AN ABSTRACT OF THE THESIS OF

LILIA ARMENDEZ IBAY__ for the degree __MASTER OF SCIENCE__ in __ENTOMOLOGY__ presented on ____July 6, 1989____

Title: __BIOLOGY OF THE TWO EXTERNAL *ACARAPIS* SPECIES__ __OF HONEY BEES: *ACARAPIS DORSALIS* MORGENTHALER__ __AND *ACARAPIS EXTERNUS* MORGENTHALER (ACARI:__ __TARSONEMIDAE)__

Abstract approved: Redacted for privacy

Dr. Dennis Michael Burgett

The biology of the two external *Acarapis* mites of honey bees, *Acarapis dorsalis* Morgenthaler and *Acarapis externus* Morgenthaler was studied. It was observed that both *Acarapis* species have similar developmental period (8-9 days) with males emerging earlier than females.

Mite load and infestation rate of *A. dorsalis* decreased as bees become older. *A. externus* remained high on bees up to 35 days old. This observation may indicate that *A. dorsalis* prefers younger bees while *A. externus* seems to maintain its population on older bees.

In nucleus colonies deliberately exposed to known populations of both external *Acarapis* species, infestation by *A. dorsalis* appears to be more rapid than *A. externus*. Introduction of 500 *A. dorsalis* established the highest rate of infestation (17.10%) in a relatively short period of time, *i.e.*, 9-12 weeks.

The highest infestations of *A. dorsalis* were during the spring months (March to June) and in mid-late summer (August and September) with the lowest infestation rates in January and July. For *A. externus*, mite population was highest in the fall (October and November). The lowest infestation was recorded in July. The average female:male ratios observed were 1.9:1 for *A. dorsalis* and 2.07:1 for *A. externus*. No relationship between nectar flow and percent mite infestation was established.

Biology of the Two External *Acarapis* Species of
Honey Bees: *Acarapis dorsalis* Morgenthaler
and *Acarapis externus* Morgenthaler
(Acari: Tarsonemidae)

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed July 6, 1989
Commencement June 1990

APPROVED:

Redacted for privacy

Associate Professor of Entomology in charge of major

Redacted for privacy

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Date thesis is presented   July 6, 1989

Typed by Lilia A. Ibay

ACKNOWLEDGMENT

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INTRODUCTION

Mites are among the most important pests in beekeeping. The mites associated with honey bees are categorized as parasitic, phoretic, or house guest mites (De Jong *et al.* 1982). Of these, parasitic mites are given more attention by researchers because they can cause devastating losses of infected honey bee colonies.

Parasitic mites include the Asiatic brood mites *Varroa jacobsoni* and *Varroa underwoodi* Oudemans, *Tropilaelaps clareae* Delfinado and Baker, *Tropilaelaps koenigerum* Delfinado-Baker and Baker and *Euvarroa sinhai* Delfinado and Baker, and the *Acarapis* species complex (De Jong *et al.* 1982, Delfinado-Baker and Styer 1983). *V. jacobsoni* is presently considered the most economically important mite parasite of honey bees on a worldwide scale.

Three *Acarapis* species (Tarsonemidae) are known to be host specific to the western honey bee (*Apis mellifera* L.). The most economically important of these is the endoparasitic mite, *Acarapis woodi* (Rennie), which also infests the Asian honey bees, *Apis indica* F. and *Apis dorsata* F. (Delfinado-Baker 1988). At present, no *Acarapis* infestation has been reported on *Apis florea* F.

Infestation by *A. woodi* was believed to be responsible for the syndrome known as Isle of Wight Disease which caused a severe loss of honey bee colonies in the British Isles between 1905 and 1919. However, in studies on *A. woodi* infestation, Bailey (1963) found that

this species slightly shortens the life span of the bees and further claimed that Isle of Wight Disease remains to be of unknown origin.

In 1934, Morgenthaler described two additional *Acarapis* species: *Acarapis externus* and *Acarapis dorsalis*. Both species are ectoparasites and now are believed to occur universally on *Apis mellifera*. A third species of external *Acarapis, Acarapis vagans*, was proposed by Schneider (1941). However he failed to show morphological characters, which differentiated the taxon. And, because the original description is inadequate and illustrations or specimens are lacking, this *Acarapis* species is considered a *nomen dubium* (Delfinado-Baker and Baker 1982).

The three *Acarapis* mites are distinct species that are most commonly identified by location on the bee host (Bailey 1963). *A. woodi* lives and breeds in the prothoracic tracheae and air sacs of the head and thorax. *A. externus* is restricted to the ventral and lateral sides of the area behind the head capsule while *A. dorsalis* dwells on the dorsal scutoscutellar groove of the thorax. All species are obligate parasites during all stages of development on adult honey bees and require a total developmental period of *ca.* 15 days (Royce *et al.* 1988).

A. woodi is the only *Acarapis* species for which detailed biological studies have been carried out. Although the external *Acarapis* are known to be hemophagic parasites (Orosi-Pal 1934), they have been largely ignored since they have been considered as relatively benign parasites. The feeding effects of *A. dorsalis* and *A. externus* on honey bees and their impact on colony health have therefore not been assessed. For this reason, biological studies were undertaken in order to better understand the interactions of the external *Acarapis* and

their honey bee hosts. Monitoring parasite populations for their damage is important in determining whether or not economic loss is likely to occur. A systematic study of the life histories of *A. dorsalis* and *A. externus* and of the relationship between parasite population and economic threshold is essential. Should these *Acarapis* species eventually be elevated to pest status, such information will be useful in formulating tactics for controlling parasite populations and/or their damage to the host.

The objectives of this study were: a) To clarify the life cycles of *A. dorsalis* and *A. externus*, external *Acarapis* parasites of *Apis mellifera*; b) To determine the variations in mite load according to honey bee age; c) To investigate the population growth rates of both species in initially mite-free nucleus colonies; d) To monitor trends of mite populations within colonies; and e) To evaluate the economic impact of *A. dorsalis* and *A. externus* on infested bees.

REVIEW OF LITERATURE

The three *Acarapis* species associated with honey bees are widely distributed. The two external *Acarapis*, *A. dorsalis* and *A. externus*, are cosmopolitan (Clark 1985), being prevalent in Europe, the South Pacific archipelagos and North America (Delfinado-Baker and Baker 1982, Shaw *et al.* 1961). In Canada, external *Acarapis* was first reported from Quebec in 1926. Both species were then found in British Columbia, Alberta and Ontario. The first discovered external *Acarapis* in the United States was in New York in 1930. These mites were subsequently found in 22 other states namely: Arkansas, California, Connecticut, Florida, Georgia, Hawaii, Idaho, Iowa, Louisiana, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Nebraska, North Carolina, Oregon, Tennessee, Texas, Utah, Vermont, and Virginia (Eckert 1961, Shaw *et al.* 1961, Delfinado-Baker and Baker 1982, Royce *et al.* 1988). In Britain, *A. dorsalis* is reported to be more prevalent than *A. externus* (Bailey 1981) but a survey done in British Columbia (Clark 1985) established *A. externus* as the more common species. Delfinado-Baker and Baker (1982) believed that the geographical distribution of *A. externus* is the same as that of *A. dorsalis*. They argued that *A. externus* has a higher population density and as such was more frequently collected and observed than *A. dorsalis*. *A. woodi* initially appeared to have a more limited distribution than the external *Acarapis*. Jeffree (1959) hypothesized that the limited distribution of this species is correlated with temperature. However, later reports established that *A. woodi* has spread to many geographical areas whose climates are not necessarily

similar to the countries surveyed by Jeffree, *i.e.*, areas enclosed between the January (24-54°F) and July (51-68°F) isotherms. Climatic factors that may influence the distribution of *Acarapis* species in certain areas remain to be established.

The three *Acarapis* species colonize different parts of the bee host, *i. e.*, *A. woodi* in the trachea, *A. dorsalis* on the dorsal groove and *A. externus* on the neck of the bee host. These microhabitat differences probably are important in allowing the coexistence of the three *Acarapis* species on a single host. However, these parasitic mites have similar food preferences, *i.e.*, hemolymph, which may reduce the probability of more than one *Acarapis* species becoming associated on a single host (Burgett *et al.* in press).

Because adult external *Acarapis* migrate from one bee to another, limited information on their life history is available. Several attempts have been made to culture *Acarapis in vitro* but none has been successful (Eckert 1967, Jaycox per. com.). By introducing marked newly emerged bees into infested colonies, the life cycles of external *Acarapis* have been estimated to be 10 days (Eckert 1961). Royce *et al.* (1988) reported a life cycle of *ca.*15 days, which approximates the duration of the life cycle of *A. woodi*. Eckert (1961) and Delfinado-Baker and Baker (1982) observed that *Acarapis* species have an unusually large egg. The larva has six legs of which one pair is well developed and the two pairs are rudimentary. The larva and adult are parasitic on adult bees while the nymph is a calyptostatic.

The three *Acarapis* species prefer young bees, *i.e.*, less than 9 days of age (Eckert 1961, Shaw *et al.* 1961, Royce *et al.* 1988). Hirschfelder and Sachs (1952) proposed that the resistance of older

bees to *A. woodi* infestation may be attributed to the stiffness of hairs surrounding the entrance to the first thoracic spiracle. Lee (1963) opposed this claim and suggested that older bees are less attractive rather than resistant to *A. woodi* infestation. Detailed preference studies with the external *Acarapis* are lacking.

The female to male ratio in *Acarapis* species varies considerably. Ratios of 1.1:1 to 3.3:1 were reported by Morgenthaler (1931) for *A. woodi* while Betts (1951) established a range of 1:1 to 3:1. Female to male ratios higher than 1:1 were reported in other studies, *i.e.*, 3.29:1 (Morison 1931), 3:1 to 4:1 (Morison 1932), 1.7:1 (Bochert 1932) and 3:1 (Otis 1988, Burgett *et al.* 1989 in press). *A. externus* has a ratio of 1:1 (Brugger 1936). Lindquist (1986) estimated female:male ratio of *A. dorsalis* and *A. externus* as 2:1 through 1:1 to 2:3. while ratios of 3:1 (*A. dorsalis*) and 2:1 (*A. externus*) were reported by Burgett *et al.* (in press). Seasonal variations of sex ratios have not been established.

Seasonal fluctuations in external *Acarapis* mite infestations have been examined by several researchers but contradictory results have been reported. Incidence of external *Acarapis* mites was reported to be high during all seasons of the year. Mite infestations were highest in spring and summer in Massachusetts (Shaw *et al.* 1961) and in western Canada (Clark 1985). In New Zealand, mite populations were observed to be highest in fall and spring and lowest in summer (Clinch 1976). Examining dead bees in winter and foraging bees in summer, Bailey (1963) claimed that the percentage of infested bees was highest in winter and lowest about midsummer in the United Kingdom. Eckert (1961) in California found that *A. dorsalis* had peaks of infestation during spring and fall. A wide fluctuation in the infestation

of *A. externus* was observed. Obviously, more studies are needed to clarify the conflicting reports on the seasonal fluctuations of mite infestations.

