Journal of Asia-Pacific Entomology 19 (2016) 589–594

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

The impact of pyriproxyfen on the development of honey bee (*Apis mellifera* L.) colony in field

Yue-Wen Chen [a], Pei-Shan Wu [a], En-Cheng Yang [b], Yu-Shin Nai [a,*], Zachary Y. Huang [c,*]

[a] Department of Biotechnology and Animal Science, National Ilan University, Ilan, Taiwan
[b] Department of Entomology, National Taiwan University, Taipei, Taiwan
[c] Department of Entomology, Michigan State University, East Lansing, MI 48824, USA

ARTICLE INFO

Article history:
Received 3 January 2016
Accepted 7 June 2016
Available online 10 June 2016

Keywords:
Pyriproxyfen
Honey bee
Insect growth regulator
Field test
Royal jelly

ABSTRACT

Pyriproxyfen (PPN) is an insect growth regulator (IGR) that interferes with insect metamorphosis. Although the side effects of PPN on honey bee larval/adult stages have been studied, the risk to honey bee larvae from PPN residue in the environment is still unclear. In this study, we evaluated the impact of PPN on larval honey bees in field colonies by using an *in vivo* feeding assay. Oral toxicity to adult honey bees were determined. Finally, influence on royal jelly production was also examined. For *in vivo* feeding assay, the highest observed PPN treatment caused 67% mortality during pupal stage and in the remaining bees, 62.3% showed abnormal eclosion. Reductions in hatching rate, capping rate and adult emergence rate and increased abnormal eclosion rate were found in the colonies fed with 10 ppm PPN syrup. Oral toxicity test revealed that adult honey bees were less susceptible to PPN. Moreover, PPN reduced not only queen cell acceptance rate but also yield of royal jelly in queen cells. These results indicate that PPN has negative impacts on both larval and adult honey bees and royal jelly production, especially under high PPN concentrations. Since PPN is harmful to the development of honey bee larvae and pupae in the natural environment, the issue of honey bee colony contamination by PPN should be addressed.

Introduction

Honey bee, *Apis mellifera*, is a major pollinator in agricultural systems (Kevan, 1999), and produces valuable products such as honey, royal jelly, and bee pollen. However, honey bees are also exposed to various pesticides when they are collecting nectar and pollen (Kevan, 1975; Crane and Walker, 1983; Lu et al., 2015). Honey bees may be influenced by the pesticide-contaminated nectar and pollen, which is brought back to bee hives from foraging on flowers that have been sprayed with pesticides, or from applications aimed to control pest problems inside the colonies by the beekeepers themselves; larvae, drones and queen will be also influenced by these pesticide-contaminated nectar and pollen (Sanchez-Bayo and Goka, 2014). Therefore, toxicity of various pesticides to honey bees needs to be addressed (Johnson et al., 2010).

Insect growth regulators (IGRs) have been widely used for pest control in past decades for their high potency and selectivity against insects and low toxicity to mammals. Therefore, there is a high potential for residual IGRs to be present in agricultural systems. IGRs such as juvenile hormone (JH) analogs affect the development of target insects by disrupting the molting process and therefore pose a higher risk to larvae than to adults in non-target insects (Tasei, 2001). Exposure to JH analogs can result in death, larval ejection by workers, or deformed larvae and adult honey bees (Tasei, 2001; Thompson et al., 2005, 2007). It has also been reported that methoprene affects behavior and temporal polyethism in adult honey bees (Robinson, 1985, 1987). Residues of IGRs in the field are a potential chronic killer of honey bees (Fourrier et al., 2015).

Pyriproxyfen (PPN) is one of the IGRs that has been widely applied for crop protection against pests from the early 1990s (Dennehy et al., 1996). PPN is classified as a JH analog, which emulates the effects of JH and thus changes JH and ecdysteroid titers in arthropods (Bitondi et al., 1998; Zufelato et al., 2000). Similar to other JH analogs, PPN can affect the homeostasis of hormones in insects and inhibit embryogenesis, egg hatching, metamorphosis and adult eclosion, and resulting in the death of insects (Glancey et al., 1990; Reimer et al., 1991; Miyamoto et al., 1993; Santos et al., 2001).

PPN is considered to be non-toxic to honey bees because LD_{50} of PPN to the honey bee adults is more than 100 μg/bee (WHO, 2001). However it has been demonstrated that both honey bee larval and pupal developments are retarded and adult emergence rate is lowered when honey bee worker larvae were treated with PPN at different stages (Bitondi et al., 1998; Zufelato et al., 2000; Santos et al., 2001; Fourrier et al., 2015).

