

***Varroa destructor* infestation impact on *Apis mellifera carnica* capped worker brood production, bee population and honey storage in a Mediterranean climate**

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Abstract – This study investigated the impact of *Varroa destructor* infestation on the amount of capped worker brood, the adult bee population and honey production of authenticated *Apis mellifera carnica* colonies kept in a Mediterranean climate. For this purpose, colonies were set-up and either maintained mite-free or artificially mite-infested and allowed to develop an infestation. Periodical evaluations of those colonies unravelled the pattern of the previously mentioned variables across the season, and allowed for comparative numerical analyses. Progressive reductions on the amount of capped worker brood, bee population and honey storage in mite-infested colonies only became increasingly evident during spring and summer, apparently associated with impressive mite population increases. By the end of the experiment, mite-infested colonies showed a unitary average reduction of 45% in the amount of capped honey they stored, meaning an average annual loss of ≈ 24 kg of honey per colony. However, the amount of capped honey stored per bee and day was found to be independent from colony *V. destructor* status, indicating a lack of direct effect of mite infestation on honey hoarding behaviour.

***Varroa destructor* / *Apis mellifera* / population dynamics / honey production**

1. INTRODUCTION

Since *Varroa destructor* Anderson & Trueman (Anderson and Trueman, 2000) was first found in Portugal, in 1987 (Belchior, 1996), it has been associated with both increased honey bee colony mor-

talidity and decreased honey production. However, this is the first study quantifying the impact of *V. destructor* infestation on capped worker brood, bee population and honey production of honey bee colonies in Portugal. Studies of this nature, conducted in a single environment (Büchler, 1992,

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1994) and covering an entire annual colony cycle (Echazarreta and Paxton, 1997), have been rare.

In temperate climates, the general pattern of colony growth throughout the year has been well described (Jeffree, 1955; Seeley, 1984; Winston, 1987). However, the uninterrupted presence of brood (except perhaps for a short period associated with swarming) in Mediterranean climates (Vieira et al., 1990; Puerta et al., 1993; Ferrer-Dufol et al., 1994; Calatayud and Verdu, 1995; Branco et al., 1999) is a major qualitative difference to that pattern. The milder winter conditions in Mediterranean climates and the availability of food during a considerable part of the winter account for this difference in the brood-rearing cycle, which, in turn, is relevant to the intrinsic growth rate of *V. destructor*. Thus, the purpose of this study was to investigate the relative impact of mite infestation on capped worker brood, bee population and, particularly, on honey production of honey bee colonies kept in a common Mediterranean environment.

2. MATERIALS AND METHODS

2.1. Colony establishment and management

Field work was started in July 1992 and ran until late August 1993. At the beginning of this experiment, 12 young (less than two month old) mated queens designated by the seller as *Apis mellifera carnica* Pollmann were imported from the USA. All queens were wing-clipped, colour coded and introduced into 1 kg (\pm 50 g) packages of *A. m. iberica* Goetze workers, which had been previously treated with Folbex® VA (bromopropylate). After queen introduction, these packages of bees were kept in five-frame Langstroth hives and received six litres of sucrose solution (50%, w/v) during the four weeks after colony set-up

(which occurred during early May). In June, these colonies were moved into 10 frame Langstroth hives and allowed to develop for approximately eight weeks, so that the progeny of the introduced queens could replace most of the initial *Apis mellifera iberica* workers [before being evaluated (mid July)]. By this time, seven honey bee colonies existed. They were kept at the Research Station of Évora University (southern Portugal; N38°34', W07°54') and were all morphometrically authenticated, based upon standards provided by the Institut für Bienenkunde (Oberursel, Germany), as *A. m. carnica* (Graça, 1996). This verified that the introduced queens had replaced most of the initial *A. m. iberica* workers with their own offspring.

