Effects of Sublethal Concentrations of Bifenthrin and Deltamethrin on Fecundity, Growth, and Development of the Honeybee Apis mellifera ligustica

Ping-Li Dai,† † Qiang Wang, † † Ji-Hu Sun, ‡ Feng Liu, † † Xing Wang, † Yan-Yan Wu, † † and Ting Zhou* † †

† Institute of Apicultural Research, Chinese Academy of Agricultural Science, Beijing, 100093, China
‡ Key Laboratory of Pollinating Insect Biology, Ministry of Agriculture, Beijing, 100093, China
§ Department of Physiology, Second Military Medical University, Shanghai, 200433, China

(Submitted 1 March 2009; Returned for Revision 16 April 2009; Accepted 11 September 2009)

Abstract—Bifenthrin and deltamethrin have been widely used as pesticides in agriculture and forestry and are becoming an increasing risk to honeybees. The honeybee, Apis mellifera ligustica, is widely recognized as a beneficial insect of agronomic, ecological, and scientific importance. It is important to understand what effects these chemicals have on bees. Effects of two pesticides at sublethal concentrations on fecundity, growth, and development of honeybees were examined with the feeding method for a three-year period (2006–2008). It was shown that both bifenthrin and deltamethrin significantly reduced bee fecundity, decreased the rate at which bees develop to adulthood, and increased their immature periods. The toxicity of bifenthrin and deltamethrin on workers of Apis mellifera ligustica was also assessed, and the results from the present study showed that the median lethal effects of bifenthrin and deltamethrin were 16.7 and 62.8 mg/L, respectively. Environ. Toxicol. Chem. 2010;29:644–649. © 2009 SETAC

Keywords—Bifenthrin Deltamethrin Apis mellifera ligustica Toxicity test Sublethal concentration

INTRODUCTION

The honeybee, Apis mellifera ligustica, is widely recognized as a beneficial insect of agronomic, ecological, and scientific importance. It produces commercially valuable products (honey, pollen, royal jelly, propolis, and wax) and plays a major role in crop pollination [1]. Because the honeybee is in contact with various pollutants during its foraging activity, it has been considered an environmental sentinel of high sensitivity [2–4]. The use of synthetic pesticides is increasing the pollution risk for honeybees in countries such as China, United States, and India, especially because the use of synthetic pesticides is increasing. The gravity of the environmental risk pesticides pose is usually assessed by both the measurement of pesticide exposure and pesticides’ effects on living organisms [5].

Bifenthrin is a member of the synthetic pyrethroid family, which is a group of nonsystemic insecticides. Because of its stability when exposed to light, low volatility in the environment, low toxicity to mammalian life, and high insecticidal potency under normal conditions, it has been widely used [6]. In China, bifenthrin has become an essential component of pest control strategies for cereals, cotton, fruits (notably orange), and vegetables. Bifenthrin usually affects insects’ central and peripheral nervous systems by interfering with sodium channels, which are characterized by a strong environmental persistence and high insecticidal activity. Deltamethrin is a type II pyrethroid insecticide whose primary target is the voltage-dependent sodium channel [7]. Deltamethrin is extensively used in agriculture and forestry because of its high efficacy in exterminating a broad spectrum of insect pests [8].

To assess the risk of a contaminant, two types of tests may be used. Toxicity assay is to examine the toxicity of contaminant at a lethal dose [9,10], and sublethal effect is to examine the effect of contaminant at a sublethal dose on insects. Many sublethal effects of toxic contaminants are known to be important within the ecological context. Sublethal doses of insecticides are known to disturb the behavior of honeybees [11], leading to poor individual performance and population dynamics disorders within the bee colony [12]. Some insecticides have been shown to decrease the production of offspring [13–15]. Furthermore, there is increasing concern among beekeepers that sublethal behavioral effects may have significant impacts on foraging activity and learning performances [16–19]. Some sublethal effects produced by deltamethrin have also been reported, such as the paralysis of foragers [20], the decrease of foraging activity [21], and a disruption of the homing flight of foragers [22].

