CHAPTER 7

A COMPARISON FOR RESISTANCE OF ARS PRIMORSKY HONEY BEE (*Apis mellifera*) AND DOMESTIC EUROPEAN HONEY BEE (*Apis mellifera*) AGAINST *Tropilaelaps clareae*

Abstract

Ten ARS Primorsky honey bee (*Apis mellifera*) and 10 domestic (Thai) colonies (*A. mellifera*), Italian honey bee hybrids, were used to compare for resistance against *Tropilaelaps clareae*. The experiment was conducted at an apiary in Chiang Mai, Thailand, during November 2001- February 2003. Each colony was started with 100 *Tropilaelaps* mites. Resistance to *T. clareae* of the Primorsky and domestic bees was evaluated on the basis of proportion of infested cells, infestation rates on adult bees, mite numbers in colonies, colony longevity, percentages of injured mites in hive debris, and the numbers of mites and progeny per infested cell. The average infestation rate of *T. clareae* on Primorsky brood (18.5±2.6%) was significantly higher (P=0.001) than that of the domestic brood (11.4±1.5%) (mean ± standard error). The mite infestation rate on the Primorsky (0.8±0.2%) and domestic (0.5±0.1%) adult bees did not significantly differ (P=0.160). The average numbers of mites through time in the Primorsky (871.5±179.5) and domestic (954.9±184.6) colonies were not significantly different (P=0.753). The number of progeny produced per infested cell of the Primorsky (1.3±0.1) and domestic (1±0.1) bees was not significantly different (P=0.092). The mite number per infested cell of the Primorsky (2.9±0.2) and domestic (2.4±0.2) bees was not significantly different (P=0.115). The longevity of the Primorsky and domestic colonies did not significantly differ (P=0.103) with means of 4.6±0.5 and 6.2±0.8 months, respectively. The injured mite percentage of the domestic and Primorsky colonies was 70.3 and 72.7%, respectively.

Key words: *Tropilaelaps clareae* / resistance / *Apis mellifera* / Primorsky / Thailand

Introduction

Tropilaelaps clareae Delfinado & Baker (Acari: Laelapidae) is the most economically important mite pest in beehives; causing serious damage to *Apis mellifera* beekeeping industry in tropical Asia (Delfinado-Baker and Peng, 1995). This mite feeds on haemolymph of bees; resulting in reduction of bee body weight and longevity (Kitprasert, 1984; Delfinado-Baker et al., 1992). *T. clareae* is an indigenous parasite of *A. dorsata* Fabr., a native honey bee species of South-East Asia (Burgett and Krantz,1984; Wongsiri et al., 1989). The regional distribution of *T. clareae* seems to be limited in tropical Asia and coincides with the indigenous areas of *A. dorsata* (Burgett et al., 1983; Burgett and Akratanakul, 1985; Delfinado-Baker and Aggarwal, 1987; Delfinado-Baker et al., 1989). However, the infestation of *T. clareae* in *A. mellifera* colonies outside the range of *A. dorsata* was reported in Afghanistan (Woyke, 1984) and South Korea (Woo and Lee, 2001). When *A. mellifera* was introduced in tropical Asia, *T. clareae* successfully switched from indigenous *A. dorsata* host to be *A. mellifera* (Rath and Delfinado-Baker, 1990) and has become a serious problem to *A. mellifera* colonies in the region (Wongsiri et al., 1989). Several studies have been conducted to find the effective control against *T. clareae* in *A. mellifera* colonies. Chemical, physical, biotechnical and combinations of chemical and biotechnical methods provide some relief to colonies but nothing offer complete control (Wongsiri and Tangkanasing, 1987; Tangkanasing et al., 1988). In addition, these methods are either labor intensive, costly, reduce bee populations or contaminate bee products. The use of resistant *A. mellifera* stocks to *T. clareae* has been thought to be a better solution to the *Tropilaelaps* problem in Asia. The benefits of using resistant honey bees to parasitic mites include: less chance of contaminating bee products with undesirable chemicals, low cost of labor and materials and less risk of the mite developing resistance to acaricides. However, no studies have been done to find resistant *A. mellifera* stocks to *T. clareae*. The ARS Primorsky honey bee (*A. mellifera*) is known to be resistant to *Varroa jacobsoni*, *V. destructor* and *Acarapis woodi* (Rinderer et al., 1997, 1999; de Guzman et al., 1996). However, resistance of the ARS Primorsky honey bee to *T. clareae* has yet to be

established. Therefore, this study was conducted to compare for resistance of ARS Primorsky and domestic (Thai) honey bees against *T. clareae*.

Materials and Methods

Ten ARS Primorsky (*A. mellifera*) and 10 domestic (an *A. mellifera* stock commercially available in Thailand) colonies were used to compare for resistance against *T. clareae*. The ARS Primorsky queen bees were received from the USDA, Honey Bee Breeding, Genetics and Physiology Laboratory in Baton Rouge, Louisiana, USA while the domestic (Thai) queen bees were provided by Supa's apiary in Chiang Mai, Thailand. This experiment was conducted at an apiary in Chiang Mai, Thailand, during November 2001- February 2003.

Establishment of the test colonies

Twenty test colonies were established in Langstroth hives in November 2001 by splitting only adult bees (workers and drones) from 10 colonies of domestic bees (8 combs of adult bees per colony) to become 20 colonies that had 4 combs of adult bees per colony. The ARS Primorsky and domestic queen bees were installed into the split colonies. Each test colony was contained a queen bee, about 9,000 adult bees, 2 frames with honey and pollen and 2 combs with foundation sheets. Since *T. clareae* cannot survive for three days on adult bees (Rinderer et al., 1994), all *Tropilaelaps* mites in the test colonies are likely to die in this broodless period because of no food (bee brood). No chemicals were applied in the test colonies throughout the experiment.

