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Original article

Geographical distribution, levels of infestation and population density of the mite *Euvarroa sinhai* Delfinado and Baker (Acarina: Mesostigmata) in *Apis florea* F colonies in Iran

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Summary — The geographical distribution, levels of infestation and population density of the parasitic mite, *Euvarroa sinhai* Delfinado and Baker, were investigated for the first time in Iran. *Euvarroa sinhai* was recorded from all the *A florea* colonies that were collected as a whole colony or sampled. The mite was found up to an elevation of 1 270 m. The mite population is generally rather low in the bee colonies. The number of live *Euvarroa* females per 1 000 worker bees was found to be 3.28 in the period from April–June when drone brood rearing is at its peak; from June–April this number increased to 5.7. The number of mites on drones at the time of emergence was 1–8, with a mean of 4.30 ± 1.66. Percentage of infested drone cells in newly constructed combs with first drone generation emerged from these cells varied from 3.15–30.43%, with a mean of 8.38%. The female mites were phoretic from 4–10.5 months, with an average of 6.4 months on adult worker bees before entering drone cells.

***Euvarroa sinhai* / *Apis florea* / infestation rate / geographical distribution / population density / Iran**

INTRODUCTION

The mite *Euvarroa sinhai* Delfinado and Baker is a parasite of drone brood of the honeybee *Apis florea* in Asia. It was first described by Delfinado and Baker (1974) as being associated with dwarf honeybee in India. Akratanakul and Burgett (1976) later identified the mite as a parasite on the drone brood of the dwarf bees in Thailand, and also observed the mite as existing only on adult drones. Koeniger *et al* (1983) found the adult female mites on adult worker bees in Sri-Lanka, but not on adult drones. Mossadegh and Komeili (1986) found the adult female mites on both adult worker and drone bees in addition to drone brood. This mite has been reported recently in hive debris from *A mellifera* colonies in India (Kapil and Aggarwal, 1987).

The object of the present study was to investigate the geographical distribution, levels of infestation and population density of *Euvarroa* in *A florea* colonies in Iran. *A florea* is distributed along the entire

southern boundary of Iran, from Beluchistan close to the border of Pakistan, along the coast of the Gulf of Oman and the Persian Gulf to Lurestan in the north west of the country. This narrow strip, beginning in the east at latitude 28° and extending as far north as 34° in the west, is ≈ 2 000 km long (Ruttner *et al*, 1985).

MATERIALS AND METHODS

For this study, 76 colonies of *A florea* from different bee distribution areas were examined during the period of October 1986 through August 1988. Forty-two whole colonies and samples of an additional 34 colonies were collected. Whole colonies were collected by cutting the entire nest from its substrate and shaking the bees in a container of 75% methyl alcohol (33 colonies) or placing the nest in a muslin bag for subsequent refrigeration (9 colonies). Samples of colonies were collected using an entomological forceps. The bees were put in 75% methyl alcohol for subsequent inspection. To study drone brood rearing and the number of mites on drones at the time of emergence, some *A florea* colonies were established in the experimental apiary station. Colonies of the bees were collected in cardboard boxes and covered with muslin. Most of the colonies were collected in December and January when the bees were in winter cluster. At the other time of the year, when bees were active, water was spread by a hand sprayer on the bees before the colony was detached from its substrate. A total of 27 colonies of *A florea* were established in the apiary during December 1986–June 1988. The number of mites on drones was counted in 2 ways: 1), drones were picked up with entomological forceps from the main colonies and were put singly in a small test tube (10 x 1 cm); 2), sealed brood drone comb section was cut off from the main comb, placed in 20 x 20 x 16-cm boxes and kept in an incubator set at 35 °C and 50 ± 5% RH. Emerging drones were collected daily and placed singly in a small test tube (10 x 1 cm). All the bees were inspected under a binocular microscope (10x). Sections of sealed brood drones were also inspected for mites, their progeny and percentage of infested cells. The brood cells were carefully uncapped and the walls, larvae or pupae were examined carefully under a binocular microscope (10x and 14x).

For detection of mites, the soaked bees in the container were carefully shaken by hand for 10 min (Ritter, 1981). The contents were poured several times through a wire screen (mesh 2 x 2 mm) to filter out the mites. Excess alcohol was passed through a cotton cloth and the mites were collected and counted. The procedure was repeated 3 times for all the samples to ensure that no mite was left on the bees.

