Journal of Invertebrate Pathology 118 (2014) 18–19

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

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

Short Communication

Thiacloprid–*Nosema ceranae* interactions in honey bees: Host survivorship but not parasite reproduction is dependent on pesticide dose

Gina Retschnig *, Peter Neumann, Geoffrey R. Williams

Agroscope, Swiss Bee Research Centre, Bern, Switzerland
Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

ARTICLE INFO

Article history:
Received 20 November 2013
Accepted 19 February 2014
Available online 1 March 2014

Keywords:
Honey bee
Apis mellifera
Nosema ceranae
Synergism
Thiacloprid
Neonicotinoid

ABSTRACT

Interactions between stressors contribute to the recently reported increase in losses of honey bee colonies. Here we demonstrated that a synergistic effect on mortality by the low toxic, commonly used neonicotinoid thiacloprid and the nearly ubiquitous gut parasite *Nosema ceranae* is dependent on the pesticide dose. Furthermore, thiacloprid had a negative influence on *N. ceranae* reproduction. Our results highlight that interactions among honey bee health stressors can be dynamic and should be studied across a broader range of combinations.

Losses of honey bee (*Apis mellifera*) colonies have been attributed to interactions between environmental stressors such as parasites and agricultural chemicals. Recent data suggest that interactions between the nearly ubiquitous gut parasite *Nosema ceranae* and neonicotinoid insecticides are dynamic and are influenced by exposure dose, time, and sequence (Alaux et al., 2009; Aufauvre et al., 2012; Vidau et al., 2011). So far these studies have only investigated neonicotinoids that are highly toxic to honey bees, despite other relatively low-toxic agrochemicals also prevalent in the environment. To fill part of this knowledge gap, we employed thiacloprid, a commonly applied neonicotinoid that was shown to elicit a synergistic effect on honey bee mortality when combined with *N. ceranae* (Vidau et al., 2011), to investigate the relationship between exposure dose of a low-toxic neonicotinoid and *N. ceranae* in honey bees.

Freshly emerged workers from four local colonies (predominantly *Apis mellifera carnica*) were randomly assigned to six treatments (control, thiacloprid$_{high}$, thiacloprid$_{low}$, *N. ceranae*, *N. ceranae* + thiacloprid$_{high}$ and *N. ceranae* + thiacloprid$_{low}$), distributed into plastic cages ($n = 4$ cages per treatment, 20 workers each), and group fed with either *N. ceranae* spore (100,000 spores/worker in 1.5 ml of 50% (weight/volume) sucrose solution) or control suspensions (1.5 ml of 50% sucrose solution). Cages were maintained in darkness at 30 °C and ⩾65% RH (Williams et al., 2013) for 14 days, provided with 50 % (w/v) sucrose solution containing either no thiacloprid, 60 μg/g (=60 ppm or 70 mg/L; thiacloprid$_{high}$), or 30 μg/g thiacloprid (=30 ppm or 35 mg/L; thiacloprid$_{low}$) *ad libitum*. Every second day mortality and food consumption was recorded, and dead workers were removed. At 14 days all surviving bees were frozen at −20 °C and used for *N. ceranae* quantification ($n = 20, 16, 15, 18, 8, 18$ for Control, thiacloprid$_{high}$, thiacloprid$_{low}$, *N. ceranae*, *N. ceranae* + thiacloprid$_{high}$ and *N. ceranae* + thiacloprid$_{low}$ treatments) following Fries et al. (2013). Thiacloprid residues were confirmed in pooled samples ($n = 20$ bees/treatment) at the USDA National Science Laboratory, Gastonia, USA following Mullin et al. (2010). Survival analyses were conducted using censored Kaplan Meier Log-Rank in SPSS 19 and synergistic interactions were assessed using χ^2-tests (Morales-Rodriguez and Peck, 2009). Food consumption and *N. ceranae* data were square-root transformed to improve fit to normality, and compared among groups using ANOVA and the Tukey HSD test in R.

