Overlooked threats to bumble bees: Entomopathogenic nematodes reduce queen hibernation success

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Introduction

Sustainable agriculture increasingly relies on biological control agents as alternatives to synthetic pesticides (Bale et al. 2008). Entomopathogenic nematodes (EPNs) are widely used to control soil-dwelling insect pests. However, many bee species also nest and develop in soil, raising concerns about unintended exposure. EPNs are typically applied from spring to autumn, overlapping with periods where soil-dwelling insects are either in hibernation or nesting. However, potential risks from EPNs such as *Steinernema* spp. and *Heterorhabditis* spp. remain poorly studied (Cappa et al. 2022, Dutka et al. 2015). Here, we test the effects of two commercial EPN species on the hibernation success of buff tailed bumble bee (*Bombus terrestris*) gynes (i.e., future queens).

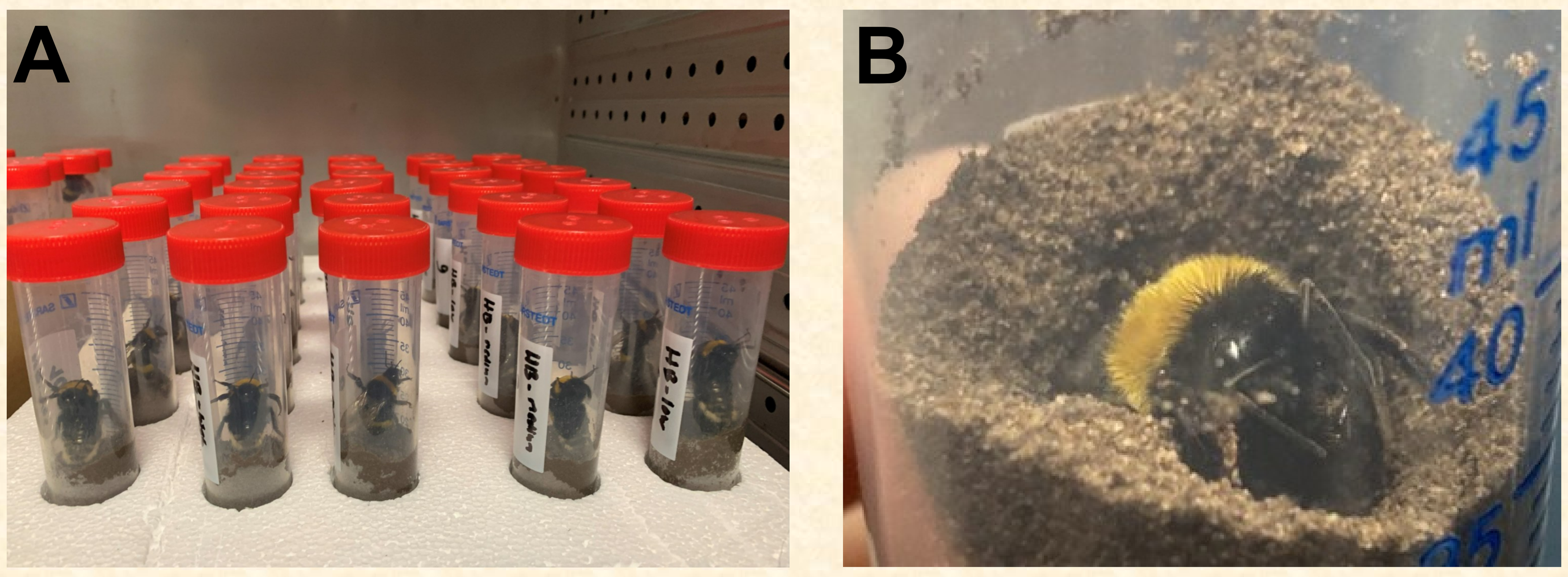

Fig. 1: Hibernation set-up with *Bombus terrestris* gynes inside soil-filled Falcon tubes **(A)**. Partially buried gyne in soil within a Falcon tube, enduring the simulated overwintering period at 14°C **(B)**.

