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Original article

Incidence rates of *Acarapis woodi* (Rennie) in queen honey bees of various ages

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Summary — Queen honey bees, *Apis mellifera* L., exhibited a rapid decline in susceptibility to infestation by the tracheal mite, *Acarapis woodi* (Rennie), with increasing age. Ten-day-old queens are invaded by 1.0 mites per queen, while queens one day of age had 6.5 mites. Laying queens removed from colonies during requeening had an *A. woodi* incidence rate of 30.6%. Newly mated queens obtained from mating nuclei infested with *A. woodi* had an incidence rate of 14.3%. Additionally, queens stored in queen banks prior to shipment may be subject to further mite pressure as we found that *A. woodi* was able to move through wire screening from infested to uninfested workers during food exchange.

queen honey bee — *A. woodi* — age — incidence

Résumé — Taux de parasitisme par *Acarapis woodi* des reines d'abeilles en fonction de leur âge. *Des reines d'abeilles (*Apis mellifica *L.) ont été utilisées dans 3 expériences pour étudier les taux d'infestation par* Acarapis woodi *(Rennie). Une quatrième expérience a été faite pour tester le passage de l'acarin à travers un grillage.*

Expérience 1. Des reines vierges âgées de 0, 5 et 10 jours ont été placées dans des cagettes avec 150 ± 20 ouvrières fortement infestées. Cinq jours plus tard les ouvrières ont été disséquées et le nombre d'acariens compté. Les reines vierges ont montré avec l'âge une diminution significative de la sensibilité à l'infestation. Chez les reines âgées de 10 jours, le taux d'infestation a été d'1,0 acarien/abeille contre 6,5 chez les reines d'un jour.

*Expérience 2. On a introduit des cellules royales dans des nuclei de fécondation et laissé 2 à 3 semaines aux reines vierges naissantes pour s'accoupler. Les reines ont été ensuite disséquées et des échantillons prélevés dans les colonies pour déterminer le taux d'infestation. Le taux d'infestation des nuclei a été de 10 à 50%, celui des reines fécondées de 14,3% (*n=35*), avec une moyenne de 1,4 acarien/reine infestée.*

Expérience 3. Un total de 86 reines ont été prélevées dans des colonies appartenant à un apiculteur professionnel, lors du remérage. Une étude randomisée des colonies a donné un taux d'infestation des colonies de 74%. 30,6% des 86 reines pondeuses étaient infestées.

Expérience 4. Des grillages de maille 12 (0,21 cm) et 8 (0,33 cm) ont été fixés sur des cages d'expédition de reines. Cent cages ont été suspendues dans le nid à couvain d'une colonies fortement infestée (100%) avec 10 ouvrières naissantes dans chacune d'elles. Celles-ci ont été disséquées 10 jours plus tard. Elles étaient infestées à 42,5%, tandis que les témoins (abeilles nais-

santes introduites dans la colonie) l'étaient à 98%. Les 2 types de grillage ont permis le transfert de l'acarien au cours des échanges alimentaires entre les abeilles de la colonie et celles des cages.

reine des abeilles* — A. woodi — *âge* — *incidence

Zusammenfassung — Häufigkeitsverteilung von *Acarapis woodi* (Rennie) bei Königinnen der Honigbiene in Abhängigkeit vom Alter. *Es wurden drei Experimente zur Bestimmung des Infektionsgrads von* Acaparis woodi *(Rennie) bei Königinnen der Honigbiene (*Apis mellifera *L.) durchgeführt. Ein viertes Experiment diente dazu, die Bewegung der Milbe durch ein Drahtgitter zu testen.*

Experiment 1 : Unbegattete Königinnen im Alter von 0, 5 oder 10 Tagen wurden in Laborkäfige mit 150 ± 20 stark infizierten Arbeitsbienen gesetzt. Nach fünf Tagen wurden die Königinnen auf-präpariert und die Anzahl an Milben festgehalten. Unbegattete Königinnen zeigen mit zunehmendem Alter einen signifikanten Rückgang der Anfälligkeit gegenüber einer Infektion mit Acarapis woodi. *Zehn Tage alte Königinnen wurden von durchschnittlich 1.0 Milbe pro Königin infiziert, einen Tag alte Königinnen von 6.5 Milben.*

Experiment 2 : Königinnenzellen wurden in Begattungskästchen gesetzt und den frischgeschlüpften Königinnen 2-3 Wochen Zeit zur Begattung gegeben. Die Königinnen wurden dann auf-präpariert und der Befallsgrad der Völkchen bestimmt. Der Befallsgrad der Begattungskästchen mit Acarapis woodi *betrug 10-50%. Die begatteten Königinnen zeigten eine Befallsrate von 14.3% (n=35) mit 1.4 Milben pro infizierter Königin.*

Experiment 3 : Insgesamt 86 Königinnen wurden bei einer Umweiselung von Völkern eines kommerziellen Imkers untersucht. Zufallsverteilte Stichproben aus den Völkern ergaben einen Befallsgrad von 74%. Von den 86 legenden Königinnen waren 30.6% infiziert.