* Corresponding authors.
E-mail address: ysnai@niu.edu.tw (Y.-S. Nai).

http://dx.doi.org/10.1016/j.aspen.2016.06.005
1226-8615/

A dose response effect is found in the larval development of the PPN treated larvae, including reduced eclosion rate (0.1 ppm), adults with deformed wings (1 ppm), 53% mortality during pupal stage (10 ppm) and 50% mortality rate during larval stage (100 ppm) (Yang et al., 2010). Since PPN is harmful to the development of honey bee larvae and pupae, the safety of PPN on the honey bee is of concern; residual IGRs in the environment can become a chronic damaging factor to honey bee populations and gradually lead to colony collapse.

The aim of this study was to evaluate PPN impacts on the development of honey bee larvae and pupae in the natural environment. We examined the impact of PPN on larval development under field conditions and determined oral and contact toxicity of PPN to adult bees. Finally, effect of PPN on royal jelly production was also examined.

Materials and methods

Honey bees

Twelve healthy honey bee (*Apis mellifera*) colonies were selected as experimental colonies from the NIU apiary (National Ilan University, Taiwan, GPS coordinates: N24.747278, E121.746200). There were nine frames in each experimental colony containing c.a. 30,000 workers and a queen.

Preparation of PPN solution in basic larvae diet (BLD)

PPN (11% emulsifiable concentrate, Sumitomo, Japan) was prepared in four stock solutions between the concentrations of 1 and 1000 mg/kg in double-distilled water (ddH_2O) and stored at 4 °C. These stocks were then diluted to a final concentration between 0.1 and 100 mg/kg in the BLD before used for the following experiments. The BLD was prepared as described by Vandenberg and Shimanuki (1987) and stored at 4 °C and pre-warmed to 35 °C before feeding the larvae.

In vivo feeding method

In vivo feeding method is similar to that of Hanley et al. (2003). Fifty cells containing 1-day-old worker larvae were randomly selected and marked for each treatment. Different concentrations of PPN (0, 0.1, 1, 10 and 100 ppm) were prepared in BLD for this experiment. For each dose, 10, 10 and 20 μL of PPN-containing BLD were pipetted to each cell at day 1, 2 and 3, respectively. Control (0 ppm) larvae received the same amount of BLD but no pesticides were added. This resulted total PPN of 4, 40, 400, and 4000 pg per larva for the four doses. After adding PPN-BLD, the treated frames were returned to the original colonies. The mortality and capping rates of the marked-larvae were assessed at day 7. At day 13, the pupae in the marked area were taken out from capped cells, and put into a 24-well tissue culture plates with double-layer tissue papers (Kimwipes) at the bottom of each well. They were kept in an incubator at 34 °C and 70% RH until emergence. A total of 4 colonies were used; the data were recorded and analyzed using LSD test ($P < 0.05$).

Toxicity testing to honey bee adults

To determine the oral toxicity of PPN on 1-day-old worker bees, 0, 0.1, 1, 10, 100, 1000, and 10,000 ppm PPN were prepared in 50% syrup in 1.5 mL micro-centrifuge tubes. For each treatment, fifty worker bees were collected in a 90-mm-diam plastic container (c.a. 250 mL) and then fed on different concentration PPN-syrup, respectively. Control bees were fed with 50% syrup. All the bees were kept at 25 °C and 70% RH and syrup solution in the micro-centrifuge tubes were replaced daily. The mortality of the honey bees was recorded for 7 days. This experiment was replicated 7 times. The LC_{50} was calculated using Calcusyn (Biosoft, USA).

Toxicity of PPN at colony level

Twelve honey bee colonies, each containing nine frames, were used in this trial. Each colony was then divided into two parts, part A (4 frames) and part B (5 frames), by a queen excluder. The queen was first moved from part A into part B for 3 days, resulting no newly laid eggs in part A. The queen was then moved back to part A for laying eggs for 24 h and a total of 100 eggs were immediately marked in a comb using a piece of transparency. After three days (day 4) post egg laying, the queen was transferred to part B for laying eggs for 24 h and a total of 100 eggs were immediately marked in a comb using a piece of transparency. Queen exchange was repeated for nine times, and 3 days/per exchange. Queen exchanges at day 1, 4, 7, 10, 13, 16, 19, 22, and 25 were designated as group 1 to 9, respectively (Fig. 1).

