

Tropilaelaps clareae females can survive for four weeks when given open bee brood of *Apis mellifera*

J WOYKE¹

Institute of Apicultural Research,
Chinese Academy of Agricultural Sciences,
Xiang Shan, Beijing, China

(Received 23 March 1993,
accepted subject to revision 24 June 1993,
accepted for publication 9 September 1993)

SUMMARY

Female *Tropilaelaps clareae* mites were released into small petri dishes without food, or on small pieces of brood comb containing several *Apis mellifera* larvae 1–4 days old. On each day of the experiment the piece of brood comb was exchanged for a new one containing larvae of the age being tested. In dishes with no food only 5.5% of mites survived for two days. Survival of gravid female mites was significantly higher than that of thin females. On bee larvae 1, 3 and 3.5 days old, 0%, 9% and 47% of *T. clareae* females survived until the second day. None survived until the fourth day. However, on bee larvae four days old, 89%, 68%, 32%, 7% and 4% of females were alive on the 2nd, 5th, 10th, 19th and 28th day respectively. Thus, *T. clareae* females can survive for up to four weeks on bee larvae four days old. The amount of brood pheromones on larvae 4–5 days old is greater than that on younger larvae, probably stimulating feeding and thereby supporting mite survival. Fertilized female mites quickly become gravid, and they must enter cells containing bee larvae to lay eggs. *T. clareae* females do not need to feed on prepupae or pupae to lay eggs and to survive for longer periods. Queen honey bees need not be caged after brood removal from the colony in order to control the parasitic mite *T. clareae*, as by the time any eggs laid have developed into four-day-old larvae the mites will have died.

Keywords: honey bees, *Apis mellifera*, larvae, parasitism, pheromones, *Tropilaelaps clareae*, survival

¹Present address: Agricultural University-SGGW, Bee Division,
166 Nowoursynowska, 02-766 Warsaw, Poland

INTRODUCTION

The parasitic bee mite *Tropilaelaps clareae* Delfinado and Baker 1961 is considered to be more dangerous for *Apis mellifera* than is *Varroa jacobsoni*. Woyke (1984, 1985) found that *T. clareae* can survive on adult *A. mellifera* worker bees for two days only, and on this basis he elaborated a chemical-free method to control the mite. Caging the queen for three weeks or depriving the colony of all brood results in all *T. clareae* mites dying within a few days. Woyke (1987) showed that *T. clareae* females enter new cells containing brood within two days after emerging from the previous cell. Therefore the question arises whether *T. clareae* females can feed and survive on unsealed bee brood for more than two days. Knowledge of the possibilities of survival on different developmental stages of the honey bee is crucial for effective control of *T. clareae*. According to Kitprasert (1984) adult *T. clareae* females survive on bee pupae for an average of 28.5 days. On combs with eggs, no mites survive for more than two days (Woyke, 1984). No records exist concerning survival of *T. clareae* females on honey bee larvae.

MATERIALS AND METHODS

The investigations were conducted at the Institute of Apicultural Research of the Chinese Academy of Agricultural Sciences in Beijing, China, in July and August 1992. *T. clareae* mites infesting the local strain of *A. mellifera ligustica* were investigated. Forceps with thin points were used to catch and move mites, the points being placed near both sides of a running mite. As a result, the mite walked on to one point of the forceps. Next, the point of the forceps was placed on the surface of the petri dish used to study mite survival. The mite quickly walked into the dish.

The survival of thin and thick (gravid) *T. clareae* females (Woyke, 1989) was compared. Gravid female mites (as judged by being thick) were collected from cells containing honey bee prepupae. Thin

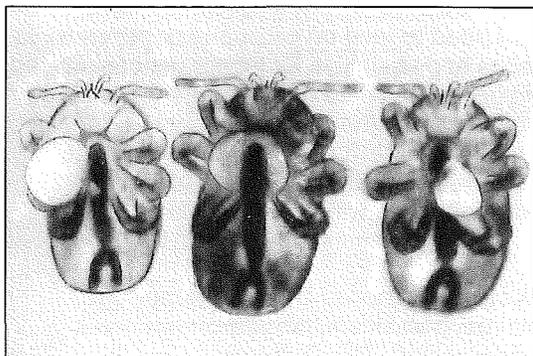


FIG. 1. *Tropilaelaps clareae* females with eggs half-way out of genital orifice. Notice the difference in the size of the females.

