Impact of *Varroa destructor* on honeybee (*Apis mellifera scutellata*) colony development in South Africa

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Abstract The devastating effects of *Varroa destructor* on European honeybee colonies (*Apis mellifera* L.) have been well documented. Not only do these mites cause physical damage to parasitised individuals when they feed on them, they also transmit viruses and other pathogens, weaken colonies and can ultimately cause their death. Nevertheless, not all honeybee colonies are doomed once *Varroa* mites become established. Some populations, such as the savannah honeybee, *A. m. scutellata*, have become tolerant after the introduction of the parasite and are able to withstand the presence of these mites without the need for acaricides. In this study, we measured daily *Varroa* mite fall, *Varroa* infestation rates of adult honeybees and worker brood and total *Varroa* population size in acaricide treated and untreated honeybee colonies. In addition, honeybee colony development was compared in order to measure the cost incurred by *Varroa* mites to their hosts. Daily *Varroa* mite fall decreased over the experimental period with different dynamics in treated and untreated colonies. *Varroa* infestation rates in treated adult honeybees and brood were lower than in untreated colonies, but not significantly so. Thus indicating a minimal benefit of treatment thereby suggesting that *A. m. scutellata* have the ability to maintain mite populations at low levels. We obtained baseline data on *Varroa* population dynamics in a tolerant honeybee over the winter period. *Varroa* mites appeared to have a low impact on this honeybee population, given that colony development was similar in the treated and untreated colonies.

Introduction

The Eastern honeybee (*Apis cerana* Fabr.) is the natural host of the ectoparasitic mite *Varroa destructor*. Co-evolution between these two species resulted in the persistence of low mite populations in colonies at levels that do not endanger their survival (Rosenkranz et al. 2010). The ability of *A. cerana* to maintain *Varroa* mite infestations at low levels is supposedly due to *Varroa* mite reproduction being limited to drone brood as well as the grooming and hygienic behaviour exhibited by their workers (Koeniger et al. 1983; Peng et al. 1987; De Jong 1988; Büchler et al. 1992; Rath 1999). Adult honeybees are able to sense mite parasitised brood and they are very efficient at killing mites and eliminating them from colonies (Peng et al. 1987; but see Fries et al. 1996). The European honeybee does not express these traits at a level at which they confer tolerance to the parasite (Rosenkranz et al. 2010). This became obvious after the mite shifted host from *A. cerana* to *A. mellifera* L. (Rath and Drescher 1990; Solignac et al. 2005) and became responsible for the mortality of a large number of honeybee colonies in several regions of the world where it has spread (see references in Dietemann et al. 2012; van Dooremalen et al. 2013; Spleen et al. 2013; Steinhauer et al. 2014). *Apis mellifera* colonies cannot survive without chemical treatment against *Varroa* mites and colonies usually die within one to three years if left untreated (Martin 1998; Rosenkranz et al. 2010). The susceptibility of *A. mellifera* to this parasite is the general rule, but there are several examples of European, Africanised and African honeybee populations that have, despite the absence of co-evolution with *Varroa* mites, survived in their presence without chemical treatment (De Jong and Soares 1997; Allsopp 2006; Fries et al. 2006; Le Conte et al. 2007; Seeley 2007; Locke and Fries 2011).

In 1956, *A. m. scutellata* queens were introduced into honeybee colonies in Brazil where their progeny hybridised with the previously introduced European honeybee sub-species (Kerr 1967; Francoy et al. 2009). The so called Africanised honeybees that resulted from this hybridisation spread to most parts of South, Central and North America (Sheppard et al. 1991; Winston 1992; Visscher et al. 1997; Page 1998; Sheppard and Smith 2000). A significant amount of research has been done on the genetic and behavioural composition of the invasive Africanised honeybee, with most of the research indicating that a high percentage of African characters are conserved within these populations (Schneider et al. 2004; Moritz et al. 2005; Whitfield et al. 2006; Kraus et al. 2007). In Brazil, *Varroa* mite infestation rates of Africanised honeybee colonies have remained very low over the years, from when the mite was first observed in the late seventies up until recent times (Rosenkranz 1999), with no reports of large scale honeybee mortality (De Jong et al. 1984; Camazine and Morse 1988; Carneiro et al. 2007; Calderón et al. 2010). In general, tolerance to *Varroa* mites in Africanised honeybees has been attributed to the presence of a large number of infertile female mites (Camazine 1986; Rosenkranz and Engels 1994), the uncapping and removal of mite infested brood (Corrêa-Marques and De Jong 1998; Guerra et al. 2000; Vandame et al. 2002) and the mortality of both male and female mite offspring, which decreases the reproductive output of the mites (Medina and Martin 1999; Mondragón et al. 2005; Mondragón et al. 2006). In African populations, tolerance mechanisms have not been studied as extensively, but possible reasons for *Varroa* mite tolerance have been attributed to a short post-capping stage, good hygienic and grooming behaviour and high absconding and swarming

rates (Moritz and Hänel 1984; Moritz 1985; Boecking and Ritter 1993; Allsopp 2006; Frazier et al. 2010). This knowledge has been gathered from a variety of sub-species, of which two occur in South Africa.

The introduction of *Varroa* mites into South Africa was a fairly recent occurrence. *Varroa* mites were introduced into the Western Cape region during 1997, and they spread to most parts of the country within a few years and infested both endemic sub-species, *A. m. scutellata* and *A. m. capensis* (Martin and Kryger 2002; Allsopp 2006). During the first few years of invasion by *Varroa* mites, typical symptoms of *Varroa* mite presence in honeybee colonies were observed and *Varroa* mite population sizes were estimated at approximately 10 000-50 000 per colony (Allsopp 2006). However, after examination of both wild and commercial honeybee colonies, Allsopp (2006) concluded that both honeybee sub-species of South Africa were able to survive without treatment in the presence of *Varroa* mites, with tolerance to mites occurring after 3-5 and 6-7 years in Cape (*A. m. capensis*) and savannah honeybees (*A. m. scutellata*), respectively.

The establishment of a balance between a parasite and its host requires a limit on the parasite's reproduction at levels that do not result in costs that could compromise the host's survival. Closer examination of the population dynamics of *A. m. scutellata* colonies and of *Varroa* mites within these colonies will give a better idea of the infestation rates compatible with host survival and therefore establishment of tolerance in honeybees that have recently encountered these mites. In this study, we also assessed the impact of the *Varroa* mite populations on colony development a decade after the hypothesised establishment of tolerance towards the mites (Allsopp 2006). This knowledge could provide reference values for selection programs destined to breed European honeybees tolerant to the parasite.

Materials and Methods

The study was conducted at the Experimental Farm of the University of Pretoria (25°45'11"S, 28°15'29"E) in South Africa from May (end of autumn) to October (middle of spring) 2011. This period included the winter season when brood rearing is reduced in savannah honeybees. It is during this period of the year that most of the damage of *Varroa* mites occurs at colony level in European honeybee populations (Rosenkranz et al. 2010). Colonies were housed in standard 10 frame Langstroth hives and placed on stands protected by oil filled cans to prevent ants from entering the colonies and removing mites. The queens were marked in July to ensure that the same colonies were monitored for the duration of the experiment. A feeding station was set up close to the colonies in both apiaries during the winter months, due to low food availability. A pollen supplement as well as a sugar and water diet (1:1 w/v) was provided on a regular basis. The colonies were only opened when honeybee colony size was estimated (see below). Adult honeybee and brood samples were collected on the same day as colony size estimation in order to keep the disturbance to a minimum. The number of colonies used for different analyses to compare treated and untreated *Apis mellifera scutellata* apiaries is presented in Table 1.

Acaricide treatment

Nine colonies originating from two sources were distributed randomly in each of the two apiaries. Colonies were also assigned to the two apiaries according to their strength (treated: 7 357 ± 2711 vs. untreated: 6 309 ± 2402 honeybees, Mann-Whitney U Test: $U = 32.5$; $Z = 0.7$; $P > 0.05$), (treated: 8.7 ± 6.8 vs. untreated: 8.4 ± 4.4 sealed brood cells (dm^2), $U = 38.0$; $Z = -0.2$; $P > 0.05$), treated: 7.6 ± 4.1 vs. untreated: 6.2 ± 5.0 unsealed brood cells (dm^2), $U = 32.5$; $Z = 0.7$; $P > 0.05$)) and *Varroa* mite infestation rates of adults and brood (see results). All the colonies in one of the apiaries received an acaricide treatment, while those in the other remained untreated. The separation of treated from untreated colonies (by roughly one kilometre) was necessary to minimise drifting of honeybees between apiaries and thus prevented contamination of the untreated colonies with the acaricide (Allsopp 2006). On the 14th of June 2011, Bayvarol® strips (Bayer Healthcare) were placed in colonies of the treated apiary, according to the manufacturer's recommendations. We divided our experiment into three periods: pre-treatment (from 21st May-14th June 2011), during treatment (15th June-30th June 2011) and the post-treatment period (1st July-4th October 2011). The 'during treatment' period encompassed the maturation period (approximately 12 days) of the worker brood capped just before treatment, which might have contained mites not exposed to the acaricide. In the last three days those *Varroa* mites emerging from the cells would have been exposed to the acaricide and killed. Daily mite fall showed that the values typically observed before treatment were again measured from the 30th of June onwards. We thus divided our experimental periods according to the *Varroa* population status rather than to the less biologically relevant presence of treatment strips in the colonies, which extended beyond these 2 weeks. In South Africa, no acaricide treatment is implemented to ensure colony survival. We could therefore exclude any resistance of the *Varroa* population towards the product used.

