Brood cell size of *Apis mellifera* modifies the reproductive behavior of *Varroa destructor*

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Abstract We undertook a field study to determine whether comb cell size affects the reproductive behavior of *Varroa destructor* under natural conditions. We examined the effect of brood cell width on the reproductive behavior of *V. destructor* in honey bee colonies, under natural conditions. Drone and worker brood combs were sampled from 11 colonies of *Apis mellifera*. A Pearson correlation test and a Tukey test were used to determine whether mite reproduction rate varied with brood cell width. Generalized additive model analysis showed that infestation rate increased positively and linearly with the width of worker and drone cells. The reproduction rate for viable mother mites was 0.96 viable female descendants per original invading female. No significant correlation was observed between brood cell width and number of offspring of *V. destructor*. Infertile mother mites were more frequent in narrower brood cells.

Keywords *Varroa destructor* · *Apis mellifera* · Brood cell width · Reproductive behavior · Foundress mother mites · Reproduction rate

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Introduction

Varroa destructor is an ectoparasitic mite of the Asian honey bee, *Apis cerana* that has become an important pest of the western honey bee (*Apis mellifera*) (De Jong et al. 1982b; Anderson and Trueman 2000). As this mite reproduces in honey bee brood cells, where temperature and humidity are tightly regulated by the bees, climate conditions appear unlikely to influence the life cycle of this parasite. However, *Varroa* reproduction can be influenced by host parameters (Ifantidis 1988; Eguaras et al. 1994).

Several studies have shown that the distribution of *Varroa* mites in brood combs differs significantly from a mathematical Poisson distribution and that it is aggregative (Donzé et al. 1996; Floris 1991; Le Conte et al. 1990). The authors of these studies indicated that aggregation of *Varroa* female mites in brood cells suggests differences in the chemostimuli triggering infestation, such as higher production of kairomones from parasitized larvae, deposition of chemical trails, or emission of an aggregation pheromone by infesting mites. However, Fuchs (1988) suspected that fluctuations in the infestation rate are the reason for the non-Poisson distribution. This was later shown by Martin (1995), by controlling for variations in infestation level. When this influence was removed, he found that the female mites entered brood cells on a fairly random basis; the resulting clustering can be described by the Poisson distribution. Based on this result, Martin (1995) suggested that the over-dispersed distribution of mites found in other studies is likely to be a result of combining data sets with varying infestation levels.

Varroa mites reproduce and mate only in capped brood cells of bees, but only adult female mites live outside the brood cells (De Jong et al. 1982a). To infest cells and reproduce, *Varroa* mites walk off their adult-bee hosts onto the comb surface and then walk into cells where mature larvae are located (Boot et al. 1991). Various studies have investigated factors that could affect the infestation rate of *V. destructor*, such as local climate conditions (De Jong et al. 1984; Ritter and De Jong 1984), bee race (Corrêa-Marques et al. 2002; Moretto et al. 1991), infertility of adult female mites (Corrêa-Marques et al. 2003), absence of the queen (De Jong 1981) and the rate of removal of infested brood (Corrêa-Marques and De Jong 1998). In addition, chemical signals related to the invasion of brood cells have been reported. Le Conte et al. (1989) and Nazzi et al. (2004) identified putative attractants in extracts of bee larvae that affect the brood cell invasion behavior of *V. destructor*. Once mites contact late larval bee instars, they are apparently retained on the larval cuticle by hexane-extractable hydrocarbons (Rickli et al. 1994). It has also been demonstrated that physical signs affect brood cell invasion by *Varroa* mites (Kuenen and Calderone 2000). The distance from a bee larva to the rim of the brood cell appears to be a key factor in the process of cell invasion by *Varroa* (Boot et al. 1995; Goetz and Koeniger 1993). Also, old honey bee brood combs are more infested by *Varroa* mite than new brood combs (Piccirillo and De Jong 2004).

Cell size is an important determinant in the invasive behavior of *V. destructor*; more parasites per cell were found in worker brood cells that are individually raised above the surrounding cells (De Jong and Morse 1988; De Ruitjer and Calis 1988). Message and Gonçalves (1995) observed higher infestation and reproduction rates of *V. destructor* in European bee combs, when compared with Africanized combs, associated with their greater comb cell width. Piccirillo and De Jong (2003) found the same pattern among combs built by different subspecies of *A. mellifera*, when compared in the same colony. However, Taylor et al. (2008) reported that brood cell width had no significant effect on mite reproduction or infestation rate. These various studies of the effect of comb cell width were made by manipulating the brood nest, introducing combs with different cell sizes into

colonies. However, how brood cell width affects the number of foundress mother mites per cell and its reproduction under natural conditions has not been studied previously.

