Insect Science (2010) 17, 67–72, DOI 10.1111/j.1744-7917.2009.01286.x

Sensitivities of three bumblebee species to four pesticides applied commonly in greenhouses in China

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Abstract This study was conducted to evaluate the effects of four pesticides (Mosplian, Kingbo, Score and Lvrtong) applied commonly in greenhouses in China, on three bumblebee species (*Bombus hypocrita*, *Bombus ignitus* and *Bombus patagiatus*). The study used a contact experiment and oral toxicity LD_{50} values. The results showed that the mortality for *B. hypocrita* after contacting the four pesticides was significantly lower than *B. patagiatus* and *B. ignitus*, but there was no significant difference between *B. patagiatus* and *B. ignitus*. The oral toxicity median lethal dose (LD_{50}) value of Mosplian to *B. hypocrita* (0.0028 μg active ingredient/bee) was significantly higher than that to *B. ignitus* (0.0023 μg active ingredient/bee) and *B. patagiatus* (0.0021 μg active ingredient/bee). Of the bee species, it can be concluded that *B. hypocrita* was the least susceptible to the four pesticides. The mortality rates of each bumblebee species after contact with Mosplian were significantly higher than for the other three pesticides and the control group. For Kingbo, the rates were significantly higher than the control group, but Score and Lvrtong exposed groups showed no significant increase in mortality relative to the control group. It can therefore be concluded that the pesticides differ in their negative influences on bumblebees, and that Mosplian is the most harmful.

Key words bumblebee, mortality, pesticide, toxicity

Introduction

Bumblebees are important pollinators of economically important crops and many wildflowers and are considered 'beneficial' insects. As a result, many species of bumblebees have been reared and used for commercial crop pollination around the world, including *Bombus terrestris*, *Bombus lucorum*, *Bombus occidentalis*, *Bombus ignitus*, and *Bombus impatiens* (Velthuis *et al*., 2006). The native Chinese bumblebees, *Bombus ignitus*, *Bombus lucorum* and *Bombus patagiatus* are promising candidates for crop pollination, and colonies have been reared in the laboratory from post-hibernating queens for scientific studies of social behavior, biology and pollination since 1996 (Liang *et al*., 1999; Peng *et al*., 2003; Guo *et al*., 2003; Wu *et al*., 2005; Li *et al*., 2006, 2008). Chinese farmers now use several hundred native bumblebee colonies per farm for the pollination of glasshouse tomato, pimiento and Chinese watermelon crops.

However, bee populations are often exposed to pesticide treatments in both natural and agricultural ecosystems (Kevan, 1975; Crane & Walker, 1983; Johansen & Mayer, 1990; Peach *et al.*, 1993; Ladurner *et al.*, 2003), and insecticide poisoning is considered one of the main causes of bee population declines worldwide (Dias *et al.*, 1999; Tepedino & Ginsberg, 2000). Given the ecological and economic importance of bees as pollinators of wild flowers and cultivated plants (Kevan, 1991; Southwick & Southwick, 1992), it is surprising that our knowledge of toxicity of pesticides to bees is so fragmented and mostly restricted to one species, the honeybee, *Apis mellifera*

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L. (Johansen & Mayer, 1990; OEPP/EPPO, 2001). Information on pesticide toxicity to non-*Apis* bees is scarce, and limited to a handful of species managed for crop pollination.

Some research on the toxicological impacts of pesticides on bumblebees have been reported in Europe, reviewing several methods to determine the impact of pesticides (Gretenkord & Drescher, 1993, 1996; Van der steen, 2001; Incerti *et al.*, 2003) and evaluating the effects of some pesticides on bumblebees (Tasei *et al.*, 1993, 1994, 2000, 2001; Wael *et al.*, 1995; Gels *et al.*, 2002; Marletto *et al.*, 2003; Morandin & Winston, 2003). In China, Wang *et al.* (2003) reported fungicides and insecticides were harmful to the bumblebees, but that the toxicity of fungicides was much lower than that of the insecticides. With this exception, no further studies of a similar nature have been reported in China.