Mite inoculation

After the introduction of the test queens about 3 months (February 2002), all test colonies were estimated for the numbers of mites and sealed brood by the method as described by Rinderer et al. (1999). About 200 worker brood and 100 drone brood cells (depending on the availability) from each colony were uncapped and scored for the

presence and numbers of *T. clareae*. Sampling was done on both sides of two different combs (50 cells per side) for worker brood and both sides of a drone comb (50 cells per side) for drone brood. To estimate the number of mites in the colony's worker brood, the number of mites per worker cell was multiplied by the numbers of sealed worker cells. Similarly, the number of mites in the colony's drone brood was estimated. A sample of about 400 adult bees (workers and drones) was taken from the brood combs of each colony and washed in 70% ethyl alcohol to remove mites. Both *T. clareae* and bee numbers in the sample were counted. The number of mites per bee was multiplied by the estimated number of bees in the colony to estimate the number of mites present on adult bees of the colony. The number of adult bees in the colony was determined as described by Burgett and Burikam (1985). The total number of mites in each colony was estimated from the sum of the estimates of mites in worker brood, drone brood and on adult bees in the colony. After the mite number estimation in each colony, *Tropilaelaps* mites were added into the test colonies to become 100 mites per colony by the use of an infested sealed brood section technique.

Infested brood comb sections with *T. clareae* were obtained from other highly infested domestic colonies. About 200 sealed worker cells on the two sides (100 cells per side) of each brood comb were uncapped and scored for the presence and numbers of *T. clareae*. The number of mites per worker cell of each brood comb was used to calculate for the number of mites in brood comb sections. The brood comb section was inserted into a frame of sealed brood in the test colony to add the total number of *T. clareae* to become 100 mites per test colony.

Evaluation for resistance to *T. clareae*

Resistance to *T. clareae* of ARS Primorsky and domestic bees was evaluated on the basis of: (1) proportion of brood infestation, (2) infestation rates on adult bees, (3) mite numbers in colonies, (4) mite numbers (including all life stages of *T. clareae*) per infested cell, (5) progeny numbers per infested cell, and (6) percentages of injured mites in hive debris.

The proportion of brood infested with *T. clareae* in each colony was determined by examining 200 worker brood and 100 drone brood cells. Sampling was done every month on both sides of two combs (50 cells per side) for worker brood and both sides of a drone comb (50 cells per side) for drone brood. Since counting of mites becomes difficult because of the quickness of *Tropilaelaps* to move around the cells, sections (6 x 10 cm.) of brood combs were cut and immediately frozen until examination. Infestation levels of *Tropilaelaps* on adult bees were determined by sampling about 400 adult bees (workers and drones) per colony every month. Adult bees were collected from brood frames and washed in 70% ethyl alcohol to remove mites. The number of mites and bees were counted to determine the levels of infestation on adult bees. The number of dead *T. clareae* in each colony was monitored every month using mite collecting boards with wire screens to prevent bees from carrying away debris. The boards were coated with a thin film of vegetable oil and maintained in the hives for periods of 1 month until the colonies died or to the end of the experiment (12 months). Dead mites on the boards were retrieved using a fine paint brush. They were examined and classified by type of injuries (using a stereomicroscope at 40X magnification). Injuries to the mites were classified as: (1) injured legs (missing legs or parts of legs), (2) injured body only, or (3) injured legs + injured body.

The numbers of *Tropilaelaps* mites and brood in the test colonies were determined as described by Rinderer et al. (1999). In brief, the area percentage of sealed worker cells on both sides of a brood frame was estimated and converted to numbers of sealed cells by the aid of cell numbers. The total number of sealed worker cells in a colony was obtained from the sum of the sealed worker cells of each frame in the colony. Where drone cells are scattered, they were counted individually. The number of mites in the test colonies was estimated using the same procedure as previously described in the section of the mite inoculation. The number of adult bees in the test colonies was estimated by the method as described by Burgett and Burikam (1985). Longevity of the test colonies was also recorded. Colonies were excluded from the experiment if either supersedure or a queenless condition was observed. All

colonies were fed with sugar syrup and pollen when nectar and pollen from natural sources were scarce.

Statistical analyses

Data in each month of brood infestation rates, infestation rates on adult bees, dead mite numbers on the bottom boards, injured mite numbers, brood numbers and adult bee numbers were analyzed using *t*-tests (SPSS statistic program). The data in each month of each bee stock were pooled together for final analysis. Data on colony longevity and the numbers of mites and progeny per infested cell were also analyzed by the use of *t*-tests (SPSS statistic program). The bee colonies were considered as the replications. Pearson correlations were used to analyze the relationships between: (1) brood infestation rates and brood numbers, (2) brood and adult bee infestation rates, (3) mite numbers in the colonies and brood infestation rates, (4) mite numbers in the colonies and brood numbers, (5) mite numbers in the colonies and adult bee numbers, (6) adult bee numbers and adult bee infestation rates, (7) dead mite numbers in hive debris and mite numbers in the colonies, (8) dead mite numbers in hive debris and brood infestation rates, and (9) longevity of the colonies and brood infestation levels during the last month prior to death of the colonies.

