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Biology and control of the parasitic bee mite *Tropilaelaps clareae*

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Introduction

After *Apis mellifera* was introduced to South-East Asia, two parasitic mites *Varroa jacobsoni* and *Tropilaelaps clareae* have been causing severe problems to beekeeping with that bee (Crane 1968).
T. clareae is considered to be more destructive to *A. mellifera*, (De Jong et al 1982; Nyein and Zmarlicki 1982; Burgett et al 1983; Woyke 1984).
The population of *T. clareae* in European bee colonies built up much faster than that of V. jacobsoni (Nuein and Zmarlicki 1982, Woyke 1984). Logically, it was believed, that the reproduction rate of T. clareae must be faster, than that of V. jacobsoni.
One of the major question regarding *T. clareae* concerns its potential escape from tropical Asia and whether it could become established on *A. mellifera* in temperate regions.
The life cycle of *T. clareae* was believed to be similar to that of *V. jacobsoni* (De Jong et al 1982). Therefore similar methods were applied to combat *T. clareae* as were practiced to control *V. jacobsoni.* However, some methods which were effective in decreasing V. jacobsoni population like Folbex treatment were ineffective in decreasing T. clareae population. (Laigo and Morse 1969 and Atwal and Goyal 1971). The reason for this was not known.
Woyke (1984, 1985a) showed, that *T. clareae* may be combated without the use of any chemicals. Reviews of earlier publications on *T. clareae* are given by (De Jong et al 1982, Burgett et al 1983, and Burgett and Akratanakul 1985).

Infestation of honeybee colonies by Tropilaelaps clareae

T. clareae parasiting honeybee colonies was reported from several countries (Crane 1968, Nixon 1983), but most of the reports are in form of short notes.
Detailed examinations conducted by Woyke (1987d) in Afghanistan in 1984, revealed up to 54% and on average 24 % of brood cells infested by Tropilaelaps clareae in May, and up to 86% and on avg. 31 % of cells infested in September (Table 1).
No V. jacobsoni mites were found in Afghanistan.
High infestation of brood by *T. clareae* - up to 62 % and on avg. 46.2 % was found in south Vietnam. V. jacobsoni infested there on the average only 4,9 % of brood cells.
In North Vietnam, average brood infestation by *T. clareae* was lower. Out of 9 apiaries examined, infestation level was below 5 % in three apiaries, 13 - 20 % in five apiaries and 35.1 % infestation was found in one apiary only.
Thus high infestation of bee brood by T. clareae was found in the more tropical conditions in Afghanistan and in south Vietnam. Low infestation occurred in the temperature conditions in north Vietnam. *V. jacobsoni* infested bee brood in low percentage in both parts of Vietnam.

Relationship between brood and adult worker bees infestation by both mites

Average infestation of adult worker bees by T. clareae was very low, mostly below 2,0 % (Table 1). Percentage of brood cell infestation was in hot climate, in Afghanistan and in south Vietnam, 13.5 % - 16.0 times higher than adult worker infestation. In temperate climate in north Vietnam, the brood infestation was even 26.1 - 36.9 times higher, than that of worker. Thus, very few mites were found outside brood cells in the temperate climatic conditions. The ratio of brood to worker infestation was in the temperate climate about twice as high as in the hot climate.
V. jacobsoni infested always higher percentage of adult workers (5 %), than *T. clareae* (1.5%), although the first infestated much less bee brood (13.5 %), than T. clareae (36.5 % Tab. 1 and 5). Between others, the ratio of B/W infestation is higher when the mites stay outside sealed brood cells for shorter time. This can be correlated with the outside temperature. In XuanLoc apiary in south Vietnam, the temperature in February was about 30C and T. clareae B/W infestation was 13.5. Temperature around Hanoi was in this time same month only 13C and B/W infestation was 27.7 being more than twice as high. The fresh air circulating inside bee hives may force the mites to enter the brood cells earlier.

Table 1 : Relationship between average percentage infestation of brood (B) and worker bees (W) by parasitic mites.

Place	Month	No. colonies	% infestation						
			B	V. jacobsoni		T. Clareae		T.B./W	
				W	B/W	B	W	B/W	V.B/W
							Afghanistan		
Kabul	5	10	-	-	-	24.0	1.5	16.0	-
Kabul	9	6	-	-	-	31.3	2.3	13.6	
							South Viet Nam		
X.Loc	2	6	4.9	6.3	0.8	46.2	3.4	13.5	17.3
							North Viet Nam		
L. Son	2	6	8.2	1.0	8.2	16.6	0.6	27.7	3.4
D. Giao	10	6	19.5	6.7	2.9	28.7	1.1	26.1	9.0
D. Giao	11	3	14.3	4.0	3.6	48.0	1.3	36.9	10.3