Daily *Varroa* mite fall

Daily mite fall is a simple, non destructive method that can be used to assess the number of mites in a honeybee population (Branco et al. 2006). All 18 honeybee colonies were equipped with screened *Varroa* bottom boards (460 x 360 x 5 mm) on which sheets of white paper were inserted to collect fallen *Varroa* mites. On each sampling occasion (daily or weekly depending on the intensity of mite fall) the paper in all colonies were removed, placed into plastic ziploc bags to allow for mite counting in the laboratory and immediately replaced with new sheets of paper. Average daily mite fall was obtained by dividing the number of mites fallen on the paper by the number of days since the previous mite count.

Varroa mite fall was recorded for 25 days in both apiaries to obtain the baseline counts in all the colonies before the chemical treatment was applied (pre-treatment period). After 25 days, adult female mites were again counted on the bottom boards daily or weekly for 16 days in both apiaries (during treatment period). Following this, the daily mite fall was recorded at regular intervals for an additional 96 days in the treated and untreated apiaries (post-treatment period).

Varroa mite infestation rates in adult honeybees and worker brood

For a more precise, but more destructive and therefore less frequent evaluation of infestation rates in the colonies, adult honeybees (129.5 ± 39.8 honeybees) and worker brood (98.1 ± 7.2 sealed cells) were collected before treatment in May and twice (July and September) after the treatment was applied. No samples were collected if colonies were too weak or did not have enough brood. *Varroa* mite infestation rates were determined as outlined in Allsopp (2006) and summarised as: number of *Varroa*/100 adult honeybees and number of *Varroa*/100 worker cells.

Honeybee colony development

In order to measure the effect of the parasitic mites on the colonies, we evaluated colony strength in the experimental apiaries. We assumed that the single acaricide treatment per se had no negative effect on colony development during the experimental period and that the sizes measured were only affected by the presence or absence of the *Varroa* mites. The number of adult honeybees, sealed and unsealed brood in a colony was assessed every month for the duration of the experiment using the Liebefeld colony size estimation method (Gerig 1983). To determine the number of adult honeybees, sealed and unsealed brood present in the colonies, brood frames were divided into eight squares of 1 dm^2 each. To get a precise estimate of the number of adult *A. m. scutellata* that completely fill 1 dm^2 square, photographs of 21 frames were taken and the number of honeybees in each of the squares was counted. Only fully occupied squares were used for the final estimate. Results showed that one fully occupied square contained on average 170 ± 19.9 honeybees. The number of fully occupied squares on both sides of the brood frames as well as on the lids and walls of the hives was counted and multiplied by 170 to obtain the number of honeybees present. The surface area containing sealed and unsealed brood on both sides of the frames was counted and expressed in dm^2. The estimation of honeybee colony development using the Liebefeld method was performed by the same individuals on every occasion. The Liebefeld method was conducted at the start of the experiment (21st of May 2011), when the treatment was applied (14th of June 2011), when the treatment was removed (14th of July 2011) and finally one (August), two (September) and three (October) months after the treatment was removed. The presence of the queen and queen cells were also recorded to monitor the general status of the colonies. Since colony development is highly variable even in the same apiary, we measure the colony development in terms of percentage change of the number of adult honeybees and the surface area of sealed and unsealed brood on a monthly basis. A value above 100% shows a growth in the adult honeybee population or brood presence and values below 100% indicate a decrease in adult numbers or brood surfaces compared to the first measure in May.

Varroa population size in adult honeybees and brood

The population size of *Varroa* mites on adult honeybees was determined in each colony by taking the total number of honeybees and multiplying it by the proportion of *Varroa* infested adult honeybees. For the population size of

Varroa mites in sealed worker brood, the number of sealed cells was multiplied by the proportion of *Varroa* infested brood. The values obtained above were then added to give the total *Varroa* population size in each colony (Dietemann et al. 2013). Three frames were used to calculate the average number of worker cells in 1 dm^2. Worker cells in ten 1 dm^2 squares were counted (Delaplane et al. 2013) and showed a density of 523 ± 16 worker cells per 1 dm^2.

Statistical analysis

A Mann-Whitney U Test was performed to compare average daily *Varroa* mite fall and total mite fall from May to October, *Varroa* mite infestation rates as well as total *Varroa* mite population size between the treated and untreated apiaries. A Pearson correlation was performed to determine whether there was a correlation between the number of adult honeybees or brood and the total *Varroa* mite population sizes in both apiaries. A Repeated measures ANOVA was used to compare honeybee colony development (number of adult honeybees, surface area of sealed and unsealed brood) between both apiaries. When colonies lost their queen or absconded, data were only considered for analyses until the queen was last seen alive or the colony last seen inhabiting the hive. To compare the total mite fall and population size of *Varroa* mites in adults and brood between the two apiaries, only the queenright colonies that were still present at the end of the experiment were considered. Gehan's Wilcoxon test was used to compare colony survival in both apiaries.

Results

In total, six honeybee colonies absconded over the monitoring period (May to October 2011). In the treated apiary, three colonies absconded before day 24, 43 and 82, respectively. In the untreated apiary, one colony absconded before day 24 and the other two colonies absconded before day 82. Consequently, these colonies could not be monitored for the entire duration of the study. There was no effect of acaricide treatment on absconding with both apiaries experiencing a loss of the same number of colonies (Gehan's Wilcoxon test, Survival T statistic^2 = - 0.29; $P > 0.05$).

Two colonies in the treated apiary became queenless during the experimental period. All other colonies in both apiaries had the originally marked queens present until study completion. During honeybee colony development assessments, we observed no obvious damage by *Varroa* mites as described by Allsopp (2006) during the initial invasion period of the parasite in South Africa.

Daily *Varroa* mite fall

Pre-treatment period

A significant difference was observed in *Varroa* mite fall between the apiaries. Daily mite fall was significantly higher (U = 17203.0; Z = - 4.8; $P < 0.01$) in the untreated apiary (9.5 ± 5.2, mean ± SD) compared to the apiary that would receive treatment in the next phase of the experiment (4.3 ± 2.2, mean ± SD, Fig. 1).

During treatment period

A significant difference was observed in *Varroa* mite fall between the treated and untreated apiaries. Daily mite fall was significantly higher (U = 5394.5; Z = 3.2; $P < 0.01$) in the treated apiary (20.2 ± 20.4, mean ± SD) compared to the untreated apiary (7.8 ± 2.2, mean ± SD, Fig. 1).

Post-treatment period

Daily *Varroa* mite fall was significantly higher in the untreated apiary compared to the treated apiary during July (U = 7760.0; Z = - 12.1; $P < 0.05$), August (U = 6492.0; Z = - 9.4; $P < 0.05$) and September (U = 9050.0; Z = - 7.2; $P < 0.05$). No significant differences in *Varroa* mite fall was found between the two apiaries during October (U = 208.0; Z = - 0.7; $P > 0.05$) (Fig. 1). Mite fall numbers might be a fraction lower than expected given that ants were occasionally found on the bottom boards in most of the colonies of both apiaries despite efforts to keep them away. Nonetheless, no significant differences were found in the number of ants (Mann-Whitney U Test: U = 16.5; Z = 1.6; $P > 0.05$) between both apiaries. The total number of mites that fell in the two apiaries over the whole experimental period was not significantly different (treated 3207 ± 199 vs. untreated 4167 ± 149, U = 432.5; Z = - 1.4; $P > 0.05$).

Adult honeybee infestation rates

Varroa infestation rates were generally higher in the untreated apiary compared to the treated apiary in which not all of the mites were eliminated. However, these differences were not significant between the treated and untreated apiaries in May (U = 30.0; Z = - 0.5; $P > 0.05$), July (U = 3.0; Z = - 1.6; $P > 0.05$) or September (U = 3.5; Z = - 1.2; $P > 0.05$, Fig. 2).