Results

T. clareae infestation on Primorsky and domestic brood

On average the infested brood rate by *T. clareae* through time of the Primorsky bees was significantly higher (t=3.344, df=106, *P*=0.001) than that of the domestic bees with means of 18.5±2.6 and 11.4±1.5% (mean ± standard error), respectively (Table 7.1). Within each month, the mite infestation rate on brood of the two bee stocks did not significantly differ. In March, the average infestation rate on the Primorsky and domestic brood was 3.8±0.8 and 2.6±0.7%, respectively. The infested brood percentage of the two stocks increased rapidly in April and May. The maximum infestation rate on both

Primorsky and domestic brood was found in May with means of 32.6±6.1 and 21±6.3%, respectively. The mite infestation rate on the Primorsky (24±6%) and domestic (10.2±3.5%) brood decreased dramatically in June. In July, the infested brood percentage of the domestic bees (11.4±3.4%) increased slightly while the infested brood rate of the Primorsky bees (21.2±9.8%) decreased slightly. Because there was only 1 survival Primorsky colony in August, thus means of the infested brood rate and resistant parameters could not be statistically compared (Table 7.1). The last colony of the Primorsky stock died in December 2002 while one domestic colony survived from *T. clareae* infestation at the end of the experiment (February 2003).

Infestation levels of *T. clareae* on adult bees

The average infestation rate of *T. clareae* on the Primorsky and domestic adult bees through time did not significantly differ (t=1.424, df=56.828, *P*=0.160) with means of 0.8±0.2 and 0.5±0.1% (mean ± standard error), respectively (Table 7.1). Within each month, the mite infestation rate on adult bees of the two stocks was not significantly different. In March, the rate of the mite infestation on the Primorsky and domestic adult bees was the same level (0.1%). The mite infestation level on the Primorsky and domestic adult bees increased continuously in April to May with means of 0.4±0.2 and 0.8±0.2% in April, 1.2±0.6 and 1±0.5% in May, respectively. In June, the mite infestation rate on the domestic adult bees (0.3±0.1%) decreased while the infestation rate on the Primorsky adult bees (1.4±0.9%) increased. The mite infestation rate on adult bees of the two stocks decreased and was the same level (0.2%) in July. In August, the mite infestation percentage on the domestic adult bees decreased again (0.1%) while no mites were found on the Primorsky adult bees sampled from one survival colony.

T. clareae populations in Primorsky and domestic colonies

Populations of *T. clareae* in each test colony were estimated from mites in sealed brood cells and on adult bees of the colony in each month. The average number of *T. clareae* adults present in the Primorsky and domestic colonies through time was not

significantly different (t=0.315, df=106, *P*=0.753) with means of 871.5±179.5 and 954.9±184.6 mites per colony (mean ± standard error), respectively (Table 7.1). The mite numbers in the Primorsky and domestic colonies increased rapidly from February (100 mites per colony) to a maximum number in May with means of 1,673.5±368.7 and 2,390.6±854.6 mites per colony, respectively. The mite populations in the Primorsky and domestic colonies decreased continuously in June to July with means of 251.5±86.6 and 388.3±142.7 mites per colony in July, respectively. In August, the mite population in the domestic colonies increased slightly (mean=452±124.6 mites per colony) while the mite number in one survival Primorsky colony was 181 mites. The amount of *Tropilaelaps* mites present in the test colonies had positive correlations with brood infestation rates (r =0.602, *P*=0.000), brood sizes (r =0.234, *P*=0.015) and the amount of adult bees (r =0.229, *P*=0.017).

Brood sizes and adult bee populations

On average brood production rate through time in the domestic colonies was significantly higher (t=3.344, df=106, *P*=0.001) than that of the Primorsky colonies with means of 5,996.4±557.2 and 3,591.3±363.8 sealed brood cells per colony (mean ± standard error), respectively (Table 7.1). The significantly larger brood size in the domestic colonies than that of the Primorsky colonies was observed in March and May. Brood sizes of the domestic and Primorsky colonies increased to a maximum in April with means of 8,108.9±1,504.6 and 5,496.7±798.2 sealed brood cells per colony, respectively. The brood sizes of the two stocks decreased continuously in May to July. In August, the domestic brood size was the similar size in July (mean=3,098.8±1,125 sealed brood cells per colony) while the brood number in one survival Primorsky colony was 3,239 brood. A negative correlation between *Tropilaelaps* infestation in brood cells and the amount of brood present inside the test colonies was detected (r =-0.314, *P*=0.001).

The average number of adult bees through time inside the domestic hives was significantly higher (t=3.604, df=106, *P*=0.000) than that of the Primorsky hives with

means of 5,207.8±492.1 and 2,917.7±322.1 bees per colony (mean ± standard error), respectively (Table 7.1). The significantly higher number of adult bees in the domestic colonies than that of the Primorsky colonies was found in March and May. In April, the adult bee population in the Primorsky colonies increased to a maximum (mean =4,062.2±780.6 bees per colony while the domestic bee population almost unchanged (mean = 5,870.4±996.3 bees per colony). The number of the domestic adult bees increased to a maximum in May (mean =7,148.4±1,346.6 bees per colony) while the Primorsky bee population decreased continuously in May to July, and only one Primorsky colony remained alive in August. The continuous decline in the domestic bee populations was observed in June to October when two domestic colonies remained alive in October. The adult bee populations in the domestic (r =0.031, *P*=0.810) and Primorsky (r =-0.229, *P*=0.126) colonies were not significantly correlated with *Tropilaelaps* parasitism levels on the adult bees.

Table 7.1 Numbers of *T. clareae*, brood and adult bees through time in ARS Primorsky and domestic colonies and infestation rates of *T. clareae* on brood and adult bees of ARS Primorsky and domestic honey bees.

