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# Effects of Clothianidin on *Bombus impatiens* (Hymenoptera: Apidae) Colony Health and Foraging Ability

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**ABSTRACT** We conducted laboratory experiments to investigate the lethal and sublethal effects of clothianidin on bumble bee, *Bombus impatiens* Cresson, colony health and foraging ability. Bumble bee colonies were exposed to 6 ppb clothianidin, representing the highest residue levels found in field studies on pollen, and a higher dose of 36 ppb clothianidin in pollen. Clothianidin did not effect pollen consumption, newly emerged worker weights, amount of brood or the number of workers, males, and queens at either dose. The foraging ability of worker bees tested on an artificial array of complex flowers also did not differ among treatments. These results suggest that clothianidin residues found in seed-treated canola and possibly other crops will not adversely affect the health of bumble bee colonies or the foraging ability of workers.

**KEY WORDS** *Bombus impatiens*, bumble bees, clothianidin, chloronicotinyl insecticide, seed dressing

SYSTEMIC INSECTICIDES SUCH AS chloronicotinyl compounds are distributed throughout plant tissues, providing cultivated crops with protection from both root and foliar pests (Mullins 1993, Tasei et al. 2000). Clothianidin, the newest member of the chloronicotinyl insecticide family, has recently been registered in Japan for foliar spray and seed treatment applications under the trade names Fullswing and Dantotsu, and registration is pending in North America and Europe under the trade names Poncho and Clutch (Jeschke et al. 2003). Clothianidin has a high activity against a broad range of insects, including sucking insects, chewing insects, and some lepidopterans (Jeschke et al. 2003).

Wild pollinators may be experiencing declines worldwide with unknown consequences for the yield of food crops. One factor contributing to pollinator declines is pesticides (Allen-Wardell et al. 1998), and clothianidin-treated crops could be hazardous to managed and wild bees that feed on pollen and nectar-containing residues. To date, only one study that includes two field trials has examined the effects of clothianidin seed-treated canola on honey bees, *Apis mellifera* L., and found no adverse effect on colony health at residue levels ranging from 0.9 to 3.7 ppb (Scott-Dupree, personal communication). However, no studies have investigated the lethal or sublethal effects of clothianidin residues on wild or other managed pollinators in the field or laboratory.

Exposure to clothianidin at sublethal levels may adversely affect the foraging ability of pollinators, because it is neurotoxic and therefore could cause coordination problems (Kiriyama and Nishimura 2002). The sublethal effects of a similar neonicotinoid, imidacloprid, have been tested on bumble bees and honey bees (Schmuck et al. 1999, 2001; Morandin and Winston 2003). *Bombus impatiens* Cresson workers exposed to imidacloprid in previous tests showed no effect at field residue levels, but at a dose 5 times higher than the highest residue levels, bees showed reduced foraging ability and trembling (Morandin and Winston 2003). In addition, Schmuck et al. (1999) found that imidacloprid reduced the ability of honey bees to recruit other foragers to nectar and pollen sources at doses >20 ppb. Although both clothianidin and imidacloprid are members of the chloronicotinyl family, clothianidin is chemically different, and its effects on bees are unknown.

The objective of our study was to investigate the lethal and sublethal effects of clothianidin on *B. impatiens* colony health and foraging ability. First, we examined the effects of clothianidin on brood development and colony size at levels similar to and higher than those found in the nectar and pollen of seed-treated crops. Second, we assessed the ability of foraging bees exposed to these levels to access artificial complex flowers. We hypothesized that the high clothianidin dose would adversely affect colony health and foraging ability of workers.

## Materials and Methods

On 16 May 2002, 24 *B. impatiens* colonies were received from Biobest Canada Ltd. (Leamington, ON, Canada). Each colony consisted of one queen and between 7 and 19 workers (first and second brood

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stage). Colonies were housed in cardboard boxes containing 20 by 28 by 18-cm plastic nesting boxes with several ventilation openings. Bottles of Biogluc (provided in colony boxes by Biobest Canada Ltd.) containing sugar water and preservative, located underneath the nest boxes, were fed to the bees ad libitum.

