Greater Wax Moth:[1] Behavior of Larvae[2,3]

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ABSTRACT

Ann. Entomol. Soc. Am. 72: 811–815 (1979)

Larval behavior of *Galleria mellonella* (L.) was observed in apiaries of the honey bee, *Apis mellifera* L., and in the laboratory. The 1st instars fed on honey, and then dispersed to all colonies of the apiary where they infested brood combs, primarily in areas of unsealed brood or pollen. Larvae tunneled into cell walls until bees capped their brood, and then fed in the capped cells. After the bees emerged, worker bees captured moth larvae as cells were cleaned and repaired. Small captured larvae were eaten by bees; larvae that escaped capture until late instars were removed from the colony. Bees tried to sting large larvae, but larvae were resistant to penetration of the sting.

Larvae of the greater wax moth, *Galleria mellonella* (L.), infest nearly all colonies of the honey bee, *Apis mellifera* L., but female moths deposit eggs in only a few colonies (Nielsen and Brister 1977). Paddock (1918) observed 1st instars in colonies, and described their activity as they burrowed from the outer surface of the cells to the midrib of the comb. However, little was known about how colonies that were not visited by adults became infested with larvae.

We report observations of wax moth larvae and the role of larval behavior on colony infestation.

Materials and Methods

Eclosion

Laboratory observations were made on larvae from both the laboratory strain of moths used by Nielsen and Lambremont (1976) and from the resident population of Louisiana. Field observations were made on larvae of the resident population. To obtain larvae of the same age for use in tests, we placed enough clusters of hatching eggs into a vial to obtain the desired number of larvae that were within 15 min of being the same age. Observations of small larvae were made with a dissecting microscope.

Feeding Behavior

Fourteen groups of 0- to 8-h-old larvae were observed on dark brood combs that contained all stages of honey bee brood, pollen, and honey. Group size was 10–200 larvae; temperature was 30°–35°C. Larvae were confined to a part of the comb by a circle of petroleum jelly, ca. 15 cm diam. Nine groups were observed continuously until more than 80% of the larvae had burrowed into the comb, and 5 groups were observed continuously until all larvae entered the comb.

Observations were also made in 3-dram (11.1 ml) vials, and in 3.79-liter (gallon) jars.

Tunnel Construction

The place where tunnels were started was tested by allowing 2000 first instars from the resident strain and the same number from the laboratory strain to burrow into dark brood combs containing ca. equal-sized areas of honey, pollen, sealed brood, and unsealed brood. Small areas of the combs contained empty cells. Tunnels started on the edge of the comb between a cell of unsealed brood and an empty cell or between unsealed brood and a cell with pollen were classified as being in the area of unsealed brood.

From 53 bee colonies, 4 were randomly selected for observations of tunnel construction by resident-strain larvae in combs of active bee colonies. Tunnels by larvae 1–10 days old were studied in a comb that was removed from each selected colony for ca. one h to allow infestation of ca. 2000 first instars in each comb. Combs were sampled daily for 10 days by randomly removing 2 combs, and a 3-cm^2 piece of comb was cut from each and placed at -18°C to kill larvae and halt tunnel construction. After all samples were taken, they were cut and pulled apart to observe tunnel construction.

From the 4 selected colonies, 3 dark brood combs were removed from each colony, numbered, infested with ca. 2000 first instars/comb, and returned to the colonies. When larvae were 10–28 days old, 2 combs were randomly sampled daily by removing a 3-cm^2 piece of comb. Samples were stored at -18°C until all samples were taken, then were cut and pulled apart to observe tunnel construction.

Dispersal

Observations on dispersal of larvae include: movement through plastic tube; barriers on plywood sheets; infestation of larvae-free colonies; and infestation of vials.

Movement Through Plastic Tube—A device consisting of two 3-dram cotton-stoppered vials connected by a plastic tube (ID 3–4 mm) with a clamp near one vial was used to test movement of 1st instars. Each vial contained dark crushed brood comb, pollen, and honey. Tube length between the vials was 10 m. Fifteen replicates were used to test the time required for the 1st larvae to reach the 2nd vial. In each test, ca. 1000 hatching eggs were placed in the vial near the clamp. Ca. one h later, the clamp was loosened, and larvae were allowed to move through the tube.

We used 25- and 50-m tubes between the 2 vials to determine distances that 1st instars can move. When the 50-m tube was used, either vial was placed in a chamber with a slight vacuum so that air flowed slowly through the tube.

Barriers on Plywood Sheets—Thirteen selected colonies were each placed on a 1.2 × 2.4-m sheet of plywood to determine if larvae move between colonies or if eggs are deposited in or on most colonies. Ten of the

[1] Lepidoptera: Pyralidae.

[2] In cooperation with the Louisiana Agric. Exp. St. Received for publication Feb. 26, 1979.