Worker brood infestation rates

Brood infestation rates in the treated apiary decreased after treatment and were lowest during September. There was also a great reduction in the infestation rates of *Varroa* mites from July to September in the untreated apiary. *Varroa* mite numbers were higher in the brood of the untreated apiary compared to the treated apiary. However, these

differences were not significant between the treated and untreated colonies in May (U = 18.00; Z = - 0.77; P > 0.05), July (U = 0.5; Z = - 1.9; P > 0.05) or September (U = 4.0; Z = - 0.5; P > 0.05) (Fig. 3).

Varroa mite and host population dynamics

No significant differences were observed in the total mite population sizes between the treated and untreated colonies during May (U = 8.0; Z = - 0.4; P > 0.05), July (U = 2.0; Z = - 1.2; P > 0.05) and September (U = 3.0; Z = - 0.9; P > 0.05). The total *Varroa* population size appeared to decrease in the treated colonies from May to September, while in the untreated colonies, the mean mite population seemed to double from May to July and then decreased thereafter (Figs. 4, 5). The total *Varroa* population size in both apiaries was at its lowest during September (even though a high number of brood cells was present during this period), with the treated and untreated colonies having 76 ± 55 (mean ± SD) and 185 ± 149 (mean ± SD) mites, respectively.

In the treated colonies, there was no correlation between the number of adult honeybees (R = - 0.03, P > 0.05) or the number of sealed brood (R = - 0.41, P > 0.05) and total *Varroa* population size. In addition, there was no correlation between the number of adult honeybees (R = - 0.06, P > 0.05) or the number of sealed brood (R = - 0.17, P > 0.05) and total *Varroa* population size in the untreated colonies.

Honeybee colony development

Adult honeybees

The percentage change in the number of adult honeybees measured from July to October was not significantly different between the treated and untreated apiaries ($F_{3,27}$ = 0.65, P > 0.05, Fig. 6). The average number of adult honeybees was highest in both apiaries during October (treated 9656 ± 5545, untreated 8798 ± 3911).

Sealed brood

The changes in surface area of sealed worker brood measured from July to October were not significantly different between the treated and untreated colonies ($F_{3,27}$ = 0.74, P > 0.05, Fig. 7). The surface area of sealed brood increased similarly in both apiaries during July and August. In the untreated colonies, the presence of sealed brood increased more rapidly than that in treated colonies from August to October. In both apiaries the surface area of sealed brood was highest in October (treated 44.6 ± 35, untreated 29 ± 13).

Unsealed brood

No significant differences were found in the changes of surface area of unsealed worker brood measured from July to October between the treated and untreated colonies ($F_{3,27}$ = 2.27, P > 0.05, Fig. 8). In both apiaries the surface area of unsealed brood increased during spring.

Discussion

The immediate effect of the acaricide on *Varroa* mites was clearly observed during the treatment period with a peak in mite fall. In the following months, daily mite fall remained low. In contrast, daily mite fall in the untreated colonies decreased steadily during the whole period. Only in October was the mite fall similar between the two groups of colonies. Unexpectedly, *Varroa* mite infestation rates in adult honeybees and worker brood were similar between the treated and untreated colonies. Accordingly, the estimated total *Varroa* population sizes were not significantly different in both apiaries during May, July and September. Also, no difference could be measured between the treated and untreated colonies with regard to the number of adult honeybees present and the surface area of sealed and unsealed brood.

A decrease in infestation rates was found by Allsopp (2006) who also measured the *Varroa* infestation rates of adult honeybees (*A. m. scutellata*) in acaricide treated and untreated colonies. After treatment, *Varroa* infestation rates decreased in both groups of colonies placed in the same apiary. Allsopp (2006) attributed the decrease of infestation rates in the untreated colonies to acaricide contamination originating from the treated colonies. In our study, it was clear that no acaricide contamination from the treated to the untreated colonies took place, as mite fall immediately rose after acaricide application in the treated apiary while it progressively decreased in the untreated apiary, but eventually reached the same low values as in the treated apiary. Treatment thus only significantly affected the timing of the mite fall, but not its extent. In this study the decrease in daily mite fall in the untreated apiary is not reflected in the assessment of total *Varroa* population size (Fig. 5). This suggests a seasonal effect on mite longevity with mites living longer (resulting in a reduced mite fall) over the cold period (Ellis 2008), as reported for mites in populations of European honeybees.

The effect of treatment on mite fall was also not reflected in the measures of adult or brood infestation rates, which were not significantly different between the treated and untreated colonies. This discrepancy could be due to the very low infestation rates in untreated colonies. These low rates could be a consequence of the host's ability to keep mite numbers at low levels, thereby minimising the treatment effect. Alternatively, mite fall could have been biased by the absence of brood (Branco et al. 2006) in some of our colonies during the coldest months of the study. We found no correlation between the amount of brood or number of adults and the total *Varroa* population size. The decrease in infestation rates observed was thus not a dilution effect due to the increase of colony strength in the spring. The absence of a correlation also indicates that an increase of brood production was not followed by an increase of mite population. These trends rather suggest that *A. m. scutellata* maintain mite numbers at low levels in their colonies.

In both our apiaries, average *Varroa* mite infestation rates were low throughout the monitoring period. We measured 1.6 and 2.2 mites per 100 adult honeybees before treatment and 0.7 and 1.6 mites per 100 adult honeybees four months after treatment in the treated and untreated apiaries, respectively. The infestation rates before treatment are lower than those measured in 1999 by Allsopp (2006) (7.7 mites per 100 adult honeybees per colony) shortly after the invasion by the *Varroa* mite in South Africa, but are similar to his measurements in 2005 when less than

one mite was found per 100 workers. There has therefore not been a further decrease of infestation rates after the 6-7 years period following invasion (Allsopp 2006) and the host-parasite relationship therefore seems to have reached equilibrium. *Varroa* population size in tolerant Africanised honeybee colonies from Mexico ranged from 1 000 to 3 500 mites per colony over a year (Medina et al. 2002). This is one order of magnitude higher than our mite population size estimate (approximately 400 mites). Although our estimates were obtained using different methods, the size of this gap in a tolerated parasite population suggests that tolerant honeybee populations rely on different mechanisms to survive in presence of the mite (Locke et al. 2011; 2012).

Despite low average infestation rates and mite numbers, the natural mite fall before treatment exceeded ten mites per day in five of the 18 colonies. In central European countries, honeybee colonies are considered in danger of collapsing due to *Varroa* once natural mite fall goes beyond ten mites per day (Le Conte et al. 2010). However, none of the colonies showed any signs that they were close to collapsing, suggesting that the damage threshold is higher in *A. m. scutellata*. The tolerance of these honeybees for higher infestation rates could be due to the absence of deleterious virus (e.g. deformed wing virus) outbreaks in the honeybee and *Varroa* populations used in this study (Strauss et al. 2013). This is in contrast to other parts of the world where *Varroa* mites and honeybee viruses are very prevalent and may have contributed to the weakening and mortality of a significant number of honeybee colonies (Berthoud et al. 2010; Genersch et al. 2010; Martin et al. 2012; Francis et al. 2013).

The number of adult honeybees and surface area of sealed and unsealed brood was similar for both our apiaries, with no significant differences observed within each of the monitoring periods. Our results suggest that the reduction of *Varroa* population size due to the use of the acaricide treatment was not significant enough to translate into a clear benefit in terms of colony development over the winter. The brood produced over the cold season thus seems only slightly, if at all, affected by the *Varroa* population tolerated by the savannah honeybee. Alternatively, given the difference in climate compared to more temperate regions, the African winter honeybees, if negatively affected, might not be as critical to the vitality and survival of the colony. However, the positive effects of acaricide treatment might have appeared over a longer period, while the debilitating effects of mite presence could have accumulated over time in untreated colonies. Given that mortality due to the mite has not been reported in the *A. m. scutellata* population 6-7 years after the initial invasion (Allsopp 2006) and recently (Strauss et al. 2013; Pirk et al. 2014), this is unlikely.

In total, six honeybee colonies absconded during the experiment. African honeybees generally abscond due to unfavourable environmental conditions and disturbance, but also due to predation pressure (wax moths, ants, small hive beetle and humans, Michener 1973; Camazine and Morse 1988; Hepburn and Radloff 1998). Indeed, when honeybees abscond, many of the pests and parasites remain in the comb and by doing this they decrease the parasite load that can affect them at their new nest site (Fletcher 1978). The high rate of absconding measured during the monitoring period is in line with the natural behaviour of the African sub-species (Fletcher 1978; McNally and Schneider 1992; Hepburn and Radloff 1998). In South Africa between 10-30% of colony losses experienced by beekeepers during a year is due to absconding (Fletcher 1975; Swart 2001). The Liebefeld method for colony size estimation does not seem to have a negative effect on colonies (Imdorf and Maquelin 1993) and since the colonies in this study were opened only once a month, it is most likely that the six colonies that did

abscond did so for other reasons. In addition, the six absconding events occurred independently of the treatment regime of the colonies.