The deleterious effect of external *Acarapis* infestation on honey bees has not been studied. However, Bailey and Lee (1959) observed that *A. woodi*-infested adult bees die earlier. No similar studies have been done with external *Acarapis*. Decreased honey production in Mexico was noted in colonies having *A. woodi* infestations exceeding 35% (Guzman-Novoa and Zozaya-Rubio 1984). Eckert (1961) observed that percent infestation of both external *Acarapis* had no apparent effect on colony population and honey production of infested colonies. His conclusion was based on a single colony infested with 40% *A. dorsalis* and 25% *A. externus* that became a strong hive and yielded *ca* 150 lbs of honey. While the presence of a parasite on a host does not necessarily signify a threat to the host's health, one observation on a single colony provides only minimal evidence in support of this claim. Furthermore, Eckert failed to show the relationship between the mite population and economic threshold. If his mite population was at a level that the honey bees could tolerate, no effect on the host would be expected. The effect of external *Acarapis* on honey production and the relationship between percent infestation and honey surplus have not been studied.

Because external *Acarapis* species feed on host hemolymph (Orosi-Pal 1934), it is possible that they have some deleterious effects on infested bees. Anticipating such effects, studies have been conducted on the control of external *Acarapis* parasites.

Very few chemicals are recommended for the control of external *Acarapis* mites and none of the recommended chemicals is registered in the U.S. Endosulfan was found to be effective in tests conducted by Clinch *et al.* (1977) in New Zealand. An 85% reduction in the number of infested bees was obtained when a single dose of 40 mg of endosulfan per infested colony was fed in 2:1 sugar syrup. A second dose given 19 days later resulted in a 98% decrease in the number of infested bees. Further study showed that fenbutatin oxide and trichlohexyltin hydroxide were more effective than endosulfan and safer than amitraz (Clinch 1979). Sulfur dioxide vapors were also found to decrease the number of external *Acarapis* (Anonymous 1932).

MATERIALS AND METHODS

Life Cycles of *A. dorsalis* and *A. externus*

Studies of the bionomics of external *Acarapis* species would be greatly facilitated if the parasites could be reared *in vitro* under defined conditions. Attempts were made to culture *A. dorsalis* in the laboratory using the method described by Giordani (1967). In brief, newly sealed larvae or pupae placed in paraffin beds were used as alternate hosts. Two female mites were inoculated on each larva or pupa and maintained in an incubator at 28°C. For the relative humidity, a pan of water was placed inside the incubator. Larvae were given a drop of a mixture of honey and pollen, which was renewed every day.

Young infested worker bees were also used in this study. These infested bees were obtained by marking newly emerged bees and introducing them into infested colonies. After two days, marked bees were recovered and examined for the presence of female mites. To prevent migration of mites from one bee to another, infested bees were immobilized by fastening the legs into pieces of cardboards using melted wax. Cardboards were then pinned down in a styrofoam. The bees were fed regularly with honey and pollen mixture on cotton pads and placed them in an incubator at 28°C.

For the *in vitro* rearing of *A. externus*, Bruce's (per. com) diet for *V. jacobsoni* was tried. Diets were placed in artificial queen cups and covered with thin parafilm. Small plastic tubes were then placed on

top of the parafilm wherein two female mites were introduced per tube.

Since the rearing methods described above were unsuccessful, estimates of parasite bionomy were therefore performed *in vivo* using cohorts of known age worker bees introduced into infested colonies at the OSU Honey Bee Laboratory.

Cohorts of newly emerged (parasite-free) worker bees were reared from capped brood in an incubator maintained at 32°C. Upon emergence, bees <24h old were marked on the abdomen, head or thorax with acrylic paint. Marked bees were then introduced into infested colonies. Thirty marked bees were collected daily for 15-22 days. The presence of different mite developmental stages on the collected bee samples was then observed. Observations of the various mite life stages were done under 20-50X magnification using a Zeiss dissecting binocular microscope.

Differentiation of male from female *Acarapis* was based on body size and the number of setae on tibia 1V. Males are usually smaller with one long seta on tibia 1V while females are bigger with two long tibial setae (Delfinado-Baker and Baker 1982, Royce *et al.* 1988). Larvae were not differentiated from pharate nymphs.

Variations in Mite Load According to Honey Bee Age

Marked cohorts of newly emerged bees were introduced into infested colonies. Fifty-bee samples were collected every 5 days over a

40-day period and examined for the presence of mites to determine the fluctuations in mite load as the bee hosts aged.

Acarapis Population Growth Rates

To follow *Acarapis* population development, known mite populations were introduced into mite-free nuclei (small colonies with a volume of 1,980 in^3). This was an attempt to establish a founding population that would enable the mites to build up in the bee population. Nucleus colonies are more manageable units than standard colonies and were kept small by removing 1-2 frames of brood every week to prevent swarming, which is an asexual reproduction of bee colonies wherein the old queen and at least half of the colony population will leave the hive to establish a new home.

Since brood is not infested by *Acarapis* mites, combs of emerging callow adult bees were used to create uninfested nucleus colonies. Capped brood was reared in an incubator set at 32°C. Newly emerged adults were used to establish 4-frame nuclei that were free of external *Acarapis*. Two frames of pollen and honey and two brood frames without adult bees were used per nucleus. Newly mated, uninfested queens were provided to the nucleus test colonies.

Acarapis mites are microscopic and are difficult to introduce directly into a colony. Therefore, infested bees (2-3 days old) with known mite populations were used as inoculum. These infested bees were obtained by marking newly emerged bees and introducing them into an infested colony. After 48-72 hours, marked bees were

recovered and examined under a dissecting microscope. Since both external *Acarapis* species are found on the wings during migration and sometimes during reproduction, mites within the wing axillaries, on the wings and on the body hairs were discarded to prevent possible species confusion. Mites found on the scutoscutellar groove of the thorax were considered to be *A. dorsalis* and those on the neck region *A. externus*.

To prevent fighting between the inoculum bees and host bees, inoculum (infested) bees with known mite loads were placed in screen cages and introduced into host colonies. Due to the paucity of adult mites, eggs also were used to attain the required number of mites per treatment. Infested bees with known parasite loads were released two days after introduction. Twelve 4-frame nuclei were used in this study and each mite species was evaluated at 50 or 500 female mites or eggs per initial infestation.

Introduction of inoculum-infested bees was done on the third to fourth week of June 1988. Sampling started 10-15 days post-introduction to give ample time for the mites to reproduce. This experiment was terminated during the second week of October 1988 due to climatic problems. All treatments were replicated 3 times and data were analysed using a factorial in Randomized Complete Block Design. Means were compared using Least Significant Difference (LSD).

Both external *Acarapis* mites may be present in a single colony. Introduction of both external *Acarapis* (25 *A. dorsalis* + 25 *A. externus* or 250 *A. dorsalis* + 250 *A. externus*) into clean nuclei also was done to

determine possible occurrence of competition between the two species.

Seasonal Population Fluctuations

Seasonal abundance of the two external *Acarapis* mites was initially investigated by monitoring six colonies for *A. dorsalis* and four colonies for *A. externus*. Colony monitoring for *A. dorsalis* was begun in August 1986 and continued until August 1988. *A. externus* colonies were monitored from August 1987 to January 1989. One colony from the *A. dorsalis*-infested hives and two colonies from the *A. externus* experimental group died during the winter of 1988.

About 100 bees from each colony were sampled at weekly intervals. Of these, 30 individual bees were examined for the presence of all stages of both mite species. Bee samples were frozen following collection for later examination. In the two *A. externus* colonies, which were concurrently infested with *A. dorsalis*, all adult mites found on wings and wing axillaries were mounted in Hoyer's solution and identified to species under a phase contrast microscope. Since males and immatures of *A. dorsalis* and *A. externus* are difficult to distinguish, all were considered to be *A. dorsalis* in this experiment since most females observed on the wings were identified as this species. Percent infestation was calculated based on the number of bees with live mites.

Effects of *A. dorsalis* and *A. externus* on Bee Mortality

     A. dorsalis and *A. externus* are adapted to parasitic feeding, although no visible symptoms of injury to the bee hosts has been reported (Eckert 1961, Definado-Baker and Baker 1982). The impact of both *Acarapis* species on infested adult bees was determined by marking cohorts of newly emerged bees and placing them in infested colonies. For the control, a cohort of marked bees was placed into a mite-free colony obtained by rearing capped brood in an incubator. Dead marked bees were collected daily using a dead bee trap, and examined for the presence of mites. Mortality of marked bees that became infested and those that remained uninfested was compared.

RESULTS AND DISCUSSION

Life Cycles of *A. dorsalis* and *A. externus*

The rearing of the two external *Acarapis* mites *in vitro* gave negative results. Out of the 48 *A. dorsalis* female mites inoculated into 24 larvae (2 mites/larva), none of them survived as the inoculum mites drowned on larval food or disappeared from the culture. However, when pupae were used as hosts, three mites out of the 24 inoculum mites lived for two days and one for 5 days without laying eggs. The removal of bee larvae and pupae from cells led to high mortality rates of the bees due to mechanical injury. The use of young infested worker bees isolated from siblings was also unsuccessful, as worker bees died after two days, with the subsequent death of the mites. When the artificial diet was used for *A. externus*, laboratory cultures failed to thrive as mites were immobilized by the tackiness of the artificial diet that leaked out of the parafilm. Eckert (1967) and Jaycox (per. com.) also were unsuccessful in maintaining and rearing mites by *in vitro* methods.

The problems encountered in rearing external *Acarapis in vitro* proved intractable. The life cycles of *A. dorsalis* and *A. externus* were therefore estimated by sampling, over time, uninfested marked bees introduced into infested colonies. Trials were conducted for two consecutive summers (1987 and 1988).

On its natural host within a colony, *A. dorsalis* has a total developmental period of 8-9 days (Table 1). Eggs appeared two days

Table 1. Life cycle comparison of A. *dorsalis* and A. *externus*.

Life Stage	Duration (days)*	
	A. *dorsalis*	A. *externus*
Egg	4	3
Larva/Nymph	4-5	5-6
Oviposition to Adult	8-9	8-9

* Average of two trials conducted during consecutive summers
  n = 1,110 bees

post-introduction of newly emerged marked bees into infested host colonies. The first larvae were observed after 6 days, suggesting a 4-day egg incubation period (Figure 1a). Newly emerged adults were first observed 4-5 days after egg hatch (8-9 days following egg deposition). Males first appeared on the 10th day after bee introduction while new females were observed on the 11th day post-introduction, which suggests that the developmental period for the male is shorter than that of the female.

