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Comparative reproduction of *Varroa destructor* in different types of Russian and Italian honey bee combs

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Abstract Earlier studies showed that Russian honey bees support slow growth of varroa mite population. We studied whether or not comb type influenced varroa reproduction in both Russian and Italian honey bees, and whether Russian bees produced comb which inhibited varroa reproduction. The major differences found in this study concerned honey bee type. Overall, the Russian honey bees had lower (2.44 ± 0.18%) levels of varroa infestation than Italian honey bees (7.20 ± 0.60%). This decreased infestation resulted in part from a reduced number of viable female offspring per foundress in the Russian (0.85 ± 0.04 female) compared to the Italian (1.23 ± 0.04 females) honey bee colonies. In addition, there was an effect by the comb built by the Russian honey bee colonies that reduced varroa reproduction. When comparing combs having Russian or Italian colony origins, Russian honey bee colonies had more non-reproducing foundress mites and fewer viable female offspring in Russian honey bee comb. This difference did not occur in Italian colonies. The age of comb in this study had mixed effects. Older comb produced similar responses for six of the seven varroa infestation parameters measured. In colonies of Italian honey bees, the older comb (2001 dark) had fewer (1.13 ± 0.07 females) viable female offspring per foundress than were found in the 2002 new (1.21 ± 0.06 females) and 1980s new (1.36 ± 0.08 females) combs. This difference did not occur with Russian honey bee colonies where the number of viable female offspring was low in all three types of combs. This study suggests that honey bee type largely influences growth of varroa mite population in a colony.

Keywords *Varroa destructor* · Reproduction · Dark comb · New comb · Resistance

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Introduction

Management of varroa mites generally requires the use of acaricides. However, injudicious use of acaricides may pose residue problems, especially in wax and honey (Lodesani et al. 1992; Bogdanov et al. 1998; Kochansky et al. 2001; Martel and Zeggane 2002). It may also lead to the development of mites resistant to acaricides. In the United States, resistance to amitraz, fluvalinate and coumaphos by varroa mites has been reported (Baxter et al. 1998; Elzen et al. 1999; Elzen and Westervelt 2002). Thus, the implementation of sound Integrated Pest Management (IPM) programs involving the use of honey bees resistant to mites and non-chemical mite control techniques may be the key to managing parasitic mite populations.

One possible contribution to a successful IPM program is the comb type provided to colonies for brood production. One attribute of comb, which varies, is cell size. Different honey bee sub-species build cells of different sizes. Larger cells built by European honey bees (EHB) supported greater varroa reproduction when compared to the smaller cells built by Africanized honey bees (Message and Goncalves 1995). However, a study comparing small and large cells both built by EHB showed no differences in varroa reproduction (J. Berry, personal communication). Combs of different ages also have different sizes. Older comb that has repeatedly been used for brood rearing has an accumulation of layers of cocoons, which reduces cell size (Chauvin 1962 as cited by Hepburn and Kurstjens 1988). In contrast to the report of Message and Goncalves (1995), higher varroa reproduction has been reported in old comb having smaller size than in new comb with AHB in Brazil (Piccirillo and de Jong 2004).

Combs also contain volatile chemicals. For example, the odor of comb increases bees' hoarding behavior (Rinderer and Baxter 1978). Semiochemicals occur in larval food (Nazzi et al. 2001) which probably help regulate larval feeding but also attract varroa mites. Other volatiles associated with cocoons (Donzé et al. 1998) and general nest odors probably accumulate in older combs. Perhaps bees that are resistant to varroa mites produce comb with volatiles that inhibit varroa reproduction.

Owing to the lipophylic nature of its wax, comb that remains in nests during acaricidal treatments for varroa is known to retain and accumulate traces of acaricides (Lodesani et al. 1992). These acaricide residues present in treated combs are known to affect varroa mite population growth (Kraus and Page 1995). Acaricides also are retained in wax used to produce commercial wax foundations (Lodesani et al. 2003; Jiménez et al. 2005; Leníček et al. 2006). High contamination levels (100 ppm fluvalinate, and 10 and 100 ppm coumaphos) caused dramatic varroa mite mortality during the first brood cycle (Fries et al. 1998). However, the accumulation of cocoons can prevent mite mortality even at high contamination levels (Fries et al. 1998).

