Life History of the Honey Bee Tracheal Mite (Acari: Tarsonemidae)

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ABSTRACT Data on the seasonal reproductive patterns of the honey bee tracheal mite, *Acarapis woodi* (Rennie), were obtained by dissecting host honey bees, *Apis mellifera* L., at intervals during their life span. Mite reproduction normally was limited to 1 complete generation per host bee, regardless of host life span. However, limited egg laying by foundress progeny was observed. Longer lived bees in the fall and winter harbored mites that reproduced for a longer period than did mites in bees during spring and summer. Oviposition rate was relatively uniform at ≈0.85 eggs per female per day during the initial 16 d of adult bee life regardless of season. In all seasons, peak mite populations occurred in bees ≈24 d old, with egg laying declining rapidly beyond day 24 in spring and summer bees but more slowly in fall and winter bees. Stadial lengths of eggs and male and female larvae were 5, 4, and 5 d, respectively. Sex ratio ranged from 1.15:1 to 2.01:1, female bias, but because males are not known to migrate they would have been overestimated in the sampling scheme. Fecundity was estimated to be ≈21 offspring, assuming daughter mites laid limited eggs in tracheae before dispersal. Mortality of adult mites increased with host age; an estimate of 35 d for female mite longevity was indirectly obtained. Daughter mites began dispersing when infested bees were ≈13 d old, and mite dispersal peaked when infested bees were 15–25 d old. The mating behavior of tracheal mites is described.

KEY WORDS *Acarapis woodi*, *Apis mellifera*, parasite

THE HONEY BEE tracheal mite, *Acarapis woodi* (Rennie), is an internal parasite of adult honey bees, *Apis mellifera* L. The life history of *A. woodi* is poorly known, in part because of its small size (≈350 × 150 μ, Delfinado-Baker and Baker 1982) and in part because of the destructive sampling needed to examine mites in the honey bee tracheae. Nevertheless, Morgenthaler (1931), Orosi-Pal (1934), and Hirschfelder and Sachs (1952) have reported on some aspects of the biology, dispersal, and feeding habits of the mite (reviewed by Delfinado-Baker and Baker 1982). Mites preferentially disperse to adult worker honey bees <3 d old (Lee 1963, Gary et al. 1989) and use host cuticular hydrocarbon cues (Phelan et al. 1991). Female mite dispersal occurs primarily at night (Pettis et al. 1992). However, no information is available concerning the number of generations per host bee or the host age at which the next mite generation begins dispersal. Attempts to rear *A. woodi* in vitro have thus far been only partially successful, with no complete mite generations reared (Giordani 1967).

Studies on 2 other species of *Acarapis* (*A. dorsalis* Morgenthaler and *A. externus* Morgenthaler) have shed some light on reproductive patterns (Ibay and Burgett 1989). However, these 2 species are external and their patterns may differ from those of the internal *A. woodi*.

Host honey bees vary in their longevity (15 to >200 d) depending on the season and the conditions under which they are reared (reviewed by Winston 1987). Varied host longevity undoubtedly has an effect on mite reproduction. Our study reports on the following 3 objectives: (1) to quantify seasonal mite reproduction with changing host longevity, (2) to quantify stadial lengths, and (3) to examine timing and duration of female mite dispersal from infested bees.

Materials and Methods

Seasonal Sampling. Adult worker honey bees infested with tracheal mites were dissected at regular intervals from emergence through the end of their lifespan. Dissections were performed during 4 consecutive seasons (winter 1987 through fall 1988) to determine seasonal incidence, sex ratios, and reproductive patterns of the mite parasite. Honey bees used in these studies were located near Weslaco, TX, and predominantly *Apis mellifera ligustica* L. Five colonies were used as the source of winter samples and 4 colonies in each of the other 3 seasons. Each colony was housed in 2, 10-frame deep Langstroth hive bodies (50 by 43

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by 25 cm). Colonies were ranked for adult bee population on a relative 1–5 scale; 1 being ≤5 frames covered in bees, 3 being ≤15 frames covered in bees, and 5 being ≥20 frames crowded with bees (Nasr et al. 1990). Only 1 colony in the winter sample was rated a 2, most colonies were rated as 3, 2 colonies rated as 4, and no colonies received a rating of 1 or 5. Honey supers were added as needed to provide ample space for honey storage and to discourage swarming. Colonies were fed a dry mixture of powdered sugar and oxytetracycline to prevent American foulbrood.