Another parameter that could possibly contribute to tolerance of honeybee host colonies is low *Varroa* mite fertility (Rosenkranz and Engels 1994). However, fertility rates can change over time, as was found in Brazilian honeybees where mite fertility was low before the late 1990's but increased after this period (Corrêa-Marques et al. 2003; Garrido et al. 2003; Carneiro et al. 2007). Even though most of the evidence obtained so far points towards *Varroa* mite tolerance in both sub-species of South Africa, Martin and Kryger (2002) found that *Varroa* mite reproductive rates were similar in *A. m. scutellata* and European honeybees, thereby suggesting that *Varroa* mites should have the same negative effect in *A. m. scutellata* as in European honeybees. Also, Martin and Kryger (2002) showed that *Varroa* mites were reproductively more successful in *A. m. scutellata* drone and worker cells compared to Africanised workers cells and that a higher percentage of fertile female mites were produced in *A. m. scutellata* compared to Africanised honeybees. Consequently, more studies are needed to give us a better idea of the reproductive potential of *Varroa* mites in both honeybee sub-species of South Africa and on the tolerance mechanisms that these honeybees developed against the parasite.

During his studies on tolerance development in the Cape honeybee, Allsopp (2006) observed that *Varroa* infestation rates of adult honeybees decreased over the years in the monitored colonies and that they became much lower than when the mite first arrived in South Africa. Cape honeybees, however, showed no direct aggression towards *Varroa* mites, nor did they exhibit grooming behaviour (Allsopp 2006). He concluded that the shorter post-capping stage (between 9.6-12 days in Cape honeybees, 10-12 days in *A. m. scutellata*, 11.5-11.6 in Africanised honeybees compared to 11.6-12 in European honeybees (Moritz and Hänel 1984; Moritz 1985; Vandame et al. 1999; Tribe and Allsopp 2001; Martin and Kryger 2002; Allsopp 2006; Calderón et al. 2010) and the ability of these honeybees to eliminate reproductive *Varroa* mites from brood (hygienic behaviour) contributed to tolerance. No studies on the mechanisms of *Varroa* mite tolerance have been done on *A. m. scutellata* honeybees in South Africa. However, there is evidence of good, but variable, hygienic behaviour in *A. m. scutellata* colonies from Zimbabwe (Fries and Raina 2003) and possibly Kenya (Frazier et al. 2010). These traits have to be investigated further to determine the role they play in providing tolerance to the host colonies.

The aim of this study was to examine the impact of *Varroa* mites on honeybee colony development and survival in order to determine whether the *Varroa* population tolerated in colonies of the savannah honeybee incurred any cost to its host at natural infestation levels. Honeybee colony development was similar in both apiaries, despite reduced infestation rates of *Varroa* mites in treated colonies. Colonies of both apiaries did not show any signs of disease or collapse and were developing normally in the presence of *Varroa* mites, suggesting a low cost of the tolerated parasite population on colony development within the period investigated. Although this study was conducted for only a short period, compared to the time usually needed for European *A. mellifera* colonies to collapse from *Varroa* infestation (one to three years, Martin 1998; Rosenkranz et al. 2010), some insights into the population dynamics of both *Varroa* mites and honeybees were gained. *Varroa* mite fall and infestation rates naturally decreased from May to October. This suggests that infestation rates in colonies decrease during the colder seasons and colonies start the warmer season with a low parasite population. This could prevent the exponential

build-up of mite populations over the years to levels that can reach the damage threshold, as is the case in European honeybee sub-species (see references in Rosenkranz et al. 2010). The mechanism behind this decrease in *Varroa* mite numbers in the absence of treatment is still unclear and requires further research over longer time periods. A more detailed analysis of *Varroa* mite reproduction and *A. m. scutellata* hygienic behaviour is also necessary to allow for a better understanding of the factors involved in *Varroa* mite tolerance and the long-term survival of these honeybees.

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References

Allsopp M (1997) *Varroa jacobsoni* in South Africa. S Afr Bee J 69:73-82

Allsopp M (2006) Analysis of *Varroa destructor* infestation of southern African honeybee populations. MSc-thesis, University of Pretoria

Berthoud H, Imdorf A, Haueter M, Radloff SE, Neumann P (2010) Virus infections and winter losses of honey bee colonies (*Apis mellifera*). J Apic Res 49:60-65

Boecking O, Ritter W (1993) Grooming and removal behaviour of *Apis mellifera intermissa* in Tunisia against *Varroa jacobsoni*. J Apic Res 32:127-134

Branco MR, Kidd NAC, Pickard RS (2006) A comparative evaluation of sampling methods for *Varroa destructor* (Acari: Varroidae) population estimation. Apidologie 37:452-461

Büchler R, Drescher W, Tornier I (1992) Grooming behaviour of *Apis cerana, Apis mellifera* and *Apis dorsata* and its effect on the parasitic mites *Varroa jacobsoni* and *Tropilaelaps clareae*. Exp Appl Acarol 16:313-319

Calderón RA, van Veen JW, Sommeijer MJ, Sanchez LA (2010) Reproductive biology of *Varroa destructor* in Africanized honey bees (*Apis mellifera*). Exp Appl Acarol 50:281-297

Camazine S (1986) Differential reproduction of the mite, *Varroa jacobsoni* (Mesostigmata: Varroidae), on Africanized and European honey bees (Hymenoptera: Apidae). Ann Entomol Soc Am 79:801-803

Camazine S, Morse RA (1988) The Africanized honeybee: The epithet "killer bee" is undeserved. Am Sci 76:464-471

Carneiro FE, Torres RR, Strapazzon R, Ramirez SA, Guerra JCV, Koling DF, Moretto G (2007) Changes in the reproductive ability of the mite *Varroa destructor* (Anderson and Trueman) in Africanized honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) colonies in southern Brazil. Neotrop Entomol 36:949-952

Corrêa-Marques MH, De Jong D (1998) Uncapping of worker bee brood, a component of the hygienic behaviour of Africanized honey bees against the mite *Varroa jacobsoni* Oud. Apidologie 29:283-289

Corrêa-Marques MH, Medina ML, Martin SJ, De Jong D (2003) Comparing data on the reproduction of *Varroa destructor*. Genet Mol Res 2:1-6

De Jong D (1988) *Varroa jacobsoni* does reproduce in worker cells of *Apis cerana* in South Korea. Apidologie 19:241-244

De Jong D, Soares AEE (1997) An isolated population of Italian bees that has survived *Varroa jacobsoni* infestation without treatment for over 12 years. Am Bee J 137:742-747

De Jong D, Goncalves LS, Morse RA (1984) Dependence on climate of the virulence of *Varroa jacobsoni*. Bee World 65:117-121

Delaplane, KS, van der Steen J, Guzman-Novoa E (2013) Standard methods for estimating strength parameters of *Apis mellifera* colonies. In: Dietemann V, Ellis JD, Neumann P (eds) The COLOSS BEEBOOK, Volume I: standard methods for *Apis mellifera* research. J Apic Res 52(1): http://dx.doi.org/10.3896/IBRA.1.52.1.03

Dietemann V, Nazzi F, Martin SJ, Anderson D, Locke B, Delaplane KS, Wauquiez Q, Tannahill C, Frey E, Ziegelmann B, Rosenkranz P, Ellis JD (2013) Standard methods for varroa research. In: Dietemann V, Ellis JD, Neumann P (eds) The COLOSS BEEBOOK, Volume II: standard methods for *Apis mellifera* pest and pathogen research. J Apic Res 52(1): http://dx.doi.org/10.3896/IBRA.1.52.1.0

Dietemann V, Pflugfelder J, Anderson D, Charrière J-D, Chejanovsky N, Dainat B, de Miranda JR, Delaplane K, Dillier F-X, Fuchs S, Gallmann P, Gauthier L, Imdorf A, Koeniger N, Kralj J, Meikle W, Pettis, JS, Rosenkranz P, Sammataro D, Smith D, Yañez O, Neumann P (2012) *Varroa destructor*: research avenues towards sustainable control. J Apic Res 51:125-132

Ellis JD (2008) Varroa Mite, *Varroa destructor* Anderson and Trueman (Acari: Varroidae)In: Capinera JL (ed) *Encyclopedia of Entomology*. Springer; Berlin. pp. 4041-4048

