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Effects of Novel Pesticides on Bumble Bee (Hymenoptera: Apidae) Colony Health and Foraging Ability

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ABSTRACT Two experiments were conducted testing for lethal and sublethal effects of the transgenic proteins Cry1Ac and chitinase, and the chemical seed and soil treatment imidacloprid on bumble bees (*Bombus occidentalis* Greene and *B. impatiens* Cresson, Hymenoptera: Apidae). In the first experiment, *B. occidentalis* colonies were exposed to realistic residue levels of Cry1Ac, chitinase, and imidacloprid found in pollen. There were no effects on pollen consumption, bumble bee worker weights, colony size, amount of brood, or the number of queens and males produced. In the second experiment, using *B. impatiens*, we tested the effects of Cry1Ac and two levels of imidacloprid. Similar colony health measures were collected as in the first experiment, but in addition foraging ability of individual bees was tested on complex artificial flowers. There were no differences in colony characteristics among treatments. However, bees in the high-imidacloprid treatment had longer handling times on the complex flowers than bees in the other treatments. No lethal, sublethal colony, or individual foraging effects of these novel pesticides were found at residue levels found in the field, suggesting that bumble bee colonies will not be harmed by proper use of these pesticides. Use of an artificial flower foraging array proved to be a sensitive method for detecting sublethal response of bees to pesticides.

KEY WORDS bumble bees, *Bombus occidentalis*, *B. impatiens*, nontarget insects, genetically modified, insecticides

PESTICIDES USED ON AGRICULTURAL crops can be harmful to pollinators (Johansen and Mayer 1990), and sprayed applications generally are restricted to nighttime or when the crop is not in bloom to minimize pollinator exposure. Recently developed insect control techniques, such as genetically modified crops with insecticidal proteins and systemic chemical seed and soil treatments, are often safer for nontarget species than broad-spectrum insecticidal sprays (Betz et al. 2000). However, potential harm could come to pollinators if the insecticide is expressed in or transported to pollen or nectar.

Bees are important pollinators of many crop species (e.g., Delaplane and Mayer 2001). Research concerning pesticide impact on non-*Apis* pollinators is scarce, in spite of a growing concern over suspected declines of nature pollinators and its effect on agricultural production and biodiversity (e.g., Allen-Wardell et al. 1998). Until recently, studies conducted on the effects of new insect control treatments on bees have focused almost exclusively on honey bees (*Apis mellifera* L.), despite data indicating that bee species differ in their tolerance to insecticides (Johansen and Mayer 1990).

We tested the effects of three new pesticides; Bt and chitinase proteins transferred into crop plants [i.e., genetically modified (GM)], and the chloronicotinoid seed treatment imidacloprid on bumble bees (*Bombus* spp. Hymenoptera: Apidae). These pesticides were chosen because they are either widely used or have potential to harm pollinators (see below). Bumble bees were chosen to test because they are ubiquitous wild non-*Apis* pollinators and also are increasingly managed for crop pollination (Delaplane and Mayer 2001).

Genetically modified crops do have human and environmental benefits, but this new technology also presents potential risks (Winston 2002). Nectar contains insignificant amounts of protein and is unlikely to contain transgenic products, but pollen is 8–40% protein and often expresses transgenic products dependent on plant species and variety, location of the inserted gene, and type of promoter (Wilkinson et al. 1997). More than 99% of commercialized, transgenic, insect-resistant crops have been transformed with genes coding for crystalline (Cry) proteins from the soil bacterium *Bacillus thurigiensis* (Bt) (ISAAA 2001). Transgenic cotton plants containing the Cry1Ac gene (Bollgard) from Bt, express the protein in pollen at a concentration of 11.5 ng/g fresh weight (EPA 2001), whereas concentrations in nectar are below detectable levels of 1.6 ng/g (EPA 2001). Studies on the effects of Bt Cry proteins on honey bees, using test doses ranging from 20 μg/ml to 625 μg/g, showed no effect on survival or feeding behavior

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(Sims 1995, Malone et al. 1999). However, few studies have investigated colony health or sublethal effects on adults, and none have examined lethal or sublethal effects on other managed and wild pollinators in either laboratory or field studies.

