THE BEE LOUSE, *BRAULA COECA* NITZSCH,
ITS DISTRIBUTION AND BIOLOGY ON HONEY BEES

by

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ABSTRACT

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Bee lice were found in 28% of Maryland apiaries and 18% of the colonies examined. In apiaries with lice, 50% of the colonies contained lice.

Laboratory tests demonstrated that bee lice had no preference between 1, 5, 15 and 30 day old honey bee (*Apis mellifera* L.) workers at 25°C while there was a preference for 1 day old workers at 34°C. Lice preferred young drones over old drones and virgin and mated queens over young drones at 25 and 34°C. Lice preferred virgin queens over old drones at 25°C while no preference was observed at 34°C. Mated queens were preferred over old drones at 25 and 34°C. There was a preference of lice for foraging age workers over old drones at 25°C while there was no preference at 34°C. Lice preferred both virgin and mated queens over random age workers at 25 and 34°C.

Louse larval tunnels were numerous in nucs (4 frame honey bee colonies) stocked with lice from May through August corresponding with periods of nectar flow when bees were capping honey. In field colonies, louse populations decreased

in the late spring to a low in early June. During July and after, populations of lice rose with the emergence of new lice. Few immature and adult lice were observed in control nucs having similar populations of bees.

In nucs, 1 or more lice were observed on 24% of the queens between August and December. Only 2% of the virgin queens contained lice during the same period. In field colonies, 62% of the queens examined from June through the rest of the season harbored lice; 58% of these lice were pale in color indicating they were less than 1 day old. One louse was observed on 98.6% of the workers with lice, while 1.2% harbored 2 lice and 0.2% had 3 lice; 4.2% of the lice were on drones.

A single bee louse was observed on 3,092 foraging honey bees sampled. One-hundred-seventeen lice were collected on 14,459 bees collected from the brood nest of the same hives. Control samples indicated a 14 to 15% loss of lice during sampling. Tests demonstrated that during visual observations of lice on bees only 49% of the lice present were observed. Fluctuation in louse population levels were similar to those found elsewhere in this study.

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CHAPTER I

INTRODUCTION

The bee louse, *Braula coeca* Nitzsch (Diptera: Braulidae), is a commensilate found in honey bee *Apis mellifera* L. (Hymenoptera: Apidae) colonies. The louse was first discovered by Reaumur (1740) and was named by Nitzsch (1818). Imms (1942) placed *Braula* in a separate family, Braulidae, near the Chamaemyiidae of the acalypterates. Presently 5 species and 1 subspecies are included in the Braulidae. This study deals only with *B. coeca*.

Adult bee lice are reddish-brown in color having a length and width of 1.5 mm and 0.75 mm, respectively. Lice can usually be seen attached to the propodium or prothorax of honey bees (Imms, 1942). They are capable of moving freely on their host and from one bee to another. Adults feed by first performing a dance on the face of their host after which they feed on honey or nectar obtained from the proboscis of the bee.

Bee lice are found on worker, drone and queen honey bees. Arnhart (1924), Hempsall-Herrod (1931) and Atakishiev (1971) stated that *Braula* are infrequently found on drones. Phillips (1925) stated that no more than 1 louse per single worker had been found in Maryland. There are numerous reports of more than one louse being present on queen honey bees especially during the late summer or fall.

Braula lay their eggs on the undersurface of wax cappings covering partially sealed honey (Imms, 1942) or on the surface of fully capped honey cells (Hassanein and Abd El-Salam, 1962). Emerging *Braula* larvae construct distinct and easily visable tunnels in the wax cappings over honey. Tunnels obtain an outside diameter of 2.4 mm and a length of 5 to 9 cm (Atakishiev, 1971). It is here in the tunnels that larvae obtain nourishment from debris within the wax. When mature, the larvae pass into a prepupal and pupal stage enclosed in the unmodified cuticle of the last larval instar. The duration of their total life cycle ranges from 18 to 23 days (Atakishiev, 1971). The adult bee louse emerges from its tunnel through a circular cut made before pupation. Newly emerged *Braula* soon make their way to a host honey bee.

Braula coeca has been found on every continent. It was probably first introduced into the United States along with the first shipments of honey bees. Phillips (1925) stated that bee lice were repeatedly introduced into the United States on imported queens. Presently the bee louse has been sporadically observed within 12 states in the United States.

There is disagreement about the amount of damage caused by *Braula* to honey bee colonies. Clausen (1940) and Frank (1969) felt that the bee louse causes little or no harm to colonies. However, most investigators feel that *Braula* does no good and is harmful to some extent to colonies.

Control measures have been developed to reduce or eliminate the bee louse. Most beekeepers practice mechanical control unknowingly by extracting honey. Braula larvae are eliminated while removing cappings before extraction. Chemical control measures have been developed in Europe and Asia. No pesticide is registered for use against the bee louse in the United States.

This study was conducted to investigate:

(1) The extent of bee lice infestation in Maryland honey bee colonies,

(2) Louse preference between worker, drone and queen honey bees under laboratory and field conditions, and

(3) Fluctuation in louse populations throughout the year.

CHAPTER II

LITERATURE REVIEW

Classification

The bee louse was first discovered by Reaumur (1740). He briefly discussed the species and its relationship to the honey bee colony. The genus and species were later described by Nitzsch (1818) who supplied the bee louse name *Braula coeca*. Nitzsch classified it in the order Diptera, Pupipara, due to the structure of its mouth parts.

Earlier, Fabricius (1794) had erroniously placed the bee louse in the genus *Acarus*, based on Reaumur's figures. Costa (1845) used the species name *entomobis*, apparently not knowing of the work of Nitzsch. Bigot (1885) suggested that the name of the genus might more appropriately be *Melitomyia*. This name is derived from the Greek name, melitta, meaning honey bee.

Errors in Nitzsch's description of the antennae and thorax were corrected by Egger (1853) thus removing some doubts as to its alliance to Diptera. Muggenburg (1892) demonstrated a relationship of the head of *Braula* to the Hippoboscidae. It was originally believed that *B. coeca* was pupiparous because of a general resemblence to the Hippoboscids, Streblids, and Nycteribiids. Borner (1908) put *Braula* in the Hemipteran genus *Thaumatoxena*, which later turned out to be a fly of the family Phoridae. Bezzi (1916) was the first taxonomist to definitely remove the bee louse from the

Pupipara. He placed it as a subfamily of the Phoridae. The Phoridae, however, lack the ptilinum possessed by Braula so Schmitz (1917) suggested that Braula was allied with the acalyptrates nearest to the Sphaeroceridae (Borboridae).

Imms (1942) placed Braula in a separate family, Braulidae, near the Chamaemyiidae of the acalyptrates based on their larval similarities. According to Imms, the following larval characteristics are common to the families of Braulidae and Chamaemyiidae indicating their mutual relationship:

(1) A sclerotized band of cuticle around the pseudocephalon. This feature does not appear to be evident in other larvae of the Schizophora and is probably the vestige of a former more complete head capsule.

(2) A fusion of the hypostomal and pharyngeal sclerites and their general similarity of form, together with the absence of pharyngeal ridges.

(3) The simple, edentate mouth-hooks with broad bases.

(4) The general form of the antennae and maxillary palpi and the associated sensoria.

(5) The tuberculated cuticle together with segmental bands of integumentary processes.

Five species of Braula have been described. Schmitz (1914) designated a second species, B. kohli, on the basis of a single male specimen collected on the African honey bee Apis mellifera adansoni Latreille in the Congo. The species was distinct in the shape of the abdomen, the number of comb teeth on the tarsi (24 to 25) and on the yellowish color. Kaschef (1960) questioned whether *B. kohli* was a separate species. Different authors had reported *B. coeca* to have 30 to 32, 29, 32, 30 to 32, and 22 to 24 comb teeth. Kaschef in his own samples, found that the comb teeth number varied from 23 to 30, depending on the individual, on the leg, on the side of the body the leg was on and on the sex. He also pointed out that young *B. coeca* are yellowish in color.

Three additional *Braula* species, *B. schmitzi* (Orosi-Pal, 1939), *B. pretoriensis* (Orosi-Pal, 1939), and *B. orientalis* (Orosi-Pal, 1963), have been described. Orosi-Pal (1966a) later added the subspecies *B. coeca angulata*. In his review of the family, Orosi-Pal (1966a, 1966b) assigned the *Braula* species, including *B. kohli*, to the following groups:

(1) Coeca group. First abdomenal sternite covered by hairs. Female cerci short, with a shallow intercercal incision; sixth abdominal sternite separated into 2 scale-like portions. Male hypopygium with 2 longer, and between them 1 shorter, appendages. Eggs have a narrow flange with a ragged, indented margin. The group contains *Braula coeca* Nitzsch and *B. coeca angulata* Orosi-Pal.

(2) Schmitzi group. First abdominal sternite wholly, or at least the major anterior portion, glabrous. Female cerci long, with a deep intercercal incision; sixth abdominal sternite undivided, but medially atrophic. Male hypopygium with 2 longer, and between them 1 short, appendages. Eggs

have a wide flange without deep grooves, marked indentations. The group contains *Braula schmitzi* Orosi-Pal and *B. orientalis* Orosi-Pal.

(3) Pretoriensis group. First abdominal sternite covered with hairs. Female cerci distinct, long, pediculate, spatulate appendages; sixth abdominal sternite dissolved into numerous, irregular small sclerites, or absent. Male hypopygium medially with 2 appendages only. Eggs have a wide flange without deep grooves, marked indentations. The group contains *Braula kohli* Schmitz and *B. pretoriensis* Orosi-Pal.

Life and Cycle and Behavior

Egg Stage: Skaife (1921a, 1921b) first observed *Braula* eggs within a honey bee colony. He described the eggs as being white in color with an oval shape. The chorion of the eggs contain a closely punctured surface with an exochorion that is developed as 2 lateral flanges. Each flange is flat and extends parallel to the other and to the long axis of the egg. The surface of these flanges is coarsely reticulated in an irregular pattern. Imms (1942) suggested that the flanges serve either for attachment to a solid substrate, or for supporting the egg upright in a fluid medium such as honey.

Skaife (1921a, 1921b), Beliavsky (1929), Hempsall-Herrod (1931) and Atakishiev (1971) stated that *Braula* eggs are 0.75 to 0.85 mm long by 0.43 to 0.56 mm wide. None of these authors clarified whether the dimensions included the lateral flanges found on eggs. Orosi-Pal (1938) measured eggs without flanges and stated they were 0.72 to 0.75 mm long and 0.23 to 0.25 mm wide. Imms (1942) measured eggs of Braula immediately after mounting in De Favre's medium. He reported the average length, without flanges, ranged from 0.78 mm to 0.81 mm and the width from 0.28 to 0.33 mm. Including the flanges, a typical egg measured 0.84 mm by 0.42 mm.

There is disagreement as to where female Braula lay their eggs. Skaife (1921a, 1921b) reported finding eggs on the wax cappings of sealed brood. Beliavsky (1929) reported finding 448 eggs on the surface of wax cappings over honey and 18 eggs in the wax and dirt found in the bottom of hives. Arnhart (1923), Argo (1926), Hempsall-Herrod (1931), Orosi-Pal (1938) and Imms (1942) all stated that eggs are laid on the inside edges of honey filled cells just prior to sealing. Alexeyenko and Bakai (1958a) reported that eggs are placed on both the outer and inner sides of cappings, the cappings over honey bee pupae and on the wooden parts of frames. Atakishiev (1971) stated that the bee louse female lays eggs on brood combs, fastened to the cappings and also on the bottom or lateral walls of empty cells.

Hassanein and Abd El-Salam (1962) found Braula eggs deposited individually on the outer surface of honey cell cappings, on brood cappings, on the walls of empty cells and a small number were on wax dirt found on the floors of bee colonies. Only eggs laid on honey cappings hatched; eggs laid in empty cells, on brood cell cappings or on wax dirt on the floors of colonies failed to hatch.

The incubation period for eggs varies according to the different seasons of the year from a minimum of 2.2 days during the summer to a maximum of 7.4 days during the winter (Hassanein and Abd El-Salam, 1962).

Larval stage: Skaife (1921a, 1921b) was the first person to observe Braula larvae which are transparent white in color. Newly emerged larvae measure 0.88 mm long by 0.25 mm wide (Hempsall-Herrod, 1931; Imms, 1942). Orosi-Pal (1938) recorded young larvae to be much larger, 2.25 mm long by 0.85 mm wide. Full grown third instar larvae measure 4.60 mm in length and are 1.30 mm in diameter according to Hempsall-Herrod (1931); Imms (1942) recorded them as being 2.25 mm long by 0.5 mm wide.

Braula larvae emerge from the attached end of their egg (Hempsall-Herrod, 1931) where they begin constructing a tunnel. Thus tunnels are not only made under the cappings but sometimes in the walls and bottoms of cells. Skaife (1921a, 1921b) observed Braula larvae beside healthy drone larvae.

The tunnels give cappings of an infested comb the appearance of being intersected with fine fractures. When viewed closely, the tunnels appear broad and quite distinct, similar to the mines of a leaf miner.

Newly hatched larvae construct tunnels by graving the inner surface of wax cappings or cell walls and moulding the separated fragments to form a tube or tunnel in which they live (Hassanein and Abd El-Salam, 1962). Tunnels are impervious to honey and the interior of tunnels remain dry. As a rule, tunnels follow the walls of cells, but occasionally tunnels of different sizes are found intersecting each other.

Argo (1926) found that tunnels attain a length of up to 5 cm while Beliavsky (1929) found the longest tunnels to extend 7 cm; Atakishiev (1971) stated tunnel length as 5 to 9 cm.

Hempsall-Herrod (1931) and Atakishiev (1971) reported the diameter of newly constructed tunnels as 0.30 to 0.40 mm inside and 0.70 to 0.80 mm outside. When the larvae are full grown, the diameter of tunnels is 1.60 mm to 1.80 mm internally and 2.40 to 2.50 mm externally. The initial thickness of tunnel walls is 0.3 mm, increasing to 0.4 mm when larvae are mature.

According to Orosi-Pal (1938), Imms (1942) and Hassanein and Abd El-Salam (1962) bee louse larvae move with facility either backwards or forwards in their tunnels. However, Atakishiev (1971) stated that *Braula* larvae can only move forward. Orosi-Pal (1938) stated that larvae are helpless outside of tunnels but Hempsall-Herrod (1931) was astonished at the rapid movements of a larvae placed on a glass slide.

Larvae evidently feed upon honey and pollen grains within the wax of their tunnels (Hempsall-Herrod, 1931). Imms (1942) found that the digestive system of larvae contained beeswax and often pollen grains. Orosi-Pal (1938) reported that the epithlial cells of the mid-intestine contain micro-organisms which he suggests may break down the wax for use by the larvae in a manner similar to what may occur in the wax moth, *Galleria mellonella* L.

Bee louse larvae pass through 3 instars. The first instar lasts from 1.1 to 2.9 days, the second larval instar lasts 1.5 to 3.9 days while the third larval period varies from 3.7 to 5.6 days. The shortest total duration of the larval stage was 7.1 days while the longest was 10.8 days (Hassanein and Abd El-Salam, 1962).

At the end of the larval stage, immature *Braula* make a fine circular cut through the wall at the anterior end of its tunnel. This cut is used as the escape route when adult *Braula* emerge from the tunnel (Hempsall-Herrod, 1931).

Imms (1942) made a detailed study of *Braula* larvae morphology. He found the general form to differ very little in successive instars. The body is composed of 3 thoracic and 8 abdominal segments together with a much reduced pseudocephalon.

The cuticle over the surface of the larvae appears granulated due to the presence of tubercles of varying sizes. The most characteristic external feature is the presence of whorls of sensoria on the first 4 segments and on the last segment. The sensoria are largest over the dorsal surface becoming smaller towards the ventral side. There are 2 such whorls on the first segment. On segments 2, 3 and 4 there is only 1 row of sensoria. The sensoria present on the eighth abdominal segment are less clearly arranged in a row. The pseudocephalon is bilobed with antennae and maxillary rudiments with palpi. A pair of very small sensoria are present between the antennae and on the inner side of the maxillary rudiments.

Two pairs of larger and more conspicuous sensoria are situated ventro-laterally. The most characteristic feature of the pseudocephalon is a band of sclerotized yellowish cuticle. The bucco-pharyngeal armature is highly characteristic with stout mouth-hooks that are curved and devoid of teeth.

