Journal of Insect Physiology 59 (2013) 487–493

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Interactive effect of reduced pollen availability and *Varroa destructor* infestation limits growth and protein content of young honey bees

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ARTICLE INFO

Article history:
Received 30 November 2012
Received in revised form 12 February 2013
Accepted 13 February 2013
Available online 21 February 2013

Keywords:
Apis mellifera
Varroa destructor
Pollen
Growth
Protein content

ABSTRACT

Varroa destructor in combination with one or more stressors, such as low food availability or chemical exposure, is considered to be one of the main causes for honey bee colony losses. We examined the interactive effect of pollen availability on the protein content and body weight of young bees that emerged with and without *V. destructor* infestation. With reduced pollen availability, and the coherent reduced nutritional protein, we expected that *V. destructor* infestation during the pupal stage would have a larger negative effect on bee development than without infestation. Moreover, when raised with ample pollen available after emergence, infested pupae were expected not to be able to compensate for early losses due to *V. destructor*. We found that both *V. destructor* infestation and reduced pollen availability reduced body weight, abdominal protein level, and increased the head to abdomen protein ratio. The availability of pollen did indeed not result in compensation for reduced mass and protein content caused by *V. destructor* infestation in young bees after 1 week of their adult life. Both *V. destructor* and nutrition are top concerns for those studying honey bee health and this study demonstrates that both have substantial effects on young bees and that ample available pollen cannot compensate for reduced mass and protein content caused by *V. destructor* parasitism.

1. Introduction

Recent colony losses in honey bees (*Apis mellifera*, L.) might be attributed to an array of causes varying from low food availability, diseases and parasites such as *Varroa destructor*, to chemical toxins in the environment (Ellis et al., 2010; Neumann and Carreck, 2010; Potts et al., 2010). Although there is a general agreement that there is no single explanation for the extensive colony losses, the presence of *V. destructor* in colonies places an important pressure on bee health (Le Conte et al., 2010). *V. destructor* in combination with one or more stressors is therefore considered to be one of the main causes for colony losses (Aizen and Harder, 2009; Ellis et al., 2010; Le Conte et al., 2010; Potts et al., 2010; Rosenkranz et al., 2010).

V. destructor reduces the health of honey bees during the pupal and adult life stages (Bowen-Walker and Gunn, 2001; Rosenkranz et al., 2010). Kovac and Crailsheim (1988) showed that infestation by *V. destructor* shortened the lifespan of honey bees during the hive-bound period as well as during the foraging period, and van Dooremalen et al. (2012) linked the lower lifespan of bees during winter to colony losses in spring. Emerging bees infested with *V. destructor* showed a lower body weight than non-infested bees (Bowen-Walker and Gunn, 2001; De Jong et al., 1982; Schneider and Drescher, 1987), less protein in the hemolymph (Amdam et al., 2004; Weinberger and Madel, 1985), and smaller hypopharyngeal glands to feed offspring (Schneider and Drescher, 1987). In contrast to protein content and body weight at emergence, protein supply during the first days of life was positively related to mean lifespan (Kunert and Crailsheim, 1988). It was suggested that adult bees are able to compensate for some larval/pupal deficiencies, and that perhaps this compensation for parasite-caused protein loss would occur more under optimal colony conditions, i.e. when sufficient food and nurse bees are available (Kovac and Crailsheim, 1988). If bees cannot compensate for larval/pupal deficiencies under optimal colony conditions, then bees infested with *V. destructor* and raised under optimal colony conditions are expected to maintain low protein content and body weight.

Besides *V. destructor* (Amdam et al., 2004; Bowen-Walker and Gunn, 2001; Brødsgaard et al., 2000; Martin, 2001; Rosenkranz et al., 2010), food quantity and diet composition have been shown to also influence honey bee vitality (Alaux et al., 2010; Brodschneider and Crailsheim, 2010; Crailsheim and Stolberg, 1989; Cremonez et al., 1998). A high protein level in the diet was positively related to the levels of vitellogenin (a major protein in worker bees) and other proteins in the hemolymph of 6 days-old adult bees (Cremonez et al., 1998), and resulted in increased development of

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http://dx.doi.org/10.1016/j.jinsphys.2013.02.006

the hypopharyngeal glands (Crailsheim and Stolberg, 1989). A decrease in dietary protein quantity, however, did not reduce baseline immune-competence, but a decrease in diet diversity did (Alaux et al., 2010). Brodschneider and Crailsheim (2010) concluded that colonies with reduced protein input or under starvation will lead only to slightly negatively affected workers, as the number of larvae reared will be first reduced by cannibalism to secure the quality of the remaining offspring. They suggested, however, that larval starvation, alone or in combination with other stressors, can weaken colonies.

Reduced pollen availability and infection with *V. destructor* can have effects at colony level, individual level and molecular level. At colony level, foragers from colonies with moderate infestations are found to carry smaller pollen loads than those from lightly infested colonies resulting in lower pollen availability in the former colonies (Janmaat et al., 2000). Moreover, lightly infested colonies exhibited a larger increase in number of pollen foragers than moderately infested colonies during the seasonal growth of the colony, suggesting that more intense mite infestations compromised forager recruitment. As a result, lightly infested colonies were rearing more brood by the end of the season and showed higher efficiency in converting pollen to brood than moderately infested colonies (Janmaat et al., 2000). Increased amount of beebread in colonies also increased the removal of infested brood cells (49%, compared to 33% removal by colonies with low amounts of beebread storage; Janmaat and Winston, 2000a).

