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The effect of temperature and humidity on grooming behaviour of honeybee, *Apis mellifera* (Hym.: Apidae) colonies against varroa mite, *Varroa destructor* (Acari: Varroidae)

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Abstract

Grooming behaviour is one of the important mechanisms of honeybee defence against parasitic mites. In addition to genetical characteristics, environmental conditions affect the behavioural traits of honeybee colonies. The main objective of this study was to examine the effect of temperature and humidity on the ability of honeybee workers to remove varroa mites from their body. One hundred worker bees from resistant and susceptible colonies were placed in cages and infested with 40 varroa mites per cage. Cages from each group of workers were randomly assigned to a combination of temperature and humidity treatments. Individual cages were held in incubators at 10, 25 and 34°C under low (20%), medium (50%) and high (80%) humidity. The entire experiment was replicated three times (18 cages per replicate). The proportion of mites falling into the base of the cages (grooming), or migrating within the closed system and the number of dead bees were monitored on day 2, 4 and 6 of experiment. At the end of experiment the proportion of mites on the live bees, dead bees and in the equipment was also determined. The results showed significant differences in the ability of the two groups to groom mites off their bodies and that the relative effectiveness of the grooming in the two groups of bees was dependent upon the combination of temperature and humidity to which they were exposed. Total mite mortality of the two groups of bees was also different; however, it was not influenced by temperature. Total mite mortality may include grooming, or the natural mite fall that cannot relocate their host. In the programs that are attempting to breed bees with increased grooming behaviour and enhanced resistance to *Varroa destructor* (Anderson & Trueman), the environmental conditions should be considered under which experiments are carried out.

Key words: varroa mite, honeybee, grooming, temperature, humidity

چکیده

رفتار نظافت‌گری یکی از مهم‌ترین مکانیزم‌های دفاعی زنبور عسل علیه کنه‌ی واروآ می‌باشد که در طرح‌های اصلاح‌نژادی برای بهبود این صفت تلاش می‌شود. علاوه بر خصوصیات ژنتیکی کلنی، شرایط محیطی نیز در بروز چنین صفات رفتاری مؤثر است. هدف این تحقیق، تعیین تأثیر دما و رطوبت نسبی روی رفتار نظافت‌گری کلنی‌های زنبور عسل علیه کنه‌ی واروآ در دو لاین مقاوم و حساس به این کنه بود. برای این منظور، صد زنبور عسل کارگر از هر یک از دو گروه در قفس‌های آزمایشگاهی قرار داده شد و هر قفس با ٤٠ کنه‌ی واروآ به طور مصنوعی آلوده گردید. این قفس‌ها داخل انکوباتور به طور تصادفی در تیمارهای گوناگون با رطوبت و حرارت متفاوت قرار می‌گرفتند که ترکیبی از حرارت‌های ١٠، ٢٥ و ٣٤ درجه‌ی سانتی‌گراد و رطوبت نسبی ٢٠٪ (کم)، ٥٠٪ (متوسط) و ٨٠٪ (زیاد) بود. آزمایشات مذکور در سه دوره‌ی زمانی تکرار شد و در هر دوره، ١٨ قفس آزمایشگاهی حاوی زنبور عسل آلوده به کنه‌ی واروآ تحت بررسی قرار گرفت. تعداد کنه‌ی ریخته‌شده در کف قفس‌ها، تعداد کنه‌های منتقل‌شده به روی قفس‌ها و تعداد زنبورهای مرده در روزهای دوم، چهارم و ششم شمارش می‌گردید. در پایان هر دوره‌ی آزمایش، تعداد کنه‌های روی زنبورهای زنده و مرده، و نیز کنه‌های داخل قفس‌ها شمارش شد. نتایج نشان داد که از نظر میزان کنه‌های دفع‌شده در دو گروه کلنی‌های مقاوم و حساس تفاوت معنی‌داری وجود دارد و درجه‌ی حرارت و رطوبت محیط قفس‌های آزمایشگاهی نیز در میزان کنه‌ی ریخته‌شده و رفتار نظافت‌گری زنبورها تأثیر گذاشته است. مجموع تلفات کنه‌ها نیز در دو گروه متفاوت بود ولی درجه‌ی حرارت روی آن‌ها تأثیری نداشت. در این تحقیق، تلفات کنه‌ها شامل کنه‌هایی می‌شد که از بدن زنبورها دفع و یا به طور طبیعی از بدن آن‌ها به کف قفس‌ها ریخته شده

و قادر به بازگشت روی بدن میزبان نبودند. نتایج این تحقیق نشان داد که در اصلاح نژاد کلنی‌های زنبور عسل بـرای بهبـود صفت نظافت‌گری و مقاومت به کنه‌ی وروآ باید به شرایط محیطی کلنی‌ها نیز توجه نمود.
واژگان کلیدی: کنه‌ی واروآ، زنبور عسل، نظافت‌گری، دما، رطوبت

Introduction

Varroa mite, *Varroa destructor* (Anderson & Trueman, 2000) is the most important pest of apiculture faces in the world that infests the colonies of several species of honey bee (Buchler *et al.*, 1992). Chemical acaricides are typically used to protect honey bee colonies against *V. destructor*, but their long term use can cause several problems like the development of acaricides resistance mites (Lodesani *et al.*, 1995; Elzen *et al.*, 1999; Milani, 1999), the build up of chemical residues in the colonies (Faucon & Flamiini, 1990; Lodesani *et al.*, 1992; Wallner, 1999), increases input costs for the producers and the possibility of outbreaks of secondary pests.