The choice of a bee brood cell to begin reproduction is an important and critical moment in the life of the *Varroa* mite. In addition, the various reports of interaction between cell size and reproduction of the mite, in the interaction between *V. destructor* and it host *A. mellifera*; provide an excellent opportunity to study how selective pressures physical cues (in this case variability in cell width) can modify the reproductive behavior of an ectoparasite. Our objectives were to examine the natural variability in brood cell width in combs of *A. mellifera* and to analyze whether this factor affects the number of foundress mother mites per cell and its reproduction rate under natural conditions. The hypothesis tested was that brood cell width of *A. mellifera* is a key factor that affects the reproductive behavior of *V. destructor* under natural conditions. This hypothesis would be supported if infestation or reproduction rates vary with brood cell width.

Materials and methods

The study was conducted at the Laboratorio de Apicultura, J.J. Nágera coastal station (38°10′06″S; 57°38′10″W) and at the Laboratorio de Artrópodos (Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata in Argentina. Drone cells were limited during the sampling period because we carried out the sampling during fall and spring 2007, when cold weather induces the bee colonies to restrict the brood area, especially the drone brood. Eleven colonies of *A. mellifera* maintained in Langstroth-type beehives were used for the experiments.

Population distribution of *Varroa destructor*

We examined the population distribution of *V. destructor* within brood cells of *A. mellifera*. One sealed brood comb from each colony was intensively sampled. All of the brood cells of each comb were opened and the number of foundress mother mites (parasitic intensity) inside each cell was registered.

Morisita's index (Brower and Zar 1977) was estimated according to the following equation:

$$\text{Is} = N\left[\left(\sum X^2 - \sum X\right)\Big/\left(\left(\sum X\right)^2 - \sum X\right)\right]$$

where:

Is = Morisita's dispersion index
N = Number of cells of *A. mellifera* inspected.
$\sum X$ = Sum of the number of *V. destructor* foundress mother mites found within the inspected cells.

Based on the characteristics of this dispersion index, if:

Is = 1, the population distribution of *V. destructor* was adjusted to Poisson.
Is < 1, the population distribution of *V. destructor* was normal.
Is > 1, the population distribution of *V. destructor* was distributed according to negative binomial law.

To evaluate if the value of Is was significantly different from 1, the following equation was employed:

$$F0 = \left(Is \left[\left(\sum X \right) - 1 \right] + N - \sum X \right)/N - 1$$

If the value of F0 was greater than the tabulated F with $n - 1$ grades of freedom and 95% of confidence, it was concluded that Is was significantly different from 1. Infestation rate and mite distribution were analyzed for each colony. Infestation rate was estimated following De Jong (2005).

Brood cell width and its effect on the number of foundress mother mites invading cells and on its reproduction rate

Six colonies of *A. mellifera* were used. A drone brood comb was collected from one colony, while a worker brood comb (with no drone brood) was sampled from the remaining five colonies. Photographs of each comb were taken with a Kodak Easy Share C340 camera. The inner width of each sealed brood cell (distance between two parallel walls) was measured in each photograph using Image J software. Diameter distributions of worker and drone cells were graphed in frequency histograms.

All of the brood cells of each comb were opened and the number of foundress mother mites inside each cell was registered. To facilitate recording the data, a colored pin of different color was assigned to each opened cell according to the following criteria: green—zero mites; yellow—one mite; light blue—two mites; red—three or more mites. The combs were photographed again with the pins. Using Image J software the two types of photographs (combs with sealed cells and those with opened cells with pins) were superimposed in order to relate the width of each sealed cell measured with the number of foundress mother mites and its reproduction rate. Reproduction rate of *V. destructor* was estimated only in worker brood cells. Only worker brood cells invaded by a single mother mite were analyzed, since multiple infestations can affect mite reproduction (Fuchs and Langenbachs 1989). Whenever possible, only brood that had been sealed for longer than 230 hours (at least at the yellow thorax stage of development) was collected. Mite eggs and protonymphs were not sexed, as this cannot be done reliably using external characteristics (Steiner 1988). Protonymphs and deutonymphs were distinguished by counting the number of sternal setae, according to Nannelli (1986). Reproduction rate was calculated for foundress mother mites according to Ifantidis (1984), by dividing the total progeny of the mites by the number of mites of the parental generation.