To obtain young experimental bees, all combs containing emerging brood were removed from each mite-infested colony and placed in wire-screen emergence cages within an incubator maintained at 35°C. As young bees emerged they were marked on the abdomen with paint (Testors, Weston, ON) and placed in screened cages (23 by 13 by 5 cm) for transfer back to each parent colony. Emerging bees were marked every 6–8 h and were thus <9 h old when returned to their colonies. Marked bees were placed on the top bars of their respective parent colonies as the colonies were lightly smoked. Paint colors were changed every 24 h and bees were marked for 4 consecutive days yielding ≈1,500 marked bees per colony. Bee marking dates began on 1 November 1987, 9 March 1988, 8 July 1988, and 18 October 1988 for each season, respectively.

To examine mite reproduction within the marked bees, a random sample of 25 (winter) or 30 marked bees was removed per colony for live dissection. Sampling began 4 d after the introduction of marked bees, and continued at 4-d intervals thereafter. Thus, at each sampling date 120–125 bees, originating from 4 to 5 colonies, were dissected. Dissections were performed on live bees according to the methods of Eischen et al. (1987), and on each bee both main prothoracic tracheal trunks from the spiracle to the 1st bifurcation (Snodgrass 1956) were examined. Mites were recorded as live or dead, and there sex and stage of development were noted.

Bees in the spring were sampled more often, every 2 d, for the first 20 d of the life of marked bees. As the marked bees began to decline with increasing age in each season, the number of bees that could be recovered decreased. Thus, later samples were not so large as the initial samples. A group of 100 marked bees introduced into one of the mite-infested colonies in the spring, was followed to serve as a control for bee longevity. These bees were counted every 4th d during the spring experiment but were not dissected.

Stadial Length Sampling. To measure stadia of *A. woodi*, infested bees were sampled daily during the initial 16 d of the life of host bees. Newly emerged honey bees were marked and introduced into a mite-infested colony (mite prevalence, 85% of workers infested) located in College Station, TX (March 1991), as described above. Exceptions to these methods were as follows: emerged bees were marked at 0800 and 1600 hours; and at 2400 hours bees were removed from combs but not marked. Experimental bees were thus 0–8 h old when marked, and color patterns were changed every 8 h. Marked bees were exposed in the mite-infested colony for 24 h and then transferred into cages (10 by 7 by 5 cm, 60 bees per cage). Cages were held in an incubator at 35°C and each cage was supplied with pollen and a 50:50 (vol:vol) sucrose to water solution. At 24-h intervals, a sample of 50 bees was dissected. Mite life stages and location within the tracheal tubes were recorded.

Dispersal Sampling. To determine when female mites disperse with respect to host age, the following experiment was conducted in Weslaco, TX, in October 1988. Newly emerged worker bees were introduced daily into small nucleus colonies that consisted of a cohort of ≈500 mite-infested bees, all of the same age. To establish the nuclei, >500 newly emerged worker bees were marked and released into a colony heavily infested with *A. woodi* (93%). Five days later, 500 marked bees were removed and placed into a small nucleus colony (20 by 14 by 14 cm). A 2nd control nucleus was started with 500 bees of mixed ages from the mite-infested colony that had held the marked bees. Each nucleus contained 4 small frames of comb (1 with stored honey) and a queen housed in a wooden mailing cage. The queens were not released for 12 d so that their brood would not emerge before the study ended and compete for mites with the young bees introduced each day. Fifty marked bees were dissected to determine initial infestation levels of the marked bees and the host colony bees. Nuclei were held in a screen wire flight cage (1.5 by 2 by 2 m) located within a building with skylights and no air conditioning. The flight cage prevented robbing and drifting from colonies outside the study. Starting when the marked bees in the uniform age nuclei were 12 d old, 30 newly emerged bees were marked and released into each nucleus every 24 h. Two colonies with open mated queens provided emerging brood, the emerging bees from the 2 colonies were mixed before marking and introduction. Sets of 30 bees were removed after 72 h of exposure within each nuclei, placed in alcohol, and later dissected to reveal the number of mites that had dispersed to these bees during 3 d of exposure within infested nuclei. These procedures were continued for 34 d, when the population of nuclei began to decline rapidly because of natural attrition. The experiment was terminated at 34 d, before emergence of the 1st brood from introduced queens.