The developmental time of A. *externus* was similar to that of A. *dorsalis* however, differences in the length of egg and larval/nymphal stadia were noted. Egg incubation took only three days in A. *externus* as compared to four days in A. *dorsalis*. The larval/nymphal stages however, required a day longer (5-6 days). Males emerged earlier than females. The observed developmental stages of both *Acarapis* species were as described by Delfinado-Baker and Baker (1982).

Eggs of A. *externus* were first observed on the second day post-introduction (Figure 1b). In contrast to A. *dorsalis*, which lays eggs singly on the dorsal groove of the bee thorax, A. *externus* eggs are
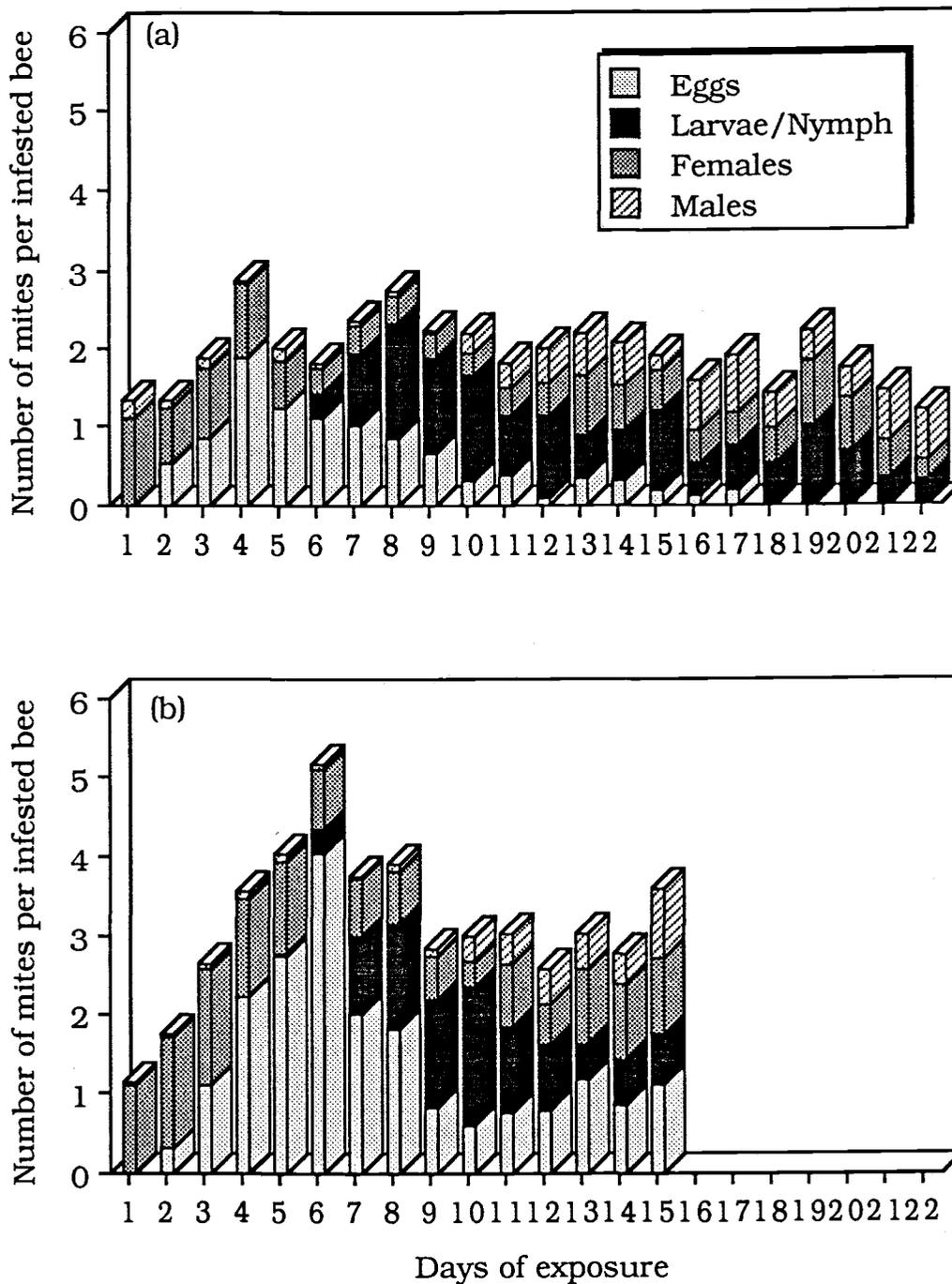
Figure 1. Life stage distribution showing the number of mites per infested bee at 24 hour intervals after the introduction of marked newly emerged bees into infested colonies: (a) *A. dorsalis* (b) *A. externus*.

glued to the neck of the bee host with a mucilaginous substance. Peak egg production was observed four days after bee introduction for *A. dorsalis*, while *A. externus* showed highest egg production on the 6th day (Figure 1).

For both species, there was a gradual decrease in the number of females and a distinct reduction in the proportion of eggs up to the 10th day of bee exposure (Figures 1a and 1b). This observation suggests that females of both species migrate to other bee hosts following egg deposition on an initial host. The decrease in the number of females and in the number of eggs may also indicate that only 1 to 9-day old bees are attractive to mites, as suggested by Royce *et al.* (1988). That females and males of both species were migratory is shown by the appearance of both sexes one day post-introduction of newly emerged bees into infested colonies. Both mite species may infest bees within a day of hosts emergence from brood cells.

Mating behavior was not observed for either mite species. The emergence of males prior to females could indicate that mating is accomplished before females migrate to young bees. Rennie (1921) and Hirschfelder and Sachs (1952) suggested that only gravid females of *Acarapis* migrate.

It is of interest to note that the number of *A. externus* found on each infested marked bee was usually higher than that of *A. dorsalis*. Variation in mite load as affected by the age of the bee hosts was studied in a separate experiment.

Variations in Mite Load According to Honey Bee Age.

Newly emerged marked bees less than 24h old were introduced into infested colonies and were monitored for 40 days. Changes in infestation levels and mite load were noted as the bees aged. Sampling was performed at five day intervals. The data showed a decrease in the percent infestation of *A. dorsalis* on the 25th day (Figure 2). This decrease coincides with the foraging age of the host bees, which peaks on the 23rd day of the bees' life (Winston 1987). Percent infestation by *A. externus* remained relatively high for bees 35 days of age. A decline in the number of mites per infested bee was observed on the 20th day for *A. dorsalis* (Figure 3a). For *A. externus*, the drastic decline in mite load was not observed until the 40th day (Figure 3b), when marked bees were approaching the end of their life span. No more marked bees were present in experimental colonies 40 days post-introduction.

From the data, it appears that *A. externus* is more capable than *A. dorsalis* in surviving on older bees. Relatively high infestation levels and mite load of both species when hosts are younger could be an indication of mite preference for younger bees. However, this observation could also be attributed to the temporal division of labor among honey bees. While old bees generally leave the hive to forage for nectar and pollen, young worker bees clean, build combs and care for the brood (Free 1965, Winston *et al.* 1982, Winston 1987). Since the majority of young bees are hive bees, there are potentially more contacts among young bees. This condition could facilitate the transmission of mites from infested to uninfested bees, with the
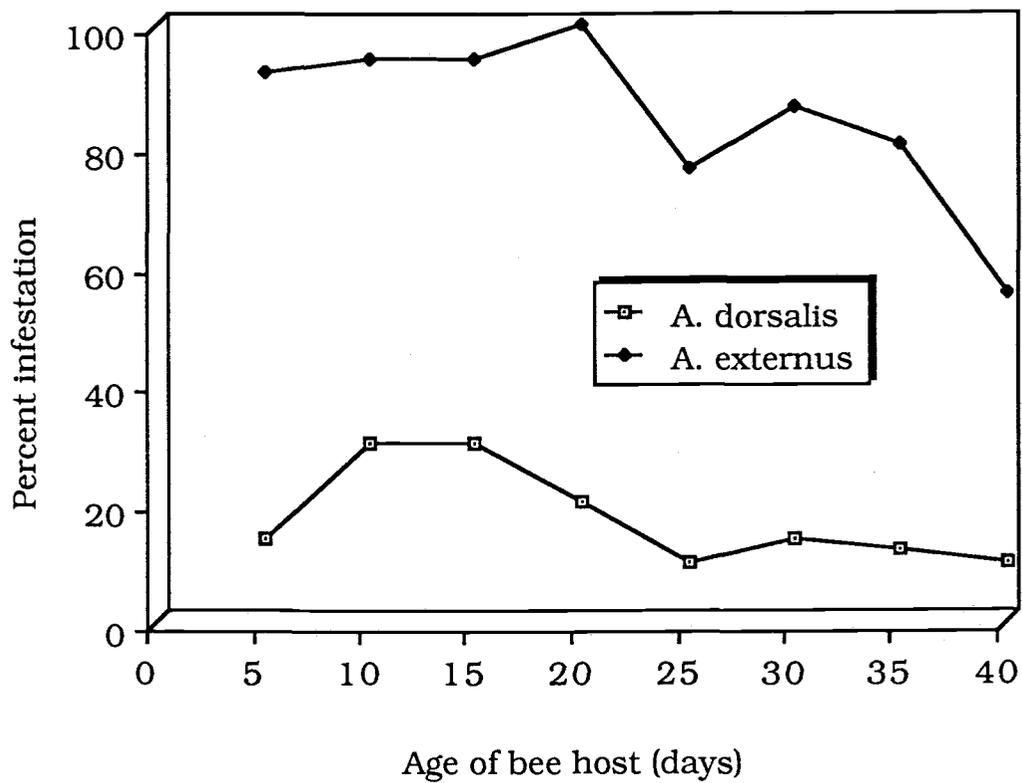
Figure 2. Infestation levels of *A. dorsalis* and *A. externus* in
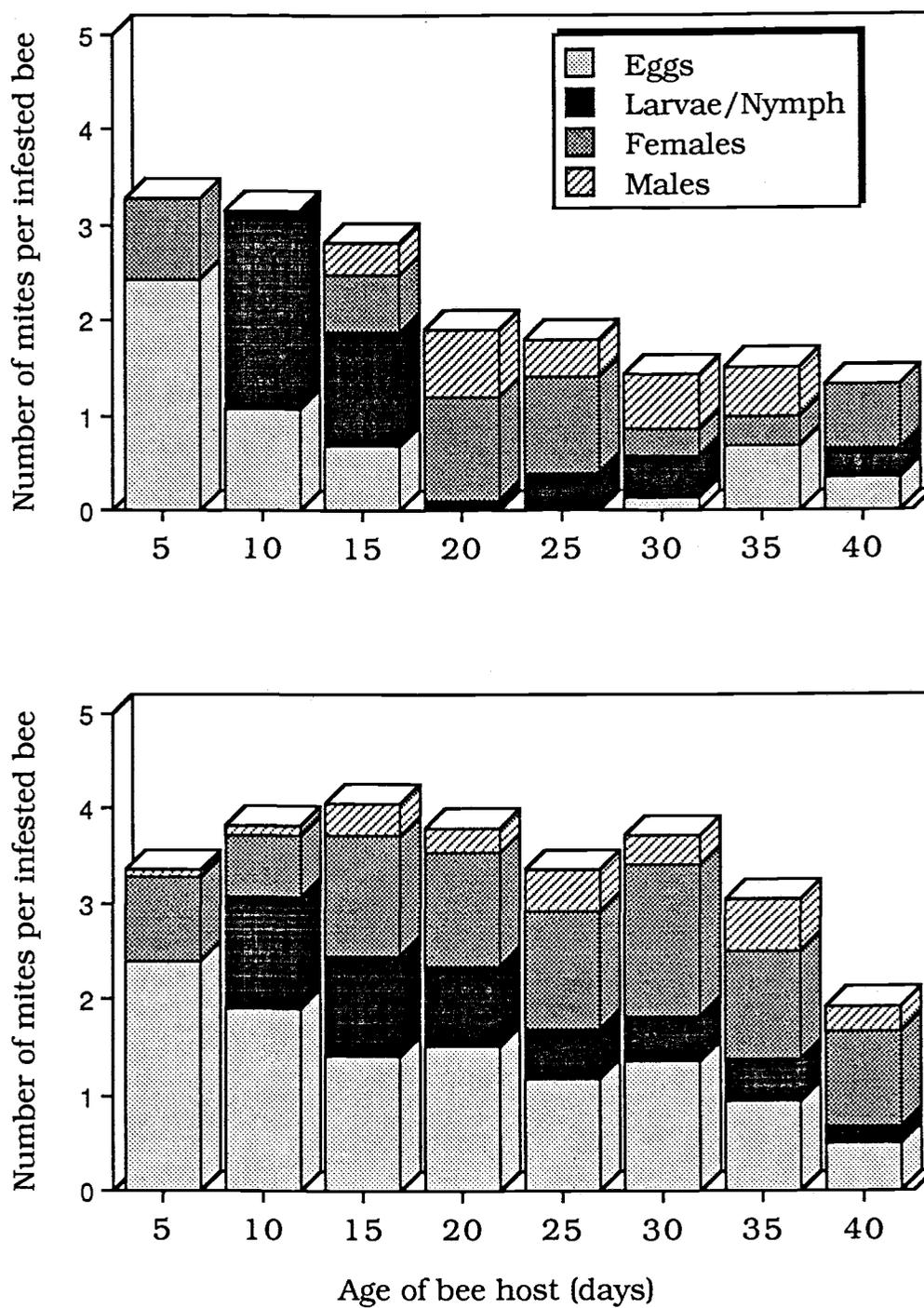honey bees of various ages.