Table 2 : Average infestation of Worker (W) and Drone (D) brood cells by V. jacobsoni and T. clareae in North Vietnam 1985

month	no. colonies	no. brood cells examined		% with V. jacobsoni			% with T. clareae		
		W	D	W	D	D/W	W	D	D/W
Hanoi, Lon son :									
Feb.	8	715	650	2.3	14.9	6.5	3.9	0.5	0.13
Thai Binh, Moc Chau :									
July Aug.	13	1300	1300	1.8	5.6	3.1	1.9	2.5	1.32
Long Son, Drog Giao :									
Nov.	2	150	75	5.5	40.0	7.2	7.0	5.0	0.71
Total	23	2165	2025	2.3	11.8	5.1	3.0	2.0	0.67

Comparison of worker and drone brood infestation by both mites

The phenomenon of higher infestation of drone than worker brood by *V. jacobsoni* is well known. This was the basis for elaborating a method to lower mite population by trapping them to drone brood which was next destroyed. Similar method is practiced in some countries to control *T. clareae.* According to Burgett et al (1983) *T. clareae* infested in Thailand 10 - 90 % of worker brood and 80 - 90 % of drone brood. Woyke (1987d) compared worker and drone brood infestation in Vietnam in 1985. Together 2165 worker and 2025 drone brood cells were examined in 23 honeybee colonies. Detailed records showed, that *V. jacobsoni* infested 3.1 - 7.2 % times more drone than worker brood (Table 2). On the average 5.1 times more drone than worker brood was infested.
Drone brood infestation by T. clareae was 0.13 to 1.32 of that for worker brood. Average, drone brood infestation by T. clareae was 0.67 of that for worker brood. Thus contrary to *V. jacobsoni, T. clareae* infested 1.5 times more worker than drone brood.
Thus, no justification was found for a method to trap *T. clareae* to drone brood, and then to destroy it in order to reduce population of that mite.

Onset of egg laying and shape change of Tropilaelaps clareae

According to direct reports (Grobov et al. 1983; Glinski & Chmielewski, 1984) or indirect ones (Kitprasert, 1984; Ritter & Schneider-Ritter, 1988; Akratanakul, unpublished), T. clareae females lay eggs in cells which contain older bee larvae, before the brood is sealed.
Woyke (1989) recorded in China the time in which brood cells were sealed in colonies infested with *T. clareae*. The cells were opened at successive 4h intervals, and bee brood developmental stage was determined as well as presence of *T. clareae* females, eggs and developmental stages. The size of *T. clareae* females was also recorded as a measure of their readiness to oviposit.

The earliest egg was found in a cell containing a prepupa 48-52 h after cell sealing. *T. clareae* eggs and larvae were then found until observations ceased, 88-92 h after sealing.
The thickness of females moving freely on combs was 0.30 mm (Fig. 1a). In cells with spinning bee larvae, the thickness of female mites increased after brood sealing as follows: 0-8 h, 0.31-0.35 mm (Fig. 1b); up to 24 h, 0.43 mm (Fig. 1c); and up to 44 h, 0.50 mm. In cells with prepupae, until 48 h, further increase up to 0.56 mm was noticed. Afterwards, until 96 h after cell sealing, the thickness of T. clareae females varied between 0.58 and 0.64 mm (Fig. 1d). Thus the thickness of the females, 48 to 96 h after cell sealing, doubled in relation to that of mites moving freely on the combs.
In this time of maximal thickness of *T. clareae* females, the first and further eggs, were found in the cell.