Colony management was conservative, meaning that no strategies or management techniques were followed to improve colony productivity or population growth. The major concern was to prevent swarming during the spring, mainly by making areas of honey storage and egg laying space (drawn comb) available approximately two weeks before it was probably needed.

No prophylactic or therapeutic actions were carried out, other than the one applied to colonies intended to be mite-free. Also, no feeding was carried out during the experimental period and no honey was removed from the experimental colonies.

2.2. Artificial *V. destructor* infestation

Three colonies were randomly selected to be kept virtually mite-free (control colonies, hereafter referred to by "non-infested" colonies), while the remaining four colonies were artificially infested with *V. destructor* (treatment colonies, hereafter referred to as "infested" colonies).

To ensure comparable initial infestation levels (in relative terms), the number of mites received by each infested colony ranged from 50 to 102 (77.0 ± 13.4 ,

$\bar{x} \pm \text{s.e.m.}$) and was kept proportional ($2.91 \pm 0.003\%$) to the area of capped worker brood (cm^2) that these colonies had at that time (mid July, 11707 ± 2028 capped worker brood cells).

V. destructor individuals were collected from honey bees of highly mite-infested colonies (by dusting with powdered sugar) and pooled together before being randomly distributed to experimental colonies previously selected to be artificially infested. Only dark-brown motile mites were selected for artificial infestation. They were introduced into colonies at sunset, onto frames with open and capped worker brood. The few individual mites (1.14 ± 0.40) that fell onto hives' bottom boards overnight were recovered on next day early morning and replaced by equal numbers of mites at sunset time.

2.3. Maintenance of non-infested colonies

Non-infested colonies were continuously treated with two strips of Apistan[®] (Fluvalinate, 800 mg per strip) to kill most incoming mites before they could start reproducing (Milani et al., 1993). Apistan[®] strips were replaced approximately every eight weeks, according to manufacturer's instructions.

2.4. Data collection

Data were collected on (i) areas of capped worker and, if existent, drone brood; (ii) honey bee colony population estimates and (iii) areas of capped honey. Colonies were assessed approximately every three weeks (except during the months of November, December and January).

All comb areas occupied with brood or capped honey were drawn onto transparent acetate sheets and were later digitized by computer assisted image analysis. All measured areas, of a given type, in every colony

frame, were added per experimental colony and observation date.

Conversion of measured areas of capped brood, into number of capped brood cells, were achieved by using the factors of 2.90 and 4.42 brood cells $\times \text{cm}^{-2}$, for drone and worker brood respectively. A colony's adult population was roughly estimated by visual comparison of each frame occupied with honey bees with a set of eight calibrated photographs (Jeffree, 1951). Evaluations were always made by the same observer in early morning or late afternoon, when most of the forager bees were expected to be in the hive. The aim of this methodology was to gain an approximation to adult bee populations, with a view to comparing among colonies.

In assessing the amount of honey stored in colonies, only areas of operculated honey were considered. Because both brood nest boxes and honey supers were of the same type and filled with ten frames each, variation in comb depths was reduced and randomly distributed between mite status. By studying eight samples (two per box type and mite status) of $\approx 25 \text{ cm}^2$ each of capped honey and fully drawn empty comb, it was concluded that each cm^2 of registered capped honey held $1.79 \pm 0.06 \text{ g}$ of honey. Honey hoarding behaviour (daily honey gain per bee) was calculated based on an estimated mean adult population at the midpoint between to consecutive observation dates, assuming linear population variations between observation dates.

Also, to follow the population dynamics of *V. destructor* without interfering excessively with mite population growth or honey bee colony development, samples of capped worker brood and adult honey bees were also collected on 15.07.92 (control sampling carried just before artificial infestation), 01.11.92, 26.02.93, 20.05.93 and 28.07.93. As far as capped drone brood was concerned, samples were only collected on 20.05.93, when most experimental colonies had sufficient capped drone brood to be

assessed without interfering excessively with drone production. These samples allowed the assessment of apparent mite infestation levels of capped brood and of adult honey bees (Pappas and Thrasyvoulou, 1988). The total mite population in all experimental colonies were then obtained, across all sampling dates, by adding up the estimated numbers of mites in capped (worker and drone) brood and on adult bees.