Pesticides have been used extensively in agriculture since the 1950s, promoted as a tool without which developing countries could not become self-sufficient (Eddleston, 2000). Unfortunately there has been a decline in the abundance of bumblebees (*Bombus* sp., Apidae) in many countries, and it is possible that this is due in part to the use of certain pesticides. Concern continues to grow about the effects of pesticides on bee populations, and pesticide restrictions have been put into force in many countries (Darren *et al.*, 2003). However, in China, application of chemical pesticides are still the dominant form of pest control.

This paper examines the toxicity to bumblebees of various pesticides that are widely used on crops in China. The main aim of our study is to provide information for farmers and legislators that can be used in making decisions about pesticides. Further, if pesticide use is unavoidable, our study will help farmers to choose pesticides of low toxicity and match these with the least susceptible commercially available bumblebees.

Materials and methods

Study pesticides and bee species

The experiments were conducted at the laboratory of the Institute of Apicultural Research, Chinese Academy of Agricultural Sciences Beijing. Fifty *B. ignitus*, 60 *B. hypocrita* and 40 *B. patagiatus* queens were caught during April and May 2006 in Beijing and Hebei province, and kept individually in wooden nest boxes (12 × 12 × 10 cm) in a climate room at 28–29°C and 60%–65% relative humidity (RH). Queens and their offspring were fed with 50% sucrose solution and pollen collected from honeybees using pollen traps and then frozen at −20°C. During the course of the experiments, pollen from rape (*Brassica campestris* L.) and apricot (*Prunus armenica* L.) were used. The pollen was replaced every second day. Sucrose solution was provided in vertical feeders and replaced every second day. The experiment involved four pesticides as follows: Mosplian (3% acetamiprid), Kingbo (0.6% Matrine aqueous solution), Score (10% difenoconazole), Lvrtong (12% copper abietate).

Contact test

In the contact experiment, the newly emerged workers of each species were collected and recorded by color codes on the thorax in order to determine their ages for use in the test. Workers aged 9–10 days after emergence were selected at random from different colonies, 450 workers of each bumblebee species were used. In each treatment and the control (without pesticides) three replicates with 30 workers per replicate were tested. Each test group was kept in an observation cage at 27°C, 60% RH, cages were wooden with five mesh sides and measured 40 × 40 × 40 cm (internal dimensions). The bottom of the observation cage was covered inside with 156 cm^2 of paper sprinkled with 20 mL test solution in water and afterwards air-dried, and test solutions according to application doses used in greenhouses were as follows: 1 : 5 000 v/v Mosplian, 1 : 5 000 v/v Kingbo, 1 : 1 000 v/v Score, 1 : 5 000 v/v Lvrtong. During the course of the experiments, sugar syrup and water (50% w : w) were supplied to the bees via 6-mL graduated gravity feeders and replaced every second day. Pollen was replaced daily. All cages were inspected every 24 h, and mortality was recorded.

Acute oral median lethal dose (LD_{50}) test

The result of the contact test showed that the mortality rates of each bumblebee species after contacting Score ($P = 0.070$) and Lvrtong ($P = 0.110$) were not significantly different than the control group, so we further examined the toxicity of Mosplian and Kingbo to three bumblebee species. The toxicity of Mosplian and Kingbo to *B. hypocrita*, *B. ignitus* and *B. patagiatus* was assessed through feeding assays on an artificial diet. Before starting this experiment, we carried out a pre-experiment to find the concentration of pesticides which lead to approximately 0% and 100% mortality. Based on this five concentrations of each pesticide were prepared in 50% sugar solution. In the experiment, 180 young workers were selected respectively from *B. hypocrita*, *B. ignitus* and

