CHAPTER 6

Effects of *Tropilaelaps mercedesae* parasitism on the weight of different stages of *Apis mellifera* (Hymenoptera: Apidae)

6.1 Introduction

In Thailand, the most destructive acarine parasites of the introduced western honeybee, *Apis mellifera*, are *Varroa destructor* and *Tropilaelaps mercedesae*, which are both ectoparasitic on honey bee brood. The adapted host for *V. destructor* is the eastern honey bee *A. cerana* (Oudemans, 1904). *T. mercedesae*'s adapted host is the giant honey bee *A. dorsata* (Burgett and Kitprasert, 1990; Burgett *et al*., 1990). Both mite species are able to utilize the introduced western honey bee species (*A. mellifera*) as an alternate, competent host. Thai *A. mellifera* beekeepers recognized the severity of acarine brood parasites ever since the introduction of this exotic species in the early 1980s (Burgett and Akratanakul, 1985). These ectoparasitic mite infestations are the primary cause for *A. mellifera* colony mortality in Thailand. The primary hosts for both mite species are the immature stadia of the honey bee with the most severe pathogenic effects experienced during the pre-pupa through pupal stages. The damage caused by *V. destructor* is well noted as it has attained a near worldwide distribution. *T. mercedesae* is still limited to its Southeast Asian distribution concurrent with the biogeography of *A. dorsata* with the exception of its inadvertent introduction to the Korean peninsula in the mid1990s (Woo and Lee, 1993), which is outside of the range of *A. dorsata*. High levels of mite parasitism will eventually result in colony death, which is ultimately brought about by the loss of replacement bees necessary to replenish the natural mortality of older worker forager bees.

Past research on *V. destructor* parasitism of *A. mellifera* has shown reduced weight of parasitized worker and drone pupa, as well as shortened life spans of adult worker bees and reduced sperm production by drones (De Jong, et al. 1982; De Jong and De Jong, 1983; Rinderer et al., 1998, Duay et al., 2002; 2003). The effects of *T.*

mercedesae parasitism on the brood of *A. mellifera* are to date unknown. In this study, I assessed the effects of *T. mercedesae* parasitism on the weights of infested *A. mellifera* brood .

6.2 Materials and methods

6.2.1 Samples

A. mellifera capped brood combs were collected from an apiary maintained by the Thai Department of Agriculture Beekeeping Extension and Development Center and from commercial *A. mellifera* apiaries around Chiang Mai in 2013-2014. Brood combs were collected from 42 colonies that were untreated with acaricides for at least three months prior to examination. Brood cells were de-capped and the immature stage noted. For infested brood, the number of adult and immature mite stages was recorded. Honey bee pupal stages were differentiated according to body and eye color and classified as follows: newly sealed larva, pre-pupa, white eyed pupa, pink eyed pupa, purple eyed pupa, tan bodied pupa, black thorax pupa, and newly emerged adult bees. Newly sealed larva was distinguished from pre-pupa by the presence of silk beneath the cell capping. Recorded mite stadia were as follows: foundress females, eggs, protonymphs, deutonymphs, and F_1 adults (male and female).

6.2.2 Data analysis

The transcription levels were normalized using SPSS version 20 for Windows (SPSS, Inc., 2012). One sample t-tests and one-way ANOVA were used to analyze the significance of different mean values for overall data to compare weights of infested and uninfested brood.

6.3 Results

In total, 11,263 *A. mellifera* brood cells were de-capped; 5,879 workers and 5,384 drones. *T. mercedesae* infestation rates were 9.2% for worker brood (Table 6.1) and 8.9% for drone brood (Table 6.2).

For drones, the mean weight for infested newly emerged adult was 161.7 ± 61.9 mg; for non-infested drones, the mean weight was 196.2 ± 66.6 mg (Table 6.4). This is a 17.6% weight loss for infested drones *vs*. non-infested and is statistically significant at the 1% level (t-test). However, no difference in weight was detected for infested and non-infested newly emerged worker bees (Table 6.3).

Table 6.1 Number of *A. mellifera* worker brood examined and the percentage of *T. mercedesae* infestation.

Honey bee stage		**Worker**		
		Uninfested brood	***T. mercedesae***	**Total**
Newly sealed larva	**NL**	179	16 (20.3%)	**195**
Pre-pupa	**PP**	391	48 (12.3%)	**439**
White eyes pupa	**WE**	671	61 (9.1%)	**732**
Pink eyes pupa	**PI**	531	69 (13.0%)	**600**
Purple eyes pupa	**PE**	596	92 (15.4%)	**688**
Tan body	**T**	787	141 (17.9%)	**928**
Black thorax	**B**	407	41 (10.1%)	**448**
Newly emerged bee	**E**	1780	69 (3.9%)	**1849**
$\sum$		5342	537 (9.2%)	**5879**

Table 6.2 Number of *A. mellifera* drone brood sampled and the percentage of *T. mercedesae* infestation.