Because of serious problems associated with chemical treatments in the long term, it is necessary to develop alternative methods to control the varroa mite. Integrated control of *V. destructor* using the host resistance in conjunction with chemical and other control methods will provide a more sustainable method to manage this parasite.

Existence of resistant lines or breeding programs for the selection of colonies against varroa mite is the basic practice of integrated control. Grooming is one of the defence mechanisms against varroa mite and worker bees groom themselves with their legs and mandibles to get rid of a mite. Grooming has been evaluated with indirect methods in honeybee colonies (Morse *et al.*, 1991; Ruttner & Hunel, 1992; Boecking *et al.*, 1993; Rosenkrans *et al.*, 1997; Bienefeld *et al.*, 1999), and through laboratory assay (Hoffman, 1993; Fries *et al.*, 1996; Szabo *et al.*, 1996; Aumeier, 2001; Spivac & Reuter, 2001).

It seems likely that a number of individual traits may be required to provide collectively *Apis mellifera* L. colonies with some levels of tolerance to varroa (Rinderer *et al.*, 2001). Grooming as a defence against *V. destructor* has been actively investigated since the behaviour was noted in *Apis cerana* Fabr. by Peng *et al.* (1987). Direct assays have been developed to evaluate self (autogrooming) and group (allogrooming) behaviour (Peng *et al.*, 1987; Hoffman, 1993; Aumeier, 2001), while other indirect assessments typically evaluate the proportion of damaged (mites that are chewed by the bees mandibles) to undamaged mites falling from the bees or quantify the number of mites with specific types of damage (Hoffman, 1993, 1995; Szabo *et al.*, 1996; Zaitoun *et al.*, 2001). These assays show that *A.*

mellifera can successfully groom varroa but the estimates of the contribution of this behaviour to successful defence against the parasite by the colony are highly variable (Buchler *et al.*, 1992; Arechavaleta-Velasco & Guzman-Novoa, 2001; Lodesani *et al.*, 2002; Moretto, 2002; Vandame *et al.*, 2002; Mondragon *et al.*, 2005).

Species and race differences have been documented and the results of some studies indicated that *A. cerana* had low infestation of varroa mite and grooming behaviour in some honeybee races were significantly higher than north American races. The effective grooming behaviour of *A. cerana* and also the number of killed and injured mites in their colonies provide a further explanation for the resistance of these colonies (Peng *et al.*, 1987; Buchler *et al.*, 1992). Africanized honeybees had higher grooming behaviour than European honeybees (Vandame *et al.*, 2002). *Apis mellifera meda* Skorikow worker bees in Iran showed that their extensive grooming and allogrooming behaviour were similar to *A. cerana* (Pourelmi, 1989) and *Apis mellifera capensis* Esch. had effective grooming activity, higher than *A. mellifera carnica* Pollmann (Moritz & Mautz, 1990).

The ability of honeybee colonies to remove the parasitic mite is affected by genotype of honeybees and most likely the environmental conditions. Quantifying the costs and benefits of a host's response to a parasite is difficult, in part, because it is often not possible to separate environmental effects from those resulting from genetic traits for specific defence mechanisms. Although, social species of insects such as *Apis* spp. regulate the environment within their hive, external environmental factors still seem to play a major role in affecting the relationship between honey bee parasites and their host. However, these interactions are poorly understood.

The objectives of this study were to examine the differences of grooming behaviour between the two honeybee lines and the effect of environmental conditions such as temperature and humidity on the ability of honeybee workers to groom varroa mite from their body.

Materials and methods

The experiments were conducted at the University of Manitoba in the Winnipeg (49 54 N, 97 14 W), Manitoba, Canada, from May to October 2004. Some preliminary experiments were performed to optimize the bioassay conditions and to select the high and low grooming colonies. The selection was based on the results of bioassays conducted in 2003 and also the

results of our pretests in 2004. Colonies were obtained from stock produced by Manitoba producers that was a part of the three years breeding project at the University of Manitoba.

