Journal of Nematology 27(3):378–381. 1995.

Nonsusceptibility of the Honey Bee, *Apis mellifera* (Hymenoptera: Apidae), to Steinernematid and Heterorhabditid Nematodes

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Abstract: We exposed honey bee workers and brood to four entomopathogenic nematode species under conditions normally encountered in the hive by spraying nematodes onto combs. Mortality of adult bees exposed to any of the nematode species was less than 10%, and there was no evidence of nematode infection when dead adults were dissected. To assess the impact of nematodes on brood, we used smaller-size honey combs placed in the second story (super) of a hive and large brood combs placed in the main section of the hive. Our results were inconsistent between these two experimental designs. The smaller honey combs sprayed with *Steinernema carpocapsae* contained the largest number of uncapped cells, those sprayed with *Heterorhabditis bacteriophora* or *S. riobravis* contained an intermediate number of uncapped cells, and control combs and those sprayed with *S. glaseri* contained the fewest number of uncapped cells. Large combs sprayed with *S. riobravis* contained more uncapped cells than controls or those sprayed with *S. carpocapsae*, although the differences were not significant. Our results do not support the hypothesis that high-temperature-tolerant species of nematodes are necessarily more infective to honey bees.

Key words: entomopathogenic nematode, *Heterorhabditis bacteriophora*, high-temperature tolerance, nontarget insect, *Steinernema* spp.

Entomopathogenic nematodes are commercially available for the control of a number of soil-inhabiting insects. To further expand their use, they are being tested against foliar and floral insect pests (5,14). This increases the potential for contact between nematodes and certain beneficial insects.

Several studies have been conducted on the susceptibility of the honey bee, *Apis mellifera* L., to nematode infection. Hackett and Poinar (8) and Cantwell et al. (2) showed that worker and brood bees are susceptible to nematode infection under laboratory conditions. However, Kaya et al. (9) demonstrated that the immature stages of the honey bee were not susceptible to *Steinernema carpocapsae* (Weiser) Poinar when the infective nematode was sprayed in the hive. A few adults became infected, but the investigators concluded that there was no effect on a colony. Kaya et al. (9) suggested that the high temperatures in the hive minimized nematode infection.

More recently, several entomopathogenic nematode species have been found to be tolerant to high temperatures. Grewal et al. (7) demonstrated that both *S. riobravis* Cabanillas, Poinar & Raulston and *S. glaseri* (Steiner) Wouts, Mrácek, Gerdin & Bedding can infect insect hosts at temperatures between 37–39 C. These temperatures are substantially higher than those required to maintain the brood (18). The objective of our experiments was to determine whether nematodes with high-temperature tolerance could be detrimental to honey bee colonies.

Materials and Methods

Nematodes and honey bees: Steinernema carpocapsae All strain, *S. glaseri* NC strain, *S. riobravis* RGV strain, and *Heterorhabditis bacteriophora* Poinar (NC Strain) were produced in *Galleria mellonella* L. larvae (3) and maintained as described by Woodring and Kaya (19). An Italian strain of European honey bee (*Apis mellifera* L.) was maintained in Langstroth hives at the Apiary of the University of California-Davis Bee Biology Facility. Bee colonies were

Received for publication 12 December 1994.

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The authors thank H. Ferris and A. M. Koppenhöfer for their comments on this manuscript.

checked for disease symptoms (e.g., American foulbrood and chalkbrood disease), and only strong, healthy colonies were selected for these experiments. Experiments were conducted in Davis, California, in April 1994, when bee colonies were actively engaged in brood rearing. During April, the mean high and low temperatures were 21 and 5 C, respectively; relative humidity averaged 69%; and 2 mm of rain fell (data obtained from the California Department of Water Resources).

Susceptibility of honey bee workers: Small combs (11.5 × 11.5 cm) containing honey-filled cells were sprayed with one of four nematode species (*S. carpocapsae, S. glaseri, S. riobravis,* or *H. bacteriophora*) suspended in a 0.01% Triton X-100 solution, or a 0.01% Triton X-100 solution without nematodes (controls). Each comb was sprayed with 5 ml/side of nematode suspension (1,000 nematodes/ml) or control solution. Each treated comb and 270 ± 20 (±S.E.) adult worker bees were placed in miniature hives (18 × 18 × 20 cm). The 20 prepared miniature colonies (five treatments, four replicates) were placed inside an incubation room in a randomized complete block (RCB) design. The conditions maintained in the incubation room were 33 ± 2 C, 80% RH, and 24 hours of dark, similar to conditions maintained around the brood within a hive (18). After 3 days, dead worker bees were collected and dissected to determine nematode infection. The experiment was repeated twice and data pooled for both experiments because data were not significantly different between experiments.

Susceptibility of honey bee brood: In the first experiment, small combs containing uncapped brood were removed from the experimental hive. Each treatment (four nematode species and one control) was applied to three combs (as described above), and combs were placed back in the super of the experimental hive in an RCB design. Five days later brood mortality was measured by an indirect method (described below). A side of a comb was considered a replicate; thus, treatments were replicated six times. This experiment was conducted once.

In a second experiment, standard-sized brood combs (23 × 28 cm) were sprayed with 25 ml/side of either *S. riobravis, S. carpocapsae,* or 0.01% Triton X-100 solution (control). Combs were placed back in the main section of the experimental hive. Five days later brood mortality was measured by an indirect method (described below). Three combs were sprayed—one comb per treatment—and each side of a comb was designated a replicate. This experiment was conducted once.