Methods

Freshly mated *B. terrestris* gynes (age ~14 days) were exposed to three field-realistic concentrations of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* - 0.25 (low), 0.5 (medium), and 1 million/m² (high) - in simulated soil hibernation chambers using 50 mL Falcon tubes (*N*=20 per treatment group; Fig. 1A&B). Gynes were kept at 14 °C for 28 days to mimic hibernation. Mortality was recorded daily. Nematode replication was assessed using White traps (White 1927; Fig. 2B) for deceased and surviving individuals (Fig. 3). Survival analyses were performed in R (4.2.2) using the survival and *surminer* packages. Survival curves were estimated using the *survfit()* function and differences among treatment groups were tested using a Log-Rank test via *survdiff()* and *pairwise_survdiff()*.

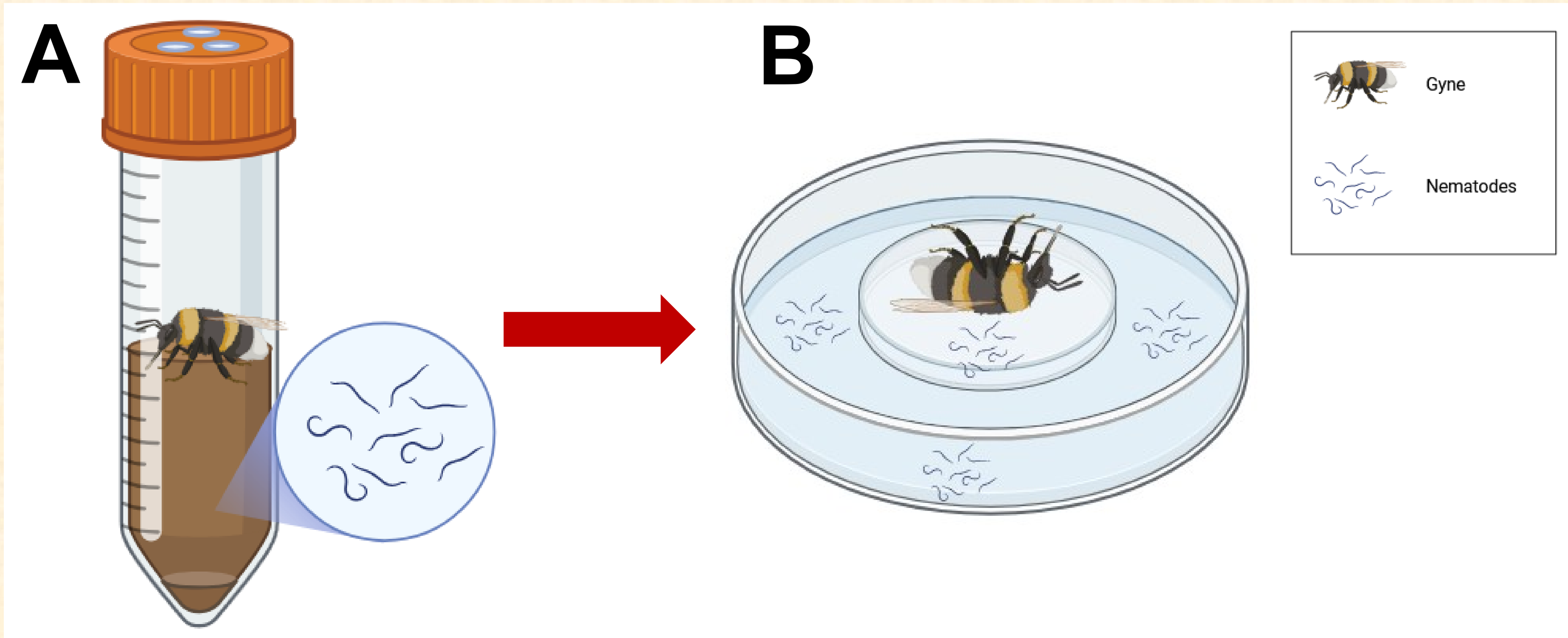

Fig. 2: Schematic representation of the hibernation set-up. Hibernation simulation at 14°C under controlled soil moisture conditions **(A)**. Dead bee on white trap for nematode collection **(B)**.