Loss of bees by swarming was appraised by checking for the presence of the young colour-coded queens during the regular visits to the colonies. Where loss of queens occurred (either by swarming, supersedure or accident), colonies were removed from the experiment.

2.5. Statistical analyses

Assumptions inherent to parametric statistics, namely variables' independent normal distributions of errors (with a mean of zero and constant variance) and experimental group (mite status) homoscedasticity (Fry, 1993), were confirmed before carrying out statistical tests. Levels of capped worker brood in the colony, adult populations, amounts of capped honey and honey hoarding behaviour were analysed by a two-way repeated-measures analysis of variance (repeated-measure ANOVA), where (i) honey bee colonies represented the subjects upon which measures were repeated, (ii) mite status was the between-subject effect and (iii) the observation date was the within-subjects effect.

3. RESULTS

When colonies were first assessed (mid July), no significant differences were found in terms of capped worker brood ($F_{[1,5]} < 0.001$, $P = 0.995$), honey bee colony population ($F_{[1,5]} = 0.038$, $P = 0.853$), or capped honey ($F_{[1,5]} = 0.037$, $P = 0.856$) that could be associated with mite status.

Although no *V. destructor* were found established in brood or on adult bees of non-infested colonies, these colonies received 0.8 ± 0.2 invading mites \times day⁻¹, throughout most of the experiment. A major increase (21 ± 6 mites \times day⁻¹) in *V. destructor* infestation pressure occurred during the second half of December 1992, apparently related with robbing of collapsing mite-infested colonies. Across the experiment, non-infested colonies received a total number of 414 ± 16 incoming mites.

In infested colonies, mean increases of approximately 1344% in established mite populations were observed within 195 days after artificial infestation (late February 1993). Colonies' mean mite population was then 1083 ± 131 (Fig. 1), corresponding to daily finite rates of mite population increase (Calatayud and Verdu, 1993) of 1.0138. On middle May 1993, infested colonies hosted an average absolute peak of 9616 ± 3877 *V. destructor*, decreasing in mite population (7505 ± 854) towards late July 1993 (Fig. 1). This corresponds to an overall experimental daily finite rate of mite population increase of 1.0123.

Compared with non-infested colonies, only moderated capped worker brood and honey bee population growths were achieved by infested colonies, particularly throughout spring and summer 1993 (Fig. 1). Considering all estimates of bee colony populations carried out across the experiment, statistically significant differences were associated with both mite status ($F_{[1,5]} = 36.73$, $P = 0.002$) and its interaction with observation date ($F_{[12,60]} = 11.24$, $P < 0.001$). Mite infested colonies only became significantly associated with reduced colony populations from April onwards. A slower accumulation of honey stores was observed in infested colonies (Fig. 2). Indeed, a repeated-measure ANOVA revealed statistically significant differences in the amounts of capped honey related to both mite status ($F_{[1,5]} = 72.42$, $P < 0.001$) and its interaction with observation date