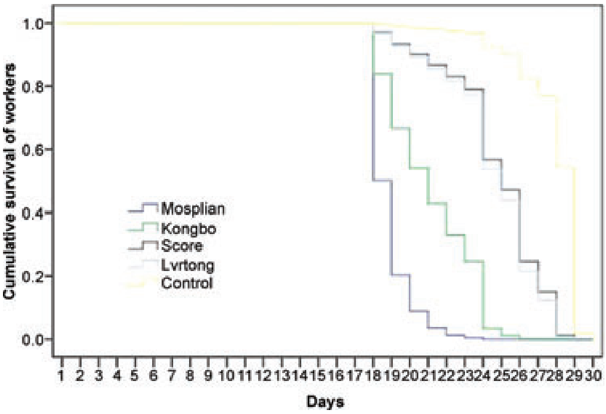

Fig. 1 Survival funciton for *Bombus patagiatus* workers after a spray application of the pesticides, Mosplian, Kingbo, Score and Lvrtong. Thirty bees are represented by each line; three replicates per cage.

B. patagiatus colonies. At each pesticide concentration and the control (50% sugar solution without pesticide) three replicates with 30 workers per replicate were tested. Each test group was kept in a box at 27°C, 60% ± 10% RH. Prior to offering the test solution for consumption, the bumblebees were starved for 2–3 h. Each test solution of 10 mL was provided in vertical feeders. Mortality was checked after 48 h, the LD_{50} were derived for the oral trials, for which the individual dose applied to each worker was the total amount of consumed solution divided by the number of 30 workers.

Statistical analysis

Statistical analysis was done in SPSS 17.0 (SPSS Inc., Chicago, IL, US). Pesticide treatments were compared among *B. hypocrita*, *B. ignitus* and *B. patagiatus*, treatment groups and the control group. A Cox-survival analysis was done. Mortality rates were analyzed using analysis of variance (ANOVA) with Tukey's post-hoc test. The LD_{50} was calculated with POLO-PC software (LeOra Software, Berkeley, CA, US).

Results

The results of contact tests showed survival function of workers over time; the percent survival 16 days after initial contact with Mosplian for three bumblebee species was the highest (Figs. 1, 2, 3). The percentage mortality 16 days after initial contact with Mosplian for *B. patagiatus* and *B. ignitus* workers was 98.33% ± 0.56% and 96.67% ± 2.33% respectively (Fig. 4). The mortality of *B. hypocrita* after contact with the four pesticides

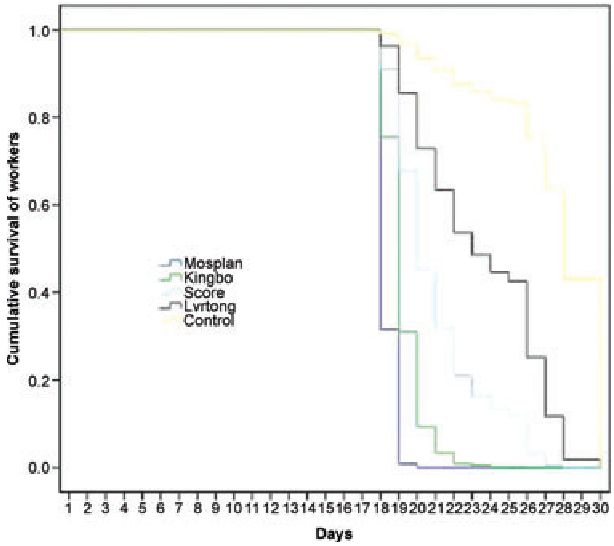

Fig. 2 Survival function for *Bombus ignitus* workers after a spray application of the pesticides, Mosplian, Kingbo, Score and Lvrtong. Thirty bees are represented by each line; three replicates per cage.