Months	Bee types	No. of survival colonies	Means ± standard error				
			Infestation rates on brood (%)	Infestation rates on adult bees (%)	No. of adult mites / colony	No. of sealed brood / colony	No. of adult bees / colony
Mar.02	Domestic	10	2.6±0.7^{a}	0.1±0.05^{a}	272.9±87.7^{a}	7,964.8±795.4^{a**}	5,917.1±733.9^{a**}
	Primorsky	10	3.8±0.8^{a}	0.1±0.06^{a}	255±54.2^{a}	5,154.5±526.5^{b}	3,769.1±671.9^{b}
Apr.02	Domestic	10	8.5±1.6^{a}	0.8±0.2^{a}	1,367.8±435.9^{a}	8,108.9±1,504.6^{a}	5,870.4±996.3^{a}
	Primorsky	10	9.8±3.0^{a}	0.4±0.2^{a}	826.5±249.5^{a}	5,496.7±798.2^{a}	4,062.2±780.6^{a}
May 02	Domestic	10	21±6.3^{a}	1±0.5^{a}	2,390.6±845.6^{a}	7,917.5±1,716.3^{a**}	7,148.4±1,346.6^{a**}
	Primorsky	10	32.6±6.1^{a}	1.2±0.6^{a}	1,673.5±368.7^{a}	3,070.1±601.4^{b}	2,790.6±499.7^{b}
Jun.02	Domestic	10	10.2±3.5^{a}	0.3±0.1^{a}	480.4±184.3^{a}	5,132.9±1,508.4^{a}	5,500.1±1,656.2^{a}
	Primorsky	8	24±6.0^{a}	1.4±0.9^{a}	1,080±703.7^{a}	2,472.5±1,089.7^{a}	2,264.9±690.1^{a}
Jul.02	Domestic	8	11.4±3.4^{a}	0.2±0.1^{a}	388.3±142.7^{a}	3,078.5±1,210^{a}	4,502.9±1,902.6^{a}
	Primorsky	4	21.2±9.8^{a}	0.2±0.2^{a}	251.5±86.6^{a}	1,201.5±652.4^{a}	891.5±284.7^{a}
*Aug.02	Domestic	5	13.2±3.4	0.1±0.06	452±124.6	3,098.8±1,125	3,460.4±974.3
	Primorsky	1	4.8	0	181	3,239	1,368
*Sep.02	Domestic	3	13.6±4.1	0.7±0.1	1,305.3±939.4	3,659±1,754	2,766.7±1,118
	Primorsky	1	5.4	1.7	179	2,216	1,228
*Oct.02	Domestic	2	24.7±7.7	0.7±0.05	971±923	2,732.5±2,551.5	1,695±1,403
	Primorsky	1	39.1	1.2	466	920	1,695
*Nov.02	Domestic	1	2.5	0.6	179	6,307	4,325
	Primorsky	1	40.3	7.3	169	200	150
*Dec.02	Domestic	1	4.4	0	201	4,602	3,215
	Primorsky	0	-	-	-	-	-
*Jan.03	Domestic	1	11.8	0	636	4,432	2,104
	Primorsky	0	-	-	-	-	-
*Feb.03	Domestic	1	7.3	0.2	103	1,193	1,660
	Primorsky	0	-	-	-	-	-
Average	**Domestic**	**N = 62**	**11.4±1.5^{a**}**	**0.5±0.1^{a}**	**954.9±184.6^{a}**	**5,996.4±557.2^{a**}**	**5,207.8±492.1^{a**}**
	Primorsky	**N = 46**	**18.5±2.6^{b}**	**0.8±0.2^{a}**	**871.5±179.5^{a}**	**3,591.3±363.8^{b}**	**2,917.7±322.1^{b}**

Similar letters in the same month and column are not significantly different at the 0.05 level.

*Means were not statistically compared.

**Means were significantly different at the 0.01 level.

Mite loads and reproductive rates of *T. clareae*

The number of progeny produced per infested cell (or reproductive rate) and the number of mites per infested cell (or mite load) were determined from all *Tropilaelaps* mites in 1,022 and 1,187 infested cells from 10 domestic and 10 Primorsky colonies, respectively (Table 7.2). Both of the mite reproductive rate (t=1.778, df=18, *P*=0.092) and mite load (t=1.657, df=18, *P*=0.115) on the Primorsky and domestic brood were not significantly different. The mean of progeny numbers produced per infested cell of the Primorsky and domestic bees was 1.3±0.1 and 1±0.1 (mean ± standard error), respectively. The mite load mean (including all developmental stages of the mites) on the Primorsky and domestic brood was 2.9±0.2 and 2.4±0.2 mites, respectively.

Colony longevity

The colony longevity did not significantly differ (t=1.719, df=18, *P*=0.103) between the Primorsky and domestic colonies (Table 7.2). On average the Primorsky and domestic colonies could survive from *T. clareae* infestation for 4.6±0.5 and 6.2±0.8 months (mean ± standard error), respectively. No significant correlation between the colony mortality and the mite infestation rate on brood in the last month before the colony died (r =-0.045, *P*=0.853) was observed.

Table 7.2 Longevity of ARS Primorsky and domestic honey bee colonies, numbers of mites and *T. clareae* progeny per infested cell of ARS Primorsky and domestic bees.