TABLE 1. Survival of thin and gravid *Tropilaelaps clareae* females in petri dishes without food.

Trial	Number of mites	Number alive	
		Day 2	Day 3
Thin females			
1	19	2	0
2	18	8	1
3	8	0	0
4	23	12	0
Total	68	22	1
Gravid females			
5	17	7	0
6	23	16	5
Total	40	23	5

mites (presumably having finished egg-laying and thus non-gravid) were collected from older bee pupae. Only small pieces of cell cappings were present in the petri dishes, to attract the mites.

For investigating survival on bee larvae, *T. clareae* mites were collected from combs containing emerging workers, or cells with workers ready to emerge. The mites were released into small petri dishes which contained a small piece of brood comb (3 × 1.5–2 cm) containing honey bee larvae. In each comb piece 10–12, 8, 8, and 5–6 bee larvae of the age 1, 3, 3.5 or 4 days old, respectively, were present. The age of larvae was judged by their size (Nelson *et al.*, 1924). Due to earlier experience in investigating honey bee larvae of an exact age (Woyke, 1963) it was easy to distinguish larvae 3.5 days old from those four days old. Bee larvae up to 3.5 days old were white, whereas larvae four days old had finished their last moult, were much larger and transparent, and their imaginal plates were visible (Woyke 1963). The combs were cut along the foundation so that larvae were present on one side of the comb only, and the length of the cells was shortened. Each day the piece of brood comb was exchanged for a new one containing larvae of the age being tested. In a pilot experiment I tried to feed the larvae with royal jelly or mixed food, however, this was not necessary since all larvae were alive the next day without additional feeding (larvae survived from the age of 4 days to the age of 6 days without feeding). Mostly, 4–5 *T. clareae* mites were released on a piece of comb, and the number of surviving mites as well as their behaviour was recorded each day. Dead mites were removed and their sex was determined based on the shape of the epigynial plate (Delfinado & Baker, 1961).

Petri dishes with the larvae were placed in an incubator at 34–34.5°C. Daily measurements showed an air temperature around 30°C and humidity around 90%, and the humidity in the petri dishes containing

