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

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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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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.

before the experiment. These bees were assumed to perform nursing tasks (Seeley, 1982) at the start of the experiment. Nursing bees were recaptured the day before the experiment started and randomly allocated to the Liebefelder cages (15 nursing bees per cage, 7–8 days of age, 10 cages per batch). All nurse bees were collected from the same colony (10-frame hive) that had a low *V. destructor* load (daily mite fall on bottom board 0.6 ± 0.3). For this experiment, we assumed that nurse bees that could not feed on pollen during the experiment would still be able to feed the test bees for another week (Crailsheim, 1990), i.e. for the group that received empty combs the nursing capacity was not nullified, but reduced.

### 2.3. Field animals

To check growth and protein content of bees in the field (June 2006), 110 emerging bees (0 mites, dissected as explained above for the laboratory bees) from a healthy bee colony (low disease levels, checked for visual symptoms), were marked and weighed. These field test bees were returned to a host colony (10 frame hive; daily mite fall on bottom board in June was 0.1 ± 0.1), to mimic the situation in the laboratory: nurse bees originated from a different colony (10-frame hive) than the test bees. After 7 days in the field, 66 marked test bees were recaptured from the host colony, weighed and stored at −20 °C until protein analysis. Of these 66 bees, one bee was lost before weighing the dry body weight ($n = 65$), two more were lost before the protein in the head was measured ($n = 63$), and another bee before the abdominal protein was measured ($n = 62$).

### 2.4. Measurements

For both the laboratory and field bees, protein level was analyzed in the 1 week old test bees. Bees were freeze-dried for 3 days and the dry weight was determined for the whole body, and then for the head and abdomen separately. Head protein was used as a measure for the protein level in the hypopharyngeal glands and the capability to feed offspring (Münch and Amdam, 2010; Standifer, 1967), as was head dry weight (Babendreier et al., 2005; Hrassnigg and Crailsheim, 1998). Abdominal protein was used as a measure for the body reserve to support lifespan (Amdam and Omholt, 2002; Crailsheim, 1990; Fluri et al., 1982). Freeze dried head and abdomen were individually homogenized in 1 ml distilled water and centrifuged at 1500*g* for 5 min. The supernatant was extracted and mixed with BioRad Dye Reagent and Bovine Serum Albumin as a standard. Protein level was determined with a spectrophotometer following the protocol of Bowen-Walker and Gunn (2001).

### 2.5. Statistics

Relatively low numbers of young bees that emerged with two co-emerging *V. destructor* mites were collected. We first tested all response variables using the distinction between bees emerging from cells with 0, 1 or 2 mites. However, not one of the tested response variables showed a difference between the one or two mites treatment. The more or less equal sample sizes and the lack of difference between the one- and the two-mites treatment in the results made us decide to pool the data for the one and two mites treatment. Response variables tested were wet and dry body weight, dry weight of the head, head protein level, abdomen protein level, and head to abdomen protein ratio (H:A ratio). Differences between groups were tested using a $2 \times 2$ ANOVA for infestation with *V. destructor* during emergence (yes/no), pollen availability (yes/no) and the interaction between *V. destructor* and pollen availability. For wet body weight, a repeated measures ANOVA was used for day 0 and day 7, emergence with *V. destructor* (yes/no), pollen availability for nurse bees (yes/no) and the interaction between time, pollen availability and *V. destructor* infestation. The H:A ratio was Ln(+0.25)-transformed. A Pearson correlation was used for the relation between head and abdomen protein levels. An additional linear regression model was used for the relationship between abdomen protein level and abdomen dry weight (dry weight was Ln-transformed for linearity of the data).

The field bees that emerged without *V. destructor* in the host colony had access to beebread. The field data was tested against the data from the laboratory bees that also emerged without *V. destructor* and had nurse bees with beebread access. The two groups were compared using an Independent Samples *t*-Test for the response variables wet body weight at emergence and day 7, dry body weight (day 7), head protein level (day 7), and abdomen protein level (day 7).