At day 13, treated colonies were fed 1 kg 50% syrup mixed with 10 or 100 mg/kg PPN (10 and 100 ppm), and the control group was fed on 1 kg 50% syrup only (Fig. 1). There were 4 replicates for each treatment, each with a different colony. The hatching rate was recorded at day 5 (0 to 1-day-old larvae) and capping rate at day 11 (6 to 7-day-old larvae). At day 17 (12 to 13-day-old larvae, pupated), the pupae were extracted and placed in 24-well culture plates with each cell lined with double-layered tissue paper (Kimwipes). All other batches of eggs also followed the same schedule for data collection. The plates were kept in an incubator at 34 °C and 70% RH until emergence to record their eclosion rate.

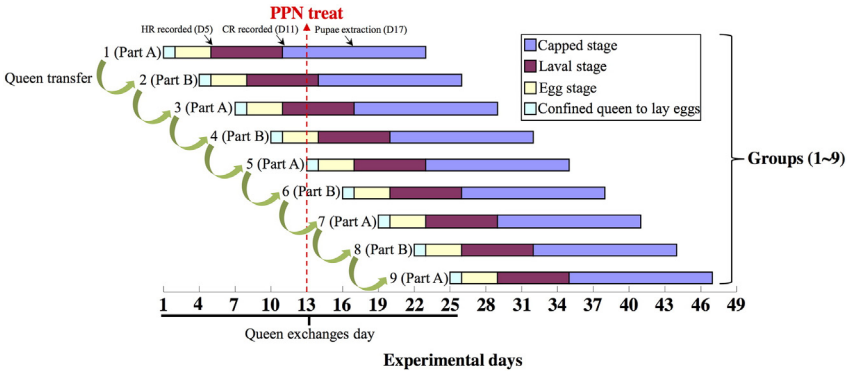

Fig. 1. Timeline for when PPN is started relative to the 9 experimental groups. Each colony was divided into part A and part B. The queen exchanges at day 1, 4, 7, 10, 13, 16, 19, 22, and 25 from part A to part B *vice versa* were designated as group 1 to 9. Different concentrations of PPN were fed at day 13 (red arrow). For each group, the hatching rate (HR) and capping rate (CR) were recorded at day 5 (D5) and day 11 (D11), respectively as arrows indicated. The pupae were extracted at day 17 (D17) as arrow indicated in each group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Effects of continued 3-days feeding of PPN on 1-day-old larvae. To each larval cell, 10, 10 and 20 μL of BLD were added from days 1 to 3, respectively. Each assay contained 25–38 larvae in a colony and 4 colonies were tested. Means ± SD are presented. Different letters in the same column are significantly different by Least Square Difference test ($P < 0.05$) after ANOVA showed a significant effect.

PPN-BLD (ppm)	Larvae No.	Larval development			
		Capping rate (%)	Days to emergence	Eclosion rate (%)	Bees with deformed wings (%)
100	140	0b	–	0	–
10	139	22.2 ± 33.2b	–	0	–
1	140	72.3 ± 17.9a	16.9 ± 0.7a	67.7 ± 17.6a	7.7 ± 5.7a
0.1	136	78.3 ± 17.5a	17.1 ± 0.8a	75.4 ± 22.8a	1.4 ± 2.8b
0 (BLD)	138	78.9 ± 5.4a	17.1 ± 0.8a	78.9 ± 5.4a	0b
Unfed control	122	87.8 ± 9.1a	17.2 ± 0.9a	86.1 ± 7.2a	0.8 ± 1.5b

Assessment of royal jelly yield

One-day-old worker larvae were grafted into queen cells; a total of 64 queen cells in a queen-rearing frame were used for royal jelly production assay. The queen-rearing frame was put into a queen-less part (of the above experiment in the same colonies) for 72 h; the acceptance rate of cells and the yield of royal jelly were recorded. Royal jelly was produced nine times, 3 days for each time as described above (larvae were grafted at day 5, 8, 11, 14, 17, 20, 23, 26, and 29). The experiment was replicated in 4 different colonies.

Statistics

Data were further analyzed using analysis of variance (ANOVA) by SAS. Least Square Difference test ($P < 0.05$) was used to analyze between treatment differences.

Results and discussion

Effects of PPN on honey bee larvae

The two high doses (10 and 100 ppm) caused high mortality during larval stage, so that capping rate was low, with 22.2% and 0%, respectively (Table 1). Both these doses had no enclosed adults so days to emergence and eclosion rates could not be measured. In the other two doses (0.1 and 1 ppm), their capping rates, days to emergence and eclosion rates were not significantly different from 0 (BLD food added) or unfed control' with only a significantly higher percentage of bees with deformed wings in 1 ppm.

