4.5 Including Bombus impatiens in the mix: Developing semi-field pesticide risk assessment methodology for the North American surrogate bumble bee

Cynthia Scott-Dupree[1], Angela E. Gradish, Tara Celetti, Chris Cutler

[1]School of Environmental Sciences, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada N1G 2W1

DOI 10.5073/jka.2018.462.044

Introduction

While standardized, tiered pesticide risk assessment protocols exist for honey bees, these protocols cannot be used for bumble bees (*Bombus* spp.) because of pronounced differences in their life history and behaviour (Thompson and Hunt 1999; Devillers et al. 2003; Scott-Dupree, Conroy and Harris 2009). To incorporate bumble bees into the regulatory process, it is imperative that risk assessment protocols be developed and validated specifically for these bees (Cabrera et al. 2016). We conducted a series of studies over 3 years aimed at contributing to the development of a semi-field (Tier II) method for assessing the risk of pesticides to *Bombus impatiens* Cresson, the species that will likely serve as a surrogate for bumble bee pesticide risk assessments in North America.

The objectives of this research were to:

1. Characterize *B. impatiens* colony development and foraging activity on flowering red clover (*Trifolium pratense*), purple tansy (*Phacelia tanacetifolia*), and buckwheat (*Fagopyrum esculentum*) to identify a potential surrogate plant that will adequately sustain colonies during semi-field trials; and
2. Characterize the impact of potential toxic insecticide reference standards –dimethoate and diflubenzuron – to *B. impatiens* colonies in semi-field trials.

Surrogate Plant Study

Methods

The study was conducted in a 6 ha field near Tillsonburg, ON. Three potential surrogate plants were investigated: buckwheat (*Fagopyrum esculentum*, var. common), red clover (*Trifolium pratense*) and purple tansy (*Phacelia tanacetifolia*). All previously identified as being attractive to bumble bees (Williams 1997; Carreck and Williams 2002; Pontin et al. 2006; Bartomeus et al. 2014). Between May (red clover) and June (purple tansy and buckwheat), 2 ha of each plant type were broadcast seeded at the highest rate recommended for sandy soil. Once a plant type was at 2nd to 4th leaf stage, 10 plots (3.5 m^2) were delineated (n= 30 plots). Plots were established at least 2 m apart in areas of the field where plants were evenly distributed, of similar density, and visibly healthy.

Bombus impatiens colonies (Biobest Biological Systems Ltd.) were placed in the field for each plant type once the plants had reached approximately 20-25% bloom by visual estimate. Upon arrival, colonies were visually inspected and weighed. One colony was then placed on a wooden stand consisting of a plywood platform (30 x 35 cm) attached to a 5 cm^2 stake in the centre of each plot with the platform approximately 10 cm above the plant canopy. A screened enclosure (3.4 x 3.4 x 2.3 m, Instant Screen House®, Coleman Canada Inc.) was then placed over each plot.

Colonies remained on the plots for 16 days, which coincided with the predicted flowering period for red clover. In the morning 3 times per week, the number of workers entering or exiting the colony for 10 min was recorded. These assessments were repeated 1 – 2 h later. Therefore, in total there were 140 bouts of foraging activity assessments for each plant type (10 plots x 7 observation days x 2 observation periods per day).

After the 16 day field period, colonies were placed in a growth cabinet at the University of Guelph and maintained in the dark at 25°C, 20-30% RH and provided with honey bee-collected pollen three times per week and nectar substitute *ad lib*. Each colony was placed in a freezer 2 weeks after the first emergence of a queen (alternatively, if a colony did not contain newly emerged

queens or queen pupae 8 weeks after it was put in the growth cabinet, it was frozen), and subsequently dissected to assess colony development by counting the number of individual live eggs, larvae, pupae (queen and worker/male pupae were differentiated), and adults (queens, workers, and males). Additionally, adult workers, males, and new queens were weighed.

Data Analysis

In the red clover colonies, 3 of 10 queens died either in the field or lab portion of the study (n=9 for foraging activity analyses; n=7 for colony development analyses). All colonies from the buckwheat and purple tansy plots retained viable queens and were included in the analyses (n=10 for each). A linear mixed model was used to analyse data on foraging activity, colony weight, and adult worker, male, and queen weight. Means were separated using Tukey's tests. Data on the number of immature and adult individuals per colony were analyzed using non-parametric Kruskal-Wallis tests. If a Kruskal-Wallis test was significant, a Wilcoxon rank sum test was performed to determine differences between means. All analyses were performed at a significance level of α=0.05.