All figures and tables show the mean ± SEM, unless indicated otherwise. Sidak post hoc tests were used for pair-wise comparisons between means. Assumptions for normality of the residuals were met for all tests.

## 3. Results

Bees (infested or not) with nurse bees without beebread during the first week of their lives (laboratory) showed, at the end of that week, a wet body weight that was on average 6.1 ± 2.1 mg lower than bees supplied with nurse bees with beebread (repeated measures ANOVA: beebread $F_{1,149} = 8.60$, $P < 0.001$). Infestation by *V. destructor* (with or without beebread) during the pupal stage resulted in on average 9.6 ± 2.1 mg lower wet body weight at the end of the first week of adult life compared to bees not infested during the pupal stage (*V. destructor* $F_{1,149} = 21.48$, $P < 0.001$; interaction beebread × *V. destructor* $F_{1,149} = 2.52$, $P = 0.11$). Bees gained or lost body weight in the first week after emergence (day $F_{1,149} = 4.16$, $P = 0.04$; interaction day × beebread × *V. destructor* $F_{1,149} = 6.73$, $P = 0.01$; Fig. 1). Without beebread for the nurse bees, young test bees lost weight between day 0 (emergence) and day 7 when infested or not infested during the pupal stage. With beebread available for the nurse bees, young test bees gained weight between emergence and day 7 when not infested, but they neither lost nor gained weight when infested during the pupal stage. At emergence (day 0), wet body weight was lower for bees infested than not infested; this was the case in both the groups provided with nurse bees with beebread and without beebread. Wet body weight at emergence did not differ between bees provided with nurse bees with or without beebread, both for the test bees infested and not infested by *V. destructor* during the pupal stage.

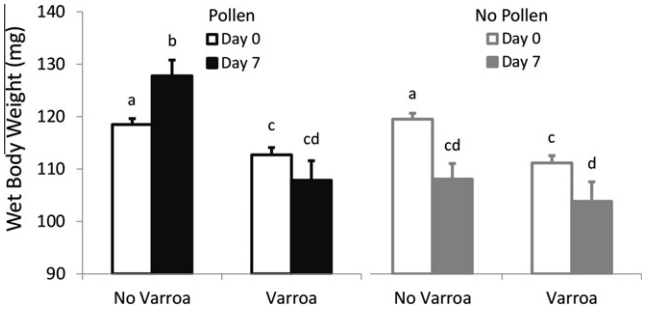


**Fig. 1.** Wet body weight (mean ± SEM) of bees at emergence (day 0) and after 1 week (day 7) for infestation with *Varroa destructor* (yes/no) and pollen availability (yes/no). Bars represent wet weight on day 0 (open bars), day 7 (closed bars), for groups with nurse bees with access to pollen (black bars) and with nurse bees without access to pollen (grey bars). Pairwise comparisons with Sidak post hoc tests were used to test for differences between means (denoted by different letters for $P < 0.05$).

Dry body weight (mg) on day 7 represented on average 29.0 ± 0.6% of the wet weight and showed a similar pattern for day 7. Infestation by *V. destructor* during the pupal stage reduced the dry body weight of the test bees, but only when beebread was available for the nurse bees. Without beebread available for the nurse bees, dry body weight was low, regardless whether the test bees were infested by *V. destructor* or not (ANOVA: beebread $F_{1,159} = 13.38$, $P < 0.001$; *V. destructor* $F_{1,159} = 4.30$, $P = 0.04$; interaction beebread × *V. destructor* $F_{1,159} = 6.18$, $P = 0.01$, Fig. 2).