Prepupal stage: Larvae pass gradually from the third larval instar to the prepupa stage within the unmodified cuticle of the last larval instar (Imms, 1942; Hassanein and Abd El-Salam, 1962). The prepupal period varies from 1 day during the summer to 2.7 days in the winter (Hassanein and Abd El-Salam, 1962). The prepupa is cream-white when viewed through the transparent larval skin.

Pupal stage: The pupae of Braula coeca is enclosed within the unmodified cuticle of the last larval instar (Skaife, 1921a, 1921b; Beliavsky, 1929; Imms, 1942; Hassanein and Abd El-Salam, 1962). Imms (1942) reported this covering remains colorless. The pupae is white (Skaife, 1921a; Beliavsky, 1929) or yellowish in color (Imms, 1942).

Hempsall-Herrod (1931) stated pupae to be 3.5 mm long by 1.18 mm wide. These figures appear to be exaggerated and the figures of Orosi-Pal (1938), 1.65 mm long by 0.7 mm wide or Imms (1942) 1.4 to 1.7 mm long by 0.5 to 0.75 mm wide are more accurate.

The pupal stage lasts a minimum of 1.1 days in the summer and a maximum of 6.1 days in the winter. If the prepupal period is added to the pupal period, the shortest duration is 3.2 days in the summer and the longest is 8.2 days in the winter. The duration of the total life cycle thus ranges from a minimum of 16 days in summer to a maximum of 24.3 days in winter (Hassanein and Abd El-Salam, 1962). Atakishiev (1971) reports that complete development of Braula occurs in 18 to 23 days at hive temperature and in 22 to 28 days in the laboratory.

Adult stage: The adult bee louse emerges from its wax tunnel by inflating its ptilinum and forcing open the circular cut it had previously made in the wax before pupation (Hempsall-Herrod, 1931; Imms, 1942). Newly emerged Braula are white in color and have soft chitin. The color of the body gradually changes from white to a permanent reddish brown. Coloration commences at the head and gradually spreads to the posterior extremity (Hempsall-Herrod, 1931; Atakishiev, 1971). Hempsall-Herrod (1931) reported that the coloration is fully completed in 12 hours while Atakishiev (1971) found that it occured in 14 to 16 hours.

Upon emergence adult bee lice quickly make their way to a host honey bee. Their usual position on bees is on the propodium or prothorax (Hempsall-Herrod, 1931; Imms, 1942). The lice are able to move about freely on hosts. Not only can they move from one bee to another, but they can move equally well on the dorsal or ventral side of the bee (Hempsall-Herrod, 1931; Dietz et al., 1971).

Bee lice are found on all castes of honey bees, however Benton (1895), Arnhart (1924), Hempsall-Herrod (1931) and Atakishiev (1971) agreed that *Braula* are infrequently found on drones. Usually only 1 louse is found per bee, although they can occur in greater numbers (Assmuss, 1865). Phillips (1925) stated that no more than 1 louse per single worker bee had been observed in Maryland. Hempsall-Herrod (1931) reported that as a rule, no more than 2 *Braula* are present on the body of workers at the same time.

There are numerous reports of more than one louse being present on queen honey bees. Hammer (1858) reported taking 187 *Braula* from a queen and then 64 from the same queen at a later date. Cheshire (1888) reported removing 6 lice from a queen in England. Benton (1895) removed as many as 75 from a single queen; however, he usually found fewer than a dozen lice per queen. Marbound (1907) removed 31 lice from a single queen, then 33 the next day, 43 two days later, and he continued removing them reaching a total of 371. Kramer and Theiler (1913) found 60 on a single queen; Argo (1926) found 35; Imms (1942) found up to 14; Stejskal (1965) found as many as 19 on one queen; Atakishiev (1971) stated finding as many as 46 on one queen.

Timm (1917) questioned the accuracy of reports in which large numbers of lice were found on queens because of the relatively large size of *Braula* compared with that of a bee. Timm stated lice may have been mistaken for mites or triungulin larvae of *Meloe*.

It does appear from the literature that a number of lice can be found on queens at certain times of the year. Losy (1902a, 1902b) and Alexeyenko and Bakai (1958a) reported that there is a migration from workers to the queen at the close of broodrearing. Argo (1926) found pale newly emerged lice on queens during September. Cory (1935) found lice to be more numerous during the early summer on worker bees than on any other caste, but in late summer, or early fall, he found them more numerous on queens than on workers. Atakishiev (1971) speculated that lice prefer queens because of their sedentary life, and because queens are fed the high protein royal jelly which lice prefer.

According to Phillips (1925), Dietz et al. (1971) and Burgett (1971) adult bee lice are 1.5 mm long by 0.75 mm wide. Hempsall-Herrod (1931) figures of 3.0 mm long by 2.0 mm wide, seem to be somewhat exaggerated. Kaschef (1960) in his detailed study of Braula dimensions found the mean head width of females to be 0.667 mm and males to be 0.645 mm. The mean width of the abdomen of females was 1.056 mm and 1.001 mm for males.

The body of Braula is covered with sensory hairs of varying lengths and diameters (Phillips, 1925; Alfonsus and Braun, 1931). According to Kaschef (1960) the head is flattened and oriented vertically on the thorax, bringing the mouth-parts toward the ventral surface of the insect. The antennae have a peculiar structure and are articulated in a deep fossa on each side of the head. Muggenberg (1892) described the mouth parts and discovered the eyes of the supposedly blind insect. Eye rudiments are present just above the antennae as pale spots on the cuticle surface surrounded by more darkly pigmented chitinous rings. No ocelli are present (Kaschef, 1960).

The thorax is discoid, very short and is connected throughout its width to the abdomen. There is no trace of wings or halters (Kaschef, 1960). Phillips (1925) stated that the legs are equal in length; however, Kaschef (1960) reported the forelegs are shorter than the hindlegs. The tarsi are 5 segmented. The last tarsal segment has 2 pulvilli that are covered with fine hairs. Also, each terminal joint contains a comb-like structure, divided in the middle, with a variable number of teeth. The combs allow Braula to attach itself firmly to the branched hairs of its host (Kaschef, 1960).

The abdomen has 5 dorsaly visable segments that occupy 60 to 75% of the whole length of the body (Phillips, 1925; Kaschef, 1960).

Feeding Behavior: The bee louse was originally thought to take its food like a true louse by sucking the haemolymph of the host bee. However, the proboscis of Braula as described by Losy (1902a, 1902b) and Massonnat (1909) is incapble of piercing the integument of the bee. Subsequently the bee louse was observed to take food from the mouth parts of its host (Perez, 1882; Losy, 1902a, 1902b; Beliavsky, 1929; Hempsall-Herrod, 1931). Argo (1926) supplied the following description of Braula feeding behavior:

"The insect runs about on the head of the

> bee, showing great agitation, and then settles down on the clypeus, fastens the last pair of tarsal combs in the hair of the bee's face, and frantically claws at the labrum of the host with the other 4 tarsi. This continues until the bee slowly straightens out its tongue. The louse quickly moves forward so that it is able to apply its mouth parts to the base of the bee's tongue just beyond the edge of the labrum. In this position it remains quiet for a time, unless the available nourishment seems to become exhausted. This causes another rather hysterical outburst and the scratching of the labrum of the bee is resumed for a time sufficient to cause more food to be emitted, after which the *Braula* feeds until satisfied, and then returns to its usual resting place on the thorax of the host."

Dietz *et al.* (1971) concluded that when the louse runs about on the face of the bee it stimulates the antennal receptors similar to the stimulations encountered when a bee begs food from another bee.

Phillips (1925) made smears of the contents of the alimentary tract of *Braula*, and found pollen grains that were the same as those found in the host bee. However, it is not known whether pollen is an important constituent of the *Braula* diet.

Seasonal cycle: Argo (1926) found lice only on workers and drones in the spring. During July and August few adults were found. Queens became heavily infested in September with pale, newly-emerged lice. Later, dark, mature *Braula* appeared on workers.

Imms (1942) found lice in all stages present from July to October, only pupae from October to November, and adults only in the winter and spring. Alexeyenko and Bakai (1958a) observed that *Braula* do not lay eggs during the winter-spring period. The authors stated that they overwintered in the adult stage. In late spring, when honey is being stored in hives, eggs are layed by females. In May-June prior to the beginning of the honey flow the least number of lice is present in hives. In August the number of Braula increases and in September and October there is a mass appearence of lice on bees. In November and December, some lice within the hive perish due to the cold, thus reducing the population of lice.

Reproductive behavior of Braula: There are no reports in the literature regarding the mating behavior of Braula. Phillips (1925) suggested without evidence, that mating takes place only on queens since lice migrate to queens during the fall of the year.

Geographical Distribution

Braula coeca has been reported in bee colonies on every continent. Records are presented in Table 1.

The bee louse was probably introduced into the United States with the first shipments of honey bees to North America. Phillips (1925) stated that Braula was repeatedly introduced into the United States on imported queens from foreign countries. Bee lice were first recorded in the United States in 1911 from bees wintered at Mankota, Blue Earth County, Minnesota (Argo, 1926). Lice were found in Grantham, Cumberland County, Pennsylvania in 1918 (Argo, 1926). The first report from Maryland was 1920, from

Table 1. World distribution of bee lice *Braula coeca* Nitzsch.

	Africa
Africa	Skaife (1921a, 1921b), Hempsall-Herrod (1931), Schmitz (1956), Orosi-Pal (1966b)
Congo	Orosi-Pal (1966b)
Egypt	Kaschef (1960), Hassanein and Abd El-Salam (1962)
Morocco	Orosi-Pal (1966b)
	Asia
India	Rajagopalachari (1948)
Soviet Union	Orosi-Pal (1966b), Smirnov (1970)
	Australia
Tasmania	Pender *et al.* (1925), Pender (1926), Nolan (1926), Nicholls (1932), Orosi-Pal (1966b)
	Europe
Europe	Hempsall-Herrod (1931), Schmitz (1956), Orosi-Pal (1966b)
Baltic Region	Assmuss (1865)
Mediterranian Countries	Benton (1895)
Pomerania	Timm (1917)
Austria	Arnhart (1923), Orosi-Pal (1966b)
Bulgaria	Velickov (1963a), Lazarov and Nedyilkov (1971)
Cyprus	Cook (1910)
Czechoslovakia	Schonfeld (1925), Orosi-Pal (1966b)
France	Donhoff (1858), Assmuss (1865), Buttel-Reepen (1925), Orosi-Pal (1966b)
Germany	Donhoff (1858), Assmuss (1865), Buttel-Reepen (1925)
German Democratic Republic	Orosi-Pal (1966b)
Great Britain	Cheshire (1888), Hempsall-Herrod (1931), Mace (1976)

Holland	Schmitz (1914)
Hungary	Orosi-Pal (1966b)
Ireland	Hillyard and Markham (1968)
Italy	Donhoff (1858), Assmuss (1865), Pender *et al*. (1925), Pender (1926), Orosi-Pal (1966b) Leporati (1974)
Netherlands	Orosi-Pal (1966b)
Poland	Orosi-Pal (1966b)
Rumania	Orosi-Pal (1966b)
Soviet Union	Donhoff (1858), Alexeyenko and Bakai (1958a, 1958b), Beliavsky (1929) Orosi-Pal (1966b), Smirnov (1970)
Spain	Gil Collado (1932)
Yugoslavia	Orosi-Pal (1966b)

North America United States

Alabama; Houston Co.	Tew (1976)
Delaware; Castle, Kent and Sussex Co.	Bowman (1976)
Illinois	Alfonsus (1932), Corrington (1951)
Maryland	Cory (1935), Corrington (1951)
Maryland; Allegany Co.	Argo (1926)
Maryland; Baltimore Co.	Argo (1926), Lindner (1970)
Maryland; Carroll Co.	Argo (1926), Lindner (1970)
Maryland; Frederick Co.	Lindner (1970)
Maryland; Howard Co.	Lindner (1970)
Maryland; Montgomery Co.	Cory (1935)
Maryland; Prince George's Co.	Argo (1926)
Maryland; Washington Co.	Argo (1926), Cory (1935), Lindner (1970)
Minnesota; Blue Earth Co.	Argo (1926), Cory (1935), Corrington (1951)
New York; Monroe Co.	Argo (1926)
North Carolina; Wake Co.	Caron (1976)
Ohio	Corrington (1951)
Pennsylvania	Clarke (1970)
Pennsylvania; Cumberland Co.	Argo (1926), Alford (1928), Steinhauer (1977)
Pennsylvania; Franklin and Juneata Co.	Collison (1977)
Tennessee; Blout Co.	Kerr (1977)
Virginia; Clarke, Fauquier, Frederick, Loudoun and Warren Co.	Powers (1976)
West Virginia	Corrington (1951)

West Virginia; Berkeley Co.	Peairs (1929)
West Virginia; Jefferson Co.	Stone (1970)
West Virginia; Kanawha Co.	Montgomery (1976)
Wisconsin	Alfonsus (1932)

South America

Argentina	Wolffhugel (1910), Bregante (1972)
Brazil	Miranda Ribeiro (1905), Ronna (1936)
Trinidad and Tobago	Lawrence and Mohammed (1974)
Venezuela	Stejskal (1967a), Shuttleworth (1977)

colonies at Uniontown, Carroll County, Maryland where Mr. Rockward Nusbaum, a large commercial beekeeper in Maryland, sent bee louse specimens taken from his bees to the Bureau of Entomology. He claims to have observed the insects at least 5 years earlier without knowing what they were (Argo, 1926).

Braula has been found in a total of 12 states to date (Table 1). It seems likely that the bee louse is present in other states but we lack such records only because no one has looked for it.

Spread of *Braula*

The bee louse is spread to other colonies by beekeeper transfer of brood combs from one colony to another. Also, lice may be transmitted from one hive to another by robber bees, drifting bees and in swarms (Alfonsus, 1932; Atakishiev, 1971; Jean-Prost, 1972).

Damage

There is disagreement as to the amount of damage caused by the bee louse. Clausen (1940) and Frank (1969) felt *Braula* causes little or no harm to honey bee colonies. Others such as Assmuss (1865), Skaife (1921b), Arnhart (1924) and Phillips (1925) believed *Braula* became harmful only if found in large numbers in the hive. Smirnov (1970) and Dell Pozo and Schopflocher (1974) reported that infected colonies are more prone to disease than noninfected colonies.

Phillips (1925) and Smirnov (1970) believed that Braula occured in colonies that are not properly housed and managed. Kramer and Theiler (1913) stated that Braula became a problem only in poor seasons. Shuttleworth (1977) found the bee louse a problem only if a colony became weak.

Most investigators agree that the bee louse does no good and may be harmful to some extent to bee colonies. The importance of Braula to the beekeeper depends on whether or not a honey crop is directly or indirectly affected by the insect. Any loss to the beekeeper comes either through the effect of Braula on adult bees, or through the effect of tunnels in capped honey made by the larvae.

Damage to Adult Bees: Bee lice constantly disturb bees (Stejskal, 1967b; Biri, 1974) and steal food that would otherwise be used by the adult bees or fed to the brood. However, since only 1 or 2 lice are usually found on workers, the amount of damage caused to individual bees is minimal (Hempsall-Herrod, 1931; Leporati, 1974). Braula tend to migrate to queen bees during the fall of the year and infested queens with B. coeca receive less food (Atakishiev, 1971) seriously disturbing their egg laying ability (Hempsall-Herrod, 1931; Alfonsus, 1932; Alexeyenko and Bakai, 1958a; Smirnov, 1970). In some cases lice may cause supersedure of queens (Cory, 1936) or cause them to perish from exhaustion (Assmuss, 1865; Arnhart, 1924; Alfonsus, 1932; Alexeyenko and Bakai, 1958b; Smirnov, 1970).

In a study by Atakishiev (1971), 50% of the bees in 25 colonies were infested with bee lice. Individual workers had up to 4 lice each while queens had up to 36 lice. The total number of eggs found daily in the apiary fluctuated from 275 to 1,125. In another apiary previously infested with B. coeca but de-loused with phenothiazine, the number of eggs ranged from 1,400 to 3,200.

Damage to Honey: Tunnels produced by the larvae of Braula coeca disfigure the surface of capped honey. Cappings are also weakened due to the tunnels. When removed from the colony, honey absorbs atmospheric moisture through the tunnels, becomes watery and the honey percolates through and trickles over the face of the comb (Hempsall-Herrod, 1931; Cory, 1935).