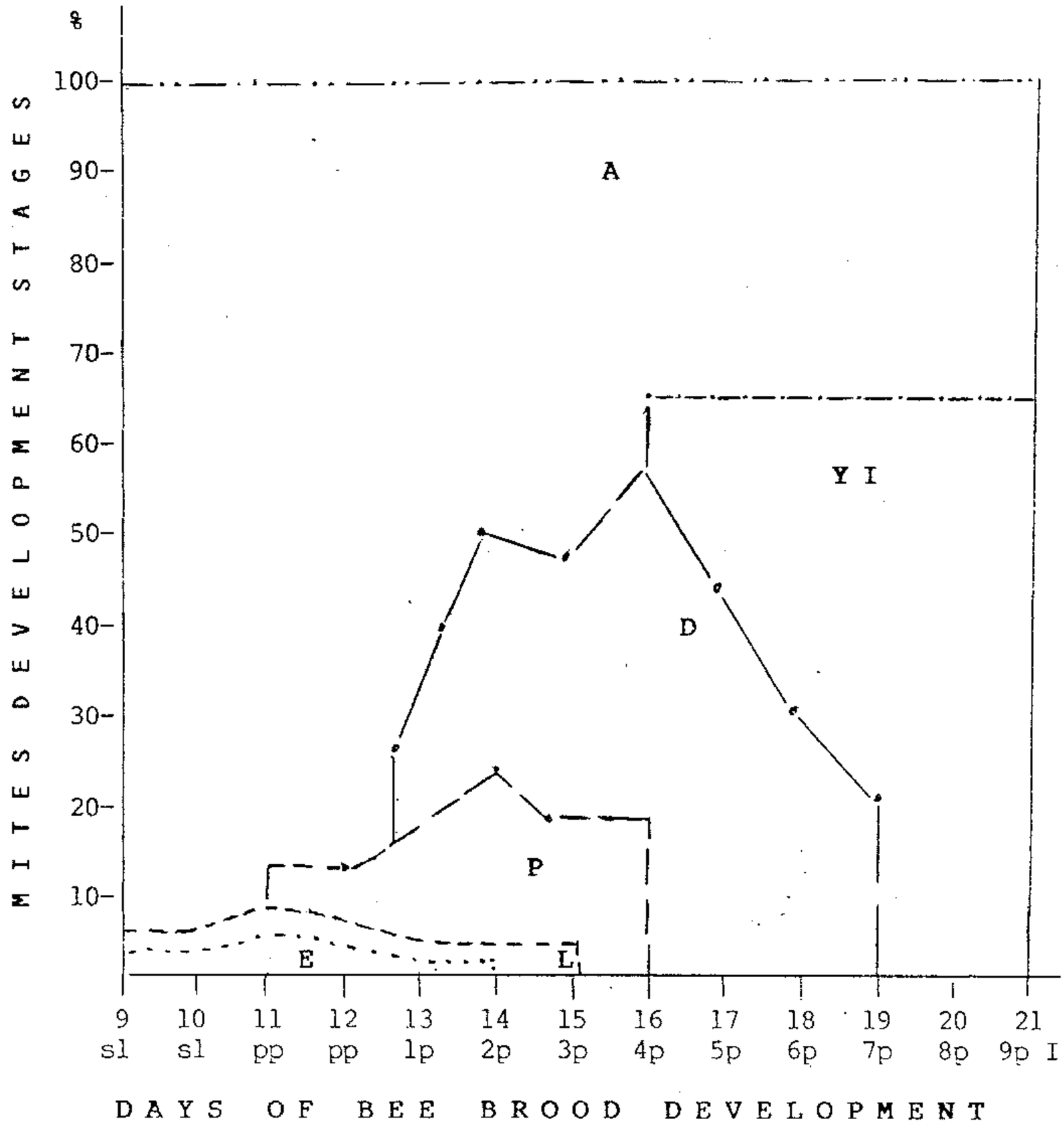

Figure 1 : Percentage contents of Tropilaelaps clareae development stages (E-egg, L-larva, P-protonymph, D-deutonymph, YI-young imago, A-adult) in comb cells with bee brood of different age (sl-spinning larva, pp-prepupa, Ap-9p-pupa 1-8 days old, I-amago).

Swollen females with an egg half-way out of the genital orifice were found, but this was never seen in thin females. Swollen females were found on older spinning larvae and prepupae as reported by Woyke (1987a, 1989) and Ritter and Schneider-Ritter (1988). No eggs were found in cells with thin females, both shortly after brood sealing as well as in those with older bee pupae. It is therefore concluded, that only swollen females lay eggs. No swollen females were found among *T. clareae* mites moving freely on brood combs. It is therefore concluded that *T. clareae* females do not lay eggs in brood cells before they are sealed, but they start oviposition 40 or 48 h after cell sealing.

Development of Tropilaelaps clareae

Only two papers describe development of T. clareae. One is an English abstract A.A.1341/85 of an unpublished M. Sci. thesis of Kitprasert (1984) written in Thai language, and the other is of Woyke (1987c).
Kitprasert investigated the development of *T. clareae* directly in the laboratory. All the eggs died, and out of 60 larvae only 4.7 % reached the imaginal stage. Woyke investigted 1161individuals of different development stages in worker brood cells in bee colonies. The development periods of successive *T. clareae* stages were calculated from the percentage participation of those stages in mites

contents of infected brood cells. Results obtained by both authors differ (Table 3). The total development period according to Kitprasert is 8.76 days (roughly 9 days) and according to Woyke only 6 days. The lengths of successive T. clareae stages were : eggs 0.4 day ; larvae 0.6 day ; protonymphs 2 days and deutonymps 3 days (Woyke 1987c).

Table 3 : Duration of development periods (in days) of successive T. clareae stages

Develpmental stages	Afghanistan 1984	Vietnam 1985	Rough average both countries	Kitprasert 1984/1985
Egg	0.35	0.47	0.4	1.05
Larva	0.78	0.35	0.6	1.85
Protonymph	2.34	1.98	2	2.11
Deutonymph	2.53	3.20	3	3.75

If the duration of *T. clareae* development was 9 days, and first imagines appeared on bee brood 16 days old (Kitprasert 198 and Woyke 1987c), than the first eggs should have been deposited on larvae 4 days old, 1.5 day before sealing of the brood. However, at this time T. clareae females are thin and do not lay eggs. Thus the duration of 8.76 days of development period of *T. clareae* reported by Kitprasert seems to be too long. This was probably caused by investigating the development of *T. clareae* in the laboratory, where the conditions were worse than in bee colony.