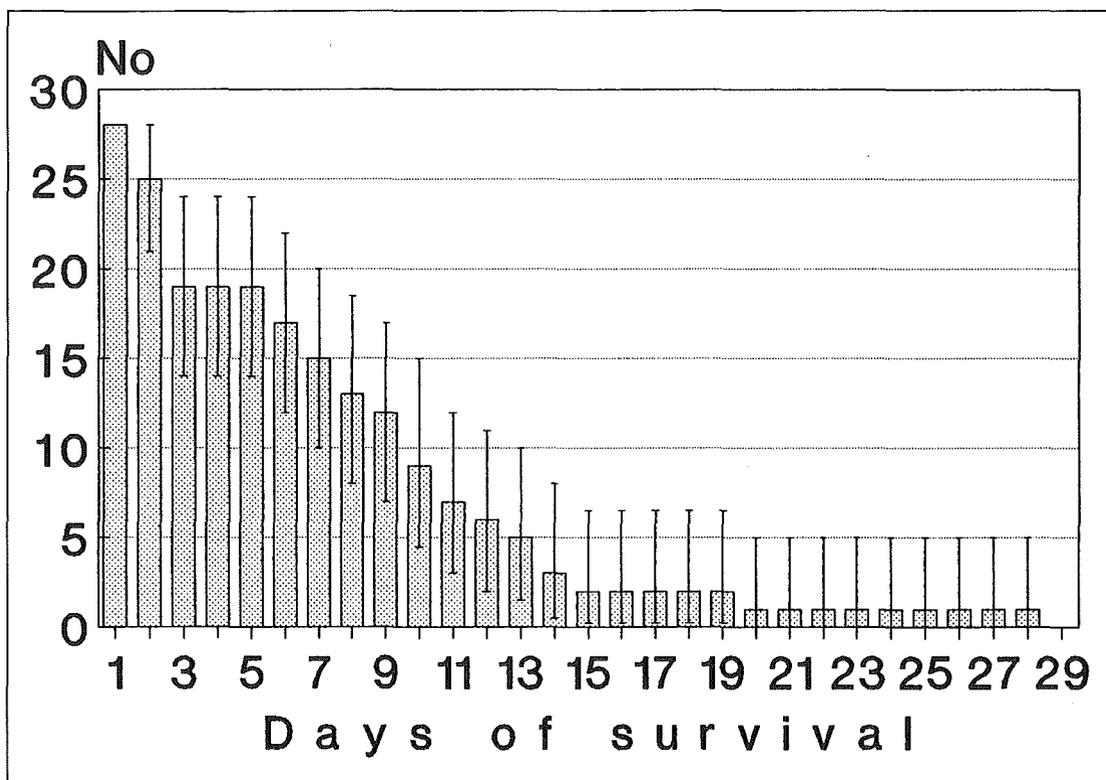


FIG. 2. Daily survival of 28 *Tropilaelaps clareae* females on honey bee larvae 4 days old. Bars represent the numbers alive. Error vectors represent the binomial 95% confidence intervals.

experiments stored in the incubator was probably a little lower.

RESULTS

Comparison of survival of thin and gravid female mites

Of 68 thin females, 22 survived one day and one survived two days; of 40 gravid females, 23 survived one day and five survived two days without food (table 1). The survival of gravid females was significantly higher than the survival of thin females ($\chi^2 = 6.76$; d.f. = 2; $P = 0.035$). In two dead gravid females, eggs protruding from the genital opening were found (fig. 1).

Survival of female mites on bee larvae of different ages

Mite survival increased on older bee larvae (table 2). No *T. clareae* females survived until the second day on bee larvae one day old; one of 11 mites was alive on the second day on larvae three days old; 16 and two mites out of 34 were alive on the second and third days on larvae 3.5 days old; 25 and 19 mites out of 28 were alive on the second and third days on larvae four days old. Thus, a significant larval age effect, with older larvae supporting survival, was detected (multivariate analysis of variance using Wilks' lambda: $F = 23.15$, d.f. = 2, $P < 0.0001$).

TABLE 2. Survival of *Tropilaelaps clareae* females on honey bee larvae 1–4 days old.

Trial	Age of larvae (days)	Number of mites	Number alive	
			Day 2	Day 3
1	1	17	0	0
2	3	11	1	0
3	3.5	34	16	2
4	4	28	25	19

On the 5th, 10th and 19th days, 19, 9 and 2 respectively of the original 28 females were alive (fig. 2). The last female was still alive on the 28th day of observation.

Why do *T. clareae* females stay outside brood cells for two days only?

After *T. clareae* females were released on the small pieces of brood combs in the petri dish, they were thin (0.30 mm) and were running rapidly on the edges of the cells. Females in trials with some males present became gravid after two days, and their thickness increased by up to 0.50–0.56 mm. The gravid females slowed in their movements and then became motionless. The females laid eggs inside the cells on walls, or on the honey bee larvae. Several times females with eggs protruding from the genital orifice were found (fig. 1).

In contrast, all seven females in one trial without males were thin and very active for 12 days. At that time, three males were added to the three females still alive. Within 4–5 days the thickness of two females which survived increased by up to 0.50 mm.

In some trials with one or two males (which died within 1–2 days) some females remained thin and very active. It seems that unfertilized females remain thin, do not lay eggs, and are very active. In a honey bee colony *T. clareae* females mate shortly after emerging from an infested cell. Within two days, they must enter cells with bee larvae ready to be sealed, because the females quickly become gravid and must lay eggs. The results show that *T. clareae* females do not need to feed on prepupae or pupae to lay eggs and to survive for long periods.