Pupa stage		**Drone**		
		Uninfested brood	***T. mercedesae***	**Total**
Newly sealed larva	**NL**	542	175 (13.8%)	**717**
Pre-pupa	**PP**	372	121 (32.5%)	**493**
White eyes pupa	**WE**	766	44 (5.7%)	**810**
Pink eyes pupa	**PI**	724	37 (5.1%)	**761**
Purple eyes pupa	**PE**	790	38 (4.8%)	**828**
Tan body	**T**	793	28 (3.5%)	**821**
Black thorax	**B**	378	17 (4.5%)	**395**
Newly emerged bee	**E**	538	21 (3.9%)	**559**
$\sum$		4903	481(8.9%)	**5384**

Average weights for uninfested brood and *T. mercedesae* parasitized brood for both workers and drones decreased relative to developmental stage as shown in Figures 6.1 and 6.2, respectively. Overall, the average weight of all brood stadia when comparing uninfested brood to infested brood for both workers (Figure 6.1) and drones (Figure 6.2) was not significantly different (F = 1.005, P = 0.485 and F = 0.672, P = 0.999, respectively).

Infested worker brood cells contained up to 11 mites. The average number of mites infesting a worker brood cell was 2.7 ± 1.6; for infested drone cells the average infestation was 1.8 ± 1.5. The maximum number of mites observed infesting a drone brood cell was 9 mites.

Table 6.3 Weight (mean±SD) of different stages of uninfested and *T. mercedesae*-infested *A. mellifera* worker.

Pupa stage		**Worker**	
		Uninfested brood	***T. mercedesae***
Newly sealed larva	**NL**	149.2±11.5	141.4±35.7
Pre-pupa	**PP**	150.1±38.5	165.6±51.8
White eyes pupa	**WE**	127.6±23.9	126.2±24.7
Pink eyes pupa	**PI**	120.6±8.0	115.0±9.8
Purple eyes pupa	**PE**	123.4±25.9	112.0±9.5
Tan body	**T**	116.1±8.8	113.3±18.3
Black thorax	**B**	113.1±8.4	99.9±18.6
Newly emerged bee	**E**	112.0±17.2	114.5±19.6

Table 6.4 Weight (mean±SD) of different stages of uninfested and *T. mercedesae*-infested *A. mellifera* drone.

Pupa stage		**Drone**	
		Uninfested brood	***T. mercedesae***
Newly sealed larva	**NL**	262.4±29.4	271.8±22.8
Pre-pupa	**PP**	295.0±45.0	264.0±37.6
White eyes pupa	**WE**	279.6±30.1	271.2±35.8
Pink eyes pupa	**PI**	265.1±32.7	258.9±27.2
Purple eyes pupa	**PE**	275.2±33.0	245.7±29.7
Tan body	**T**	265.6±31.1	237.6±32.1
Black thorax	**B**	252.0±21.1	228.1±22.2
Newly emerged bee	**E**	196.2±66.6	161.7±61.9

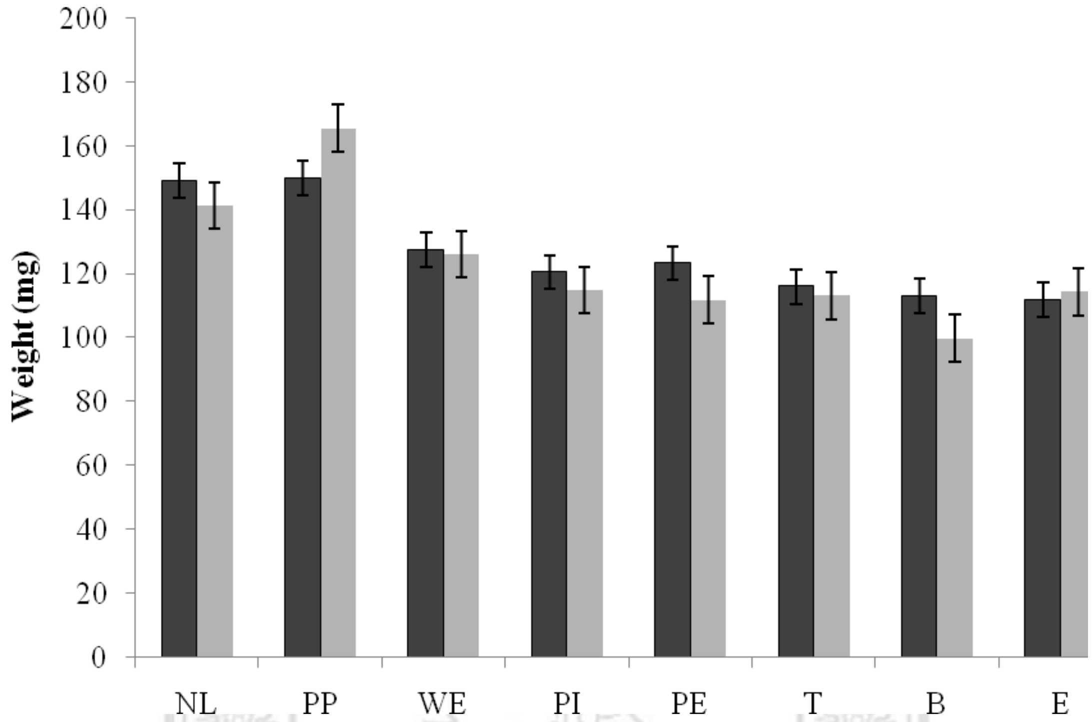

Figure 6.1 Weight (mean±SE) of different stages of uninfested (dark gray bars) and *T. mercedesae*-infested (light gray bars) *A. mellifera*.workers.