Fletcher DJC (1975) New perspectives in the causes of absconding in the African bee (*Apis mellifera adansonii* L.). Part 1. S Afr Bee J 47:11-14

Fletcher DJC (1978) The African bee, *Apis mellifera adansonii*, in Africa. Annu Rev Entomol 23:151-171

Francis RM, Nielsen SL, Kryger P (2013) Varroa-virus interaction in collapsing honey bee colonies. PLoS One 8: e57540

Francoy TM, Wittmann D, Steinhage V, Drauschke M, Müller S, Cunha DR, Nascimento AM, Figueiredo VLC, Simões ZLP, De Jong D, Arias MC, Gonçalves LS (2009) Morphometric and genetic changes in a population of *Apis mellifera* after 34 years of Africanization. Genet Mol Res 8:709-717

Frazier M, Muli E, Conklin T, Schmehl D, Torto B, Frazier J, Tumlinson J, Evans JD, Raina S (2010) A scientific note on *Varroa destructor* found in East Africa; threat or opportunity? Apidologie 41:463-465

Fries I, Raina S (2003) American foulbrood and African honey bees (Hymenoptera: Apidae). J Econ Entomol 96:1641-1646

Fries I, Huazhen W, Wei S, Jin CS (1996) Grooming behavior and damaged mites (*Varroa jacobsoni*) in *Apis cerana cerana* and *Apis mellifera ligustica*. Apidologie 27:3-11

Fries I, Imdorf A, Rosenkranz P (2006) Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a Nordic climate. Apidologie 37:564-570

Garrido C, Rosenkranz P, Paxton RJ, Gonçalves LS (2003) Temporal changes in *Varroa destructor* fertility and haplotype in Brazil. Apidologie 34:535-541

Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen W, Gisder S, Meixner M, Liebig G, Rosenkranz P (2010) The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41:332-352

Gerig L (1983) Lehrgang zur erfassung der volksstärke, Schweiz. Bienen-Zeitung 106:199-204

Guerra JCV, Gonçalves LS, De Jong D (2000) Africanized honey bees (*Apis mellifera* L.) are more efficient at removing worker brood artificially infested with the parasitic mite *Varroa jacobsoni* Oudemans than are Italian bees or Italian/Africanized hybrids. Genet Mol Biol 23:89-92

Hepburn HR, Radloff SE (1998) Honeybees of Africa. Springer, Berlin

Imdorf A, Maquelin C (1993) Volksschätzung im zeitigen Frühjahr, Schweiz. Bienenztg 116:392-395

Kerr WE (1967) The history of the introduction of African bees to Brazil. S Afr Bee J 39:3-5

Koeniger N, Koeniger G, Delfinado-Baker M (1983) Observations on mites of the Asian honeybee species (*Apis cerana*, *Apis dorsata*, *Apis florea*). Apidologie 14:197-204

Kraus FB, Franck P, Vandame R (2007) Asymmetric introgression of African genes in honeybee populations (*Apis mellifera* L.) in Central Mexico. Heredity 99:233-240

Le Conte Y, de Vaublanc G, Crauser D, Jeanne F, Rousselle JC, Bécard J-M (2007) Honey bee colonies that have survived *Varroa destructor*. Apidologie 38:566-572

Le Conte Y, Ellis M, Ritter W (2010) *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? Apidologie 41:353-363

Locke B, Fries I (2011) Characteristics of honey bee colonies (*Apis mellifera*) in Sweden surviving *Varroa destructor* infestation. Apidologie 42:533-542

Locke B, Le Conte Y, Crauser D, Fries I (2012) Host adaptations reduce the reproductive success of *Varroa destructor* in two distinct European honey bee populations. Ecol Evol 2:1144-1150

Martin SJ (1998) A population model for the ectoparasitic mite *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies. Ecol Model 109:267-281

Martin SJ, Kryger P (2002) Reproduction of *Varroa destructor* in South African honey bees: does cell space influence *Varroa* male survivorship? Apidologie 33:51-61

Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, Powell M, Nikaido S, Schroeder DC (2012) Global honey bee viral landscape altered by a parasitic mite. Science 336:1304-1306

Mcnally LC, Schneider SS (1992) Seasonal cycles of growth, development and movement of the African honey bee, *Apis mellifera scutellata*, in Africa. Insectes Soc 39:167-179

Medina LM, Martin SJ (1999) A comparative study of *Varroa jacobsoni* reproduction in worker cells of honey bees (*Apis mellifera*) in England and Africanized bees in Yucatan, Mexico. Exp Appl Acarol 23:659-667

Medina LM, Martin SJ, Espinosa-Montaño L, Ratnieks FLW (2002) Reproduction of *Varroa destructor* in worker brood of Africanized honey bees (*Apis mellifera*). Exp Appl Acarol 27:79-88

Michener CD (1973) The Brazilian honeybee. BioScience 23:523-527

Mondragón L, Martin SJ, Vandame R (2006) Mortality of mite offspring: a major component of *Varroa destructor* resistance in a population of Africanized bees. Apidologie 37:67-74

Mondragón L, Spivak M, Vandame R (2005) A multifactorial study of the resistance of honeybees *Apis mellifera* to the mite *Varroa destructor* over one year in Mexico. Apidologie 36:345-358

Moritz RFA (1985) Heritability of the postcapping stage in *Apis mellifera* and its relation to varroatosis resistance. J Hered 76:267-270

Moritz RFA, Hänel H (1984) Restricted development of the parasitic mite *Varroa jacobsoni* Oud. in the Cape honey bee, *Apis mellifera capensis* Esch. Z Angew Entomol 97:91-95

Moritz RFA, Härtel S, Neumann P (2005) Global invasions of the western honey bee (*Apis mellifera*) and the consequences for biodiversity. Ecoscience 12:289-301

Page RE (1998) Blessing or curse? *Varroa* mite impacts Africanized bee spread and beekeeping. Calif Agric 52:9-13

Peng YS, Fang Y, Xu S, Ge L (1987) The resistance mechanism of the Asian honey bee *Apis cerana* Fabr., to an ectoparasitic mite *Varroa jacobsoni* Oudemans. J Invertebr Pathol 49:54-60

Pirk CWW, Human H, Crewe RM, vanEngelsdorp D (2014) A survey of managed honey bee colony losses in the Republic of South Africa - 2009 to 2011. J Apic Res 53:35-42

Rath W (1999) Co-adaptation of *Apis cerana* Fabr. and *Varroa jacobsoni* Oud. Apidologie 30:97-110

Rath W, Drescher W (1990) Response of *Apis cerana* Fabr. towards brood infested with *Varroa jacobsoni* Oud., and infestation rate of colonies in Thailand. Apidologie 21:311-321

Rosenkranz P (1999) Honey bee (*Apis mellifera* L.) tolerance to *Varroa jacobsoni* Oud. in South America. Apidologie 30:159-172

Rosenkranz P, Engels W (1994) Infertility of *Varroa jacobsoni* females after invasion into *Apis mellifera* worker brood as a tolerance factor against varroatosis. Apidologie 25:402-411

Rosenkranz P, Aumeier P, Ziegelmann B (2010) Biology and control of *Varroa destructor*. J Invertebr Pathol 103:96-119

Schneider SS, DeGrandi-Hoffman G, Smith DR (2004) The African honey bee: factors contributing to a successful biological invasion. Annu Rev Entomol 49:351-376

Seeley TD (2007) Honey bees of the Arnot Forest: a population of feral colonies persisting with *Varroa destructor* in the northeastern United States. Apidologie 38:19-29

Sheppard WS, Smith DR (2000) Identification of African-derived bees in the Americas: A survey of methods. Ann Entomol Soc Am 93:159-176

Sheppard WS, Soares AEE, De Jong D, Shimanuki H (1991) Hybrid status of honey bee populations near the historic origin of Africanization in Brazil. Apidologie 22:643-652

Solignac M, Cornuet J, Vautrin D, Le Conte Y, Anderson D, Evans JD, Cros-Arteil S, Navajas M (2005) The invasive Korea and Japan types of *Varroa destructor*, ectoparasitic mites of the Western honey bee (*Apis mellifera*), are two partly isolated clones. Proc R Soc Biol Sci Ser B 272:411-419

Spleen AM, Lengerich EJ, Rennich K, Caron DM, Rose R, Pettis JS, Henson M, Wilkes JT, Wilson ME, Stitzinger J, Lee K, Andree M, Snyder R, vanEngelsdorp D (2013) A national survey of managed honey bee 2011-2012 winter colony losses in the United States: results from the Bee Informed Partnership. J Apic Res 52:44-53