In the production of section honey, injury by larval tunnels causes considerable damage (Alfonsus, 1932; Hillyard and Markham, 1968) and often makes it unfit for sale (Nolan, 1926; Hempsall-Herrod, 1931; Cory, 1935). Cory (1935) felt it doubtful whether a producer of extracted honey need be very concerned with the presence of Braula tunnels in cappings.

Control

There have been considerable efforts in developing prophylactic and remedial measures to rid honey bee colonies of bee lice. Most control measures are aimed at destroying adult Braula; however investigators have attempted to destroy the immature stage of the pest as well. Smirnov (1970) made note of the fact that where lice are a problem all possible prophylactic and theraputic measures must be employed to keep honey bee colonies free of bee lice.

Control of adult Braula coeca

Early control methods were aimed at destroying adult Braula on bees, especially queens. Cook (1910) felt the only way to remove lice from queens was to remove them with a knife, scissors or forceps. Arnhart (1923) recommended using a small pointed stick dipped in honey to remove Braula. Lice were also removed from queens with a small fine brush moistened with honey (Smirnov, 1970; Atakishiev, 1971) or alcohol (Smirnov, 1970).

Early control methods for entire colonies included smoking bees with saltpeter or Lycopodium (Boise, 1890). These materials not only caused lice to fall off their hosts but also resulted in stupified bees. The use of oil of turpentine on a cloth (Hommell, 1919) or incense powder (Clement, 1905) placed on the bottom board was also supposed to kill lice.

Another control method employed was to open infested colonies in the evening and sprinkle or spray the bees thoroughly with dilute honey (annonymous, 1923; Nicholls, 1932). In cleaning up the honey the bees supposedly removed the Braula.

Tobacco Smoke: Bertrand (1904), Bautier (1926), Hempsall-Herrod (1931), Atakishiev (1971) and Del Pozo and Schopflocher (1974) recommended the use of tobacco smoke on queens infested with Braula coeca. Infested queens were removed from colonies and smoked gently. This caused stupefied Braula to drop off after which they were destroyed. Since the queens collect more lice when returned to the hive, it was recommended that removal and smoking of the queens be repeated.

Phillips (1925) pointed out that smoking a queen with tobacco smoke may cause the bees to ball her when she is returned to the hive. Atakishiev (1971) suggested reintroducing queens in cages or smearing them with honey so they will be accepted.

Tobacco smoke has also been recommended for use in delousing entire colonies (Cowan, 1911; Hempsall-Herrod, 1931; Argo, 1932; Smirnov, 1970). Some authors (Del Pozo and Schopflocher, 1974; Biri, 1974) still prefer this method although it is the opinion of Velickov (1963a), Atakishiev (1971) and Leporati (1974) that better control methods are available.

Smirnov (1970) suggested fumigating with tobacco smoke in the evening. Before fumigation, spare combs are removed from the hive so the remaining frames can be arranged more sparsely in such a way that bees sitting on one frame do not touch the bees on adjacent frames. The bottom board is covered with paper. Tobacco smoke is directed into the entrance of the infested hive. The colony is closed and the smoke kept in the hive for 3 to 5 minutes, after which the hive is opened and ventilated. The paper on the bottom board now containing bee lice is removed and burned. Fumigation should be repeated in 3 successive days and repeated every 10 days until all lice are destroyed.

Leporati (1974) reported tobacco smoke is currently the most common method of controlling Braula in Italy. He recommended a 30 second smoking with tobacco. Since the smoke does not kill Braula some may reattach themselves to bees if not removed after treatment. For this reason, Leporati felt tobacco smoke cannot be considered an efficient material for controlling Braula.

When smoking colonies with tobacco, the bees become quite demoralized and roar loudly (Argo, 1932).

Naphthalene: Naphthalene has been used to delouse bee colonies (Clement, 1905; Ludwig, 1906; Hommell, 1919; Biri, 1974).

Smirnov (1970) recommended placing 10 to 20 g of naphthalene on a piece of gauze placed on a pieceof paper on the bottom board of the hive in the evening. Cracks in the hive are plugged and the hive entrance is reduced. The naphthalene should be removed in the morning and the paper with fallen bee lice burned. The treatment should be repeated twice and then repeated every 10 days until all lice are destroyed.

A variable amount of naphthalene is recommended because of the different qualities of naphthalene sold. Dosage also depends on air temperature; with a higher temperature, dosage should be somewhat reduced.

Ronna (1936) suggested that naphthalene placed in the corners of the hive can prevent infestations of lice by acting as a repellent. However, Zander (1921) pointed out that naphthalene may not only dislodge Braula but may also drive bees from the hive. Baranichenko (1938) reported naphthalene is ineffective at temperatures below 15°C. According to Krasnopeyav (1936) naphthalene often causes poisoning of the bees. At concentrations of 200 to 500 mg/m^3 of air, naphthalene causes nearly 10% of the bees to perish (Alexeyenko and Bakai, 1958b). Poltev (1948) reported honey absorbs the naphthalene odor.

Because of such problems Velickov (1963a) and Atakishiev (1971) considered naphthalene to be unsatisfactory for use in controlling *Braula coeca* in honey bee colonies.

Camphor: Smirnov (1970) reported camphor can be used to control *Braula* in honey bee colonies, although he stated that it is less effective than other control methods. Velickov (1963a) and Atakishiev (1971) reported camphor has not proved to be very effective.

To treat hives with camphor, the bottom boards of hives are covered with paper and pieces of camphor weighing 3 to 5 g, wrapped in clean gauze, are placed in the bottom of hives in the evening. The bee lice fall to the bottom at night, and in the morning they are destroyed. Treatment with camphor is repeated 3 successive days, every 10 days until all lice are killed (Smirnov, 1970).

Phenothiazine (thiodiphenylamine): Chukanov (1963) found that lice could be destroyed with phenothiazine. Velickov (1963a, 1963b) recommended wrapping 3 g of phenothiazine in a piece of paper and placing it in a hot smoker. When white smoke begins to come out of the smoker, 2 colonies of bees should be smoked simultaneously through hive entrances. During 30 seconds, 30 puffs of smoke are given to the first colony and then to the second one; then 20 puffs are given to the first colony followed by the second. Smoked lice fall off the bees and perish.

Stejskal (1965) obtained good results using Velickov's recommendations except he administered 40 total smoke puffs to colonies.

Atakishiev (1971) recommended 8 g phenothiazine with 40 to 50 seconds of smoke to colonies. After treating 3 colonies, 30 additional puffs of smoke were administered and colony entrances closed for 10 to 16 minutes.

Leporati (1974) suggested using 3 g of phenothiazine to treat 2 or 3 colonies. Colonies are given 20 puffs of smoke in 30 seconds or if there is a heavy infestation, 30 puffs of smoke may be given. After treatment the hive entrance is kept closed for 15 to 20 minutes.

Fumigation of colonies with phenothiazine is performed in the evening to prevent robbing that might occur. Best results are obtained when the air temperature is between 10 and 15°C (Velickov, 1963a, 1963b). At temperatures above 26°C the effect of the chemical is considerably reduced (Smirnov, 1970). For best results the smoker should have a piece of tube attached to insure that the smoke enters the hive.

According to Smirnov (1970), phenothiazine is the most effective of all chemical control agents against Braula. Tsivilev (1968), Smirnov (1970) and Leporati (1974) advised that colonies must be treated 3 days in a row with phenothiazine. Treatment is repeated every 10 days until lice vanish. Smirnov (1970) advocated the removal of 1 or 2 frames from the hive and said the hive should be tightly closed for 10 minutes following fumigation.

Fumes from burning phenothiazine show no noticeable toxic effects on bees or brood in honey bee colonies (Velickov, 1963a, 1963b; Boldyrev, 1964; Smirnov, 1970). It does have a narcotic effect on bees making them sluggish; however, they return to normal after several minutes of fresh air (Smirnov, 1970).

The best time to treat with phenothiazine is in April, May and the beginning of June in the Soviet Union. The treatments are less effective in October and November due to lowered air temperatures (Atakishiev, 1971).

<u>Folbex (chlorobenzilate)</u>: According to Stejskal (1967b), Kulikov reported that folbex also is useful in delousing colonies. Strips the depth of frames and 3 cm wide are moistened with folbex and suspended between the upper combs of the hive (1 strip between each frame). The hive entrance is then closed for 1/2 hour. Lice drop from bees and perish while no injury to adult bees, larvae or eggs has been observed.

Smirnov (1970) reports a slightly different means of using folbex. In this treatment several frames are removed from a hive and 2 or 3 cardboard strips of folbex evenly suspended in the resulting spaces are burned. A piece of polyethylene or similar material is placed at the hive top under the cover and the hive entrance is closed for 30 minutes. When necessary, treatments are repeated at 5, 12 and 21 day intervals until lice are eradicated.

Folbex should not be used after September because queen loss has been observed when colonies are treated in the fall (Smirnov, 1970).

Thymol: Alexeyenko and Bakai (1958b) and Volcinschi (1964) suggested using thymol for fumigation of bees with lice. However Velichkov (1963) and Atakishiev (1971) found thymol unsatisfactory because of its toxicity to bees. Smirnov (1970a) reported thymol was less effective in treating colonies then folbex. Thymol is used in doses of 60 to 100 g per colony. During fumigation, hives are tightly closed for 2 to 3 hours after which the lice are gathered on paper placed in the bottom of the hive. The treatment is repeated 3 successive days at 10 day intervals (Smirnov, 1970).

Tedion: Atakishiev (1971) listed tedion as a modern drug for the control of *Braula coeca*. However he gave no details regarding its use.

Other chemicals: Alexeyenko and Bakai (1958a) conducted laboratory tests to determine the insecticidal activity of 14 ethers and esters, chlorobenzene, chloroform, chloramine B, ethyl bromide, the herbicides 2, 4-DU and -DU and thymol against *Braula*. The volatile substances of horse radish (*Cochlearia amoracea*) garlic (*Allium sativum*) and summer savory (*Satureia hortensis*) were also tried against bee lice. Various concentrations of these substances were tested and compared to naphthalene. Results showed that thymol had the best insecticidal action against Braula in concentrations of 200 to 500 mg/m^3 of air. Bees were not affected. Thymol however is more toxic to lice than naphthalene. Chloromethyl-acetate (5 to 10 ml/m^3 of air) and the volatile substances of horse-radish (200 to 500 ml/m^3 of air) also showed toxic effects on Braula; however higher concentrations and prolonged exposures were toxic to the bees as well. Less toxic action on Braula was noted for adipodimethyl ester (50 ml/m^3 of air), cinnamalisoamyl ester (20 ml/m^3 of air), herbicides 2, 4-DU and -DU (100 to 150 mg/m^3 of air), and chloramine (200 mg/m^3 of air). The remaining substances tested were not toxic enough to kill lice and most rendered narcotic and toxic action on bees.

Control of Immature forms of Braula

Mechanical Control: Cory (1935) reported that in badly infested apiaries, the best procedure for comb honey producers is to extract all honey and render out the wax in the fall of the year thus destroying bees and lice. After thoroughly scraping and sterilizing hives one should start new the following spring with package bees that are free of bee lice.

Vidano and Cantone (1973) suggested uncapping sealed honey when Braula larvae are present. Many beekeepers practice this control measure while extracting honey. Smirnov (1970) reported that capped honey containing Braula immatures could be heat-treated by spraying combs with hot water between 70° and 90°C or by flame-treatment with a blow torch held at a distance of 35 to 40 cm. The flame is quickly passed over the comb's surface.

Chemical Control: Beliavsky (1929) found that fumigation of combs with carbon disulfide or formalin killed *Braula coeca* larvae and possibly eggs. Smirnov (1970) advocated treating brood combs from infected colonies with carbon disulfide or formalin every 3 weeks. Combs should be placed in tightly closed hive bodies and fumigated several times at a rate of 50 g of sulfur/cm^3. When disinfecting with formalin, combs in a closed box should be sprayed with a 4% solution at a temperature of not less than 20°C and left for 4 hours. Comb can also be treated with fumes of boiling formalin. The vapor is directed into a box with comb for 30 minutes. Temperatures should not exceed 55°C. The odor of formalin is removed by washing the combs with water and drying them in open air or by sprinkling the combs with ammonium hydroxide.

Smirnov (1970) found that the gas methyl bromide or a mixture of ethylene oxide and methyl bromide effectively killed *Braula*. A single treatment given in a closed chamber using methyl bromide at 80 g/cm^3 or a mixture of ethylene oxide and methyl bromide at 50 g/cm^3 gives effective results.

The Relationship of Braula to Similar Organisms

The bee louse is totally dependent upon the honey bee for its existence. Burgett (1971) described the bee louse as an inquiline of the hive or a "guest in the house."

Braula transmits no bee diseases so far as is known and is not a predator as was once believed. However *Braula* does not find the bee a willing commissariat, since its host tries to rid itself of the unwelcome guest by scraping it away with its legs (Hempsall-Herrod, 1931).

The bee louse was once thought to be associated with the Dipteran family Phoridae, due to its superficial resemblance to *Thaumatoxena*. There are some similarities. Both insects live in the dark, they are epizoic and both live under very uniform environmental conditions; in one case in the nest of the bee hive and in the other case in the nest of termites (Imms, 1942). *Thaumatoxena* is similar to *Braula* in that it has lost its wings and is host specific. Phoridae larvae live as scavengers of commensals in the nest of ants, bees, wasps and termites (Oldroyd, 1964). Phorids, like *Braula*, are unable to suck blood from their hosts. *Metopina pachycondylae* lives on the larvae of the ant *Pachycondyla harpax* where it steals food brought by worker ants to feed the larvae.

Two Dipteran families, Streblidae and Nycteribidae, are ectoparasites of bats. These are wingless, small, spiderlike insects similar to the bee louse.

The fly family Hippoboscidae, or louse flies, also includes wingless forms like *Braula*. These are the only flies likely to be found living on birds. The sheep ked, *Melophagus ovinus* L. is a fairly common wingless louse fly. It is 4 times as long as the bee louse, has a reddish brown flattened body similar to *Braula* and is a parasite of sheep

(Oldroyd, 1964; Borror and DeLong, 1971).

The insect order Mallophaga, or chewing lice, are small, usually flattened, wingless external parasites of birds and mammals. Unlike bee lice, they have chewing mouth parts and feed on bits of hair, feathers or skin of their host. They are similar to *Braula* in that the transmission from one host to another usually occurs when 2 hosts come in contact. Also these lice are unable to survive away from the host and most are quite host specific (Borror and DeLong, 1971).

The sucking lice of the insect order Anoplura, are small, wingless, external parasites that feed by sucking blood. Anoplurans are parasites of mammals and are host specific like the bee louse. Some of these insects are important vectors of disease (Borror and DeLong, 1971).

There are 2 external mites that live in somewhat similar situations as the bee louse. *Varroa jacobsoni* (Oudemans) and *Tropilaelaps clareae* (Delfinado and Baker) are similar in size to *Braula coeca* and are found in honey bee colonies. However unlike the bee louse, they are parasitic on living or dead larvae, pupae and adult honey bees. These mites lay their eggs in cells of larvae just before they are capped. Nymphs feed on prepupae or pupae killing the brood or causing deformed bees to emerge. Unlike *Braula*, adult males are free living on the combs of the hive while females attach themselves to bees feeding on haemolymph. Adults are able to move about very quickly on bees in a manner similar to *Braula* (Gochnauer *et al*., 1975; Shabanov *et al*., 1978; Shimanuki, 1977).

CHAPTER III

MATERIALS AND METHODS

Maryland Survey for Bee Lice

A survey for bee lice in honey bee colonies in Maryland was conducted during the active season of 1976. Colonies were examined for the presence of lice during regular inspections of hives conducted for the Maryland Department of Agriculture Apiary Inspection service. The author along with the help of five regional inspectors and the chief apiary inspector conducted the survey.

Observations were made for the presence of adult bee lice on honey bees or for the distinctive tunnels made by bee louse larvae in the cappings of sealed honey.

Bee Louse Host Selection Tests

Laboratory tests were conducted to determine louse host preference for different ages and castes of honey bees. Five lice were introduced into cages containing 16 or 20 bees, depending on the test group and replicated 3 to 5 times. After a period of 7 to 10 hours, bees were examined for lice.

