CHAPTER 4

Seasonal abundance and interspecific competition between *Varroa* and *Tropilaelaps* mites when infesting *Apis mellifera*

4.1 Introduction

In the early twentieth century, *Varroa* was first described from Java, Indonesia (Oudemans, 1904) as an ectoparasitic mite of the native Asian honey bee, *Apis cerana* and was assigned the nomen *Varroa jacobsoni*. *V. jacobsoni* has become a major parasite of *A. mellifera* on a worldwide scale. *V. jacobsoni* has been recognized to be a complex of at least two different species. Anderson and Trueman (2000) identified a new *Varroa* species *V. destructor*.

Tropilaelaps was firstly discovered from the Philippines as a brood parasite of *A. mellifera* (Delfinado and Baker, 1961). However, *Tropilaelaps* was later recognized as the obligate brood parasite of the giant honey bee (Laigo and Morse, 1968). Recently, a new species of *Tropilaelaps* has been redefined as *T. mercedesae,* and is sympatric with *T. clareae* around its SE Asian range (Anderson and Morgan, 2007).

Varroa and *Tropilaelaps* have been co-infesting *A. mellifera* colonies for about 50 years in Asia (Delfinado, 1963). When infesting *A. melli*fera of *Tropilaelaps* infestations were observed to be more frequently higher than those of *Varroa* in Thailand (Burgett et al., 1983). Woyke (1987c) and Woyke (1989) had similar observations in Afghanistan and Vietnam. In Northern Thailand, Kavinseksan (2003) observed *T. clareae* (probably referring to *T. mercedesae*) infestations in mite-inoculated colonies of Primorsky bees (=Russian honey bees, RHB) and Thai *A. mellifera*. The author found that RHB colonies (mean = 18.5%) supported higher brood infestation than the local bees (mean = 11.4%) with the highest infestations observed in May (RHB = 33%, Thai *A. mellifera* = 21%). Factors that influence population fluctuations of both *Varroa* and *Tropilaelaps* in concurrently infested *A. mellifera* colonies have not been studied in any detail. On the other hand, in the Philippines,

Fajardo and Cervancia (2004) found that *A. mellifera* colonies had higher infestations of *Tropilaelaps* than *Varroa* in April and these colonies had higher *Varroa* than *Tropilaelaps* infestations in September.

Although both *Varroa* and *Tropilaelaps* are observed infesting colonies, *Tropilaelaps* is considered to be a more serious problem for *A. mellifera* colonies than *Varroa* mites in Northern Thailand (Burgett et al., 1983; Anderson and Morgan, 2007). Such discrepancy in severity may be due to differences in their abilities to compete for honey bee hosts and reproduce within brood cells. This differential competitive ability may determine the limited concurrent infestation of brood cells by both mite species or the abundance of *Tropilaelaps* over *Varroa*. For this study, I investigated the build-up and synchronization in both *Varroa* and *Tropilaelaps* populations in concurrently infested *A. mellifera* colonies. Whereas both mites cause damage to infested bees, such information is essential in deciding the best time to take action or may help in developing management strategies for both mite species. Reproduction was also estimated in naturally infested brood and in brood cells intentionally infested with both mite species to see if variation in reproduction exists. The results on differential reproduction may help interpret population fluctuations, competitive advantage or virulence of one mite species.

4.2 Materials and methods

These experiments were conducted at the Thailand Department of Agriculture Beekeeping Extension and Development Center for Chiang Mai Province and the Chiang Mai University campus (18° 44' Lat., 98° 55' Long.), September 2011-September 2012.

4.2.1 Seasonal abundance of *Varroa* and *Tropilaelaps* in *A. mellifera* colonies in Northern Thailand

Sixteen standard 10-frames Langstroth hives were utilized for the experimental trials. All miticidal treatments were ceased at least two months before the commencement of observations. All queens came from the same genetic source, based on the Italian honey bee (*A. m. ligustica*). All colonies were

equalized for strength and food stores before starting observations. Brood comb areas (cm^2) were determined by visual estimation of brood comb area according to Rogers et al. (1983). Mite infestations were determined by examining 50-100 worker brood cells on a monthly basis. Drone brood were examined when available, however the sample size for drone brood was so small it was insufficient for statistical analysis. The number of sampled brood per colony was based on an estimate of overall colony strength. The number of *Varroa* and *Tropilaelaps* in brood cells were derived from counts of adult mites in infested brood cells and the total number of sealed brood cells in each colony (Rinderer et al., 2001; de Guzman et al., 2007). The stages of mite development were differentiated and recorded.