In the present study, the toxicity of bifenthrin and deltamethrin on the honeybee Apis mellifera ligustica was evaluated under laboratory conditions, and the fecundity, growth, and development of honeybees from colonies fed with bifenthrin and deltamethrin at low concentrations was compared with that of bees from control colonies.

MATERIALS AND METHODS

Materials

Bifenthrin (2-methylbi phenyl-3-ylmethyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropylcarboxylate) was obtained from FMC in the form of 2.5...
emulsifiable concentrates (EC). Deltamethrin, (S)-alpha-cyano-3-phenoxynbenzyl(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropancarboxylate, was obtained from Bayer, in the form of 2.5 EC.

Experimental design

Worker bees (Apis mellifera ligustica) were captured from honey and pollen combs in the hive for bioassays. Bees were held for a short period of time in 9 × 9 × 6-cm cages. All bifenthrin and deltamethrin solutions were prepared in a liquid mixture of 1:1 sucrose:water. Treated bees were fed pesticide solution with different concentrations that were arranged geometrically. Concentrations of bifenthrin were 4.0, 7.9, 15.5, 30.6, and 60.2 mg/L, and deltamethrin concentrations were 20.0, 36.0, 64.8, 116.6, and 210.0 mg/L. Control bees were fed a liquid mixture of 1:1 sucrose:water. In each experiment, a treatment included three cages of 20 bees each. The cages were placed in the incubator (30 ± 1°C, 60 ± 10% relative humidity, darkness). Three cages of 20 bees were used for each concentration of bifenthrin and deltamethrin. Experiments were replicated at least three times. Control mortality was less than 5% in all experiments. Mortality was recorded at 48 h after the feeding.

Experiments to examine the effect of the bifenthrin and deltamethrin at sublethal dose on fecundity, growth, and development of honeybees commenced on May 15, 2006 and continued on July 20, 2007 and September 1, 2008. The plants around the apiary did not have nectar throughout the experimental period. New colonies were used each year, a pair of sister queens were used in the same year, and all treatments had five colonies. Each treated colony was fed pesticide solution (400 ml per day) at an estimated five percent lethal concentration (LC5) that was derived from the toxicological tests. Considering the influence of secondary metabolites, the queen was directly fed 5 µl of pesticide solution every 5 d. Pesticides were in a liquid mixture of 1:1 sucrose:water. Control colonies were fed a liquid mixture of 1:1 sucrose:water. All of the colonies were fed for 20 d. The stored honey and syrup were taken out every 3 d to avoid the effects of pesticides concentrated in the stored honey. Pesticides were not sprayed onto plants around the apiary. The queen laid eggs on a new comb within 1 d in the queen excluder. The number of eggs per female per day and their weight was recorded. Frames containing a patch of 200 eggs (24 h old) were mapped on a transparency and placed in the supers. The same patch of eggs residing on the patch of 200 eggs (24 h old) were mapped on a transparency and placed in the incubator. The larva weight was measured within mapped frames was then checked when the eggs hatched, and placed in the supers. The same patch of eggs residing on the patch of 200 eggs (24 h old) were mapped on a transparency and placed in the supers. The egg weight, the larva weight, the hatch rate, the cap rate, the egg stage, the sealed brood stage (= feeding larvae), the unsealed brood stage (= postfeeding larvae and pupae), and the immature stage were all measured and determined at the endpoints. Because these parameters are considered to be the most biologically meaningful because they cover the main stages of the life cycle, they served as a measurement of the effects of bifenthrin and deltamethrin on bees at the population level.

Statistical analysis

The median lethal concentration (LC50) and LC5 values were determined by the probit analysis program of Russell et al. [23]. Statistical analyses were performed using SAS® (Cary, NC, USA) [24]. The fecundity and the development period for each year were analyzed separately using one-way analysis of variance (ANOVA) with type of pesticide (bifenthrin, deltamethrin, and control) as the independent variable. Each colony was treated as an experimental unit. When significant differences were found (p ≤ 0.05), multiple comparison procedures were performed by Tukey’s honestly significant difference (HSD) tests. The developmental rate was compared between pesticide and control by χ² tests.