Mating Behavior. During the dissections of live bees, pharate adult mites were often seen moving within the larval skin before ecdysis. A pharate female and adult males, dissected from a single tracheal tube, were held on a glass slide with the broken remains of the tracheal tube. A video tape was made: the female ecdysed, and a male guarded her

Table 1. Data from cohorts of marked honey bees infested with tracheal mites, 1987–1988

Season	No. bees introduced	% bees recovered	% bees infested, range	Foundress mites/ infested bee, mean ± SEM	Sex ratio, ♀:♂
Winter	5,310	33.9	09–44	0.89 ± 0.2	1.41:1
Spring	6,047	23.1	05–65	1.47 ± 0.4	2.01:1
Summer	5,910	24.3	00–53	0.97 ± 0.4	1.15:1
Fall	7,280	20.3	13–90	3.01 ± 0.5	1.46:1

Bee samples were dissected at 4-d intervals during the life span of the cohort. Each season, marked bees originated from 4 to 5 colonies located near Weslaco, TX.

before ecdysis and then mated with her. The video tape was reviewed and descriptions and timing of various behaviors recorded.

Results and Discussion

Seasonal Sampling. The length of time over which marked bees were sampled covered a wide range; 132, 42, 56, and 92 d, for winter, spring, summer, and fall bee populations, respectively. Approximately 25 percent of the marked bees were recovered, foundress female mites ranged from 1 to 3 mites per infested bee over the 4 seasons (Table 1). Female to male sex ratios ranged from 1.15:1 to 2.01:1. These ratios overestimate males because males are not known to migrate and thus there numbers increase over the life of the bee, and females are the dispersal form and thus may not have been counted as adults before dispersal. Live mites were found throughout the life of the bee, with older bees harboring primarily live males, few immature stages, and varying numbers of dead adults.

Mite life stages found in infested bees at each dissection date during the 4 seasons are represented in Fig. 1. The mite reproductive period is extended in the longer-lived fall and winter bees. In the spring the length of time that the marked bees were available for sampling corresponded closely with the longevity of marked control bees that had been introduced into one of the mite infested colonies (Fig. 2). Based on the data presented in Fig. 2, marked bees were sampled well past their mean life span. Fifty percent of the con-