Average food consumption did not differ among treatments that received thiacloprid, regardless of dose (all p-values > 0.13). Control and *N. ceranae* + thiacloprid$_{high}$ treatments showed significantly lower and higher honey bee mortality, respectively, than all other treatments (Kaplan–Meier Log-Rank, all p-values < 0.001); no significant differences were observed among these

* Corresponding author.
E-mail address: gina.retschnig@gmail.com (G. Retschnig).

http://dx.doi.org/10.1016/j.jip.2014.02.008
0022-2011/

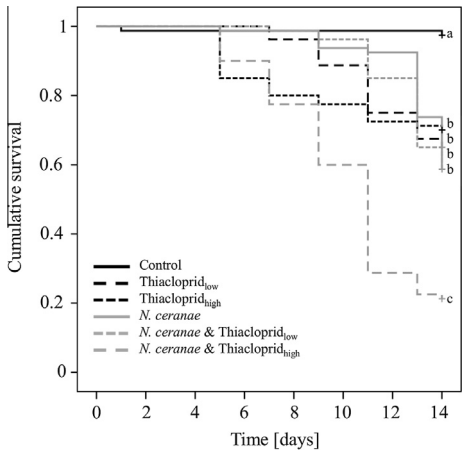

Fig. 1. Cumulative survival of worker honey bees exposed to various combinations of thiacloprid and *Nosema ceranae*. Significant differences ($p < 0.05$) among treatments are indicated by different letters.

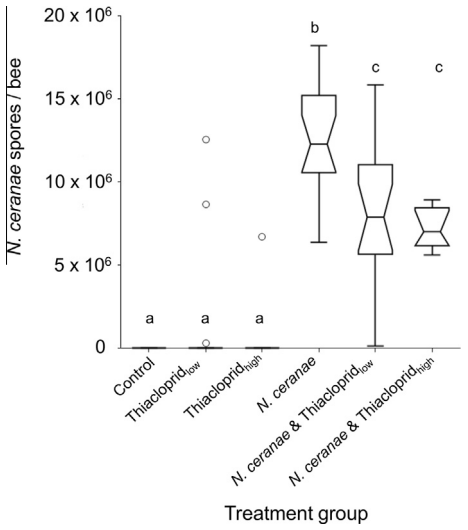

Fig. 2. *Nosema ceranae* spores per bee at day 14 in surviving workers exposed to various combinations of thiacloprid and *Nosema ceranae* displayed as boxplots. Significant differences ($p < 0.05$) among treatments indicated by different letters.

latter-mentioned treatments (all p-values $\geqslant 0.43$; Fig. 1). Challenge by *N. ceranae* + thiacloprid$_{high}$ induced a synergistic effect compared to the sum of effects by *N. ceranae*-only and thiacloprid$_{high}$-only treatments ($\chi^2 = 6.71$, theoretical $\chi^2 = 6.635$, df = 1, $p = 0.001$).

These data suggest that a synergistic effect of *N. ceranae* and thiacloprid on bee survivorship is dose-dependent; only the higher thiacloprid dose elicited such a response. This contrasts reported synergistic effects by *N. ceranae* and thiacloprid (Vidau et al., 2011) at a pesticide dose (5.1 mg/L) much lower than the ones used here (35 and 70 mg/L). Thiacloprid doses for this experiment were chosen to highlight possible interactions between low toxic pesticides and parasites. It is possible that differential induction of detoxification enzymes according to toxic metabolite level (Suchail et al., 2000) can account for this biphasic mortality pattern, or that immunity of older workers used by Vidau et al. (2011) (exposure to *N. ceranae* and thiacloprid 5 and 15 days post-emergence, respectively) differed from the younger workers used here (exposure to *N. ceranae* and thiacloprid 1 and 2 days post-emergence, respectively). Alaux et al. (2009) reported a dose-dependent synergism between *N. ceranae* and imidacloprid on worker longevity, however, an additive effect on longevity during simultaneous low pesticide exposure and parasite infection was observed while none was observed here. This difference could be because honey bee metabolism and detoxification pathways differ among pesticide substances; the P450 enzyme group appears important for thiacloprid but not imidacloprid (Iwasa et al., 2004).