To determine whether brood cell width affected the number of *V. destructor* foundress mother mites in the brood, a generalized additive model (GAM) (Hastie and Tibshirani 1990; Zuur et al. 2007), which is based on Binomial and Poisson distributions, was used for both worker and drone cells.

To determine whether brood cell size affected *V. destructor* reproduction rate, we used a Pearson's correlation and a Tukey test to calculate the relationship between the total number of mites infesting each brood cell (original adult and offspring) and the width of the brood cell. Each foundress mother mite was classified into one of the following three categories: (1) viable mother mite (a mother mite that produced live female and male offspring); (2) inviable mother mite progeny (a mother mite that produced incomplete offspring due to dead female or male offspring or only a male produced); (3) infertile mother mite (mother mite did not reproduce). Tukey test was used to compare cell width means whenever a one-way ANOVA indicated significant differences between cell width means for the three categories of mother mites.

Results

Population distribution of *Varroa destructor*

From the eleven colonies, 6,394 worker brood cells were inspected. The distribution of *V. destructor* adjusted to the negative binomial distribution in all colonies. Morisita's index was significantly greater than one ($P < 0.05$), in all colonies (Table 1). When data for all colonies were pooled, Morisita's index was 1.344 and was significantly different from 1 (F0 = 1.32 > Ft; $P < 0.001$). Infestation rates for each colony varied from 16.1 to 75.6% (Table 1). The mean infestation rate (±standard deviation) for all colonies was 39.6 ± 19.8% Frequency distribution of mean numbers of mites per cell per colony and standard deviations for pooled data are shown in Fig. 1.

Brood cell width and its effect on the number of foundress mother mites invading cells and on its reproduction rate

A total of 2,899 worker cells and 552 drone cells from the six colonies were examined. The mean width of the worker brood cells was 5.26 ± 0.51 mm (range = 4.17–6.86 mm), while for the drone brood cells it was 6.4 ± 0.5 mm (range = 5.05–8.07 mm). Worker and drone cell widths overlapped at from 5.5 to 6.8 mm (Figs. 2 and 3). A unimodal distribution of cell width was observed for both castes, with low relative frequencies for the extreme values (Figs. 2 and 3). Drone cell mean widths were 1.22 times greater than worker cell mean widths, although some worker cells were larger than the mean drone cell size.

Parasite intensities were 1.46 ± 0.34 foundress mother mites/worker cell (mean infestation frequency = 40.87%) and 2.16 ± 0.56 foundress mother mites/drone cell (mean infestation frequency = 75.36%).

GAM analysis for worker brood cells was significant ($\chi^2 = 104.4$; df = 2,786; $P < 2e\text{-}16$); it explained 5.15% of the deviance. The results presented here showed that the probability to find parasites inside cells worker brood cells was positively correlated with worker brood cell width. The relation was sigmoidal: the probability to find parasites inside worker brood cells increased lineally from cells of 4 mm to 5.9 mm where it became asymptotic (Fig. 2). GAM analysis for the drone brood cells was significant ($\chi^2 = 163.4$; df = 2,399; $P < 2e\text{-}16$); it explained 14.3% of the deviance. The number of parasites inside drone cells was positively correlated with cell width. The relation was sigmoidal: the number of foundress mother mites of *V. destructor* increased linearly from 5 to 6.8 mm, after which it became asymptotic (Fig. 3).