Steinhauer NA, Rennich K, Wilson ME, Caron DM, Lengerich EJ, Pettis JS, Rose R, Skinner JA, Tarpy DR, Wilkes JT, vanEngelsdorp D (2014) A national survey of managed honey bee 2012-2013 annual colony losses in the USA: results from the Bee Informed Partnership. J Apic Res 53:1-18

Strauss U, Human H, Gauthier L, Crewe RM, Dietemann V, Pirk CWW (2013) Seasonal prevalence of pathogens and parasites in the savannah honeybee (*Apis mellifera scutellata*). J Invertebr Pathol 114:45-52

Swart DJ (2001) Specialized management. In: Johannsmeier MF (ed) Beekeeping in South Africa, Plant Protection Handbook No. 14, Agricultural Research Council, Pretoria, pp 85-94

Tribe GD, Allsopp M (2001) Life history of the honeybee colony. In: Johannsmeier MF (ed) Beekeeping in South Africa, Plant Protection Handbook No. 14, Agricultural Research Council, Pretoria, pp 17-26

van Dooremalen C, Stam E, Gerritsen L, Cornelissen B, van der Steen J, van Langevelde F, Blacquière T (2013) Interactive effect of reduced pollen availability and *Varroa destructor* infestation limits growth and protein content of young honey bees. J Insect Physiol 59:487-493

Vandame R, Colin ME, Otero-Colina G (1999) Africanized honey bees tolerance to *Varroa* in Mexico: Mite infertility is not the main tolerance factor. Apiacta 1:12-20

Vandame R, Morand S, Colin ME, Belzunces LP (2002) Parasitism in the social bee *Apis mellifera*: quantifying costs and benefits of behavioral resistance to *Varroa destructor* mites. Apidologie 33:433-445

Visscher PK, Vetter RS, Baptista FC (1997) Africanized bees, 1990-1995: Initial rapid invasion has slowed in the US. Calif Agric 51:22-25

Whitfield CW, Behura SK, Berlocher SH, Clark AG, Spencer Johnston J, Sheppard WS, Smith DR, Suarez AV, Weaver D, Tsutsui ND (2006) Thrice out of Africa: Ancient and recent expansions of the honey bee, *Apis mellifera*. Science 314:642-645

Winston ML (1992) The biology and management of Africanized honey bees. Annu Rev Entomol 37:173-193

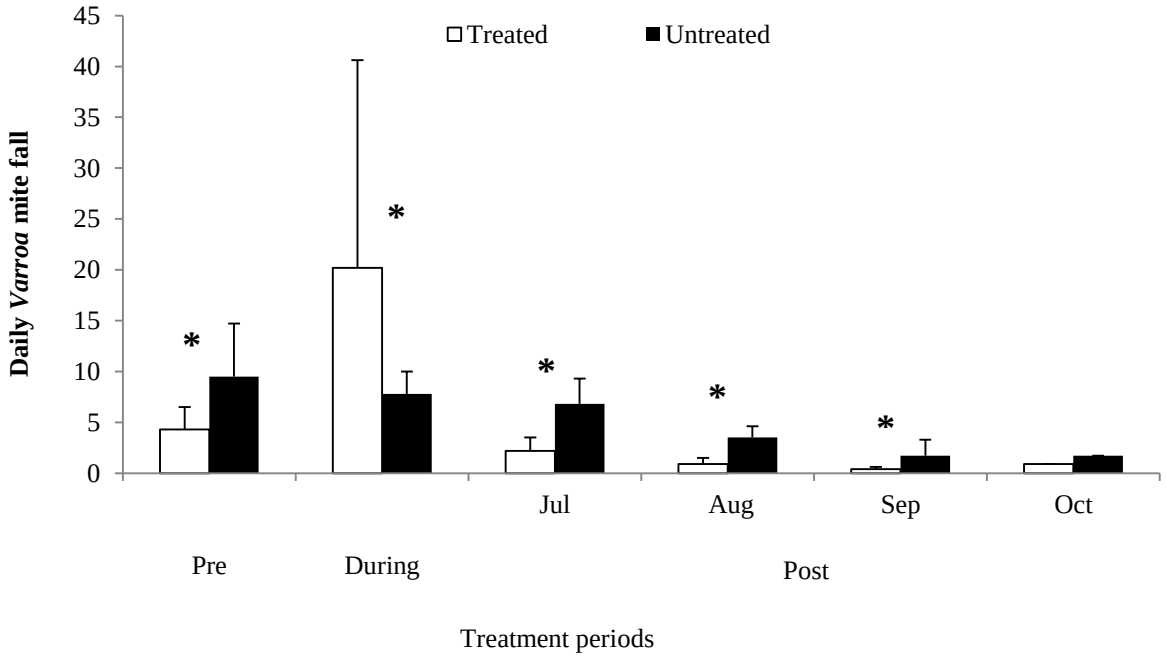

Fig. 1 Daily *Varroa* mite fall (mean ± SD) in treated and untreated colonies before (21st May-14th June 2011), during (15th June-30th June 2011) and four months (1st July-4th October 2011) after treatment. *Indicates significant differences between treated and untreated colonies (Mann-Whitney U test, $P < 0.05$).

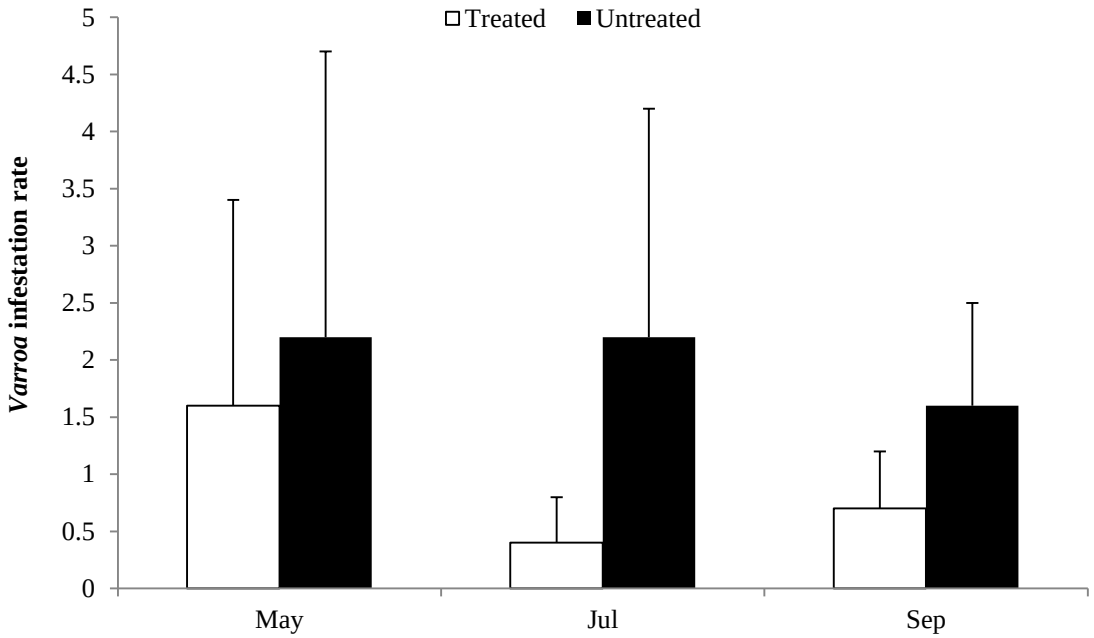

Fig. 2 *Varroa* mite infestation rates (mean ± SD) per 100 adult honeybees in the treated and untreated colonies measured before treatment (May) and after treatment (July and September).