DISCUSSION

T. clareae females cannot survive on bee larvae up to 3.5 days old. However, many of them can survive for four weeks on larvae four days old. The females are surely able to pierce the epidermis of larvae younger than four days, since they can pierce the much harder epidermis of older pupae, so the thickness of the epidermis cannot be the cause of the differential survival of mites. Trouiller *et al.* (1991) showed that the amounts of pheromones and kairomones in the cuticle of worker honey bee larvae five and six days old are higher than they are in younger larvae. *T. clareae* female mites released in this investigation on bee larvae 4–4.5 days old remained on them until the next day when the larvae were 5–5.5 days old. Perhaps ectohormones are different on the epidermis of larvae 4–5 days old, stimulate feeding, and thereby support mite survival. Kairomones on 4–5-day old larvae are those which normally attract *T. clareae* females to enter into brood cells, and to feed on bee larvae of that age.

The results showed that *T. clareae* females can feed and survive on unsealed four-day-old larvae for more than 24 days. The length of that period is more than double the length of the reproductive cycle of the mite in sealed honey bee brood cells. Nevertheless, female mites enter the brood cell within two days of emerging. Thus, mated females quickly become gravid and must enter the bee brood cells to lay eggs.

One method to control *T. clareae* without chemicals is to remove all the brood from a bee colony (Woyke, 1984, 1985, 1993a). This investigation suggests that the queen does not need to be caged after brood removal, since eggs she may lay at the time of brood removal will not support mite survival until they become four-day-old larvae. By that time, all mites will have died. Perhaps even larvae 1–2 days old may be left, since even they will not support the survival of *T. clareae* mites.

ACKNOWLEDGEMENTS

I thank Prof Jin Zhen-Ming, the director of the Institute of Apicultural Research of the Chinese Academy of Agricultural Sciences in Beijing, for providing me the facilities to conduct the investigations, Prof Huang Shuang-Xiu, the vice-director of that institute, for her inestimable help in every respect, and Mr Zhang Yao-Gang for technical assistance.

REFERENCES

The numbers given at the end of references denote entries in *Apicultural Abstracts*.

- DELFINADO, M D; BAKER, E W (1961) *Tropilaelaps*, a new genus of mite from the Philippines (Laelapidae (s. lat.): Acarina). *Fieldiana Zoology* 44(7): 53–56. 866/63
- KITPRASERT, C (1984) [Biology and systematics of the parasitic bee mite, *Tropilaelaps clareae* Delfinado and Baker (Acarina: Laelapidae).] MSc thesis; Kasetsart University, Thailand; 70pp (in Thai).
- NELSON, J A; STURTEVANT, A P; LINEBURG, B (1924) *Growth and feeding of honeybee larvae*. US Department of Agriculture; Bulletin No. 1222; 38 pp.
- TROUILLER, J; ARNOLD, G; LE CONTE, Y; MASSON, C (1991) Temporal pheromonal and kairomonal secretion in the brood of honeybees. *Naturwissenschaften* 78: 368–370. 494/93
- WOYKE, J (1963) Rozpoznawanie płci żywych larw pszczołelich. *Pszczelnicze Zeszyty Naukowe* 7(1): 23–31. 780/64
- WOYKE, J (1984) Survival and prophylactic control of *Tropilaelaps clareae* infesting *Apis mellifera* colonies in Afghanistan. *Apidologie* 15(4): 421–433.
- WOYKE, J (1985) Further investigations into control of the parasitic bee mite *Tropilaelaps clareae* without medication. *Journal of Apicultural Research* 24(4): 250–254. 969/86
- WOYKE, J (1987) Length of stay of the parasitic mite *Tropilaelaps clareae* outside sealed honeybee brood cells as a basis for its effective control. *Journal of Apicultural Research* 26(2): 104–109. 253/89

- WOYKE, J (1989) Change in shape of *Tropilaelaps clareae* females and the onset of egg laying. *Journal of Apicultural Research* 28(4): 196-200. 278/91
- WOYKE, J (1993a) Practical control method of the parasitic bee mite *Tropilaelaps clareae*. *American Bee Journal* 133(7): 510-511.
- WOYKE, J (1993b) Adult *Tropilaelaps clareae* males can feed and survive for two weeks. *Apidologie* (in press).
-