Based on the assay, we found honey bee larvae were more sensitive to high concentrations of PPN (10 and 100 ppm). Under these two concentrations of PPN, the treated larvae died at the pupal stage. Effects of PPN were mainly thought to be related to development and physiology. High PPN exposure levels could induce mortality and abnormalities, especially during development (Bitondi et al., 1998; Pinto et al., 2000; Zufelato et al., 2000). These studies also revealed that low concentrations of PPN would cause melanization of pupae. This may be due to increased phenoloxidase activity to regulate melanization and pupation (Bitondi et al., 1998; Zufelato et al., 2000; Santos et al., 2001). However, honey bee larvae in our study were fed with, and may have contact with PPN from the first day of being hatched, which might be one of factors that caused large impacts and lead to a low emergence rates.

It was demonstrated that there was a delay in differentiation of flight muscles in honey bees when honey bee larvae were fed with a PPN contaminated diet (Correa Fernandez et al., 2012). From our data, we also found that adult honey bees appeared with deformed wings and the proportion of deformed winged bees increased with higher doses of PPN (Table 1). This might be due to change hormonal levels during metamorphosis because wings are formed during that critical stage.

Hanley et al. (2003) used syrup to deliver transgenic pollen or a positive control pesticide diazinon and found that workers removed little, if any, of the added food to brood cells. We used BLD instead, but based on the observed positive effect and a dose response, we assumed that nurses also did not remove the artificially added BLD to the brood cells.

Toxicity of PPN to adult honey bees

The results of oral toxicity of adult honey bees showed that only 10,000 ppm and 1000 ppm PPN treatments resulted in obvious increasing mortality, and the LC_{50} of PPN on adult honey bees was around 1000 ppm (Fig. 2). According to previous data of oral and contact toxicity, the LD_{50} of PPN on adult bees was more than 100 μg/bee (WHO, 2001) and is therefore considered as non-toxic to bees. It has previously been reported that bumblebee, *Bombus terrestris*, colonies developed normally after feeding on PPN syrup of 20 ppm (de Wael et al., 1995). In comparison, our data also showed low toxicity to adult honey bees with the LC_{50} oral toxicity on adult bees around 1000 ppm. However, though many pesticides (*e.g.* insect growth regulator) have low toxicity to adult insects, there is still a high risk to larval development (Bitondi et al., 1998; Pinto et al., 2000; Zufelato et al., 2000). Furthermore, it was reported that behavioral changes (high rejection rate and low performance of social tasks) appeared at the adult stage after subjecting the larvae with sub-lethal doses of PPN (Fourrier et al., 2015). This data shows the problems that should be addressed and more effort is required to assess the impact of pesticides on the development of different stages in honey bee colonies and honey bee health.

Toxicity of PPN at colony level

In our field studies, we observed the effects of PPN on the rates of hatching, capping and eclosion to evaluate PPN impact on honey bees in the natural environment (Fig. 3). After the colonies were fed 1 kg syrup with 10 or 100 ppm PPN, a large number of pupae died with black cuticle or by failure to emerge. Some newly emerged adults of 10 ppm treatment survived, however, at 100 ppm PPN treatment, the

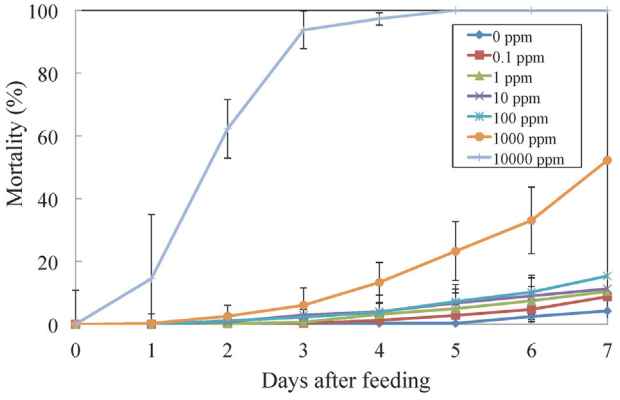

Fig. 2. Mortality of 1-day-old worker bees fed with different concentrations of PPN syrup. Only 10,000 ppm and 1000 ppm PPN treatments resulted in obvious increasing mortality.