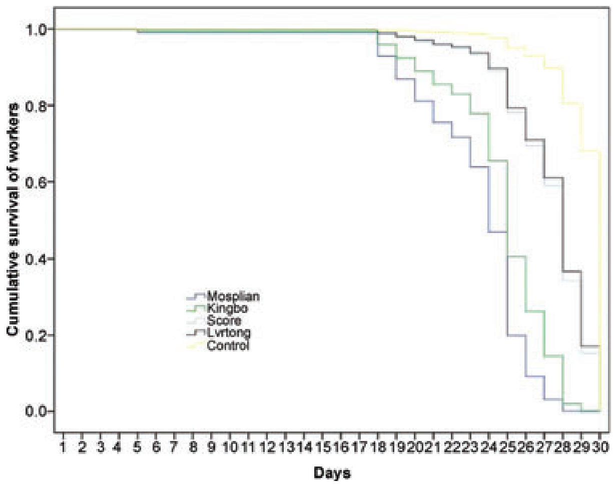

Fig. 3 Survival function for *Bombus hypocrite* workers after a spray application of the pesticides, Mosplian, Kingbo, Score and Lvrtong. Thirty bees are represented by each line; three replicates per cage.

was significantly lower than *B. patagiatus* ($P = 0.023$) and *B. ignitus* ($P = 0.016$), but there was no significant difference between *B. patagiatus* and *B. ignitus* ($P = 0.963$) (Table 1). The mortality after contact with Mosplian was significantly higher than after contact with Score ($P = 0.047$), Lvrtong ($P = 0.004$) and the control treatment ($P = 0.003$). The mortality after contact with Kingbo was significantly higher than in the control

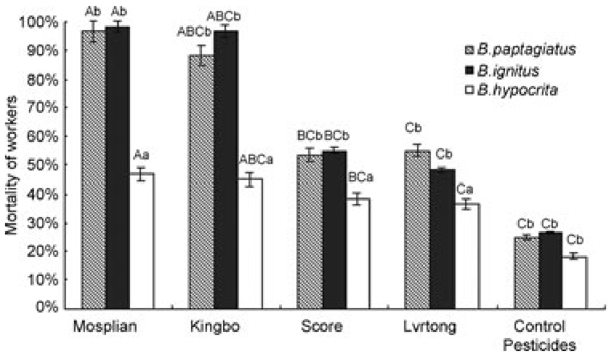

Fig. 4 The percentage mortality of the three bumblebee species 16 days after contact with each of four pesticides. Thirty workers are represented by each bar, with three replicates per treatment. Dates followed by the same capital letters indicate no difference between different pesticides at the $P > 0.05$ level; the different capital letters indicate significant difference at the $P < 0.05$ level. The same small letters indicate no difference between different pesticides at the $P > 0.05$ level; the different small letters indicate significant difference at the $P < 0.05$ level.

treatment ($P = 0.047$). Mortality rates after contact with Score ($P = 0.070$) and Lvrtong ($P = 0.110$) were higher than in the control group, but this was not significantly different (Table 1).

The oral toxicity LD_{50} value of Mosplian to *B. hypocrita* (0.002 8 μg active ingredient/bee) was significantly higher than that to *B. ignitus* (0.002 3 μg active ingredient/bee) and *B. patagiatus* (0.002 1 μg active ingredient/bee). The LD_{50} value of Kingbo to *B. hypocrita* (0.001 9 μg active ingredient/bee) was also significantly higher than that to *B. ignitus* (0.000 5 μg active ingredient/bee) and *B. patagiatus* (0.000 5 μg active ingredient/bee) (Table 2).

Discussion

Our results showed that the bumblebee *B. hypocrita* was the least susceptible species to the four pesticides. The pesticide Mosplian was the most toxic, and Kingbo treatment gave higher mortality than the control treatment.

Table 1 Analysis of variance (ANOVA) table and post-hoc tests for the mortality of bumblebee workers 16 days after contact with the four pesticides.