[3] Mention of a proprietary product in this paper does not constitute an endorsement of the product by the USDA.

colonies were selected because no moths were observed entering them during 10 nights of observations, and 3 colonies were selected because 7–12 ♀ moths visited them each night. For 5 days, the surface of the plywood was periodically examined for larvae. No larval barrier was used the 1st day, but on the 2nd day a petroleum jelly barrier was placed around the edge of each plywood sheet to prevent larvae from entering or leaving. Observations on the 2nd day were not started until 4 h after the barrier was in place to allow time for 1st instars present in a colony to burrow into the comb. Thus, any observed larvae would be from eggs deposited on or in the enclosed colony.

Infestation of Larvae-Free Colonies.—Bee colonies free of moth larvae were placed in an apiary during the day to determine if colonies became infested by larval movement during the day when moths were not active. Hives were held at -18°C for 48 h to kill moth eggs or larvae present and were kept within a petroleum jelly barrier to prevent larval infestation prior to the test. Each hive contained honey and pollen in 4 dark 13 × 19-cm combs. Bees were made larvae-free by placing them in a clean colony for 24 h to allow any transferred larvae to burrow into the comb. A queen and enough bees to cover the 4 combs were transferred to each of 10 larvae-free hives, and bees were confined during the test. After colonies contained bee eggs and unsealed brood, they were placed at least 3 m from any resident colony in the apiary from 0800 to 1600 h for 4 days. A barrier of petroleum jelly was placed around each of 5 larvae-free control colonies, and a test colony without a barrier was placed near each control. After 4 days in the apiary, bees were removed, and hives were incubated at 33°–34°C for 22 days. Moth larvae were hand picked from combs and counted as they developed enough to be found.

Infestation of Vials.—Three-dram vials containing crushed comb, pollen, and a drop of honey were placed in the apiary during the day to further test larvae movement. Vials were closed with a cotton stopper and held at -18°C for 24 h to kill eggs or larvae prior to testing. Cotton stoppers were removed, and a ring of petroleum jelly was placed on the outside of 250 control vials, but not on the 250 test vials. Vials were then placed at least 3 m from any of 54 standard-size bee colonies in the apiary. A control vial was placed within 2 cm of each test vial. After 8 h (at 1600 h) the cotton stopper was placed on each vial, and vials were examined for moth larvae. Vials were incubated for 14 days at 33°–34°C and then examined for presence of larvae missed in the previous examination.

Honey Bee Colony Defense

Interaction of honey bees and moth larvae was studied by observations in large standard-size bee colonies and in 4 heavily infested observation hives. Larvae of various ages were placed in the hives and response of bees was observed.

In 20 large colonies, 10 groups of 10 late-stage larvae were placed inside the top of each colony, and larvae were observed and counted as they were removed from the hive by the bees. Fifty larvae removed from each colony were placed in vials and observed to determine mortality.

Wax Moth Strain Differences

Resident and laboratory strains were studied for the need of honey in the diet. Four diets were tested for the number of adults that developed from 15-mg eggs (ca. 475 larvae)/replicate, 10 replications. The diets included: crushed dark comb, pollen, and honey; crushed dark comb and pollen; artificial medium (Dutky et al. 1962); and artificial medium and honey. Treatments that contained honey had only a small drop of honey that was provided for newly emerged larvae.

Differences in where tunnels were started were observed by allowing 2000 larvae from each strain to burrow into brood combs containing honey, pollen, and all stages of brood.

The device described above, consisting of two 3-dram vials connected by a 10-m tube, was used to test temperature preference of larvae. One vial was placed at 22° and the other at 32°C, and ca. 475 (15 mg) hatching eggs were placed in either vial for 10 replications in each for each strain. For 5 days larvae were allowed to select the desired temperature.

Results

Eclosion

Newly emerged larvae moved very little for ca. 10–20 min and then, by running on their true legs, they moved rapidly over combs or in vials to which they were confined. Young 1st instars were found in 53 or 54 observed bee colonies.

Feeding Behavior

Larvae 10–20 min old moved over the comb and sometimes stopped at the bottom of cells containing the larvae, but it could not be determined if they fed on liquid food in those cells. Within 15–30 min, they stopped at open cells of honey or drops of honey and fed for 10–30 min. After feeding, they moved over the comb, sometimes stopping to feed on pollen. When larvae were ca. 2 h old they again fed on honey for 5–10 min. When honey was available, larvae always fed at least twice on honey and often fed on pollen before tunnelling into the comb.

Newly emerged larvae in vials fed similarly to those placed on comb if honey was available. The 1st day larvae fed in gregarious groups between the food and the vial, but by the 2nd day they started to form individual tunnels. As larvae developed, the tunnels which were lined with silk, particles of food, and feces were enlarged.

After larvae had fed for ca. 1 day, they did not move readily to different areas. Vials with cotton stoppers removed were placed in 3.79 liter jars containing larval food, and very few larvae moved out of the vials though plenty of food was just 1 cm away. Larvae developed in the vials until they were crowded, and many were cannibalized before the remaining larvae moved 1 cm to food outside the vial. However, when food from the jar was placed next to food and larvae in the vials, the larvae moved out of the vials and into the jar.