Figure 3. Variations in *Acarapis* mite load in honey bees of various ages: (a) *A. dorsalis* (b) *A. externus.*

subsequent increase in infestation level and mite load.

For *A. dorsalis*, the decline in the rate of infestation and mite load as the bees become older may be advantageous for the mites. At the onset of foraging activity (23 days), bee mortality increases markedly, thereby drastically affecting mite population in the colony. Having high mite load in older bees for *A. externus* may be advantageous for mite dispersal through drifting of older foraging adults.

Natural introduction of mites into uninfested colonies is probably caused not only by drifting of older foraging adults but by lost young bees as they stray into other hives during orientation flights. Additional factors which should be considered include swarming and management techniques such as division of colonies, joining of weak colonies, and migratory beekeeping.

Acarapis Population Growth Rates

The population growth dynamics of *A. dorsalis* and *A. externus* were studied for 13 weeks (first week of July to second week of October 1988) in initially uninfested nuclei deliberately inoculated with known *Acarapis* populations. Analysis of variance revealed that population growth was significantly affected by mite species (P<0.0001), number of mites introduced (P<0.0001) and the combination of the two variables (P<0.0260).

Regardless of the number of mites introduced, *A. dorsalis* showed higher rate of infestation (12.71%) than *A. externus* (1.49%) (Table 2). Introducing 500 mites established a higher rate of mite infestation (9.89%) faster than introducing 50 mites (4.31%) of either *Acarapis* species. Introduction of 500 *A. dorsalis* resulted in the highest infestation rate (17.10%) of any treatment combinations.

Table 2. Percent infestation as affected by the *Acarapis* species involved, and the number of mites introduced.

Acarapis species	No. of mites introduced		Sum	Mean
	50	500		
Acarapis dorsalis	8.31	17.10	25.41	12.71
Acarapis externus	0.31	2.67	2.98	1.49
Sum	8.62	19.77	28.39	
Mean	4.31	9.89		7.10

Standard error = 5.16
n = 12 nucleus colonies

Infestations by *A. dorsalis* can increase tremendously in a relatively short period of time, *i.e.*, 9-12 weeks after mite introduction (Figure 4). This suggests that *A. dorsalis* can establish itself more rapidly in a colony than *A. externus*. It may also be the reason why *A. dorsalis* is frequently collected at the OSU apiary, in a commercial apiary in Washington (Burgett *et al.* in press), and in Britain (Bailey 1981). Whether this ability is due to colonization ability or the capacity for increase of *A. dorsalis* cannot be ascertained with the data available.
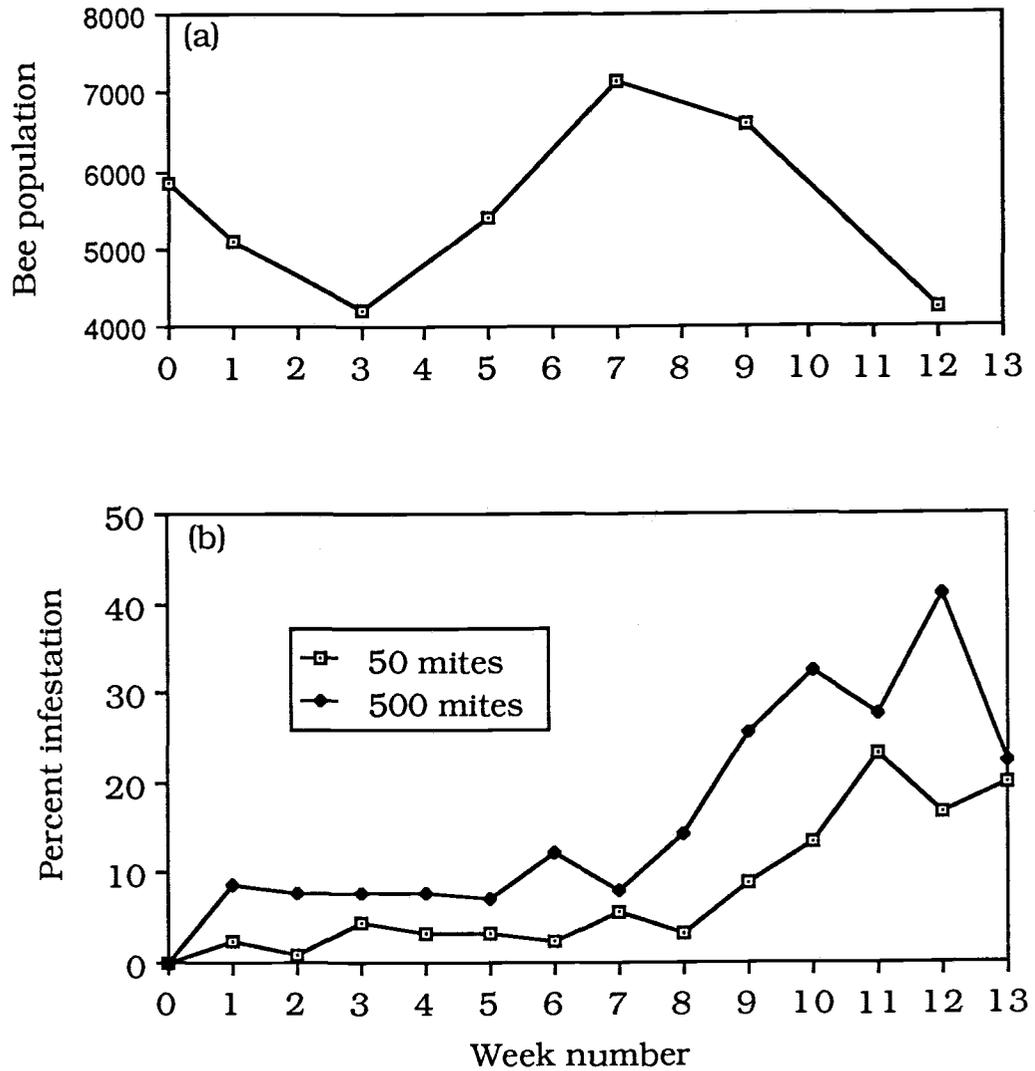
Figure 4. (a) Average adult bee population in the nucleus
colonies. (b) Percent infestation of *A. dorsalis*
over a 13-week period following introduction as
affected by the number of mites introduced.

The apparent dominance of one external *Acarapis* species over the other may also be associated with the hive management practice involved. In the course of looking for a possible source of *A. externus* for this experiment, bee samples were taken from three hobbyists and one commercial beekeeper. Examination of these samples revealed that only *A. dorsalis* was present on the hobbyists' bees, while samples from the migratory beekeeper had both mite species, with *A. externus* as the more prevalent species.

Nuclei deliberately infested with only *A. dorsalis* maintained this species as their sole parasite, whereas nuclei infested with *A. externus* were eventually invaded by *A. dorsalis* (Figure 5). This invasion may be due to drifting of foraging bees from *A. dorsalis*-infested colonies at the apiary.

Higher abundances of *A. dorsalis* were also observed when an equal number of each species (25 *A. dorsalis* + 25 *A. externus* or 250 *A. dorsalis* + 250 *A. externus*) were introduced into clean nuclei (Figure 6). A low level of infestation by *A. dorsalis* and no *A. externus* were observed 1.5 months after 25 mites of each species were introduced into nucleus colonies (Figure 6a). At higher initial levels of infestation, *A. dorsalis* always had a higher infestation rate than *A. externus* in all nuclei observed. This suggests that populations of *A. dorsalis* have a higher growth rate than *A. externus*. It is also possible that *A. dorsalis* outcompetes *A. externus* in these colonies. However, interaction between these two *Acarapis* needs further study.