Source	Type III Sum of Squares	df	Mean square	*F*	Significant
Intercept	45 375.000	1	45 375.000	311.466	0.000
Species	2 440.000	2	1 220.000	8.374	0.011
Pesticides	6 744.611	4	1 686.153	11.574	0.002
Error	1 165.456	8	145.682	–	–
Total	55 725.067	15	–	–	–
Corrected total	10 350.067	14			

		Subset		
Tukey B Species	n	1	2	3
Bombus hypocrita	5	37.000 0	–	
Bombus ignitus	5	–	63.000 0	
Bombus patagiatus	5		65.000 0	
Pesticides		–		
Control	3	23.333 3	–	–
Lvrtong	3	45.556 7	–	–
Score	3	48.886 7	48.886 7	–
Kingbo	3	–	77.223 3	77.223 3
Mosplian	3	–	–	80.000 0

Post-hoc comparisons were conducted with the Tukey test using variables: species, pesticides. Means for groups in homogeneous subsets are displayed and based on Type III Sum of Squares, the mean difference is significant at the $P = 0.05$ level. Means within the same column are not significantly different at the $P = 0.05$ level; the different columns indicate significant difference at the $P = 0.05$ level. R-squared = 0.887 (adjusted R-squared = 0.803), $^{*}P < 0.05$.

Table 2 Oral toxicity of Mosplian and Kingbo to the three bumblebee species.

Species		LD_{50} [μg. a. i./bee]	95% CI	Slope ± SE
Bombus ignitus	Mosplian	0.002 3	0.002 1–0.002 4	2.253 0 ± 0.144 0
	Kingbo	0.000 5	0.000 3–0.001 3	4.431 0 ± 0.772 1
Bombus hypocrita	Mosplian	0.002 8	0.001 8–0.003 1	1.102 8 ± 0.552 0
	Kingbo	0.001 9	0.001 8–0.002 0	2.332 1 ± 0.540 1
Bombus patagiatus	Mosplian	0.002 1	0.002 0–0.002 3	0.973 9 ± 0.435 0
	Kingbo	0.000 5	0.000 3–0.001 4	0.997 3 ± 0.777 3

While there was a trend for greater morality in the Score and Lyrtong treatments, they did not differ significantly from the control.

Some authors have reported that there is a significant correlation between the size of a bumblebee and its susceptibility to a pesticide, the larger the bumblebee the less susceptible (Drescher & Geusen-Pfister 1991; Van der Steen, 1994, 2001). However, our experiments showed that *B. hypocrita* was the least susceptible to the four pesticides despite having the smallest individuals. Therefore, it is possible that the effect of species on susceptibility to a pesticide is more complex than simply the effect of the size of individuals.

Furthermore, we found that the toxicity of Score (P = 0.070) and Lvrtong (P = 0.110) to bumblebees was not significantly different than the control treatment. Thus, we can suggest the use of such low toxicity pesticides as Score and Lvrtong in areas where contact with bumblebees is likely.

The four pesticides were differentially harmful to bumblebees (Table 1), with Mosplian being the most harmful. Although no studies have reported a decline in the abundance of bumblebees in China, we found that mortality of native bumblebees used for pollination of greenhouse crops was high when exposed to certain commonly used pesticides. The application of chemical pesticides is still the dominant method of pest control in China. We suggest that care should be taking when choosing and applying pesticides, especially in areas where bumblebees play a vital role in pollination.

Acknowledgments

We would like to thank Dr. Regula Schmid-Hempel and Dr. Ben Sadd for their help in revising this paper, and Zhan-Bao Guo, Yue-Ming Tong, Hai-Yan Shi and Wen-Feng Chen for help and advice in the lab. This work was financially supported by grants from Chinese National Natural Science Foundation for Young Scholars (NO. 30700606), the R&D Special Fund for Public Welfare Industry (agriculture) (nyhyzx07-041) and China Application of Advances in Agricultural Science and Technology Projects (NO. 2006GB23260392).

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Accepted June 5, 2009