In addition to Bt proteins, plants are being engineered with chitinases that naturally play a role in plant antifungal defense (Hou et al. 1998), including crops such as corn, grape, apple, strawberry, soybean, tomato, rapeseed, onion, alfalfa, potato, and tobacco (APHIS 2001). Chitin is present in the epithelial gut cells of insects (Kramer and Koja 1986) and in the exoskeleton (Boller 1988). Therefore, chitinases may have insecticidal activity and potentially could harm pollinators. No data are available on expression levels of chitinase in transformed plants, but based on pollen protein content, chitinase could be present at concentrations of 0.6 μg/g fresh weight (Picard-Nizou et al. 1997). Picard-Nizou et al. (1997) found no acute toxicity to honey bees when fed 11 μg per bee. A paucity of information is available on the potential effects of chitinase on honey bees or other pollinators.

Foliar treatments of pesticides can be restricted to application only when the crop is not in bloom, minimizing pollinator exposure. However, new chloronicotinyl compounds used as seed and soil treatments, such as imidacloprid, are systemic, dispersing throughout the plant and potentially exposing bees orally through residues in nectar or pollen. In 1999, the French Ministry of Agriculture suspended use of the imidacloprid product Gaucho on sunflower crops because of a suspected relationship between honey bee losses and imidacloprid use (summarized in Coordination des Apiculteurs de France 2000, Suchail et al. 2000). A number of laboratory and field studies by Bayer and independent researchers have shown no adverse effect to honey bees at levels of imidacloprid <20 ppb (Schmuck 1999, C. Scott-Dupree, personal communication, Schmuck et al. 2001). Analyses of residue levels of imidacloprid in canola and sunflower pollen have shown levels always <8 ppb, and usually at undetectable quantities below one ppb (Schmuck 1999, C. Scott-Dupree, personal communication, Rogers and Kemp 2002). Above 20 ppb, honey bees exhibit a decreased ability to recruit foragers to food sources (Schmuck 1999). Although field residue levels of imidacloprid in nectar and pollen have not demonstrated harm to honey bees, only one study has been published on the effects of imidacloprid on non-*Apis* pollinators. Tasei et al. (2001) exposed bumble bee (*Bombus terrestris* L.) colonies to imidacloprid-treated sunflowers in the field and concluded that proper application of imidacloprid would not effect worker behavior or colony development.

The purpose of the current experiments was to test for lethal and sublethal effects of novel pesticides on bumble bee colonies, and to assess a new method of testing sublethal foraging effects of pesticides on individual bees. Two experiments were conducted. First, the effects of Cry1Ac, chitinase, and imidacloprid on colony health in the bumble bee *B. occidentalis* Greene, at levels that could be found in pollen of field crops, were examined. In the second experiment, the effects of Cry1Ac and two concentrations of imidacloprid on *B. impatiens* Cresson colony health and individual bee foraging ability were tested. In this experiment, the higher concentration of imidacloprid tested was above the no-effect level established for honey bees. Our hypothesis was that this treatment would result in detrimental effects to colony health and bee foraging.

Materials and Methods

Experiment 1: Colony Health

Twenty-four *B. occidentalis* colonies were obtained from Biobest Canada Ltd (Leamington, Ontario, Canada). Upon delivery, each colony contained a queen and approximately 5 to 10 workers ("first brood" stage). Colonies were housed in plastic containers $\approx 20 \times 28 \times 18$ cm, surrounded by an outer cardboard casing and equipped with a bag containing a nectar substitute that bees could access freely.

The isolated proteins and insecticide were added to non-GM pollen at levels that realistically could be found in transgenic pollen or imidacloprid-treated plants (e.g., see Picard-Nizou et al. 1997, EPA 2001, C. Scott-Dupree, personal communication). Colonies were divided into four treatment groups with six colonies per treatment: (1) Control: pollen and 30% sucrose solution; (2) Imidacloprid: control plus technical imidacloprid (98%) from Bayer AG (Leverkusen, Germany) at 7 ng ([AI])/g fresh pollen; (3) Chitinase: control plus chitinase (30%) from Sigma-Aldrich (Oakville, Ontario) at 0.6 μg ([AI])/g pollen; and (4) Cry1Ac: control plus Cry1Ac (19%) from Monsanto (St. Louis, MO) at 11 ng ([AI])/g pollen. All pesticide concentrations represent the level of active ingredient that would be possible to find in dry pollen.