No. of cells examined (worker+ drone)	No. of infested cells (worker+ drone)	No. of progeny	Average numbers of progeny / infested cell	No. of mites*	Average numbers of mites / infested cell	Colony number	Colony longevity (months)
						Domestic	
615	35	21	0.6	55	1.6	1	5
684	79	80	1	179	2.3	2	5
609	115	189	1.6	353	3.1	3	4
2,443	231	218	0.9	513	2.2	4	12
1,179	96	92	1	230	2.4	5	6
930	19	6	0.3	27	1.4	6	4
1,694	85	96	1.1	245	2.9	7	7
970	179	264	1.5	591	3.3	8	6
1,312	110	88	0.8	246	2.2	9	8
572	73	69	0.9	178	2.4	10	5
11,008	1,022	1,120	mean=1±0.1[a]	2,617	mean=2.4±0.2[a]	Total	mean=6.2±0.8[a]
						Primorsky	
460	80	89	1.1	190	2.4	1	3
614	126	93	0.7	290	2.3	2	3
1,348	175	196	1.1	433	2.5	3	9
555	30	27	0.9	59	2	4	4
656	94	134	1.4	262	2.8	5	4
769	153	190	1.2	537	3.5	6	5
895	166	231	1.4	458	2.8	7	5
525	45	54	1.2	116	2.6	8	4
539	107	208	1.9	369	3.4	9	5
599	211	377	1.8	888	4.2	10	4
6,960	1,187	1,599	mean=1.3±0.1[a]	3,602	mean=2.9±0.2[a]	Total	mean=4.6±0.5[a]

Similar letters in the same column are not significantly different at the 0.05 level.
*Including all developmental stages of *T. clareae*

Mites in ARS Primorsky and domestic debris

The total numbers of 18,098 and 14,593 *Tropilaelaps* mites were collected on bottom board traps from 10 domestic and 10 Primorsky colonies, respectively (Table 7.3). The average number of dead mites through time in the Primorsky and domestic debris was not significantly different (t=0.083, df=121, *P*=0.934) with means of 270.2±82.2 and 262.3±55.7 mites per colony, respectively. The number of dead mites per colony of the two bee stocks increased continuously from March to May. In June to

August, the average dead mite number per colony in the domestic debris decreased continuously while the average number of dead mites per colony in the Primorsky debris fluctuated. In September, the average number of dead mites per colony (mean =169.6±132.3) collected from 5 domestic colonies increased again while 84 dead mites were collected from the debris of one survival Primorsky colony. The amount of dead mites in the Primorsky and domestic debris was significantly correlated with the infested brood rates in the Primorsky (r =0.496, *P*=0.000) and domestic (r =0.653, *P*=0.000) colonies, and correlated with the amount of mites present in the domestic colonies (r =0.336, *P*=0.000) but not for the Primorsky colonies (r =0.269, *P*=0.071).

The number of injured mites through time in the Primorsky and domestic debris did not significantly differ (t=0.180, df=121, *P*=0.858) with means of 196.4±58.2 and 184.4±37.8 mites per colony (Table 7.3). The amount of injured mites followed the similar pattern with the amount of the dead mites. The percentage of injured mites in the debris from the domestic and Primorsky colonies was 70.3 and 72.7%, respectively. For injured types, the percentage of mites that had injured legs only from the Primorsky and domestic debris was 82.4 and 87.2%, respectively. Mites that had injured both legs and body in the Primorsky and domestic debris were 17.5 and 12.8%, respectively. Less than 1% of the injured mites in the debris of the two bee stocks had injured body only.

Table 7.3 Numbers and percentages of dead and injured *T. clareae* in debris of ARS Primorsky and domestic honey bee colonies.

Months	Bee types	No. of colonies collected	Total mites	Means ± standard error		No. of injured mites (%)			
				No. of dead mites / colony	No. of injured mites / colony	Total	Injured types		
							Legs	Body	Legs + body
Mar.02	Domestic	10	428	42.8±10.4[a]	34.2±8.6[a]	342	246	1	95
	Primorsky	10	404	40.4±9.5[a]	28.8±6.9[a]	288	218	0	70
Apr.02	Domestic	10	1,980	198±56.2[a]	157.3±44.9[a]	1,573	1,364	0	209
	Primorsky	10	2,170	217±451.7[a]	159.6±103.3[a]	1,596	1,276	4	316
May 02	Domestic	10	6,242	624.2±232.8[a]	414.4±143.9[a]	4,140	3,719	0	421
	Primorsky	10	4,505	450.5±274.1[a]	310.1±185.4[a]	3,101	2,812	3	286
Jun.02	Domestic	10	4,160	416±174.4[a]	310.1±136[a]	3,101	2,749	1	351
	Primorsky	10	3,729	372.9±184.7[a]	283.6±137.1[a]	2,836	2,208	1	627
Jul.02	Domestic	9	950	105.6±72.1[a]	76.4±51.4[a]	688	563	0	125
	Primorsky	6	3,242	540.3±443[a]	389±317.3[a]	2,334	1,911	0	423
Aug.02	Domestic	8	370	46.3±13.3[a]	38.3±11.3[a]	306	234	0	72
	Primorsky	4	129	32.3+17.9[a]	26.5+15.5[a]	106	69	0	37
*Sep.02	Domestic	5	849	169.6±132.3	102.2±77.4	512	431	0	81
	Primorsky	1	84	84	72	72	53	0	19
*Oct.02	Domestic	3	2,030	676.7±652.8	457.7±442.3	1,373	1,213	0	160
	Primorsky	1	232	232	195	195	139	0	56
*Nov.02	Domestic	1	386	386	277	277	210	0	67
	Primorsky	1	96	96	78	78	55	3	20
*Dec.02	Domestic	1	171	171	110	110	105	0	5
	Primorsky	1	2	2	2	2	2	0	0
*Jan.03	Domestic	1	44	44	30	30	21	0	9
	Primorsky	0	-	-	-	-	-	-	-
*Feb.03	Domestic	1	488	488	275	275	246	0	29
	Primorsky	0	-	-	-	-	-	-	-
Total	Domestic	N = 69	18,098	mean=262.3+55.7[a]	mean=184.4±37.8[a]	12,727 (70.3)	11,101 (87.2)	2 (0.02)	1,624 (12.8)
	Primorsky	N = 54	14,593	mean=270.2±82.2[a]	mean=196.4±58.2[a]	10,608 (72.7)	8,743 (82.4)	11 (0.1)	1,854 (17.5)

Similar letters in the same month and column are not significantly different at the 0.05 level.