Reproduction

Reproduction of *T. clareae* was investigated by Woyke (1987d) in Afghanistan in 1984 and in Vietnam in 1985. Average infestation of brood cells in colonies investigated in Hanoi, Vietnam was 26 % and was considered as low and in 4 colonies in Kabul, Afghanistan was 72% and was considered as high. Thus parameters of reproduction in high and low infested colonies could be compared.
Results present in Table 4 show, that in low infested colonies in Hanoi, on the average 1.3 females were found per cell. In the high infested colonies in Kabul, on the average 2.3 females were found per cell. Thus 1.8 times more females per cell were found in the higher infested colonies.
In the low infested colonies in Hanoi, 18.3 % of females did not, and 81.7 % did, to reproduce. Fertile females produced on the average 1.6 descendants only, and the average for all (lying and non laying) females was 1.3 descendants. In the high infested colonies in Kabul, 7,3 % of females only did not and as many as 92.7 % did to reproduce. On the average 2.1 descendants were produced by one fertile female. The average for all females was 1.9 offspring. Thus 1.5 times more offspring was produced in high infested colonies than in the low infested ones.
Concerning the total number of all development stages, on the average, 2.9 mites per cell were found in the low infested colonies. In the high infested colonies in Kabul, on the average 5.1 mites per cell were found. Thus 1.8 times more mites per cell were found in the high infested colonies. Up to 22 or 23 mites in one brood cell were found in some other bee colonies in Kabul.
Thus high brood infestation is characteristic not only by high percentage of brood cells infested, but also by higher number of adult females per cell, by higher reproduction rate, and by higher total number of mites per cell. Many bee prepupae on which 3 or more adult female mites were feeding did not pupae and died.

Sex ratio

Together 1284 males and 5138 females were found among 6422 *T. clareae* mites collected from bottom boards of bee colonies with queens caged for several days in Kabul. The sex ratio of males to females was 1 :4. Within those mites old females as well as their offspring were present. One old female produced in Kabul roughly two descendants (Table 4). Thus the ratio had to be 1 old female to 1.4 young female to 0.6 young males. This agrees with the proportion 1 old female to 2 descendants, as well as 1 male to 4 females. Within the offspring, the ratio of males to females was 1 :2.3. Sex ratio within mites running freely on combs in China was 1 male : 1.8 females (Woyke 1989). Thus the sex ratio within the offspring is probably 1 male to 2 females.

Survival of adult Tropilaelaps clareae

According to Woyke (1984) adult *T. clareae* mites survived on adult worker bees for 2 - 2.5 days. Kitprasert (1984/85) reported an average survival of 2.7 days. Thus results of both authors agree quite well. Woyke concluded, that adult mites are unable to feed on hemolymph of adult bees. On this

Table 4: Comparison of Tropilaelaps clareae populations in honeybee colonies with brood of low (Hanoi 26%) and high (Kabul 72%) infestation

Place	No. of mites average														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14 number
*															
Hanoi	-	77	20	2	1										1.3
Kabul	-	40	28	15	8	6	1	1					1		2.3
**															
Hanoi	18	39	30	10	2										1.3
Kabul	7	24	39	26	3										1.9
***															
Hanoi	-	11	34	26	18	8		2	1	1					2.9
Kabul	-	4	8	15	25	7	13	13	4	4	1	1	13	3	1 5.1

* - % Frequency of no. of adult female mite in 1 comb cell infested
** - % Frequency of no. of offspring per 1 female.
*** - % Frequency of total no. of mites in 1 comb cell infested.

basis Woyke (1984, 1985a) worked out a methods to combat T. clareae without the use of any medicine.

Table 5: percentage infestation rates (in %) of total cells examined of worker brood cells (B) and adult worker bees (W) by V. jacobsoni and T. clareae in Viet Nam.

Month	No. of colonies	V. jacobsoni			T. clareae		
		% b	% W	B/W	% B	% W	B/W
February	12	6.6	3.7	1.8	31.4	2.0	15.7
October	6	19.5	6.7	2.9	28.7	1.1	26.7
Novemb.	3	14.3	4.0	3.6	48.0	1.3	36.9
Average		13.5	4.8	2.8	36.0	1.5	26.4

Length of T. clareae stay outside sealed honeybee brood cells

The question how long *T. clareae* stays outside sealed bee brood before it enters again a cell to parasitize the brood is crucial for effective control of that mite.
The length of *T. clareae* stay outside sealed bee brood was investigated in an indirect and direct way (Woyke 1987b).
For indirect study, the ratio of brood to adult worker bee infestation by *V. jacobsoni and T. clareae* was compared. The results showed, that *T. clareae* must stay outside sealed cells for about 1.3 day only.
In the direct investigations, all bees and mites from infested colonies were brushed onto one comb with uninfested bee brood. Ten days later, the combs were removed from bee colonies, the sealed cells were open and the age of infected brood was determined. This way it was possible to determine the day in which the mite entered the cell.
The results showed, that most and probably all *T. clareae* mites entered brood cells again within 2-3 days after emerging. The average stay outside sealed brood cells was 1.6 day.

Why population of Tropilaelaps clareae grows faster than that of Varroa jacobsoni ?