Pollen was collected from pollen traps on honey bee colonies in British Columbia, Canada, cleaned of dead insects and debris, and frozen for later use. The packed pollen lumps collected by honey bees were ground using an electric food processor before being mixed with the sucrose solution. Purified protein powders and imidacloprid were added to pollen by first being dissolved in distilled water, then added to 30% sucrose solution in distilled water and stirred for 5 min. The sucrose solution was then added to the pollen in a 2:1 pollen to sucrose solution mixture calculated by weight. Bees were fed pollen from the appropriate treatment twice weekly, ad libitum. At each feeding time, old pollen was removed and weighed, and weight of fresh pollen added was recorded.

Colonies were received on 18 May 2001 and monitored until 8 August 2001. At the beginning of the experiment, all bees were removed from colonies, cooled at 4°C for ≈10 min, weighed on an Ohaus Explorer electronic balance (Ohaus Company, Florham Park, NJ) to 0.01 g, and marked with a standard color pattern using Fast Drying Liquid Paper of various colors. Each week, all newly emerged bees were removed, cooled, weighed, and marked with a Liquid

Paper color pattern unique to their emergence week. The numbers of workers, amount of brood (defined as number of egg masses, larval masses, larval cells, and pupae), number of queens, and number of males were assessed weekly in each colony.

Data Analysis. For all analyses, bumble bee colony was treated as the replicate. The amount of pollen consumed by each colony, from feeding to removal was calculated twice weekly and divided by the estimated number of adult bees in the colony. The mean difference in pollen weight per bee for each treatment was used to estimate pollen use and consumption, and was compared among treatments using analysis of variance (ANOVA) (SPSS 1999). Weights of newly emerged workers each week were compared using a repeated-measures ANOVA (SAS Institute 2000). The number of bees that emerged from each colony each week was highly variable, ranging from 0 to more than 60. Because of this, the number emerging by week was included in the model. Weekly mean number of workers, eggs, larval masses, larval cells, pupae, queens, and males were $\log_{10}$ transformed to meet the assumptions of ANOVA and were compared among treatments using a multivariate repeated-measures ANOVA (SPSS 1999). The measures were repeated each observation day, and the test was multivariate because of the multiple measures quantified. All reported values are from the nontransformed data.

Experiment 2: Colony Health and Foraging Ability

In the second experiment, similar colony health variables were monitored as in the first experiment, although worker weights were not measured. In addition, individual bees were assessed for their ability to forage on complex artificial flowers. Preliminary experiments with *B. occidentalis* suggested that this species did not forage well in an artificial array, so 24 *B. impatiens* colonies were obtained from Biobest Canada (Leamington, Ontario), beginning on 27 September 2001. All colonies were at the first brood or early second brood stage at hive receipt. Hive design was the same as for the *B. occidentalis* colonies. As soon as the colonies were received and throughout the entire experiment, they were fed pollen from one of the following treatment groups, prepared in the same manner as the first experiment: (1) Control: pollen and 30% sucrose solution; (2) Cry1Ac: control plus Cry1Ac (19%) from Monsanto (St. Louis, MO) at 11 ng ([AI])/g pollen; (3) Imidacloprid low: control plus technical imidacloprid (98%) from Bayer AG (Leverkusen, Germany) at 7 ng ([AI])/g fresh pollen; and (4) Imidacloprid high: control plus technical imidacloprid (98%) from Bayer AG (Leverkusen, Germany) at 30 ng ([AI])/g fresh pollen.

Pollen was replaced biweekly and the amount of pollen consumed was calculated for each colony. The number of worker bees, males, queens, egg masses, larval masses, larval cells, and pupae in each hive were counted weekly.

All adult bees were marked with Liquid Paper on the abdomen 20 d after the experiment began. Marked individuals were not used in the foraging experiment, ensuring that all tested bees were of similar age and had consumed treated pollen throughout their developmental stages and as adults.

Bees were tested for their ability to access complex artificial flowers. The simple artificial flowers were designed from 1.5-ml clear micro tubes (Sarstedt, Newton, NC) with the caps removed. An artificial foraging array was created by imbedding ≈30 tubes into a 60-cm × 60-cm green Styrofoam base. Flowers were in rows, with each flower 10 cm apart. Rows were staggered, 5 cm between each, resulting in flowers ≈7 cm from their nearest neighbor. Hives were connected to a 1.2 × 1.2 × 1-m mesh flight cage by a 20-cm gated mesh tunnel. Each flight cage contained one foraging array. Throughout the experiment, two flight cages were used, each with only one colony connected at a time. Because only two colonies could be connected to flight cages at a time, four of the six colonies from each treatment were chosen for testing. To ensure that test colonies would have enough foragers for the experiment, colonies tested from each treatment were selected because they were judged the healthiest in each group based on worker number and amount of brood.