To examine adult mite phoresy and concurrent infestation of individual brood hosts, a separate set of three colonies was utilized. These were also equalized for brood and adult worker bee strength. To determine *Tropilaelaps* and *Varroa* infestation, different stages of worker and drone brood were examined from each of the three colonies. About 400-500 adult bees per colony were sampled for the presence of phoretic mites (both species). Bee samples were placed in a jar and washed with soapy water (2 ml detergent/1L water) for three times to remove mites *as per* Rinderer et al. (2004). The phoretic mites were collected and differentiated according to species. *Tropilaelaps* and *Varroa* were then counted to determine the percentage of phoresy on adult *A. mellifera*. Additionally, any correlations between infestation parameters and average monthly temperature and relative humidity data were analyzed.

4.2.2 Interspecific competition between *Varroa* and *Tropilaelaps*

During the observation of seasonal abundance of *Varroa* and *Tropilaelaps* in *A. mellifera* colonies experiment, it was rare to find co-infestations of *Tropilaelaps* and *Varroa* in the same host brood cell. We experimentally examined the condition of joint species infestations on single hosts by deliberately introducing both mite species into the same brood cells. To provide colonies as free from mites as possible, eight *A. mellifera* colonies that had been treated with

tau-fluvalinate were used in this study. These eight colonies were separate from the colonies used in determining the seasonal abundance of *Varroa* and *Tropilaelaps*. These colonies consisted of two to three brood frames, and adult bees on two comb sides of seven to eight frames. To obtain brood of the uniform age, each queen was caged over an empty comb for 24 h by using an 8 mesh screen push-in cage providing a brood area of about 400 brood cells. Eight days following queen oviposition, one foundress *Tropilaelaps* and one foundress *Varroa* were inoculated into the same brood cell. All introduced foundress mites were collected from tan-bodied pupae of highly infested *A. mellifera* colonies. The inoculated *Tropilaelaps* were initially examined under a dissecting microscope to exclude males. The mite transfer technique used to inoculate newly sealed larvae was *as per* Garrido and Rosenkranz, 2003; Kirrane et al., 2011; Khongphinitbunjong et al., 2013. The tan-bodied pupal were assessed for mite reproduction on the 9^{th} day following mite inoculation. All mite stages were differentiated.

4.2.3 Mite reproductive status

For experiment 4.2.1, reproductive *Tropilaelaps* and *Varroa* foundresses were those that produced at least one progeny. Experiment 4.2.2 used two criteria to evaluate the proportion of non-reproductive foundresses to compare with previous published studies (de Guzman et al., 2007; de Guzman et al., 2008; Khongphinitbunjong et al., 2013). For criteria 1, the reproductive foundresses *Varroa* were those that produced an adult male and daughter or viable offspring (de Guzman et al., 2007; de Guzman et al., 2008). As *Tropilaelaps* have shorter life cycle when compared to *Varroa*, it is possible that *Tropilaelaps* foundresses may lay more eggs which are able to develop to adulthood by the time of bee emergence (Sihag, 1988; Sammataro et al., 2000). Woyke (1987c, 1994) stated that *Tropilaelaps* are able to mate outside the host brood cell. So *Tropilaelaps* reproductive foundresses are those that produce at least one progeny (Khongphinitbunjong et al., 2013). For criteria 2, regardless of the mating

behavior of foundress, reproductive foundress *Varroa* or *Tropilaelap*s are those that produced ≥1 progeny.

4.2.4 Mite sample identification

Tropilaelaps species used in these studies were confirmed by DNA analysis as described in 3.2.2, Chapter 3. DNA analyses were also conducted to verify the genetic identity of *Varroa* (Warrit *et al.*, 2006). Samples of both mite species were held at -20°C until DNA extraction. DNA analyses were conducted to verify the genetic identity of *Varroa* (Warrit *et al.*, 2006). The PCR product was amplified with a *COI* 51-F primer (5′-GTAATTTGTATACAAAGAGGG-3′) and a *COI* 1400R primer (5′-CAATATCAATAGAAGAATTAGC-3′). The thermal cycling conditions were as follows: preheat lid of thermocycler to 103˚C in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, USA) (White et al., 1990), one cycle of initial denaturation at 94˚C for 1 min; 35 cycles of [94˚C for 40 sec; 40˚C for 1 min 30 sec; then increase to 72˚C at rate of 0.3˚C per sec; finally 72˚C for 2 min]; followed by final extension 72˚C for 5 min; then hold PCR product at 4˚C. Afterwards, the PCR product was digested 10ul with *XhoI* and 10ul with *SacI*. All *V. jacobsoni* and *V. destructor* should have the *SacI* site (positive control), only *V. destructor* has *XhoI* site.