Technical grade clothianidin, C(*E*)-*N*-((2-chloro-5-thiazolyl)methyl)-*N*′ –methyl-*N*″-nitroguanidine, with a purity of 99.75%, was obtained from Bayer AG (Leverkusen, Germany). The 24 colonies were randomly assigned to the following three treatments: 1) control, 2) low clothianidin, and 3) high clothianidin. The control colonies were fed a mixture of pollen and sugar water, whereas the low and high clothianidin treatments had clothianidin concentrations of 6 and 36 ppb in the pollen/sugar water mixtures, respectively. The low-dose clothianidin represented a realistic level of active ingredient found in nectar and pollen after seed treatment of canola (Scott-Dupree, personal communication). The high-dose clothianidin was similar in concentration to the chloronicotinyl insecticide imidacloprid tested in previous colony health and foraging ability studies by Morandin and Winston (2003).

Pollen traps were used to collect pollen from honey bee colonies at Simon Fraser University (Burnaby, BC, Canada) during May and June 2002. Chalk brood and dead insects were cleaned from the collected pollen. After cleaning, the pollen was ground up with an electric food processor and frozen for future use. A clothianidin solution was prepared by performing a 10,000-fold dilution of clothianidin in distilled water. The solution was mixed for 8 h in the dark, and stored at 4°C in a refrigerator. Before pollen preparation, the clothianidin solution was stirred at room temperature for 2 h to ensure all clothianidin was dissolved. The 6 and 36 ppb clothianidin doses were added to 30% sucrose solutions made with distilled water and stirred for 5 min. The sucrose solutions were added to the mashed pollen in a 2:1 pollen to solution ratio and stirred for an additional 5 min. Pollen mixtures were stored frozen and samples were sent to Bayer AG (Monheim, Germany) for verification of treatment doses. The analytical results of the pollen samples confirmed that treatment doses were 5.8 ± 0.2 and 35 ± 0.7 ppb. Colonies were fed pollen mixtures biweekly ad libitum. During each feeding, old pollen was weighed and removed from the dishes and replaced with a preweighed amount of new pollen.

**Colony Health.** During the first week of the experiment, 10 workers, or all workers if there were fewer than 10, were placed in vials and weighed on an Ohaus Explorer electronic balance (Ohaus Company, Florham Park, NJ) to 0.01 g. In subsequent weeks, a maximum of three newly emerged workers, identified by their white coloration, were removed from each colony weekly, placed in vials, and weighed. It was not always possible to obtain three weight measurements, because the number of bees emerging each week was variable. The number of workers, brood (egg masses, larval masses, larval cells, and pupae), queens, and males were recorded weekly. In addition, weekly counts of the number of dead workers, queens, and males were conducted.

**Foraging Assay.** Twenty-one days after experiment initiation, all workers were marked with white Liquid Paper. These workers were excluded from foraging trials to ensure that all foraging tests were performed on workers that had been exposed to clothianidin throughout their entire development and were of similar age.

Foraging experiments began 2 July 2002, 48 d after the start of exposure. Colonies were connected to a 1.2 by 1.2 by 1-m mesh flight cages by a 3 by 3 by 32-cm mesh tunnel with two entrance gates. An artificial foraging array was set up inside each flight cage (Morandin and Winston 2003). The array was comprised of 30 artificial flowers made from 1.5-ml clear microtubes (Sarstedt, Newton, NC) set in a 60 by 60 by 5-cm styrofoam foundation covered with green cardboard. Artificial flowers were set 10 cm apart in rows. The rows were placed 5 cm apart and staggered, resulting in a distance of ≈7 cm between flowers.

First, workers were trained to forage on an array of simple artificial flowers made from microtubes with lids completely removed. The training process began by removing the nectar supply and connecting the colony to a flight cage. An array of 30 simple flowers filled with 30% sucrose solution was placed in the flight cage. Ten to 20 workers making repeated foraging trips were marked on their abdomen and thorax with distinct Liquid Paper color combinations. Once the foragers were marked, the flight cage entrance gates were closed and all bees were returned to the hive. The array of simple flowers was removed and replaced with an array of 17 complex flowers made from microtubes with lids folded over to create openings of ≈4 mm (Gegear and Laverty 1998). A 100-μl syringe and PB600 2-μl dispenser (Hamilton Company, Reno, NV) was used to add 2 μl of 30% sucrose solution to each of the complex flowers. The entrance gates were opened to allow one marked forager into the flight cage. The forager was videotaped for a minimum of 35 successful flower visits. A flower visit was considered to be successful when the bee's entire body was inside the flower, and the 2 μl of sucrose solution was consumed. Flowers were refilled with 2 μl of the 30% sucrose solution after each successful flower visit. After the bee had completed a minimum of 35 successful flower visits, it was returned to the hive. Each forager was only tested once on the complex array of flowers.