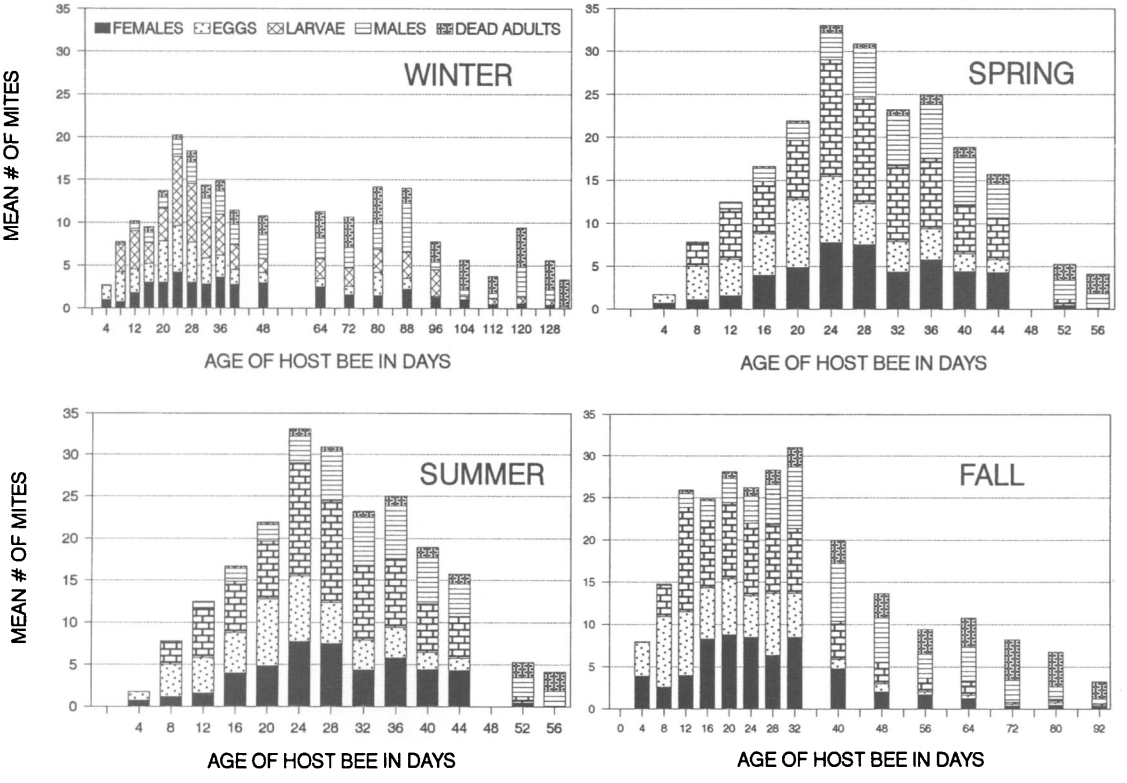

Fig. 1. Mean number of *A. woodi* life stages observed in trachea of host *A. mellifera* during 4 seasons (winter 1987–fall 1988) when founding mite populations averaged ≈1, 1.5, 1, and 3 mites per infested bee for the 4 seasons, respectively. Colonies were located near Weslaco, TX, and ≈25 infested bees are represented per dissection date.

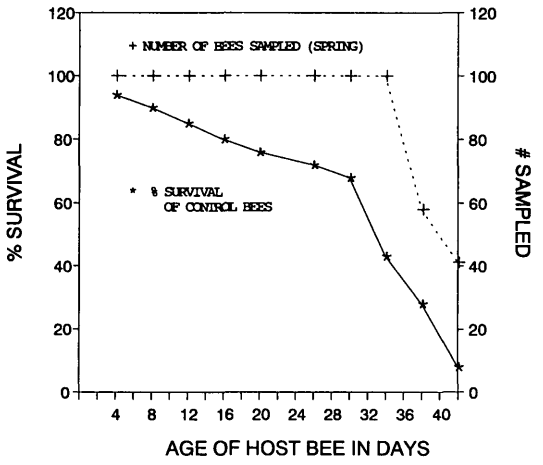

Fig. 2. Number of marked bees (*A. mellifera*) sampled for tracheal mites (*A. woodi*) and percentage of survival of control mite infested bees at 4-d intervals, Weslaco, TX, March–April 1988.

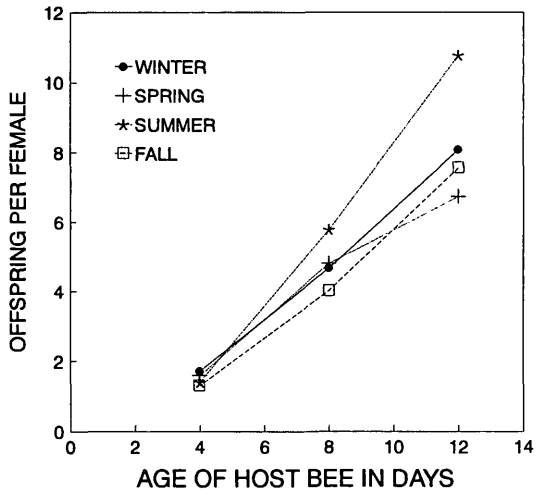

Fig. 3. Mean number of *A. woodi* offspring (eggs, larva, and adult males) per foundress observed in tracheae from honey bees during 4 seasons. Colonies were located near Weslaco, TX, 1987–1988.

trol bees had died by day 32, whereas 200 marked bees (22% of the spring sample) were dissected beyond day 32. Constant sampling surely reduced the number of bees present at subsequent sampling periods, but, based on the spring sample (Fig. 2) marked bees were sampled over the majority of their lifespan.