4.2.5 Data analyses

For experiment 4.2.1, only worker brood cells infested with either *T. mercedesae* or *V. destructor* were considered for statistical analyses. Only 15 concurrently infested brood cells were observed throughout the study and thus were excluded from the analyses. Prior to analyses, data on the percentage infestation and percentage non-reproduction (NR) were transformed using an arcsine square-root transformation. A repeated measures analysis of variance (ANOVA) with observation dates and mite type as the main effects were performed to determine differences in infestations of both mite species through time. To assess *Varroa* reproduction, observations on infestations using purple eyed pupae (PE) and tan-bodied pupae (TB) were used. *V. destructor* were considered reproductive if the foundress had at least one adult male and one

viable daughter (de Guzman et al., 2007; Kirrane et al., 2011). *T. mercedesae* were considered reproductive if the foundress produced ≥1 progeny (Khongphinitbunjong et al., 2013). However, observations using white eyed pupae (WE), purple eyed pupae (PE), and tan-bodied pupae (TB) were considered for analyses. A z-test for proportions was used to compare the overall non-reproductive status for both mite species. The average proportion of NR was calculated by determining the percentage of NR foundress for each colony on each observation date and then calculated the average by mite species and by observation date. A mixed model ANOVA was performed with observation dates and mite species as the main effects. A two-way ANOVA with replication and mite species as the effects was used to determine differences in the number of progeny between the mite species and/or the reproduction types. A one-way ANOVA was used to determine infestation trends of *V. destructor* and *T. mercedesae* through time.

For interspecific competition between *Varroa* and *Tropilaelaps* experiment, a z-test for two proportions was used to compare reproductive success between the two mite genera. Comparisons between the two mite species were also made for each trial. Differences in the reproductive status for each trial were compared using the Marascuillo procedure for multiple proportions (http://www.itl.nist.gov/div898/handbook/prc/section4/prc474.htm). A paired sample *t*-test was used to compare differences in the progeny number produced by foundress *Tropilaelaps* and *Varroa*.

4.3 Results

4.3.1 DNA identification

When compared in the Gen Bank database using the BioEdit program (Hall, 1999), the DNA sequences (501-552 bp) derived from our samples (Accession number KP774521, KP774523-KP774526) showed a 99% similarity to sequences from *T. mercedesae* collected from China [HQ533165.1] and Sri Lanka [EF025472.1]. Consequently *Tropilaelaps* used in this study were identified as *T.*

mercedesae (Anderson and Morgan, 2007). *Tropilaelaps* used in this study were identified as *T. mercedesae* (Anderson and Morgan, 2007) and *Varroa* were identified as *V. destructor* (Anderson and Trueman, 2000).

4.3.2 Seasonal abundance of *Varroa* and *Tropilaelaps* in *A. mellifera* colonies in Northern Thailand

Of the 18,250 worker brood cells (newly sealed larvae to tan-bodied pupae) examined throughout this experiment, only 13 brood cells (<0.1%) were found to be concurrently infested with both *V. destructor* and *T. mercedesae* (Table 4.1). These 13 brood cells were excluded from the analyses. Of the 970 infested brood cells, 24% were infested with *Varroa* while 76% were infested by *Tropilaelaps*.

Table 4.1 *T. mercedesae* and *V. destructor* infestations in different stages of *A. mellifera* worker brood.