Foraging tests were conducted from 10 July 2002 to 6 August 2002. Foragers from two colonies were trained concurrently, because two flight cages were used throughout the experiment. Only three colonies from each treatment were used in the foraging trials because the life span of colonies was too short to continue testing additional colonies. Foraging trials were divided into three blocks, defined as the completion of foraging trials by bees from one colony of each of three treatments. The treatment order for each block was as follows: 36 ppb clothianidin, 6 ppb clothianidin, and control. New colonies were used in each block and the treatment order remained the

same. Foraging tests were performed on four to six bees from each colony. A total of 18, 15, and 11 bees were tested from the control, 6 ppb clothianidin, and 36 ppb clothianidin treatments, respectively. Each colony was trained and tested over a period of 5–8 d.

Access times for the 35 successful flower visits were recorded from foraging videos for each bee. Access time was defined as the time a forager spent in contact with flowers before making a successful flower visit. A stopwatch was used to measure access time to one hundredth of a second.

**Data Analysis.** Each colony represented one replicate for all data analyses. To compare weekly pollen consumption per bee, we performed a repeated measures analysis of variance (ANOVA) (JMP 2001) with treatment as the main effect and time as the repeated factor. Univariate ANOVA (PROC MIXED, SAS Institute 1999) with treatment as the main effect was used to analyze the weekly average weights of newly emerged bees for each colony. PROC MIXED procedures were used for this analysis because they are robust to missing values. Because the design was unbalanced, i.e., there was an unequal number of bees weighed per treatment, the test statistics did not follow an exact F distribution, so approximate *P* values were computed using an F approximation with fractional degrees of freedom (Satterthwaite approximation, SAS Institute 1999). Repeated measures ANOVA (JMP 2001) with treatment as the main effect and time as the repeated factor was used to test for differences in the total amount of brood and number of workers over time among treatments. Univariate ANOVA (JMP 2001) with treatment as the main effect was used to compare the total number of queens and males produced among treatments. All colony health variables were log transformed to improve homoscedasticity; reported means and SE are from the nontransformed data.

A repeated measures ANOVA (JMP 2001) with treatment as the main effect and flower number as the repeated factor was used to compare the mean access time among treatments for flower visits 1–35. Univariate ANOVA (JMP 2001) with treatment as a main effect and block as a random factor was used to compare learning rates and mean access time among treatments for both the first 10 flowers and flowers 20–35, respectively. The learning rate was defined as the difference in mean access time between flower visits 1–10 and 20–35. Block was included in the model to control for the effects of colony age.

## Results

**Colony Health.** The mean weekly pollen consumption per bee (±SE) was 0.26 ± 0.02, 0.27 ± 0.02, and 0.23 ± 0.01 g in the control, 6 ppb clothianidin, and 36 ppb clothianidin treatments, respectively, and was not different among treatments ($F = 0.66$; df = 2, 21; $P = 0.53$). There were no interactions between treatment and time for the main effects newly emerged bee weights ($F = 1.49$; df = 12, 117; $P = 0.14$), number of workers ($F = 0.96$; df = 24, 20; $P = 0.54$), and brood ($F = 0.85$; df = 24, 20; $P = 0.65$). The mean weights of newly emerged workers were not different among treatments ($F = 0.41$; df = 2, 21.8; $P = 0.67$; Fig. 1), and mean number of workers and brood also did not differ ($F = 2.83$; df = 2, 21; $P = 0.08$ and $F = 0.59$; df = 2, 21; $P = 0.56$, respectively; Fig. 2). In addition, there was no difference in the total number of males or queens produced among treatments ($F = 1.25$; df = 2, 21; $P = 0.31$ and $F = 0.38$; df = 2, 21; $P = 0.69$, respectively; Fig. 3).

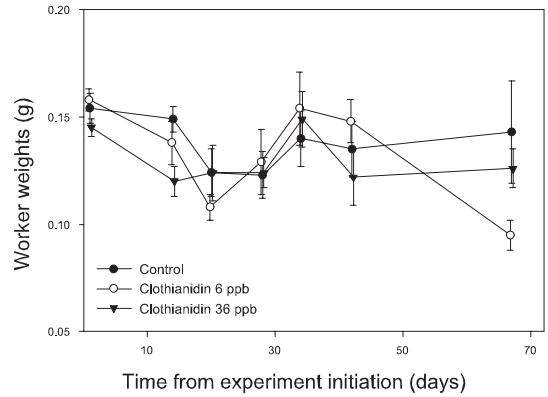


**Fig. 1.** Mean (±SE) worker weights for eight *B. impatiens* colonies from each of the three treatments; control, 6 ppb clothianidin, and 36 ppb clothianidin.