Results

Survival in the control group remained high, with 95% of gynes surviving 28 days. Irrespective of exposure level, all *S. carpocapsae* treatments significantly reduced survival compared to the control (log-rank test: χ^2 = 13.0, df = 3, *P* = 0.005; Benjamini-Hochberg corrected pairwise comparisons, *P*<0.05), while no significant differences were detected among exposure levels (*P*>0.05, Fig. 4A). For *H. bacteriophora*, survival differed significantly among treatments (log-rank test: χ^2 = 9.9, df = 3, P = 0.02), with high and medium doses showing significantly lower survival than the control (Benjamini-Hochberg corrected pairwise tests, *P*<0.05), while no significant differences were detected among the treatment doses (*P*>0.05, Fig. 4B). Both nematode species successfully reproduced in bee cadavers, with median EPN replications being 22’350 per host.

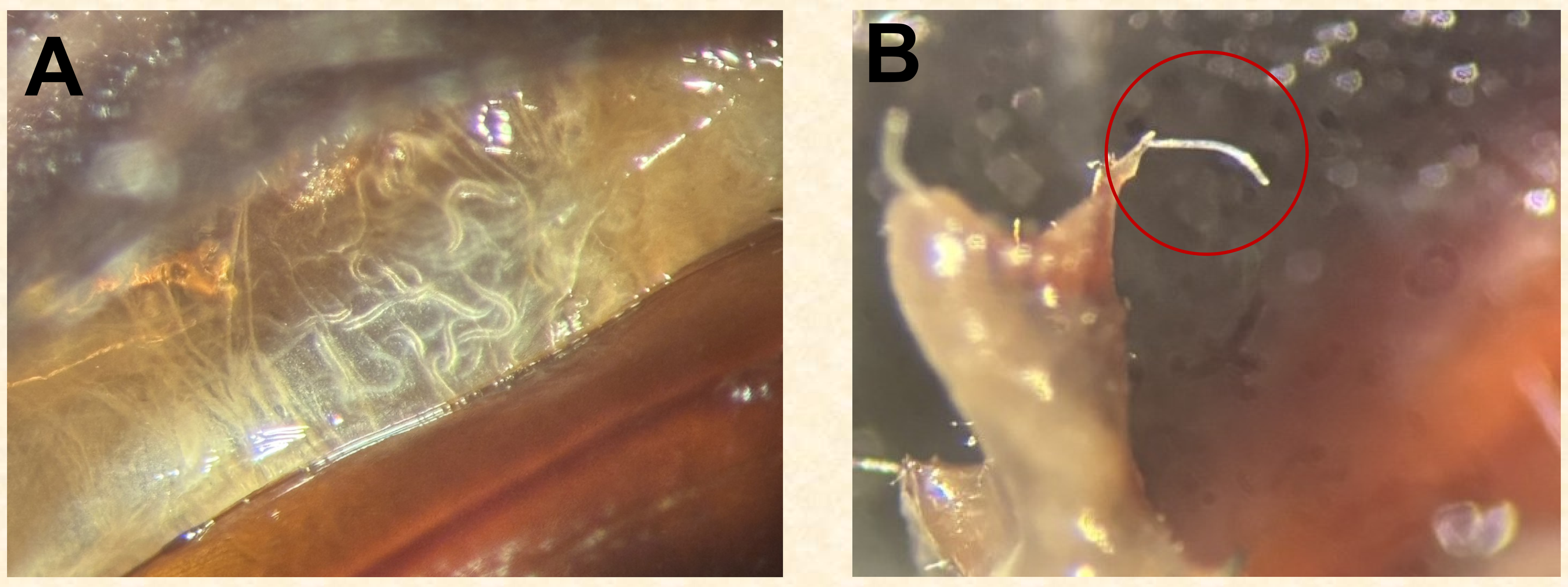

Fig. 3: **(A)** Entomopathogenic nematodes inside the abdomen of a *Bombus terrestris* cadaver. **(B)** Infective juvenile nematode attached to the leg of a *Bombus terrestris* queen after emerging from the insect cadaver, searching for a new host.