Brood stage	Total brood cells examined	Total brood cells infested	*Varroa*		*Tropilaelaps*		*Varroa* and *Tropilaelaps*	
			# brood cells infested	%	# brood cells infested	%	# brood cells concurrently infested	%
Newly sealed	5,510	335	76	1.4	259	4.7	0	0
Prepupae	142	8	2	1.4	6	4.2	0	0
White-eyed	1,860	64	22	1.2	42	2.3	0	0
Pink to purple-eyed	5,012	180	40	0.8	140	2.8	4	0.1
Tan-bodied	5,726	383	94	1.6	289	5.1	9	0.2
Total	**18,250**	**970**	**234**	**1.3**	**736**	**4.0**	**13**	**0.1**

The results showed significant effects of both mite type ($F_{1,13} = 42.75$, $P < 0.0001$) and date of observations ($F_{12,137} = 5.80$, $P < 0.0001$) for the infestation rate of both mite species. As there was a significant interaction between mite type and date of observation ($F_{12,136} = 4.39$, $P < 0.0001$), the differences in infestation rates between mite species were determined for each date of observation. Initially

in September 2011, the colonies began with a significantly higher infestation of *Tropilaelaps* (10.6 ± 2.8%) than *Varroa* (2.1 ± 1.0%) ($F_{1,136}$ = 20.94, P < 0.0001) (Figure 4.1). Thereafter, infestation levels of both mite genera decreased significantly although *Tropilaelaps* infestations remained higher than those of *Varroa* from October, November and December 2011 ($F_{1,136}$ = 23.55, P < 0.0001; $F_{1,136}$ = 8.76, P = 0.0036; $F_{1,136}$ = 4.74, P = 0.0311, respectively). Infestation by *Tropilaelaps* significantly decreased in January 2012, slightly increased in February 2012 with a small peak in March 2012, a gradual decrease in April 2012 and a steep decline in May 2012. Infestations by *Tropilaelaps* and *Varroa* were similar during these months (January, $F_{1,136}$ = 0.22, P = 0.6374; February, $F_{1,136}$ = 0.41, P = 0.5253; April, $F_{1,136}$ = 3.06, P = 0.0823; May,$F_{1,136}$ = 0.72, P = 0.3987) except in March 2012 when *Tropilaelaps* had a higher infestation than *Varroa* mites ($F_{1,136}$ = 4.24, P = 0.0413). Infestations by both mite species remained low in June 2012 ($F_{1,136}$ = 5.26, P = 0.0233). At this time, only four of the 15 surviving colonies were infested. Although infestations by both mite genera increased in July 2012, no difference in the rates of infestation between the mite species was observed ($F_{1,136}$ = 2.54, P = 0.1135). Infestation by both mite species similarly decreased in August ($F_{1,136}$ = 2.82, P = 0.0957) when only 11 colonies were sampled because several colonies were too weak to sample. Infestations increased again in September 2012 with *Tropilaelaps* having a higher rate of infestation than *Varroa* ($F_{1,136}$ = 15.21, P = 0.0002). Infestations during this month were also higher than the initial infestation observed in September 2011 for both mite species. However, only four colonies remained alive or strong enough to sample at this time. There was a significant negative correlation between brood area (cm^2) and *Tropilaelaps* infestation (r = -0.248; P = 0.0007). No correlation between brood area (cm^2) and *Varroa* infestation was detected (r = 0.023; P = 0.752). Only nine colonies (out of 16 colonies) produced drone brood during the entire experiment period. In total, 506 drone brood cells were produced throughout the experiment and all were examined. Only 13 drone cells (2.6%) were infested with *Tropilaelaps* and 78 (15.4%) were infested with *Varroa* mites.