Test cages were constructed of a 1.2 cm thick plywood frame having internal dimension of 2.0 x 8.0 x 14.0 cm with an area of 224 cm^3. Upper sides of cages were covered with nylon window screen having a 5.4 x 6.9 mesh per cm. The floors of cages consisted of beeswax comb foundation.

For convenience, cages were placed on 0.32 cm thick tempered hardboard having dimensions of 18 x 20 cm. In the center of the cage side, a 1.6 cm hole was bored in which a number 5 cork was placed. (Figure 1). One plastic queen cup[1/] filled with 0.5 ml of queen candy was placed upright in the center floor of each cage to provide food for the bees during the test. The queen candy was a standard mixture of invert sugar and powdered confectioners sugar in approximately a 1:2.5 to 3.0 ratio (Laidlaw and Eckert, 1962).

Tests were conducted without light in Precision Scientific Instrument Incubators Model 805.[2/] A 200 ml beaker of water was placed in the incubators before the start of each test cycle to raise the humidity to 60 to 75% absolute humidity. All tests were conducted at both 25 and 34°C. Two temperatures were tested for the following reasons: Kaschef (1959) had determined that lice collect chiefly in the region of 24 to 27.5°C when subjected to a linear gradient of temperature. However, the internal temperature within honey bee colonies is usually above this temperature. Kaschef (1959) found greater numbers of lice on nurse worker bees near the brood cells. The temperatures within the brood nest of colonies fluctuates from about 35°C in the center to 31.8 ± 3.5°C in the outer areas.

1/ R.C. Daniels and Company, Picayune, Mississippi.

2/ Precision Scientific Company, Chicago, Illinois.

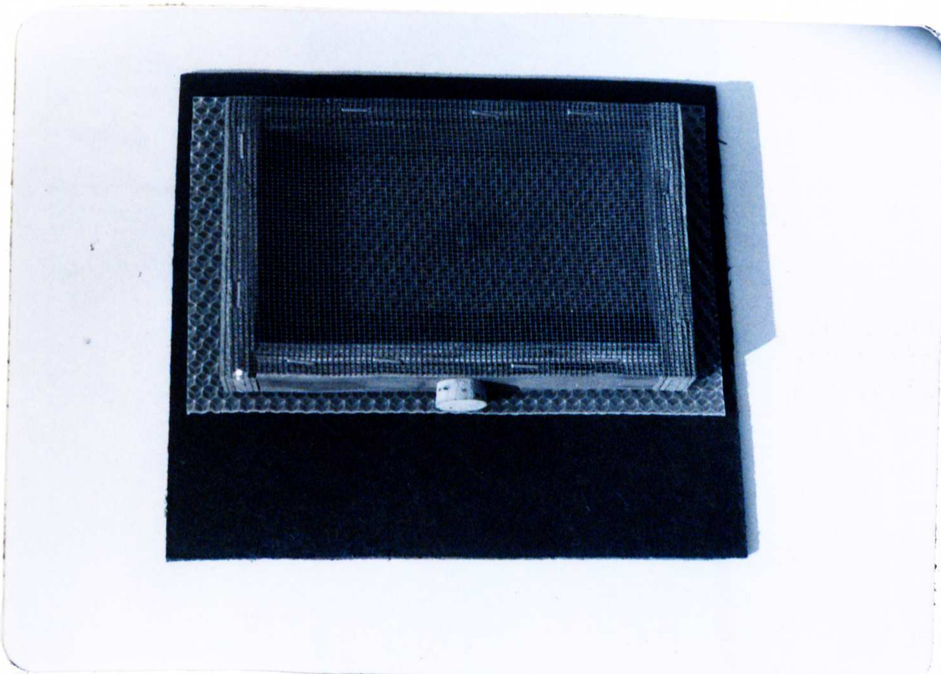

Figure 1. Cage for louse host selection test.

In preparing for each test, test bees and bees with lice were collected and stored in an incubator at 34 ± 1.5°C until needed. Bees were fed queen candy in 0.5 ml queen cups placed inside cages and invert sugar syrup that was periodically placed on the outside of cages.

Lice were placed in test cages in the following manner. Heads were removed from worker bees having lice. If more than one louse was present on a headless worker, the lice were coaxed onto other headless workers without lice. Five headless worker bees each with a louse were then introduced into each test cage through the hole in the cage side. Following the introduction, the time was recorded and the test period was initiated by placing the cage into the incubator.

At the completion of tests, cages were removed from the incubator one at a time. Bees were quickly isolated from one another by placing 37 ml clear plastic cups over each bee. The location of lice and the number of lice per bee were recorded. Braula were listed as either being on live test beees, or lice that were on dead bees or other locations, as being on the test cage. Each cage was examined before subsequent cages were removed from the incubator. The time was recorded after removal of each cage.

A chi-square goodness of fit test was run for each test group. Preference for one group of test bees over another was considered significant when the probability of a random distribution was 5% or less.

The following tests were conducted to determine louse host selection. See Table 2 for summary.

Test I: Louse host selection between workers of different ages. Lice were introduced into cages containing 20 worker bees of the following age groups: 5 workers 30 days old; 5 workers 15 days old; 5 workers 5 days old; 5 workers 1 day old.

Worker bees of known age were obtained by placing 2 frames of emerging brood in separate cages in an incubator at a temperature of 34 ± 1.5°C. One to 3 days after brood was placed in the incubator, wing marks using Testors[3] enamel paint were placed on newly emerged bees. 1,200 bees were marked with white paint 29 days before the test, 800 bees were marked with yellow paint 15 days before the test and 500 bees were marked with gold paint 5 days before the test. Marked bees were placed in a one-story active queenright hive with free flight until the day of the test when they were collected for use. Sufficient one day old bees were marked and placed in a cage a minimum of 2 hours before they were needed on the day of the test.

Test II: Louse host selection between young and old drones. Lice were introduced into cages containing 10 young drones and 10 old drones. Young drones were between 0 and 5 days old, while old drones of flying age were 6 or more days old (Oertel, 1956).

3/ The Testor Corporation, Rockford, Illinois.

Table 2. Summary of louse host selection tests.

Test Date	Test No.	No. Test Bees: Worker Age[a/] 1	5	15	30	Random	Foragers	Drones: Young	Old	Queens: Virgin	Mated	Temp. °C: 25°	34°	No. Replications
4-19-77	I	5	5	5	5							X		5
4-19-77		5	5	5	5								X	5
8-22-77	II							10	10			X		5
8-22-77								10	10				X	5
8-22-77	III							15		1		X		5
8-22-77								15		1			X	5
7-25-77	IV							15			1	X		3
7-25-77								15			1		X	5
8-22-77	V								15	1		X		4
8-22-77									15	1			X	5
8- 8-76	VI								15		1	X		5
9- 4-77									15		1		X	5
8- 7-76	VII						10		10			X		5
9- 4-77							10		10				X	5
8- 8-76	VIII					15				1		X		5
8-16-77						15				1			X	4
8- 8-76	IX					15					1	X		5
9-25-77						15					1		X	5

a/ Indicates age in days

Young drones were obtained by placing 2 frames of emerging drone brood and 1 frame emerging worker brood in a single cage in an incubator at 34 $\pm$ 1.5^{o}C 5 days before the test. Sufficient young drones were collected on the day of the test and marked with red Testors enamel paint on wings at least 2 hours before the test.

Old drones were collected the day of the test by placing drone traps on the front of strong honey bee colonies. Drones were collected and wings marked with blue Testors enamel paint at least 2 hours before the test.

Test III and IV: Louse host selection between young drones and a virgin or mated queen. Test III consisted of introducing lice into cages with 15 drones between 0 and 5 days old and 1 virgin queen 8 days old. Test IV consisted of introducing lice into cages with 15 drones 0 to 2 days old and 1 mated queen.

Young drones were obtained in the same manner as described in Test II. Virgin queens and all other queens used in this study were obtained by rearing queens using the following modified Doolittle (1915) method: One day old larvae were grafted into commercial beeswax queen cups that had been previously fastened to wooden cell bases on a modified Hoffman frame. One or 2 frames containing 36 grafted queen cells each were placed in a starting colony immediately after grafting. Starting colonies consisted of either a queenless colony with free flight or a closed swarm box, made up 1 to 24 hours before use. One day after queen cells were placed in a starting colony, they were either removed and placed in a queenright finishing colony above a queen excluder or left in the queenless colony with free flight. Starting and finishing colonies were fed sugar syrup and water in a 1:1 ration, by volume, during queen rearing. Queen cells were placed in separate cages and stored in an incubator at 34 $\pm$ 1.5^{o}C until needed.

In the louse host selection tests, cages with queen cells were provisioned with queen candy to provide food for emerging queens. After queens emerged, empty cells were removed and the queens in separate cages were placed in a queenright hive above a queen excluder until needed for the test.

Mated queens for Test IV were obtained by placing queen cells in mating nucs instead of placing the queen cells in the incubator to emerge. Those queens that successfully mated were used for tests after normal brood was observed in the colony. All queens used were less than 3 months old.

Test V and VI: Louse host selection between old drones and a virgin or mated queen. Lice were introduced into cages with 15 old drones of flying age at least 6 to 8 days old and 1 virgin queen 8 days old or 1 mated queen. Drones and queens were obtained as described in Test II, III and IV.

Test VII: Louse host selection between old drones and foragers. Lice were introduced into cages with 10 old drones of flying age at least 6 to 8 days old and 10 forager worker bees. According to Gary (1975) foraging worker bees are 14 or more days old.

Old drones were obtained as described in Test II. Foraging honey bees were obtained by closing the entrance to a strong hive during the day and collecting returning foraging bees with an insect net. Foragers were caged and set aside until needed.

Test VIII and IX: *Louse host selection between random age workers and a virgin or mated queen*. Lice were introduced into cages with 15 random age workers and 1 virgin queen 2 or 4 days old or 1 mated queen.

Random age workers were obtained from a hive by shaking bees from brood frames into a cage. Collection of bees was done on a cloudy day or either in the early morning or evening when few bees were flying.

Virgin queens were obtained as described in Test III except that queens were stored in an incubator at 34 ± 1.5°C until they were needed. Mated queens were obtained as described in Test IV.

Bee Louse Host Selection and Biology Field Tests

Honey bee colonies were established during July, August and September, 1976 and April, May and June of 1977. Colonies were placed in double nuc boxes constructed from half-depth supers (Figure 2). The 16.8 cm deep supers were fitted with 1.2 cm thick plywood dividers so that 1 super housed 2 separate colonies. Standard bottom boards were modified to have 2.2 X 10.5 cm entrances at opposite ends. Two 20.5 X 50.5 cm inner covers made from 0.3 cm thick temp-

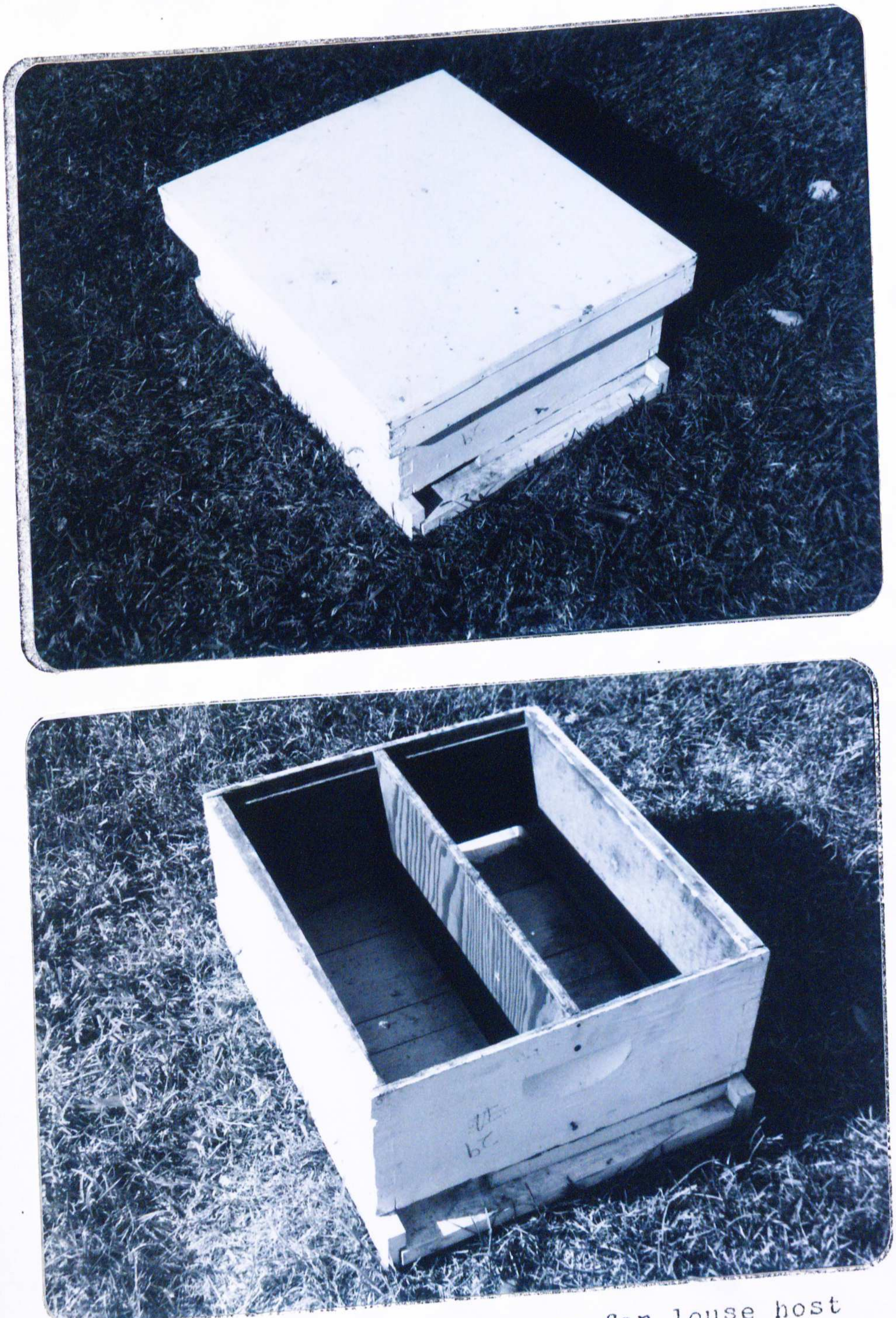

Figure 2. Double nuc boxes for louse host selection and biology field tests.

ered hardboard were placed over each nuc. A standard outer cover was used on top of the double nucs.

Nucs were stocked with bees and brood from the University of Maryland Apiary located on the campus at College Park. Nucs were given 1 frame of brood, except one which was given only 1/2 frame of brood and 6 nucs which were given 1 frame of nectar and no brood. Colonies were stocked with bees covering between 1 to 4 frames. Nucs were given 1 or 3 frames of sealed honey and 0 or 2 empty frames containing drawn comb. Total frames in each nuc was 4.

Colonies were made up during the day, screened and moved a distance of 7 miles to the University of Maryland farm at Beltsville. Entrance screens were then removed. All colonies were given queen cells the same day the nucs were made or the following day except for 6 nucs which were given laying queens.

Laying queens for 6 nucs were obtained from standard colonies. Queens were caged, placed in nucs, and released 5 days after introduction.

Zero to 50 lice were introduced to each nuc. Table 3 summarizes this test. Lice were collected on bees the day before they were needed for introduction into nucs. Bees were fed queen candy in several plastic queen cups inside the holding cage and invert sugar syrup placed on the outside of the holding cage.

Lice introduction cages were constructed of a 1.2 cm thick plywood frame having internal dimensions of 2.0 x 8.0

Table 3. *Summary of bee louse host selection and biology field tests.*

Date Nucs Established	No. of Nucs	No. Frames Placed in Nucs: Brood	Nectar	Honey	Empty	Queen Condition: Queen Cell	Mated Queen	No. Lice Introduced: 0	30	36	40	50
7-12-76	1	1		1	2	X						X
	1	1		1	2	X					X	
	1	1		3		X					X	
	1	1		1	2	X					X	
	1	1		3		X				X		
	1	1		3		X			X			
8-18-76	2	1		3			X					X
	1	1		1	2		X					X
	1	1		3			X					X
	2	1		1	2		X					X
9-17-76	1		1	3		X						X
	1	0.5		3		X						X
	1	1		1	2	X						X
	2		1	1	2	X						X
	1	1		1	2	X						X
	1		1	3		X		X				
	3	1		1	2	X		X				
	2		1	3		X		X				
4-18-77	6	1		1	2	X						X
	6	1		1	2	X		X				

5-15-77	3	1	1	2	X		X
	3	1	1	2	X	X	
6-23-77	2	1	1	2	X		X
	2	1	1	2	X	X	

x 14.0 cm with an area of 224 cm^3. The sides of the cages were covered with nylon window screen having a 5.4 x 6.9 cm mesh. In the center of the cage side, a 1.6 cm hole was bored in which a number 5 cork was placed (Figure 3).