The number of mite eggs present within bees of increasing age indicates a change in oviposition in bees 18–28 d old (Fig. 1). Evidence of a 2nd generation is indicated between days 18 and 28 when the number of eggs found within the tracheal tubes often nearly doubled. The fall season was an exception (Fig. 1). At 18–20 d, daughters of founding females would have had sufficient time to mate, and develop an egg, following ecdysis at days 12–14. In the fall sample, no increase in egg number occurred at day 20. The fall bees had on average 3 foundress mites per bee compared with <1.5 foundress mites in the other 3 seasons (Table 1). The higher level of parasitism in the fall could have been sufficient to cause daughter mites to find the bee unsuitable for further reproduction. Unsuitability could have been caused by crowding, scarring, and brittleness of the tracheae, or other factors. Egg census data suggest that a 2nd or partial 2nd generation per host bee occurs in bees that contain 1 or 2 foundress mites per tracheal tube.

Oviposition over the initial 12 d was ≈0.85 eggs per day per female, and results were consistent with earlier work (Royce et al. 1988) (Fig. 3). Regression analysis of egg number on host age showed that the slopes were not significantly different among the 4 seasons ($P < 0.05$). Females present beyond day 12 could have been either foundress females or their female progeny, and thus only data from days 4 to 12 were used.

The number of total progeny per female cannot be directly derived from this data set. An estimate is extrapolated from the initial oviposition rate of 0.85 eggs per day multiplied over the estimated oviposition period ≈25–30 d. These calculations gave an estimate of 21–25 progeny per female.

An estimate of foundress female mite longevity (≈30–35 d) was obtained indirectly from adult mortality counts (Fig. 4). The longevity estimate of 30–35 d is the point at which the mean number of dead mites equalled the mean number of foundress females. Additionally, the percentage of tracheae that contained live female mites dropped

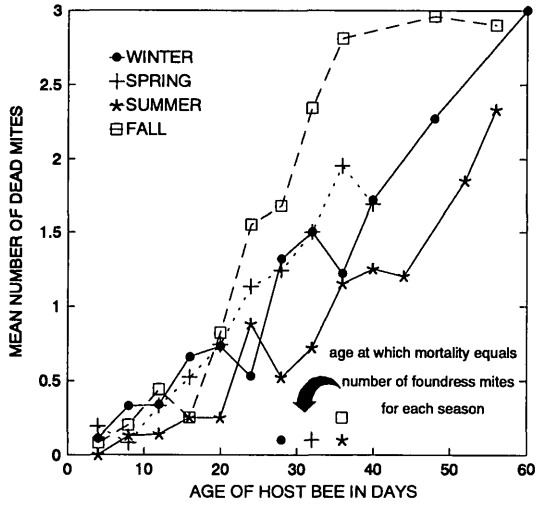

Fig. 4. Mean number of dead adult tracheal mites found in host bees during 4 seasons. An estimate of female longevity is obtained when the mean adult mortality equals the mean number of foundress females in each season.