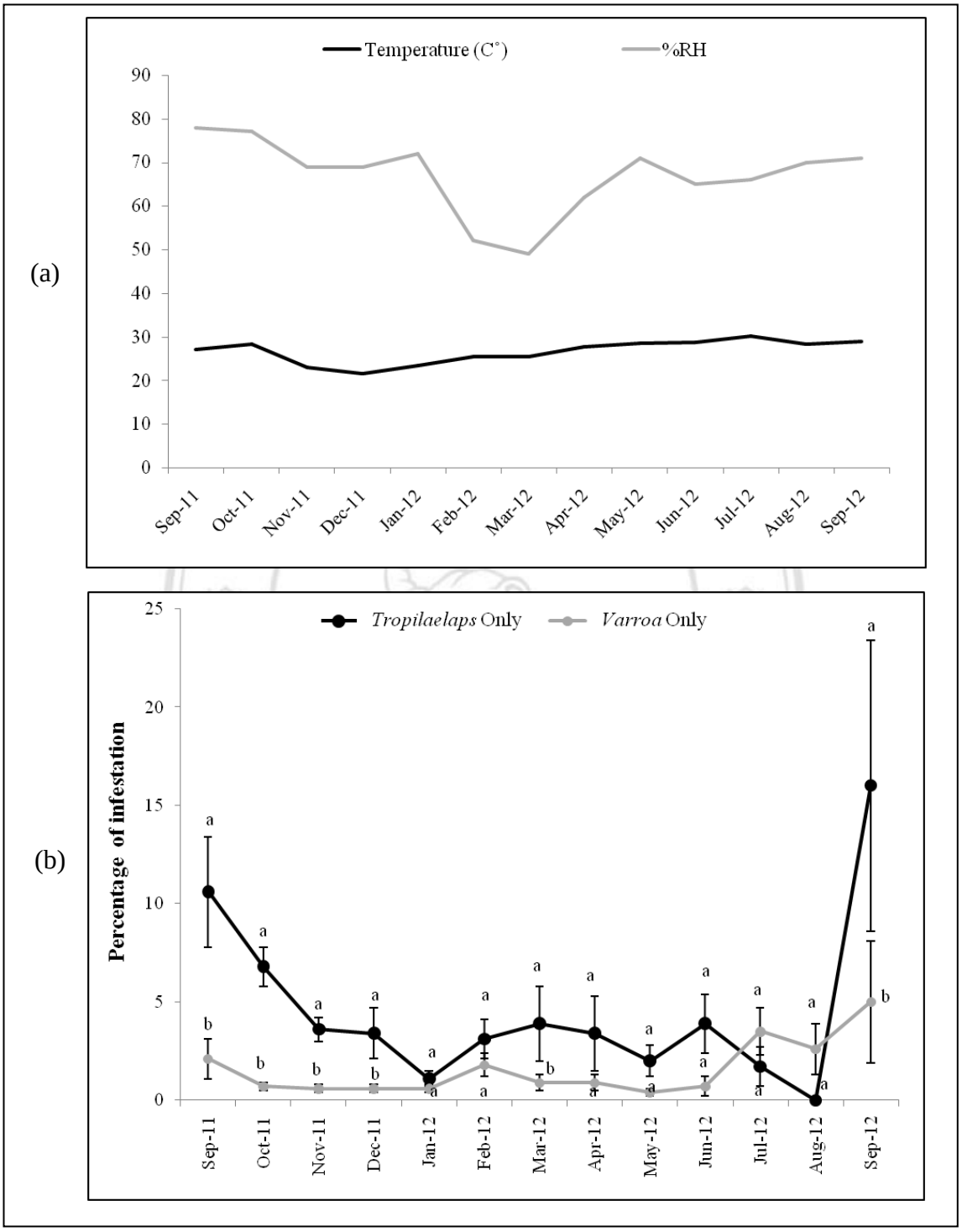
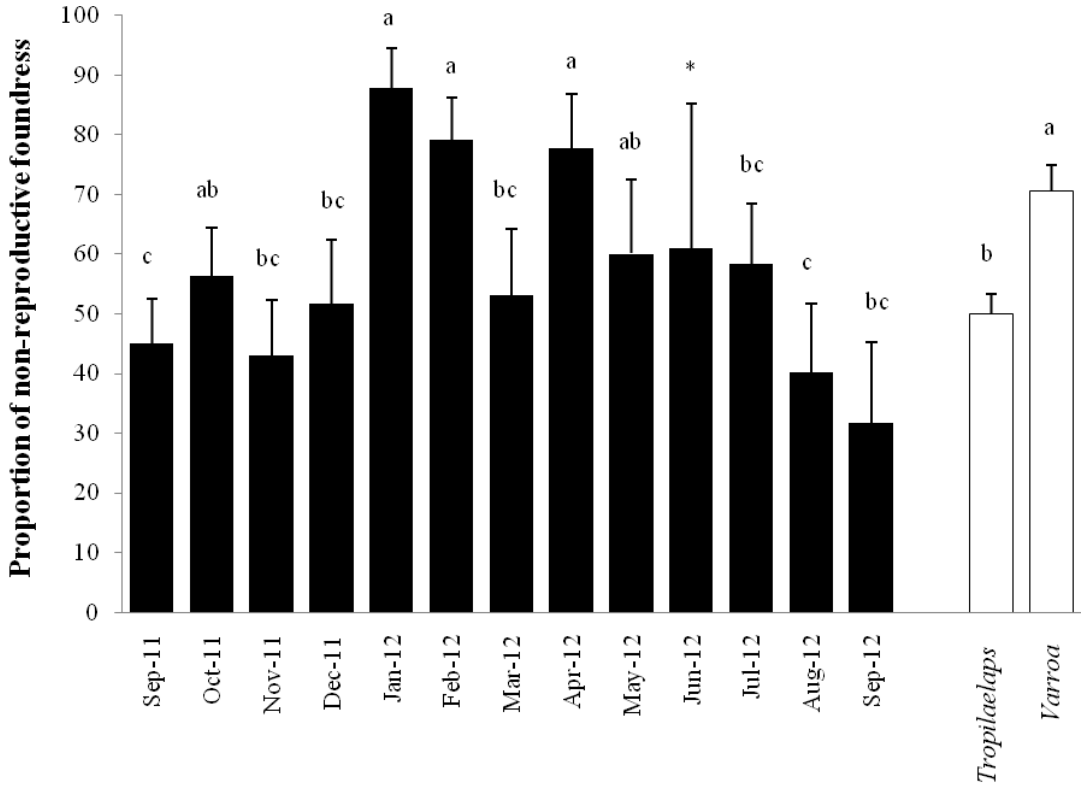