The Russian honey bees program began in 1997 when queens from the Primorky region were imported into the United States (Rinderer et al. 2000). Series of experiments were then conducted and showed that Russian bees are resistant to varroa mites (Rinderer et al. 2000, 2001). Growth of varroa mites in Russian honey bee colonies is consistently slow which was supported by lower percentages of infested worker and drone brood cells, lower frequency of brood cells infested with multiple mites, lower mite reproduction and an extended phoretic period (de Guzman et al. 2007). Other mechanisms such as comb type or cell size may also be involved. To explore these possibilities, we studied whether or not older comb supported less varroa reproduction in both Russian and Italian honey bees, and whether Russian bees produced comb which inhibited varroa reproduction.

Materials and methods

Experiment 1—Reproduction of varroa in different types of combs built and used by the same honey bee type through four brood-rearing cycles

Thirty colonies (15 Russian and 15 Italian) were established on May 1, 2002 using the large package technique (Harbo and Hoopingarner 1997). Russian queens were obtained from the Russian honey bee breeding program while the Italian queens were purchased from a queen breeder in California who advertises Italian queens. All colonies used combs that were drawn and used by the honey bee type that produced them such that Italian colonies only used combs drawn by the Italian bees and Russian colonies exclusively used combs that were produced by Russian bees. When drawn combs were unavailable, frames with wax foundation were used. All colonies received one drone comb to accelerate varroa population growth. The initial mite infestation of packages was 81 ± 6 mites (mean ± SD) per 1.4-kg package.

This experiment began in July 2002 when test bees populated the hives. Each colony received three types of test combs: (a) dark comb (cell size = 5.38 mm) which started from wax foundation (purchased in 2001), built and then used extensively for brood rearing by the same honey bee types during the 2001 season, (b) new comb (cell size = 5.35 mm), drawn by the same honey bee type using wax foundation purchased in 2002, and (c) new comb (cell size = 5.35 mm), drawn by the same honey bee type from wax foundation purchased in the early 1980s before the discovery of tracheal (1984) and varroa (1987) mites in the U.S. All dark combs were in storage for about 6 months prior to use. None of the combs used in test colonies received any form of chemical treatment.

For each comb type, varroa mite reproduction was monitored during four brood cycles. Each brood cycle requires about 17 days from egg oviposition to tan-bodied pupae. We presumed that all cells examined supported four brood cycles since all test combs had about 85–90% sealed brood during each observation period. Evaluation was conducted from July to September 2002.

Experiment 2—Reproduction of varroa in combs built and used by other honey bee type

Colonies from Experiment 1 were also used in this study. Each colony received two test combs from the other honey bee type being tested: Russian honey bee colonies received combs used and built by Italian colonies and vice versa. The two test combs were introduced simultaneously in the middle of the brood nest for egg-laying. For comparison, reproduction of varroa mites in each host colony was also assessed using two combs that contained brood of similar age as the introduced test combs. Two trials were conducted.

Mite reproduction evaluation

For both experiments, brood cells were examined until 30–40 infested cells containing purple-eyed or tan-bodied pupae were obtained per comb type per sampling time or until 500 brood cells were examined. For Experiment 1, the total numbers of cells examined were as follows: (a) 2001 dark comb, Italian = 20,618 (971 infested; 906 singly-infested) and Russian = 23,142 cells (516 infested; 498 singly-infested); (b) 2002 new comb, Italian = 18,730 (977 infested; 898 singly-infested) and Russian = 20,353 cells (424

infested; 400 singly-infested); and (c) 1980s new comb, Italian = 20,901 (1,060 infested; 989 singly-infested) and Russian = 21,358 cells (560 infested; 538 singly-infested).