Collection of foraging data began on 14 November 2001, 6 wk after the colonies began receiving treated pollen. Testing of bees in a colony began by disconnecting the colony's nectar supply. The hive was then connected to a flight cage and bees were allowed to forage on the artificial flowers containing 30% sucrose solution. Ten to 15 bees making regular foraging trips were marked with a unique Liquid Paper color combination. All bees then were returned to the hive and bee access gates to the flight cage were closed. The array of centrifuge tubes was removed and replaced with a similar array containing 17 complex artificial flowers designed using the method of Gegear and Laverty (1998). The complex flowers were constructed using clear centrifuge tubes with caps bent over the top, creating a 4-mm opening. Two microliters of 30% sucrose solution were put into each flower using a 100-μl syringe with a PB600 2-μl repeating dispenser (Hamilton Company, Reno, NV). One marked forager was released into the cage and videotaped for the duration of 40 successful flower visits. A flower visit was determined to be successful if the entire bee entered the tube and accessed the solution at the base of the flower. From initial observations, it was determined that bees completely drained the 2 μl of solution on each successful visit. Immediately after a bee had successfully accessed a flower, it was refilled with 2 μl sucrose solution. If a bee returned to the colony before completing at least 30 flowers, it was let back into the cage after voiding its sucrose solution into the colony.

Five bees from each colony were tested in the following treatment order: control, imidacloprid 7 ppb, Cry1Ac, and imidacloprid 30 ppb. The order was repeated four times with new colonies each round, resulting in a potential total of 20 bees from four colonies for each treatment. At times it was not pos-

sible to get a complete test for all five bees from a colony, and, thus, the actual number of bees included in the analyses was 20, 14, 17, and 20 in the control, Cry1Ac, imidacloprid 7 ppb, and imidacloprid 30 ppb, respectively. Each colony took 3 to 6 d to test, so the foraging experiment was conducted over a 6-wk period. Consequently, colonies tested later in the experiment were older and round (i.e., the set of four colonies, one from each treatment) was included as a factor in the statistical tests.

Access time for the each of the 40 visits was calculated for each bee from videotape data using a handheld stopwatch accurate to 0.01 s. Access time was measured as the total amount of time that a bee spent touching any of the flowers until it touched the nectar at the bottom of a tube (successful access). Time spent between flowers was not included in access time estimates. Foraging rates were estimated for each bee by the total time taken to access 10 flowers, including inter-flower time, from the 21st to 30th flowers. Access times generally did not decrease substantially after the 15th flower accessed, therefore foraging rate estimates taken from flowers 21–30 were considered to be rates of experienced foragers. Foraging rates were expressed as the number of flowers accessed per minute.

Data Analysis. Colony health variables were analyzed using repeated-measures ANOVA and multivariate repeated-measures ANOVA (SPSS 1999) with colony as the replicate. Data were $\log_{10}$ transformed to meet the assumptions of ANOVA. All reported means and graphs are from the nontransformed data.

Access times were compared among treatments by repeated-measures ANOVA with flower number as the repeated measure (SPSS 1999). Variation in foraging rates among treatments was tested using univariate ANOVA followed by Tukey's pair-wise comparison test (SPSS 1999).

Results

Experiment 1: Colony Health

Mean estimated daily pollen consumption per bee (±SE) was 0.042 ± 0.006, 0.047 ± 0.008, 0.046 ± 0.008, and 0.043 ± 0.005 g in the control, chitinase, Cry1Ac, and imidacloprid treatments, respectively, and was not different among treatments ($F = 0.11$; df = 3, 20; $P = 0.95$). Repeated-measures ANOVA on mean weights of newly emerged workers over time indicated no differences among treatments ($F = 0.52$; df = 3, 20; $P = 0.68$; Fig. 1). There was no effect of treatment on number of workers, amount of brood (eggs, larval cells, larvae, and pupae) (Fig. 2), number of queens, number of males (Fig. 3) (multivariate repeated-measures ANOVA; $F = 1.17$; df = 12, 57; $P = 0.362$).