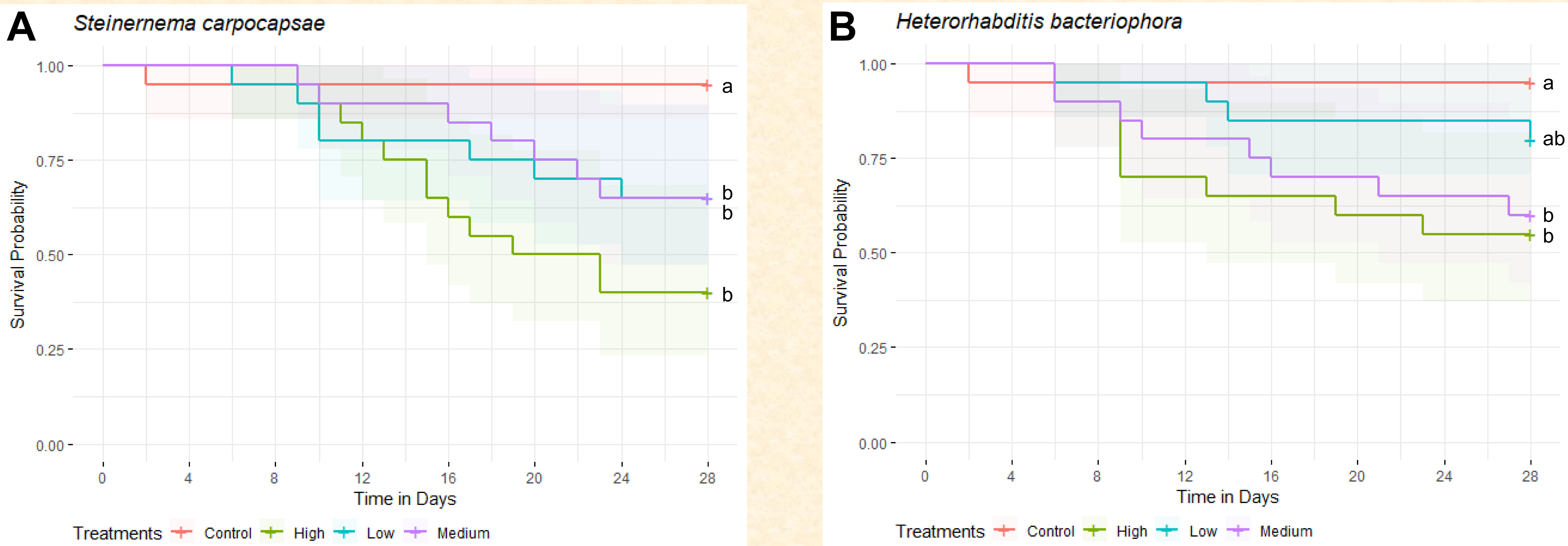

Fig. 4 Kaplan–Meier survival curves of bees: Survival Probability of *Bmobus terrestris* gynes following exposure to soil treated with **(A)** *Steinernema carpocapsae* and **(B)** *Heterorhabditis bacteriophora*. Pairwise comparisons of survival curves with Log-Rank tests show significant differences (*P<0.05*, post-hoc Benjamini-Hochberg corrected) in survival of treatment groups and are indicated by lower case letters (a and b).

Conclusion

Our results show that hibernating *Bombus terrestris* queens are vulnerable to the biocontrol nematodes *S. carpocapsae* and *H. bacteriophora*, which successfully reproduced in queen cadavers. Considering that these nematodes are commonly applied in spring and autumn, exposure during queen hibernation is a realistic yet overlooked risk. This highlights the need to systematically evaluate non-target effects of EPNs on pollinators and other soil-dwelling non-target invertebrates. Our experimental approach provides a feasible method that could support future regulatory assessments. Given that numerous bumble bee populations are declining worldwide, nematode-based biocontrol, if inadequately applied and controlled for, may represent a further inadvertent stressor. Safeguarding pollinators requires careful integration of pest management strategies with biodiversity protection.

References:

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