During examination, a variety of data related to reproduction were recorded. Non-reproduction was measured as described by Harbo and Harris (2001). Mites are considered non-reproductive when they enter the cells but: (a) produce no progeny, (b) produce males only, (c) produce progeny too late to mature, or (d) when the foundress dies before she can reproduce. Proportions of non-reproductive females, numbers of progeny, numbers of viable female offspring and dead mites were estimated using brood cells infested with only a single foundress mite. For the total number of mites per infested cell, both singly- and multiply-infested cells were analyzed. Colonies with less than eight singly-infested cells were excluded from the analyses of the proportion of non-reproductive mites.

Data analyses

Data from Experiment 1 were subjected to ANOVA for repeated measures using a compound symmetry covariance structure using the MIXED procedure. Honey bee type, comb type and brood cycle were included in the model with brood cycle being the repeated effect. No parameter had a significant three-way interaction for comb type, honey bee type and brood cycle. Mean separations were conducted by Tukey's test for multiple mean comparisons while *t*-test was used to compare means of the two honey bee types (SAS version 8.2, SAS Institute Inc 2001).

For Experiment 2, data were analyzed using a one-way ANOVA comparing the four honey bee type-by-comb combinations. Before analyses, data on the proportion of brood infested and non-reproductive mites were transformed using the arcsine transformation. Mean separations were conducted by Tukey's test (SAS version 8.2, SAS Institute Inc 2001).

Results

Experiment 1

For the percentage of brood infested (PI), ANOVA revealed no significant differences among comb type ($F = 1.23$; df = 2, 236; $P = 0.296$). However, a significant interaction between honey bee type and brood cycle was detected ($F = 13.29$; df = 3, 235; $P < 0.0001$) (Fig. 1). PI varied among the four brood cycles within the Italian ($F = 47.77$; $P < 0.0001$) and Russian ($F = 4.14$; $P = 0.007$) honey bee colonies. For both honey bee types, the highest infestations were observed in the 3rd and 4th cycles with the lowest infestation recorded in the first cycle. Within brood cycles, both honey bee types had similarly low levels of infestation during the 1st cycle. Thereafter, PI significantly increased with the Italian honey bees having higher infestations than the Russian honey bees. On average, the Italian honey bees had higher PI than the Russian honey bees ($F = 13.47$; df = 1, 28.4; $P < 0.0001$).

Analysis of the proportion of multiply-infested cells (MI) showed no significant effect of comb type ($F = 1.8$; df = 2, 235; $P = 0.168$). However, a significant interaction between honey bee type and brood cycle ($F = 2.91$; df = 3, 234; $P = 0.035$) was detected (Fig. 2). For the Italian honey bee colonies, the highest MI was observed during the 3rd

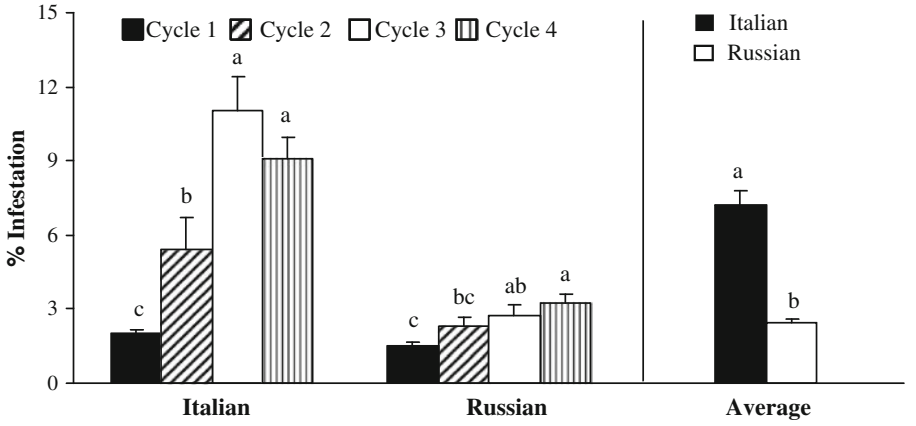

Fig. 1 Average percentage (mean ± SE) of worker brood infested in colonies of Italian and Russian honey bees through four brood cycles and overall average for each honey bee type. For each honey bee type, means followed by different letters are significantly different at $P < 0.05$ according to ANOVA for repeated measures followed by Tukey's test. Means for the two honey bee types were compared using a *t*-test. In total, 60,249 Italian and 64,853 Russian worker brood cells were examined