Experiment 2: Colony Health and Foraging Ability

The number of workers and amount of brood (eggs, larval cells, larvae, and pupae) (Fig. 4) were not different among treatments (multivariate repeated-measures ANOVA; $F = 0.695$; df = 12, 57; $P = 0.75$). When

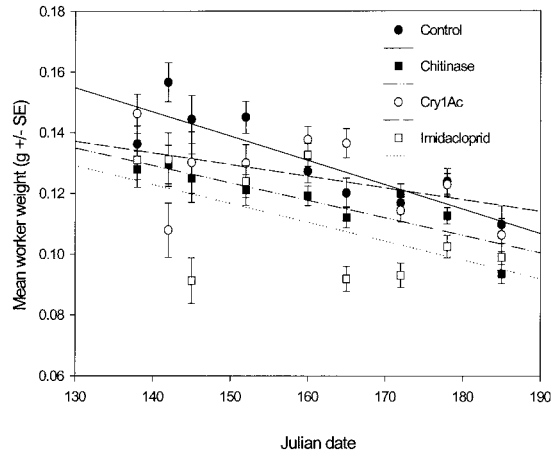

Fig. 1. Mean weights (±SE) of workers from six *B. occidentalis* colonies in each of four treatments: control, chitinase, Cry1Ac, and imidacloprid 7 ppb. Regression lines were generated by ordinary least squares regression.

presented with an artificial array of complex flowers, bees in all treatments combined successfully accessed a mean ± SE of 46.8 ± 1.0 flowers before returning to the colony. There was no difference in the number of successful flowers accessed per foraging trip among treatments ($F = 1.28$; df = 3, 67; $P = 0.290$).

There were missing values in access times for some bees after 30 flowers, hence the analysis included only flowers 1–30 for each bee. The interaction between the repeated measure of flower access time (1–30), round (1–4), and treatment was not significant ($F = 0.979$; df = 243, 1485; $P = 0.576$; 1-$\beta = 1.00$). There was an interaction between repeated access times of flowers and treatment ($F = 1.531$; df = 81, 129; $P = 0.015$; Fig. 5). Pair-wise comparisons of repeated access times over the 30 flowers indicated that foragers in the imidacloprid 30 ppb treatment took longer to access the flowers than in the other three treatments (control: $P < 0.001$, cry 1Ac: $P = 0.012$, imidacloprid 7 ppb: $P = 0.011$). No other pair-wise comparisons were significant. Foragers in the imidacloprid 30 ppb treatment spent a mean ± SE of 6.59 ± 0.37 s accessing flowers, 42.6% more time than control, Cry1Ac-, and imidacloprid 7 ppb-treated bees (4.27 ± 0.37, 4.76 ± 0.44, and 4.84 ± 0.40 s, respectively, overall mean ± SE for these three treatments = 4.62 ± 0.18 s).

Access times rapidly decreased over the first 10 flowers, and foragers were considered "experienced" after they had successfully accessed 20 flowers. Access times of experienced foragers (flowers 21–30) were different among treatments (repeated-measures ANOVA flower number*treatment: $F = 1.649$; df = 27, 183; $P = 0.029$). Pair-wise comparisons among treatments showed that access times of foragers in the imidacloprid 30 ppb treatment were significantly greater than in each of the other treatment groups ($P < 0.001$, Fig. 6).

Foraging rates of experienced foragers also were different among treatments ($F = 10.94$; df = 3, 69; $P <$