Head dry weight (mg) was increased when beebread was available for the nurse bees, but was not affected by infestation with *V. destructor* (ANOVA: beebread $F_{1,156} = 6.32$, $P < 0.01$; *V. destructor* $F_{1,156} = 0.25$, $P = 0.62$; interaction beebread × *V. destructor* $F_{1,156} = 2.07$, $P = 0.15$; Table 2). The H:A ratio was higher in test bees that were infested (0.39 ± 0.04) than when not infested (0.29 ± 0.02) during the pupal stage by *V. destructor* and when no beebread was available (0.37 ± 0.03) compared to when beebread was available (0.26 ± 0.03) for the nurse bees (ANOVA: beebread $F_{1,155} = 6.07$, $P = 0.01$; *V. destructor* $F_{1,155} = 4.58$, $P = 0.03$; Table 2; the interaction was excluded from the analysis as it was not significant, and interfered with the variation explained by beebread: ANOVA: beebread $F_{1,154} = 2.98$, $P = 0.09$; *V. destructor* $F_{1,154} = 5.13$, $P = 0.02$; beebread × *V. destructor* $F_{1,154} = 0.97$, $P = 0.33$), which was mainly due to changes in the protein level of the abdomen and not of the head. Head protein content was not affected by any of the treatments (ANOVA: beebread $F_{1,156} = 1.41$, $P = 0.24$; *V. destructor* $F_{1,156} = 1.07$, $P = 0.30$; interaction beebread × *V. destructor* $F_{1,156} = 0.21$, $P = 0.65$; Fig. 3). In the abdomen, however, infestation by *V. destructor* during the pupal stage reduced the abdomen protein level of the test bees at day 7, but only when beebread was available for the nurse bees. Without beebread available for the nurse bees, the abdomen protein level was low, regardless whether the test bees were infested by *V. destructor* or not (ANOVA: beebread $F_{1,157} = 17.92$, $P < 0.001$; *V. destructor* $F_{1,157} = 8.87$, $P < 0.01$; interaction beebread × *V. destructor* $F_{1,157} = 4.45$, $P = 0.04$, Fig. 4). The protein level in the head and abdomen were negatively related (Pearson correlation: $r = 0.99$, $n = 4$, $P < 0.01$). The protein level in the abdomen increased linearly with the dry weight of the abdomen (Ln-transformed) (Linear regression: $R^2 = 0.44$, $F_{1,159} = 38.48$, $P < 0.001$).

Fewer bees died in the laboratory (61 bees at day 0 and 61 at day 7 alive: 0% died) compared to the field (110 bees introduced at day 0 and 66 recaptured at day 7: 40% assumed dead). However, adult bees that emerged without *V. destructor* and with access to beebread fed nurse bees in the laboratory gained less weight and protein during their first week of adult life than field bees (Table 3).

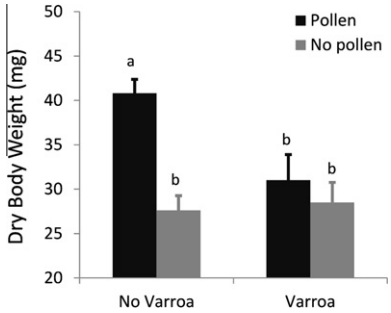


**Fig. 2.** Dry body weight (mean ± SEM) of bees on day 7 as a function of infestation with *Varroa destructor* (yes/no) and pollen availability (yes/no). Bars represent dry weight (mg) for groups with nurse bees with access to pollen (black bars) and with nurse bees without access to pollen (grey bars). Sidak post hoc tests were used to test for differences between means (denoted by different letters for $P < 0.05$).

**Table 2**
Dry weight of the head (mg) and the head to abdomen protein ratio (H:A ratio) for the treatments for *Varroa destructor* (infested during the pupal stage yes/no) and beebread availability (for the nurse bees yes/no).

| *Varroa* | Pollen | Head dry weight | | H:A ratio[c] | |
|---|---|---|---|---|---|
| | | Mean | SEM | Mean | SEM |
| No | No | 2.90 | 0.08[a] | 0.36 | 0.03[c,†] |
| No | Yes | 3.57 | 0.18[b] | 0.23 | 0.03[c,*] |
| Yes | No | 3.05 | 0.14[a] | 0.40 | 0.05[d,†] |
| Yes | Yes | 3.27 | 0.20[b] | 0.38 | 0.06[d,*] |

[a,b]Different letters denote significant differences within columns (Sidak post hoc test $P < 0.05$).
[c,d]Different letters denote significant differences within columns for *V. destructor* (Sidak post hoc test $P < 0.05$).
[†,*]Different symbols denote significant differences within columns for beebread availability (Sidak post hoc test $P < 0.05$).