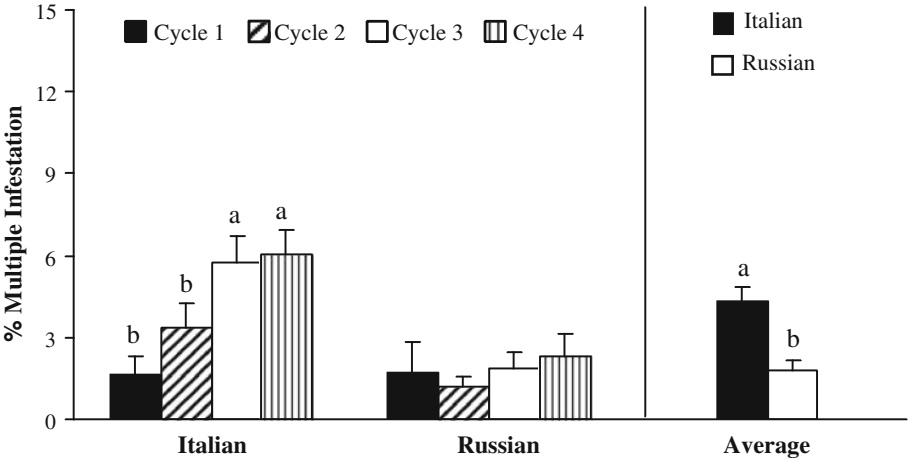

Fig. 2 Average percentage (mean ± SE) of multiply-infested worker brood in colonies of Italian and Russian honey bees through four brood cycles and overall average for each honey bee type. For each honey bee type, means followed by different letters are significantly different at $P < 0.05$ according to ANOVA for repeated measures followed by Tukey's test. Means for the two honey bee types were compared using a *t*-test

and 4th cycles while the 1st and 2nd cycles showed the lowest MI. In the Russian honey bees, MI remained consistently low throughout the four brood cycles. MI was comparable between the two honey bee types during the 1st and 2nd cycles. The Italian honey bees had more MI than the Russian honey bees in both the 3rd and 4th brood cycles. Overall, Italian honey bees had higher MI than Russian honey bees ($F = 9.07$; df = 1, 24.6; $P = 0.006$).

For the proportion of non-reproductive (NR) mites, no two-way interactions and no comb type ($F = 0.38$; df = 2,175; $P = 0.684$) or brood cycle ($F = 2.41$; df = 3,172; $P = 0.069$) effects were detected. NR between the two honey bee types diverged but not