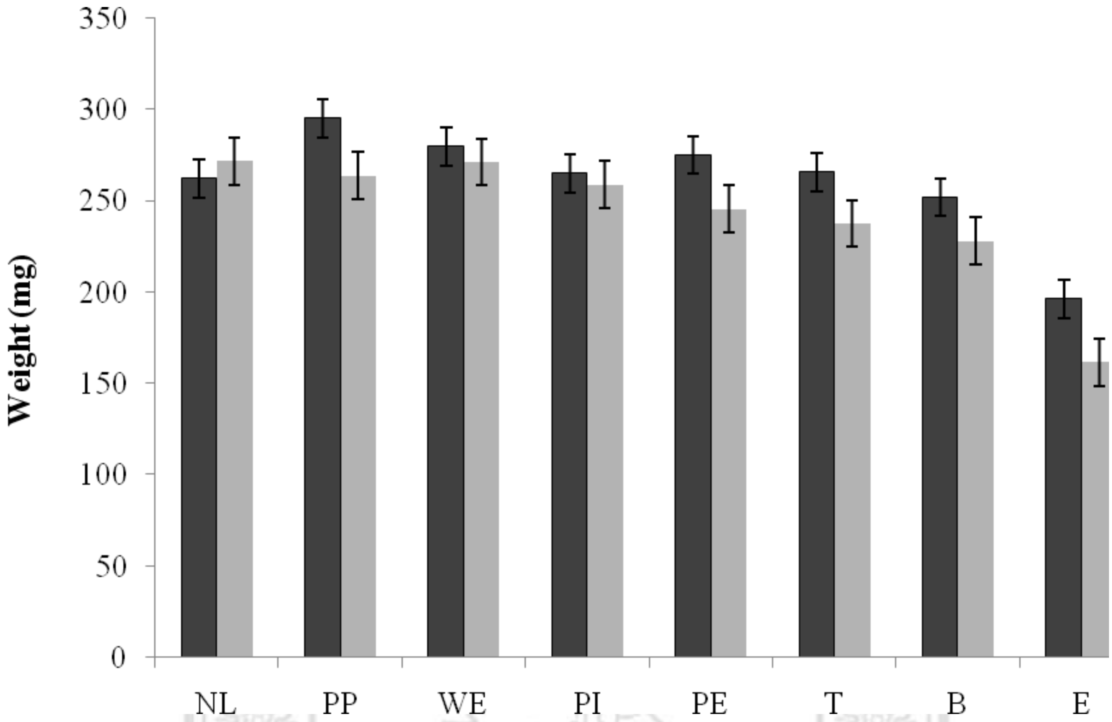

Figure 6.2 Weight (mean±SE) of different stages of uninfested (black line) and *T. mercedesae*-infested (gray line) *A. mellifera*.drone.

6.4 Discussion

Varroa parasitism is known to have negative effects on drone's health, survival and reproductive fitness (Rinderer et al. 1999; Duay et al., 2003). While Rinderer et al. (1999) reported about 7% weight loss, Duay et al. (2003) reported an average weight loss of 10% for newly emerged infested adult drones relative to uninfested controls. In this study, I present the first evidence for weight loss effects of *T. mercedesae* parasitism on *A. mellifera* drones. On average, *T. mercedesae*-infested drone lost an average weight of 17.6% relative to uninfested drones. Although, I did not monitor drone survival and sperm production, this study demonstrates one harmful effect of *T. mercedesae* infestation on *A. mellifera*.

Similarly, De Jong et al. (1982) reported that *Varroa* mites (most likely *V. destructor*) negatively affect weights of newly emerged Africanized worker bees. Their results showed a significant difference in the weights of newly emerged infested workers as 6.3 to 25% less than non-infested workers, which was correlated with the number of mites per infested host. My results with *T. mercedesae* infestation of worker brood did not show a statistically significant difference in weight between infested and uninfested newly emerged workers. These studies were conducted on brood examined during a 12 month observation period during which seasonal differences in the overall state of colony nutritional health will vary. Because of the potential season fluctuation, it can be expected that a wider range of variation in brood weight especially for workers (both infested and non-infested) will occur. Drone production is seasonal and thus, a narrower range of variation is expected.

These results demonstrate statistically significant differences in weight of developing brood stadia of both workers and drones of *A. mellifera* (more pronounced in drones), which is the non-adapted host of *T. mercedesae*. Further studies of the effects of parasitism on adult longevity in both workers and drones, and sperm production of drones would be beneficial.