Worker bees were collected from the brood frames of high and low grooming colonies, anesthetized with CO_2 and introduced into bioassay cages (9 cages per group and totally 18 cages). Approximately one hundred worker bees (13.3 grams of bees) of mixed age were established in each cage. Cages had outer dimensions of 20.5 × 8.0 × 11.2 cm. The bees were confined on a piece of plastic comb within a 15.8 × 5.0 × 8.0 cm space inside the cage, the back and top of the cage were solid wood, the front and removable floor was made from a wire screen (3 squares per cm). There was a drawer beneath the floor that could be opened for counting the fallen mites without allowing live bees to escape. It was shallow enough to allow live mites that fell into the drawer to pass back and forth through the screen. The distance between the floor of the drawer and the screened bottom of the cage was 1.5 cm. Two 50 ml centrifuge feeder tubes were placed into 3.5 cm. diameter holes on the top of the cage. Feeders had 2 cm diameter holes cut into the lid that were covered with a fine mesh cloth (28 squares per cm) to allow the bees to feed. One feeder tube contained distilled water and the other contained 67% sucrose in distilled water. Sugar syrup and water were fed at libitum. The caged bees were maintained in an incubator at 30°C for 24 hours before inoculating the cages with mites.

Mites for bioassay were collected from highly infested colonies by a modification of the CO_2 method of Ariana *et al.* (2002). Mites infested bees were exposed to a constant flow of CO_2 at 5 L/min during 5 minutes exposure.

Bees were placed on the hardware 8 mesh insert and agitated on orbital shaker table at 400 RPM for 5 minutes. The mites were picked up with the tip of a soft paint brush and placed on the wet paper towel. Mites quickly recovered and were introduced into bioassay cages. Forty mites were introduced into each bioassay cage.

Nine cages from each group (18 cages in total) were randomly assigned to a treatment combination everyone consisted of three levels of temperature (10, 25 or 34°C) and maintained with air consisted of three levels of ambient humidity as follow: low (20%), medium (50%) and high (80%). The cages were confined in a sealed 45 × 55 cm Mylar bag according to Underwood & Currie (2003) (Reckitt and Colman Inc., Toronto, Canada) and provided with a constant flow of humid air through inlet and outlet tubes mounted on the top of the bag. Humidity of the inflow air in each bag was regulated by passing air over different mixtures of potassium hydroxide and water that were mixed to provide solutions for

regulating the inflow air at 20, 50 and 80% humidity (Solomon, 1951). The range of temperature and humidity within each Mylar bag were monitored at the base and top of the cage within each bag using Hobo C-8 (Onset Computer Corp, Bourne, U.S.A.) data loggers. Air flow was maintained at a constant rate of 0.708 L/min with a Dwyer RMN-3-SSV (Dwyer Instruments Inc., Michigan City, U.S.A.) air metering valve to exhaust the excess CO_2 and moisture produced by bee metabolism. Cages were randomly distributed within incubators and that were maintained at 10, 25 or 34°C during the experiment.

The entire experiment was replicated on 12 July, 26 July and 1 August 2004. On days 2, 4 and 6, the bags were opened briefly to record the number of dead bees fallen onto the floor of the cage, the number of mites in the drawer of the cage (grooming) and the number of mites migrating within the closed system. All the counted mites were also scored as live or dead. After taking the final sample on day 6, the bees were removed from the cages and the mean abundance of mites on live bees and dead bees (mites per 100 bees) (Bush *et al*., 1997) was assessed by the alcohol shake method (Gatien & Currie, 2003). The total number of dead mites remained in the equipment was also quantified.

The effects of grooming level, humidity and temperature treatments on the change in within-cage temperature, within-cage humidity, daily mite fall, proportion of migrating mites and daily bee mortality over the time were analyzed using repeated measures ANOVA with a heterogeneous autoregressive covariance structure (PROC MIXED, (SAS Institute Inc., 1999)). Grooming level of workers, temperature, humidity and the date of trial were the main effective factors in the model and observations over the time were treated as repeated measures. Mean abundance of varroa on adult workers at the end of the experiment was analyzed by the same method excluding the time as a factor in the model. All data were arcsine transformed prior to analyses to improve the equality of variance (Snedecor & Cochran, 1980). The differences between means were compared by Bonferoni corrected contrasts whenever any significant interactions were observed between treatment factors (PROC MIXED, (SAS Institute Inc., 1999)).

Results

The results showed that daily mite fall was greater in the high grooming group of bees than in the low grooming group of bees, nevertheless, the degree of difference between groups was dependant upon temperature (temperature × grooming level, $F = 3.7$; $df = 2, 20$; $P < 0.04$) (fig. 1) and humidity (humidity × grooming level × time, $F = 4.5$; $df = 4, 36$; $P < 0.005$)

(fig. 2). The highest differences in mite fall between the two groups were observed at 25°C (fig. 1). Significant differences in mite fall were also found between the high and low grooming groups at 34°C but not at 10°C (fig. 1).

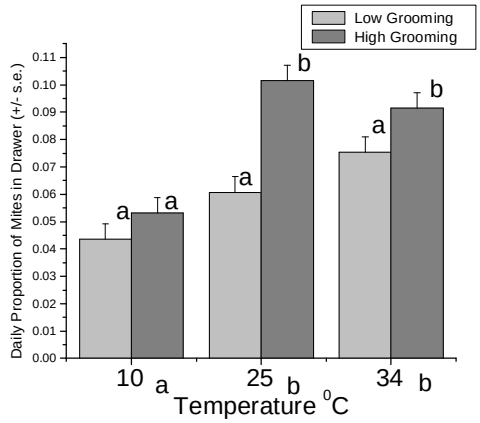

Figure 1. The effect of temperature on the mean (± S.E.) daily rate of mite fall (percentage of total mite population per day) onto the bottom of cages containing the high or low grooming bees that were held in an incubator at 10, 25 or 34°C. Within temperatures or grooming level of bees, means followed by the same letter are not significantly different ($P < 0.05$), ($N = 9$ cages per treatment combination).

