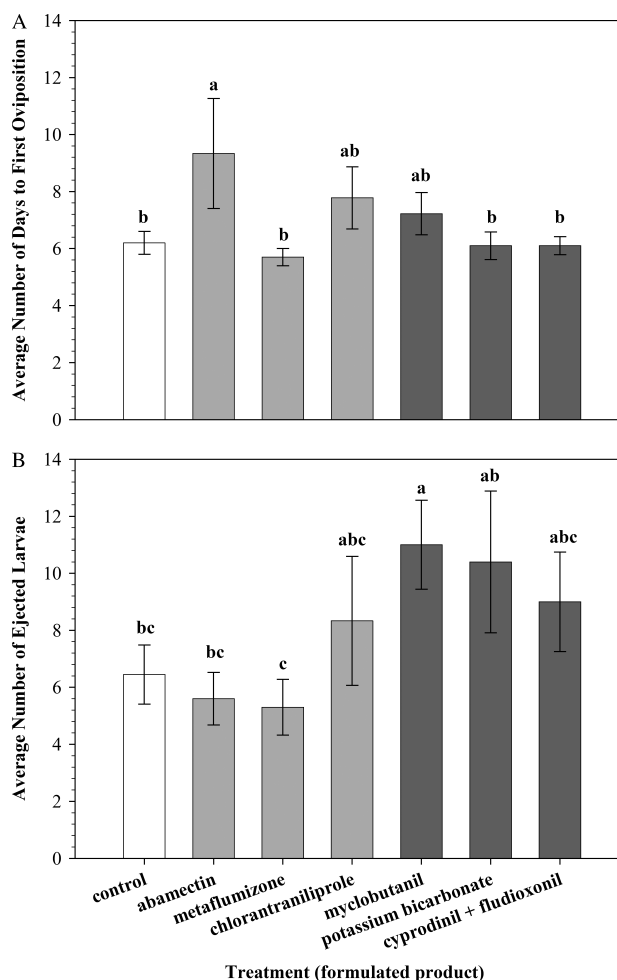


Figure 3. (A) Average number of days to first oviposition and (B) average number of larvae ejected by *Bombus impatiens* workers from micro-colonies provided with pollen contaminated with formulated abamectin, metaflumizone, chlorantraniliprole, myclobutanil, potassium bicarbonate or cyprodinil + fludioxonil. Control colonies were provided with pollen mixed with water and honey. Micro-colonies treated with imidacloprid did not initiate oviposition. Columns with the same letter are not significantly different ($\alpha = 0.05$).

is particularly useful for studying the oral toxicity of pesticide-contaminated pollen on bumble bee vitality and brood production, as it allows accurate comparison between small, easily handled, standardized colonies.¹¹ Additionally, the use of micro-colonies is more cost effective than purchasing and treating large numbers of commercial colonies. This means that the number of replications, and therefore statistical power, can be greatly increased. Other studies have successfully used micro-colonies to determine the effect of pesticides on *B. terrestris*.^{11,20} Our study is the first to use micro-colonies of *B. impatiens* and, using this method, we successfully determined the effect of some pesticides used or with promise for use in greenhouse vegetable production on worker lifespan, pollen consumption and some aspects of reproduction.

In our study, imidacloprid was harmful, causing acute worker mortality following direct contact or oral exposure. Abamectin also was lethal when applied directly to adult workers and caused some sub-lethal effects. Incerti *et al.*,⁶ Marletto *et al.*⁷ and Scott-Dupree *et al.*²¹ reported that imidacloprid caused mortality of bumble bee (*B. terrestris* or *B. impatiens*) workers following direct contact. In other studies, imidacloprid was reported to cause sub-lethal

changes in bumble bees including trembling,⁶ reduced brood production and vitality,^{8,11} and impaired foraging ability.²² To avoid contact with bumble bees, imidacloprid is typically applied in greenhouses late in the season when pollination is no longer required. Marletto *et al.*⁷ found that abamectin was topically and orally toxic to *B. terrestris*. In our study, metaflumizone was lethal by direct contact at high concentrations; however, no sub-lethal effects were observed. Chlorantraniliprole, however, was harmless in both experiments. Currently, there is no published literature on the impact of either metaflumizone or chlorantraniliprole on bumble bees.

There were no observable negative effects on bumble bees following exposure to fungicides. Similarly, Malone *et al.*²⁰ reported that captan had no negative impact on *B. terrestris* worker survival, pollen consumption, larval ejection, oviposition or male bee production. Interestingly, micro-colonies in our study provided with pollen contaminated with myclobutanil ejected more larvae than some other treatments, including control colonies. In general, as bumble bee brood size increases, workers are motivated to remove more larvae to provide the remaining individuals with adequate resources,¹¹ which suggests that myclobutanil stimulated brood production. However, bumble bee larval ejection rates are highly inconsistent and naturally vary between 0% and 100%.¹¹ Additional study is required to determine if myclobutanil has a consistent hormetic effect on brood size.