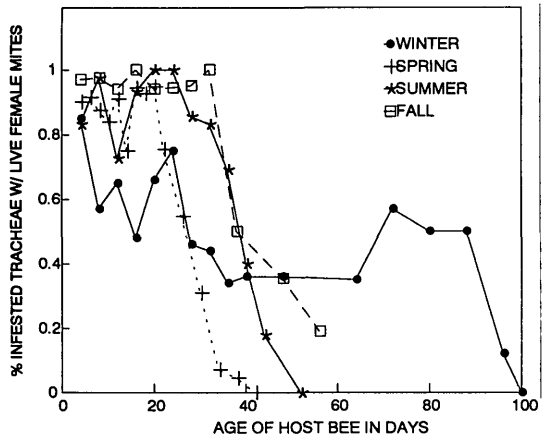

Fig. 5. Percentage of infested honey bee tracheal tubes containing live female tracheal mites. Dissections were performed at intervals during the life span of the bees and during 4 seasons.

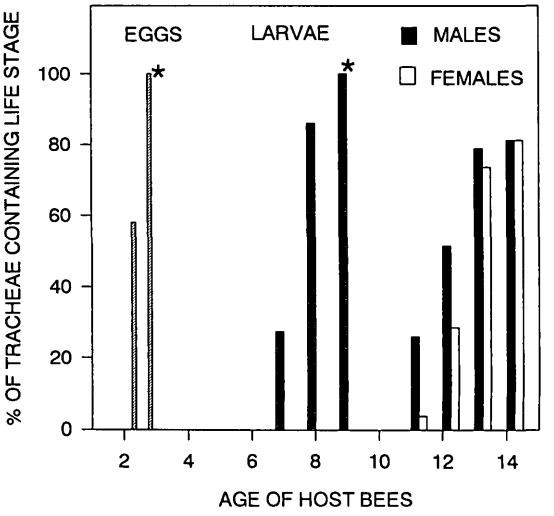

Fig. 6. Sequential appearance of life stages of *A. woodi* from worker honey bees dissected at 24-h intervals following exposure for the initial 24 h of adult life in a colony infested with tracheal mites. Bees were removed after 24 h and held in sets of 60 within an incubator until dissected. *, 100% of trachea beyond this time were occupied by eggs (day 3) and larva (day 9), respectively.

drastically around day 35 in all 4 seasons (Fig. 5). After day 24 of the life of host bees, fewer and fewer mites are present because of death of foundress females and dispersal of female progeny.

Infested tracheae were discolored in all seasons, the darkening beginning at about day 10 and increasing with increasing age of the host bees. At first the darkening appeared as rings following the curvature of the taenidia. In older infested bees, the dark areas were often continuous, and the tracheae noticeably more brittle than tracheae from uninfested bees of the same age. Liu et al. (1989) showed that a secondary film or coating is present on the interior of the tracheae from infested bees, but its composition, cause, and effects on mite reproduction are unknown.

Stadial Length Sampling. From the daily sampling of infested bees, the following estimates of stadial lengths for *A. woodi* were obtained: preoviposition ≥3 d, egg 5 d, larval male 4 d, larval female 5 d (Fig. 6). There were no eggs in the bees for the first 2 d (48 h); at 60 h, 58% of the infested tracheae contained eggs; and, by 72 h, eggs were present in 100% of the tracheal tubes. The minimal preoviposition period was estimated to be ≥3 d, as female mites would require 1 d to mate and disperse to a new host, coupled with 2 d within a new host. Nymphal stages are hidden within the larval cuticle and thus larval stadia include nymphal development time, the adult mites ecdysing through the larval cuticle. Mite stadial lengths could change with season or health of host bees. No gross changes in stadial lengths were observed in the seasonal sampling, but dissection intervals were too wide to detect small shifts in stadial lengths.

Live male mites, dead mites, and exuviae constituted the tracheal fauna of older bees nearing death. Female mites apparently respond to the physiological status of the host bees and disperse. Reproduction by daughter mites is perhaps dictated by as yet undetermined consequences of bee aging, such as nutrient changes in the hemolymph, deterioration of the structural integrity, or changes in intra-tracheal environment of the host caused by mite feeding or other factors.