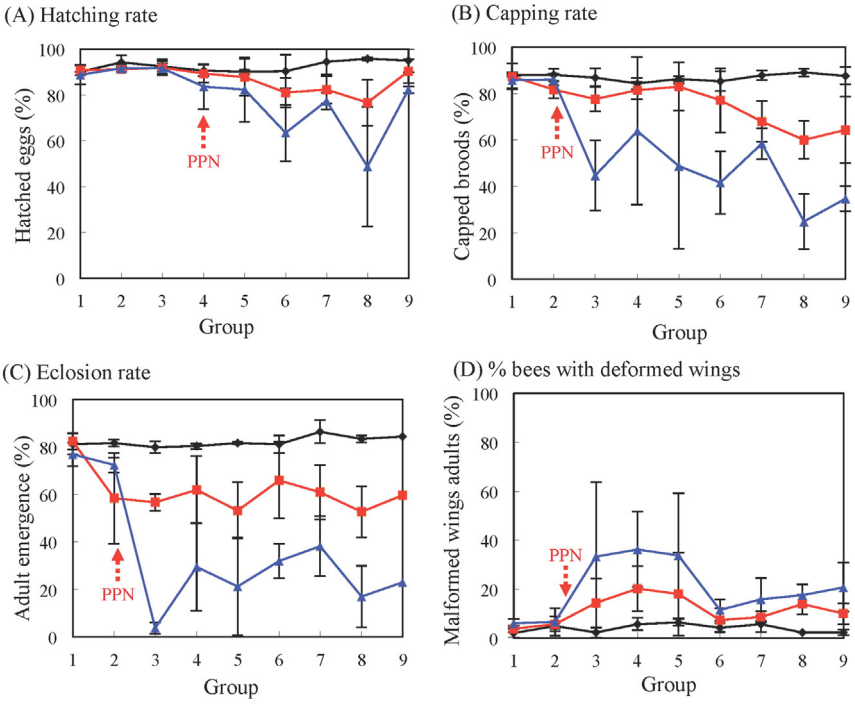

Fig. 3. Development of honey bee larvae before and after feeding 1 kg PPN syrup into tested bee colonies. Egg numbers were recorded every 3 days; 100 eggs of each were labeled and then development of the honey bees was continuously recorded until emergence; A total of nine groups were surveyed in this experiment. (A) Hatching rate; (B) Capping rate; (C) Eclosion rate; (D) % of bees with deformed wing rate; Arrows indicate the time during which PPN may start acting; on bees. Queen exchange at day 1, 4, 7, 10, 13, 16, 19, 22, and 25 were marked as group 1 to 9, respectively; Black line = 0 ppm; Red line = 10 ppm; Blue line = 100 ppm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

capped cells were uncapped and some of the injured pupae were removed from the colony by workers (Fig. 4).

In field studies, egg hatching rates were decreased in both 10 and 100 ppm PPN treatments. Higher dose (100 ppm) PPN treatment reduced hatching rate earlier and in a larger magnitude than the 10 ppm PPN treatment (Fig. 3A). Hatching rate of 100 ppm treated colonies was significantly reduced in groups 6–9, but in 10 ppm treated colonies, only group 7 and 8 showed a reduction (Fig. 3A).

Reduction of larval capping rates followed a similar pattern (Fig. 3B). Capping rates were significantly ($P < 0.05$) reduced starting from group 3 in 100 ppm fed colonies but in 10 ppm colonies, only group 2, 3, 7, 8 and 9 groups were reduced and their reduction was much smaller (Fig. 3B).

Reduction of eclosion rates was also similar to capping rates. Colonies treated with both doses were significantly different in groups 2 to 9. However the reduction is about 2× more in the 100 ppm dose compared to the 10 ppm (Fig. 3C).

Deformed winged bees were found in the newly emerged adult bees of 10 and 100 ppm PPN treatments (Fig. 3D). In the 10 ppm PPN treatment, groups 4 and 9 showed a significant increase ($P < 0.05$) in deformed wing adults. In the 100 ppm treatment, groups 4, 7 and 9 were significantly higher.

The honey bee is a social insect, with the colony being maintained by the workers (Seeley, 1982; Calderone and Page, 1992). Honey bees can be easily poisoned by various pesticides when they are collecting nectar and pollen (Kevan, 1975; Crane and Walker, 1983; Chauzat et al., 2006; Lu et al., 2015) and further influence other members in the colony. Pesticide-contaminated nectar and pollen more heavily affect the larvae, drones and queen (Sanchez-Bayo and Goka, 2014).