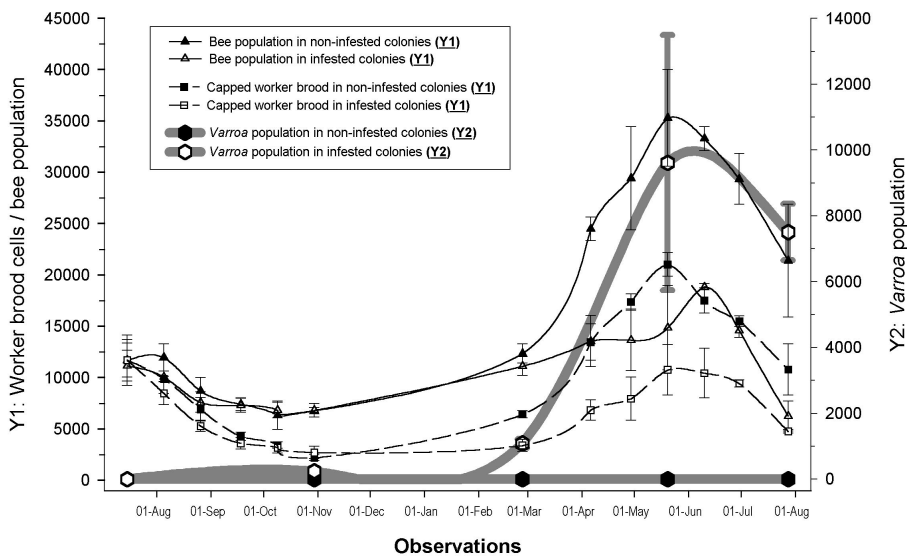


Figure 1. Adult bee populations, capped worker brood cells and *Varroa destructor* populations.

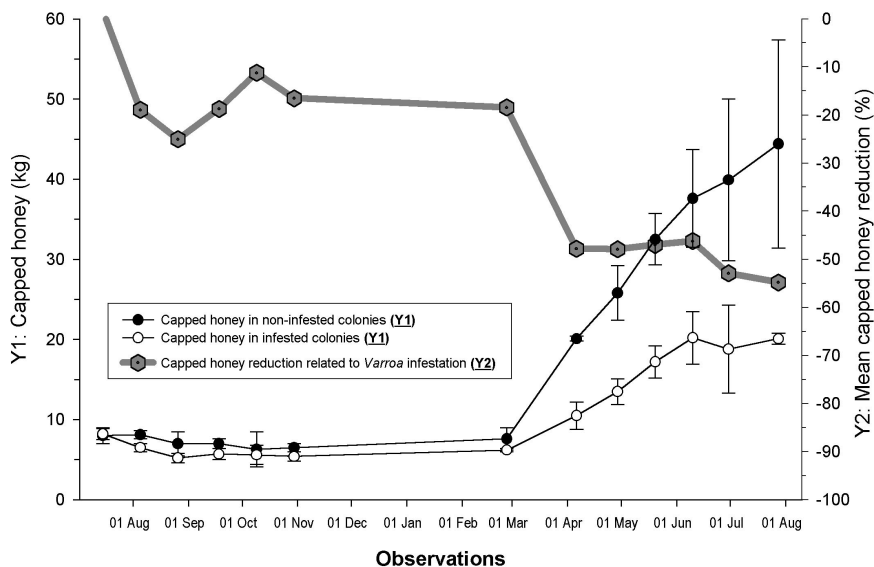


Figure 2. Capped honey and mean honey reduction associated with *Varroa destructor* infestation.

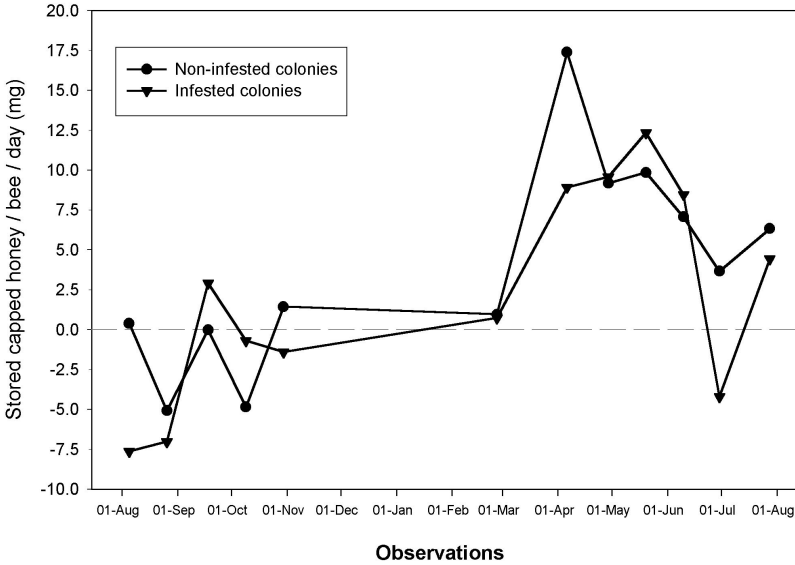


Figure 3. Mean amount of capped honey stored per bee and day.