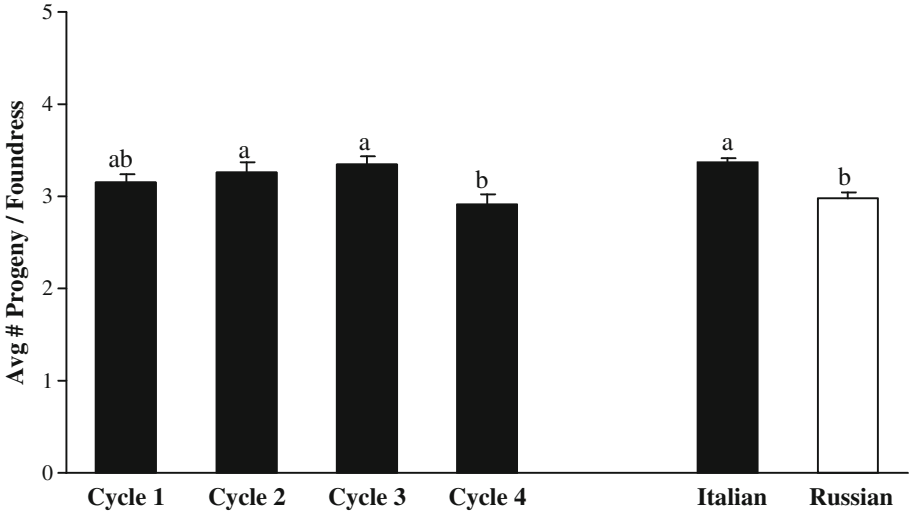

Fig. 3 Average number (mean ± SE) of progeny per foundress through different brood cycles and in Italian and Russian honey bee colonies. Means for the four brood cycles followed by different letters are significantly different at $P < 0.05$ according to ANOVA for repeated measures followed by Tukey's test. Means for the two honey bee types were compared using a *t*-test

significantly ($F = 2.67$; df = 2,21.7; $P = 0.117$) with the Italian and Russian honey bees having means of 36.26 ± 1.30% and 42.71 ± 2.03%, respectively.

No two-way interactions or no comb type ($F = 0.82$; df = 2, 238; $P = 0.44$) effect were detected for the average number of living progeny per female. However, significant honey bee type ($F = 16.98$; df = 1, 23.8; $P = 0.0004$) and brood cycle ($F = 4.07$; df = 3, 236; $P = 0.008$) effects were observed (Fig. 3). Overall, mites infesting Italian honey bees produced significantly more progeny than the mites infesting Russian honey bees. More mite progeny were recorded during the first three brood cycles than in the 4th cycle, which was similar to that of cycle 1.

The number of viable female offspring per foundress also showed a significant interaction between honey bee type and comb type ($F = 4.35$; df = 2, 240; $P = 0.014$) (Fig. 4). Within comb type, both honey bee types had a similar number of viable female offspring in the dark combs ($F = 2.29$; $P = 0.133$). In the 2002 new ($F = 20.73$; $P < 0.0001$) and 1980s new ($F = 28.96$; $P < 0.0001$) combs, the Italian honey bees supported more viable female offspring than did the Russian honey bees. Within honey bee type, the number of viable female offspring differed significantly among the comb types ($F = 3.61$; $P = 0.029$). In the Italian honey bees, 1980s new combs had the highest number of viable female offspring per foundress and the dark combs had the lowest. In the Russian honey bee colonies, dark combs had more viable female offspring than the two new (2002 and 1980s) combs. However, the difference was not strong ($F = 2.59$; $P = 0.077$). Overall, Italian honey bees had more ($F = 44.32$; df = 1, 23.9; $P < 0.0001$) viable female offspring per foundress than the Russian honey bees. Brood cycle had no effect on the number of viable female offspring per foundress ($F = 2.05$; df = 3, 236; $P = 0.108$).

When numbers of mites per infested cell were analyzed, no significant comb effect ($F = 0.55$; df = 2, 240; $P = 0.576$) or two-way interactions were detected. However, a