RESULTS

Euvarroa sinhai was found in all *A florea* colonies that were either collected as a whole colony or sampled in Iran. The mite was found up to an elevation of 1 270 m.

The mite population is generally rather low in *A florea* colonies (table I). As 1 or 2 generations of drones are produced in the spring and sometimes 1 in the autumn, mite reproduction is limited to this time of the year. In early spring and autumn when the production of drones began, most or all of the mites were in the drone cells and none or few on the worker bees (table II). Of the 21 colonies of *A florea* with drone brood cells which were examined, the drone brood of all colonies were infested by *Euvarroa*. The percentage of infested first generation drone cells in newly constructed combs varied from 3.15–30.43% with a mean of 8.38% (table III). The infestation was identified by tiny fecal white spots in the cells.

The number of mites found on drones at the time of emergence was 1–8 with a mean of 4.30 ± 1.66 (tables IV–VI). This was 1–2 for drones collected from the whole drone population in the colonies. When the infested newly-emerged drones were kept with uninfested newly-emerged

Table I. Population of adult female *E sinhai* mites in 31 whole *A florea* colonies collected in various parts of Iran.

Date collected	*No of adult workers and adult mites on workers*		*No of adult drones and adult mites on drones*	
	Workers	*Mites*	*Drones*	*Mites*
11-15-1986	10 650	4	–	–
11-15-1986	11 400	3	–	–
12-16-1986	3 184	2	–	–
05-06-1987	2 840	10	132	*
06-08-1987	4 409	10	24	*
05-10-1987	9 540	312	208	*
05-16-1987	3 150	22	82	–
05-23-1987	2 450	7	1	–
05-29-1987	1 615	21	–	–
05-20-1987	10 420	54	268	3
06-03-1987	13 439	59	21	2
07-06-1987	12 600	297	562	211
10-05-1987	4 460	7	–	–
10-05-1987	1 014	1	12	–
10-05-1987	8 845	2	388	25
10-26-1987	15 380	13	–	–
11-04-1987	3 800	–	152	33
11-08-1987	28 700	116	–	–
11-16-1987	19 600	79	–	–
12-05-1987	9 400	32	–	–
12-10-1987	10 580	140	–	–
12-10-1987	17 600	53	–	–
05-03-1988	19 610	98	112	*
05-03-1988	22 800	4	383	95
05-17-1988	17 840	8	427	107
06-08-1988	2 106	15	7	–
06-12-1988	18 950	15	449	260
06-17-1988	11 500	52	40	*
07-11-1988	12 360	17	266	54
07-11-1988	3 488	13	47	1
08-24-1988	7 794	58	4	–

* Counted with workers.

workers, many mites soon changed host at the time of feeding the drones, and were found on workers after 4 h. There were 1–6 with a mean of 1.72 mites on the body of such workers after 24 h (table VII). The mites were found attached to the venter and dorsum of the bee's abdomen, at the scutellar region or pleural area of the thorax, between the thorax and abdomen, on the neck region, the wings and the leg bases. When the mites are attached to the bee's abdomen especially on the ventral side, they are hidden between the abdominal segments and are difficult to detect.

Table II. Number of adult female mites on adult workers and in sealed drone cells in spring and autumn.

Date collected	*No of adult workers and adult female mites on worker bees*		*No of adult drones and adult female mites on drones*	
	Workers	*Mites*	*Drones*	*Mites*
10- 5-1987	8 845	2	388	25
11- 4-1987	13 800	–	152	33
5- 3-1988	22 800	4	383	95
5-17-1988	17 840	8	427	107
6-11-1988	12 360	17	266	54
6-12-1988	18 950	15	449	260

Table III. Number and percentage of infested drone cells in 7 *A florea* colonies.

No of drone cells examined	*No of infested cells*	*% of infested cells*
495	18	3.63
552	25	4.52
730	23	3.15
890	70	7.86
662	21	3.17
299	91	30.43
152	9	5.92

Examination of colony workers showed that the number of live *Euvarroa* females per 1 000 bees was low (3.28) in the period from April–June when drone brood rearing is at its peak and many of the mites are in the sealed drone brood cells or on the adult drones; from June–April this number increased to 5.7 (table VIII).