Humidity affected the amount of mite fall in two groups too; however, it only happened in low humidity conditions and during the first two days of experiment (fig. 2). Under low humidity, mite fall in cages with the high grooming group of bees was more than low grooming group ($P < 0.05$). The mean rate of daily mite fall decreased with time in both groups of bees ($F = 55$; $df = 2, 36$; $P < 0.0001$) (fig. 2). However, there was no significant level × time interaction in low grooming group and low humidity grooming ($F = 1.32$; $df = 2, 36$; $P > 0.28$). Cumulative mite fall on day 6 was 34.9 ± 2.0 percent in the low grooming group and 47.5 ± 2.0 percent in the high grooming group. Cumulative mite fall ranged from 21.7 ± 6.1 percent in the low grooming group under high humidity at 10°C to a high of 72.9 ± 6.5 percent in the high grooming group under low humidity at 25°C.

The proportion of mites that migrated outside of the cage did not differ between the high and low grooming groups of bees ($F = 0.2$; $df = 1, 20$; $P > 0.66$). However there was a

significant temperature × time interaction that affected the proportion of migrating mites (F = 45.7; df = 4, 36; P < 0.0001). The proportion of mite migration increased with temperature in the first two days of the experiment (fig. 3). But there were no significant differences in the rate of mite migration in days 4 and 6 (fig. 3).

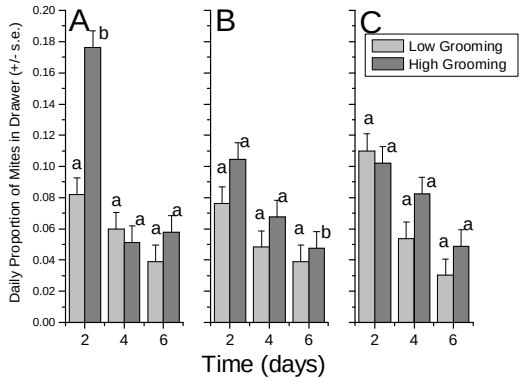

Figure 2. The effect of humidity on the daily rate of mite fall (percentage of total mite population per day ± S.E.) on the bottom of cages held under low (A) medium (B) or high (C) humidity. Within days, means followed by the same letter are not significantly different (P < 0.05), (N = 9 cages per treatment combination).

The proportion of varroa remained on live bees (mean abundance) at the end of the experiment showed that bees in cages containing the high grooming group had fewer mites than bees in cages with the low grooming group (F = 7.53; df = 1, 24; P < 0.001) (fig. 4).

Mean abundance of *V. destructor* on workers were decreased with increasing temperature (F = 16.6; df = 2, 24; P < 0.0001) but the difference between grooming level treatments was consistent across all temperatures as indicated by the lack of significant grooming level × temperature interaction (F = 1.19; df = 2, 24; P > 0.32). The mean abundance of varroa on workers was similar under the three levels of humidity (F = 0.08; df = 2, 24; P < 0.92). There were no significant two or three-way interactions among grooming level, temperature and humidity (P > 0.05).

The mean abundance of *V. destructor* on dead bees at the end of the experiment decreased with increasing temperature (F = 4.08; df = 2, 24; P < 0.02); however, it was not affected by grooming level (F = 0.02; df = 1, 24; P > 0.88) or humidity (F = 1.06; df = 1, 24;

P > 0.36). The mean abundance of *V. destructor* on dead bees was 28.6 ± 3.8, 19.3 ± 3.8 and 13.5 ± 3.8 for the 10, 25 and 34°C treatments, respectively. The varroa infestation of dead bees was higher at 10°C than at 34°C (P < 0.05). There were no significant two or three-way interactions among grooming level, temperature and humidity (P > 0.05).

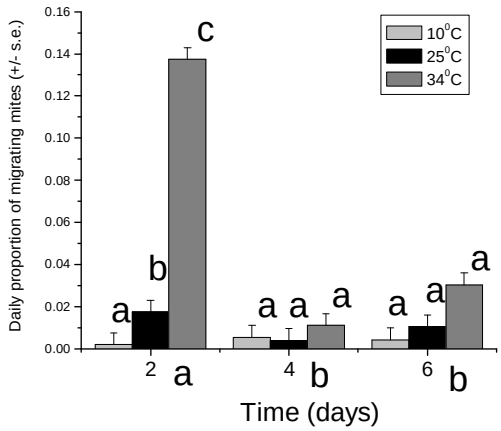

Figure 3. Mean (± S.E.) daily rate of varroa mite migration (percentage of total mite population per day found outside the cage) at temperatures of 10, 25 and 34°C on days 2, 4 and 6 of the experiment. Within days or temperatures within days, means followed by the same letter are not significantly different (P < 0.05), (N = 27 cages per treatment combination).