($F_{[12,60]} = 9.83, P < 0.001$). Again, an association between mite infested colonies and lower amounts of capped honey only became statistically significant from April onwards. Although the infested colonies arrived at the end of the experiment with only 45.2% (≈ 20 kg) of the capped honey stored in non-infested ones (≈ 44 kg), a direct effect of mite infestation on honey hoarding behaviour was not observed (Fig. 3), neither through the whole experiment (repeated-measure ANOVA; $F_{[1,5]} = 3.35, P = 0.14$) nor across the last six observation dates (repeated-measure ANOVA; $F_{[1,5]} = 2.52, P = 0.19$), when higher colony honey gains were observed.

4. DISCUSSION

This study meant to look into the impact of *V. destructor* infestation on capped worker brood, bee population and, particularly, on honey production of honey bee colonies maintained in a common Mediter-

anean environment. Considering that all colonies entered this experiment in similar conditions, the results obtained are essentially related to the interaction between mite population growth and their host colony development, rather than with large sources of uncontrolled initial variation. On the other hand, non-infested colonies were probably weakened by the negative effect of repeated fluvalinate applications (Barbattini et al., 1989; Slabezki et al., 1991; Liu, 1992), meaning that the magnitude of the differences between non-infested and infested colonies may have been underestimated. Considering the entire experimental period, the daily finite rates of mite population increase were slightly lower (1.0123) than the ones also obtained in Mediterranean conditions by Kraus and Page [(1995); 1.0137 to 1.0214], Calatayud and Verdú [(1993, 1995); 1.021 to 1.027] or Branco et al. [(1999); 1.0189 to 1.0347]. These differences may have arisen from environmental [e.g. geographical location, honey bee colony management or mite

immigration rates (Branco et al., 1999; Delaplane and Hood, 1999)] or genetic (bee strain) variations.

Continuous increases in *V. destructor* populations in infested colonies were observed up to the penultimate observation, decreasing afterwards towards the last observation date (as colonies were collapsing). Some of the reasons that can partly explain this fact [also mentioned by Branco et al. (1999)] include limitations imposed by host conditions upon mite reproduction and survival rates and, eventually, a considerable increase in desertion of highly mite-infested adult honey bees (that may occur much more frequently in the terminal phase of *V. destructor* infestation). This feed-back mechanism of host terminal condition in restricting mite population growth has been ignored by relevant models of mite population dynamics (Fries et al., 1994; Fries, 1996; Martin, 1998). Furthermore, the observed pattern of *V. destructor* population dynamics is incongruent with the exponential growth / decay cycles hypothesised to occur in *A. mellifera* colonies in temperate climates (Fries et al., 1994; Fries, 1996). In Mediterranean climates, there are no such successive cycles and most acaricide-untreated mite-infested colonies can be expected to die within approximately one year after becoming infested (Calatayud and Verdú, 1992; Rinderer et al., 1993; Murilhas, 1994; Branco et al., 1999).