Results

Among the three plant types, different patterns in foraging activity was observed over time. On buckwheat plots, the number of foragers entering or exiting the colony increased over time. In contrast, foraging activity on purple tansy increased until observation day 5 and then decreased. After a small initial increase, foraging activity on red clover plots plateaued. Overall, foraging activity was significantly higher on buckwheat plots ($F = 89.7$; $df = 2, 402$; $P < 0.0001$; Fig.1). Colonies regardless of plant type initially lost weight on average and then stabilized until transfer to the lab (Fig. 2). After transfer to the lab, colonies gained weight on average for the remainder of the study (Fig. 2). During the field portion of the study, colonies on red clover plots lost weight more quickly on average than colonies on buckwheat or purple tansy plots, while during the lab portion of the study, colonies from purple tansy plots gained weight more quickly on average than those from buckwheat or red clover plots (Fig. 2). Plant type had no effect on the number of immature stages or adults observed per colony during dissections with one exception: Colonies from purple tansy plots contained significantly more adult workers compared with colonies from buckwheat ($w = 82.5$; $P = 0.0155$) or red clover ($w = 70.0$; $P = 0.0001$) plots (Table 1).

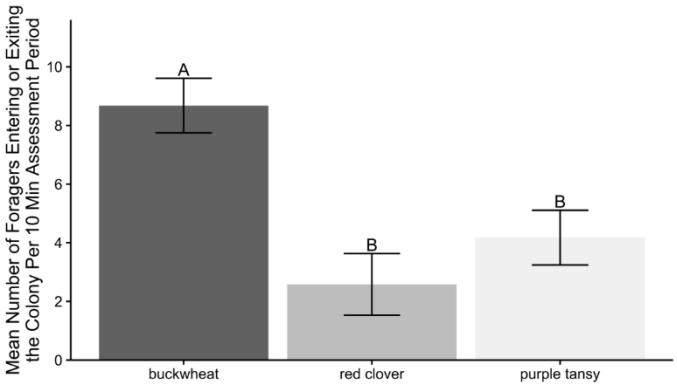

Figure 1. Mean (±SE) number of *Bombus impatiens* workers entering or exiting colonies confined to flowering buckwheat (n = 10), red clover (n = 9), or purple tansy (n = 10) per assessment period. Two assessments were performed on seven separate observation days.

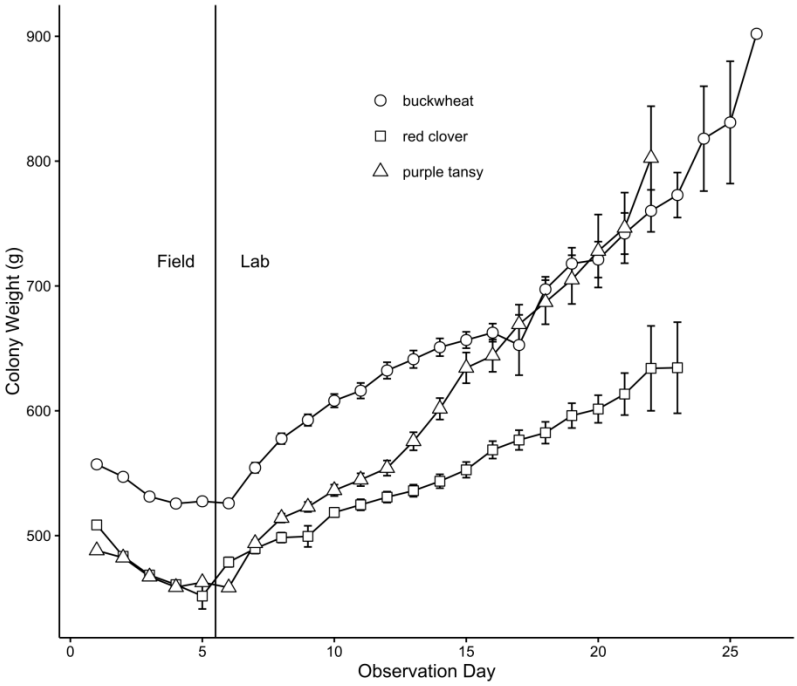

Figure 2 Mean weight (g) (±SE) of *Bombus impatiens* colonies by observation day. Colonies were initially confined to flowering field plots of buckwheat (n = 10), red clover (n = 9), or purple tansy (n = 10). After 16 days, which included 7 observation days, colonies were brought to the lab and maintained in a growth cabinet until 2 weeks after the first emergence of a new queen.