In this trial, we fed honey bee colonies with PPN-syrup and observed the development when adult honey bees took the syrup and presumably fed the queen and larvae. It was reported that PPN had low toxicity to non-target insects like honey bees (Sullivan and Goh, 2008) or bumble bees *B. terrestris* (de Wael et al., 1995). However, we found that the

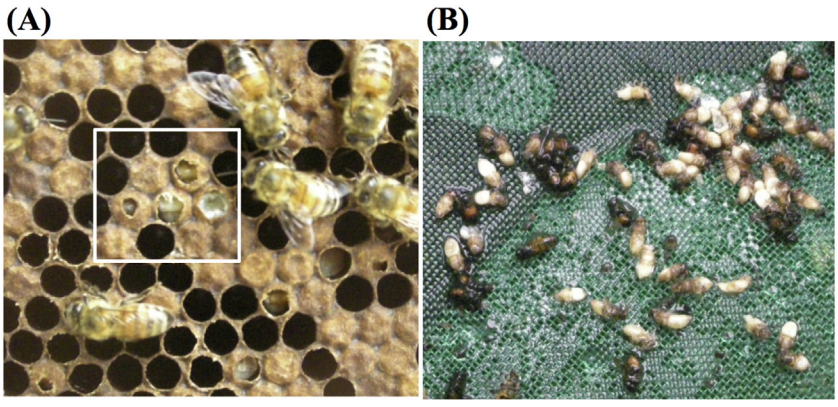

Fig. 4. 100 ppm PPN syrup treated bee colony showed (A) Uncapped cells and presumably deformed pupae; (B) Black and deformed pupae were removed by workers.

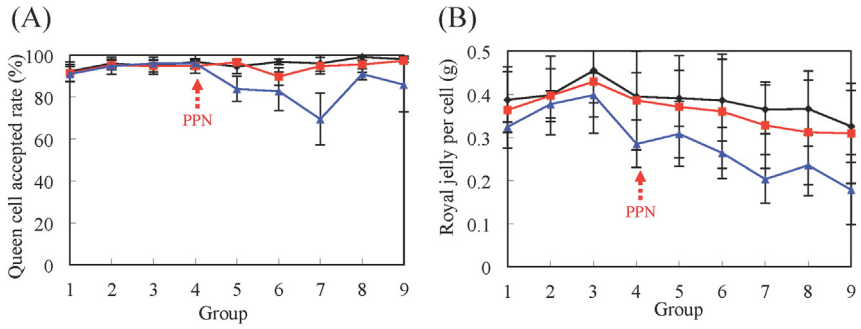

Fig. 5. Effects of 1 kg PPN syrup feeding on the production of royal jelly. (A) Accepted queen cells; (B) Weight of royal jelly per cell; PPN, timing that PPN may start acting. Black line = 0 ppm; Red line = 10 ppm; Blue line = 100 ppm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

development of bee colonies was influenced by feeding PPN syrup to the honey bee colony. In both doses (10 and 100 ppm) we found reductions in hatching rate, capping rate, eclosion rate, and increased rate of deformed winged bees. Even though in group 2, old larvae (last day or two) had opportunity to be exposed to PPN (Fig. 1), it did not show any impact except a reduction of adult eclosion (Fig. 3C). But clearly with the exception of hatching rate, all other parameters started to show a significant difference in the higher dose (100 ppm) starting on group 3 (Fig. 3B, C, D). The PPN showed impact not only on the growth and development of young honey bee larvae, but also a delayed impact on adults. Recently, it has also been reported that PPN impacts on the social behavior in adult honey bees when they have been exposed to PPN at larval stage (Fourrier et al., 2015). Therefore, it should be noted that PPN residue in the environment is harmful to honey bees.

Reduced royal jelly yields

The 100 ppm PPN treatment significantly decreased acceptance rate of queen-cells at groups 5 to 8 (10.5%, 14.1%, 26.6%, and 8.2% respectively, $P < 0.05$). However, there was no significant decrease in the 10 ppm PPN treatment (Fig. 5A). There was also a dramatic reduction in the weight of royal jelly per cell found at 100 ppm PPN treatment (Fig. 5B). The weight of royal jelly per cell decreased from groups 4 to 9 compared to the control ($P < 0.05$) but no reduction was found in the 10 ppm PPN treatment (Fig. 5B).

There are three possible mechanisms for decreased royal jelly production. Royal jelly is secreted from hypopharyngeal glands and mandibular glands of 5 to 15-day-old worker bees and is used for the rearing of all three castes of larvae (Huang et al., 1989). It was reported that JH analog treatments could inhibit hypopharyngeal gland development (Jaycox et al., 1974; Huang et al., 1994) and larval exposure to a high dose of PPN induced a significant negative effect on hypopharyngeal gland development of the resulting adults (Fourrier et al., 2015).