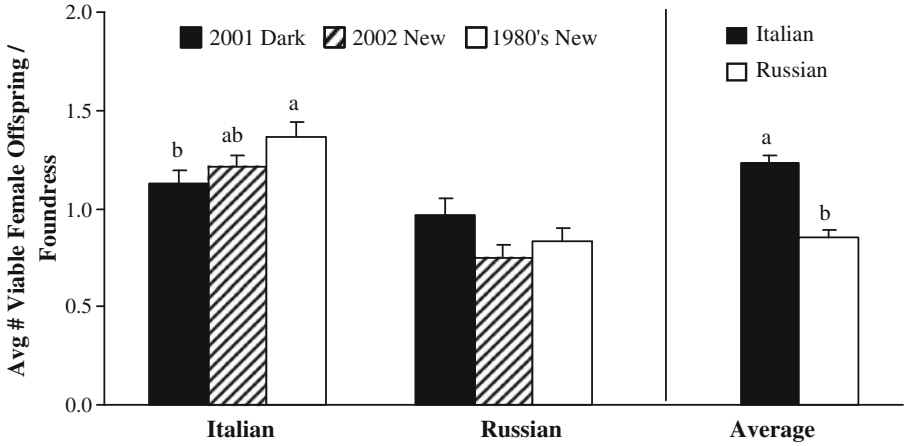

Fig. 4 Average number (mean ± SE) of viable female offspring per foundress in different comb types of Italian and Russian honey bees. For each honey bee type, means followed by different letters are significantly different at $P < 0.05$ according to ANOVA for repeated measures followed by Tukey's test. Means for the two honey bee types were compared using a *t*-test

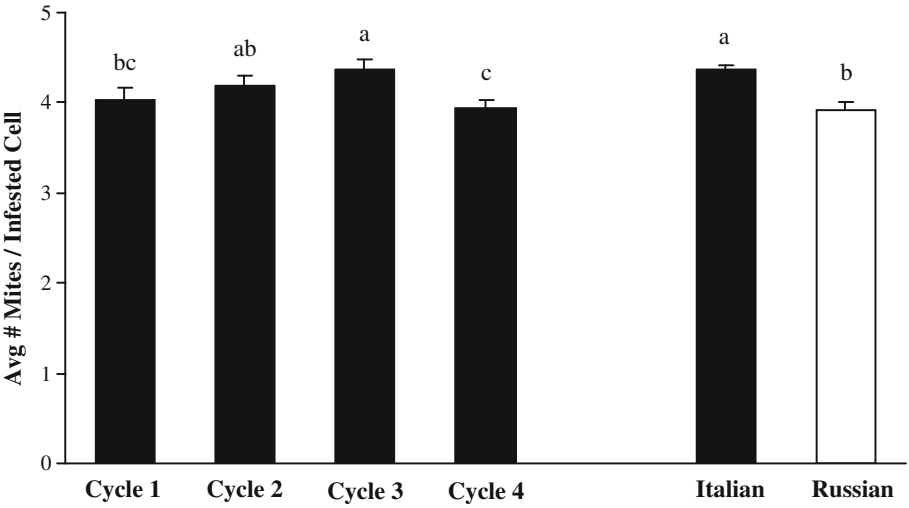

Fig. 5 Average number (mean ± SE) of mites per infested cell through different brood cycles and in Italian and Russian honey bee colonies. Means for the four brood cycles followed by different letters are significantly different at $P < 0.05$ according to ANOVA for repeated measures followed by Tukey's test. Means for the two honey bee types were compared using a *t*-test

significant influence of brood cycle ($F = 3.16$; df = 3, 238; $P = 0.025$) on the number of mites per infested cell was detected (Fig. 5). The highest numbers of mites were recorded during the 2nd and 3rd cycles with the lowest number recorded in the 4th cycle. Honey bee type effect was also detected with the Italian honey bees having higher numbers of mites per infested cell than the Russian honey bees ($F = 14.88$; df = 1, 23.5; $P = 0.0008$).

For the average number of dead mites, no honey bee type ($F = 0.19$; df = 1, 25; $P = 0.665$), comb type ($F = 0.16$; df = 2, 238; $P = 0.854$) or two-way interaction effects were

