3.12 Comparative chronic toxicity of three neonicotinoids on New Zealand packaged honey bees

Sarah C. Wood, Ivanna V. Kozii, Roman V. Koziy, Tasha Epp, Elemir Simko

Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada

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Abstract

Thiamethoxam, clothianidin, and imidacloprid are the most commonly used neonicotinoid insecticides on the Canadian prairies. There is widespread contamination of nectar and pollen with neonicotinoids, at concentrations which are sublethal for honey bees (*Apis mellifera* Linnaeus). We compared the effects of chronic, sublethal exposure to the three most commonly used neonicotinoids on honey bee colonies established from New Zealand packaged bees using colony weight gain, brood area, and population size as measures of colony performance. From May 7 to July 29, 2016 (12 weeks), sixty-eight colonies received weekly feedings of sugar syrup and pollen patties containing 0, 20 (median environmental dose), or 80 (high environmental dose) nM of one of three neonicotinoids (thiamethoxam, clothianidin, and imidacloprid). Colonies were weighed at three week intervals. There was a significant negative effect ($P<0.01$) on colony weight gain (honey production) after 9 and 12 weeks of exposure to 80 nM neonicotinoids and on cluster size ($P<0.05$) after 12 weeks. A significant effect of neonicotinoid exposure was not observed for brood area or number of adult bees, but these analyses lacked adequate (>80%) statistical power due to marked variation within treatment groups. Thus, continued reliance on colony-level parameters such as brood area and population size for pesticide risk assessment may not be the most sensitive method to detect sublethal effects of neonicotinoids on honey bees.

Reference

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3.13 Tank mixtures of insecticides and fungicides, adjuvants, additives, fertilizers and their effects on honey bees after contact exposure in a spray chamber

Anna Wernecke*, Malte Frommberger, Abdulrahim Alkassab, Jakob H. Eckert, Ina P. Wirtz, Jens Pistorius

Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Bee Protection, Messeweg 11-12, 38104 Braunschweig, Germany
*corresponding author: anna.wernecke@julius-kuehn.de

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Abstract

In agriculture honey bees may be exposed to multiple pesticides. In contrast to single applications of plant protection products (PPP), the effects of tank mixtures of two or more PPP on honey bees are not routinely assessed in the risk assessment of plant protection products. However, tank mixes are often common practice by farmers. Mixtures of practically non-toxic substances can lead to synergistic increase of toxic effects on honey bees, observed for the first time in 19921 in combinations of pyrethroids and azole fungicides. 2004 Iwasa et al. already reported that ergosterol-biosynthesis-inhibiting (EBI) fungicides strongly increase the toxicity of neonicotinoids in laboratory for the contact exposure route. Furthermore, in agricultural practice additives, adjuvants and fertilizers may be added to the spray solution. For these additives usually no informations on potential side effects on bees are available when mixed with plant protection products. Therefore, it is considered necessary to investigate possible additive or synergistic impacts and evaluate potentially critical combinations to ensure protection of bees. Here, we investigated the effects on bees of combinations of insecticides, fungicides and fertilizers under controlled laboratory conditions. A spray chamber was used to evaluate effects following contact exposure by typical field application rates. Subsequently, mortality and behaviour of bees were monitored for at least 48 h following the OECD acute contact toxicity test 2143. Dependencies of synergistic effects and the time intervals between the applications of the mixing partners were evaluated.

Introduction

In agriculture the use of tank mixtures containing two or more mixing partners (e.g. insecticides, fungicides, growth regulators, bonding agents or fertilizers) in bee-attractive crops like oilseed rape or fruit production is common practice. It allows farmers to reduce the amount of work, to be more costs efficient and to extend the spectrum of pests, which can be controlled with one application. For most tank mixtures no negative side effects to bees are known and the use is legally permitted if all label instructions are obeyed. However, some combinations cause additive or synergistic effects like mixtures of certain insecticides and EBI-fungicides1,2,4. Nevertheless, tank mixing effects are so far not systematically investigated and for newer substances no information on potential synergism is available. To detect combinations which result in synergistic or additive effects on honey bees in the laboratory, a simple and potentially more field realistic application method, compared to OECD 214 procedure was developed. It considers the use of an application chamber to simulate a field realistic contact exposure and assessments of side effects on honey bees following OECD Guideline 2143.