Experiment 4 : Zwei verschiedene Drahtgitter mit der Maschenweite 12 (0.21 cm) und 8 (0.33 cm) wurden um Königinnenversandkäfige herum angebracht. Je 10 frischgeschlüpfte Arbeitsbienen wurden in 100 Käfige gegeben und in den Brutraum von stark infizierten Völkern (100%) gehängt. Nach 10 Tagen wurden die Bienen zur Präparation herausgenommen. Durchschnittlich 42.5% der Bienen waren infiziert. Bei der Kontrollgruppe (frischgeschlüpfte Bienen, die frei im Volk herumlaufen konnten) waren 91% infiziert. Beide Gittergrößen ließen während des Futteraustauschs zwischen den Stockbienen und den Bienen im Käfig eine Milbenübertragung zu.

Königinnen der Honigbiene* — A. woodi — *Alter* — *Befallsgrad

Introduction

The incidence or spread of *Acarapis woodi* (Rennie) around the world has been rapid since its discovery in honey bee colonies in Great Britain in 1919 (Rennie, 1921; Cromroy & Kloft, 1980). Nixon (1982) stated that 37 countries have confirmed infestations of *A. woodi.* Delfinado-Baker (1984) recently reported the discovery of mites in the United States. By transporting infested bees, especially queens, around the world, mankind has accelerated the natural dissemination of *A. woodi* (Kaeser, 1960; Nixon, 1982; Wilson, 1982).

Migrating mites prefer to invade bees younger than 5 days of age (Morgenthaler, 1931; Lee, 1963; Gary, 1988). Thus, if queens cannot become infested after 6—10 days of age, the time they spend in the mating nuclei is most important in interactions between queens and mites. A queen that is not infested 5 days after emergence could remain mite-free for the remainder of her life.

Virgin queens in the United States are usually mated in small mating nuclei (Datant, 1975). This process spans the first 12—16 days of a queen's life (Laidlaw and Eckert, 1962). After mating, queens are often stored in queenless colonies cal-

led queen banks. Queens are held behind wire screening and are fed by workers through the screen. Morgenthaler (1931) reported that mites did not transfer across a wire gauze during feeding. If aging of virgin queens proves to be effective in lowering susceptibility to *A. woodi*, the most convenient means of aging would be behind wire screening.

It has been established that queens can become infested (Morgenthaler, 1930; Kaeser, 1960; Giordani, 1977), but it is unknown if they are as susceptible as workers. Giordani (1977) reported 20 of 39 queens from infested colonies as being infested. In addition to Giordani (1977), Rennie (1923) and others found some queens from infested colonies to be free of mites.

This research was designed to : (1) examine the effect of aging on the susceptibility of queens to infestation by *A. Woodi*; (2) determine incidence rates in newly mated queens; (3) examine incidence rates of queens heading established colonies; and (4) determine if *A. woodi* can traverse wire screening.

Materials and Methods

All experiments were carried out in General Teran, Nuevo Leon, Mexico (250° 18' N; 99.5° 3'N); however, some data are from mite surveys of colonies located in the state of Tamaulipas, Mexico. All specimens unless noted were preserved in alcohol and dissected according to the method described by Morland (1936). Uninfested workers for experiments 1 and 4 were obtained by allowing bees to emerge in the incubator. Samples of bees dissected (> 300 examined) confirmed their mite-free status.

Experiment 1 : aging studies

The laboratory cages used contained a piece of comb measuring 8 x 10 cm and 150 ± 20 infested workers. The overall dimensions of the wooden laboratory cage were 15 x 14 x 19 cm, with a screened top to allow for ventilation and feeding. To obtain infested workers, empty combs were placed in the honey supers of a colony with a 100% worker incidence rate and left until the comb surface was covered with 150 ± 20 infested bees. Laboratory cages with infested bees were maintained at 34°C and supplied with high fructose corn syrup (42%) and water. Each laboratory cage received a virgin queen (0, 5, or 10 days old). Five days later queens were removed and examined. Twenty-four hours later, a second group of queens were added. Bees were held in the laboratory cages for 12 days.