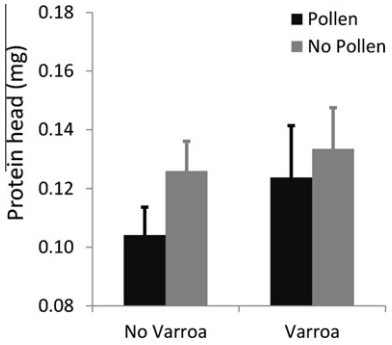


**Fig. 3.** Protein in the head (mg, mean ± SEM) of 7-day-old bees as a function of infestation with *Varroa destructor* (A, yes/no) and for pollen availability (B, yes/no). Bars represent head protein (mg) for groups with nurse bees with access to pollen (black bars) and with nurse bees without access to pollen (grey bars). Sidak post hoc tests showed no differences between means.

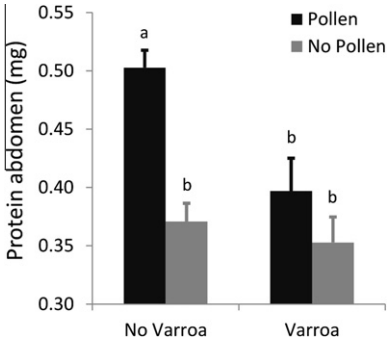


**Fig. 4.** Protein in the abdomen (mean ± SEM) of 7-day-old bees as a function of infestation with *Varroa destructor* (yes/no) and pollen availability (yes/no). Bars represent abdominal protein (mg) for groups with nurse bees with access to pollen (black bars) and with nurse bees without access to pollen (grey bars). Sidak post hoc tests were used to test for differences between means (denoted by different letters for $P < 0.05$).

## 4. Discussion

In the laboratory experiment, we found a general pattern of decreased wet and dry body weight and protein level in the abdomen (on day 7) in response to infestation by *V. destructor* during the pupal stage followed by reduced pollen (beebread) availability in the first week of the bee's adult life. Infestation by *V. destructor* during the pupal stage not only set the bees back in weight at emergence, as was also found by Bowen-Walker and Gunn (2001), but also hampered growth and protein build-up during the first week of

**Table 3**
Mean (mg) and standard error of the mean (SEM) of the laboratory ($n$ = 61) and field ($n$ = 62–66, see method) test bees for the wet body weight on day 0 and day 7, and the dry body weight, protein content of the head, protein content of the abdomen on day 7. The laboratory bees were not infested by *Varroa destructor* during pupation and were fed by nurse bees that had access to beebread. The field bees were not infested by *Varroa destructor* during pupation and were nursed during the first week of their life by a host colony with a low *Varroa destructor* infestation.

| | Laboratory | | Field | | Test values | | |
|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | df | $t$ | $P$ |
| *Day 0* | | | | | | | |
| Wet weight (mg) | 119 | 1 | 118 | 1 | 112[a] | 0.22 | 0.82 |
| *Day 7* | | | | | | | |
| Wet weight (mg) | 128 | 3 | 139 | 2 | 125 | −3.19 | <0.01 |
| Dry weight (mg) | 41 | 2 | 50 | 1 | 124 | −3.94 | <0.001 |
| Protein head (mg) | 0.10 | 0.01 | 0.15 | 0.01 | 94[a] | −3.48 | <0.001 |
| Protein abdomen (mg) | 0.50 | 0.02 | 0.58 | 0.03 | 113[a] | −2.31 | 0.02 |