Figure 4.1 (a) The average temperature (C°) and %RH in Chiang Mai metropolis (b) Seasonal fluctuation (mean ± SE) of *T. mercedesae* and *V. destructor* in worker brood cells of concurrently infested in *A. mellifera* colonies, September 2012-September 2013.

A separate examination of three concurrently infested colonies showed a similar trend. Once more, the co-infestation of *Tropilaelaps* and *Varroa* mites in the same brood cell was rare. Out of 1,230 worker brood cells examined, only four cells (0.3%) were concurrently infested. *Tropilaelaps* was more predominant than *Varroa* in worker brood cells with an average infestation of 19.9% (*Tropilaelaps*) and 0.7% (*Varroa*). Drone brood (n = 481 cells) infestation was low; *Varroa* (2.5%) infestation was higher than *Tropilaelaps* (1.9%), and no concurrent infestations were observed in the drone brood. Also, adult bee phoresy (average = 484 bees/colony) in these three colonies was very low: *Tropilaelaps* = 0.31%; *Varroa* = 0.16%.

Regarding the proportion of non-reproductive mites, ANOVA revealed significant mite species ($F_{1, 13} = 15.05$; $P = 0.0019$) and date of observation ($F_{12, 95} = 3.29$; $P = 0.0005$) effects for the proportion of NR foundress (Figure 4.2) mites. However, no interaction between mite species and date of observation for the proportion of NR foundresses was detected ($F_{11, 24} = 1.41$; $P = 0.2311$). Regardless of mite species, the highest proportion of NR foundresses were observed in January 2012 which is comparable to the proportions recorded in December 2011, and February 2012 to July 2012 except for June 2012 when only four of the colonies were infested and at very low levels. The lowest NR was detected in November 2011 and was comparable to the proportions observed in nearly all the observation dates except for January and February 2012. Overall, there were more *Varroa* mites that did not reproduce as compared to *Tropilaelaps*. Further, both mites produced similar numbers of progeny when observed in the purple-eyed and tan-bodied pupal stages **(***Tropilaelaps* = 1.48 ± 0.05; *Varroa* =1.69 ± 0.14 progeny per foundress) ($t = 0.88$, $P = 0.381$). A similar trend was observed when younger brood (prepupae and older) were included ($t = 1.42$, $P = 0.156$): *Tropilaelaps* = 1.34 ± 0.05; *Varroa* =1.45 ± 0.13 progeny per foundress. Of the 13 brood cells naturally infested with both mites, seven (54%) supported reproduction of both mite species.



*June is non-estimable in the means comparison because of low sample size (infested colonies = 4).

**Black bars indicate the proportions of NR both *T. mercedesae* and *V. destructor* foundresses in each month. White bars represent the average NR proportions for *T. mercedesae* and *V. destructor* throughout the year (infested colonies = 16).

Figure 4.2 Proportion (mean ± SE) of non-reproductive foundress (did not produce any progeny) in naturally infested brood cells.