Table 1 Number of inspected cells, parasitized cells, infestation rate and Morisita's index for each colony sampled

	Colony										
	1	2	3	4	5	6	7	8	9	10	11[a]
Total of inspected cells	809	917	304	443	470	536	641	685	442	585	552
Cells containing one or more mites	507	510	49	115	223	171	117	170	226	156	417
Infestation rate	62.7	55.6	16.1	25.9	47.4	32	18.2	24.4	51.2	26.7	75.6
Morisita's index	1.55	1.43	1.22	1.26	1.3	1.29	1.23	1.27	1.4	1.26	1.6

[a] In colony 11 a drone brood comb was sampled. Colonies from 6 to 11 were used to relate brood cell width with its effect on the number of foundress mother mites per cell and its reproduction rate
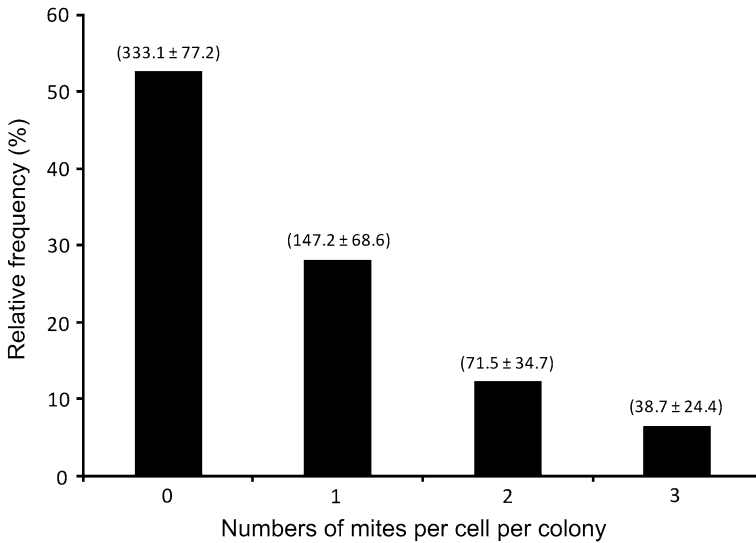
Fig. 1 Frequency distribution of mean numbers of *Varroa* mites per cell per colony and standard deviations for all *Apis mellifera* colonies sampled
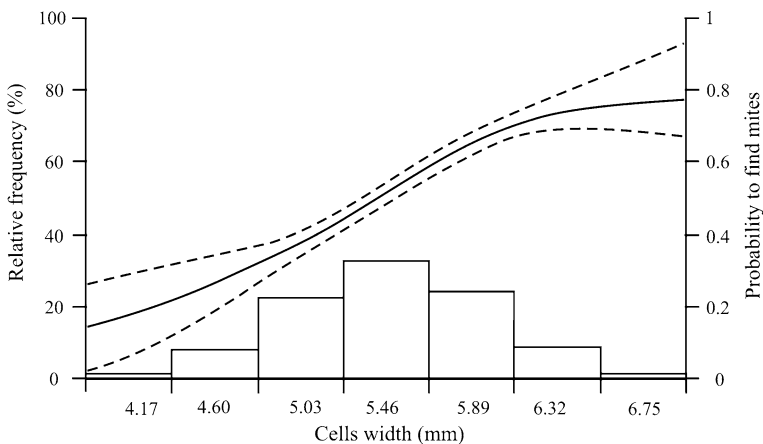

Fig. 2 Relative frequency distribution of worker brood cells width of *Apis mellifera* by means of class intervals and its relation with the probability to find a foundress mother mite inside them. Generalized additive modelling using a Binomial distribution ($\chi^2 = 104.4$; df = 2,786; $P < 2e\text{-}16$). *Upper* and *lower lines* represent superior and inferior fiducial limits, respectively ($n = 2,899$)

Foundress mother mites per cell varied with the proportion of available worker and drone brood cells (Figs. 2 and 3). The number of mites per cell increased positively and linearly with the width of worker and drone cells, until it became constant due to a decrease in the availability of wider cells.

