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Underestimated adverse effects of entomopathogenic nematodes on honey bees

Angélique Rüfenacht[1], Lars Straub[1], Daniela Grossar[2], Lukas Jeker[2]
1 Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, CH
2 Swiss Bee Research Centre, Agroscope, Bern, CH

Introduction

There is much interest in finding sustainable plant protection products to safeguard biodiversity and our ecosystem. Entomopathogenic nematodes (EPNs) have received considerable attention as alternative biological-control agents to conventional synthetic agrochemicals (Erler et al., 2022). EPNs live parasitically and are mainly applied as soil treatments or foliar sprays where they infect various insect pests (Labaude & Griffin, 2018). However, as nematodes are considered natural enemies, authorities are faced to approve commercial products based on limited or no data (EU Commission, 2001). Here, we assess whether foliar application of a commerical EPN can pose a risk to honey bees, *Apis mellifera*.

Methods

Under laboratory conditions (**Fig.1A**), newly emerged worker honey bees and greater wax moth (*Galleria mellonella*) larvae were exposed to either dry or wet spray residues on foliage at a field-realistic low (0.25 Mio/m^2) and high (0.5 Mio/m^2) concentrations of *Steinernema carpocapsae* colonised with the bacteria *Xenorhabdus spp*. Three replicates of each of the following experimental groups were made: Direct overspray wax moth larvae (wet), Dried residue wax moth larvae (dry), Direct overspray honey bees (wet), Dried residue honey bees (dry)) per Nematode concentration (low & high) and Controls. Mortality was assessed over 96h and nematode reproduction (i.e., total number offspring) was evaluated for all dead individuals (**Fig. 1B&C**). Generalized linear regression models (GLMs) were applied to analyse that data using STATA 17 statistical software.

Results

EPN exposure resulted in an 80% increase in wax moth larval mortality (p<0.001; **Fig. 2A**). Honey bee mortality was significantly affected by EPN exposure (p<0.001; **Fig. 2B**), however the effect was dose-independent. Both low and high direct overspray lead to a significant decrease in survival of ~55% (p<0.001) where as the dry high and low did not significantly differ from the control treatment groups (p>0.3; **Fig. 2B**). Nematode reproduction was significantly higher in wax moths than in honey bees (p<0.001). Irrespective of the treatment group, mean nematode reproduction per wax moth larvae and honey bee was 1,127 and 41, respectively; representing a 27-fold increase in wax moths. (**Fig. 2C&D**). In honey bees, the high treatment groups lead to a significant increase in nematode reproduction compared to the low exposure (p's<0.05; **Fig. 2D**); where the high wet treatment significantly differed from all the remaining treatments showing the highest nematode counts (p<0.01; **Fig. 2D**).

Fig. 1 A laboratory cage designed simulating EPN overspray application on bees (**A**). Nematode infection and quantification was assessed 15 days post exposure. Nematodes were found on (e.g. tarsus-claw) and replicating in honey bees (White traps) (**B, C**).