There may be a critical mite population required for both species to establish a viable population. The introduction of 500 *A.*

Figure 5.   Percent infestation of *A. externus* over a 13-week
period following introduction as affected by the
number of mites introduced: (a)   50 mites
(b)   500 mites. Also shown are the percent
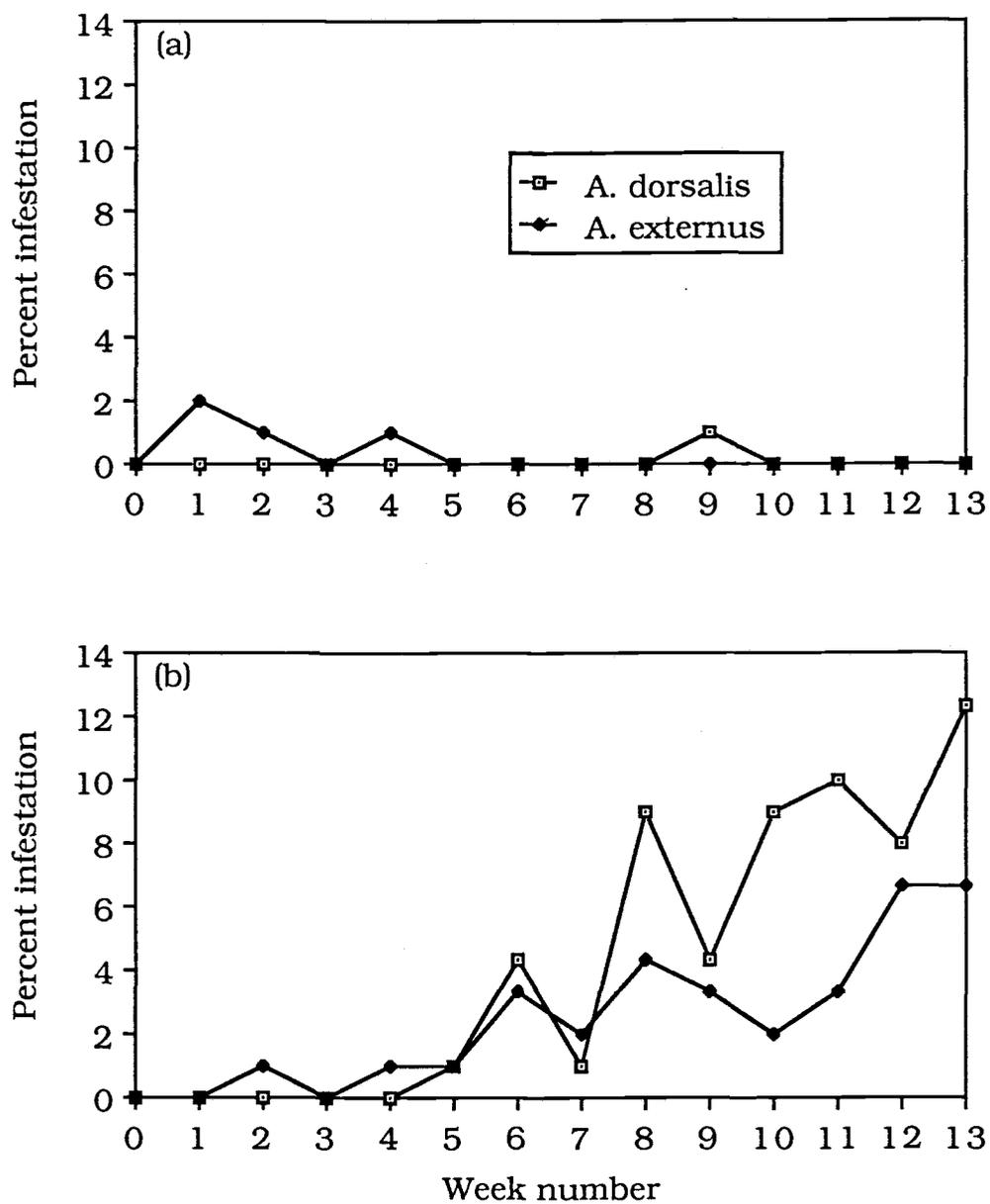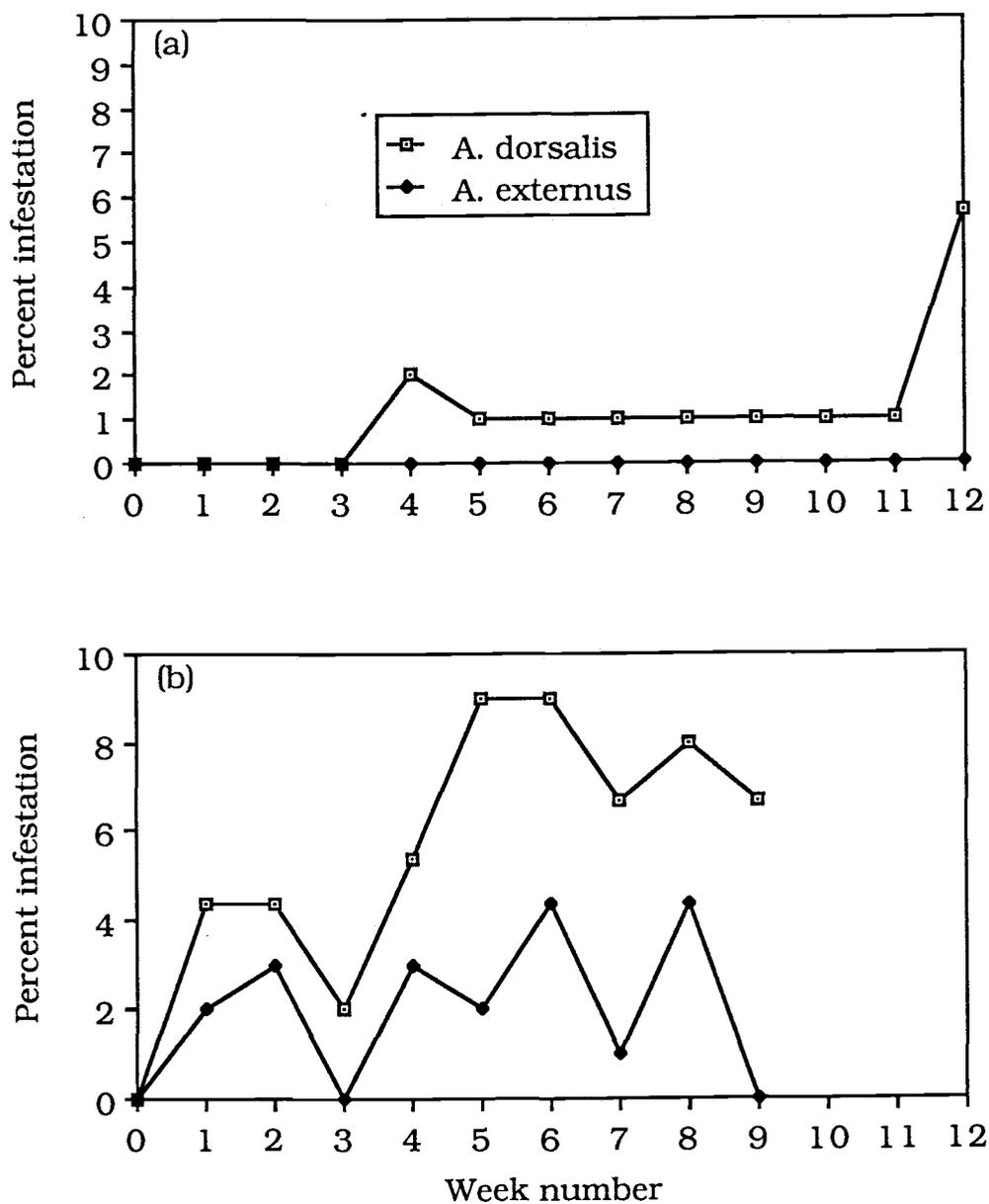infestations by invading *A. dorsalis*.

Figure 6. Percent infestation of *A. dorsalis* and *A. externus* over a 12-week period following introduction as affected by the number of mites introduced: (a) 25 *A. dorsalis* + 25 *A. externus* (b) 250 *A. dorsalis* + 250 *A. externus*.

externus may be too small an initial population, so that *A. dorsalis* can easily outcompete them. *A. dorsalis* may dominate a host colony at lower mite populations. In short, the critical founder population level of *A. externus* may be higher than 500, the maximum number used for this experiment.

As noted earlier, where uninfested bees were introduced into a mite-infested colony, *A. externus* appeared to have higher rate of increase than *A. dorsalis*. The reverse seemed to be true for the rate of infestation in initially clean nuclei inoculated with infested bees. This observation suggests that a critical population must be attained by *A. externus* to establish itself in a hive. Unless this critical population is reached prior to invasion by *A. dorsalis*, *A. externus* appears to be unable to maintain its population in the hive.

The experiments described here did not reveal the critical mite population requirement for either mite species but from the available data, *A. externus* probably requires an initial population higher than 500, the maximum number used in the experiments. For *A. dorsalis*, introduction of 50 mites was probably sufficient for establishing a viable parasite population in a clean hive. This estimate is supported by data from the following section: *A. dorsalis* populations showed a maximum of 30% infestation, a level very close to the highest infestation reached by introducing 50 *A. dorsalis* into clean nuclei with an average adult bee population of 5,193.

A) *Acarapis dorsalis*

The seasonal phenology of *A. dorsalis* is shown in Figure 7a. The infestation rate was 22 percent in August 1986 when monitoring began, and gradually decreased to less than 5 percent in January of 1987. The same trend was observed during the second year. The initial infestation rate of 22% in August 1986 was never reestablished during the remainder of the observation period, but relatively high infestations were recorded in the spring months (March to June) and during mid-late summer (August-September). This period coincided with the increased and decreased brood rearing activities of a honey bee colony during the active foraging season. Infestation levels were generally low in the winter months (December, January), when little brood was present in colonies, and when only the longer-lived bees populated the hives. Death of bees due to aging, and to unfavorable conditions inside the hive (*e.g.*, condensing water not removed in the course of bee housekeeping activities) is a normal occurrence in winter months. Flights to void fecal matter also contribute to host bee mortalities during the winter period.