When needed bees with lice were removed from the holding cage and isolated singly under 37 ml plastic cups. Lice on bees were counted and then bees with lice were placed in lice introduction cages through the hole in the cage side. Cages contained 1 plastic 0.5 ml queen cup filled with queen candy and invert sugar syrup was placed on the cage screen to provide food for the bees.

The lice introduction cages with the selected number of bees and lice were each placed in separate paper bags and transported to the nucs at the Beltsville farm. Corks in cages were removed and replaced with loose fitting grass that was later slowly removed by the bees. Cages were placed with the hole upward between the center 2 frames of the nucs.

Identical lice introduction cages were placed in nucs that did not receive lice. Cages were stocked with 30 worker bees without lice.

Lice introduction cages were removed the day following introduction if the bees were released from the cages. If bees were still trapped in the cages, the grass in the hole was removed and the cage was removed the next day.

Nucs were observed several times the first week after establishment and then approximately once a week thereafter for the remainder of the experiment. Colonies were examined

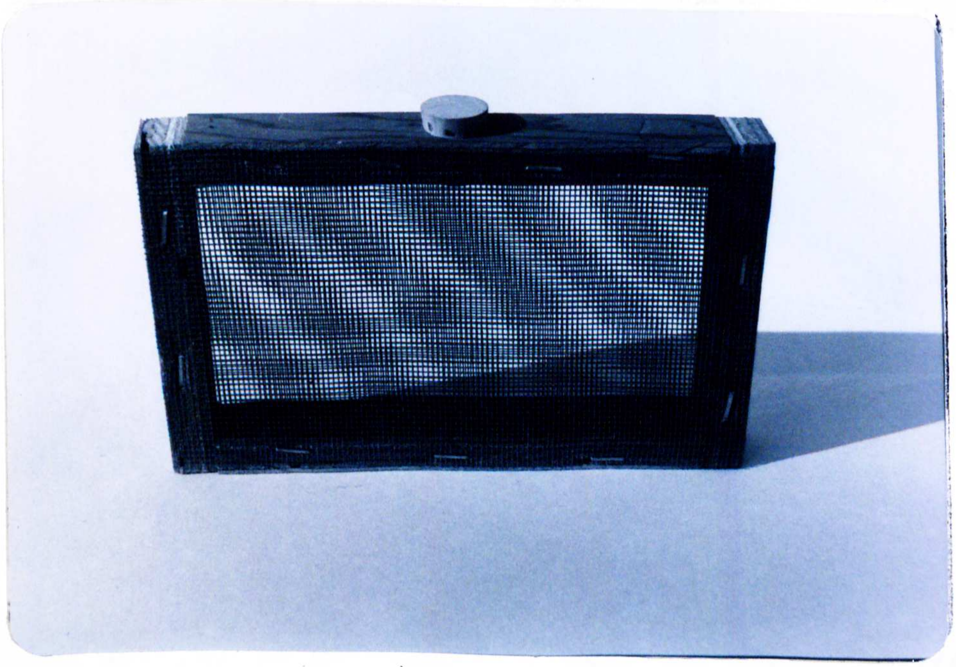

Figure 3. Louse introduction cage.

for size, amount of brood, amount of capped honey, number of louse larval tunnels, number of lice on 1 frame and the number of lice present on the queen. An attempt was made to determine louse host selection and reproduction cycles of the bee louse.

During inspections of nucs, colony population was recorded as the number of frames or parts of frames containing bees. The amount of brood in colonies was recorded as the number of half frames containing any amount of brood. Honey in colonies was estimated as the number of frames or parts of frames filled with capped honey.

Louse larval tunnels were individually counted in each hive. When few tunnels were present an accurate count of larval tunnels was obtained but counts made when tunnels were abundant are somewhat less accurate in number.

The number of lice on workers and drones on 1 frame was determined during the inspection of each colony. The second frame from the center divider of each nuc was used when counting lice if it contained brood (when present in colonies) and bees. When brood or bees were not on this frame another frame was used to determine the number of lice present.

An attempt was made to locate the queen in each nuc during inspections. If found, the queen was picked up by her wings and closely examined for lice.

Bee Louse Movement on Caged Honey Bees

A total of 1,357 honey bees harboring 1,392 bee lice for introduction into experimental nucs were observed for the location of lice after caging. Bees contained 1 louse each at the start of tests except approximately 10 bees which had no lice and 35 bees which harbored 2 lice each.

Bees with lice were placed in a holding cage with internal dimensions of 3.6 X 10.6 X 10.6 cm. The sides of the cage were covered with a 20.3 cm mesh nylon screening. The holding cage was fitted with a sliding top for introduction of bees with lice (Figure 4). Several plastic queen cups filled with queen candy were placed in the cage to provide food for bees. Invert sugar syrup was also placed on the side of the cage to provide food for bees shortly before placing the cage in an incubator.

Bees with lice were collected between 10 A.M. and 4 P.M. They were brought back to the lab and placed in an incubator at 25 or 34 $\pm$ 1.5^{o}C. Bees with lice were removed the following day after a period of 20 $\pm$ 4 hours. Bees were isolated by placing bees under 37 ml plastic cups and then counting the number of lice present per bee.

Field Observations on Bee Lice

Colonies owned by Mr. Alvey Myers of Myersville, Maryland were observed during the active season between May, 1976 and July, 1977. All colonies in his apiary harbored bee lice.

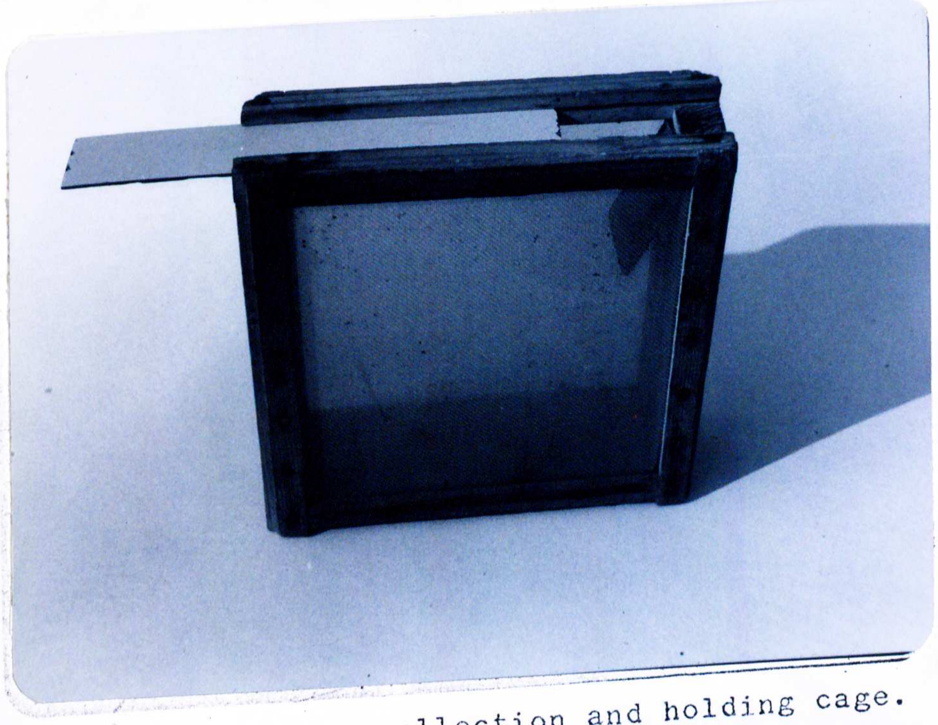

Figure 4. Louse collection and holding cage.

Colonies were observed once every 2 to 4 weeks between April and October of 1976 and between April and July of 1977. Colonies were examined for the number of lice on 1 random brood frame and the number of lice on the queen when the queen could be found.

Hive bees and foraging bees from random colonies with bee lice were sampled for the presence of lice once a month, July through October, 1976 and April through July, 1977.

Foraging bee samples were taken by closing the entrances of colonies and collecting returning foraging bees with an insect net. The end of the net containing the bees was placed in a 2.2 l bucket. Bees were anesthetized with carbon dioxide and transferred to a cardboard container.

Hive bee samples were obtained by removing a brood frame with clinging bees from the hive to be sampled. After making certain the queen wasn't present, some of the bees were brushed off with a bee brush to obtain a proper number of sample bees on the frame. The frame was examined for the number of lice present on bees. The frame was then placed in a glass observation hive. The bees were anesthetized with carbon dioxide and placed in a 0.47 l cardboard container.

1976 bee samples were placed in a freezer to starve to death. Samples were left frozen until examined. Sample bees were individually examined for lice and counted. 1977 bee samples were collected in containers that were fitted with a 3.2 cm mesh screen placed inside. Screens were elevated 1 to 2 cm from the container bottoms. Sample bees were kept at room temperature and allowed to starve to death.

Dead bees were removed and lice that had fallen under the screen were counted and collected. Sample bees were spread out on white paper and any lice observed on the paper were counted and collected. Sample bees were then counted.

Honey bee samples were collected from a hive apparently free from lice at the University of Maryland campus apiary to serve as a control. Five foraging bee samples were collected with an insect net from the front of a closed colony. Ten lice on 6 to 9 bees were placed inside the net while collecting each sample. Five additional foraging bee samples had no bees with lice placed in the net. Samples were counted in the same manner as during 1977.

Fifteen hive bee control samples were collected. Ten lice each on 4 to 8 bees were introduced to 10 samples before anesthetizing sample bees in the glass observation hive. Five samples were counted in the same manner as during 1976 and 5 samples were counted as during 1977. Five additional samples containing no added lice were similarly counted.

CHAPTER IV

RESULTS AND DISCUSSION

Maryland Survey for Bee Lice

1,881 honey bee colonies were examined for bee lice in 272 Maryland apiaries located in 20 counties during 1976. Three-hundred-forty-three colonies in 76 apiaries from 11 counties were found to harbor *Braula coeca*. Anne Arundel, Carroll, Frederick and Washington counties contained the highest infestation levels of lice. The results of the survey are presented in Table 4.

The survey indicates a high infestation level in Maryland honey bee colonies. 27.9% of all apiaries examined contained lice and 18.1% of the total colonies examined were found to be infested with *Braula*. 49.9% of the colonies in apiaries with lice, were infested with bee lice. Phillips (1925) estimated that probably no more than 10% of colonies in infested apiaries contained lice. However it appears that once *Braula* becomes introduced into an apiary the bee lice spread to many other colonies in the same apiary.

The occurence of lice in bee colonies in the 20 counties surveyed is probably greater than actually observed. Reasons lice may not have been observed when they were actually present are as follows: (1) Adult bee lice are difficult to find in bee colonies unless they are numerous;

Table 4. Maryland bee louse survey by counties, 1976.

County	No. Apiaries Examined	No. Apiaries with Lice	% Apiaries with Lice	No. Colonies Examined	No. Colonies with Lice	% Colonies with Lice	No. Colonies in Apiaries with Lice	% Colonies with Lice in Apiaries with Lice
Allegany	16	0	0.0	203	0	0.0	---	---
Anne Arundel	19	7	36.8	87	12	13.8	47	25.5
Baltimore	37	4	10.8	234	7	3.0	37	18.9
Calvert	1	0	0.0	3	0	0.0	---	---
Caroline	3	0	0.0	83	0	0.0	---	---
Carroll	6	6	100.0	31	13	41.9	31	41.9
Cecil	3	0	0.0	95	0	0.0	---	---
Charles	7	2	28.6	21	2	9.5	9	22.2
Dorchester	11	0	0.0	99	0	0.0	---	---
Frederick	10	7	70.0	77	65	84.4	74	87.8
Garrett	1	0	0.0	1	0	0.0	---	---
Harford	34	9	26.5	266	21	7.9	122	17.2
Howard	5	1	20.0	19	1	5.3	4	25.0

Montgomery	53	17	32.1	192	32	16.7	75	42.7
Prince George's	33	8	24.2	131	12	9.2	60	20.0
Queen Anne's	2	0	0.0	6	0	0.0	---	---
St. Mary's	8	2	25.0	34	5	14.7	11	45.5
Talbot	2	0	0.0	3	0	0.0	---	---
Washington	15	13	86.7	237	173	73.0	218	79.4
Wicomico	6	0	0.0	59	0	0.0	---	---
Totals	272	76	27.9	1881	343	18.2	688	49.9

(2) Many colony inspections were made in the spring when some colonies had no capped honey. During this time colonies without capped honey would not have any bee louse larval tunnels. (3) The main purpose of colony inspections was to check for the presence of foulbrood; often only a small portion of capped honey was examined for louse larval tunnels.

Bee Louse Host Selection Tests

Laboratory tests were conducted to determine louse host selection for different ages and castes of honey bees. The results obtained from Tests I through IX are presented in Table 5. Records of each test are presented in Appendices I through IX.

Test I: Louse host selection between workers of different ages. Lice were randomly distributed on test bees held at 25°C while lice preferred one day old worker bees at 34°C. The different results may be partially explained by honey bee activity.

Honey bees held at the lower temperature were less active than those kept at 34°C. This limited activity of bees may have resulted in random distribution of lice. Lice preference for young bees was expected and follows the results of Kaschef (1959).

Test II: Louse host selection between young and old drones. Bee lice preferred young drones over old drones at both 25 and 34°C.

During the test at 34°C there was a high mortality rate among drones; 62% of the young drones and 82% of the old drones died. At the end of the test only 24% of the lice were on the test bees. Many of the lice preferred to move

Table 5. *Results of bee louse host selection tests.*

Test No.	Temp. °C 25°	Temp. °C 34°	Test Bees: Worker Age[a/] 1	5	15	30	Random	Foragers	Drones Young	Drones Old	Queens Virgin	Queens Mated	Value of P
I	X		XO[b/]	XO	XO	XO							>.05
		X	XP[c/]	XO	XO	XO							<.01
II	X								XP	XO			=.05
		X							XP	XO			<.01
III	X								XO		XP		<.01
		X							XO		XP		=.05
IV	X								XO			XP	<.01
		X							XO			XP	<.01
V	X									XO	XP		<.01
		X								XO	XO		>.05
VI	X									XO		XP	<.01
		X								XO		XP	<.01
VII	X							XP		XO			<.01
		X						XO		XO			>.05

VIII	X		XO	XP		<.01
		X	XO	XP		<.01
IX	X		XO		XP	<.01
		X	XO		XP	<.01

a/ Indicates age in days

b/ No preference

c/ Indicates preference

onto the cage instead of remaining on drones.

Free (1957) stated that drones are fed by workers during the first days of their lives. Older drones largely feed themselves and stay out of the brood area. In this test both the young and old drones fed very little, if any, on the queen candy in cages. Little feeding plus the fact that drones often remain stationary on the comb in hives (Free, 1957) probably accounted for the low lice recovery on drones.

Test III and IV: Louse host selection between young drones and a virgin or mated queen. Bee lice preferred virgin or mated queens over young drones at both temperatures. As in Test II there was high drone mortality. Forty-four percent of the drones died during Test III and 22.5% died during Test IV. Only 25% of the lice in Test III were recovered on test bees while 73% of the lice were on bees at the conclusion of Test IV.

Although the results of Test III indicate a preference of lice for the virgin queen, she is not highly attractive since 75% of the lice were on the test cage or unaccounted for. Weiss (1967) reported that isolated virgin queens can feed themselves on sugar-honey candy. Thus the queens in the test should have fed themselves and been able to feed any lice on them.

The results of Test IV agree with Argo (1926) and Cory (1936) who observed numerous lice in the field on mated queens in the late summer or early fall.

Test V and VI: Louse host selection between old drones and a virgin or mated queen. Bee lice preferred virgin queens over old drones at 25°C while at 34°C there was no preference observed. Lice preferred mated queens over old drones at both temperatures as expected.