Queens were reared according to the Dolittle method (Laidlaw and Eckert, 1962). Emerging virgins were either placed directly into laboratory cages (*i. e.*, controls, 0 days of age) or aged in Benton cages for 5 or 10 days. The Benton cages were provisioned with queen candy, and 4—6 (laboratory emerged) uninfested workers. Queens in Benton cages were held at 35 ± 2°C, and supplied with a drop of water twice daily. Queens were dissected to determine the incidence rates of tracheal mites.

Experiment 2 : mating nuclei

Six frame nuclei (n = 19) were made up by splitting populous colonies in May 1985; each contained 2 combs of brood and one frame of honey, all with adhering bees. To each nucleus a "ripe" queen cell was added. Approximately 2 weeks later 19 mated queens were removed, and sample of 20 bees was taken to determine the infestation levels of each nucleus. New queen cells were added 2 days later for a second mating cycle. The nuclei were then examined 3 weeks later, and 16 mated queens removed. Three queens failed to mate during the second cycle. Queens were dissected to determine incidence rates.

Experiment 3 : established colonies

A total of 86 laying queens were removed during requeening from colonies belonging to a commercial beekeeper located in the States of Nuevo Leon and Tamaulipas, Mexico. Random colony surveys of the beekeeper's operation were conducted in December, 1984 and January, 1985, to determine colony incidence rates of *A. woodi.*

Experiment 4 : transfer across screen wire

Two sizes of wire screening, 12 mesh (0.21 cm) and 8 mesh (0.33 cm) were fastened onto (Benton) queen mailing cages (3 x 2 x 8 cm). Ten replications of each mesh size were utilized. During February, 1985, 10 newly emerged bees (0—12 h old) were placed in each cage and suspended in the brood chamber of a heavily infested colony (100%). One hundred young marked bees were released into the colony to serve as contols. All bees were removed after 10 days for subsequent dissection.

Results

Experiment 1 : aging studies

Virgin queens exhibited a significant decline in susceptibility to infestation by *A. woodi* with increasing age (Table I). All control queens (*i.e.*, newly emerged virgins, 0 days), were infested with an average of 6.5 mites per infested queen (n = 32). Fifty-four percent of virgins aged for 5 days were infested, with an average of 2.3 mites per infested queen (n = 22). Virgins aged for 10 days had an incidence rate of 35% with 1.0 mites per infested queen (n = 20).

Experiment 2 : mating nuclei

Virgins maintained with infested bees in mating nuclei showed an incidence rate of 14.3% (n = 35). These queens had an average of 1.4 mites per queen (Table II). The incidence rate in nuclei ranged from 10% to 50%.

Experiment 3 : established colonies

Of laying queens removed from colonies during requeening 30.6% were infested (n = 83). Of the 83 queens, 3 were dissected alive to determine the presence of live and dead mites : determination was based on movement and color of individual mites (Eischen *et al.*, 1987). Only one live mite (male) was found among 131 dead mites. Individual tracheae of queens were heavily infested (tracheal trunks and air sacs occupied) and discolored, more so than the workers examined in this study.

A random survey of colonies (n = 73) from the beekeeper cooperating in the study revealed that 74% of his colonies had tracheal mites. The average incidence rate within each colony examined was 42.1%.

Experiment 4 : transfer across screen wire

The results are summarized in Table III. Sixty percent of control bees recovered from the colonies, and no mortality occur-

Table I. Infestation levels and numbers of invading mites, *Acaparis woodi* in queen honey bees held in cages for 5 days with 150 ± 20 (100% infested) worker bees. All were virgin queens and 0, 5, or 10 days of age when placed with infested bees in laboratory cages.

	Total virgin queens	*% of queens infested*	*Total No. of mites*	*Mites / queen (x ± SD)*
Controls aged 0 days	32	100	207	6.5 ± 6.2
Virgins aged 5 days	22	54	28	2.3 ± 1.2*
Virgins aged 10 days	20	35	7	1.0 ± 0.0*

* Significantly different from the preceding value ($P < 0.05$).