As expected, overall worker mortality rates were higher at lower temperatures than at higher temperatures (F = 32.6; df = 2, 30; P < 0.0001) (fig. 5). Nevertheless, there were differences in worker mortality rates between the high and low-grooming treatment levels. The daily morality rate of worker bees in the high-grooming group was greater than the rate in the low-grooming treatment group. This was just at 10°C and only during the first two days of the experiment (P < 0.05).

Discussion

This research pointed out the impact of temperature and humidity on grooming behaviour of honeybee colonies against varroa mite in high and low grooming groups. Worker mortality rate of high grooming group was greater than low grooming group only in

low temperature in the first two days but totally, bee survival was not improved by grooming

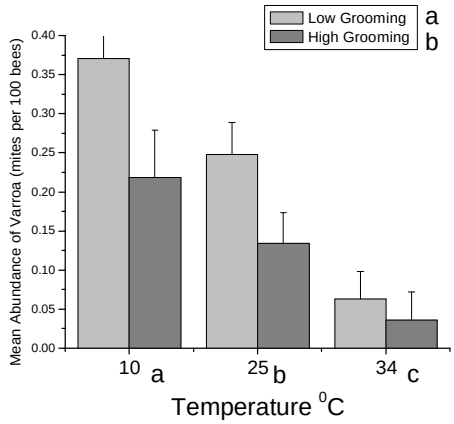

Figure 4. Mean abundance (± S.E.) of varroa at the end of the experiment in the high and low grooming groups at temperatures of 10, 25 and 34°C. Within main effect treatments of grooming level of bee and temperature, means followed by the same letter are not significantly different (P < 0.05), (N = 9 cages per treatment combination).

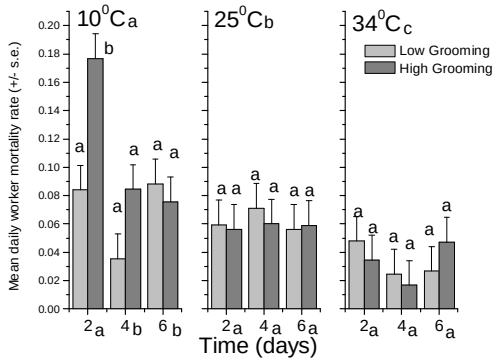

Figure 5. Mean daily rate of worker mortality of the high and low grooming groups of bees that were inoculated with varroa and held under temperatures of 10, 25 or 34°C for periods of 6 days. Means within grooming levels, temperatures or times within temperatures that are followed by the same letter are not significantly different (P < 0.05), (N = 9 cages per treatment combination).

in this study.

The results showed that in high grooming group the ability of bees for removing the mites from their body in different conditions was higher than low grooming group (figs 1-2). The significant differences of fallen mites in the different groups and in various temperatures or humidity were in agreement with the results of the experiments reported by several authors (Moritz & Mautz, 1990; Szabo *et al.*, 1996; Arechavaleta-Velasco & Guzman-Novoa, 2001; Aumeier, 2001).

The part of difference could be traced back to genotypic effects and the other part of the difference could be related to external influences of environmental conditions (Boecking & Spivak, 1999). We selected humidity and temperature treatment levels that were similar to those in a broodless honeybee cluster (VilKa, 1996; Stabentheiner *et al.*, 2003). Results of this experiments suggested that differences of fallen mites in experimental conditions could be explained mainly by the worker ability to remove the mites infesting adult bees, presumably by grooming behaviour, since this was the only measuring character that showed significant differences between groups. Cumulative mite fall ranged from 22 % in low grooming groups of bee held at 10°C and high humidity, to 73% in high grooming groups held at 25°C and low humidity. Results also showed that mite grooming in the low grooming group was increased regularly in the higher temperatures but in the high grooming group the fallen mites were increased but not regularly with increasing the temperature (fig. 1). Present results on the effect of temperature on grooming behaviour confirmed previous observations of more mites fall in the hot weather (Engels & Rosenkrans, 1992; Webster *et al.*, 2000). Similarly, the proportion of remaining mites on the live bees in low temperature was more than high temperature. Actually there were less remained mites on live bees in high temperature (34°C) and more left mites on live bees in low temperature (10°C) (fig. 4).

Overall success in reducing the mite loads in both grooming level treatments increased with increasing the temperature. Relative success of high and low grooming groups did not follow the same pattern. The greatest differences in mite removal between high grooming and low grooming groups occurred at 25°C that was within the range of temperatures that would be expected in a broodless cluster of bees during winter (Stabentheiner *et al.*, 2003). It is not known if worker bees groom *V. destructor* more effectively at 25°C or if the mites "less inclined" relocate their hosts as Patzold & Ritter (1989) has shown that *V. destructor* prefers temperatures between 26-31°C to 35°C when placed in a temperature gradient. Therefore, groomed mites that leave their host when the ambient temperature is 25 may not attempt to

relocate their hosts as quickly as mites at higher temperatures. The levels of impact on the grooming populations of mite are likely to change with season and are predicted to be higher in broodless period (Wilkinson *et al.*, 2001; Wilkinson & Smith, 2002; Mondragon *et al.*, 2005). Models predict that a 50% increase in the efficiency of grooming of phoretic mite in broodless bees during winter would reduce mite population growth by 25% (Wilkinson *et al.*, 2001; Wilkinson & Smith, 2002).