Once again there was a high mortality of drones during both tests. Forty percent of the drones died during Test V while 25% died during Test VI.

During Test V at the lower temperature, the drones were probably less active than the virgin queen, while at 34°C the increased activity of drones may have produced the random distribution of lice on the test bees. The fact that drones probably do not feed themselves should have resulted in a limited preference for virgin queens at both temperatures. Under hive conditions when drones are not starving it is possible that lice may exhibit a preference for drones over virgin queens.

Test VII: Louse host selection between old drones and foragers. Bee lice were found to prefer foraging bees over old drones at 25°C but had no preference for either group at 34°C.

At the lower temperature both workers and drones were less active. Drones were probably underfed as a result of this limited activity and the lice preferred worker honey bees as hosts. At 34°C there was increased activity, probably resulting in the random distribution of lice observed.

Benton (1895), Arnhart (1924), Hempsall-Herrod (1931) and Atakishiev (1971) reported that Braula are infrequently found on drones. However, since most beekeepers make an effort to keep drone populations low through the use of worker cell foundation there is less opportunity for lice to be on drones. Therefore it is likely that lice are randomly distributed between workers and drones within honey bee colonies which agrees with field observations made in this study.

Test VIII and IX: Louse host selection between random age workers and a virgin or mated queen. Lice were found to prefer virgin or mated queens over random age workers. Preference for mated queens was expected since much of the literature on Braula emphasizes their being found on queens. In the louse host selection and biology field tests, few lice were found on virgin queens while at the same time lice were found to be present on mated queens. Although more attractive than workers, it appears that virgin queens are less attractive to lice than mated queens.

Bee Louse Host Selection and Biology Field Tests

A total of 46 nucs were established during July, August and September, 1976 and April, May and June, 1977. A summary of the results of this test are presented in Table 6 and 7, and Appendicies X through IXX.

During 1976, louse populations in test nucs remained relatively constant after an apparent initial drop the first month. Louse populations continued to drop in 1977 during April through July. When the tests were terminated in July, 1977, no lice were found in 6 of 11 nucs that had 50 lice introduced to them earlier in the season.

Table 6. Summary of bee lice examinations in 29 nucs each receiving 30 to 50 bee lice, Beltsville apiary 1976 - 1977.

Month	No. Inspect-ions	Average Colony Strength			Average Adult Lice			Average Louse Larval Tunnels		Queens	
		Bees[a]	Brood[b]	Honey[c]	No. on 1 Frame[d]	No. in Nuc[e]	Total No. on Queens	No. Ob-served	Per Frame[f]	Total Ob-served	Total with Lice
April	18	1.9	1.0	0.67	8.61	16.35	0	0.00	0.00	6	0
May	48	2.5	1.4	0.81	5.23	13.08	5	52.75	65.12	36	4
June	42	3.0	1.9	0.68	3.39	10.17	1	104.29	153.36	34	1
July	57	3.0	1.8	0.90	2.14	6.42	9	36.85	40.94	48	5
August	41	2.2	1.5	1.31	3.70	8.14	43	13.77	10.51	37	10
September	67	2.2	1.2	1.42	4.79	10.63	43	6.70	4.72	59	19
October	47	2.2	0.9	1.08	3.11	6.84	18	3.19	2.95	41	12
November	41	1.9	0.1	0.68	4.24	8.06	33	1.21	1.78	37	19
December	13	1.6	0.0	0.58	3.39	5.42	3	**	**	10	3
Totals	374	2.3	1.1	0.90	4.29	9.87	155	27.35	30.38	308	73

a/ Number frames with bees (1 frame = 1300 bees).

b/ Number frames containing brood (counted as the number of half frames containing any amount of brood).

c/ Number frames honey (1.00 = 560 cm^2 capped honey).

d/ On workers and drones.

e/ Number lice, on workers and drones, on one frame multiplied by number frames bees in nuc.

f/ Equivalent number tunnels per 1 frame capped honey.

** Not recorded.

Table 7. Summary of bee lice examinations in 17 nucs not receiving bee lice, Beltsville apiary 1976 - 1977.

Month	No. Inspections	Average Colony Strength			Average Adult Lice			Average Louse Larval Tunnels		Queens	
		Bees[a/]	Brood[b/]	Honey[c/]	No. on 1 Frame[d/]	No. in Nuc[e/]	Total No. on Queens	No. Observed	Per Frame[f/]	Total Observed	Total with Lice
April	18	1.5	0.8	0.33	0.00	0.00	0	0.00	0.00	10	0
May	49	2.0	2.3	0.80	0.04	0.08	0	0.18	0.23	38	0
June	42	2.6	1.7	0.67	0.00	0.00	0	0.19	0.29	38	0
July	25	3.1	2.6	0.44	0.00	0.00	0	0.49	1.11	38	0
August	--	--	--	--	--	--	--	--	--	--	--
September	12	2.3	0.5	2.00	0.08	0.18	0	0.00	0.00	11	0
October	18	2.2	0.7	1.81	0.11	0.24	0	0.00	0.00	13	0
November	15	2.1	0.4	1.19	0.20	0.42	0	0.00	0.00	13	0
December	5	1.7	0.0	0.81	0.20	0.34	0	**	**	4	0
Totals	184	2.2	1.1	1.01	0.08	0.18	0	0.12	0.12	165	0

a/ Number frames with bees (1 frame = 1300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames honey (1.00 = 560 cm^2 capped honey).
d/ On workers and drones.
e/ Number lice, on workers and drones, on 1 frame multiplied by number frames bees in nuc.
f/ Equivalent number tunnels per 1 frame capped honey.
** Not recorded.

A portion of the initial drop in observed lice can be attributed to problems in detecting lice on bees. It was common during inspections to observe lice move to the ventral surface of bees where they were undetectable.

Louse population levels remained relatively constant in nucs established during 1976 because lice were probably from the current years brood. Some of the lice introduced to nucs were pale in color, indicating they were less than 1 day old (Hempsall-Herrod, 1931; Atakishiev, 1971). Lice introduced to nucs during 1977 were dark in color and had probably overwintered from the previous year. Therefore, it can be assumed that most of them were old and many died after introduction into nucs.

Observation of louse larval tunnels indicate that many lice were ovipositing. As many as 669 louse larval tunnels were observed in 1 hive established April, 1977. However, few larvae, if any, reached the adult state. No pale lice were observed in nucs after the initial introduction of lice. Also, after finding many larval tunnels, the number of adult lice continued to drop when they should have been increasing due to adult emergence. Lice perished during development for an unknown reason.

Louse larval tunnels were observed during both 1976 and 1977. Oviposition by female lice is dependent on periods of nectar flow when bees are actively capping ripened honey. *Braula coeca* females place eggs either in honey cells under the surface of wax cappings before they are completed by bees (Arnhart, 1923; Argo, 1926; Hempsall-Herrod, 1931; Orosi-Pal, 1938; and Imms, 1942) or on the outer surface of capped honey (Alexeyenko and Bakai, 1958a; Hassanein and Abd El-Salam, 1962). Alexeyenko and Bakai (1958a) and Atakishiev (1971) also reported that eggs are deposited on brood combs, but there are few reports of developing larval of B. coeca over the cappings of sealed brood.

In this study, reproduction of bee lice was apparently limited to times of nectar flow when bees were actively capping honey. These periods occured during August and September of 1976 and portions of May, June and a small nectar flow in July of 1977.

Few tunnels were observed in previously capped honey that was placed in nucs at the beginning of tests. Those that were observed in this honey may have been started where the bees had begun uncapping the honey. During periods when bees were consuming honey faster than they were producing it, many louse larval tunnels were destroyed.

Queens were observed 307 times in the 29 nucs receiving lice. Seventy-three queens (24%) were observed with 1 or more bee lice primarily during August to September. A summary of the findings of lice on queens is presented in Table 8.

One bee louse was found on a single virgin queen of 49 observed. Since virgin queens were observed without lice during periods when lice were observed on mated queens, the single observation indicates that lice seldom select virgin queens as hosts.

Table 8. Number and percent lice on queens, test nucs receiving lice, Beltsville apiary 1976 - 1977.

Month	No. Queens Observed	No. Queens with Lice	% Queens with Lice	Total Lice on Queens	Average No. Lice on Queens with Lice	No. Virgin Queens Observed	No. Virgin Queens with Lice	% Virgin Queens with Lice
April	6	0	0.0	0	---	6	0	0.0
May	36	4	11.1	5	1.25	11	0	0.0
June	34	1	2.9	1	1.00	5	0	0.0
July	48	5	10.4	9	1.80	15	1	6.7
August	37	10	27.0	43	4.30	0	0	0.0
September	59	19	32.0	43	2.26	8	0	0.0
October	41	12	29.3	18	1.50	4	0	0.0
November	37	19	51.4	33	1.74	0	0	0.0
December	10	3	30.0	3	1.00	0	0	0.0
Totals	308	73	23.7	155	2.12	49	1	2.0

Bee Louse Movement on Caged Honey Bees

After 20 ± 4 hours at 25 or 34 ± 1.5°C, 61.4% of the bees initially harboring lice retained lice. As many as 9 lice were found on a single bee. The results are presented in Table 9.

The probability of the observed results being a random distribution is less than 1%. The preference of lice probably was for younger bees as observed earlier in this study.

Field Observations on Bee Lice

Fourteen colonies owned by Mr. Alvey Myers of Myersville, Maryland, were observed between April and October, 1976 and April and July, 1977 for bee lice. Results are presented in Figures 5 and 6 and Tables 10 to 12.

Figure 5 indicates a rise in louse populations during April for an unknown reason. During this period, no pale lice were observed in hives to indicate oviposition. Adult bee lice populations decreased during May probably as overwintered lice died following oviposition. There was a peak in July due to the emergence of the first adult lice. August samples were reduced for an unknown reason when populations were expected to continue rising. Late in the season the number of lice in colonies continued to climb, due to the emergence of young lice.

Adult bee lice were first found on queen honey bees during June (see Table 10). The percentage of queens with lice was as high as 100% in the fall months. Over half of the lice on queens were pale in color indicating they were less than 1 day old. This indicates an obvious attraction of young lice to queen honey bees.

Table 9. Records of bee lice movement on caged honey bees after 20 hours.

	Number of Lice per Bee										
	0	1	2	3	4	5	6	7	8	9	Total
No. Bees with Lice[a/]	524	480	226	71	43	7	4	1	0	1	1,357
% Bees with Lice	38.6	35.4	16.7	5.2	3.2	0.5	0.3	0.1	0.0	0.1	
Total No. Lice on Bees	---	480	452	213	172	35	24	7	0	9	1,392

[a/] All bees had 1 louse each when caged except approximately 10 bees which had no lice and 35 bees which harbored 2 lice each.

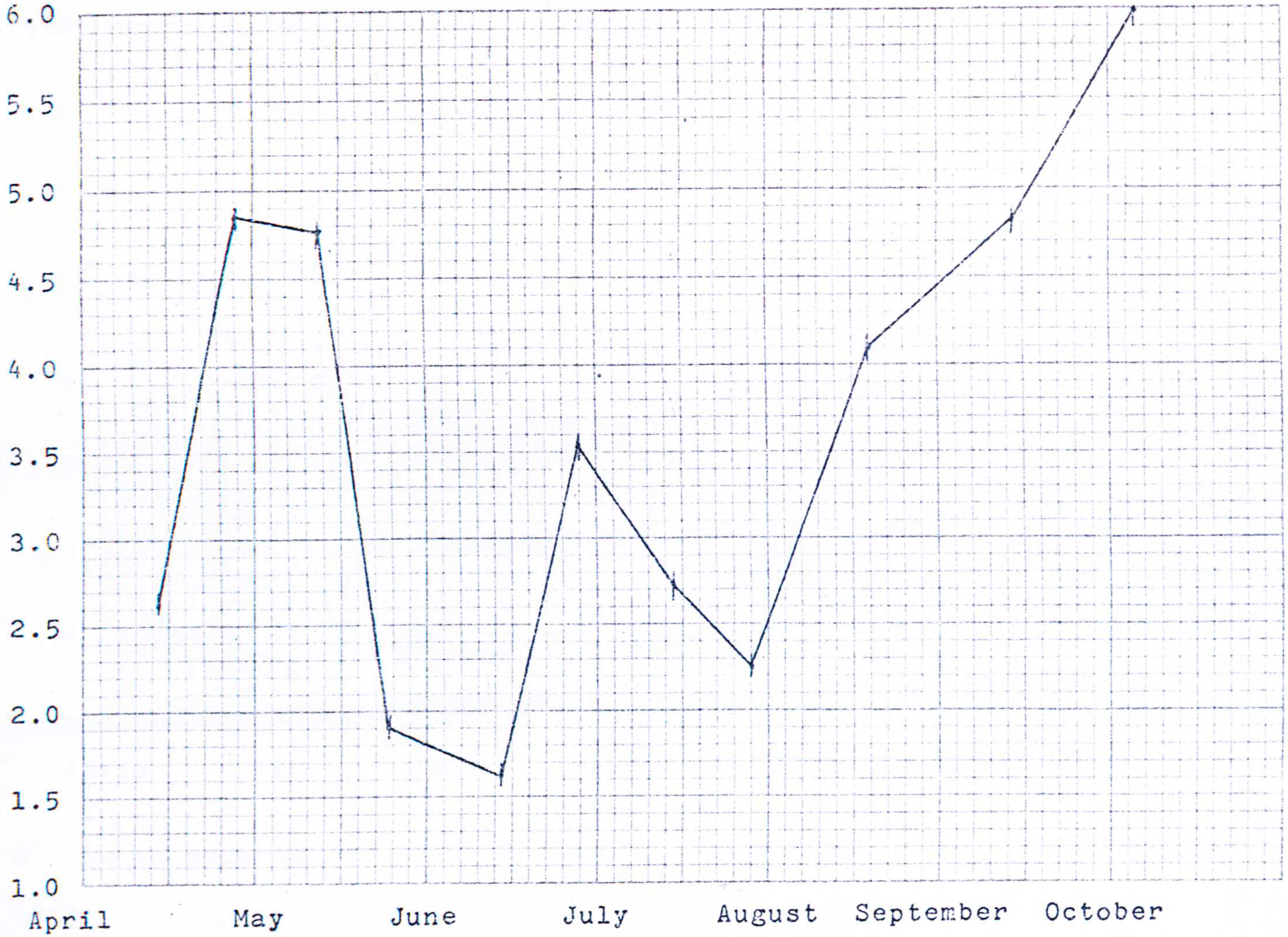

Figure 5. Average number of lice per frame of bees observed during inspections of Myers' apiary, 1976 - 1977.