*Means were not statistically compared

Discussion

The ARS Primorsky honey bee is used for beekeeping industry in the USA because this honey bee stock has resistant genetics and natural defensive mechanisms to *V. jacobsoni* and *V. destructor* (Danka et al., 1995; Rinderer et al., 1997, 1999). The natural *Varroa* infestation rate on the Primorsky brood (0-1.1%) and adult bees (0-0.7%) in the present study was lower than those of the domestic brood (0-5.7%) and adult bees (0-6.1%) (did not show data). This is the first time for introducing the ARS Primorsky bees to Thailand to compare with a honey bee stock commercially available in Thailand (domestic bees) for their resistance to *T. clareae*. In this study, the mite infestation rates on adult bees, mite numbers per colony (Table 7.1), colony longevity, progeny numbers per infested cell, mite numbers per infested cell (Table 7.2), numbers of dead and injured mites (Table 7.3) of the Primorsky and domestic honey bee colonies were not significantly different. Also, the similar percentage of injured mites was detected in the Primorsky (72.7%) and domestic (70.3%) debris (Table 7.3). In addition, the same level of hygienic behavior and *T. clareae* non-reproduction in the Primorsky and domestic colonies was previously reported by Kavinseksan et al. (in press a). These results suggest that the Primorsky and domestic bees had resistance or tolerance against *T. clareae* in the same level. Although the average infestation rate of *T. clareae* on the Primorsky brood was significantly higher than that of the domestic brood, this should have come from the significantly smaller brood size of the Primorsky colonies than that of the domestic colonies (Table 7.1). It was observed that the test colonies with small sizes of brood had higher infested brood rates than the colonies with large sizes of brood.

Since *A. mellifera* was introduced in tropical Asia, *T. clareae* successfully switched host from its natural host, *A. dorsata*, to be *A. mellifera* and has become a dangerous pest of *A. melliera* (as compared to *A. dorsata*) (Wongsiri et al., 1989; Rath and Delfinado-Baker, 1990). Both *Tropilaelaps* and *Varroa* mites have been considered to be the most important limiting factors to the development and expansion of *A. mellifera* beekeeping in Thailand and tropical Asia (De Jong et al., 1982; Neyin and Zmarlicki, 1982). However, several researchers suggest that *T. clareae* may be more

destructive than *Varroa* mites to *A. mellifera* colonies because the numbers of *T. clareae* are often higher than those of *Varroa* mites in Thailand (Burgett et al., 1983; Wongsiri et al., 1989). The result here showed that the population growth of *T. clareae* in the Primorsky and domestic colonies increased rapidly to a maximum level in the third months (May) (Table 7.1). Almost test colonies died due to *Tropilaelaps* infestation within 5-6 months (Table 7.2). This observation was consistent with the previous report by Crane (1990) that *Tropilaelaps* can kill untreated *A. mellifera* colonies within a few months. From the data obtained, the average infestation rate of *T. clareae* through time on the domestic and Primorsky brood (11.4-18.5%) was much higher than that on the adult bees (0.5-0.8%) (Table 7.1), similar to Woyke (1984). This suggests that most *Tropilaelaps* mites were in brood cells to reproduce. According to Woyke (1984, 1987), *T. clareae* adults stay outside sealed brood cells for short periods of time. They re-entered brood cells to reproduce within 2-3 days after their emerging and *Tropilaelaps* mites can produce 2 generations of offspring within a month. Thus, the population growth of *T. clareae* in this study increased rapidly although the average number of progeny produced per infested cell (1 to 1.3) was low (Table 7.2). This finding agrees well with the previous report by Delfinado-Baker and Peng (1995) that *T. clareae* is capable of increasing population within a short time in response to host constraints, which may result in high mortality.

Woyke (2001) reported that *A. mellifera* worker bees were able to detect the presence of *Tropilaelaps* mites in sealed brood cells and to remove them by uncapping the cells. About 94% of the uncapped cells showed a heavy infestation with *T. clareae* (few infested cells with *Varroa* were uncapped by *A. mellifera* worker bees) (Ritter and Schneider-Ritter, 1988). In the present study, the brood peak in the Primorsky and domestic colonies was found before the peaks of the brood infested and the mite number about 1 month (Table 7.1). The increase of brood infestation coincided with the significant decrease in the amount of the Primorsky and domestic brood (r=-0.314). Brood areas in heavily infested Primorsky and domestic colonies soon declined because the worker bees opened the infested cells to remove the parasitized brood and mites. A large number of cells uncapped by the bees were found in the heavily infested colonies.

The pupae were either undamaged or eaten so that only abdomen or parts of the pupae remained in the opened cells, similar to Atwal and Goyal (1971), Woyke (1984) and Ritter and Schneider-Ritter (1988). Thus, colony strength decreased rapidly and colonies died within a short time. These suggest that the Primorsky and domestic colonies died because the worker bees opened the infested-*Tropilaelaps* brood cells to remove the parasitized brood and mites from their colonies.