RESULTS

Toxicity assay

In toxicological studies, each pesticide concentration was tested using three cages of 20 individuals, and each study was replicated three times. The LC50 and LC5 values of bifenthrin and deltamethrin in honeybees obtained with oral tests are summarized in Table 1. The slopes of dose–response curves are 3.77 and 3.56, respectively.

Sublethal effects

Daily fecundity. The ANOVA for the daily fecundities indicated that the mean of fecundities from control, bifenthrin, and deltamethrin were significantly different across the three-year period (p < 0.0001 for 2006; p < 0.0001 for 2007; p < 0.0001 for 2008). The Tukey’s HSD test indicated that bifenthrin- and deltamethrin-treated females produced significantly fewer (p ≤ 0.05) eggs than the control in 2006, 2007, and 2008 (Fig. 1A).

Egg weight. The ANOVA for egg weights indicated that the means of egg weights from control, bifenthrin, and deltamethrin were significantly different across the three-year period (p < 0.0001 for 2006; p < 0.0001 for 2007; p < 0.0001 for 2008). The Tukey’s HSD test indicated that significantly greater (p ≤ 0.05) egg weights were found for colonies fed bifenthrin compared with the control in 2006 and 2007. There were significant differences (p ≤ 0.05) in egg weight between the control and deltamethrin across the three-year period (Fig. 1B).

Fresh larval weight. The ANOVA for fresh larval weights indicated that the means of fresh larval weights from control, bifenthrin, and deltamethrin were significantly different in 2006

<table>
<thead>
<tr>
<th>Oral LC50 (mg/L)</th>
<th>Oral LC5 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.7 [12.4-22.6]</td>
<td>6.9 [3.0-9.9]</td>
</tr>
<tr>
<td>60.8 [53.6-69.0]</td>
<td>21.6 [16.5-26.3]</td>
</tr>
</tbody>
</table>
and 2008 ($p < 0.0001$ for 2006; $p = 0.3483$ for 2007; $p < 0.0001$ for 2008). The larval weight of colonies fed deltamethrin was significantly lower (Tukey’s HSD, $p \leq 0.05$) in 2008 than the control. The larval weight of colonies fed deltamethrin was significantly higher ($p \leq 0.05$) in 2006 and lower ($p \leq 0.05$) in 2008 than the control (Fig. 1C).

**Hatch rate.** There were no significant differences in hatch rate between bifenthrin and the control in 2006 and 2007, but the difference between the two rates was significant in 2008, and colonies fed bifenthrin had a lower hatch rate compared with the control ($p = 0.7943$ for 2006; $p = 0.6621$ for 2007; $p < 0.0001$ for 2008; Table 2). Significantly lower hatch rates were found.
for colonies fed deltamethrin compared with control across the three-year period ($p < 0.0001$ for 2006; $p < 0.0001$ for 2007; $p < 0.0001$ for 2008; Table 2).

**Cap rate.** Although there was no significant difference in the cap rate between control and bifenthrin in 2008, significantly lower cap rates were found for colonies fed bifenthrin compared with control in 2006 and 2007 ($p < 0.0001$ for 2006; $p = 0.0048$ for 2007; $p = 0.7237$ for 2008; Table 2). The cap rate of colonies fed deltamethrin was significantly lower than that of control over the 3-year period ($p < 0.0001$ for 2006; $p = 0.0036$ for 2007; $p = 0.0036$ for 2008; Table 2).

**Emergence rate.** The emergence rate of colonies fed bifenthrin was significantly lower than the emergence rate of the control in 2007 and 2008 and was not significant in 2006 ($p = 0.0508$ for 2006; $p < 0.0001$ for 2007; $p = 0.0050$ for 2008; Table 2). There were no significant differences in the emergence rate between deltamethrin and the control in 2006 and 2008, but there was a significant difference in 2007, and colonies fed deltamethrin had a lower emergence rate compared with the control ($p = 0.0549$ for 2006; $p = 0.0002$ for 2007; $p = 0.9387$ for 2008; Table 2).