When the hosts do not survive to become adult, many of the female mites are able to leave the cells by making a small rectangular hole in the middle of the cell cap with their mouthparts. The mite first makes a small hole with the sharp teeth of

Table IV. Daily total number of the adult female mites on emerged drones in 8 drone comb sections.

	Days									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
	76	19	24	33	–	–	–	–	–	–
	13	9	8	25	–	–	–	–	–	–
	7	–	–	–	–	–	–	–	–	–
	102	57	73	17	10	2	1	–	–	–
	11	5	7	4	9	12	7	23	–	–
	3	8	2	9	2	30	–	8	20	2
	33	5	2	6	8	6	4	8	20	–
	10	17	154	15	–	–	–	–	–	–
Total	255	120	270	109	29	50	12	39	40	2

Table V. Number of *Euvarroa* female mites on drones at the time of emergence in 8 drone comb sections.

	Euvarroa *female No*								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
No cases observed	323	64	25	9	4	5	2	2	–
Total No	323	128	75	36	20	30	14	26	–

Table VI. Number of daily *Euvarroa* female mites on drones at the mite of emergence in 8 drone comb sections.

	Day										
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*Total*
No of mites	255	120	270	109	29	50	12	39	40	2	926
No of drones	108	79	106	49	20	25	10	13	21	1	433

Table VII. Number of *Euvarroa* mite on uninfested workers kept with infested drones for 24 h.

	No of mites					
	1	*2*	*3*	*4*	*5*	*6*
Adult workers						
No of cases observed	33	9	8	3	–	1

her movable digit chelicera in the middle of the cap and then enlarges it sideways by pushing aside the wax. When the hole became large enough, the mite passed through it sideways and out of the cell; it took 24–48 h for the mites to make such a hole and leave the capped cells. Out of 99 sealed drone cells with dead pupae, 15 were found with such holes and with no live female mites in these cells. The mites left in the cells were either dead parental females or living or dead males. Many mites leave the cells when the adult drones begin to chew their cell cappings. The mites leave the cells and wander over the comb surface searching for a new cell or a carrier host.

As reported by Mossadegh and Komeili (1986), the mite spends the winter as an adult on worker bees and feeds on clusteri-

Table VIII. Number of live *E sinhai* adult females per 1 000 worker adult bees measured during November 1986–August 1988.

Date	*No of colonies examined*	*Total No of worker bees*	*Total No of adult female mites*	*No of mites per 1 000 worker bees*
April–June	12	116 280	381	3.28
June–April	12	143 436	814	5.70

Table IX. Phoresy duration time of adult female *E sinhai* mites on adult workers in 8 *A florea* colonies that were under constant observation.

Date of colony establishment	*Date of drone brood rearing*	*Nos of adult workers and adult mites worker bees*		*Nos of adult drones and adult mites on drone*		*Phoresy duration time*
		Workers	*Mites*	*Drones*	*Mites*	
12-31-1987	4-27-1988	17 840	8	427	107	4 months
11-22-1987	5- 6-1988	18 950	15	449	260	5 1/2 "
11-01-1987	5- 5-1988	19 610	98	112	*	6 1/2 "
11-26-1987	5-25-1988	13 500	6	395	76	6 1/2 "
11-27-1987	5-29-1988	11 500	52	40	*	6 1/2 "
11-28-1987	5-25-1988	12 360	17	266	54	6 1/2 "
10-16-1987	5-12-1988	22 800	4	383	95	7 1/2 "
5-29-1987	4-18-1988	20 340	9	466	266	10 1/2 "

* Counted with workers.

ng bees during the cold season when brood rearing has ceased. More information was obtained on phoresy of the adult female mites in the course of this investigation. It was found that the mite, in colonies that were under constant observation, can spend from 4–10.5 months with an average of 6.38 months on worker bees before entering drone cells (table IX).

The male mites do not die soon after mating in the sealed brood cells. 78.74% of a total of 127 males in 99 sealed drone cells were found alive; up to 3 live male mites were found in a single cell. The bees in the pupal stage were dead in these cells, but had not dried. Some of these cells were uncapped up to 13 d after the last drone had emerged from the comb. All live female mites in these cells had swollen bodies and were ready to lay eggs.