Royal jelly production could also be reduced if PPN treatment reduces vitellogenin levels in workers because of higher JH levels are usually associated with lower levels of vitellogenin (Pinto et al., 2000; Amdam et al., 2003; Nelson et al., 2007). Royal jelly production could also decrease due to the death of larvae and pupae by PPN treatment which eventually results in fewer nurses. Based on our results, it was demonstrated that the production of royal jelly per cell decreased at 100 ppm of PPN treatment. It is possible that that PPN contaminated food from the environment may cause negative effects on not only production of royal jelly, but also affect honey and pollen collection.

Conclusions

In this study, we evaluated the impact of PPN on larval honey bees, adult honey bees and the influence on royal jelly production. *In vivo* PPN feeding showed that the highest concentration of PPN treatment caused pupal death and deformed winged bees. We also found deformed winged bees and reduced rates in hatching, capping, and eclosion in field colonies. Moreover, PPN also reduced the queen-starter-cell acceptance rate and the weight of royal jelly. These results indicate that PPN has negative impacts on both the growth stages from larva to adult honey bees and bee products, especially under high PPN concentrations. Based on our data, PPN has a high risk to the development of honey bee larvae and pupae in the natural environment. The issue of honey bee colony contamination by PPN should be seriously addressed.

Acknowledgements

This research was supported by Grant 101AS-10.3.1-BQ-B1 from the Bureau of Animal and Plant Health Inspection and Quarantine, the Council of Agriculture, Executive Yuan and Grant NSC102-2313-B-197-005 from the National Science Council of Taiwan.

References

Amdam, G.V., Norberg, K., Hagen, A., Omholt, S.W., 2003. Social exploitation of vitellogenin. Proc. Natl. Acad. Sci. U. S. A. 100, 1799–1802.
Bitondi, M.M., Mora, I.M., Simoes, Z.L., Figueiredo, V.L., 1998. The *Apis mellifera* pupal melanization program is affected by treatment with a juvenile hormone analogue. J. Insect Physiol. 44, 499–507.
Calderone, N.W., Page Jr., R.E., 1992. Effects of interactions among genotypically diverse nestmates on task specialization by foraging honey bees (*Apis mellifera*). Behav. Ecol. Sociobiol. 30, 219–226.
Chauzat, M.P., Faucon, J.P., Martel, A.C., Lachaize, J., Cougoule, N., Aubert, M., 2006. A survey of pesticide residues in pollen loads collected by honey bees in France. J. Econ. Entomol. 99, 253–262.
Correa Fernandez, F., Da Cruz-Landim, C., Malaspina, O., 2012. Influence of the insecticide pyriproxyfen on the flight muscle differentiation of *Apis mellifera* (Hymenoptera, Apidae). Microsc. Res. Tech. 75, 844–848.
Crane, E., Walker, P., 1983. The Impact of Pest Management on Bees and Pollination. IBRA, Cardiff, UK.
de Wael, L., De Greef, M., van Laere, O., 1995. Toxicity of pyriproxyfen and fenoxycarb to bumble bee brood using a new method for testing insect growth regulators. Agric. Res. 34, 3–8.
Dennehy, T.J., Ellsworth, P.C., Nichols, R.L., 1996. The 1996 whitefly resistance management program for Arizona cotton. University of Arizona IPM Vol. 8, p. 16.
Fourrier, J., Deschamps, M., Droin, L., Alaux, C., Fortini, D., Beslay, D., Le Conte, Y., Devillers, J., Aupinel, P., Decourtye, A., 2015. Larval exposure to the juvenile hormone analog pyriproxyfen disrupts acceptance of and social behavior performance in adult honey bees. PLoS One 10, e0132985.
Glancey, B.M., Reimer, N., Banks, W.A., 1990. Applied Myrmecology: A World Perspective. Westview Press.
Hanley, A.V., Huang, Z.Y., Pett, W.L., 2003. Effects of dietary transgenic Bt corn pollen on larvae of *Apis mellifera* and *Galleria mellonella*. J. Apic. Res. 42 (4), 77–81.
Huang, Z.Y., Otis, G.M., Teal, P.E.A., 1989. Nature of brood signal activating the protein synthesis of hypopharyngeal gland in honey bees, *Apis mellifera* (Apidae: Hymenoptera). Apidologie 20, 455–464.
Huang, Z.Y., Robinson, G.E., Borst, D.W., 1994. Physiological correlates of division of labor among similarly aged honey bees. J. Comp. Physiol. A. 174, 731–739.
Jaycox, E.R., Skowronek, W., Guynn, G., 1974. Behavioral changes in worker honey bees (*Apis mellifera*) induced by injections of juvenile hormone mimic. Ann. Entomol. Soc. Am. 67, 529–534.
Johnson, R.M., Ellis, M.D., Mullin, C.A., Frazier, M., 2010. Pesticides and honey bee toxicity-USA. Apidologie 41, 312–331.
Kevan, P.G., 1975. Forest application of the insecticide fenitrothion and its effect on wild bee pollinators (Hymenoptera: Apoidea) of lowbush blueberries (*Vaccinium* SPP.) in Southern New Brunswick, Canada. Biol. Conserv. 7, 301–309.

