

Effect of an immune challenge with *Paenibacillus larvae* in honeybees

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Introduction

In recent years studies have documented that honeybees have only two thirds of the genes involved in immune defense at their disposal as compared to other invertebrates (1). Nevertheless, the activation of the immune system of honeybees produced during an infectious process also leads, as reported for other species of insects, to an up-regulation of genes codifying for several antimicrobial peptides (2,3). Here we investigated whether honeybees, as a consequence of a previous immunological insult, are capable of showing protection upon secondary exposure to the same pathogen. This effect of immune priming has been proven in bumble bees, where reinfection of individuals with same pathogen cause then lower mortality rates (4). For our purpose, *Paenibacillus larvae* (PI), the causing agent of American foulbrood, was chosen as immune elicitor to challenge honeybees and subsequently to infect the bees with a defined dose of viable bacteria.

Materials and Methods

Two independent experiments were carried out with either winter bees (February) or freshly emerged bees in summer (July). The experiments were repeated once, a total of 266 bees in winter and 521 in summer were investigated. In each assay one group was injected with 2 µl of Ringer saline solution into the hemolymph and the second group was injected with 2µl of heat-killed bacteria (90° C, 10 minutes) of *Paenibacillus larvae* at a concentration of 10⁸ CFU (colony forming units)/ml (photo 1). A third group composed of naive bees was also included for the summer trials. Bees were kept in wooden cases in an incubator (photo 2).

At day 8th after the first challenge, all groups of winter bees were injected for a second time with either 2µl of Ringer saline solution or 2µl of a defined dose of viable bacteria (400 CFU/ bee). Experimental groups were as follow: Ringer-challenged bees injected with Ringer for a second time (R-R) or viable bacteria (R-PI), and bacteria-challenged bees injected with Ringer for a second time (PI-R) or viable bacteria (PI-PI). For freshly emerged bees (baby bees), the experiment was repeated in the same way with the inclusion of a naive group (N-PI) and by using a higher dose of bacteria (800 CFU/bee) for the second injection. Survival data was then recorded during the following days.

For monitoring of proliferation of bacteria in hemolymph, 1µl of hemolymph of bees from the summer experiment was extracted from N-PI (n=17), R-PI (n=14) and PI-PI (n=23) bees 24 hours after infection and plated in MGYDP-Agar for counting of CFU.

Results

In both experimental setups, winter bees (Fig. 1) and summer bees (Fig. 2) showed significant differences between R-PI groups and PI-PI groups ($p = 0,018$ and $0,000$ respectively, Kaplan-Meier Test). Besides, for summer bees no significant differences were found between ringer challenged group and naive groups upon infection with viable bacteria.

Additionally, hemolymph samples plated on agar revealed that 24 hours after infection, 52% of the PI-PI challenged bees had less than 350 CFU/0,5 µl of hemolymph whereas 88,2% of N-PI and 85,3% of R-PI showed more than 350 CFU/0,5 µl of hemolymph, corroborating the differences between groups obtained in the survival experiment (data not shown, photo 3).

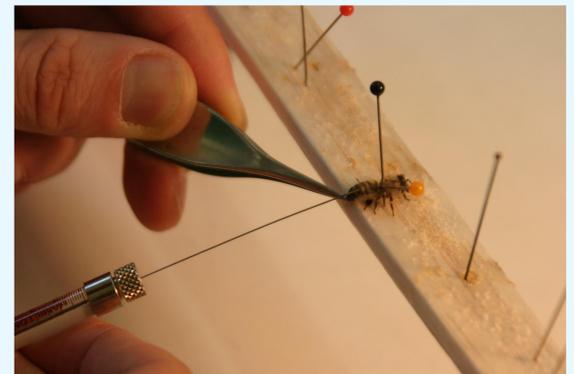


Photo 1: Challenge of bees



Photo 2: Maintenance of bees in cages

Survival rate for winter bees

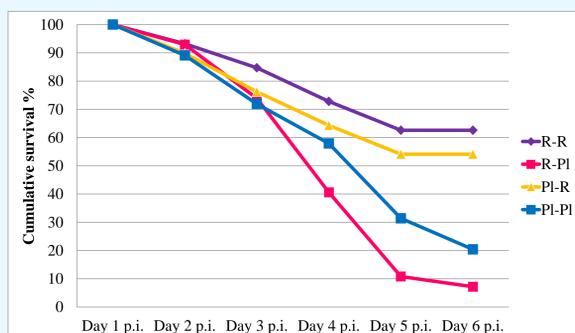


Fig. 1: Survival curve of winter bees. Groups; R-R (n=59), R-PI (n=84), PI-R (n=59) and PI-PI (n=64).

Survival rate for summer bees

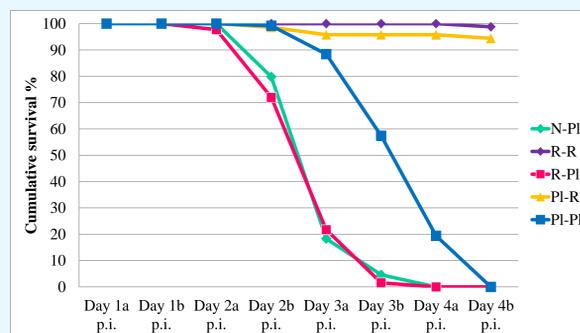


Fig. 2: Survival curve of summer bees. Groups; R-R (n=83), R-PI (n=129), PI-R (n=71), PI-PI (n=129) plus inclusion of a group composed of naive bees N-PI (n=109).

PI growth in hemolymph of summer bees



Photo 3: From left to right, bacterial counts in hemolymph of PI-PI bees (n=23), R-PI bees (n=14) and N-PI bees (n=17).

Discussion

Here we have demonstrated that honeybees show a different long-term survival curve pattern when an infection with bacteria occurs upon a previously activation of their immune system. However, whether this is the consequence of a long-term immune activation (one week between challenge and infection) or the production of a stronger immune reaction upon infection, cannot be concluded.

Moreover, these experiments seem to reveal significant differences between honeybees and bumble bees. The first ones are only capable of increasing the life span for about 24 hours upon infection without altering final survival figures (eventually they all perish). This behavior was also observed in 3-4 months old winter bees. In contrast, bumble bees are not only capable of increasing their life span but also developing a long-lasting protection that reflects a better survival rate in time (4). These differences could potentially be a consequence of the reduction of genes involved in immune processes that have been demonstrated in honeybees (1).

- Literatur -

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