4.3.3 Interspecific competition between *Varroa* and *Tropilaelaps* co-infesting individual brood hosts

A total of 254 tan-bodied pupae inoculated with both mite species, as newly sealed larvae, were recovered and examined for mite reproduction after nine days. These observations found that the reproduction of *Tropilaelaps* and *Varroa* was similar in brood cells deliberately infested with both mite species ($z = 1.84$, $P < 0.01$). By using criteria 1, 45% of the co-infested brood hosts displayed reproduction by both mite species. Utilizing criteria 2, 52% of the co-infested brood hosts displayed reproduction by both mite species (Table 4.2). Only 15% of the inoculated brood cells had both mites that did not produce any progeny. When both mites were artificially inoculated into the same brood cell, *V. destructor*

produced more progeny per foundress (2.2 ± 0.1) than *T. mercedesae* (1.5 ± 0.1) (t = 5.31, $P < 0.0001$).

Table 4.2 Reproduction of *T. mercedesae* (T) and *V. destructor* (V) when co-infested single host pupa (n = 254 brood cells that were deliberately infested; NR= non-reproductive mite; R= reproductive mite).

Reproductive status	Criteria 1	Criteria 2
VNR, TNR	18.1% (n = 46)	15.0% (n = 38)
VNR, TR	24.0% (n = 61)	17.3% (n = 44)
VR, TNR	13.0% (n = 33)	16.1% (n = 41)
VR, TR	44.9% (n = 114)	51.6% (n = 131)

4.4 Discussion

V. destructor and *T. mercedesae* have been concurrently infesting *A. mellifera* colonies for more than 50 years in Asia (Delfinado, 1963). These experiments assist in explaining why *T. mercedesae* is competitively superior to *V. destructor* in co-infested *A. mellifera* colonies in Northern Thailand. The infestation rates by both mite genera fluctuated throughout the 13-month study period and showed almost identical patterns. Although infestations by *Tropilaelaps* were consistently higher than that of *Varroa*, infestations in January to August except in March were not significantly different. Based on our two assessments, the abundance of *Tropilaelaps* over *Varroa* observed in this study agreed with previous observations that *Tropilaelaps* outcompetes *Varroa* when co-infesting the non-adapted host *A. mellifera* colonies (Burgett et al. 1983; Pettis et al., 2012). Our observations are in contrast to what has been shown in South Korea where *V. destructor* infestation rates are greater than *Tropilaelaps* infestations (Lee et al., 2005). We offer several explanations as to why *Tropilaelaps* was more abundant than *Varroa*. The *V. destructor* haplotypes for South Korea (K1) and Thailand (J1) (Navajas et al., 2010) may result in the different infestation rate of *Varroa* since K1 is

considered to be more virulent than J1 (Solignac et al., 2003). It is also possible that the dramatically different climatic conditions for Korea (temperate) compared to Thailand (tropical) contribute to this discrepancy in prevalence. Extended brood production in tropical biomes (*i.e.* Thailand) offers a constant supply of hosts for mite reproduction (de Guzman et al., 2007). In contrast, a break in brood production during cold weather (*i.e.* South Korea) forces *Tropilaelap*s to have a longer phoretic period (Woyke, 1987b; Koeniger and Muzaffar, 1988; Rinderer et al., 1994) since *Tropilaelaps* has a limited ability to feed on adult bees. Unlike *Varroa*, the gnathosomal structure of *Tropilaelaps* is only meant to puncture soft integuments (Griffiths, 1988) such as those of a developing bee. In addition, Khongphinitbunjong et al. (2013) suggested that a phoretic phase may not be required for successful *Tropilaelaps* reproduction as compared to *Varroa.* Having a short life cycle may also facilitate the abundance of *Tropilaelaps* (Woyke, 1987b). Emergence of *Tropilaelaps* F_1 sons and daughters has been observed at the red brown-eyed pupal stage (Woyke, 1987b; Ritter and Schneider-Ritter, 1988).