V. jacobsoni female produces mostly 4 to 5 descendants. However, only 1-2 young *V. jacobsoni* females reach the adulthood at the time the worker bee emerges from the cell. *T. clareae* female produces mostly 1-2 and up to 4 eggs. All young T. clareae mites reach the imago stage before the worker bee emerges from the cell (Woyke 1987c).
Some females do not reproduce. Therefore the reproduction rate for one passage through the brood cell for *V. jacobsoni* female was 0.71 (Ifantidis 1984) or 1.3 (Schulz 1984). Average reproduction rate for T. clareae female in Kabul was 1.4 young females (Woyke 1987d). Thus the reproduction rate for one passage through brood cell was only little higher for T. clareae than for *V. jacobsoni*. All

this shows, that the reproduction rate is not the main factor responsible for the raid increase of *T. clareae* population.
Schultz (1984) reported, that *V. jacobsoni* females stay outside sealed brood cells on the average for 13 days. Woyke (1984, 1987b) concluded, that *T. clareae* females stay outside sealed brood cells on the average for 1.5 day. Thus, with similar reproduction rate, and the same 12 days stay in sealed brood cells, T. clareae can produce within 25 days, two generations while *V. jacobsoni* one only. So population of *T. clareae* increases in geometrical progresssion in ratio to that of *V. jacobsoni*. Thus the shorter period of *T. clareae* stay outside sealed brood cells must be considered as the main factor responsible for the more rapid increase of *T. clareae* population.

Control of Tropilaelaps clareae

T. clareae can be controlled both ways, without and with medication.
Woyke (1984) found that *T. clareae* can survive on adult worker bees in the absence of bee brood, for up to 2.5 days only. This phenomenon allowed to elaborate a method to combat the mite without the use of any drugs. There suffices to deprive bee colonies of all the brood for short period. Woyke (1984, 1985a) investigated three modifications of that method. The results obtained were as follows :

1. Queens were caged for more than 21 days. Within three days after the last worker bees emerged from the comb cells, the number of dying mites decreased considerably, and few days later no more mites were found on adult bees (Table 6).

Table 6 : infestation of brood cells (B) and adult workers (W) by T. clareae, and no. of mites falling to bottom board (FR) after the queen was caged.

Successive days	colony no.					
	2		3		4	
Infestation of bee colonies						
	% B	% W	% B	%W	% B	
	12	0.8	6+27	2.6	40	3
No. of mites falling to bottom board and worker infestation						
	no. fb	% W	no. fb	% W	no. fb	
1-3	66		109		86	
4-6	51		389		99	
7-9	142		417		247	
10-12	442	9	593		805	
13-15	622		733		666	
16-18	493		792		446	
19-21	555		395		307	
22-24	284		170		104	
25-27	11		18	0	7	0
26-30	2	0	0		7	
					0	

2. Queens were caged for nine days and then brood cappings were cut off, and all brood was shaken out. The number of dying mites falling on the bottom board decreased considerably within three to four days. Later no more mites were found on the adult workers (Table 7).
3. All brood was removed from the colonies. The number of dying mites decreased considerably within one to three days and no more mites were found on the workers few days later (Table 8). Thus *T. clareae* can be combated without the use of any medicine, just by depriving bee colonies of all the brood.
Beekeepers often do not wish to deprive bee colonies of brood by caging the queens or byremoving the brood out of the hive. They believe the colony will weaken too much. Therefore efforts ware made to combat *T. clareae* in the presence of bee brood. However 12 weekly treatments with Folbex conducted by Laigo and Morse (1969) as well as 6 weekly treatments applied by Atwal and Goyal (1971) were ineffective. Woyke (1987a) found out in Vietnam, that Folbex kills *T. clareae* in the same degree as it kills *V. jacobsoni* (Table 9). The efforts were ineffective in decreasing *T. clareae* popu-

Table 7: infestation of brood cells (B) and adult workers (W) by T. clareae and no. of mites falling to bottom board (FB) after the queen was caged and sealed brood was shaken out after nine days.

Successive days	colony no.					
	7		10		17	
	Infestation of bee colonies (% B)					
	54		86		72	
	no. mites	falling	to bottom		board and worker infestation	
	no. fb	% W	no. fb	% W	no. fb	% W
9-11	1072	19.3	1937	15.0	2443	19.3
12-14	283		1101		580	
15-17	21		220		128	0
18-20	17	0	22	0	31	
21-23			16			

Table 8: Infestation of brood cells (b) and adult workers (W) by T. clareae and No. of mites falling to bottom board (Fb) after the brood was removed from colony with queen free or caged.

Successive days	Queen Free					Queen Caged			
	colony no.								
	A	B	D	27		12		13	
	Infestation of bee colonies								
	% B	% B	% B	% B	% W	% B	%W	% B	%W
	8	8	43	47		46	3.9	74	2.0
	No. mites falling to bottom board and workers infestation								
	No. Fb	No. Fb	No. Fb	No. Fb	No. W	No. Fb	No. W	No. Fb	No. W
-3-1						1352		2974	
1-3	4	58	49	18		31		368	
4-6	4	8	7	8		11		38	
7-9	1	0	4	4		7		10	
10-12	1	0	2	2		5	0	7	0
13-15	1	0	2	7	0	2		15	

lation because *T. clareae* stays outside sealed brood cells for 2 days only. Thus, when treatments in weekly intervals were applied, *T. clareae* mites entered the cells again before the next treatment. In the presence of bee brood in the colonies, all short acting chemicals like, fumigation with Folbex, amitraz, or phenotiazine can not control *T. clareae*, even when their application is repeated several times. *T. clareae* can be controlled successfully in the presence of brood only by chemicals with prolonged action like formic acid or fluvalinate.