These observations seem to illustrate the classic case of an obligate parasite adapting its population levels to that of its host. In periods where no new hosts were emerging (*i.e.*, winter), *A. dorsalis* was maintained at a low population level. During these months (December and January), no new hosts were available. Consequently, limited food resources slowed the reproduction rate of the parasite.
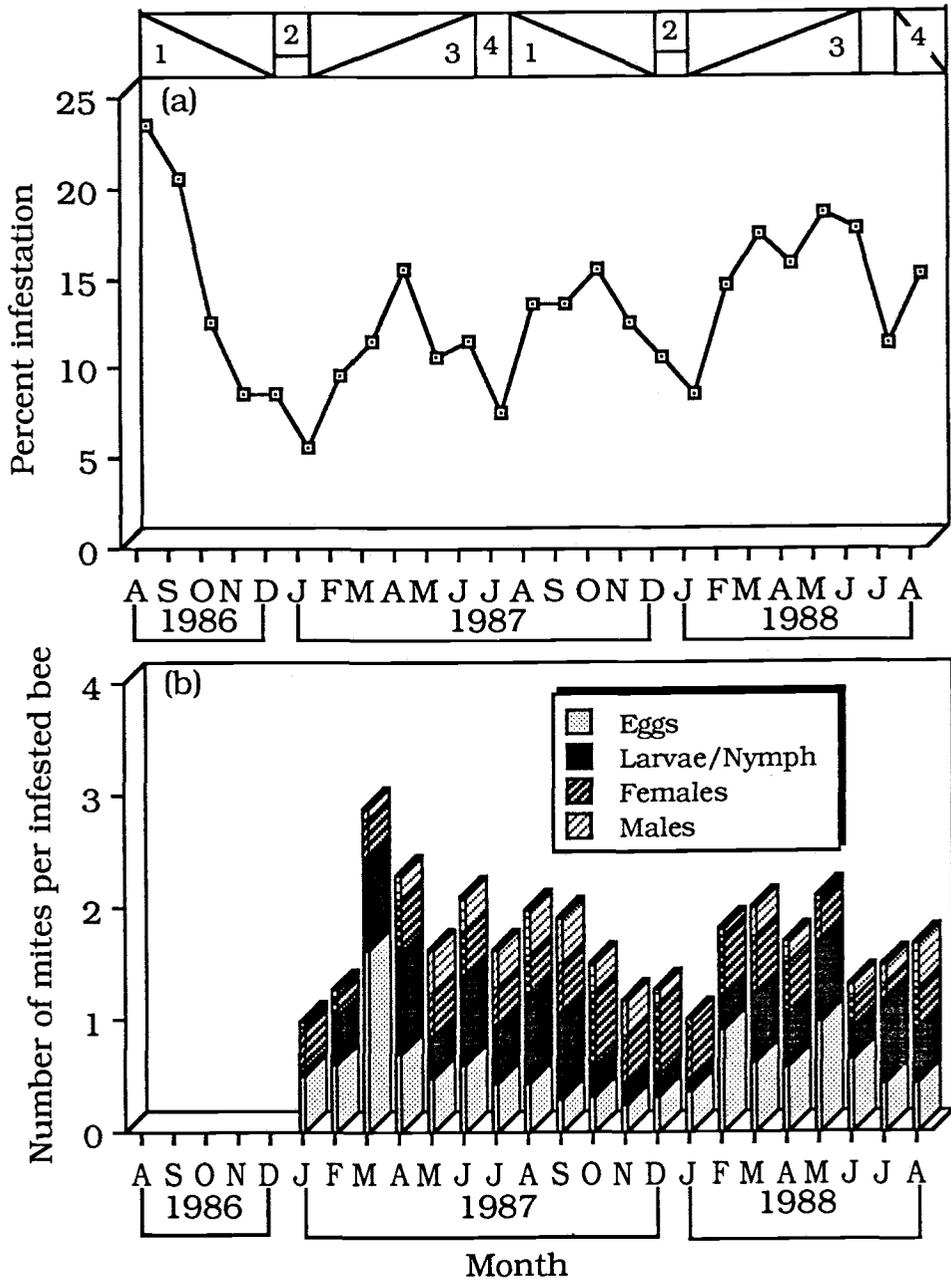
Figure 7. (a) Seasonal population fluctuation of *A. dorsalis*
in relation to bee brood rearing activity. (b) Life
stage distribution of *A. dorsalis* at monthly
intervals. 1 - brood rearing declines, 2 - little
or no brood, 3 - brood rearing increases,
4 - peak adult bee population.

From March to June, new brood began to emerge, more new hosts were available, and mite populations increased.

A distinct drop in infestation level was apparent in July of both 1987 and 1988. This month coincided with peak bee emergence in the hive, and might reflect the dilution of mite populations due to a high number of new, uninfested hosts emerging from cells.

All the life stages of *A. dorsalis* were found throughout the sampling period (Figure 7b). The constant presence of immatures suggests that the mites reproduce continuously throughout the year. However, fecundity was higher in the spring (March 1987 and February 1988) than in the fall and winter months.

It is of interest to note that the months of high infestations and high host populations (March to June) coincided with months of high mite load per infested bee. However, periods of low infestation levels did not always coincide with low mite loads. This observation may indicate that mite populations increase with the population increase of the host, but the actual transfer of mites from infested bees to new hosts lags behind host population increases.

B) *Acarapis externus*

Figure 8 shows the population fluctuation of *A. externus* from two colonies during the period August 1987 to January 1989. It should be noted that these colonies were also infested with *A. dorsalis*. In these colonies, *A. externus* was more abundant, *i.e.*, its population was higher at both colony and individual host levels than was that of *A.*
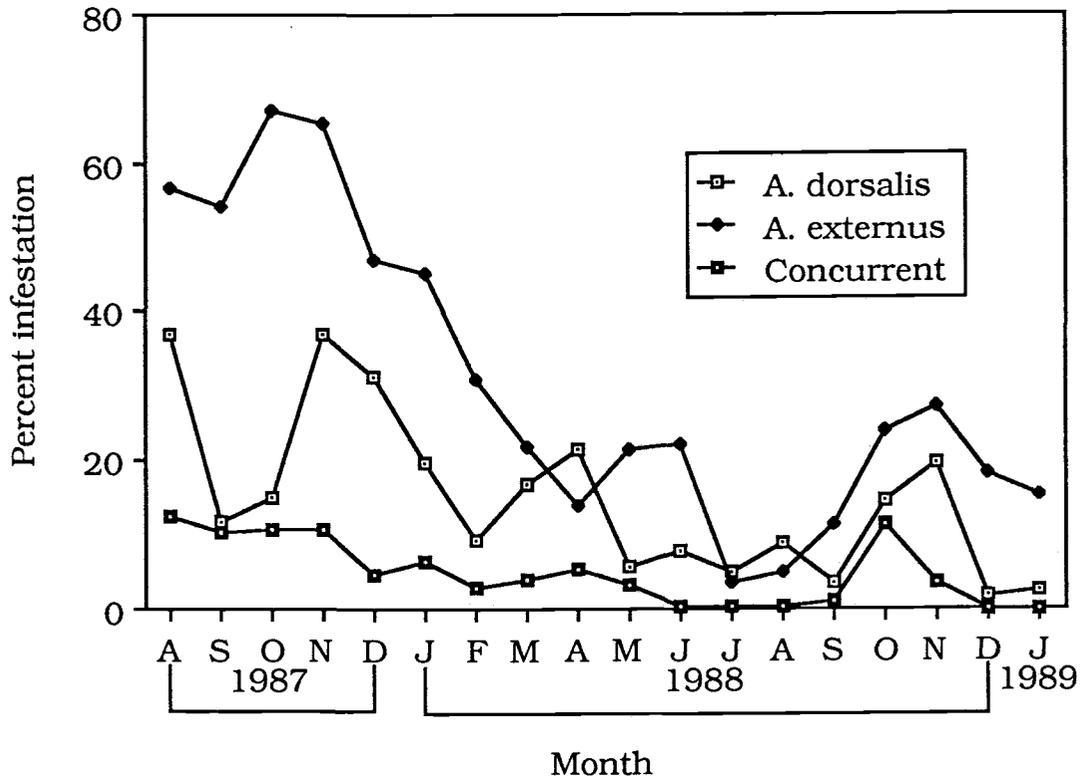
Figure 8. Seasonal population fluctuation of *A. dorsalis* and
A. externus in concurrently infested hives.

dorsalis. A relatively small percentage of worker bees were parasitized by both species, a phenomenon also noted by Burgett *et al.* (in press).

The infestation rates of both species in these two colonies fluctuated throughout the experimental period and mite incidence showed almost identical patterns. The rate of infestation for both species began to decrease in December, reaching a minimum in July. All the developmental stages were found throughout the year and fecundity of both species decreased in fall and increased again in winter (Figures 9a and 9b). These results do not agree with previous reports (Anonymous 1932, 1933) stating that *A. externus* increases reproduction during late fall and winter because this period is when the winter cluster forms within the colony.

Percent infestation by *A. externus* was highest in October and November 1987, and declined until the end of the observation period (January 1989). The level of infestation was lowest in July 1988. This trough was followed by a slight increase in percent infestation, and a small peak in November 1988. *A. externus* loads per infested bee were also relatively high during these months, a time when bee colonies had a higher proportion of older bees as brood production and emergence of young bees were on the decline. It thus appears that, for *A. externus*, the age of the host has little effect on mite population.

Unfortunately, the presence of *A. dorsalis* in hives designated for the study of *A. externus* population dynamics complicated data collection. Because of possible interspecific influences, any conclusions based on the available data should be made with caution.
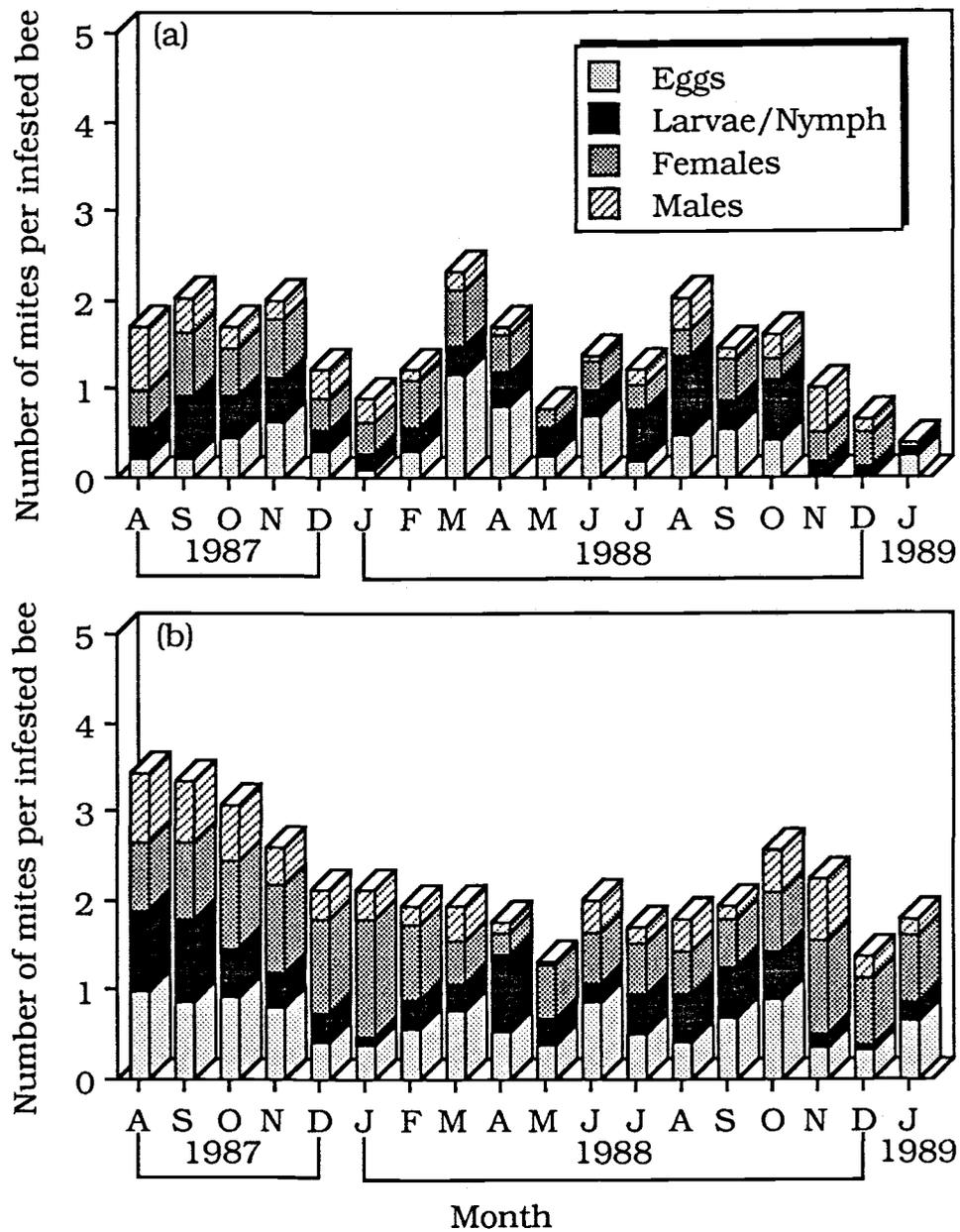
Figure 9. Life stage distribution: (a) *A. dorsalis* in colonies with *A. externus* (b) *A. externus* in colonies with *A. dorsalis*.