Table 1 Mean (±SE) number of immature stages (eggs, larvae, and pupae) and adult workers, males, and queens in *Bombus impatiens* colonies that were restricted to foraging on flowering buckwheat (n=10), red clover (n=7), or purple tansy (n=10) for 16 days at the beginning of their colony cycle. Colonies were then maintained in a growth cabinet until 2 weeks after the first emergence of a new queen and then dissected. Means within columns with the same letter are not significantly different at α=0.05.

	Mean (±SE) Number of Immature Stages and Adults per Colony						
Plant Type	Eggs	Larvae	Queen Pupae	Male or Worker Pupae	Adult Workers	Adult Males	Adult Queens
Buckwheat	29±5.8a	93±12.6a	3±2.1a	36±4.9a	56±12.0b	28±9.9a	9±2.5a
Red Clover	29±7.1a	60±18.1a	1±0.72a	27±8.0a	28±5.0b	41±13.1a	9±4.1a
Purple Tansy	29±3.4a	98±16.2a	4±1.9a	35±6.9a	108±15.7a	33±16.7a	11±2.5a

Discussion and Recommendation

Bombus impatiens foraging activity differed with plant type: the number of foragers entering or exiting colonies were at least 2x higher on buckwheat plots compared with colonies on purple tansy and red clover. This was surprising, as all three plants are known forage plants for bumble bees and other bees. Buckwheat plants outside of the screened enclosures were continuously heavily visited not only by wild bumble bees, but also honey bees, carpenter bees and various species of solitary bees. However, consistent with the low foraging rates we observed on red clover plots, we observed a lack of bee visitation, in terms of both numbers of individuals and species, to red clover plants outside of the screened enclosures.

All colonies from buckwheat and purple tansy survived for the duration of our study and appeared to develop normally. However, the foundress queen in three colonies from red clover plots died.

The reason(s) for these queen deaths are unclear. Among colonies with surviving foundress queens, the number of individuals per colony was similar on all three plant types. In particular, the number of queens produced per colony, a critical endpoint recommended for assessing the impacts of pesticides on bumble bee colonies, did not differ between plant types. However, two purple tansy colonies did not produce any new queens. At the time of dissection, these two colonies also contained an abundance of workers (181 and 189) compared to all other colonies in our study. These two colonies also inflated the mean number of workers per colony we observed for purple tansy (Table 2). Corresponding to the number of individuals per colony, we did not observe a difference in colony weight due to plant type. Interestingly, all colonies, regardless of plant type, initially lost weight during the field portion of our study (Figure 9). During this time, we did not observe a concurrent loss of workers or brood, and thus weight loss did not seem to reflect a decline in colony health. However, colony weight loss generally corresponded with an increase in foraging activity (Figures 5 and 6), and thus part of the loss likely can be attributed to the foragers that were absent from the colony during weighing. The remaining weight may have been lost as colonies initially or continually consumed stored honey and pollen to compensate for a lack of incoming food resources.

In conclusion, our results suggest that buckwheat, red clover, and purple tansy are not equally appropriate as surrogate plants in semi-field studies using small screened enclosures with *B. impatiens*. Therefore, to ensure forager pesticide exposure, adequate colony development, and favourable plant growth, we recommend buckwheat as an optimal surrogate plant for use in semi-field pesticide toxicity assessments with *B. impatiens* (Gradish et al. 2016)

Toxic Insecticide Reference Standards Study

Methods

These studies were conducted in 2016 and 2017 at the same site used for the surrogate crop study, and the entire field was broadcast seeded with buckwheat at a rate of 23 kg seed/ha. In 2016, plots were sprayed with dimethoate (Lagon® 480E; 400 g a.i./ha) or diflubenzuron (Dimilin® 25%WP; 257 g a.i./ha). The application rate of 400 g a.i./ha for dimethoate was chosen because it had been used on *Bombus terrestris* with no indication of acute toxicity. Insecticides were mixed with water and applied at a spray volume of 0.5 L/plot (approximately 1300 L/ha). Control plots were treated with water only.

Plots were set up in the same way as for the surrogate plant study. Treatments were applied to plots once plants had reached 90-95% bloom (6 days after colonies were placed in the enclosures). On the day of application, the entrance/exit on each colony was closed approximately 30 min before dawn. Approximately 1 h later, colonies and enclosures were removed from all plots. Each plot was then sprayed with its corresponding treatment using a CO_2 powered backpack sprayer fitted with a four nozzle (TeeJet® VisiFlo Flat Spray 800 2VS), 2 m handheld boom, at a pressure of 60 psi. Enclosures and colonies were placed back on the plots once plants had dried completely (approximately 30 min after application).