As expected, low humidity enhanced the effectiveness of grooming. Differences in grooming success between high and low grooming groups of bees at low humidity were only apparent during the first two days of the experiment .Mites are very susceptible to desiccation (Bruce *et al.*, 1997; Yoder *et al.*, 1999). So it is likely that the low humidity prevents the fallen mites to relocate a host before they die because of water loss. Since proportionately more mites fell from the high grooming group this would enhance the success of grooming in the low humidity treatment.

It is noticeable that one part of difference in remained mites on live bees is related to the grooming and the other part is based on the migration of mites onto the equipments bags or cages. The proportion of migrating mites on the bags and equipments was more in high temperature rather than in low temperature (fig. 3). The mite migration on the bags or cages was not different in two honeybee groups. However, there was significant temperature × time interaction on the migration. Actually, migration had increased with temperature in the first two days (fig. 3).

The remained mites on the live bees were affected by temperature but humidity had not significant effect on the remained mites on the live bees. The mean abundance of the left mite on the bees decreased with increasing temperature (fig. 4). The mean abundance of the left mites on the live bees in the high grooming group was fewer than low grooming group (fig. 4). Szabo *et al*. (1996) reported significant differences of left mites in the different lines. The results of their tests showed the average of the left mites on the live bees in three different lines were 30, 43 and 62% after 8 days respectively. The level of varroa left at the end of experiment on the dead bees was not affected by the grooming level and decreased with increasing temperature.

De Guzman *et al*. (1993) reported that mites can survive on the dead bees for up to three days. This would explain why not all of the mites had fallen after 6 days in our test. It seems more mites can return to the live bees in high temperatures.

In this study, bee worker mortality was affected by temperature and grooming level. Daily mortality rate at low temperature (10°C) was greater than high temperatures (25 and 34 °C). Engels & Rosenkrans (1992) reported the temperature tolerance of bees. They believed bees and broods could be injured at 40-50°C and higher temperature. Currie & Gatin (2006) believed in the long term studies exposing the colonies to the mean abundance of 40 mites per 100 bees (similar to the present work) would normally result in substantial mortality in broodless period during the winter. Our bioassay showed that worker mortality of the two groups also differed and was greater in high grooming group but only in the day 2 at 10°C. So we can conclude the six days bioassay were not functional enough to clarify the effect of lower mite infestation on worker longevity in the high grooming group completely. Though, there were significant interactions among the grooming level of bees, temperature and time.

High mortality could result if: (i) a high cost biochemical or physiological trait (Rigby *et al.*, 2002), associated with the maintenance of the grooming mechanisms resulted in reduced cold tolerance in the high grooming worker bees; (ii) worker lifespan were shortened as a result of the increased activity level associated with active grooming as would be predicted by (Neukirch, 1982); or (iii) there was increased risk of death associated with misallocation to defence (Jokela *et al.*, 2000) grooming at the expense of cluster formation. Because grooming behaviour seems to have relatively high costs and moderate efficacy, tolerance also might be expected to be favoured under low parasite infestation levels (Jokela *et al.*, 2000; Vandame *et al.*, 2002). This tolerance was not measured in this study.

The study showed that environment had a significant impact on the relative effectiveness of defence against *V. destructor* in high and low grooming groups of worker bees. Under low humidity conditions, the relative effectiveness of mite removal in high grooming group of bees was greater at 25°C than at higher or lower temperatures and this was slightly higher than at higher levels. Efficiency of mite removal in the high grooming treatment increased in comparison with the low grooming treatment group by 41%, 46% and 43% in the 10, 25 and 34°C treatments, respectively. However, bee mortality rates in the high grooming group were greater than in the low-grooming group under low temperatures indicating there may be a relatively high biological cost associated with the grooming behaviour at low temperature there.

It seems the additional studies are necessary to confirm the actual contribution and importance of grooming behaviour in different environments with various populations of honeybee.

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References

Anderson, D. & Trueman, J. V. A. (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology* 24, 165-189.

Arechavaleta-Velasco, M. G. & Guzman-Novoa, E. (2001) Relative effect of four characteristic than restrain the population growth of the mite *Varroa destructor* in honeybee (*Apis mellifera*) colonies. *Apidologie* 32, 157-174.

Ariana, A., Ebadi, R. & Tahmasbi, Gh. (2002) Laboratory evaluation of some plant essence to control *Varroa destructor* (Acari: Varroidae). *Experimental and Applied Acarology* 27, 319-327.