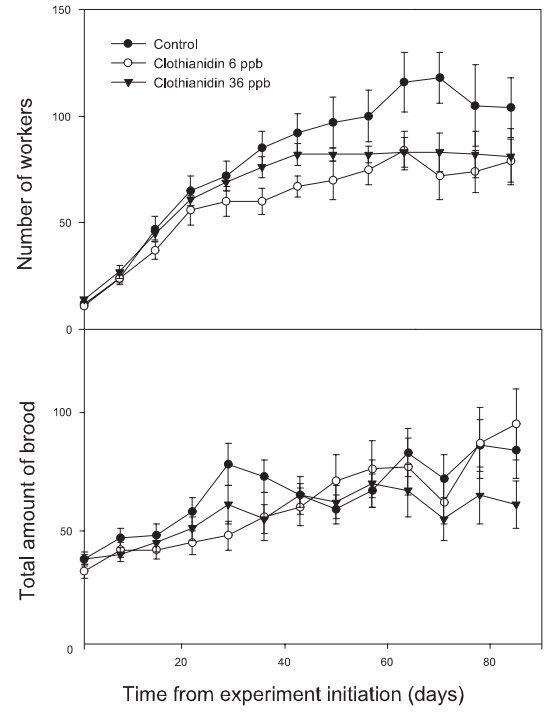


**Fig. 2.** Mean (±SE) number of workers and mean total amount of brood in eight *B. impatiens* colonies from each of the three treatments; control, 6 ppb clothianidin, and 36 ppb clothiandin.

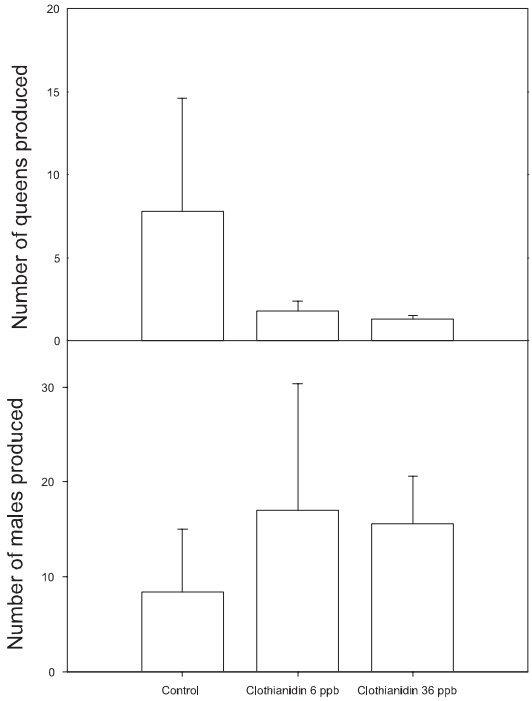


**Fig. 3.** Mean (±SE) total number of males and queens produced in eight *B. impatiens* colonies from each of three treatments; control, 6 ppb clothianidin, and 36 ppb clothiandin.

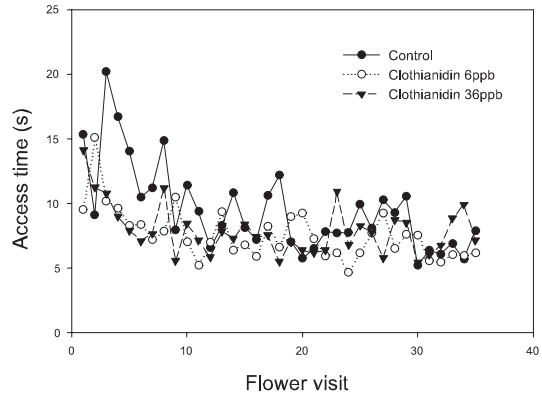


**Fig. 4.** Mean access times for flower visits 1–35 in three *B. impatiens* colonies from each of three treatments; control, 6 ppb clothiandin, and 36 ppb clothiandin. Foraging tests were performed on 18, 15, and 11 foragers, respectively. Access time measured the time a forager spent in contact with artificial complex flowers before successfully entering a flower and consuming the sucrose solution.