The percent infestations of both *Acarapis* species from the two colonies that died in the winter of 1988 and the two surviving colonies are shown in Figure 10. A higher rate of infestation by the two *Acarapis* species was observed in the dead colonies than in the surviving ones. There was an increase in the percent infestation by *A. dorsalis* (50.38%) with a relatively high number of bees infested concurrently by both species (45.5%). Infestation by *A. externus* remained at high levels although a slight decrease was noted during the winter months. The death of the two colonies probably was caused by winter stress (chilling), aggravated by the increasing infestation of *A. dorsalis* and concurrent infestation by many *A. externus*. The last sampling of the two dead colonies revealed a mean percent infestation of 64% by *A. dorsalis*, and 62% by *A. externus*. A total of 49% were parasitized by both species. In the two surviving colonies (Figure 10b), the percent infestation by both species declined in February, which may have lessened the effects of chilling and starvation that the bees were experiencing at that time.

Percent mite infestation of *A. dorsalis* in relation to nectar flow can be deduced from Figure 11. Nectar flow at the apiary was based on the gain or loss of weight of a scale hive taken at weekly intervals. Figure 11 shows that there was no relationship between percent mite infestation and nectar flow. Decreased infestation rates in July (Figure 11b) were observed in both years of observation irrespective of gain or loss in the weight of the scale hive. No research has been done on *A. dorsalis* and *A. externus* to directly confirm this finding. However, literature on *A. woodi* and nectar flow suggests that a good season reduces or keeps the infestation rate low (Clark 1985, Bailey 1981).
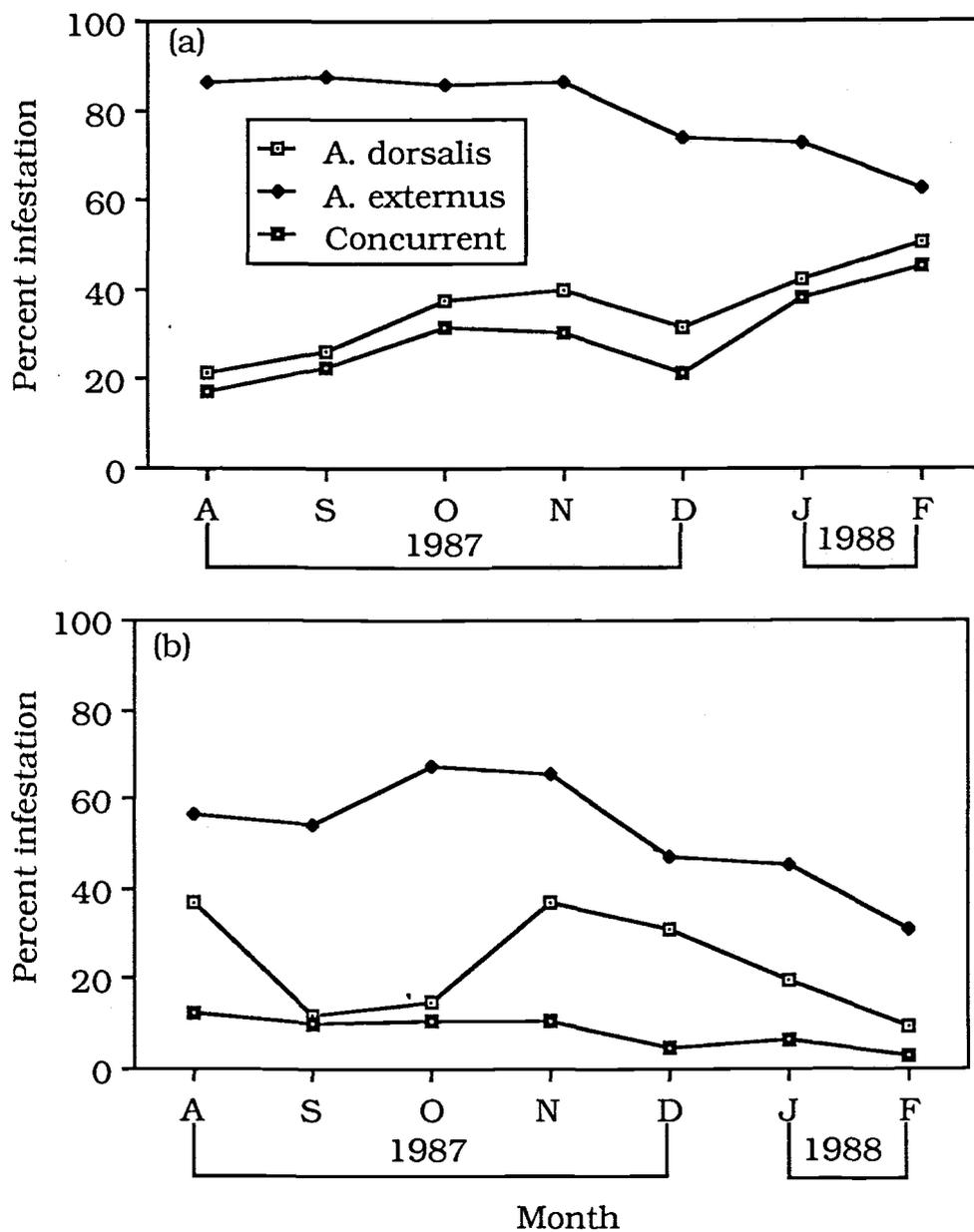
Figure 10. Percent infestation of *A. dorsalis* and *A.*
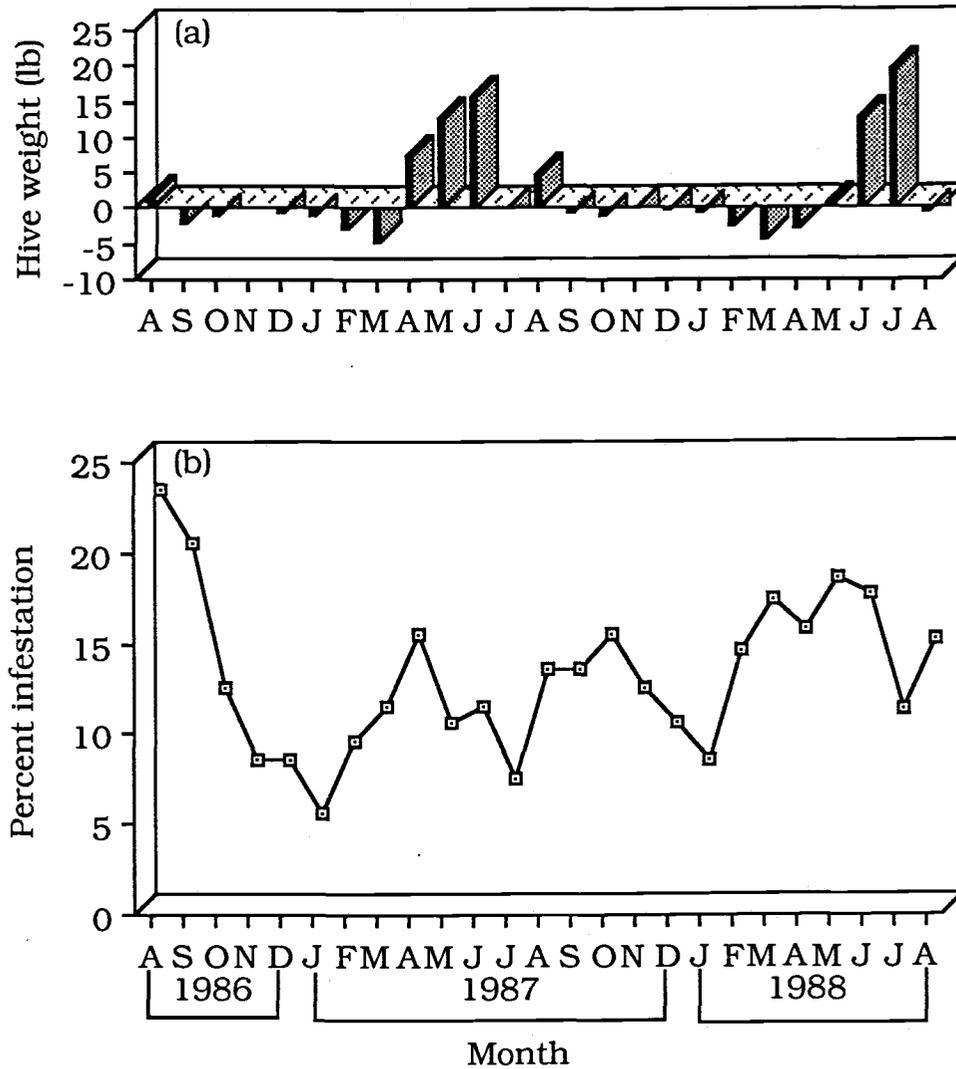*externus*: (a) two dead colonies (b) two
surviving colonies.