The dominance of *Tropilaelaps* over *Varroa* may also be influenced by their ability to reproduce. Both mite species produce similar numbers of progeny on average. However, higher proportion of *Tropilaelaps* (70%) than *Varroa* (50%) produced at least one progeny. This ability to reproduce even just one progeny may increase the population of *Tropilaelaps* faster than *Varroa* mites. Woyke (1987c) speculated that *Tropilaelaps* have the ability to mate outside their natal cells. Thus, mating failure is probably very limited in *Tropilaelaps*. In contrast, *Varroa* needs to mate within the host cells before the adult bees emerge. The lack of males within brood cells, often as a result of hygienic activities, will result in mating failure in *Varroa* (Kirrane et al., 2011). Also, all *Tropilaelaps* progeny reach adulthood before the bee emerges (Ritter and Schneider-Ritter, 1988). In contrast, not all progeny of *Varroa* develop to adulthood at bee emergence. Further, *Varroa* only lays one male egg while *Tropilaelaps* lay male and female eggs in equal numbers (Ritter and Schneider-Ritter, 1988), which may increase cross breeding or mating success. Thus, the overall population increase of *Tropilaelaps* at the colony host level is greater than that for *V. destructor* when parasitizing *A. mellifera*, the non-adapted host.

When infesting *A. mellifera*, *Varroa* is known to prefer drone brood over worker brood (Fuchs and Lagenbach, 1989). The low infestation rate of *Varroa* mites on drones may be associated with the minimal production of drone brood during this study. Of the total number of potential brood hosts examined in this study (18,756), only 506 were drone brood (2.7%). Although we found more drones infested with *Varroa* than *Tropilaelaps*, we cannot conclude whether or not *Tropilaelaps* prefer worker over drone brood because of the limited production of drones. From our results, the infestation rate of drone brood by *Varroa* mites was 3.6 times more than worker brood. For *Tropilaelaps* mites, the infestation rate of worker brood was 9.4 times greater than that of drone brood. Woyke (1987a) reported that worker brood experienced a similar level of *T. clareae* (most probably *T. mercedesae*) infestation as drone brood while drone brood showed a higher *Varroa* infestation. In addition, when infesting its indigenous host, *A. dorsata*, *T. mercedesae* does not exhibit an immature host sex preference, *i.e.*, drone and worker brood experienced similar infestation rates (Buawangpong et al., 2013).

The removal of *Varroa* infested brood interrupts mite reproduction in *A. mellifera* VSH bees (Harbo and Harris, 2009). I did not monitor brood removal in my colonies. Nevertheless, increased removal of *T. mercedesae* inoculated brood has been observed in *A. mellifera* colonies in Northern Thailand (Khongphinitbunjong et al., 2013). In addition, decapped *Tropilaelaps*-infested brood is frequently observed by Thai beekeepers (Pettis et al., 2012). The removal of *Tropilaelaps* infested brood at early pupal stages when all mite progeny are still young may affect mite population growth (Khongphinitbunjong et al., 2013). However, merely decapping brood may not have a negative impact on *Tropilaelaps* population since all mite progeny can continue to develop or may have reached adulthood in older pupae. Adult female *Tropilaelaps* that are released following eclosion of callow bees from brood cells may invade new hosts and continue to reproduce. The high mobility of *Tropilaelaps* may partially protect them from adult bee aggression. In Thailand *A. mellifera* were not as responsive to phoretic *Tropilaelaps* as *A. cerana* or *A. dorsata* in cage experiments (Khongphinitbunjong et al., 2013).

The co-infestation of a single host by *Tropilaelaps* and *Varroa* is rare; an observation also reported by Ritter and Schneider-Ritter (1988) and Burgett et al. (1989) with the *Acarapis* species complex. In this study, avoidance of an infested cell may be one of the reasons for such a low mixed-genera infestation. It is possible that a blend of chemicals or volatiles produced by the resident *Tropilaelaps* itself or from their feces and wounds to honey bee hosts deters *Varroa* mites from invading. *Varroa* submerge in the larval food of a L4 larvae after invasion. Thus, we are unsure if they too are able to produce these volatiles while being submerged. It is also unlikely that the mites are competing for food or space since infestations by both mite species were generally low and that brood was available. We found that both mite species reproduced similarly when introduced together in the same brood cell. The reproductive fecundity of *T. mercedesae* may contribute to their higher prevalence, an indication of increased virulence of this mite species for *A. mellifera* colonies in Thailand. Also, possible infections from other pathogens vectored by *Tropilaelap*s *e.g.* DWV virus (Dainat et al., 2009, Forsgren et al., 2009, Khongphinitbunjong et al., 2013) can have synergistic effects on the overall health of infested colonies and should be of consideration for further studies.