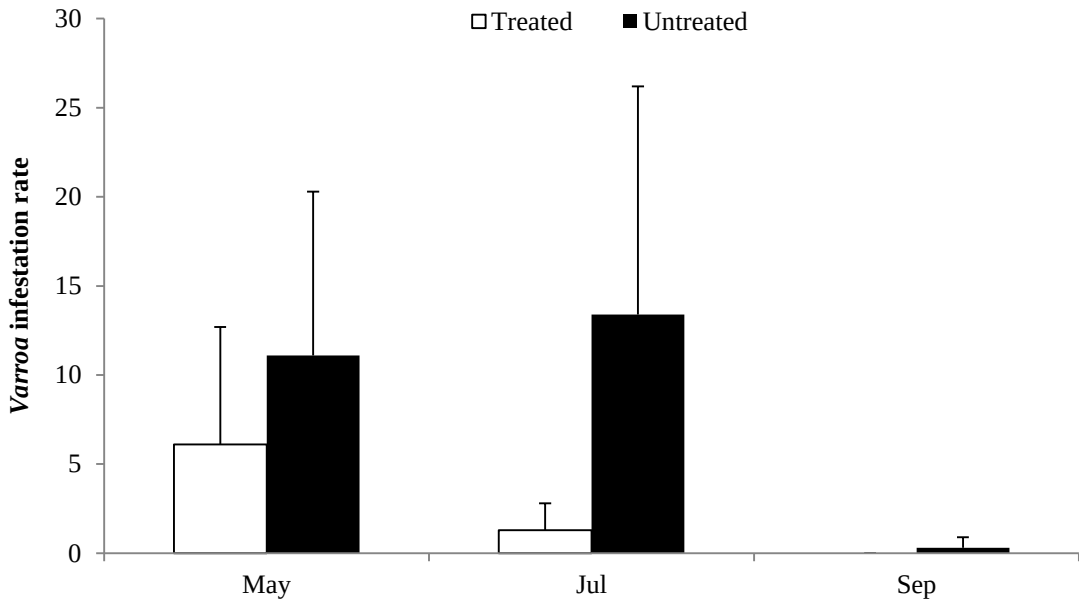

Fig. 3 *Varroa* mite infestation rates (mean ± SD) per 100 worker brood cells in the treated and untreated colonies measured before treatment (May) and after treatment (July and September).

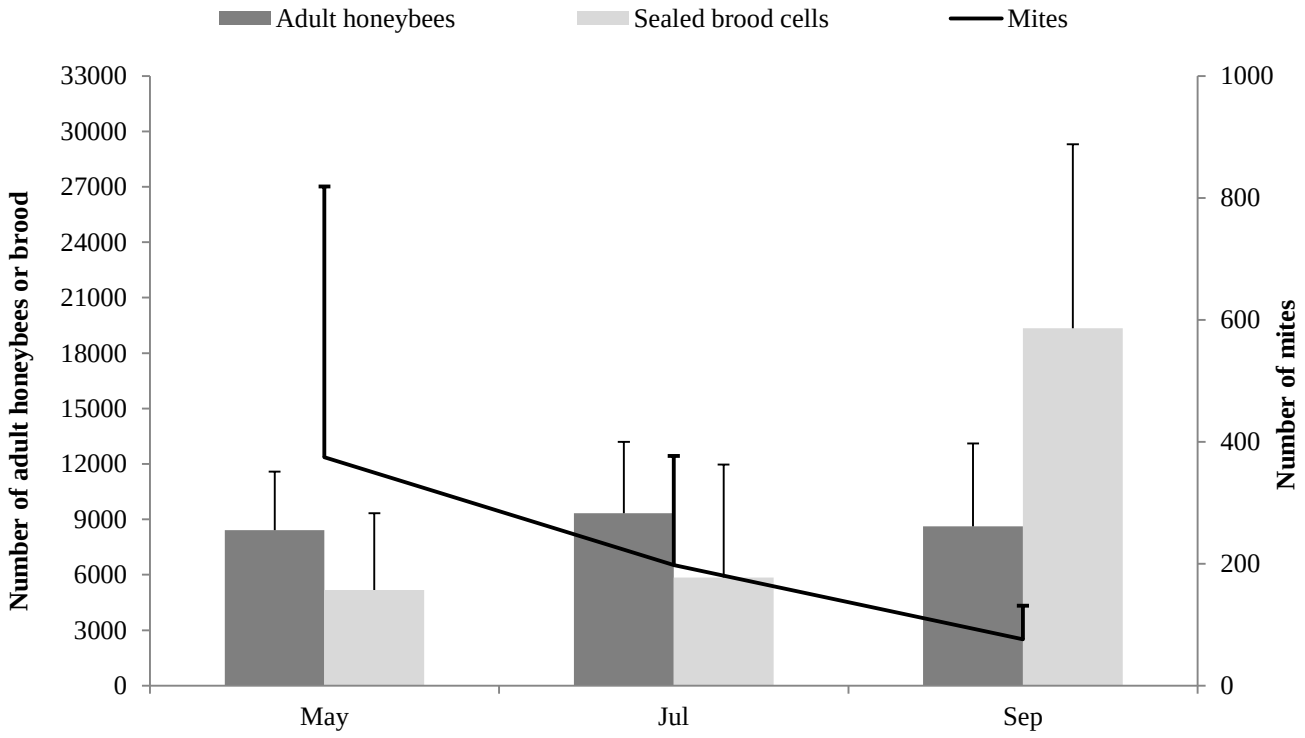

Fig. 4 The total number of adult honeybees, sealed brood cells and *Varroa* mites (mean ± SD) measured in treated colonies during May, July and September.

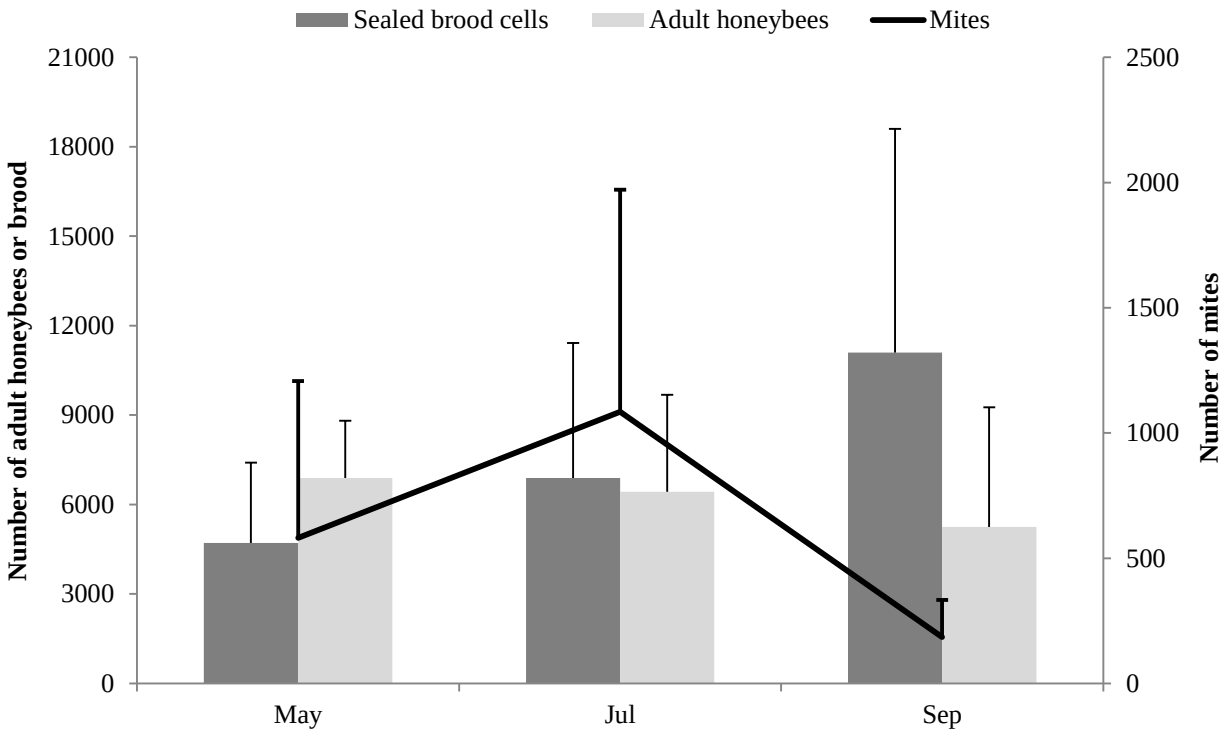

Fig. 5 The total number of adult honeybees, sealed brood cells and *Varroa* mites (mean ± SD) measured in untreated colonies during May, July and September.