[a] Unequal variances assumed.

their adult life. The interaction with reduced pollen availability, in the form of beebread for the nurse bees, caused a large negative effect on wet weight, dry weight and abdominal protein level in the test bees. The first 8–10 of the bees' adult life are, however, essential for the build-up of the protein level for the rest of their lives (Amdam et al., 2004; Amdam and Omholt, 2002). The maximum protein level at 10 days of age is strongly related to honey bee lifespan, and determines their capacity to nurse brood, the capacity to forage, and the chance to survive winter (Amdam et al., 2004; Kovac and Crailsheim, 1988).

Although it was suggested that bees could compensate for some pupal deficiencies when sufficient food and nurse bees are available (Kovac and Crailsheim, 1988), we found that after 1 week of their adult life, young bees infested by *V. destructor* and ample pollen had lost body weight and did not gain body weight and abdominal protein as much as bees not infested during the pupal stage reared under similar conditions. Hence, bees cannot compensate for the pupal deficiencies caused by infestation with *V. destructor* even when sufficient food and nurse bees are available. Our results are supported by the results of Schneider and Drescher (1987), who found that worker bees were not able to compensate for weight losses caused by *V. destructor* when they developed in a colony without mites. Our results are also supported and explained by Alaux et al. (2011), who found that at molecular level the negative impacts of *V. destructor* on the bee metabolism and immune functions could not be reversed by pollen feeding due to incorrect assimilation and use of pollen nutrients required for their physiological development at least until 8 days after emergence. They suggested that by inhibiting the macromolecule metabolism (notably proteins), *V. destructor* parasitism prevented bees from accessing the beneficial effects of pollen. This inhibition is most likely also related to their observation that pollen feeding increased virus prevalence in *V. destructor* infested bees. *V. destructor* is an efficient vector of bee viruses by promoting their transmission and development (Bowen-Walker et al., 1999). Alaux et al. (2011) hypothesized that because viruses such as Deformed Wing Virus multiply in the bee's fat body and this tissue is more developed in pollen-fed bees, bee viruses are able to increase under high food availability. These severe effects of Deformed Wing Virus interacting with *V. destructor* have been confirmed by Nazzi et al. (2012), who found that *V. destructor* can destabilize the within-host dynamics of Deformed Wing Virus via a widespread immunosuppression resulting in increased bee mortality.

It seemed that, in our experiment, more bees infested with *V. destructor* during pupation died in the first week, which agreed with the pattern of weight loss and other parameters, and was confirmed by Nazzi et al. (2012). Also, the availability of pollen to nurse bees did not enable the test bees to compensate for the negative effects of infestation. On the contrary, most bees died when both the parasite and pollen were present. This observation could be virus-related, as viruses may be able to increase under high food availability (see above). Bee mortality did not seem to be causally related, however, to the number of mites with which the bees emerged (as most bees emerged with one and not two mites). In general, fewer bees died during the laboratory experiment compared to the field experiment, where 40% of the bees were assumed dead after the first week of their adult life. We know from bees in the field, that they leave the hive for the first time around 6.2 days (S.E. 0.18 days) for an orientation flight (Capaldi et al., 2000). Not all of these bees may return due to their high naivety (Capaldi and Dyer, 1999).

Infestation with *V. destructor* decreased the pollen load size per foraging flight (Janmaat et al., 2000), which could well be a consequence of the limited growth and protein build-up in the first week as adult bees as we observed in our study. Janmaat and Winston (2000b) found that foraging age decreased and lifespan tended to decrease (but not significantly) by infestation by *V. destructor* and low pollen availability during brood rearing. This decrease in foraging age and (almost) reduced lifespan supports our findings on reduced weight at emergence and protein level in bees subjected to reduced pollen availability and infestation by *V. destructor*, as limited growth and reduced protein level could both cause decreased foraging age and lifespan (Amdam and Omholt, 2002; Amdam et al., 2004). It also agrees with Alaux et al. (2011), who found that reduced pollen availability as well as infestation with *V. destructor* induced a forager profile based on the expression of vitellogenin (which enhances longevity) and malvolio (encodes a manganese transmembrane transporter involved in sucrose responsiveness), suggesting that both factors induce aging.