Figure 11. Relationship between nectar flow and percent
infestation of *A. dorsalis*: (a) Monthly change
in weight of a scale hive (b) Percent infestation
of *A. dorsalis*.

Guzman-Novoa and Zozaya-Rubio (1984) found that low honey production was related to infestation rates above 35 per cent.

C) Sex Ratio

The average female:male ratio of *A. dorsalis* was established at 1.9:1 (Figure 12a). The highest number of males was recorded during the summer, while more females were observed in the fall. Female:male ratios were always higher than 1:1 during all seasons except summer.

The sex ratio of *A. externus* also was skewed in favor of females (Figure 12b) with a ratio of 2.07:1. This estimate is not in agreement with the data of Brugger (1936), which established a 1:1 sex ratio. A varying sex ratio of 2:1 through 1:1 to 2:3 by *A. dorsalis* and *A. externus* was cited by Lindquist (1986). The apparent abundance of females in both species may suggest that males are shorter-lived than females.

Effects of *A. dorsalis* and *A. externus* on Bee Mortality

The effect of the two external *Acarapis* species on infested bees is presented in Table 3. Very few dead marked bees were recovered from any treatment colonies throughout the experimental period. This experience agrees with that of Gary (1975), who noted that most bees die outside of the hive. Out of the 730 marked bees introduced into an *A. dorsalis*-infested colony, only 46 dead marked bees were
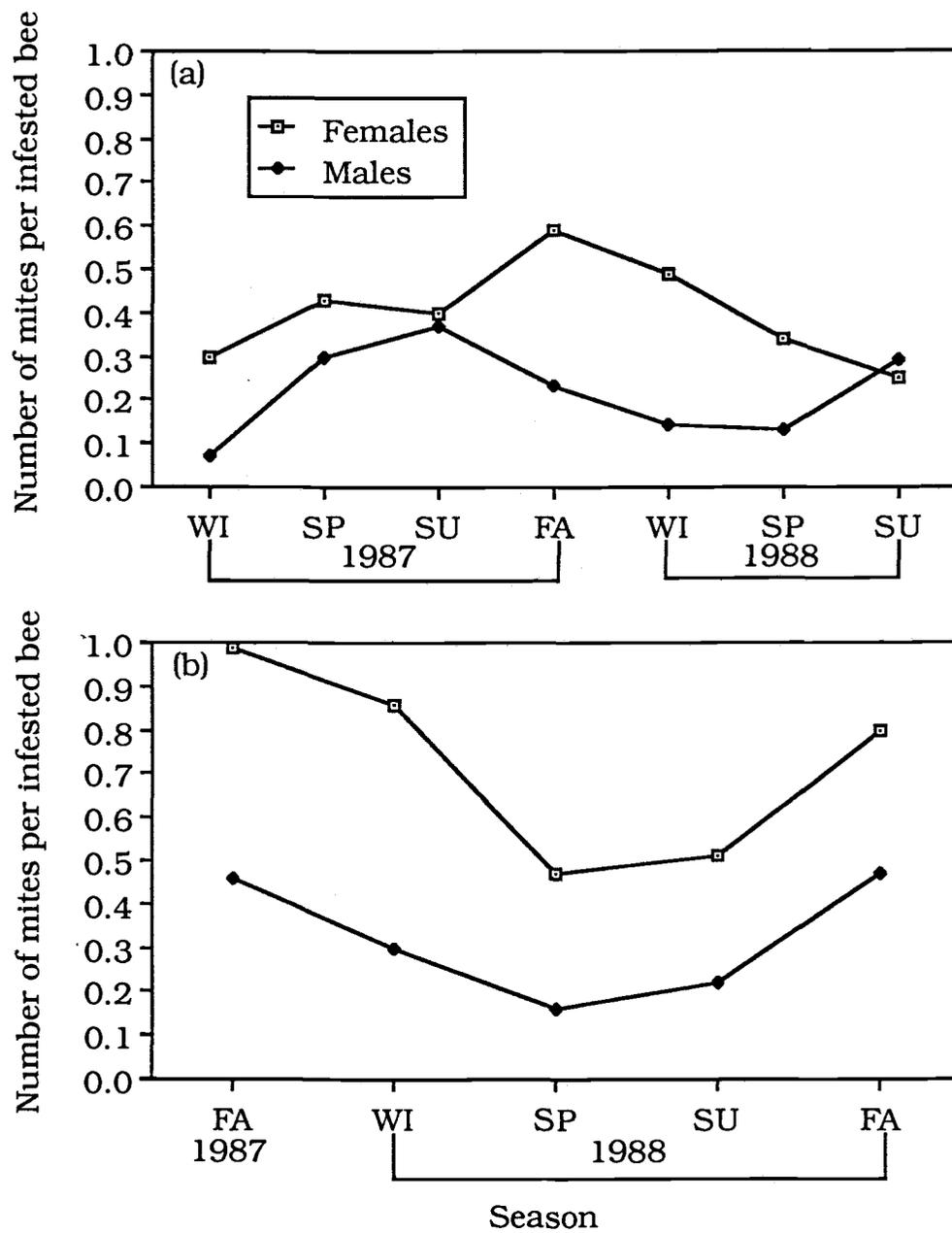
Figure 12. Seasonal sex ratio: (a) A. dorsalis (b) A. externus.
WI - Winter, SP - Spring, SU - Summer, FA - Fall.

retrieved. Of these, 16 bees were infested and 30 were uninfested. Of the 52 dead marked bees recovered from the 1,217 marked bees introduced into an *A. externus*-infested hive, 26 were infested and 26 uninfested. Sixty three dead bees out of the 1,090 marked bees placed in the control colony were recovered, of which only one was infested.

On March 23, 1989, all live marked bees that survived the winter were collected. Very few marked bees were retrieved from the *A. dorsalis*-infested hive (84 bees) or the control colony (4 bees), while 392 bees were recovered from the hive infested with *A. externus*. Most bees apparently died outside of the hive while foraging. The discrepancy among colonies in the proportion of the total number of dead marked bees and the number of marked bees that survived through the winter may be due to the inequality of colony strength during the winter months. The *A. externus*-infested colony had an abundant supply of honey and remained strong, while the colony with *A. dorsalis* and the control colony were weak and had little honey stores.

It is difficult to conclude from this experiment whether or not the mites have a pathogenic effect on bees. Many variables may have affected conclusions that may be derived from the data. Among these were colony strength and food stores. Variations in these factors would have strongly influenced the ability of the colonies to successfully survive the winter. Moreover, some colonies may have had a greater proportion of older bees at the onset of winter. A major difficulty in research protocol was the exclusive maintenance of one

species of *Acarapis* mites in a colony. All *Acarapis* species were found in the apiary and drifting of bees could not be completely controlled.

Table 3. Number of infested and uninfested bees from dead marked bee samples.

Treatment	Month	Date and no. of bees introduced	No. of dead marked bees		
			Total	Infested	Uninfested
Acarapis	October	Sept. 23,	6	3	3
dorsalis	November	1988	4	0	4
	December		5	1	4
	January	(730 bees)	7	3	4
	February		8	4	4
	March		16	5	11
		Total	46	16	30
Acarapis	October	Sept. 22,	8	3	5
externus	November	1988	3	2	1
	December		6	3	3
	January	(1,217	8	6	2
	February	bees)	26	11	15
	March		1	1	0
		Total	52	26	26
Control	October	Sept. 21,	6	0	6
	November	1988	18	0	18
	December		21	0	21
	January	(1,090	3	0	3
	February	bees)	14	1	13
	March		2	0	2
		Total	63	1	62

SUMMARY AND CONCLUSIONS

Acarapis dorsalis and *A. externus* both completed their development in 8-9 days. *A. dorsalis* required four days for embryogenesis (egg hatch) and 4-5 days more before emergence of adults. *A. externus* needed only three days for embryogenesis, but the immature stages lasted for 5-6 days. In both species, males emerged earlier than females.

Changes in infestation levels and mite loads were monitored in newly emerged bees introduced into infested colonies. A decrease in the percent infestation of *A. dorsalis* was observed on bees older than 25 days, which coincided with the mean foraging age of the marked bees. *A. externus* infestation remained high on bees up to 35 days old. Mite loads in *A. dorsalis*-infested bees declined on the 20th day. For *A. externus*, the decline in mite load was not observed until the 40th day. It appears that *A. dorsalis* prefers only younger bees while *A. externus* seems to maintain its numbers on older bees.

The infestation rates of *A. dorsalis* and *A. externus* were also studied in initially uninfested nucleus colonies of bees. The infestation rates were significantly affected by the species of mite introduced, the number of mites introduced, and the interaction of these two variables. Invasion by *A. dorsalis* appears to be more rapid than that of *A. externus*. Introduction of 500 *A. dorsalis* rapidly established the highest rate of infestation noted. The available data indicate that a critical mite population level is required for either species to establish a viable population. Introduction of 500 *A. externus* may not be enough to accomplish establishment. For *A. dorsalis*, 50 mites

probably is sufficient for establishing a viable population in a non-infested colony. However, an accurate estimate of this founder population could not be made with the available data. Furthermore, nuclei deliberately exposed to *A. dorsalis* maintained this mite as their exclusive parasite while those infested with *A. externus* eventually showed concurrent infestation by *A. dorsalis*. Such cross-infestation was attributed to drifting of infested forager bees between colonies.

Seasonal fluctuations in populations of *A. dorsalis* and *A. externus* were monitored in full strength colonies. *A. dorsalis* had the highest infestation levels recorded in the spring months (March to June), when suitable hosts were emerging, and during mid-late summer (August and September) when brood rearing activity was on the decline. For *A. externus*, infestation was highest in the fall (October and November), when there was a higher proportion of older bees. This is another indication that *A. externus* can maintain itself on older bees. *A. externus*-infested hives used for this study eventually were invaded by *A. dorsalis*.

The lowest populations of *A. dorsalis* were recorded in January when no suitable hosts for the mites were available and in July, which coincided with the peak adult bee population and dilution of the mite population inside the colony. *A. externus* also had the lowest infestation during the month of July. Both species were observed to be multivoltine since all developmental stages were present year round. Fecundity decreased during the winter months (December-January). The average female:male ratios in colonies used for seasonal fluctuation studies (6 for *A. dorsalis* and 2 for *A. externus*)) were 1.9:1 for *A. dorsalis* and 2.07:1 for *A. externus*. This may indicate that males

are shorter-lived than females. No relationship between nectar flow and percent mite infestation was established.

An attempt was made to study the effects of external *Acarapis* on the mortality of marked bees in infested hives. The recovery of dead marked bees was too low to allow collection of meaningful data. The question of whether or not the effect of these mites is really of economic importance has yet to be resolved.

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