Aumeier, P. (2001) Bioassay for grooming effectiveness towards *Varroa destructor* mites in Africanized honeybees. *Apidologie* 32, 81-90.

Bienefeld, K., Zautke, F., Pronin, D. & Mazedd, A. (1999) Recording the proportion of *Varroa jacobsoni* Oud. in the debris of honeybee colonies (*Apis mellifera*). *Apidologie* 30, 249-256.

Boecking, O., Rath, W. & Drester, W. (1993) Grooming and removal behaviour strategies of *Apis mellifera* and *Apis cerana* bees against *Varroa jacobsoni*. *American Bee Journal* 133, 117-119.

Boecking, O. & Spivak, M. (1999) Behavioural defences of honeybee against *Varroa jacobsoni* Oud. *Apidologie* 30, 141-158.

Bruce, W. A., Needham, G. R. & Potts, W. J. E. (1997) The effects of temperature and water vapor activity on water loss by *Varroa jacobsoni* (Acari: Varroidae). *American Bee Journal* 137, 461-463.

Buchler, R., Dreschler, W. & Torrier, I. (1992) Grooming behaviour of *Apis cerana, Apis mellifera* and *Apis dorsata* and it's effect on the parasite mite *Varroa jacobsoni* and *Tropilealaps clareae. Experimental and Applied Acarology* 16, 313-319.

Bush, A. O., Lafferty, K. D., Lotz, J. M. & Shostak, A. W. (1997) Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Journal of Parasitology* 83, 575-583.

Currie, R. W. & Gatin, P. (2006) Timing acaricide treatments to prevent *Varroa destructor* (Acari: Varroidne) from causing economic damage to honeybee colonies. *Canadian Entomology* 138, 238-252.

De Guzman, L. I., Rinderer, T. E. & Beaman, L. D. (1993) Longevity of *Varroa jacobsoni* away from a living host. *Proceeding of First International Conference on the Asian Honeybee and Bee Mites*, 434-491.

Elzen, P. J., Eischen, F. A., Baxter, J. R., Elzen, G. W. & Wilson, W. T. (1999) Detection of resistance in U. S. *Varroa jacobsoni* Oud. (Mesostigmata: Varroidae) of the acaricides fluvalinate. *Apidologie* 30, 13-17.

Engels, W. & Rosenkrans, D. (1992) Therapy of Varroatosis and its effects. *Apidologie* 23, 379-381.

Faucon, J. P. & Flamiini, C. (1990) Residue of fluvalinate dans la cire et dans le mile (residue of fluvalinate in honey and wax). *Fante Pobeille* 118, 182-189.

Fries, I. W. H., Wei, S. & Jin, C. S. (1996) Grooming behaviour and damaged mites (*Varroa jacobsoni*) in *Apis cerana cerana* and *Apis mellifera ligustica*. *Apidologie* 27, 3-11.

Gatien, P. & Currie, R. W. (2003) Timing of acaricide treatments for control of low level populations of *Varroa destructor* (Acari: Varroidae) and implications for colony performance of honey bees. *Canadian Entomologist* 135, 794-763.

Hoffman, S. (1993) The occurrence of damaged mites in cage test and under field conditions in hybrids of different Carniolan lines. *Apidologie* 24, 493-495.

Hoffman, S. (1995) Registration of damaged varroa mites in small colonies for the assessment of grooming behaviour. *Apidologie* 26, 322-323.

Jokela, J., Schmid-Hempel, P. & Rigby, M. C. (2000) Dr. Pangloss restrained by the Red Queen – steps towards a unified defence theory. *Oikos* 89, 267-274.

Lodesani, M., Colombo, M. & Spreafica, M. (1995) Ineffectiveness of Apistan treatment against the mite *Varroa jacobsoni* Oud. in several distinct of Lombardy (Italy). *Apidologie* 26, 67-72.

Lodesani, M., Craishleim, C. & Moritz, R. F. A. (2002) Effect of some characters on the population growth of mite *Varroa jacobsoni* in *Apis mellifera* L. colonies and results of a bi-directional selection. *Journal of Applied Entomology* 126, 120-137.

Lodesani, M., Pellacani, A., Bergomi, S., Carpana, E., Rabitti, T. & Lasagmi, P. (1992) Residue determination for some products used against varroa infestation in bees. *Apidologie* 23, 257-272.

Milani, N. (1999) The resistance of *Varroa jacobsoni* Oud. to acaricides. *Apidologie* 30, 229-234.

Mondragon, L., Spivak, M. & Vandame, R. (2005) A multifactorial study of the resistance of Africanized and hybrid honey bees *Apis mellifera* to the mite *Varroa destructor* over one year in Mexico. *Apidologie* 36, 345-358.

Moretto, G. (2002) Mortality of *Varroa destructor* in broodless Africanized and Carnica honey bee (*Apis mellifera* L.) colonies. *Interciencia* 27, 702-704.

Moritz, R. F. A. & Mautz, D. (1990) Development of *Varroa jacobsoni* in colonies of *Apis mellifera capensis* and *Apis mellifera carnica*. *Apidologie* 21, 53-58.