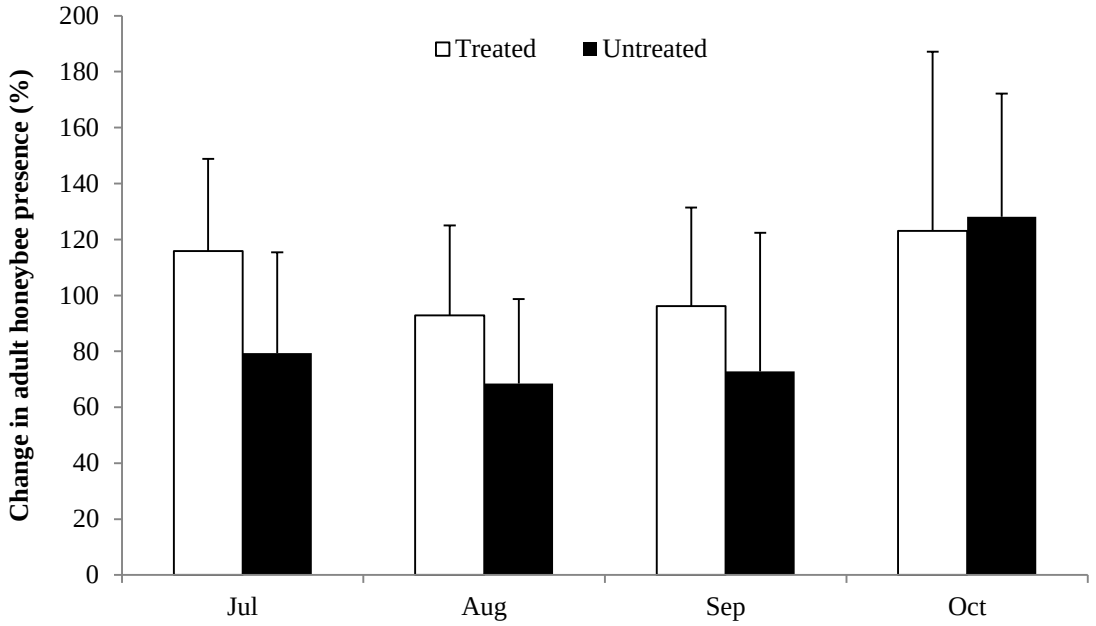

Fig. 6 Percentage change in adult honeybee populations (mean ± SD) measured in the treated and untreated colonies from July to October 2011.

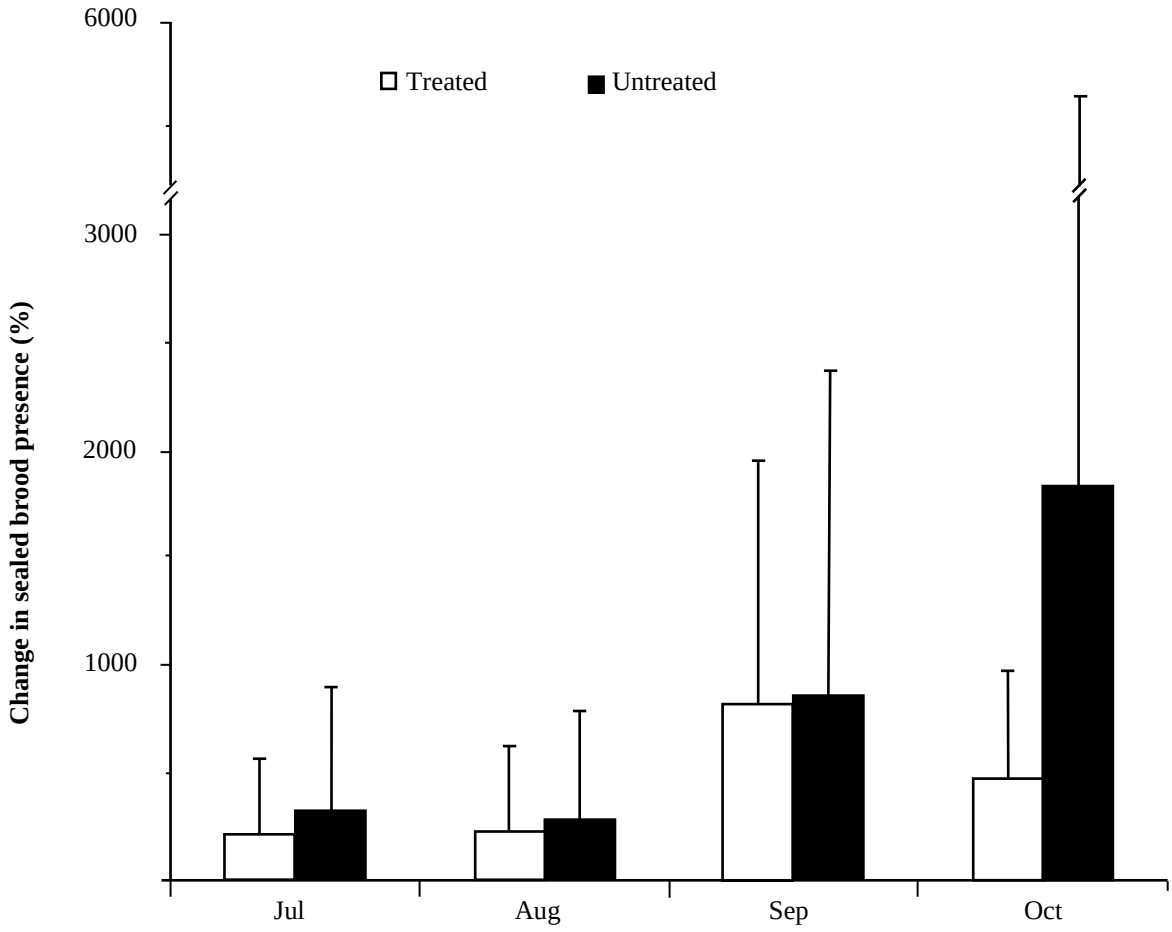

Fig. 7 Percentage change in surface area of sealed worker brood (mean ± SD) in the treated and untreated apiaries from July to October 2011.

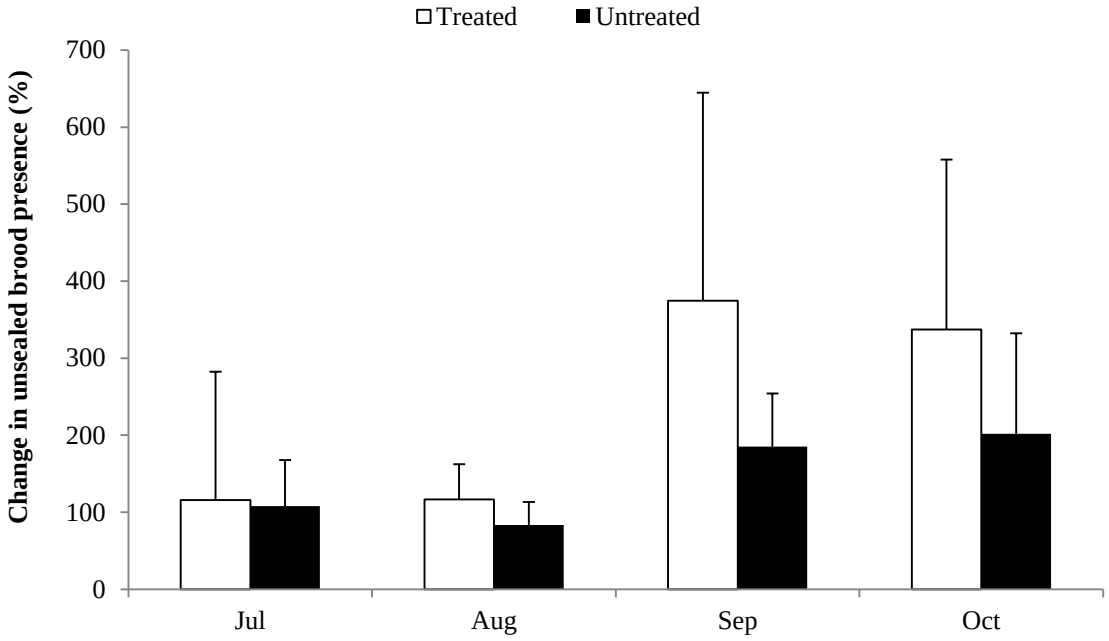

Fig. 8 Percentage change in surface area of unsealed worker brood (mean ± SD) in the treated and untreated colonies from July to October 2011.

Table 1. The number of colonies used for different analyses to compare treated and untreated *Apis mellifera scutellata* apiaries. Sample size varies according to the availability of adults, brood, loss of queen in the colonies and absconding.

	Treated				Untreated			
	Pre-treatment	**During treatment**	**Post-treatment**		**Pre-treatment**	**During treatment**	**Post-treatment**	
Daily mite fall	7-9	7	5-6		8-9	8	6-8	
	May	**Jul**	**Sep**		**May**	**Jul**	**Sep**	
Varroa infestation rates (Adults)	9	4	4		8	5	4	
Varroa infestation rates (Brood)	7	3	4		7	5	3	
Varroa population size (Adults)	5	4	4		6	4	4	
Varroa population size (Brood)	4	3	4		5	4	3	
Varroa population size (Total)	4	3	4		5	4	3	
	Jul	**Aug**	**Sep**	**Oct**	**Jul**	**Aug**	**Sep**	**Oct**
Percentage change in adult honeybees, sealed and unsealed brood	6	5	5	5	8	6	6	6
	May-Oct				**May-Oct**			
Total mite fall	5				6			