Table 9: Infestation of bee colonies by T. clareae (Tr) and V. jacobsoni (V) and results of Folbex (chlorobenzilate) treatment.

Colony No.	% brood infestation		% adult worker infestation before treatment		NO. mites killed		% mites killed	
	Tr	V	Tr	V	Tr	V	Tr	V
1	60	16	0.8	1.2	13	19	-	-
6	44	16	1.0	3.9	8	11	-	-
11	40	11	1.5	7.0	36	166	100	90.7

Table 10 : infestation of bee colonies by V. jacobsoni (V) and T. clareae (Tr) mites and results of chemical treatment.

Phenothiazine treatment								
Colony no.	brood		Adult workers		No. killed		% killed	
	% V	% Tr	% V	% Tr	V	Tr	V	Tr
57	6.8	62.0	8.0	3.0	10	12	3.8	60.0
34	1.4	51.7	1.3	0.7	3	8	46.2	57.1
44	5.6	21.1	2.6	0.9	2	2	69.2	55.6
Average	4.6	44.9	4.0	1.5	5.0	7.3	57.7x	56.4x
Amitraz treatment								
Colony no.	brood		Adult workers		No. killed		% killed	
	% V	% Tr	% V	% Tr	V	Tr	V	Tr
25	5.7	48.6	7.1	4.7	120	82	94.4	76.7
49	7.2	40.0	9.5	6.8	266	123	92.6	67.6
21	2.5	53.5	9.2	4.5	292	188	100.0	93.3
Average	5.1	47.4	8.6	5.3	226	131	95.7	79.2

For which climatic zones T. clareae might be dangerous ?

Fears were expressed, that *T. clareae* can escape from tropical Asia and become established on *A. mellifera* in temperate climate. Results presented above show, that *T. clareae* may become a dangerous parasite for *A. mellifera* in all tropical and subtropical zone. However, *T. clareae* will not become a severe pest of honeybee in temperate zones in which winter interruption of brood rearing occurs.