At individual level, infestation by *Varroa jacobsoni* (presumably this was *V. destructor*) and low pollen availability during brood rearing both advanced the onset of foraging age (Janmaat and Winston, 2000b), and both factors appeared to affect workers in a similar fashion, presumably through a decrease in worker protein concentration. Additionally, foragers uninfested as pupae and reared in a colony with high pollen availability tended to live longer than workers infested as pupae and reared in a colony with low pollen availability, but these results were not significant (Janmaat and Winston, 2000b).

At molecular level, pollen nutrition enhanced macromolecule metabolism, activated the pathways for tissue growth and development, and stimulated the expression of genes involved in longevity, like genes coding for vitellogenin (Alaux et al., 2011). However, in contrast to the suggestion that bees could compensate for larval/pupal deficiencies under optimal colony conditions (Kovac and Crailsheim, 1988), the negative impacts of *V. destructor* on the bee metabolism and immune functions could not be reversed by pollen feeding (Alaux et al., 2011). *V. destructor* inhibited the digestion and/or use of protein and hampered lipid metabolic processes, oxidative phosphorylation, and generation of precursor metabolites and energy, all being indicators that infested bees cannot correctly assimilate and use the pollen nutrients required for their physiological development (Alaux et al., 2011).

To date, the interactive effect of reduced pollen availability and infection with *V. destructor* on honey bee growth and protein build-up has not been studied. Whereas Alaux et al. (2011) investigated this interactive effect on molecular level, our aim was to study this interactive effect on the physiology of individual adult honey bees. Therefore, we examined under laboratory conditions the interactive effect of reduced pollen (beebread) availability on the physiological development of young honey bees that emerged with and without *V. destructor* infestation. We expected that under marginal conditions, i.e. reduced pollen availability, infestation with *V. destructor* will have a larger negative effect on bee development than under optimal food conditions (compared with emergence without *V. destructor* infestation). Both protein level and growth are expected to be reduced when bee prepupae were infested with *V. destructor* and subsequently raised in cages with reduced pollen availability. Bees that were infested during the pupal stage were expected not to be able to fully compensate for early weight and protein losses due to *V. destructor* when raised in cages with ample pollen available after emergence (Kovac and Crailsheim, 1988 vs. Alaux et al., 2011). Additionally, we checked *in vivo* the effect on growth of non-infested young bees nursed in a host colony with low *V. destructor* infestation to get an insight in the differences between bees being nursed in the laboratory or in colonies in the field.

2. Method

2.1. Set-up

For the experiment (June 2006), 40 Liebefelder cages were used in the laboratory, each containing five test bees (*A. mellifera* L.) from a highly infested colony and 15 nurse bees from a healthy colony. For the pollen treatment, half of the cages were provided with a small portion of comb with beebread (mean number of cells with pollen 42 ± 1, collected from one colony), while the other half of the cages were provided with portions of empty comb. The combs were obtained from the donor colony just before the nurse bees were put in the cages. Each cage was fitted with a feeder containing 10 ml of sugar solution (invert sugar solution 63%, BeeFit), which was refilled *ad libitum* every 2–3 days. Cages were placed inside an incubator at 35 °C with an open source of water to increase air humidity to 40–60% (Oertel, 1949). At the end of the experiment, 7 days after emergence, test bees were individually weighed and stored at −20 °C until protein analysis.

2.2. Laboratory animals

In preparation of the experiment, a brood comb was placed in an incubator (35 °C) 1 week before emergence. At the beginning of the experiment, the test bees were dissected from this comb, just before they emerged themselves. During dissection, the number of adult female *V. destructor* mites per cell was counted. To be sure all mites were still in the cell during dissection, cells with autonomously emerging bees were ignored (a "natural" hole in the cap). Bees were allocated to the Liebefelder cages based on the number of co-emerging adult mites (0, 1 or 2 mites, five bees per cage, Table 1). All test bees were collected from the same colony (10-frame hive). To obtain sufficient amounts of test and nurse bees, the experiment was performed in series: once a week 10 cages, for 4 weeks in June. To obtain the nursing bees, 200 newly emerged bees (0–1 days of age) were marked in the field a week

Table 1
Sample size information for the Liebefelder cages. The table shows the treatments for *Varroa destructor* (infested during the pupal stage yes/no) and pollen availability (for the nurse bees yes/no), the number of bees alive at day 7 (Bees #), the number of bees that died within the first week (Bees dead #), and the percentage of bees that died from all bees that emerged (Bees dead %).

Varroa	Pollen	Bees (#)	Bees dead (#)	Bees dead (%)
No	No	55	2	4
No	Yes	61	0	0
Yes	No	29[a]	5[b]	15
Yes	Yes	18[c]	8[d]	31

[a] In this group, 21 bees emerged with one mite and 8 bees emerged with two mites.
[b] In this group, 5 bees emerged with one mite and 0 bee emerged with two mites.
[c] In this group, 10 bees emerged with one mite and 8 bees emerged with two mites.
[d] In this group, 6 bees emerged with one mite and 2 bees emerged with two mites.