Ninety-five of the 2,899 worker brood cells that we examined contained a single mother mite (3.3% single infestation). No significant correlation (r = 0.25; $P = 0.2$) was observed between cell width and the number of *V. destructor* offspring. Fifty-eight percent of the
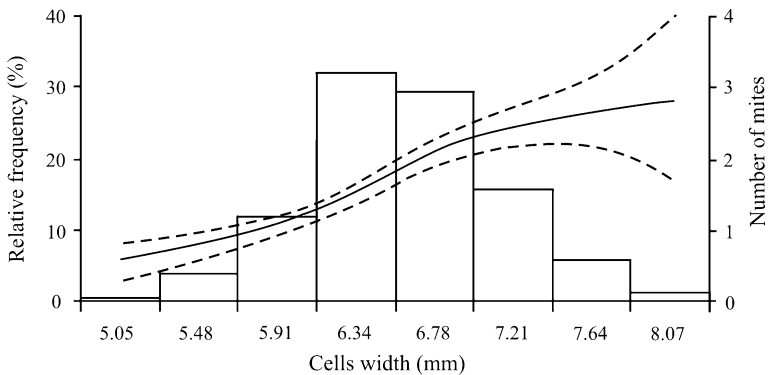
Fig. 3 Relative frequency distribution of drone brood cells width of *Apis mellifera* by means of class intervals and its relation with the number of foundress mother mites inside them. Generalized additive modelling using a Poisson distribution ($\chi^2 = 163.4$; df = 2,399; $P < 2e-16$). *Upper* and *lower lines* represent superior and inferior fiducial limits, respectively ($n = 552$)

singly-infested worker cells had viable offspring, 26% had inviable offspring and 16% contained infertile mother mites. The rate of increase for viable mother mites was 0.96 viable female descendants. Mean cell width was significantly different among the categories of mother mite progeny (ANOVA, F = 6.343, P = 0.003); the mean widths of cells with infertile females, and viable and inviable progeny were 4.92, 5.4 and 5.38 mm, respectively.

Discussion

According to Poulin (1998), parasite habitats are not spatially continuous; they consist of discrete cells or "islands". Hosts constitute patches of suitable habitat in an otherwise inhospitable environment. In addition, parasites are aggregated among available hosts, such that most hosts harbor no or few parasites and few hosts harbor many parasites. The tendency towards parasite aggregation suggests that similar processes may be acting to generate the same pattern in different host-parasite systems, i.e., reproduction of parasites within hosts, density-dependent parasite mortality and parasite-induced host mortality (Anderson and Gordon 1982).

We found the population distribution of *V. destructor* within brood combs of *A. mellifera* to be aggregated; it was explained partially by the distribution of cell widths. Other studies have shown that the distribution of *Varroa* mites in cells differs significantly from a random distribution and that it is aggregative (Donzé et al. 1996; Floris 1991; Le Conte et al. 1990). These authors indicated that the aggregation of *Varroa* female mites in brood cells suggests differences in the chemostimuli triggering infestation, such as higher production of kairomones by parasitized larvae, deposition of chemical trails, and production of an aggregation pheromone by infesting mites. However, brood cell dimensions also play an important role in the cell invasion of *V. destructor* (De Jong and Morse 1988; De Ruitjer and Calis 1988; Message and Gonçalves 1995; Piccirillo and De Jong 2003). We found that the distribution of cell widths among worker and drone cells in a brood comb of *A. mellifera* under natural conditions is not uniform and that it is highly variable, resulting in habitat patches that can be preferentially colonized by *V. destructor*. We found

the variability in the width of cells to have a direct effect on the aggregation of *V. destructor* within brood combs. Although Martin (1995) concluded that the overdispersed distribution of mites is likely to be a result of combining data sets with varying infestation rates, we found that all our colonies had overdispersed mite distributions, independent of the infestation rates. Moreover, it was only used data from one brood for each colony, so it was impossible that the overdispersed distribution found in this study was the result of combining data sets. There is controversy about which factors, including physical and chemical elements, are important in triggering the invasion of brood cells by *V. destructor* and consequently affect its aggregation. Calderone and Lin (2001) proposed four *V. destructor* invasion models based on physical interactions, chemical interactions, both physical and chemical interaction, or due to passive mechanisms (Message and Gonçalves 1995). Kuenen and Calderone (2000) proposed that physical factors are the most important. Among physical factors, brood cell size has been well studied; it has been documented that this factor can affect the invasion and reproduction rates of *Varroa* mites. Higher parasite infestations were found to occur in worker cells that were individually raised above the surrounding brood cells (De Jong and Morse 1988; De Ruitjer and Calis 1988). Piccirillo and De Jong (2003) found that when comparisons were made within the same colony, the largest category of brood cells, which were Carniolan bee combs (mean width of 5.3 mm), were about 38% more infested than Italian comb brood cells (mean width 5.15 mm), which in turn were about 13% more infested than the self-built Africanized combs (mean width of 4.8 mm). Message and Gonçalves (1995) found similar results when infestation rates of *V. destructor* were compared between European and Africanized honey bee combs in colonies of Africanized bees. Worker brood that develops in large cells should receive more visits from nurse workers, increasing the probability of the mites transferring to the cells to infest the brood. These factors could increase the probabilities of the female mites entering into the cells, and in turn such cells would contain a larger bee larva and consequently offer more food to the mite.