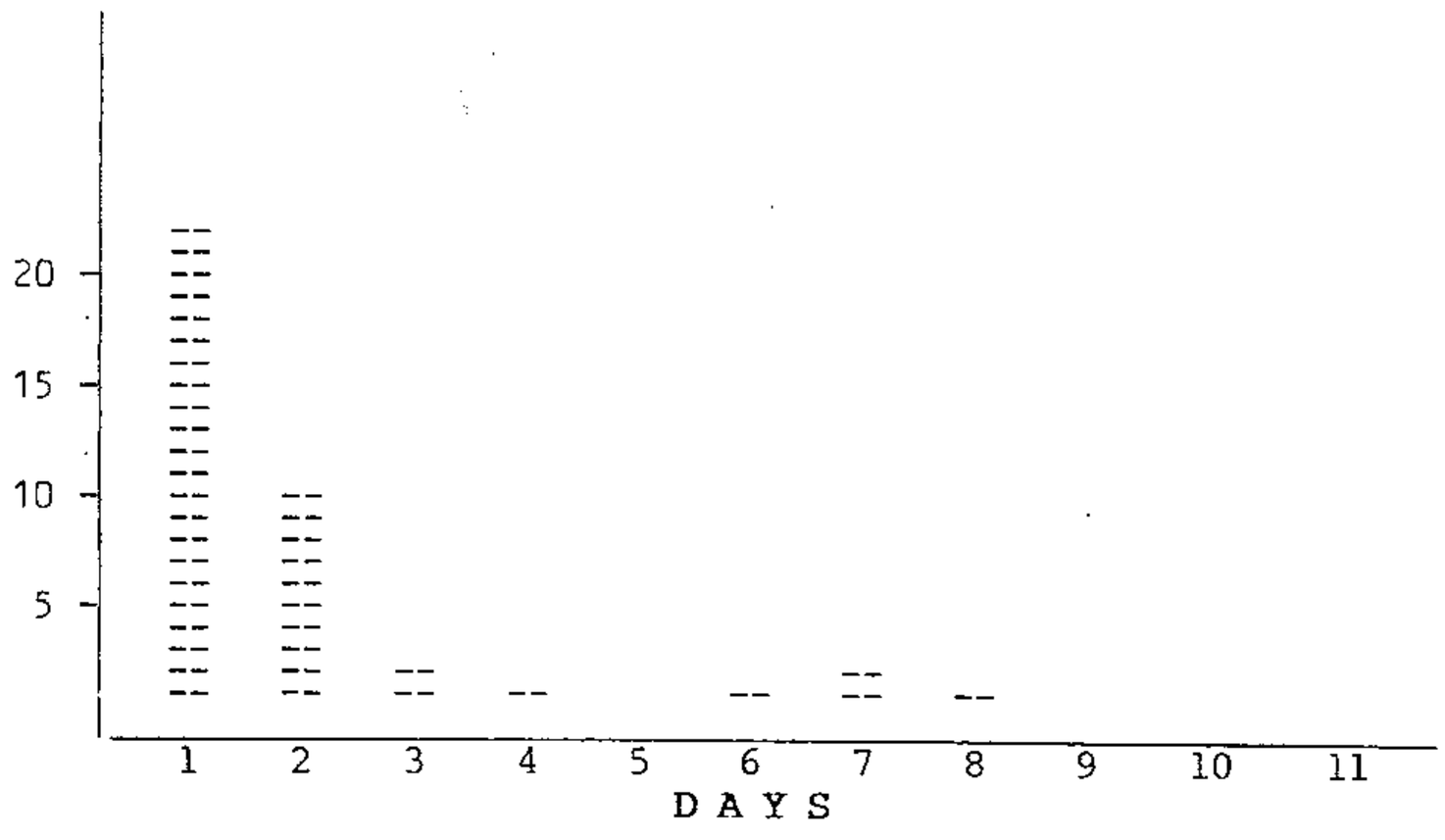

Figure 2 : Number of cells with T. clareae which were sealed in determined days, after an uninfested brood comb was introduced to bee colonies with mites 0-? hrs. old.

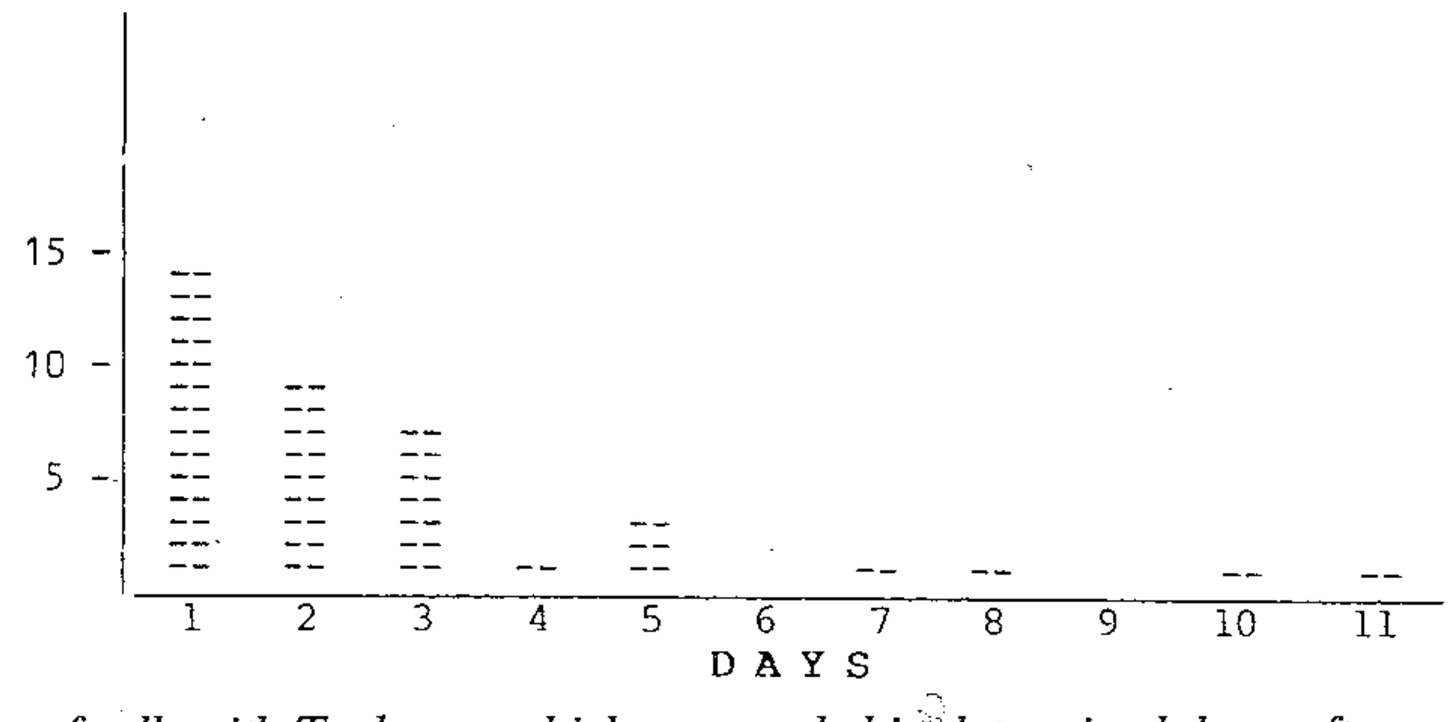

Figure 3 : Number of cells with T. clareae which were sealed in determined days, after an uninfested brood comb was introduced to bee colonies with mites which emerged within last 24 hours.