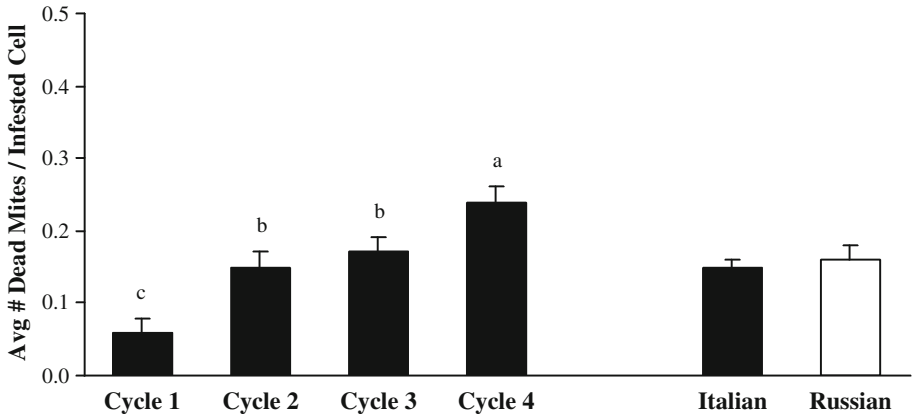

Fig. 6 Average number (mean ± SE) of dead mites per infested cell (only singly-infested) through different brood cycles and overall average for each honey bee type. Means for the four brood cycles followed by different letters are significantly different at $P < 0.05$ according to ANOVA for repeated measures followed by Tukey's test. Means for the two honey bee types were compared using a *t*-test

observed. The number of dead mites was influenced by brood cycle ($F = 10.73$; df = 3,237; $P < 0.0001$) (Fig. 6).The highest mite mortality was recorded during the 4th cycle, followed by 2nd and 3rd cycles with the lowest mortality observed during the 1st cycle.

Experiment 2

Analyses showed significant differences among the four honey bee type-by-comb combination groups for PI, NR, and numbers of progeny and viable offspring per foundress (Table 1). After examining >50,000 brood cells, we found that Italian honey bee colonies had higher PI than the Russian honey bees regardless of comb origins ($P < 0.0001$). For NR, the Russian combs in the Russian colonies supported the highest NR ($P = 0.008$) with the other three groups having similarly lower NR. Varroa mites reproduced more in the Italian colonies (whether in foreign or local combs) and in the Italian combs hosted by Russian honey bee colonies as indicated by having more progeny ($P = 0.0001$) and viable female offspring ($P = 0.0009$) per foundress. The combs built by Russian bee colonies in the Russian honey bee colonies supported the lowest reproduction of varroa. No differences among the four groups for the number of dead mites per infested cell ($P = 0.304$) were detected.

Discussion

The major differences found in this study concerned honey bee type and generally confirmed that Russian honey bees are more resistant to varroa mites than Italian honey bees (Rinderer et al. 2001; de Guzman et al. 2007). The Russian colonies had a lower percentage of brood cells that were infested. This probably led to also having a lower percentage of multiply-infested brood cells. One factor that contributed to Russian colonies having fewer mites was the comparatively reduced number of viable female offspring per foundress. This comparison was based only on brood cells having a single foundress. Since non-reproducing foundress mites were included in the estimation of the number of viable

Table 1 Varroa infestation parameters (mean ± SE) in combs having Russian or Italian colony origins

Comb type	Total number of cells examined (# cells infested)	% Infestation	% Non-reproduction (# infested cells with one foundress)	# Progeny per foundress	# Viable female offspring/foundress	# Dead mites
Russian combs in Italian colonies	13,267 (1,275)	13.32 ± 124^{a}	37.60 ± 1.72^{b} (1,136)	3.26 ± 0.08^{a}	1.31 ± 0.06^{a}	0.30 ± 0.04
Italian combs in Italian colonies	11,096 (973)	13.44 ± 2.15^{a}	39.84 ± 2.57^{b} (882)	3.00 ± 0.13^{ab}	1.21 ± 0.07^{a}	0.29 ± 0.03
Italian combs in Russian colonies	13,272 (726)	5.75 ± 0.90^{b}	39.70 ± 2.97^{b} (696)	2.85 ± 0.16^{b}	1.13 ± 0.09^{a}	0.26 ± 0.04
Russian combs in Russian colonies	12,564 (509)	5.33 ± 1.18^{b}	51.06 ± 3.44^{a} (484)	2.31 ± 0.18^{c}	0.82 ± 0.09^{b}	0.38 ± 0.05
		$P < 0.0001$*	$P = 0.008$*	$P < 0.0001$*	$P = 0.0009$*	$P = 0.304^{ns}$

* For each column, means followed by different letters are significantly ($P < 0.05$) different using one-way ANOVA followed by Tukey's test

ns Not significant

female offspring, non-reproduction is a chief contributor to the difference between the two honey bee types for the number of viable female offspring per foundress.