Quantification of *N. ceranae* spores revealed a significantly higher spore intensity in surviving workers from the *N. ceranae* only treatment compared to those of *N. ceranae* + thiacloprid$_{high}$ and *N. ceranae* + thiacloprid$_{low}$ treatments (both p-values < 0.05; Fig. 2), indicating a negative effect on *N. ceranae* reproduction in surviving workers regardless of pesticide dose. No differences were detected between the groups that were exposed to both *N. ceranae* and thiacloprid ($p = 1.00$; Fig. 2). This contrasts with Vidau et al. (2011), whereby *N. ceranae* reproduction was promoted. This might be due to differential allocation of detoxification and disease resistance resources (e.g. Alaux et al., 2009 vs. Pettis et al., 2012). Because reduction enzymes and reactive oxygen species play a vital role in host detoxification and immunity (James and Xu, 2012), it is possible that their induction during higher exposure to thiacloprid doses compared to Vidau et al. (2011) promoted defence against *N. ceranae*, or simply created unsuitable conditions for parasite development. A further explanation may be differences between dying and surviving bees. Follow-up studies are therefore required to investigate these dynamic interactions under a broad range of concentrations and substances, as well as by assessing the development of *N. ceranae* during the entire period of pesticide exposure.

Acknowledgments

We thank Patrick Obrist, Marion Mehmann and Sandra Lanz for laboratory assistance. Funding was provided by the EU 7th Framework Programme BEE DOC (BEes in Europe and the Decline Of Colonies, 244956 CP-FP), and the Ricola and Vinetum foundations.

References

Alaux, C., Brunet, J.L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M., Brillard, J., Baldy, A., Belzunces, L.P., Le Conte, Y., 2009. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environ. Microbiol. 12, 774–782.

Aufauvre, J., Biron, D.G., Vidau, C., Fontbonne, R., Roudel, M., Diogon, M., Vigues, B., Belzunces, L.P., Delbac, F., Blot, N., 2012. Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honeybee. Sci. Rep. 2, 1–7.

Fries, I., Chauzat, M.P., Chen, Y.P., Doublet, V., Genersch, E., Gisder, S., Higes, M., McMahon, D.P., Martín-Hernández, R., Natsopoulou, M., Paxton, R.J., Tanner, G., Webster, T.C., Williams, G.R., 2013. Standard methods for Nosema research. J. Apic. Res. 52, 1–28.

Iwasa, T., Motoyama, N., Ambrose, J.T., Roe, R.M., 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop Prot. 23, 371–378.

James, R.R., Xu, J., 2012. Mechanisms by which pesticides affect insect immunity. J. Inv. Pathol. 109, 175–182.

Morales-Rodriguez, A., Peck, D.C., 2009. Synergistic interactions between biological and neonicotinoid insecticides for the curative control of the white grubs *Amphimallon majale* and *Popillia japonica*. Biol. Control 51, 169–180.

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., Pettis, J.S., 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS ONE 5, e9754.

Pettis, J., vanEngelsdorp, D., Johnson, J., Dively, G., 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. Naturwissenschaften 99, 153–158.

Suchail, S., Guez, D., Belzunces, L.P., 2000. Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. Environ. Toxicol. Chem. 19, 1901–1905.

Vidau, C., Diogon, M., Aufauvre, J., Fontbonne, R., Viguès, B., Brunet, J.L., Texier, C., Biron, D.G., Blot, N., El Alaoui, H., Belzunces, L.P., Delbac, F., 2011. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. PLoS ONE 6, e21550.

Williams, G.R., Alaux, C., Costa, C., Csáki, T., Doublet, V., Eisenhardt, D., Fries, I., Kuhn, R., McMahon, D.P., Medrzycki, P., Murray, T.E., Natsopoulou, M.E., Neumann, P., Oliver, R., Paxton, R.J., Pernal, S.F., Shutler, D., Tanner, G., van der Steen, J.J.M., Brodschneider, R., 2013. Standard methods for maintaining adult *Apis mellifera* in cages under in vitro laboratory conditions. J. Apic. Res. 52, 1–36.