Kevan, P.G., 1999. Pollinators as bioindicators of the state of the environment: species, activity and diversity. Agric. Ecosyst. Environ. 74, 373–393.
Lu, C., Chang, C.H., Lin, T., Chen, M., 2015. Distributions of neonicotinoid insecticides in the Commonwealth of Massachusetts: a temporal and spatial variation analysis for pollen and honey samples. Environ. Chem. http://dx.doi.org/10.1071/EN15064 (Published online 24 July 2015).
Miyamoto, J., Hirano, M., Takimoto, Y., Hatakoshi, M., 1993. Insect growth regulators for pest control, with emphasis on juvenile hormone analogs. ACS Symposium Series, pp. 144–168.
Nelson, C.M., Ihle, K.E., Fondrk, M.K., Page, R.E., Amdam, G.V., 2007. The gene vitellogenin has multiple coordinating effects on social organization. PLoS Biol. 5, e62.
Pinto, L.Z., Bitondi, M.M., Simoes, Z.L., 2000. Inhibition of vitellogenin synthesis in *Apis mellifera* workers by a juvenile hormone analogue, pyriproxyfen. J. Insect Physiol. 46, 153–160.
Reimer, N.J., Glancy, B.M., Beardsley, J.W., 1991. Development of *Pheidole megacephala* (Hymenoptera: Formicidae) colonies following ingestion of fenoxycarb and pyriproxyfen. J. Econ. Entomol. 84, 56–60.
Robinson, G., 1985. Effects of a juvenile hormone analogue on honey bee foraging behaviour and alarm pheromone production. J. Insect Physiol. 31, 277–282.
Robinson, G., 1987. Regulation of honey bee age polyethism by juvenile hormone. Behav. Ecol. Sociobiol. 20, 329–338.
Sanchez-Bayo, F., Goka, K., 2014. Pesticide residues and bees-a risk assessment. PLoS One 9, e94482.
Santos, A.E., Bitondi, M.M., Simoes, Z.L., 2001. Hormone-dependent protein patterns in integument and cuticular pigmentation in *Apis mellifera* during pharate adult development. J. Insect Physiol. 47, 1275–1282.
Seeley, T.D., 1982. Adaptive significance of the age polyethism schedule in honey bee colonies. Behav. Ecol. Sociobiol. 11, 287–293.
Sullivan, J.J., Goh, K.S., 2008. Environmental fate and properties of pyriproxyfen. J. Pestic. Sci. 33, 339–350.
Tasei, J.N., 2001. Effects of insect growth regulators on honey bees and non-*Apis* bees. A review. Apidologie 32, 527–545.
Thompson, H.M., Wilkins, S., Battersby, A.H., Waite, R.J., Wilkinson, D., 2005. The effects of four insect growth-regulating (IGR) insecticides on honey bee (*Apis mellifera* L.) colony development, queen rearing and drone sperm production. Ecotoxicology 14, 757–769.
Thompson, H.M., Wilkins, S., Battersby, A.H., Waite, R.J., Wilkinson, D., 2007. Modelling long-term effects of IGRs on honey bee colonies. Pest Manag. Sci. 63, 1081–1084.
Vandenberg, J.D., Shimanuki, H., 1987. Technique for rearing worker honey bees in the laboratory. J. Apic. Res. 26, 90–97.
WHO, 2001. Pyriproxyfen-Acute Contact and Oral Toxicity Tests with Honey Bees (*Apis mellifera*).
Yang, E.C., Wu, P.S., Chang, H.C., Chen, Y.W., 2010. Effect of sub-lethal dosages of insecticides on honey bee behavior and physiology. Proceedings of International Seminar on Enhancement of Functional Biodiversity Relevant to Sustainable Food Production in ASPAC, Tsukuba, Japan.
Zufelato, M.S., Bitondi, M.M., Simoes, Z.L., Hartfelder, K., 2000. The juvenile hormone analog pyriproxyfen affects ecdysteroid-dependent cuticle melanization and shifts the pupal ecdysteroid peak in the honey bee (*Apis mellifera*). Arthropod Struct. Dev. 29, 111–119.