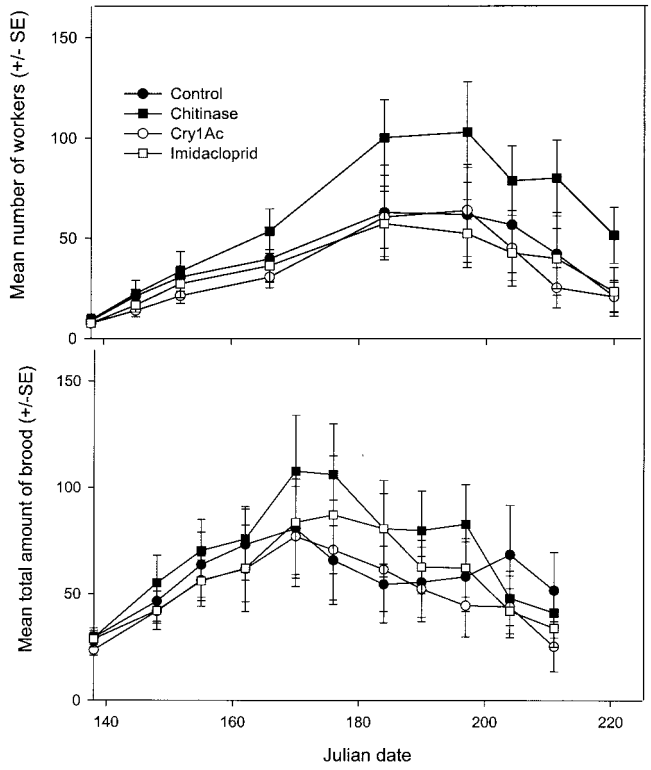

Fig. 2. Mean number of adult workers (±SE) and mean amount of brood (number of egg masses, larval masses, and pupae) (±SE) from six *B. occidentalis* colonies in each of four treatmentsL control, chitinase, Cry1Ac, and imidacloprid 7 ppb.

0.001). Foraging rate for bees in the imidacloprid 30 ppb treatment ± SE was 3.07 ± 0.14 flowers per minute, less than the foraging rates for control (4.04 ± 0.14 flowers per minute), Cry1Ac (3.75 ± 0.16 flowers per minute), and imidacloprid 7 ppb (3.98 ± 0.14 flowers per minute) individuals (Tukey's pair-wise comparison test, $P < 0.01$). Foragers in the imidacloprid 30 ppb treatment were 27.7% slower than foragers in the other three treatments, successfully accessing approximately one flower less per minute than bees in the other treatments.

Using the above data, bees in the control treatment took 14.8 s from exiting one flower to exiting the next flower (one flower cycle). Flower access time for bees in the control group averaged 3.0 s, leaving 11.8 s during which the bees were engaged in other activities such as flying above the array, walking on the array, uptake of sucrose solution, and exiting the flower. Bees in the imidacloprid 30 ppb treatment took an average of 19.5 s between successive flower exits, and using the average access time from the treatment of 4.6 s, the bee was engaged in activities other than flower handling for an average of ≈14.9 s of each flower cycle.

Discussion

There were no measurable effects on bumble bee colony or individual bee health from exposure to Cry1Ac, chitinase, or imidacloprid at concentrations similar to and above the highest residue levels found in pollen, consistent with previously published results for honey bees (Sims 1995, Picard-Nizou et al. 1997, Schmuck 1999, Schmuck et al. 2001, Scott-Dupree and Spivak 2001). The pesticide concentrations that we tested on *B. occidentalis* and *B. impatiens* colonies were chosen to reflect levels present in or higher than pollen of treated or modified commercially grown crops. Results suggest that genetically modified crops and imidacloprid seed treatments, expressing field levels of the proteins and pesticide as tested, will not harm wild bumble bee colonies.

The Bt protein Cry1Ac did not cause any lethal or sublethal effects to *B. impatiens* colonies. Access times and foraging rates did not differ from those of control bees, indicating that plants transformed with the Cry1Ac gene should be safe for bumble bees in the field. Previous studies on honey bees have found no acute toxic effects or colony health effects when individuals or colonies were exposed to the Bt proteins Cry1Ac, Cry1Ab, Cry9C, Cry3A, Cry3B, and Cry1Ba (summarized in Malone and Pham-Delegue 2001). The current study provides the first evidence that Bt proteins fed to bees throughout their development and as adults will not disrupt colony health or foraging ability.