Dispersal Sampling. The dispersal of daughter mites to the introduced young bees began when the host bees were ≥12 d of age (Fig. 7). The percentage of introduced bees that became infested rose over the first 3 dissection days from 20 to 100%. Numbers of mites transferring appeared to follow a fairly consistent pattern; peaks in dispersal occurred for both nuclei during days 5–15, when bees in nucleus number 3 were 17–27 d of age. Nucleus number 3 had fewer mite dispersals during the first 2 d, because daughter female mites were just beginning to ecdyse within host bees (12 d old) on day 1 of the experiment. As expected, both nuclei began a consistent decline in dispersal late in the experiment as host bees aged and contained fewer and fewer mites. By day 28 of the experiment (age 40 d of the uniformly aged bees), the decline in available mites reached a uniform low of 1 mite per infested bee for the remainder of the study. Female mites began to disperse when host bees were ≥12 d old, peak dispersal occurring in bees 17–27 d old.

Mating Behavior. A pharate adult female was videotaped over 40 min, during which she appeared to be guarded by a male, ecdysed, and then mated with the same male. Within the larval cuticle, the adult female moved her legs and gnathosoma, and flexed her opisthosoma dorsoventrally. A male mite from the same tracheal tube posi-

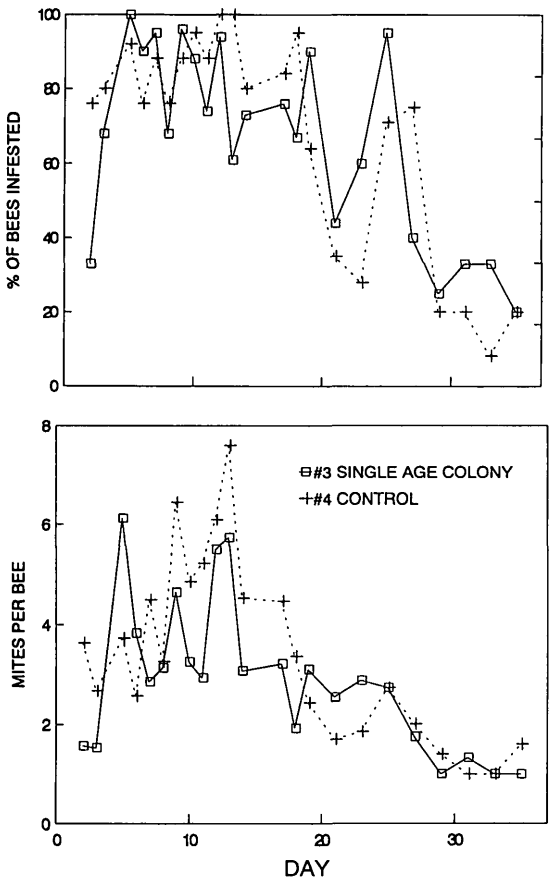

Fig. 7. Mean number of honey bee tracheal mites and percentage of target bees infested, from 3-d-old bees that had been marked upon emergence and introduced daily into 2 nuclei containing 500 bees. Thirty bees were introduced per day for 34 d, and removed after 3 d within the nuclei (October 1988). Nucleus 3 contained bees all of the same age (12 d old on day 1 of the experiment), whereas nucleus 4 contained bees of mixed ages (nucleus 3 and 4 were determined to be 80 and 88% infested, respectively, at the begining of the experiment).

tioned himself at a 90° angle to the female, with his gnathosoma and forelegs covering her opisthosoma. He approached her directly from ≈2 mm away, settled over her immediately upon contact, and did not break contact for 21 min. The female took only 45 s to clear herself from the larval exuviae. The male remained in place on the exuviae for an additional 9 min following ecdysis until the female walked over him and he moved. At 11 min after ecdysis he climbed onto the female, remained on her dorsum for 2.5 min, and moved off and positioned himself with his posterior to hers. The female, 20 s later, moved her posterior into contact with the male and they appeared to copulate for ≈25 s, after which both male and female began to move about freely on the glass slide. They were observed for an additional 5 min, and no further significant contacts were made. Guarding behavior is common in the free-living members of this family (Lindquist 1986) and is apparently retained by this parasite species. Sibling matings appear to be common in *A. woodi*, because only 1 female mite infests a tracheal tube when mite prevalence is low within a population of bees.

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