Our results suggest that imidacloprid and abamectin have the potential to severely impact bumble bee colony health and reproduction, and therefore greenhouse pollination. In contrast, the new, reduced-risk insecticides metaflumizone and chlorantraniliprole could be safer alternatives for greenhouse insect pest management. Finally, myclobutanil, potassium bicarbonate and cyprodinil + fludioxonil had no impact on colony health or reproduction and are safe to apply for greenhouse powdery mildew management in the presence of bumble bees. The queen-less micro-colony design is an accurate and valuable bioassay design for determining the sub-lethal effects of pesticides on bumble bees, and further identification of pesticides with minimal impact on bumble bees will allow growers to modify their management practices to conserve greenhouse vegetable pollination programs.

ACKNOWLEDGEMENTS

We thank Dale McArthur and Dr Ian Scott from SCPFRC-AAFC, London, ON, and Shannon Burnett, Caleigh Irwin and Ida Podrug for guidance and assistance conducting the bioassays. Funding for this project was provided by a Canada Ontario Research and Development (CORD) IV grant administered by the Agricultural Adaptation Council and provided to the Ontario Greenhouse Vegetable Growers and a grant from the Minor Use Research Initiative, Pest Management Centre, AAFC.

REFERENCES

- 1 van Ravestijn W and van der Sande J, Use of bumblebees for the pollination of glasshouse tomatoes. Sixth International Symposium on Pollination. *Acta Hort* **288**:204–209 (1991).
- 2 Shipp JL, Whitfield GH and Papadopoulos AP, Effectiveness of the bumble bee, *Bombus impatiens* Cr. (Hymenoptera: Apidae), as a pollinator of greenhouse sweet pepper. *Sci Hortic* **57**:29–39 (1994).
- 3 Ontario Ministry of Agriculture, Food and Rural Affairs. *Growing Greenhouse Vegetables*. Publication 371 (2005).
- 4 Thompson HM, Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). *Apidologie* **32**:305–321 (2001).
- 5 Thompson HM and Hunt LV, Extrapolating from honeybees to bumblebees in pesticide risk assessment. *Ecotoxicology* **8**:147–166 (1999).
- 6 Incerti F, Bortolotti L, Porrini C, Micciarelli Sbrenna A and Sbrenna G, An extended laboratory test to evaluate the effects of pesticides on bumblebees. Preliminary results. *Bull Insectol* **56**:156–164 (2003).
- 7 Marletto F, Patetta A and Manino A, Laboratory assessment of pesticide toxicity to bumblebees. *Bull Insectol* **56**:155–158 (2003).
- 8 Gels JA, Held DW and Potter DA, Hazards of insecticides to the bumble bees *Bombus impatiens* (Hymenoptera: Apidae) foraging on flowering white clover in turf. *J Econ Entomol* **95**:722–728 (2002).
- 9 Thompson HM and Barrett KA, Assessing the effects of glasshouse application of a novel insect growth regulator on bumble bee colonies, in *Hazards of Pesticides to Bees: Proceedings of the 7th ICPBR Bee Protection Symposium*: Avignon, France. L'institute National de la Recherche Agronomique (INRA), Paris, France, pp. 227–228 (1999).
- 10 Morandin LA, Winston ML, Franklin MT and Abbott VA, Lethal and sub-lethal effects of spinosad on bumble bees (*Bombus impatiens* Cresson). *Pest Manag Sci* **61**:619–626 (2005).
- 11 Tasei JN, Lerin J and Ripault G, Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. *Pest Manag Sci* **56**:784–788 (2000).
- 12 Potter C, 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 (1952).
- 13 Tomlin CDS (ed.), *The Pesticide Manual Twelfth Edition*, British Crop Protection Council, Farnham, Surrey, pp. 3–4 (2000).
- 14 BASF Agricultural Products, Metaflumizone Worldwide Technical Brochure. (2006).
- 15 Cutler GC. Potential utility of novaluron in Colorado potato beetle, *Leptinotarsa decemlineata* (Say), management. University of Guelph, Guelph, ON (2006).
- 16 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
- 17 SAS Institute. *PROC Users Manual*. Cary, NC (2005).
- 18 Sterk G, Hassan SA, Baillod M, Bakker F, Bigler F, Blumel S, *et al*, Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS working group 'Pesticides and Beneficial Organisms'. *BioControl* **44**:99–117 (1999).
- 19 Stark JD, Jepson PC and Mayer DF, Limitations to use of topical toxicity data for predictions of pesticide side effects in the field. *J Econ Entomol* **88**:1081–1088 (1995).
- 20 Malone LA, Scott-Dupree CD, Todd JH and Ramankutty P, No sub-lethal toxicity to bumblebees, *Bombus terrestris*, exposed to Bt-corn pollen, captan and novaluron. *NZ J Crop Hortic Sci* **35**:435–439 (2007).
- 21 Scott-Dupree CD, Conroy L and Harris CR, Impact of currently used or potentially useful insecticides for canola agro-ecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymenoptera: Megachilidae) and *Osmia lignaria* (Hymenoptera: Megachilidae). *J Econ Entomol* **102**:177–182 (2008).
- 22 Morandin LA and Winston ML, Effects of novel pesticides on bumble bee (Hymenoptera: Apidae) colony and foraging ability. *Environ Entomol* **32**:555–563 (2003).