The particularly rapid increase in *V. destructor* populations in honey bee colonies in Mediterranean climates, more than being caused by fast mite population growth during the summer [even in cold climates, the number of mites can increase up to 100 times during one summer (Fries et al., 1991)], seems to be determined by the lack of a period of non-reproduction and high mite losses associated with clustered broodless colony overwintering (Korpela et al., 1992). It is known that, in temperate climates, a large proportion of mites [randomly distributed among honey bees in

broodless overwintering colonies (Ritter et al., 1989)], die over winter along with numerous adult bees (Kovac and Crailsheim, 1987; Fries et al., 1991; Fries and Perez-Escala, 2001). In regions with cold winters, at least a 50% reduction in a colony's honey bee population can be expected to occur during winter (Avitabile, 1978) and one can therefore expect that, in these conditions, approximately half the *Varroa destructor* individuals will also die over winter. This situation does not happen in Mediterranean climates and thus the honey bee colony life cycle places fewer restraints on mite population growth before colonies approach collapse (Branco et al., 1999). Under such a context, infested colonies tended to hold considerably less capped worker brood, to be significantly less populous (from lower egg laying rates, higher brood mortality or shorter mean life expectancies) and to store less honey than non-infested ones (particularly during spring and summer). However, an effect of *V. destructor* infestation on honey hoarding behaviour was either non-existent or insignificant. This suggests that mites do not directly interfere with average variations in the amounts of honey daily stored per bee, independently of the over-riding indirect effect it plays (through colony adult bee population reductions) on colony foraging potential. The reasons for this may be related with the fact that severe mite infestations frequently lead either to the death of individuals or to an increased probability for them to become detached from their colonies earlier (by expulsion, predation or disorientation). In this case, *V. destructor* infestations can induce considerable colony population reductions without directly interfering with the honey hoarding behaviour of its remaining population. On the other hand, in low to moderate mite infestations, many of the parasitized workers survive long enough to forage for their colonies. In this case, Schneider and Drescher (1987) reported decreases in survival rate of bees beyond 25 days, proportionally related to the level of infestation

they suffered during their immature stages and particularly during late summer (De Jong and De Jong, 1983; Kovac and Crailsheim, 1988; Moretto et al., 1991). Nevertheless, no firm relationship has been demonstrated between the level of mite parasitism and the mean life expectancy of a bee. Some groups of severely infested workers may show long mean life expectancies and equal foraging capacity (in terms of frequency and mean time of flight duration) compared with non-infested workers of the same colony (Kovac and Crailsheim, 1988). As Ball (1994) pointed out, this suggests that the level of mite infestation is not the only (and perhaps not the primary) cause contributing to the reduction of life expectancies of *V. destructor* – infested workers. Factors such as climate, forage availability, season of the year, and the presence and level of secondary infections may all interact and contribute decisively to the outcome of infested individuals. Apparently, colony effects of all these individual outcomes should have been relevant to explain why no direct effect was found between colony mite status and the amount of capped honey they stored per bee per day.

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Résumé – Impact de l’infestation par *Varroa destructor* sur la production de couvain d’ouvrières operculé, la population d’abeilles et les réserves en miel chez *Apis mellifera carnica* en climat méditerranéen. En climat méditerranéen, les conditions hivernales douces et la possibilité de récolter de la nourriture durant une grande partie de l’hiver expliquent que l’activité d’élevage du couvain soit permanente dans les colonies d’abeilles, ce qui en retour