Diseased brood is normally removed from brood cells by workers (15). Brood that died from nematode infection would be removed from their cells by worker bees, and these cells would remain uncapped; uninfected brood would be capped. Our estimate of brood mortality was the number of uncapped cells. Before the experiment, a sheet of clear acetate was placed over the brood comb and a circle drawn on the acetate around 60–70 uncapped cells in the first experiment or around 100–120 cells in the second experiment. This map was used to later locate these cells, which all contained healthy brood before nematode application. Mortality of brood was inferred from the number of uncapped cells in a sample of 52 cells (first experiment) or 100 cells (second experiment) within the area outlined on the brood map. Differences in the number of uncapped cells among treatments within an experiment were subjected to analysis by Kruskal-Wallis test (10). In addition, five cells on each side of a comb were uncapped to determine if capped cells contained healthy brood.

Results and Discussion

Susceptibility of honey bee workers: Temperature within the miniature hives within the incubators averaged 32.5 ± 1.0 C (S.E.), and relative humidity averaged 72% ± 0.7. Worker bee mortality did not differ among treatments ($F = 1.6$; $df = 4, 15$; $P > 0.05$). An average of 9.8 ± 0.4 and 5.3 ±

3.7 bees died after exposure to the high-temperature-tolerant nematodes *S. riobravis* and *S. glaseri,* respectively; 6.0 ± 4.5 and 5.5 ± 2.7 bees died after exposure to the high-temperature-sensitive nematodes *S. carpocapsae* and *H. bacteriophora,* respectively. An average of 3.0 ± 1.3 bees died in control treatments. No nematodes were found in any of the dead bees, and no evidence of infection was found, i.e., organs and muscle tissue in thorax and abdomen were intact and appeared normal. *Steinernema carpocapsae* (9) will infect worker bees when applied directly to the bees in the hive. However, temperature and relative humidity within hives, similar to those used by Kaya et al. (9), can fluctuate between 20–33 C and 50–70% (M. E. Baur, pers. obs.). In addition, worker bees exposed to *S. carpocapsae* (8) or *S. scapterisci* (11) in honey or water at about 25 C can become infected. Therefore, worker bees exposed to nematodes at temperatures below 33 C can be infected. None of the nematode species used in the present study infected workers when temperatures were 32 C or higher.

Susceptibility of honey bee brood: In the first experiment, the number of uncapped cells differed among treatments ($H = 14.6$; $df = 4$; $P < 0.01$). Out of 52 cells, 26.5 ± 4.6 remained uncapped in combs treated with *S. carpocapsae;* this number was higher ($Z = 3.2$, $P < 0.001$) than in any other treatment. Combs treated with *S. glaseri* (1.5 ± 0.3) and controls (2.0 ± 0.4) had fewer ($Z = 2.1$, $P < 0.02$ and $Z = 1.8$, $P < 0.04$, respectively) uncapped cells than those treated with *S. riobravis* (10.3 ± 3.7) or *H. bacteriophora* (15 ± 4.4). No capped cells were found to contain infected brood.

Using standard-sized brood combs, we found no variation in the number of uncapped cells among treatments ($H = 4.6$; $df = 2$; $P > 0.1$). Although 30.2 ± 7.1 out of 100 cells were uncapped in the combs treated with *S. riobravis* and only 13.3 ± 0.7 and 15.7 ± 0.3 were uncapped in *S. carpocapsae*-treated and controls, respectively, our sample sizes may have been too small to detect differences in the number of uncapped cells between treatments. No capped cells were found to contain infected brood.

Our results do not support the hypothesis that nematode species with tolerance to high temperature are more infective to honey bees. We predicted that *S. riobravis* and *S. glaseri* would be more infective than *S. carpocapsae* and *H. bacteriophora.* In the small-comb experiment, *S. carpocapsae* appeared to be the most infective, followed by *S. riobravis* and *H. bacteriophora; S. glaseri* was not infective. The results between small-comb and large-comb experiments were inconsistent; *S. carpocapsae* caused higher mortality than the high-temperature-tolerant species when sprayed on the small combs, but *S. riobravis* caused higher mortality when sprayed on the large combs.

Overall, because mortality of worker bees was low and only moderate brood loss was observed after high inoculum levels, it is unlikely that the nematode species tested in the present study will have a negative impact on the honey bee. Although individual adults may become infected at the nectar source, the nematodes could not persist and infect brood or additional bees within the hive.

Although honey bee colonies are not affected by nematodes, some eusocial hymenoptera colonies are susceptible to nematodes. For instance, colonies of the common yellowjacket (*Vespula vulgaris* (Linnaeus) and *V. pennsylvanica* (Saussure)) (4) and imported red fire ant (*Solenopsis invicta* Burren) (6) can be suppressed using nematodes. Different hive conditions (temperature, moisture) maintained by the different species, behavioral differences between species (1), or physiological differences between species may account for the differences in susceptibility to nematode infection. If hive conditions are important in resistance to nematode infection, then solitary species of pollinator bees in North America, such as species in the genera *Nomia, Osmia,* and *Megachile* (12,13, 16,17), should be susceptible to nematode infection.

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