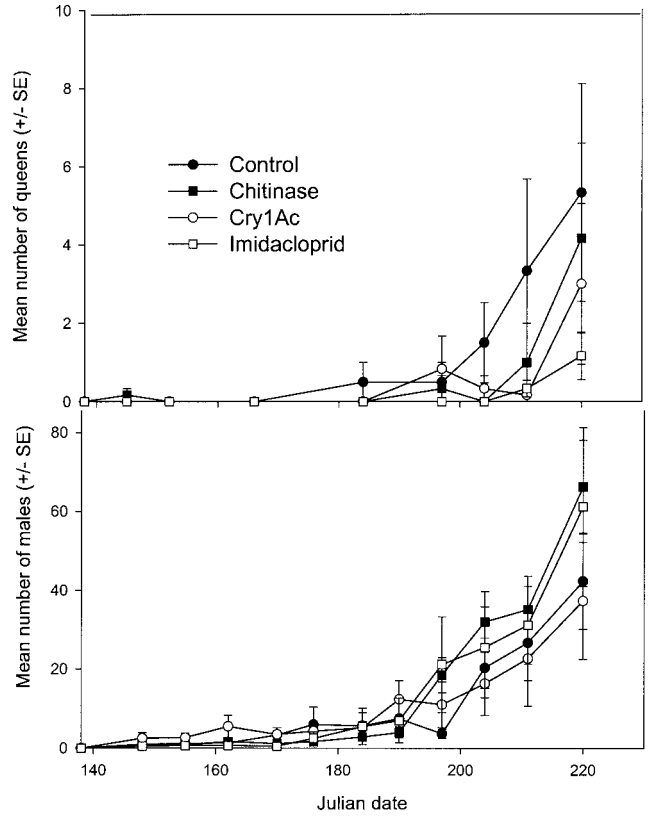

Fig. 3. Mean number of queens (±SE) and males (±SE) from six *B. occidentalis* colonies in each of four treatments: control, chitinase, Cry1Ac, and imidacloprid 7 ppb.

Picard-Nizou et al. (1995, 1997) conducted a series of studies on the effects of chitinase on honey bees including acute toxicity tests, standard conditioned proboscis extension assays, and foraging trials on control and transformed oilseed rape. Similar to the current study, Picard-Nizou et al. (1995, 1997) found no detrimental health or other effects on bees exposed to chitinase proteins.

In the current two experiments, *B. occidentalis* and *B. impatiens* colonies exposed throughout colony life to purified imidacloprid at 7 ppb did not exhibit detrimental effects. In addition, access times and foraging rates of individual *B. impatiens* bees on artificial complex flower arrays were not affected by long-term exposure to the pesticide at that concentration. However, when *B. impatiens* colonies were exposed to imidacloprid at 30 ppb, access times and foraging rates of individual bees were slower than bees exposed to 7 ppb imidacloprid or controls. Bees in the imidacloprid 30 ppb treatment may have spent longer in activities such as flying above the array and uptake of sucrose solution, in addition to spending more time handling flowers. Additional testing would be required to determine what, in addition to longer access times, caused bees in the imidacloprid 30 ppb treatment to have lower foraging rates than bees in the other treatments. Lower foraging rates for bees in the imidacloprid 30 ppb treatment of almost one less flower accessed per minute could mean that wild bumble bees if exposed to this level of pesticide may either take longer for each foraging trip, or possibly collect less pollen or nectar each trip, potentially affecting colony health.

Analysis of imidacloprid residue levels in nectar and pollen of plants grown from treated seeds, or plants grown in fields after soil treatments, have shown low, and, in most cases, undetectable levels of imidacloprid. C. Scott-Dupree, personal communication, analyzed levels of imidacloprid and its metabolites in honey bee pollen collected from treated plants and found detectable levels (limit of detection: 0.3 ppb) in two of eight samples; 7.6 and 4.4 ppb. Schmuck et al. (2001) tested nectar and pollen of sunflowers grown in greenhouses from seeds treated with imidacloprid and found no detectable levels (limit of detection: 1 ppb) of imidacloprid or its metabolites. Rogers and Kemp (2003) analyzed nectar and pollen from wild flowers and clover in years after soil application of the imidacloprid product Admire. They found no detectable residues of imidacloprid or its metabolites in clover and wild flowers or in honey bee collected pollen and nectar (limit of detection: 2 ppb). The conclusion of

