

Effect of rapid heat hardening of honeybee mother queens (*Apis mellifera*) on the immune response of their daughter workers during heat stress

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Global warming has become a clear threat to beekeeping worldwide. Rapid heat hardening in organisms, also known as "acclimation", is defined as a physiological process that includes fast pre-exposure to non-lethal temperatures for minutes to hours, these processes involve the induction of several heat stress proteins (HSP) and other stress proteins. To assess the effect of queen larval preheat hardening on the immune response of daughter workers during heat stress, twelve accepted mother queen (MQ) larvae with the age of 4 days were selected and divided into two treatment groups (n = 6). The non-heat-treated mother queens (nH-T MQ) were kept for 15 min at 34.5 °C and 70% relative humidity (RH), and the pre-heat-treated mother queens (pH-T MQ) group was subjected for 15 min to 41 °C and 70% RH. About 500 workers were collected from the brood combs of each treatment group. The worker bees in their cages were incubated for 1 h in digital-controlled thermo-incubators at the following temperatures: 35, 40, 45, 50, and 55 °C. The expression of several immune pathways genes in the intestine was assessed by relatively quantitative Real-Time qPCR (RT-qPCR). In general, pre-heat treatment of the mother queen's larvae changes the basal and dynamic expression of all tested genes in immune pathways, which significantly increases the expression of Toll-1, Spz, Df, Cact-1, Rel, Hem, PPO, and Apid-1 after heat stress in several temperatures, decreased the expression of Tab-3 and Aba, had no effect at all on the expression of Myd88 gene, and had a fluctuated effect on the expression of Hym and Def-1 in both thermoneutral and heat stress conditions of daughter workers. Furthermore, it decreased the death rate under heat stress probably indicating a significant improvement in honeybees' immune response and thermotolerance acquisition in hot areas.