Morse, R. A., Miska, D. & Robinson, G. E. (1991) *Varroa resistance* in U. S. honeybees. *American Bee Journal* 131, 433-434.

Neukirch, A. (1982) Dependence of the life span of the honeybee (*Apis mellifera*) upon flight performance and energy consumption. *Journal of Comparative Physiology B* 146, 35-40.

Patzold, S. & Ritter, W. (1989) Studies on the behaviour of the honey-bee mite, *Varroa jacobsoni* O., in a temperature gradient. *Journal of Applied Entomology* 107, 46-51.

Peng, S. C., Fang, Y., Xu, S. & Ge, L. (1987) The resistance mechanism of the Asian honeybee, *Apis cerana* Fabr., to an ectoparasite mite, *Varroa jacobsoni* Oudemans. *Journal of Invertebrate Pathology* 49, 54-60.

Pourelmi, M. R. (1989) Die mogliche resistanz der bien *Apis mellifera* meda gegenuber der Varroa-Milbe. *Bienenbesamung* 1, 17-18.

Rigby, M. C., Hechinger, R. F. & Stevens, L. (2002) Why should parasite resistance be costly? *Trends in Parasitiology* 18, 116-120.

Rinderer, T., De Guzman, L., Delatte, G. T., Stelzer, J. A., Lancaster, V. A., Kuznetsov, V., Beman, L., Watts, R. & Harris, J. W. (2001) Resistance to the parasitic mite *Varro destructor* in honey bees from far-eastern Russia. *Apidologie* 32, 381-394.

Rosenkrans, P., Fries, I., Boecking, O. & Sturmer, M. (1997) Damaged varroa mites in the debris of honeybee (*Apis mellifera* L.) colonies with and without hatching brood. *Apidologie* 28, 429-437.

Ruttner, F. & Hunel, H. (1992) Active defence against varroa mites in a carniolan strain of honeybee (*Apis mellifera carnica* Pollmann). *Apidologie* 23, 173-187.

Snedecor, G. W. & Cochran, W. G. (1980) *Statistical methods.* 7th ed. 507 pp. Iowa State University Press, Ames, Iowa.

Solomon, M. E. (1951) Control of humidity with potassium hydroxide, sulphuric acid, or other solutions. *Bulletin of Entomological Research* 42, 543-554.

Spivac, M. & Reuter, G. S. (2001) *Varroa destructor* infestation in untreated honeybee *Apis mellifera* (Hymenoptera: Apidae) colonies selected for hygienic behaviour. *Journal of Economic Entomology* 94(2), 326-331.

Stabentheiner, A., Pressl, H., Hrassnigg, N. & Crailsheim, K. (2003) Endothermic heat production in honeybee winter clusters. *Journal of Experimental Biology* 206, 353-358.

Szabo, T. I., Timothy Walker, C. K. & Muller, A. E. (1996) Grooming behaviour as a varroa resistance characteristic in honeybee colonies. *American Bee Journal* 136, 515-517.

Underwood, R. M. & Currie, R. W. (2003) The effects of temperature and does of formic acid on treatment efficacy against *Varroa destructor* (Acari: Varoidea), a parasite of *Apis mellifera* (Hymenoptera: Apidae). *Experimental and Applied Acarology* 29, 303-313.

Vandame, R., Morand, S., Colin, M. E. & Belzunces, L. P. (2002) Parasitism in the social bee *Apis mellifera*: quantifying costs and benefits of behavioural resistance to *Varroa destructor* mites. *Apidologie* 33, 443-445.

Vilka, E. E. (1996) Relative humidity and the honeybee cluster in the winter. *American Bee Journal* 136, 349-351.

Wallner, K. (1999) Varroacides and their residues in bee products. *Apidologie* 30, 235-248.

Webster, T. C., Thaker, E. M. & Vorisec, F. E. (2000) Live *Varroa jacobsoni* (Mesostigmata: Varroidae) fallen from honeybee (Hymenoptera: Apidae) colonies. *Journal of Economic Entomology* 93(6), 1596-1601.

Wilkinson, D. & Smith, G. C. (2002) A model of the mite parasite *Varroa destructor* on honeybees (*Apis mellifera*) to investigate parameters important to mite population growth. *Ecological Modeling* 148, 263-275.

Wilkinson, D., Tompson, H. M. & Smith, G. C. (2001) Modeling biological approaches to controlling varroa populations. *Ecological Modeling* 141, 511-516.

Yoder, J. A., Sammataro, D., Peterson, J. A., Needham, G. R. & Bruce, W. A. (1999) Water requirements of adult females of the honey bee parasitic mite, *Varroa jacobsoni* (Acari: Varroidae) and implications for control. *International Journal of Acarology* 25, 329-335.

Zaitoun, S. T., Al-Ghzawi, A. & Shannag, H. K. (2001) Grooming behaviour of *Apis mellifera syriaca* towards *Varroa jacobsoni* in Jordan. *Journal of Applied Entomology* 125, 85-87.

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