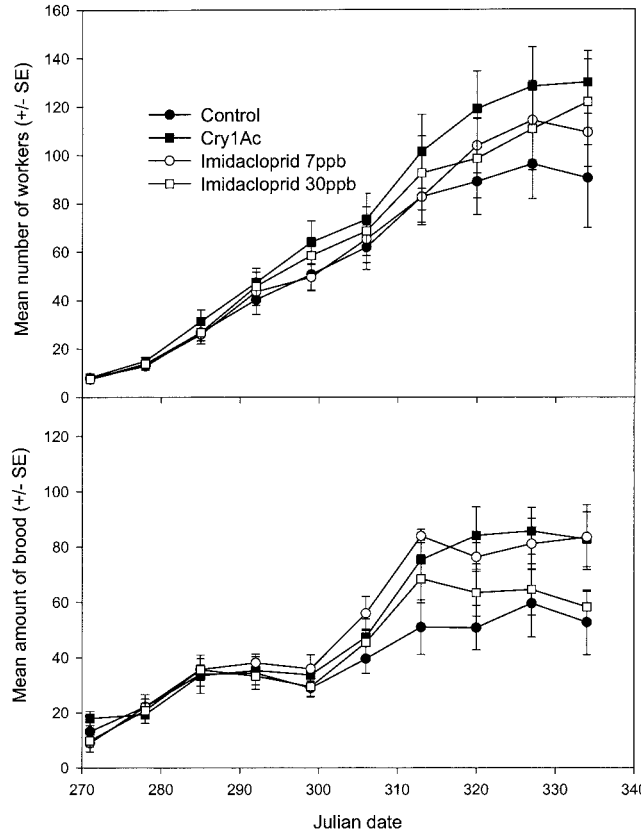

Fig. 4. Mean number of adult workers (±SE) and mean amount of brood (number of egg masses, larval masses, and pupae) (±SE) from six *B. impatiens* colonies in each of four treatments: control, Cry1Ac, imidacloprid 7 ppb, and imidacloprid 30 ppb.

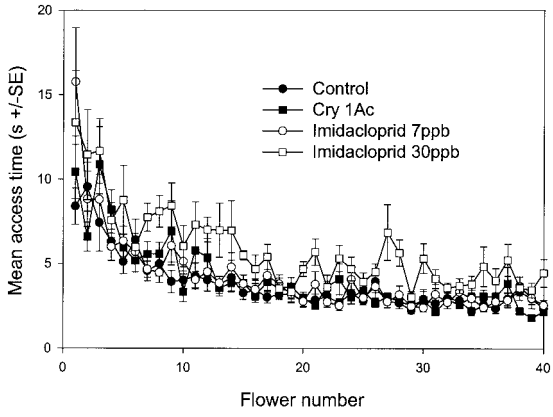

Fig. 5. Flower access times (±SE) for *B. impatiens* on artificial complex flowers from four colonies in each of four treatments: control, Cry1Ac, imidacloprid 7 ppb, and imidacloprid 30 ppb. The number of bees tested from each treatment was 20, 14, 17, and 20, respectively. Access times for each flower were calculated as the total amount of time bees spent touching flowers before successfully entering a flower.

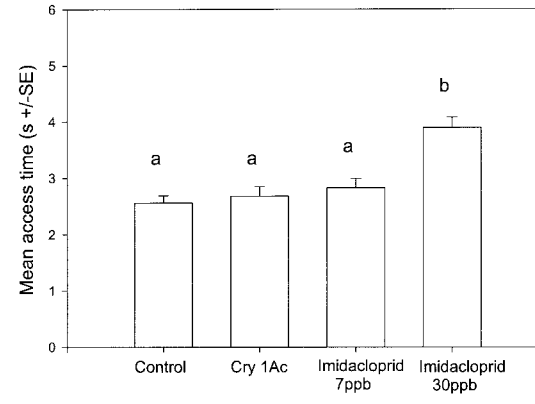

Fig. 6. Mean (+ SE) foraging rates of 'experienced' bees (see Materials and Methods) from four colonies in each of four treatments: control, Cry1Ac, imidacloprid 7 ppb, and imidacloprid 30 ppb. The number of bees tested from each treatment was 20, 14, 17, and 20, respectively. Different letters above bars indicate a significant difference in mean access time ($P < 0.05$).

our study suggests that levels of imidacloprid at or below 7 ppb in pollen will not harm bumble bee colony health or foraging ability, whereas concentrations of 30 ppb, approximately four times the highest residue level recorded in any study to date, may have sublethal effects on foraging.

Use of complex flower artificial arrays was found to be a sensitive method for testing for sublethal impacts of pesticides. Negative impacts of pesticides that might not be observed in acute toxicity tests may be detectable on artificial foraging arrays. For example, no measurable impact of 30 ppb imidacloprid on colony characteristics was found, although the foraging array revealed a sublethal behavioral effect at that higher dose. This method provides a practical and useful measure of foraging ability that could supplement or replace more expensive and logistically difficult field experiments. By altering flower design or tasks required to access a reward, artificial arrays could be modified to test for negative effects of pesticides on different aspects of foraging behavior and on different types of bees.

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