DISCUSSION

The life cycle of *E sinhai* is similar to that of *Varroa jacobsoni* Oudemans, *ie*, the mated female enters the cell of a late larval bee prior to capping, and attaches her eggs to developing bee larva or pupa. All stages live inside the capped brood cells of the bee host, and only the adult female mites leave the cells (Akratanakul, 1976). The mite reproduces on drone brood and apparently does not enter worker brood cells.

As drone brood rearing is limited both in quantity and period of time, the mite population is low in *A florea* colonies. Infestation of drones is always higher at the time of their emergence, but is reduced within 24 h. Infestation of the adult worker bees is

higher when there is no drone brood in colonies.

This study also demonstrates that the adult female mites are phoretic on adult workers and feed on bees when drone brood rearing is absent in the colony. As soon as the brood rearing starts, the mites enter the cells and reproduction begins.

Résumé — Répartition géographique, niveaux d'infestation et densité de population de l'acarien *Euvarroa sinhai* Delfinado and Baker dans des colonies d'*Apis florea* F en Iran. Pour la première fois ont été étudiés en Iran la répartition géographique, les niveaux d'infestation et la densité de population de l'acarien parasite *Euvarroa sinhai*. *Euvarroa* était présent dans les 76 colonies d'*A florea* prélevées en totalité ou échantillonnées. Il a été trouvé jusqu'à 1 270 m d'altitude. La population d'acariens était généralement assez faible (tableau I). Entre avril et juin, période pendant laquelle l'élevage du couvain est à son maximum, le nombre moyen de femelles d'*Euvarroa* était de 3,28 par 1 000 ouvrières d'abeilles. De juin à avril il est passé à 5,7 (tableau VIII). Le nombre d'acariens présents sur les mâles d'abeilles au moment de l'émergence a varié de 1 à 8 (moyenne 4,30 ± 1,66). Le pourcentage de cellules de mâles infestées dans les rayons nouvellement construits à l'émergence de la première génération de mâles a varié entre 3,15 et 30,43% (moyenne 8,38%) (tableau III). Les acariens femelles sont phorétiques (restent sur les abeilles adultes) durant 4 à 10 mois et demi (moyenne 6,4 mois) avant de pénétrer dans les cellules de mâles.

***Euvarroa sinhai* / *Apis florea* / taux de parasitisme / densité de population / répartition géographique / Iran**

Zusammenfassung — Geographische Verbreitung, Befallsgrad und Populationsdichte der Milbe *Euvarroa sinhai* bei Völkern von *Apis florea* im Iran. Zum ersten Mal wird hier über geographische Verbreitung, den Befallsgrad und die Populationsdichte der parasitischen Milbe *Euvarroa sinhai* Delfinado & Baker im Iran berichtet. *Euvarroa sinhai* wurde bei allen Völkern von *Apis florea* gefunden, die entweder als ganzes Volk oder als Probe untersucht wurden. Die Milbe wurde bis zu einer Höhe von 1270 m gefunden. Die Population war in den Bienenvölkern im allgemeinen ziemlich klein (Tabelle I). In der Zeit zwischen April und Juni, wenn sich die Drohnenaufzucht am Höhepunkt befindet, wurden im Durchschnitt 3.28 lebende *Euvarroa*-Weibchen pro 1000 Arbeitsbienen gefunden. Zwischen Juni und April stieg diese Zahl auf 5.7 (Tabelle VIII). Die Zahl der Milben, die sich zur Zeit des Schlüpfens auf Drohnen befanden, schwankte zwischen 1-8 mit einem Mittelwert von 4.30 ± 1.66.

Der Anteil befallener Drohnenzellen in neugebauten Waben mit der ersten ausschlüpfenden Drohnengeneration schwankte zwischen 3.15% bis 30.43% (Mittel 8.38%) (Tabelle III). Die weiblichen Milben waren zwischen 4 bis 10.5 Monate phoretisch (dh auf erwachsenen Bienen ansitzend) mit einer mittleren Dauer von 6.4 Monaten, bevor sie in Drohnenzellen eindrangen.

***Euvarroa sinhai* / *Apis florea* / Befallsgrad / geographische Verbreitung / Populationsdichte / Iran**

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