**Foraging Assay.** Fewer foragers were available to assess as the dose of clothianidin increased; however, the data collected was too sparse to quantify a potential pesticide effect. The mean access time (±SE) for flower visits 1–35 was 9.50 ± 1.29, 7.50 ± 0.37, and 8.00 ± 0.93 s in the control, 6 ppb clothianidin, and 36 ppb clothianidin treatments, respectively. The mean access time (±SE) for flower visits 1–10 was 13.11 ± 1.34, 9.34 ± 0.82, and 9.27 ± 0.77 s and for flower visits 20–35 was 7.59 ± 0.89, 6.67 ± 0.46, and 7.47 ± 0.86 s in the control, 6 ppb clothiandin, and 36 ppb clothianidin treatments, respectively. The interaction between the repeated measure of access time (1–35), round (1–3), and treatment was not significant ($F$ = 0.85; df = 136, 10.6; $P$ = 0.69). Overall, there was no difference in the mean access time between treatment groups ($F$ = 2.42; df = 2, 35; $P$ = 0.10; Fig. 4) or when visits were compared for flowers 1–10 ($F$ = 2.64; df = 2, 4; $P$ = 0.19) and 20–35 ($F$ = 0.49; df = 2, 4; $P$ = 0.64). The learning rate of foragers also was not different among treatments ($F$ = 3.47; df = 2, 4; $P$ = 0.13).

## Discussion

We found that clothianidin did not harm bumble bee colony health at levels at or below 36 ppb in pollen and also had no detrimental sublethal effects on the foraging ability of worker bees. The doses of clothianidin we tested represented levels equal to or higher than those found in nectar and pollen of seed-treated crops. These results provide the first evidence that clothianidin residues will not harm bumble bees foraging on seed-treated crops and suggest that clothianidin may have less potential for impact on bumble bees compared with imidacloprid.

Consistent with our results, Scott-Dupree (personal communication) found clothianidin-treated canola with pollen and nectar residues between 0.9 and 3.7 ppb to have no effect on honey bee colony health. Imidacloprid, a neonicotinoid developed before clothianidin, has been tested more extensively on bumble bees and honey bees and has also been shown to have no detrimental effects at doses comparable with field exposure (Schmuck 1999, Schmuck et al. 2001, Morandin and Winston 2003). In contrast, some previously developed insecticides harm honey bee colony health at low doses (Johansen and Mayer 1990). For example, malathion and diazinon reduce honey bee longevity, and methoxychlor reduces brood rearing in honey bee colonies (Johansen and Mayer 1990). These results suggest that clothianidin and possibly other new generation neonicotinoids may pose less of a risk to the health of bumble bee colonies than earlier insecticides.

The foraging ability of workers was not affected by long-term exposure to 6 or 36 ppb clothianidin. Similar access times and learning rates were obtained for workers in each of the three treatments. In contrast, *B. impatiens* workers exposed to imidacloprid in a previous study with comparable testing procedures showed increased access times at doses of 30 ppb (Morandin and Winston 2003). This difference may reflect a modest reduction of neurotoxic symptoms induced by clothianidin relative to imidacloprid. Kiriyama and Nishimura (2002) compared the effects of these two compounds in the cockroach *Periplaneta americana* (L.) injected with 3 times the minimum lethal dose and observed trembling in cockroaches exposed to imidacloprid, whereas clothianidin caused

no such effects. Suchail et al. (2000) found imidacloprid to induce trembling in honey bees after oral and contact exposure. In previous tests on imidacloprid, we observed trembling in *B. impatiens* workers exposed to levels of 30 ppb throughout their lifetime. In the current study, no trembling behavior was observed in foragers exposed to 6 or 36 ppb clothianidin. These results together suggest that clothianidin may have an increased margin of safety compared with imidacloprid at doses 6 to 10 times those found in treated crops.

The toxicity of many insecticides, in addition to application rate, is influenced by formulation and application method (Stark et al. 1995). Residue analysis may be of interest to determine levels in nectar and pollen of other selected crop species and in plants grown after soil or foliar treatments, because it is possible that levels higher than those tested in this study could be hazardous to bumble bee health and foraging ability. However, clothianidin and other chloronicotinyl compounds seem to offer safer alternatives for pest management than many earlier insecticides. In addition, the sensitive methods for testing sublethal foraging effects of insecticides that we used may allow compounds to be more accurately assessed for impacts on wild pollinators before being used on agricultural crops.

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