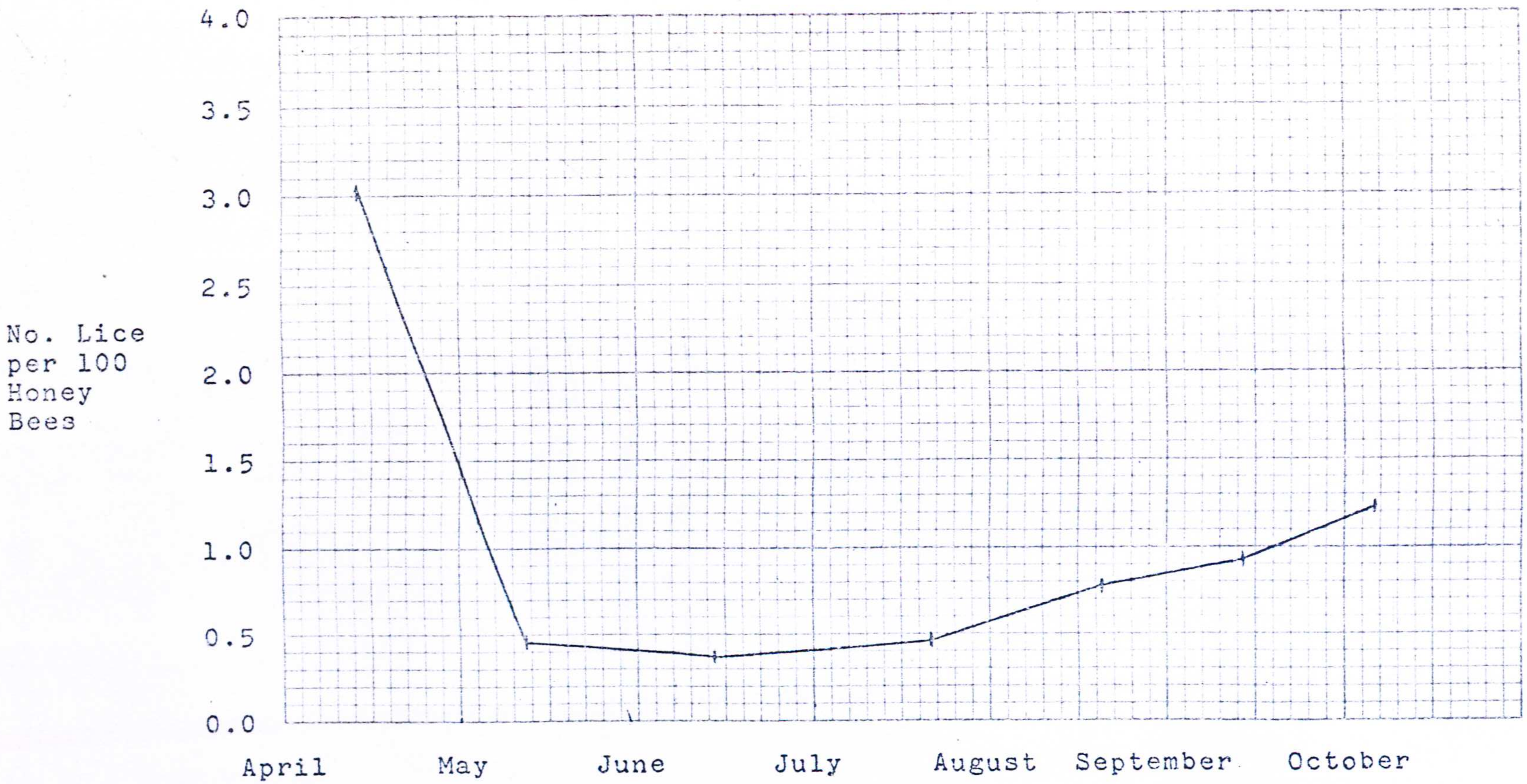

Figure 6. Louse populations observed in hive bee samples taken from Myers' apiary, 1976 - 1977.

Table 10. Queens found with and without lice, Myers apiary 1976 - 1977.

Month	No. Queens Observed	No. Queens with Lice	% Queens with Lice	No. Lice Observed on Queens	Average No. Lice Found on Queens with Lice	Color of Lice on Queens	
						No. Pale	No. Dark
April	13	0	0.0	---	---	---	---
May	13	0	0.0	---	---	---	---
June	20	9	45.0	19	2.11	10*	2*
July	24	14	58.3	104	7.43	31*	26*
August	6	6	100.0	52	8.67	33	19
September	6	4	66.7	27	6.75	17	10
October	4	4	100.0	58	14.50	28	30
Total	86	37	43.0	260	7.03	119	87

* Color of some lice on queens not recorded. Number indicates only lice observed for color.

Table 11. Number and percent of *Braula* found on foraging and hive bees, Myers' apiary 1976 - 1977.

Date	Foraging Bee Samples			Hive Bee Samples				
	No. Samples	No. Bees in Samples	No. Lice in Samples	No. Samples	No. Bees in Samples	No. Lice Observed in Samples	% Lice Observed in Samples	Total Lice in Samples
April	3	245	0	3	1,160	15	42.9	35
May	3	624	0	3	1,809	8	100.0	8
June	2	185	0	3	1,634	4	66.7	6
July	5	754	1	5	4,345	7	46.7	15
August	3	429	0	3	2,178	6*	40.0*	17
September	3	416	0	3	1,948	8	44.4	18
October	3	439	0	3	1,385	9	52.9	17
Totals	22	3,092	1	23	14,459	57	48.3	118

* One sample taken in August was not observed for lice before collecting sample.

Table 12. Average number and percent of added lice recovered from foraging and hive bee samples, 1977.

No. Replications		Average No. Bees in Sample	No. Lice Put in Each Sample	Average No. Lice Recovered
Foraging Bees	Hive Bees			
5		152	0	0.0
5		108	10	8.6
	5	394	0	0.0
	10	382	10	8.5

The highest number of lice observed on a single queen honey bee was 29. Lice were found on all parts of her body including dorsal and ventral surfaces. There are numerous reports in the literature of more than 1 louse being present on queen honey bees. Some of the more recent reports state that 35 lice (Argo, 1925), 14 lice (Imms, 1942), 19 lice (Stejskal, 1965) and 46 lice (Atakishiev, 1971) were observed on single queens.

A total of 638 worker honey bees were observed to harbor bee lice. Of those observed, 629 (98.6%) had 1 louse, 8 (1.2%) had 2 lice each and only 0.2% had 3 lice. These findings contradict Phillips (1925) who stated that no more than 1 louse per single worker bee had been observed in Maryland. However, they agree with Hempsall-Herrod (1931) who said as a rule, no more than 2 lice are found on worker honey bees.

A total of 577 workers and drones were observed to have bee lice during April 15 to September 30, 1976 and April 15 to July 13, 1977. 95.8% of the louse observations were on worker honey bees while 4.2% were found on drones. Twenty-three drones (95.8%) had a single louse and 1 drone had 2 lice (4.2%).

Benton (1895), Arnhart (1924), Hempsall-Herrod (1931), and Atakieshiev (1971) reported that Braula are infrequently found on drones. However, since most beekeepers attempt to reduce drone population by using worker cell foundation, there is a reduced opportunity for lice to select drones as hosts. Since 4.2% of the lice observed in this study were on drones, it can be concluded that there is a random distri- bution of lice found on worker and drone honey bees.

Samples for bee lice were taken from 5 random colonies during August, September and October, 1976 and May, June and July, 1977. Results are presented in Figure 6 and Tables 11 and 12.

A single louse was found on 3,092 foraging honey bees constituting 22 samples (Table 11). This is a strong indication that lice do not prefer foraging bees and there- fore rarely leave the hive on honey bees under normal colony conditions.

One hundred seventeen lice were collected on 14,459 honey bees sampled from within the brood area of the same sample hives (Table 11). These observations agree with Kaschef (1959).

Inaccuracies of observations were recorded from samples taken. 48.7% of the bee lice in the hive samples were ob- served during collection of samples (Table 11). This was probably due to some of the lice clinging to the ventral sur- face of bees during observations.

Control samples indicate that 14 to 15% of the lice collected were lost or missed when counting samples. The controls indicate there was no difference in the types of counting techniques used (See Table 12).

Fluctuation in louse populations observed in samples are presented in Figure 6. Changes were as expected and are similar to those observed in Figure 5.

SUMMARY

A survey was conducted to determine the extent of infestation of bee lice in Maryland apiaries. Of 272 apiaries examined, 28% were found to harbor Braula. Eighteen percent of the colonies examined contained bee lice and 50% of the colonies in apiaries with lice were infested. These results indicate bee lice are common in Maryland and spread to many of the colonies once present in apiaries.

Laboratory tests were conducted to determine louse preference between worker, drone and queen honey bees. Bee lice had no preference between workers 1, 5, 15 and 30 days old at 25°C while there was a preference for 1 day old workers at 34°C. Lice preferred young drones over old drones at both 25 and 34°C. Lice were found to prefer virgin and mated queens over young drones at both 25 and 34°C. Virgin queens were preferred by lice over old drones at 25°C while at 34°C no preference was observed. Lice preferred mated queens over old drones at both 25 and 34°C. There was a preference for foraging age workers over old drones at 25°C but no preference at 34°C. Bee lice preferred both virgin and mated queens over random age workers at both 25 and 34°C.

Twenty-nine honey bee colonies established in double nuc boxes during July, August and September, 1976 and April, May and June, 1977, received 30 to 50 lice each while 17 control colonies received no lice. Louse larval tunnels were observed in newly capped honey in nucs receiving lice.

Tunnels were most numerous from May through August during periods that corresponded with nectar flows when bees were capping honey. Larval Braula apparently perished for an unknown reason before emerging. Queens in nucs receiving lice were observed 307 times. Seventy-three queens (24%) were observed having 1 or more lice primarily between August and December. One bee louse was found on a virgin queen of 49 observed during periods when lice were present on mated queens. A very small number of louse larval tunnels and adult bee lice were observed in control nucs. Colony populations in controls were similar to other nucs in the test.

Bee louse movement was observed on honey bees after holding 1,357 bees with 1 or 2 lice each in a cage for 20 hours. Zero to 9 lice were found on single bees at the end of the test period.

Field cbservations were made on 14 colonies owned by Mr. Alvey Myers of Myersville, Maryland. Louse populations were observed to decrease in the late spring to a low in early June due to the death of overwintered lice. By July populaticns began increasing with the emergence of new lice. Lice were first observed on queens during June and were found on queens throughout the rest of the year. As many as 100% of the queens examined during this period contained Braula. The highest number observed was 29 on a single queen. Fifty-eight percent of the lice present on queens were pale in color indicating an affinity of young lice for queens. Of 638 worker bees observed with lice, all had 1 louse each except 1.2% with 2 lice each and 0.2% with

3 lice each. Five-hundred-seventy-seven workers and drones were observed with lice between April 15 and September 15; 95.8% of the lice were on workers and 4.2% were drones.

Honey bees were sampled from hives known to harbor lice. A single bee louse was observed on 3,092 foraging honey bees constituting 22 samples. One-hundred-seventeen lice were collected in 23 samples containing 14,459 bees collected from within the brood nest of the same hives. Samples indicate a strong preference of lice for hive bees over foraging bees. Twenty-five control samples indicate a 14 to 15% loss of lice during sampling. Also only 49% of the lice sampled from within hives were observed during collecting. Fluctuation in louse populations were similar to those found elsewhere in this study.

APPENDIX I. Test I. Louse host selection tests between workers of different ages. Five Lice were introduced to 20 total workers 30, 15, 5, and 1 days old.

Replication No.	Date	Temp. °C	Time Length of Test	Numbers Recovered: Workers, Listed by Age: 30 Days	15 Days	5 Days	1 Days	Lice	Location of Lice: Workers, Listed by Age: 30 Days	15 Days	5 Days	1 Day	Cage
1	4-19-77	25	8:07[a/]	5A[b/]	5A	5A	5A	5A	0	2	1	2	0
2	4-19-77	25	8:09	5A	5A	5A	5A	5A	0	1	2	2	0
3	4-19-77	25	8:20	5A	5A	5A	5A	5A	1	3	0	1	0
4	4-19-77	25	8:23	5A	5A	5A	5A	5A	2	0	2	1	0
5	4-19-77	25	8:20	5A	5A	5A	5A	4A 1D[c/]	2	0	1	1	1D
6	4-19-77	34	8:30	5A	5A	5A	5A	5A	1	0	0	4	0
7	4-19-77	34	8:08	5A	5A	5A	5A	5A	0	0	1	3	1A
8	4-19-77	34	8:15	5A	5A	5A	5A	4A 1U[d/]	0	0	0	4	0
9	4-19-77	34	8:21	5A	5A	5A	5A	9A*	1	2	0	6	0
10	4-19-77	34	8:20	5A	5A	5A	5A	4A 1U	0	2	0	2	0

a/ Hours and Minutes.
b/ Live individuals.
c/ Dead individuals.
d/ Unaccounted for.
* Nine lice introduced into test cage.

APPENDIX II. Test II. Louse host selection tests between young drones and old drones. Five lice were introduced to 10 drones 1 to 5 days old and 10 drones at least 6 to 8 days old.

Replication No.	Date	Temp. °C	Time Length of Test	Numbers Recovered: Drones Young	Numbers Recovered: Drones Old	Numbers Recovered: Lice	Location of Lice: Drones Young	Location of Lice: Drones Old	Location of Lice: Cage
1	8-22-77	25	8:13[a/]	7A[b/] 3D[c/]	8A 2D	6A**	1	1	4
2	8-22-77	25	8:16	8A 2D	10A	5A	0	5	0
3	8-22-77	25	8:17	10A	10A	4A 1U[d/]	4	0	0
4	8-22-77	25	8:13	6A 4D	8A 2D	4A 1U	4	0	0
5	8-22-77	25	8:14	10A	9A 1D	5A	2	2	1
6	8-22-77	34	8:12	3A 7D	1A 9D	4A 1D	1	0	3A 1D
7	8-22-77	34	8:15	4A 6D	1A 9D	3A 2U	1	1	1A
8	8-22-77	34	8:18	4A 6D	3A 7D	3A 2U	2	0	1A
9	8-22-77	34	8:15	3A 7D	3A 7D	4A 1D	0	0	4A 1D
10	8-22-77	34	8:15	5A 5D	1A 9D	3A 2U	1	0	2A

a/ Hours and minutes.
b/ Live individuals.
c/ Dead individuals.
d/ Unaccounted for.
** Five lice introduced, 6 lice recovered.

APPENDIX III. Test III. Louse host selection tests between young drones and a virgin queen. Five lice were introduced to 15 drones 1 to 5 days old and 1 virgin queen 8 days old.

				Numbers Recovered			Location of Lice		
Replication No.	Date	Temp. °C	Time Length of Test	Queen	Drones	Lice	Queen	Drones	Cage
1	4-19-77	25	9:23[a/]	1A[b/]	11A 4D[c/]	4A 1D	0	0	4A 1D
2	4-19-77	25	9:16	1A	12A 3D	2A 3U[d/]	0	0	2A
3	4-19-77	25	9:17	1A	13A 2D	5A	1	2	2A
4	4-19-77	25	9:18	1A	11A 4D	4A 1U	0	0	4A
5	4-19-77	25	9:17	1A	10A 5D	3A 1D 1U	0	1	2A 1D
6	4-19-77	34	8:59	1A	9A 6D	1A 1D 3U	1	0	1D
7	4-19-77	34	8:58	1A	5A 10D	1A 2D 2U	0	0	1A 2D
8	4-19-77	34	9:10	1A	5A 10D	2A 3U	1	1	0
9	4-19-77	34	8:58	1A	4A 11D	2A 3U	1	0	1A
10	4-19-77	34	9:06	1A	4A 11D	1A 2D 2U	0	0	1A 2D

a/ Hours and minutes.
b/ Live individuals.
c/ Dead individuals
d/ Unaccounted for.

APPENDIX IV. Test IV. Louse host selection tests between young drones and a mated queen. Five lice were introduced to 15 drones 2 days old and 1 mated queen.

Replication No.	Date	Temp. °C	Time Length of Test	Numbers Recovered			Location of Lice		
				Drones	Queens	Lice	Drones	Queen	Cage
1	7-25-77	25	9:55[a]	13A[b] 2D[c]	1A	4A 1U[d]	0	4	0
2	7-25-77	25	9:41	11A 4D	1A	5A	3	2	0
3	7-25-77	25	9:47	14A 1D	1A	4A 1U	0	3	1A
4	7-25-77	34	9:35	9A 6D	1A	4A 1U	1	0	3A
5	7-25-77	34	9:33	11A 4D	1A	5A	3	1	1A
6	7-25-77	34	9:19	12A 3D	1A	1A 4U	0	0	1A
7	7-25-77	34	9:36	10A 5D	1A	5A	2	2	1A
8	7-25-77	34	9:39	13A 2D	1A	5A	3	2	0

a/ Hours and minutes.
b/ Live individuals.
c/ Dead individuals.
d/ Unaccounted for.

APPENDIX V. Test V. Louse host selection tests between old drones and a virgin queen. Five lice were introduced to 15 drones at least 6 to 8 days old and 1 virgin queen 8 days old.

				Numbers Recovered			Location of Lice		
Replication No.	Date	Temp. °C	Time Length of Test	Drones	Queen	Lice	Drones	Queen	Cage
1	8-22-77	25	9:29[a]	13A[b] 2D[c]	1A	5A	4	1	0
2	8-22-77	25	9:38	12A 3D	1A	5A	4	0	1A
3	8-22-77	25	9:27	2A 13D	1A	3A 1D 1U[d]	1	2	1D
4	8-22-77	25	9:24	11A 4D	1A	3A 1D 1U	2	1	1D
5	8-22-77	34	9:47	9A 6D	1A	4A 1U	4	0	0
6	8-22-77	34	9:49	5A 10D	1A	2A 1D 2U	2	0	1D
7	8-22-77	34	9:53	15A	1A	5A	4	0	1A
8	8-22-77	34	9:44	9A 6D	1A	3A 1D 1U	2	0	1A 1D
9	8-22-77	34	9:46	5A 10D	1A	4A 1D	2	1	1A 1D

a/ Hours and minutes.
b/ Live individuals.
c/ Dead individuals.
c/ Unaccounted for.