Table II. Incidence of tracheal mites in young queen honey bees (n = 35) removed from mating nuclei infested with tracheal mites. Queens were exposed for a 2—3 week mating period in May 1985 in General Tehran, Nuevo Leon, Mexico.

Mite incidence in mating nuclei	*% of queens infested* (n)		*No. of mites per infested queen*
0 — 10%	06	(16)	2.0
11 — 20%	20	(15)	1.0
21 — 30%	66	(3)	2.0
31 — 40%	—	—	—
41 — 50%	00	(1)	0.0

Correlation coefficient between level of mites in the nuclei and the likelihood of a queen becoming infested was 0.24.

red in the experimental cages. The infestation levels between the controls and the confined bees in the experimental cages were significantly different ($P < 0.001$). There were no significant differences between infestation levels of bees held behind the 2 mesh sizes studied ($P < 0.05$).

Discussion

A decrease in queen susceptibility with increasing age had been postulated by Morgenthaler (1931). It is apparent that aging reduces queen susceptibility. Queens and workers exhibit decreasing susceptibility to infestation by *A. woodi* with increasing age. Morgenthaler (1931) stated that after 5 days, workers were unlikely to become infested in a free flying colony. Also when he introduced uninfested laying queens into colonies with infested bees he found that they remained uninfested (n = 5) (Morgenthaler, 1929). We made a similar observation in Mexico, when 5 mated queens remained uninfested 2 months after introduction into col-

Table III. The incidence rate of tracheal mites in newly emerged honey bees held in Benton cages (two sizes of screen used), and placed into a colony whose workers were all infested with *Acarapis woodi*. Marked control bees were released into the colony and allowed free movement. Bees were held in the colony for 10 days.

	Total No. of bees	*% of bees infested*	*No. of invading mites*	*Mites per infested bee* ($x \pm SD$)
Controls	60	95	475	8.63 ± 5.7
Large screen	100	40	86	2.15 ± 1.2*
Small screen	100	44	73	1.66 ± 0.96**

* Significantly different from preceding value ($P < 0.001$).
** Not significantly different from preceding value ($P > 0.05$).

onies with heavily infested bees (Pettis, personal observations). While the finding of 10 uninfested queens is not conclusive evidence, it does support the view that older queens remain uninfested. Migrating mites in our laboratory test cages only had a single, uninfested young queen available to them in contrast to a colony where many young worker hosts exist. Even 10-day-old queens were still likely to be the youngest bees available in the lab cages as compared to workers that were collected from the honey supers. Migrating mites, being unable to find a preferred young host, will most probably select the youngest available bee, *i. e.* the aged queens. A migrating mite should select a young bee to allow time for her offspring to mature before the death of her host. The preference for a young host was evident by the number of mites invading control queens as compared to aged queens. Control virgins had, on average, 6.5 mites per queen which compares closely with 8.6 mites per bee found in control workers from the wire screen experiment (Expt. No. 4). In both cases 100% of the attending bees were infested with tracheal mites. It seems that queens may be equally as attractive as workers, but direct comparisons are needed.

Higher incidence rates of mites in the nuclei resulting in increasing queen infestations would be expected, although the one observation at the 50% level did not result in an infested queen. A mating nucleus with mite levels below 10% should produce virtually mite-free queens.

The precise origin of queens removed during the requeening process is unknown. However, none of the queens came from commercial queen breeders and thus they were reared by the bees in the colonies, or in 6 frame nuclei originating from populous colonies (D. Cardoso-Tamez, personal communication). These mated queens thus originated from colonies 74% of which had been infested during the winter survey. A second random survey conducted in the spring showed that the percentage of colonies infested had only dropped slightly to 65%. The age of the queen and level of infestation of the colony at the time she was mated could not be determined.

Tracheae of queens examined were more heavily melanized than the trachea of workers examined in this area (Pettis *et al.*, 1987). Perhaps the longer life span of queens allows for multiple generations of mites in the same host.

The movement of mites across wire screening has implications for the storage of queens prior to shipment. Morgenthaler's findings (1931) of no transfer across screens could have been the result of maintaining bees in an unnatural environment (cages). Bees in this study were placed between frames within an established colony. Mite transfer was reduced by a screen, but *A. woodi* can indeed transfer through wire screening to infest workers. This suggests that queens may also become infested in this manner.

Queens were found to be infested by *A. woodi* in proportions similar to those of workers from this area. Mite prefer young queens to old queens. The question remains as to the means by which a mite perceives a host as young or old.

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