**Success rate of development.** The success rate of development within the colonies treated by bifenthrin was lower than within the control across the three-year period ($p = 0.0002$ for 2006; $p < 0.0001$ for 2007; $p < 0.0001$ for 2008, Table 2). The success rate of development in the colonies treated by deltamethrin was also lower than the control colonies ($p < 0.0001$ for 2006; $p < 0.0001$ for 2007; $p = 0.0001$ for 2008, Table 2).

**Egg stage.** The ANOVA for the duration of the egg stage indicated that the mean of the duration of the egg stage from the control, bifenthrin, and deltamethrin varied significantly in 2006 and 2007 ($p < 0.0001$ for 2006; $p < 0.0001$ for 2007; $p = 0.0599$ for 2008). The duration of the egg stage exposed to bifenthrin and deltamethrin was significant longer (Tukey’s HSD, $p < 0.05$) than that of the control in 2006 and 2007 (Fig. 2A).

**Unsealed brood stage.** The ANOVA for the unsealed brood stage indicated that the mean of the unsealed brood stage from the control, bifenthrin, and deltamethrin groups were significantly different from one another across the three-year period ($p = 0.0001$ for 2006; $p < 0.0001$ for 2007; $p < 0.0001$ for 2008). Tukey’s HSD test indicated that there were significant differences between the treatments.

### Table 2. The hatch rate, the cap rate, the emergence rate, and the success rate of development of the honeybee *Apis mellifera ligustica* on control, bifenthrin, and deltamethrin in 2006, 2007, and 2008. Within a row, samples not included under the same letter (a, b, or c) were significantly different ($p \leq 0.05$) by $\chi^2$ tests.

<table>
<thead>
<tr>
<th>Test time</th>
<th>Control</th>
<th>Bifenthrin</th>
<th>Deltamethrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatch rate %</td>
<td>2006</td>
<td>84.0 a</td>
<td>83.1 a</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>96.3 a</td>
<td>95.6 a</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>88.1 a</td>
<td>71.4 c</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>92.1 a</td>
<td>75.9 b</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>97.9 a</td>
<td>92.9 b</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>92.2 a</td>
<td>91.4 a</td>
</tr>
<tr>
<td>Emergence rate %</td>
<td>2006</td>
<td>97.4 a</td>
<td>92.1 a</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>98.6 a</td>
<td>85.6 b</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>97.3 a</td>
<td>92.3 b</td>
</tr>
<tr>
<td>Success rate of development %</td>
<td>2006</td>
<td>75.3 a</td>
<td>58.1 b</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>93.0 a</td>
<td>76.0 b</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>79.0 a</td>
<td>60.3 b</td>
</tr>
</tbody>
</table>

Fig. 2. The development period (mean ± standard error) of the honeybee *Apis mellifera ligustica* on the Control, Bifenthrin and Deltamethrin colonies in 2006, 2007, and 2008. (A) The egg stage. (B) The unsealed brood stage. (C) The sealed brood stage. (D) The immature stage. Within a site, bars not included under the same letter (a, b, or c) were significantly different ($p \leq 0.05$) by Tukey’s honestly significant difference tests, after an analysis of variance indicated a significant treatment effect.
differences \( p < 0.05 \) in the unsealed brood stage between the bifenthrin and control groups in 2006 and 2008. The unsealed brood stage of the group fed deltamethrin was significantly longer \( p < 0.05 \) than that of the control in 2007 (Fig. 2B).

**Sealed brood stage.** The means of the sealed brood stage from the control, bifenthrin, and deltamethrin groups were significantly different across the three-year period \( p = 0.0002 \) for 2006; \( p < 0.0001 \) for 2007; \( p < 0.0001 \) for 2008. A significantly longer (Tukey’s HSD, \( p < 0.05 \)) sealed brood stage was found for colonies fed bifenthrin compared with the control in 2006. There was also a significant difference \( p < 0.05 \) in the sealed brood stage between the control and deltamethrin during the three years (Fig. 2C).