Several researchers reported that the growth and development of *T. clareae* in *A. mellifera* colonies depends on several factors such as non-reproduction, hygienic and grooming behaviors of the bee hosts (Wongsiri et al., 1989; Spivak and Reuter, 1998; Buchler and Drescher, 1990; Rath and Delfinado-Baker, 1990; Woyke, 1990). For grooming behavior, *A. cerana* and *A. dorsata* have an effective grooming behavior to remove mites from their body and kill them (Koeniger and Koeniger, 1980; Wongsiri et al., 1989). The result here showed that the Primorsky and domestic bees exhibited grooming behavior in the similar level as indicated by the percentages of total injured mites and injured types (Table 7.3). The total injured mite percentage (70.3-72.7%) in this study was higher than that of *A. mellifera* colonies (8.5%) in Thailand from the previous reported by Wongsiri et al. (1989) but lower than that (73-94.7%) in *A. dorsata* debris (Rath and Delfinado-Baker, 1990; Koeniger et al., 2002; Kavinseksan et al., in press b). For injured types, the dead mites in the Primorsky and domestic debris did not show severe damages. Almost dead mites had injured legs only (82.4-87.2%) by missing legs or parts of legs, while the percentage of severely damaged mites on both legs and body (12.8-17.5%) was lower than that in *A. dorsata* debris (48%) in the previous report by Kavinseksan et al. (in press b). These suggest that the Primorsky and domestic bees had less efficiency to destroying *Tropilaelaps* mites by grooming behavior than *A. dorsata*.

In Thailand, *A. mellifera* was introduced for the first time in the early 1940s and for the second time in 1953 but did not succeed to maintain the bee until the early 1970s (Wongsiri and Chen, 1995). Thus, *A. mellifera* in Thailand (domestic bees) and *T. clareae* have coexisted and evolved for about 30 years. This time period might be too

short for the domestic bees to develop a high level of resistant genetics or natural defensive mechanisms to regulate *T. clareae* populations in their colonies. For the example, the Primorsky bees have coexisted with *V. jacobsoni* more than 150 years to development a high level of resistant genetics and natural defensive mechanisms to regulate *Varroa* populations in the bee colonies (Danka et al., 1995; Rinderer et al., 1997, 1999). Although this is the first time for the Primorsky bees to encounter with *T. clareae*, they could show resistance or tolerance to *Tropilaelaps* mites in the same level with the domestic bees that coexisted with *T. clareae* for about 30 years. This should be a good opportunity to developing resistant genetics against *T. clareae* of the Primorsky bees in the future.

Acknowledgements

I am grateful to the Thailand Research Fund (TRF) for the Royal Golden Jubilee Ph.D. Program and Bansomdejchaopraya Rajabhat University for financial support. I thank USDA, ARS Honey Bee Breeding, Genetics and Physiology Laboratory for providing some ARS Primorsky honey bee queens and Supa Yawilert for providing some domestic honey bee queens and the apiary site for the experiment.. I also thank Mr. Surachai Leephitakrat and Ms.Piyamas Nanork for their assistance in collecting samples.

References

Atwal, A. S., and Goyal, N. P. 1971. Infestation of honey bee colonies with *Tropilaelaps* and its control. J. Apic. Res. 10: 137-142.

Buchler, R., and Drescher, W. 1990. Variance and heritability of the capped developmental stage in European *Apis mellifera* L. colonies and its correlation with increased *Varroa jacobsoni* Oud. infestation. J. Apic. Res. 29: 172-176.

Burgett, D. M., and Akratanakul, P. 1985. *Tropilaelaps clareae* the little know honey bee brood mite. Am. Bee J. 125:112-114.

Burgett, D. M., Akratanakul P., and Morse R. A. 1983. *Tropilaelaps clareae*: a parasite of honeybees in southeast Asia. Bee Wld. 64: 25-28.

Burgett, D. M., and Burikam, I. 1985. Number of adult honey bees (Hymenoptera: Apidae) occupying a comb: A standard for estimating colony populations. J. Econ. Entomol. 78: 1154-1156.

Burgett, D. M., and Krantz, G. W. 1984. The future of the European honey bee (*Apis mellifera* L.) in Southeast Asia: constraints of parasitism. Proceedings of the expert consultation on beekeeping

Crane, E. 1990. Bees and beekeeping. Ithaca, New York: Comstock publishing associates.

Danka, R.G., Rinderer,T.E., Kuznetsov,V.N., and Detatte, G.T. 1995. A USDA-ARS project to evaluate resistance to *Varroa jacobsoni* by honey bees of Far-eastern Russia. Am. Bee J. 135(11): 746-748.

Delfinado-Baker, M., and Aggarwal, K. 1987. A new *Varroa* (Acari: Varroidae) from the nest of *Apis cerana* (Apidae). Int. J. Acarol. 13: 233-237.

Delfinado-Baker, M., Baker, E. W., and Phoon, A. C. G. 1989. Mites (Acari) associated with bees (Apidae) in Asia, with description of a new species. Am. Bee J. 129: 609-610, 612-613.

Delfinado-Baker, M., and Peng, C. Y. S. 1995. *Varroa jacobsoni* and *Tropilaelaps clareae*: A perspective of life history and why Asian bee-mites preferred European honey bees. Am. Bee J. 135: 415-420.

Delfinado-Baker, M., Rath, W., and Boecking, O. 1992. Phoretic bee mites and honeybee grooming behavior. Int. J. Acarol. 18(4): 315-322.