Regardless of the mechanism, the comb built by Russian honey bees contributes to the increase in the rate of non-reproduction and decrease in the average number of viable female offspring. In the comparison of combs having Russian or Italian colony origins in Experiment 2, Russian honey bee colonies had fewer numbers of viable female offspring in Russian honey bee comb. This difference did not occur in Italian colonies. Hence, there may be an interaction between characteristics of the comb and brood rearing. Perhaps the effect results from the combined action of inhibitory compounds emanating from both the comb and the brood. It has been reported that chemicals that attract or influence varroa mite reproduction may come from larval food (Nazzi et al. 2001). Therefore, it is possible that semiochemicals from the larval food can be found in the cocoon. Cocoons have also been documented to contain semiochemicals, which is used for the deposition of varroa feces used for aggregation, egg laying and molting by nymphs (Donzé and Guerin 1994). Russian comb in Russian honey bee colonies also was associated with an increase in the percentage of non-reproductive mites. Measures of non-reproduction are known to be increased by varroa sensitive hygienic behavior (Harbo and Harris 2005). The Russian comb in Russian honey bee colonies may have: (1) stimulated an increase in the rate of hygienic behavior, (2) through some interactive effect involving brood directly increased the rate of non-reproduction in infesting mites, or (3) stimulated both hygienic behavior and non-reproduction.

Differences in the age of comb (=cell size) in this study had mixed effects. Older comb with cocoons produced similar responses for six of the seven varroa infestation parameters. In colonies of Italian honey bees, the older comb had fewer viable female offspring per foundress than were found in both new (2002 and 1980s) combs .This difference did not occur with Russian honey bee colonies where the number of viable female offspring was low in all three types of combs. In AHB, higher varroa infestations were recorded in old brood combs that have reduced cell size due to accumulation of cocoons than in new brood combs (Piccirillo and de Jong 2004). However, EHB combs (larger cells) are reported to support higher varroa reproduction than AHB combs which have smaller cells (Message and Goncalves 1995). These discrepancies suggest that honey bee genotype largely influences varroa mites' ability to reproduce in the brood cells. Perhaps, AHB produces comb that negatively affects varroa reproduction through means not related to cell size.

Acaricide residues have been detected in commercial wax foundations (Lodesani et al. 2003; Jiménez et al. 2005; Leníček et al. 2006) and can have negative effects on varroa mites. Fries et al. (1998) documented that comb drawn from foundations treated with 100 ppm fluvalinate, and 10 and 100 ppm coumaphos caused dramatic varroa mortality during the first brood cycle. In this study, no residue analysis was conducted. However, the low number of dead mites we observed during the first brood cycle may suggest either the absence of residues in the foundations or their presence at levels that did not cause high mortality of varroa mites. Fries et al. (1998) observed low varroa mortality in foundations treated with 1 and 10 ppm fluvalinate, and 1 ppm coumaphos. The authors also observed that the presence of two layers of cocoons prevented deaths of foundress mites even at the highest contamination level of 100 ppm. Thus, the increase in the percentage of brood cells infested, percentage of multiple infestations and number of progeny per foundress throughout the four brood cycles maybe accelerated by the accumulation of $\geq$4 layers of cocoons inside the brood cells.

The only comb difference was older comb having fewer viable female offspring per foundress in Italian honey bee colonies. This difference did not occur with Russian honey

bee colonies where the number of viable female offspring was low in all three types of combs. Our observations confirm earlier reports that honey bee type largely influence growth of varroa mite population in a colony.

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