autorise un taux de croissance intrinsèque élevé des populations de l’acarien *Varroa destructor*. Dans ce contexte l’étude visait à rechercher l’impact de l’infestation par l’acarien sur la quantité de couvain operculé, la population d’abeilles adultes et la production de miel chez des colonies d’*Apis mellifera carnica* authentifiées et élevées en climat méditerranéen typique (N38°34’, W07°54’). Pour ce faire, des colonies ont été établies et soit maintenues indemnes de *V. destructor*, soit artificiellement infestées par l’acarien avec développement libre de l’infestation. Des évaluations périodiques ont éclairci les relations entre le développement de la colonie, le couvain operculé, la population d’abeilles adultes et les provisions de miel selon la saison et permis des analyses numériques comparatives. Toutes les colonies expérimentales ont montré un déroulement dans le temps semblable avec un fort accroissement des variables étudiées seulement en début du printemps (Fig. 1). Simultanément les colonies infestées ont vu des réductions croissantes du couvain d’ouvrières operculé, de la population d’abeilles et de la production de miel au cours du printemps et de l’été par rapport aux colonies non infestées (Figs. 1 et 2). Ces réductions étaient apparemment associées à des augmentations impressionnantes de la population d’acariens dans ces colonies là (Fig. 1). A la fin de l’expérience, les colonies infestées par *V. destructor* présentaient des réserves de miel diminuées en moyenne de 45 % (Fig. 2), ce qui correspond à une perte annuelle de 24 kg de miel par colonie, par rapport aux 44 kg engrangés par les colonies non infestées. Néanmoins la quantité de miel operculé stocké par abeille et par jour était indépendante de l’état d’infestation de la colonie (Fig. 3). Ceci indique que l’infestation par *V. destructor* n’a pas d’effet direct sur le comportement d’amasement du miel.

***Varroa destructor* / *Apis mellifera* / dynamique des populations / rendement en miel**

Zusammenfassung – Auswirkungen des Befalls durch *Varroa destructor* auf die Produktion von verdeckelter Arbeiterinnenbrut, Bienenpopulation und Honigvorrat bei *Apis mellifera carnica* im Mittelmeerklima. Die milderen Winterbedingungen des Mittelmeerklimas und die Möglichkeit, während eines erheblichen Teils des Winters Futterquellen zu besammeln bedingt eine durchgehende Brutfähigkeit der Bienenvölker, die wiederum für die hohe intrinsische Wachstumsrate der Populationen von *Varroa destructor* in diesen Gegenden verantwortlich zu machen ist. In diesem Zusammenhang war es das Ziel der vorliegenden Untersuchung, die Auswirkung des Milbenbefalls auf die Menge verdeckelter Arbeiterinnenbrut, die Population adulter Arbeiterinnen und die Honigproduktion in authentisierten *A. m. carnica* Völkern zu erfassen, die in einem typischen Mittelmeerklima gehalten wurden (N38°34', W07°54'). Hierzu wurden Völker aufgestellt und entweder milbenfrei gehalten oder künstlich mit Milben infiziert und die Entwicklung einer Milbenpopulation zugelassen. In regelmäßigen Zeitabschnitten wiederholte Untersuchungen zeigten das Muster der Volksentwicklung in Bezug auf verdeckelte Brut, die Population adulter Bienen und Honigvorräte über die Saison und ermöglichten eine numerische Analyse. Alle Versuchsvölker zeigten einen ähnlichen zeitlichen Verlauf, währenddessen nur im frühen Frühjahr ein starker Zuwachs der Untersuchungsvariablen zu beobachten war (Abb. 1). Gleichzeitig wurden bei den varroabefallenen Völkern im Vergleich zu den unbefallenen Völkern während des Frühjahres und Sommers zunehmende Einschränkungen bei verdeckelter Arbeiterinnenbrut, Bienenpopulation und Honigvorräten beobachtet (Abb. 1 und 2). Augenscheinlich waren diese mit eindrucksvollen Zuwächsen der Milbenpopulationen in diesen Völkern verbunden (Abb. 1). Zu Ende des Experiments zeigten die milbeninfizierten Völker eine gleichmäßige Reduktion von 45 % bei den Honigvor-

räten (Abb. 2), was einem jährlichen Verlust von 24 kg Honig im Vergleich zu den 44 kg Honig in nichtinfizierten Völkern entspricht. Nichtsdestoweniger war die Menge von Honig pro Bienen und Tag unabhängig von dem Befallstatus des Volkes (Abb. 3), was darauf hindeutet, dass es keinen direkten Effekt des Befalls durch *Varroa destructor* auf das Honigeintragverhalten gibt.

***Varroa destructor* / *Apis mellifera* / Populationsdynamik / Honigproduktion**

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