APPENDIX VI. Test VI. Louse host selection tests between old drones and a mated queen. Five lice were introduced to 15 drones at least 6 to 8 days old and 1 mated queen.

Replication No.	Date	Temp. °C	Time Length of Test	Numbers Recovered			Location of Lice		
				Drones	Queen	Lice	Drones	Queen	Cage
1	8-8-76	25	8:05[a/]	14A[b/] 1D[c/]	1A	4A 1U[d/]	1	2	1A
2	8-8-76	25	8:17	10A 5D	1A	5A	1	4	0
3	8-8-76	25	8:17	10A 5D	1A	5A	1	3	1A
4	8-8-76	25	8:22	11A 4D	1A	5A	3	2	0
5	8-8-76	25	8:24	14A 1D	1A	4A 1U	0	4	0
6	9-4-77	34	8:31	8A 7D	1A	4A 1U	2	2	0
7	9-4-77	34	8:20	9A 6D	1A	5A	0	5	0
8	9-4-77	34	8:24	12A 3D	1A	5A	1	3	1A
9	9-4-77	34	7:57	10A 5D	1A	4A 1U	0	4	0
10	9-4-77	34	7:58	14A 1D	1A	3A 1D 1U	2	1	1D

a/ Hours and minutes.
b/ Live individuals.
c/ Dead individuals.
d/ Unaccounted for.

APPENDIX VII. Test VII. Louse host selection tests between old drones and forager worker bees. Five lice were introduced to 10 drones at least 6 to 8 days old and 10 forager worker bees at least 14 days old.

Replication No.	Date	Temp. °C	Time Length of Test	Number Recovered			Location of Lice		
				Drones	Workers	Lice	Drones	Workers	Cage
1	8-7-76	25	8:25[a/]	10A[b/]	10A	4A 1U[d/]	0	4	0
2	8-7-76	25	8:24	10A	8A 2D[c/]	4A 1D	0	4	1D
3	8-7-76	25	8:33	10A	10A	5A	0	5	0
4	8-7-76	25	8:32	10A	10A	5A	1	4	0
5	8-7-76	25	8:40	10A	10A	5A	0	5	0
6	9-4-77	34	8:16	9A 1D	10A	4A 1U	1	3	0
7	9-4-77	34	8:12	8A 2D	10A	4A 1D	2	2	1D
8	9-4-77	34	8:05	6A 4D	10A	5A	0	2	3A
9	9-4-77	34	8:07	10A	10A	5A	3	2	0
10	9-4-77	34	7:59	8A 2D	10A	5A	2	3	0

a/ Hours and minutes.
b/ Live individuals.
c/ Dead individuals.
c/ Unaccounted for.

APPENDIX VIII. Test VIII. Louse host selection tests between random age workers and a virgin queen. Five lice were introduced to 15 random age workers and 1 virgin queen 2 to 4 days old.

Replication No.	Date	Temp. °C	Time Length of Test	Number Recovered: Workers	Number Recovered: Queen	Number Recovered: Lice	Location of Lice: Workers	Location of Lice: Queen	Location of Lice: Cage
1	8-8-76	25	8:04[a/]	14A[b/] 1D[c/]	1A	4A 1D	3	1	1D
2	8-8-76	25	8:05	14A 1D	1A	4A 1D	3	1	1D
3	8-8-76	25	8:08	15A	1A	4A 1D	2	2	1D
4	8-8-76	25	8:16	15A	1A	5A	5	0	0
5	8-8-76	25	8:19	15A	1A	6A***	5	1	0
6	8-16-77	34	8:18	15A	1A	5A	4	0	1
7	8-16-77	34	8:13	14A 1D	1A	5A	3	2	0
8	8-16-77	34	8:11	15A	1A	5A	4	1	0
9	8-16-77	34	8:07	15A	1A	5A	4	1	0

a/ Hours and minutes.
b/ Live individuals.
c/ Dead individuals.
*** 6 lice introduced into test cage.

APPENDIX IX. Test IX. Louse host selection tests between random age workers and a mated queen. Five lice were introduced to 15 random age workers and 1 mater queen.

Replication No.	Date	Temp. °C	Time Length of Test	Number Recovered			Location of Lice		
				Workers	Queen	Lice	Workers	Queen	Cage
1	8-8-76	25	8:13[a]	14A[b] 1D[c]	1A	5A	4	1	0
2	8-8-76	25	8:10	15A	1A	5A	5	0	0
3	8-8-76	25	8:10	15A	1A	5A	4	1	0
4	8-8-76	25	8:25	15A	1A	5A	4	1	0
5	8-8-76	25	8:35	15A	1A	5A	1	4	0
6	9-25-77	34	8:29	15A	1A	5A	1	3	1A
7	9-25-77	34	8:13	15A	1A	5A	3	1	1A
8	9-25-77	34	8:08	15A	1A	2A 1D 2U[d]	2	0	1D
9	9-25-77	34	8:18	15A	1A	4A 1U	3	1	0
10	9-25-77	34	8:25	15A	1A	4A 1U	2	1	1A

a/ Hours and minutes.
b/ Live individuals.
c/ Dead individuals.
d/ Unaccounted for.

APPENDIX X. Summary of colony examinations of 6 nucs, each with 30 to 50 added lice, established July, 1976.

Month	Average Colony Strength			Average Adult Lice			Average Louse Larval Tunnels		Queens		
	Bees[a/]	Brood[b/]	Honey[c/]	No. on 1 Frame[d/]	No. in Colony[e/]	Total No. on Queens	No. Observed	Per Frame[f/]	Total Observed	Total with Lice	% with Lice
July	2.9	0.7	2.00	3.28	9.51	9	0.16	0.08	24	5	20.8
August	3.2	2.1	0.82	1.90	6.08	5	27.53	33.57	30	5	16.7
September	3.1	2.2	0.64	2.34	7.25	6	19.26	30.09	28	6	21.4
October	2.8	1.0	0.40	1.73	4.84	4	7.33	18.33	15	4	26.7
November	2.5	0.0	0.04	3.25	8.13	6	2.00t	50.00	12	5	41.7
December	2.1	0.0	0.00	2.75	5.78	1	----	---**	4	1	25.0
Totals	2.8	1.0	0.65	2.54	7.11	31	11.26	17.32	113	26	23.0

a/ Number frames with bees (1 frame = 1,300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames capped honey (1.00=560 cm^2 capped honey).
d/ On workers and drones.
e/ Number lice, on workers and drones, on one frame multiplied by number frames bees in nuc.
f/ Equivalent number tunnels per 1 frame capped honey.
** Not recorded.
t Average for 11-1-76 only.

APPENDIX XI. Summary of colony examinations of 5 nucs, each with 50 added lice, established August, 1976.

Month	Average Colony Strength			Average Adult Lice			Average Louse Larval Tunnels		Queens		
	Bees[a/]	Brood[b/]	Honey[c/]	No. on 1 Frame[d/]	No. in Colony[e/]	Total No. on Queens	No. Ob-served	Per Frame[f/]	Total Ob-served	Total with Lice	% with Lice
August	1.1	1.0	1.80	5.50	6.05	38	0.00	0.00	7	5	71.4
September	1.4	0.9	1.80	4.52	6.33	34	0.52	0.29	21	12	57.1
October	1.7	0.8	1.48	3.38	5.75	12	1.31	0.89	11	6	54.5
November	1.5	0.1	0.78	4.33	6.50	11	0.33t	0.42	12	5	41.7
December	1.1	0.0	0.60	2.67	2.94	1	---**	----	2	1	50.0
Totals	1.4	0.6	1.29	4.08	5.71	96	0.54	0.42	53	29	54.7

a/ Number frames with bees (1 frame = 1,300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames capped honey (1.00 = 560 cm^2 capped honey).
d/ On workers and drones.
e/ Number lice, on workers and drones, on one frame multiplied by number frames bees in nuc.
f/ Equivalent number tunnels per 1 frame capped honey.
** Not recorded
t Average for 11-1-76 only.

APPENDIX XII. Summary of colony examinations of 6 nucs, each with 50 added lice, established September, 1976.

Month	Average Colony Strength			Average Adult Lice			Average Louse Larval Tunnels		Queens		
	Bees a/	Brood b/	Honey c/	No. on 1 Frame d/	No. in Colony e/	Total No. on Queens	No. Ob- served	Per Frame f/	Total Ob- served	Total with Lice	% with Lice
September	2.3	0.5	1.83	7.50	17.25	3	0.33	0.18	10	1	10.0
October	2.1	0.8	1.37	4.22	8.86	2	0.94	0.69	15	2	13.3
November	1.7	0.3	1.23	5.14	8.74	16	1.30t	1.06	13	9	69.2
December	1.4	0.0	1.14	4.75	6.65	1	---**	----	4	1	25.0
Totals	1.9	0.4	1.39	5.40	10.26	22	0.86	0.62	42	13	31.0

a/ Number frames with bees (1 frame = 1,300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames capped honey (1.00 = 560 cm^2 capped honey).
d/ On workers and drones.
e/ Number lice, on workers and drones, on one frame multiplied by number frames bees in nuc.
f/ Equivalent number tunnels per 1 frame capped honey.
** Not recorded.
t Average for 11-1-76 only.

APPENDIX XIII. Summary of colony examinations of 6 nucs, without added lice, established September, 1976.

Month	Average Colony Strength			Average Adult Lice		Average Louse Larval Tunnels	Queens	
	Bees[a/]	Brood[b/]	Honey[c/]	Number on 1 Frame[d/]	Number in Colony[e/]	Number Observed	Total Observed	Total with Lice
September	2.3	0.5	2.00	0.08	0.18	0.00	11	0
October	2.2	0.9	1.81	0.11	0.24	0.00	13	0
November	2.1	0.4	1.19	0.20	0.42	0.00t	13	0
December	1.7	0.0	0.81	0.20	0.34	---**	4	0
Totals	2.1	0.5	1.45	0.15	0.32	0.00	41	0

a/ Number frames with bees (1 frame = 1300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames capped honey (1.00 = 560 cm^2 capped honey).
d/ On workers and drones.
e/ Number lice, on workers and drones, on 1 frame multiplied by number frames bees in nuc.
** Not recorded.
t Average for 11-1-76 only.

APPENDIX XIV. Summary of colony examinations of 6 nucs, each with 50 added lice, established April, 1977.

Month	Average Colony Strength			Average Adult Lice			Average Louse Larval Tunnels		Queens		
	Bees[a/]	Brood[b/]	Honey[c/]	No. on 1 Frame[d/]	No. in Colony[e/]	Total No. on Queens	No. Ob- served	Per Frame[f/]	Total Ob- served	Total with Lice	% with Lice
April	1.9	1.0	0.67	8.61	16.36	0	0.00	0.00	6	0	0.0
May	2.7	1.8	0.62	4.78	12.91	5	96.83	156.18	29	4	13.8
June	3.5	2.5	0.51	1.33	4.65	1	177.88	348.78	18	1	5.6
July	3.8	3.0	0.55	0.50	1.90	0	88.72	161.31	11	0	0.0
Totals	3.0	2.1	0.59	3.81	11.43	6	90.86	53.61	64	5	7.8

a/ Number frames with bees (1 frame = 1300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames capped honey (1.00 = 560 cm^2 capped honey).
d/ On workers and drones.
e/ Number lice, on workers and drones, on 1 frame multiplied by number frames bees in nuc.
f/ Equivalent number tunnels per 1 frame capped honey.

APPENDIX XV. Summary of colony examinations of 6 nucs, without added lice, established April, 1977.

Month	Average Colony Strength			Average Adult Lice	Average Louse Larval Tunnels		Queens	
	Bees a/	Brood b/	Honey c/	Number on 1 Frame d/	No. Observed	Per Frame f/	Total Observed	Total with Lice
April	1.5	0.8	0.33	0.00	0.00	0.00	10	0
May	2.3	1.6	0.37	0.00	0.36	0.97	30	0
June	3.0	2.5	0.30	0.00	0.25	0.83	20	0
July	3.6	3.2	0.25	0.00	0.20	0.80	9	0
Totals	2.6	2.0	0.31	0.00	0.20	0.65	69	0

a/ Number frames with bees (1 frame = 1,300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames capped honey (1.00 = 560 cm^2 capped honey).
d/ On workers and drones.
f/ Equivalent number tunnels per 1 frame capped honey.

APPENDIX XVI. Summary of colony examinations of 3 nucs, each with 50 added lice, established May, 1977.

	Average Colony Strength			Average Adult Lice		Average Louse Larval Tunnels		Queens	
Month	Bees[a/]	Brood[b/]	Honey[c/]	No. on 1 Frame[d/]	No. in Colony[e/]	No. Observed	Per Frame[f/]	Total Observed	Total with Lice
May	2.3	1.0	1.00	5.67	13.04	8.67	8.67	7	0
June	3.1	2.4	0.60	1.83	5.67	135.00	225.00	12	0
July	3.9	3.1	0.24	0.44	1.72	58.33	243.04	9	0
Totals	3.1	2.2	0.61	2.65	8.22	67.33	110.38	28	0

a/ Number frames with bees (1 frame = 1,300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames capped honey (1.00 = 560 cm^2 capped honey).
d/ On workers and drones.
e/ Number lice, on workers and drones, on one frame multiplied by number frame bees in nuc.
f/ Equivalent number tunnels per 1 frame capped honey.

APPENDIX XVII. Summary of colony examinations of 3 nucs, without added lice, established May, 1977.

Month	Average Colony Strength			Average Adult Lice		Average Louse Larval Tunnels		Queen	
	Bees[a/]	Brood[b/]	Honey[c/]	Number On 1 Frame[d/]	Number in Colony[e/]	Number Observed	Per Frame[f/]	Total Observed	Total With Lice
May	1.7	1.0	1.23	0.08	0.14	0.00	0.00	8	0
June	2.6	1.6	0.80	0.00	0.00	0.33	0.41	12	0
July	3.3	2.7	0.47	0.00	0.00	0.78	1.66	9	0
Totals	2.5	1.8	0.83	0.03	0.08	0.37	0.45	29	0

a/ Number frames with bees (1 frame = 1,300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames capped honey (1.00 = 560 cm^2 capped honey).
d/ On workers and drones.
e/ Number lice, on workers and drones, on one frame multiplied by number frames bees in nuc.
f/ Equivalent number tunnels per 1 frame capped honey.

APPENDIX XVIII. Summary of colony examinations of 2 nucs, each with 50 added lice, established June, 1977.

Month	Average Colony Strength			Average Adult Lice		Average Louse Larval Tunnels		Queens	
	Bees[a/]	Brood[b/]	Honey[c/]	No. On 1 Frame[d/]	No. in Colony[e/]	No. Ob-served	Per Frame[f/]	Total Ob-served	Total With Lice
June	2.3	0.7	0.94	7.00	16.10	0.00	0.00	4	0
July	1.6	0.3	0.80	4.34	6.94	0.17	0.21	4	0
Totals	2.0	0.5	0.87	5.67	11.34	0.09	0.10	8	0

a/ Number frames with bees (1 frame = 1,300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames capped honey (1.00 = 560 cm^2 capped honey).
d/ On workers and drones.
e/ Number lice, on workers and drones, on one frame multiplied by number frames bees in nuc.
f/ Equivalent number tunnels per 1 frame capped honey.

APPENDIX XIX. Summary of colony examinations of 2 nucs, without added lice established June, 1977.

Month	Average Colony Strength			Average Adult Lice	Average Louse Larval Tunnels		Queens	
	Bees[a/]	Brood[b/]	Honey[c/]	Number on One Frame[d/]	Number Observed	Per Frame[f/]	Total Observed	Total with Lice
June	2.1	1.0	0.87	0.00	0.00	0.00	6	0
July	2.3	1.9	0.59	0.00	0.50	0.85	5	0
Totals	2.2	1.5	0.73	0.00	0.25	0.34	11	0

a/ Number frames with bees (1 frame = 1,300 bees).
b/ Number frames containing brood (Counted as the number of half frames containing any amount of brood).
c/ Number frames capped honey (1.00 = 560 cm^2 capped honey).
d/ On workers and drones.
f/ Equivalent number tunnels per 1 frame capped honey.

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