All colonies on dimethoate-treated plots were dead 24 h after treatment. None of the colonies on the diflubenzuron-treated plots were negatively affected, despite being exposed to the highest label rate. Therefore, for the 2017 study we decided to focus on determining lower dimethoate rates that were not acutely toxic to *B. impatiens* colonies.

In 2017, we set the study up again using the same methods as in 2016. Insecticide-treated plots were sprayed with dimethoate (Lagon® 480E) at 40, 80, or 200 g a.i/ha, abbreviated hereafter as D40, D80, and D200, respectively. Control plots were sprayed with water only. Treatments were applied to plots once plants had reached 90-95% bloom, 7 days after colonies were placed in the enclosures.

Foraging activity increased over the first two observation days after the colonies were placed on the plots and was similar among all treatments. However, the foundress queen and all workers

from one D40, eight D80, and eight D200 colonies were dead 24 h after treatment. The following day, another five colonies from D40-treated plots had died, and by 72 h after treatment, all remaining colonies from all dimethoate-treated plots were dead. Although a few dead workers were observed on the ground, most workers and all foundress queens were found dead inside the colonies. In contrast, all control colonies survived until the end of the study.

Our 2017 results indicate the rates of dimethoate tested in our study are not suitable as reference standards for use in semi-field pesticide risk assessments with *B. impatiens*. The results from our two studies with dimethoate indicate an important potential species difference between *B. impatiens* and *B. terrestris*. In a semi-field study conducted by Bayer in Monheim, dimethoate was applied in tunnels at 400 g a.i./ha while *B. terrestris* colonies were actively foraging, and exposed colonies only experienced a reduction in workers and larvae. Furthermore, the current ICpPR ring test protocol for higher tier tests with *B. terrestris* indicates that 800 g a.i./ha of dimethoate be applied. In contrast, following exposure to dried residues of dimethoate at 400 g a.i./ha and rates up to an order of magnitude lower, the *B. impatiens* colonies in our 2016 and 2017 studies died. This indicates that *B. impatiens* is much more susceptible than *B. terrestris* to dimethoate and highlights the need for species-specific risk assessment protocol development and validation for bumble bees.

References

Bartomeus, I., S.G. Potts, I. Steffan-Dewenter, B.E. Vassiere, M. Woyciechowski, K.M. Krewenka, Tscheulin, S.P.M. Roberts, H. Szentgyorgyi, C. Westphal and R. Bammarco. 2014. Contribution of insect pollinators to crop yield and quality varies with agricultural instensification. PeerJ, DOI 10.7717/peerj.328.

Cabrera, A.R., M.T. Almanza, G.C. Cutler, D.L. Fischer, S. Hinarejos, G.Lewis, D. Nigro, A. Olmstead, J. Overmyer, D.A. Potter, N.E. Raine, C. Stanley-Stahr, H. Thompson and J. Der Steen. 2016. Initial recommendations for higher-tier risk assessment protocols for bumble bees, *Bombus* spp. Integrated Environmental Assessment and Management 12:222-229.

Carreck, N.L. and I.H. Williams 2002. Food for insect pollinators on farmland: insect visits to flowers of annual seed mixtures. Journal of Insect Conservation 6:13-23.

Devillers, J., A. Decourtye, H. Budzinski, M.H. Pham-Delegue, S. Cluzeau and G. Maurin. 2003. Comparative toxicity and hazards of pesticides to *Apis* and non-*Apis* bees. A chemometrical study. Sar and Qsar in Environmental Research 14:389-403.

Gradish, A.E., G.C. Cutler, A. J. Frewin and C.D. Scott-Dupree. 2016. Comparison of buckwheat, red clover, and purple tansy as surrogate plants for use in semi-field pesticide risk assessment with *Bombus impatiens*. PeerJ, DOI 10.77 17/peerj.2228.

Pontin, D.r., M.R. Wade, P. Kehrli and S.D. Wratten. 2006. Attractiveness of single and multiple species flower patches to beneficial insects in agroecosystems. Annals of Applied Biology. 148:39-47.

Scott-Dupree, C.D., L. Conroy and C.R. Harris. 2009. Impact of currently used potentially useful insecticides for canola agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae); *Megachile rotundata* (Hymenoptera: Megachilidae) and *Osmia lignaria* (Hymentoptera: Megachilidae). Journal of economic Entomology 102:177-182.

Thompson, H.M. and L.V. Hunt. 1999. Extrapolating from honeybees to bumblebees in pesticide risk assessment. Ecotoxicology 8:147-166.

Williams, C.S. 1997. Nectar secretion rates, standing crops and flower choice by bees on *Phacelia tanacetifolia*. Journal of Apicultural research 36:23-32.