The weight of the head of the bees has been shown to relate to the size of the acini in the hypopharyngeal glands (Babendreier et al., 2005; Hrassnigg and Crailsheim, 1998). In our study, we observed that only the availability of pollen affected the weight of the head of the bees and thereby the development of the hypopharyngeal glands, as was observed by Crailsheim and Stolberg (1989), but not by the infestation with *V. destructor*. The lack of effect due to *V. destructor* was in contrast to Schneider and Drescher (1987), who found that mite infestation during pupation resulted in reduced size of acini 7 days after emergence. In our study, the protein content of the head was not affected by any of the treatments, while infestation by *V. destructor* during the pupal stage reduced the abdomen protein level of the test bees at day 7, but only when beebread was available for the nurse bees. Moreover, protein of the head and abdomen were negatively related. As a result, the protein content of the head, protein used to feed offspring via the hypopharyngeal glands (Münch and Amdam, 2010; Standifer, 1967), most likely was preserved at the cost of abdominal protein. When colony conditions are suboptimal, i.e. reduced pollen availability and *V. destructor* infestation, these social insects may pre-

serve their nutritional reserves more for traits that are allocated to nurse their offspring than to optimize their survival (Kirkwood, 1977; Münch and Amdam, 2010). Preservation of nutritional reserves for offspring at the cost of survival was previously seen in Drosophila and Braconida species (Ellers and van Alphen, 1997; Fowler and Partridge, 1989). This allocation mechanism found in individual honey bees could function as a feedback mechanism on colony level to maintain nursing offspring, for instance when food resources are scarce or *V. destructor* infestation is high (Amdam and Omholt, 2002; Münch and Amdam, 2010).

The lower weight and protein levels of young bees in the absence of pollen could be explained by either the lack of food or the inefficiency of the nursing bees to properly feed the young bees. We assume for our study it was the lack of food, as Crailsheim (1990) showed that nurse bees under starvation are able to nurse nest mates for another week. However, we did observe that bees reared in the laboratory performed less than bees reared in the field, which suggests that results from laboratory studies cannot one to one be extrapolated to field situations or to a colony level. First, the stress of being kept at the laboratory could have amplified the effect of our treatments in the laboratory. Second, there may be a certain minimum of body weight and protein below which bees will die. As conditions in the field are expected to be harsher, this minimum may actually be higher compared to the laboratory, i.e. the weakest field bees may for example die during their first orientation flight, increasing the average body weight and protein levels of the remaining test bees. If true, this required minimal body weight and/or protein level would also explain why the body weight and abdominal protein level on day 7 of test bees raised by nurse bees without pollen available was not lower in bees infested than in bees infested by *V. destructor*, as was expected. More bees died when infested by *V. destructor* and only living bees that survived the experiment were analyzed for body weight and protein level, which would increase the average body weight and protein level of the survivors.

Bowen-Walker and Gunn (2001) suggested that losses of metabolic reserves due to infestation with *V. destructor* were not judged to be serious enough to be directly responsible for the high bee mortality and ultimate colony loss that are associated with the arrival of *V. destructor* in a colony. Our study shows however that *V. destructor* in interaction with other stressors, in this study reduced pollen availability, should be considered to be a potential main cause for the decline of honey bees (Ellis et al., 2010; Le Conte et al., 2010; Potts et al., 2010; Rosenkranz et al., 2010). In the context of colony loss, interactions between different stressors are indeed likely to be involved, and the presence of *V. destructor* in a colony does place an important pressure on bee health (Le Conte et al., 2010). 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 the availability of abundant pollen cannot compensate for reduced mass and protein content caused by *Varroa* parasitism.

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