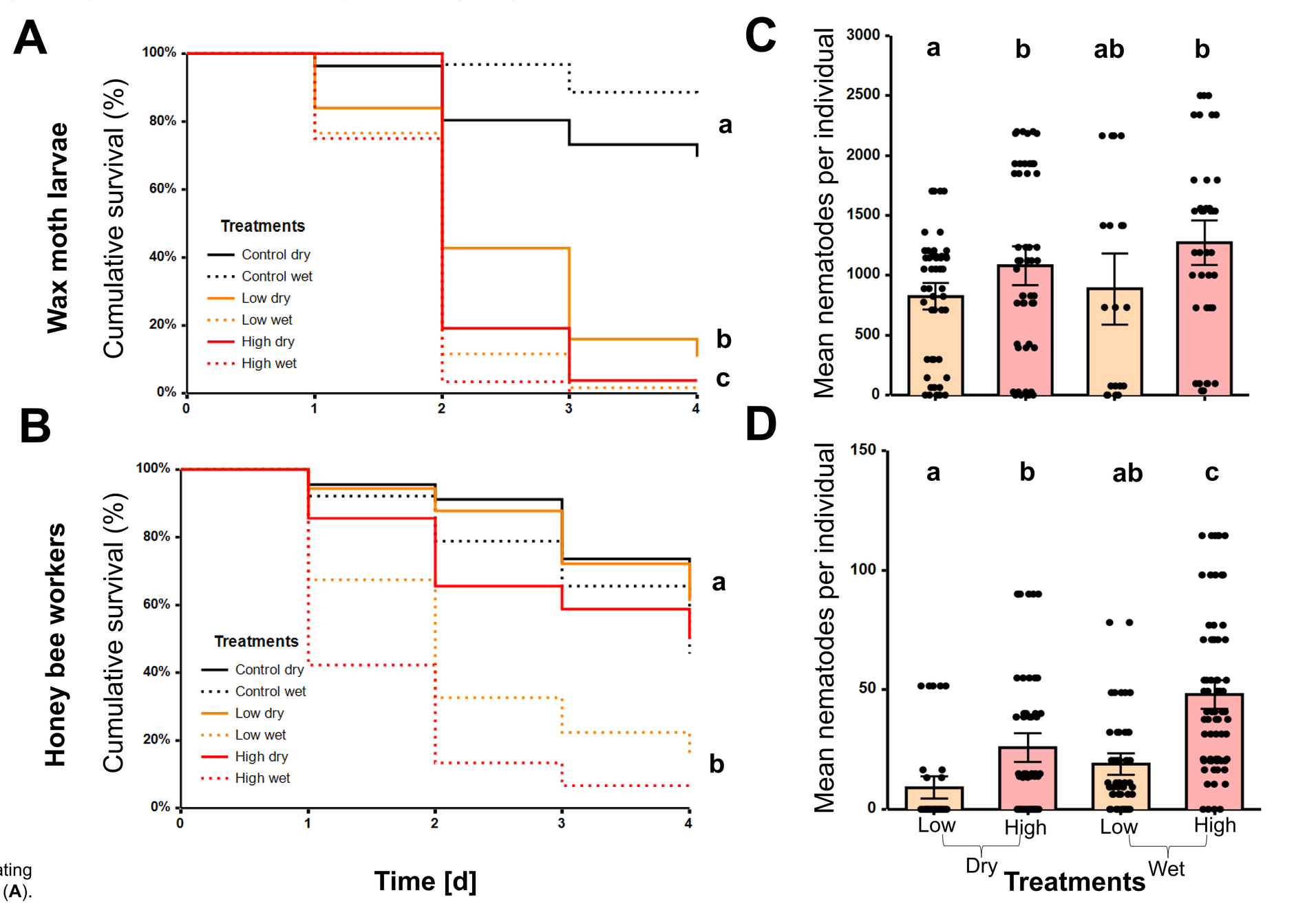

Fig. 2 Kaplan-Meier survival and *Steinernema carpocapsae* reproduction analyses. Survival was recorded over 96 h post *S. carpocapsae* exposure where as nematode reproduction was assessed in dead individuals 15 days post exposure. Survival and mean nematode reproduction post exposure per individual wax moth larvae (**A&C**) and honey bee worker (**B&D**).

Discussion and conclusion

Here we show clear evidence that foliar exposure to a commercial EPN product can cause lethal effects and that the nematodes can successfully replicate within the carcasses of adult bees. Given the lack of data on potential adverse effects of EPNs on non-target pollinating insects, our results highlight the urgent need to be cautious when applying foliar application of EPNs to crops. As dry residues of our EPN treatments imposed lower lethality and decreased nematode proliferation in honey bees when compared to direct (wet) exposure, foliar treatments with EPNs should ideally be applied when pollinators are not active (i.e., early evenings) to reduce the likelihood of exposure. Additional research is urgently required to adequately investigate the potential risk of EPNs to ground-nesting bees and other non-target insect species during foliar and soil application.

References:

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b UNIVERSITÄT BERN

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