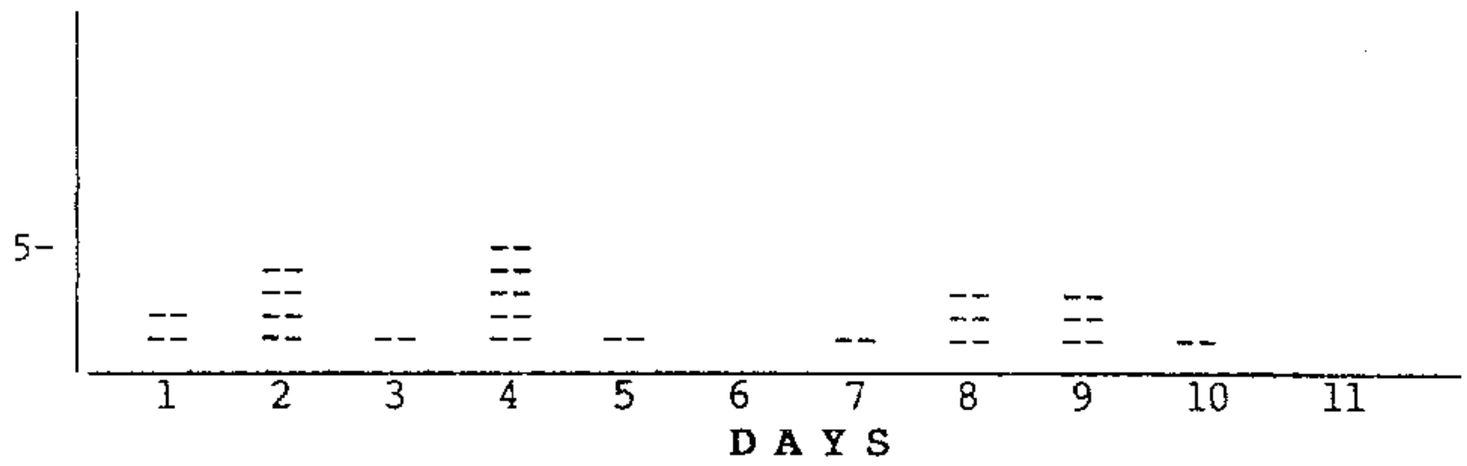

Figure 5: Number of cells with V. jacobsoni which were sealed in determined days, after an uninfested brood comb was introduced to bee colonies with mites which emerged within 24 hours.

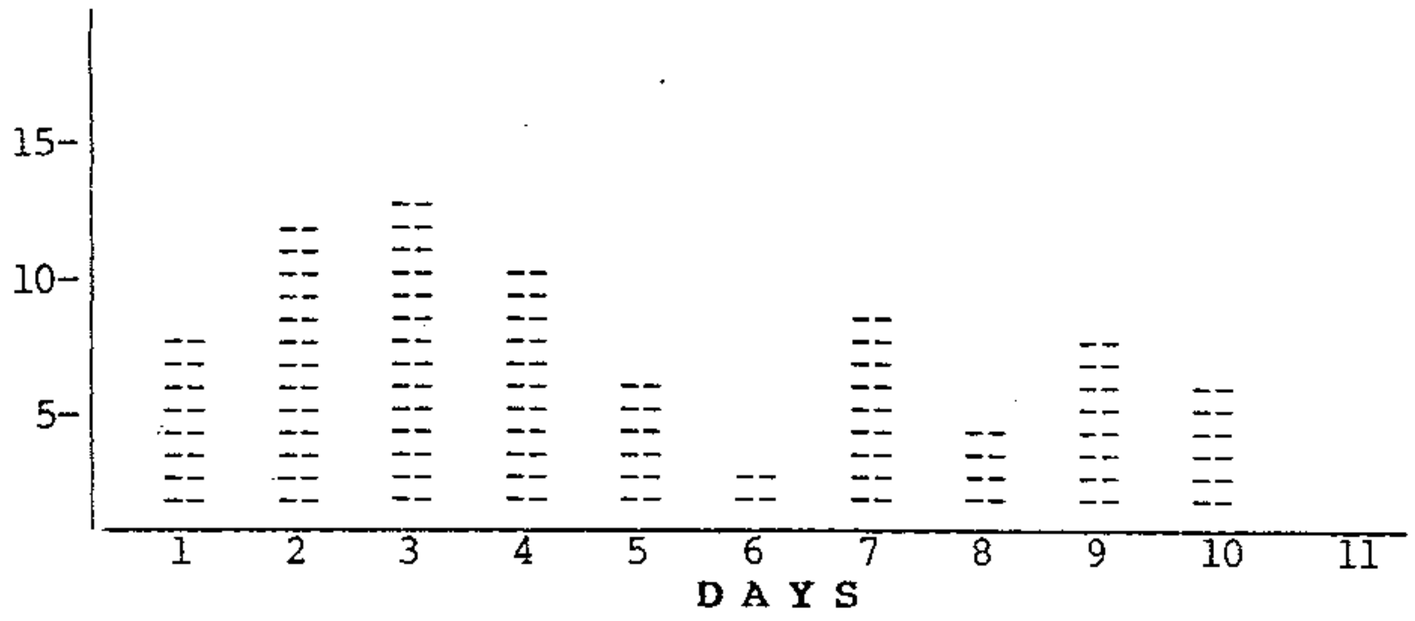

Figure 4: Number of cells with V. jacobsoni which were sealed in determined days, after an uninfested brood comb was introduced to bee colonies with mites 0-? hrs. old.

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