De Guzman, L. I., Rinderer, T. E., Delatte, G. T., and Macchiavelli, R. E. 1996. *Varroa jacobsoni* Oudemans tolerance in selected stocks of *Apis mellifera* L. Apidologie 27: 193-210.

De Jong, D., Morse, R .A., and Eickwort, G .C. 1982. Mite pests of honey bees. Ann. Rev. Entomol. 27: 229-252.

Kavinseksan, B., Wongsiri S., Rinderer, T. E., and de Guzman, L. I. (in Press a) Comparison of the hygienic behavior of Thai commercial and ARS Russian honey bees. Science Asia.

Kavinseksan, B., Wongsiri, S., de Guzman, L. I., and Rinderer, T. E. (in Press b) Population and sex ratio of *Tropilaelaps clareae* in debris and on adult bees of *Apis dorsata* in Thailand. Apidologie.

Kitprasert, C. 1984. Biology and systematics of the parasitic bee mite, *Tropilaelaps clareae* Delfinado and Baker (Acarina : Laelapidae). M. S. thesis, Kasetsart University, Thailand. (In Thai)

Koeniger, G., Koeniger,N., Anderson, D.L., Lekprayon, C., and Tingek, S. 2002. Mites from debris and sealed brood cells of *Apis dorsata* colonies in Sabah (Borneo) Malaysia, including a new haplotype of *Varroa jacobsoni*. Apidologie 33: 15-24.

Koeniger, N., and Koeniger, G. 1980. Observations and experiments on migration and dance communication of *Apis dorsata* in Sri Lanka. J. Apic. Res.19(1): 21-34.

Neyin, M. M., and Zmarlicki, C. 1982. Control of mites in European bees in Burma. Am. Bee J. 122: 638-639.

Rath, W., and Delfinado-Baker, M. 1990. Analysis of *Tropilaelaps clareae* populations from the debris of *Apis dorsata* and *Apis mellifera* in Thailand. Proceedings of the apimondia symposium recent research on bee pathology, Gent, Belgium, pp. 86-89.

Rinderer, T. E., Delatte, G. T., De Guzman, L. I., Williams, J., Stelzer, J., and Kuznetsov, V. N. 1999. Evaluations of the *Varroa*-resistance of the honey bees imported from Far-Eastern Russia. Am. Bee J. 139(4): 287-290.

Rinderer, T. E., Oldroyd, P. B., Lekprayoon, C., Wongsiri, S., Boonthai, C., and Thapa, R. 1994. Extended survival of the parasitic honey bee mite *Tropilaelaps clareae* on adult workers of *Apis mellifera* and *Apis dorsata*. J. Apic. Res. 33(3): 171-174.

Rinderer,T. E., Kuznetsov, V. N., Danka, R. G. and Detatte, G. T. 1997. An importation of potentially *Varroa* - resistant honey bees from Far-Eastern Russia. Am. Bee J. 137(11): 787-789.

Ritter, W., and Schneider-Ritter, U. 1988. Differences in biology and means of controlling *Varroa jacobsoni* and *Tropilaelaps clareae*, two novel parasitic mites of *Apis mellifera*. In Needham, G. R., Page, R. E., Delfinado-Baker, M., and Bowman, C. E. (eds.), Africanized honey bees and bee mites, New York, pp. 387-395.

Spivak, M., and Reuter, G. S. 1998. Honey bee hygienic behavior. Am. Bee J. 138(4): 283-286.

Tangkanasing, P., Wongsiri, S., and Vongsamanod, S. 1988. Integrated control of *Varroa jacobsoni* and *Tropilaelaps clareae* in bee hives in Thailand. In G. R. Needham, R. E. Page, M. Delfinado-Baker and C. E. Bowman (eds.), Africanized Honey Bee and Bee Mites. New York: John Wiley & Sons, pp. 409-412.

Wongsiri, S., and Chen, P. 1995. Effects of agricultural development on honey bees in Thailand. Bee Wld. 76(1): 3-5.

Wongsiri, S., and Tangkanasing, P. 1987. Bee mites control in Thailand. In Workshop on parasitic bee mites and their control, Pulawy, Poland, 27 August-1September 1987.

Wongsiri, S., Tangkanasing, P., and Sylvester, H. A. 1989. The resistance behavior of *Apis cerana* against *Tropilaelaps clareae*. Proceedings of the First Asia-Pacific Conference of Entomology, Chaing Mai, Thailand: pp. 828-836.

Woo, K. S., and Lee, J. H. 2001. Current status of honey bee mites in Korea. Proceedings of the Third Asian Apicultural Association Conference on Bee Research and Beekeeping Development, Hanoi, Vietnam, 1996: pp. 168-171.

Woyke, J. 1984. Survival and prophylactic control of *Tropilaelaps clareae* infesting *Apis mellifera* colonies in Afghanistan. Apidologie 15(4): 421-434.

Woyke, J. 1987. Length of successive stages in the development of the mite *Tropilaelaps clareae* in relation to honeybee brood age. J. Apic. Res. 26(2): 110-114.

Woyke, J. 1990. Biology and control of the parasitic bee mite *Tropilaelaps clareae*. Proceedings of the apimondia symposium recent research on bee pathology, Gent, Belgium, pp. 90-99.

Woyke, J. 2001. Different reaction of *Apis dorsata* and *Apis mellifera* to brood infestation by parasite mites. Proc. 3rd AAA conf. on bee res. and beekeeping dev., Hanoi, Vietnam, 1996, pp. 172-175.