We found that brood cell width in *A. mellifera* colonies affected the invasion and reproduction rate of *V. destructor* under natural conditions. The parasite invasion rate increased positively and linearly with the width of worker and drone brood cells (probably because brood developed in large cells will receive more visits from nurse workers, increasing the probability of the mites transferring to the cells to infest the brood), until it became constant when it reached a certain cell width. Moreover, it was found that infertile mother mites were more common in the smaller cells. Earlier studies had reported that larger cells promoted higher parasite reproductive success than smaller ones (Fuchs and Langenbach 1989; Message and Gonçalves 1995; Schulz 1984), but there are no reports that cell width can stop *Varroa* mite reproduction. Taylor et al. (2008) found that brood cell width had no significant effect on mite reproduction and they concluded that factors that are attributable to the race of bee, in combination with small brood cells, reduce the mite infestation and production of offspring. We found no correlation between cell width and the number of offspring of *V. destructor*. Nevertheless, cells with infertile mother mites (without offspring) were significantly smaller than cells with viable and unviable offspring. These results showed that cell width can drastically influence the invasion and the reproduction rates of the mites. Donzé and Guerin (1994) reported that *V. destructor* had several behavioral adaptations to ensure its reproduction inside brood cells, because the free space inside the cells is continually changing during the bee's metamorphosis. In addition, Martin and Kryger (2002) showed that a reduced space between the bee pupa and the cell wall in worker brood cells negatively impacts on the reproductive success of the mite because it can not move normally inside cells. Cell width plus a stress effect

(e.g., nutrition effect) could be the connection between the lack of mite reproduction and the size of the cell that we found. Previous researches have reported that the nutrition quality improves mite reproduction (Rodriguez et al., 1961; Steiner et al., 1995). It was reported that *Varroa* mite reproduction can be suppressed under stress conditions due to severe reproductive competition, which can result in egg resorption by the foundress mother mite (Steiner et al., 1995). Also, Alberti and Hanel (1986) showed that mites that had not laid eggs often had problems with prosperm maturation in the reproductive tract of the female mites. Like egg resorption, problems with prosperm maturation could be due to stress conditions. Tewarson (1983) reported that reproduction of this parasite occurs only after intake of a number of blood meals; some bee hemolymph proteins are absorbed without digestion. Steiner et al. (1995) demonstrated that oogenesis is faster in drone brood cells than in worker brood cells, probably due to greater food availability for the foundress mother mite. Based on this line of reasoning, the smaller cells could contain smaller larvae, which would provide a smaller quantity of nutrients for the initiation of mite reproduction. Harris and Harbo (1999) suggested that mites that had not laid eggs had problems with mating or a failure of the prosperm to mature in the reproductive tract of the female mites. However, they were not able to distinguish between nonmating and failure of sperm maturation.

If the cell width distributions found in this study (where bigger cells are less numerous) are considered, there could be a trade-off between the time necessary to search for optimal cells and the higher reproductive success obtained in such cells: *Varroa* females try to find bigger cells because they do not reproduce as efficiently in the small ones (probably because of a food stress factor); although finding big brood cells would insure better conditions for parasite reproduction, the associated costs with searching for infrequent cells would not be compensated by the benefits of finding such cells, making this strategy inefficient (Optimal Foraging, Charnov 1976). This hypothesis is supported by the evidence presented by Boot et al. (1994), who found that the invasion rate increases with the number of brood cells available. Moreover, to search for bigger cells, with the higher number of foundress mother mites inside, as we report here, could result in a reduction in the reproductive success of *Varroa* female mites because of a negative density-dependent effect (Eguaras et al. 1994; Fuchs and Langenbach 1989). These hypotheses could be tested in future experiments.

This study is the first report that cell width can restrict the reproduction of *V. destructor*. Moreover, we demonstrated that an aspect of the physical environment can modulate the reproductive behavior of this parasite, resulting in new hypotheses on relations between nutrition and reproductive success of the mites.

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