Materials and methods

In order to evaluate critical combinations systematically, the Institute for bee protection (JKI) established an application method in the laboratory and tested several tank mixtures with regards to their contact toxicity. For the tests, honey bees (Apis mellifera L., Buckfast) were taken from the honey chamber one day before application. The bees were briefly anesthetized by CO2 and transferred into cages (overnight acclimatisation period). Each cage contained 10 bees (≥ 3 replicates) and was monitored under controlled conditions (24°C, 50-70 % relative humidity, darkness). Feeding was conducted ad libitum with sucrose solution. Two hours before application the bees were cooled down (4°C) until immobilization. For each treatment (test substances Tab. 1) the bees were placed on petri dishes in the application chamber and sprayed by standard nozzles as used by farmers. This application method provides a more realistic exposure scenario compared to the standard procedure for contact testing following OECD Guideline 2143. Subsequently, behaviour and mortality were monitored for at least 48 h. Thereby this method allows a comparative and quick screening process (Fig. 1). Fisher's exact test (SPSS, Chicago, IL, USA) was used to evaluate the mortality between the control and treatments ($p<0.05$).

Tab. 1 Test substances (TS).

TS	Trade name	Type	Aktive substance (a.s./l or kg)	Application rate/ha
TS1	Biscaya*	Insecticide	Thiacloprid (240 g/l)	0.3 l
TS2	Cantus Gold*	Fungicide	Boscalid (200 g/l), Dimoxystrobin (200 g/l)	0.5 l
TS3	Solubor DF	Fertilizer	Boron 17.5 % as sodium borate	3.0 kg
TS4	Bor 150	Fertilizer	Boron 11.0 % as boron ethanolamine	3.0 l
TS5	Mirage 45 EC*	Fungicide	Prochloraz (450 g/l)	1.5 l
TS6	Folicur*	Fungicide	Tebuconazole (250 g/l)	1.5 l
TS7	Matador*	Fungicide	Tebuconazole (225 g/l), Triadimenol (75 g/l)	1.5 l
TS8	Karate Zeon*	Insecticide	Lambda-cyhalothrin (100 g/l)	0.075 l
TS9	Efilor*	Fungicide	Metconazole (60 g/l), Boscalid (133 g/l)	1.0 l

*classified as non-hazardous to bees up to maximum application rate as stated for authorisation

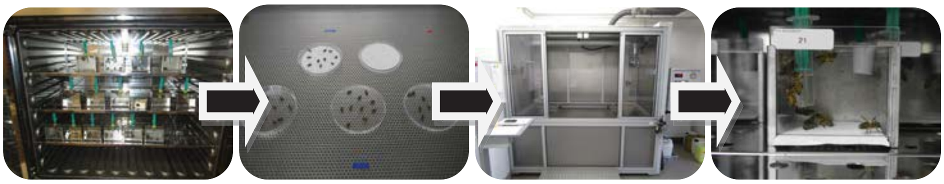

Fig. 1 Application process.

Results

Tank mixes containing boron fertilizers

Single applications of thiacloprid, boscalid, dimoxystrobin natriumborat or borethanolamin at the maximum permitted application rates had no adverse effects on bee mortality. The combination of thiacloprid and boscalid and dimoxystrobin or the addition of boron fertilizers to the spray solution did not increase the mortality or cause other apparent impairments such as behavioural abnormalities (**Fig. 2**).

Tank mixes containing neonicotinoids and EBI-fungicides

Single applications of thiacloprid, prochloraz, tebuconazole and triadimenol at the maximum permitted application rates showed no adverse effects on bee mortality following contact exposure. In contrast, tank mixtures containing thiacloprid and EBI-fungicides caused strong synergistic effects on survival capability of bees within 48 hours. All three combinations showed significant differences compared to control (**Fig. 3**).

Time interval between pyrethroids and EBI-fungicides

Single applications of tebuconazole and metconazole at the maximum permitted application rates showed no adverse effects on bee mortality following contact exposure. In contrast, a single application of lambda-cyhalothrin did induce a significant enhancement in mortality. As expected a combination of lambda-cyhalothrin and the EBI-fungicides tebuconazole and metconazole caused significant synergistic effects. A time interval of 24 h between solo applications of lambda-cyhalothrin and the EBI-fungicides did not result in an attenuation of synergistic effects (**Fig. 4**).

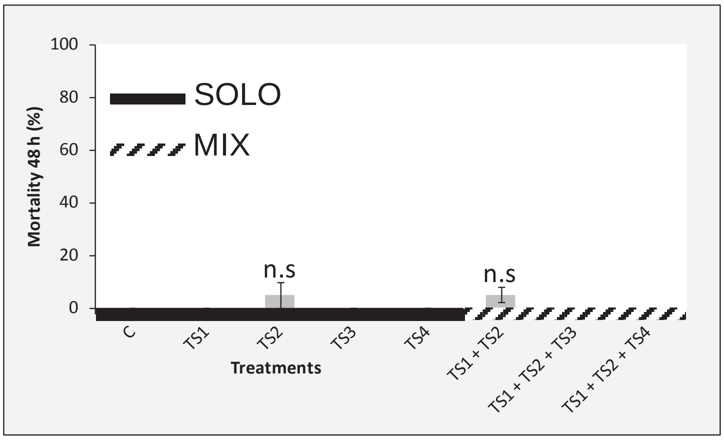

Fig. 2 Bee mortality (48 h) after solo- and tank mixture application in application chamber for thiacloprid, boscalid, dimoxystrobin and boron fertilizer (N=4; n=40). Bars indicate the mean ±SE. Fisher's exact test, $p<0.05$.