**Immature stage.** The immature stages from the control, bifenthrin, and deltamethrin colonies were significantly different across the three-year period \( p = 0.0002 \) for 2006; \( p < 0.0001 \) for 2007; \( p < 0.0001 \) for 2008. Tukey’s HSD test indicated that the immature stage was significantly longer \( p < 0.05 \) for colonies fed bifenthrin compared with that of the control colonies for 2006. There was a significant \( p < 0.05 \) difference in the immature stage between the control and deltamethrin-fed colonies during the three years (Fig. 2D).

**DISCUSSION**

Pesticide toxicity to bees was ranked according to the LC50 values. It was found that bifenthrin was highly toxic and deltamethrin was moderately toxic to honeybees. Some studies have shown high toxicity in acute toxicity tests [25]. The results of the present study can be extrapolated to pesticide exposure of bees in the field situation. The recommended concentration of bifenthrin for pest control in agriculture is approximately 20 to 100 mg/L, which is higher than the LC50. The recommended concentration of deltamethrin for pest control in fields is approximately 12.5 to 25 mg/L, roughly the same amount as the LC5. However, the exposure of associated repellent effects is likely to be well below the recommended concentration.

Sublethal doses of agrochemicals are known to disturb bee reproductive behaviors [13]. The queen supersede rate in colonies treated with very low doses of cypermethrin (80%) was significantly greater than in the controls (30%) [26]. Treatment of female leafcutter bees (*Megachile rotundata*) with a pyrethroid (20% of the median lethal dose [LD50]) resulted in 20% less eggs laid throughout a six-week period after dosing [27]. Results indicated that bifenthrin and deltamethrin significantly reduced the fecundity of the queen.

Honeybees are subjected to seasonal variations that may result in changes to their physiology and behavior [28–33]. In the present study, there were inconsistencies between years in the toxic effects of pesticides (e.g., egg weight, larval weight, hatch rate, cap rate, emergence rate, and every development stage following bifenthrin and larval weight, cap rate, emergence rate, and unsealed brood stage following deltamethrin). All of these inconsistencies might be a result of the changes of honeybee physiology. However, the effects of pesticides on the fecundity and the success rate of development within the colony were not inconsistent between years.

The impact of pesticides on the colony may be severe, including effects on egg laying, queen supersede, and the ability of the colony to requeen. Developmental effects in larvae may affect the behavior of adults and therefore affect colony survival. All of these effects potentially have a large impact on colony population.

Pyrethroids are probably the best known repellent pesticides in use. Both the phenylacetate-ester and cyclopropanecarboxylate pyrethroids exhibit repellency through similar modes of action [34–36]. The effects of pyrethroids as repellents have been suggested to be due to sublethal effects after contact exposure of the tarsi and ventral abdomen [37]. The pyrethroids are highly irritating to the bees with transfer from the tarsi to the proboscis and antennae during grooming, which results in irritation. The bees then return to the colony to recover, before they receive a lethal dose, in a similar manner to the knockdown effects of pyrethroids in other insects. Their impact should be studied under more realistic exposure. Further field studies are needed, based on residue analysis of the pesticides found on crops where and when the bees are foraging. Sublethal behavioral effects should be observed in bees after their exposure to pesticides. Assessment of the longer term consequences of sublethal changes in colonies would further reveal the effects of pesticides on honeybee colonies.

Acknowledgement—We thank Shi-Yu Yang, Shu-Fa Xu and two anonymous reviewers for comments that improved the manuscript. Gui-Rong Li for assistance in the laboratory; and Ping-Hong Wang for competent beekeeping.

This research was supported by the National Natural Science Foundation of China (No. 30571408), the Major State Basic Research Development Program of China (No. 2006BA06B04), the Special Public Sector Research of Agriculture Ministry (No. nyhyzx07-041), the Fund for Modern Agro-industry Technology Research System (nyctyx-43-kj06) and the Basic Scientific Program of Chinese Academy of Agricultural Science (No. 2007015).

**REFERENCES**


P.-L. Dai et al.