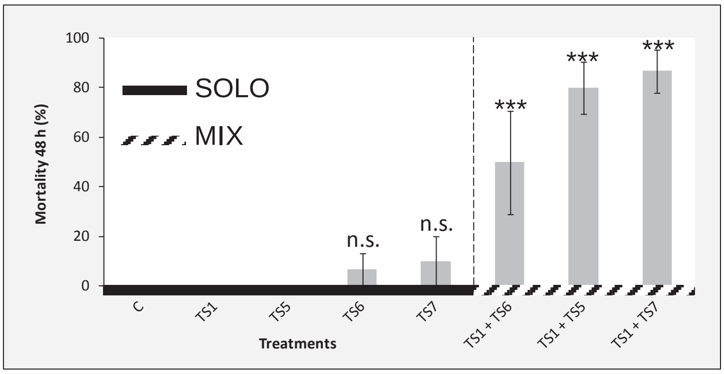

Fig. 3 Bee mortality (48 h) after solo- and tank mixture application in application chamber for thiacloprid and EBI-fungicides (N≥3; n≥30). Bars indicate the mean ±SE. Asterisks indicate significant differences compared to control. Fisher's exact test, p<0.05.

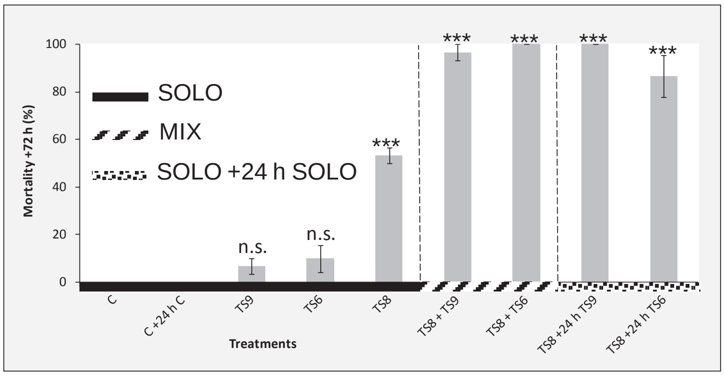

Fig. 4 Time interval (24 h) between solo application of lambda-cyhalothrin and EBI-fungicides caused similar effects as tank mixtures (N=3; n=30). Bars indicate the mean ±SE. Asterisks indicate significant differences compared to control. Fisher's exact test, p<0.05.

Conclusion

The laboratory trials demonstrated that tank mixtures do not generally cause an increase in bee mortality. However, combinations of thiacloprid with ergosterol biosynthesis inhibiting fungicides and combinations of lambda-cyhalothrin with EBI-fungicides caused significant synergistic impacts. While the biochemical mechanisms of these synergistic effects are known to be related to the inhibition of P450-mediated detoxification2,5, the level of effect is determined by the mixing partners, their nature and dosing6. This indicates that the likelihood of synergisms needs to be reflected in the course of the registration of new plant protection products or increases of application rates of already registered plant protection products which are classified as non-hazardous to bees. In conclusion, this method has proven to be effective for screening processes of wide ranges of combinations to evaluate contact toxicities under laboratory conditions and to identify combinations of concern to be further tested in higher tier semi-field and field trials. Furthermore, effects from sequential applications were investigated which are likely to result in additional risk mitigation measures and the establishment of appropriate waiting periods between single applications of insecticide-insecticide or insecticide-fungicide combinations.

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462

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Pieter A. Oomen, Jens Pistorius (Editors)

Hazards of pesticides to bees

13th International Symposium of the ICP-PR Bee Protection Group

18. - 20. October 2017, València (Spain)

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2nd Symposium, Hohenheim, Germany, 1982
3rd Symposium, Harpenden, UK, 1985
4th Symposium, Řež, Czech Republic, 1990
5th Symposium, Wageningen, the Netherlands, 1993
6th Symposium, Braunschweig, Germany, 1996
7th Symposium, Avignon, France, 1999
8th Symposium, Bologna, Italy, 2002
9th Symposium, York, UK, 2005
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Group photo of all symposium participants, standing in front, from left:

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Job & Margreet van Praagh with award,
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Foto

Pieter A. Oomen (Bumble bee *Bombus lapidarius* on thistle)

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