Cannibalism started when larvae were crowded enough that larvae could not enlarge their tunnels without disturbing the tunnel of another larva. Cannibalism did not

occur in less crowded conditions. Cannibalistic larvae grew very fast and sometimes became aggressive enough so they would attack larvae of any size.

When 10–15 days old, larvae moved out of the vial and into the 3.79 liter jars where they made tunnels between the food and the jar. In crowded conditions, larvae extended their tunnels upward on the side of the jar as much as 5 cm above the food and stayed in the tunnels above the food except when feeding. When larvae finished feeding, they moved away from the food to spin cocoons.

Tunnel Construction

Tunnels were generally started at the edge of the comb in the walls of open cells containing bee eggs or unsealed bee larvae or in open cells of pollen that were close to bee brood. Only 5 of 2000 larvae started tunnels in areas of honey, sealed brood, or empty comb.

It required 6–8 days for larvae to extend their tunnels to the mid-rib of the comb, and during this time they pushed small mounds of debris from the tunnel entrances. Mounds could be observed at tunnel entrances as soon as brood combs were removed from colonies, but more mounds developed during a few hours that combs were kept away from the cleaning activities of bees.

As bee larvae developed, bees capped their cells, and as they did so they remolded the wax at the edge of the cells, closing the entrance of tunnels before the moth larvae reached the center of the comb. When this occurred, larvae made holes into the capped cells where they fed until the bees emerged.

Moth larvae sometimes deposited enough silk and debris around abdomens of bee pupae so that when bees became adults they could not emerge from their cells. Groups of young adult bees were found trapped on many combs, but this was most evident in the 1st cycle of brood reared in newly made combs. When comb foundation was placed in a brood nest, 1st instars were attracted to the comb as it was being built, resulting in trapped adults of the 1st cycle of brood. However, after the 1st cycle of brood was reared, larvae were again well-distributed in combs containing brood.

From areas of capped brood, larvae moved outward, cutting holes and making silk-lined tunnels across the comb. Tunnels were made within a few hours if bees were removed from brood combs, and they were occasionally found on combs that were in strong colonies. In one very strong colony, a 7 cm long tunnel was found in a part of comb that contained bee eggs and bee larvae that were less than 2 days old. One h later the tunnel had been removed and the comb was repaired. Tunnels served to protect larvae from bees, allowing them to move back into protected areas when disturbed.

Full-grown larvae were found in tunnels across the top of capped pupae. Larvae in the tunnels caused the caps to be slightly raised in a straight line for several centimeters. Bee pupae were not disturbed enough to prevent their development.

Dispersal

Movement Through Plastic Tube.—In the 15 replications in which 1st instars were allowed to move through a 10-m tube between 2 vials containing food, the 1st larvae reached the 2nd vial in a mean of 16.53 min, SD 1.26, range 14.1–19. Larvae did not always travel in one direction but some moved through the 1st part of the tube as fast as 90 cm/min.

Tube length between the 2 vials containing larval food was increased from 10 to 25 and 50 m to determine distances that 1st instars move. Larvae moved readily through the 25-m tube, and no mortality occurred. Larvae also found their way through the 50-m tube, but larvae changed direction less often, and less mortality occurred when a light flow of air was passed in either direction through the tube.

Barriers on Plywood Sheets.—Observations of larvae on 13 plywood sheets where selected bee colonies were placed indicated that 1st instars moved between colonies in an apiary. During the 1st day before the larval barrier was placed around each colony, larvae were observed running on every plywood sheet. After the larval barrier was in place, no larvae were observed at any time near 9 of the 10 colonies that did not appear to attract moths. However, on the plywood near 1 of the 10 colonies, 1, 8, and 2 larvae were observed on the 2nd, 3rd, and 4th days, respectively. Thus, a few eggs must have been deposited on or in the hive though no moths were observed near the colony. After the barrier was in place, larvae were too numerous to count on the plywood near the 3 colonies that 7–12 moths entered each night. Movement of larvae on the plywood was stopped by heavy dew or rain and was inhibited or stopped by heat from direct sunlight. Larvae move freely in shaded areas and in direct sun when the plywood surface was not hot.

Infestation of Larvae-Free Colonies.—All test colonies that were made free of moth larvae became infested when placed in the apiary during the day when moths were not active. The 5 test colonies became infested with 29, 40, 51, 55, and 67 moth larvae ($\bar{x}$ = 48.4, SD = 14.52) compared with no moth larvae in the 5 control colonies.

Infestation of Vials.—In field tests, a total of 38 greater wax moth larvae infested vials containing crushed comb, pollen, and honey. Larvae entered the vials during the 8 h that 250 test vials and 250 control vials were open when vials were at least 3 m from any colonies in the apiary. On the 1st examination, 37 first instars were found, and all were in the test group. On the 2nd examination 14 days later, another larva was found in the test group that had not been detected earlier, and 3 found earlier had died. No larvae were found in the control vials.

Honey Bee Colony Defense

The 1st evidence of colony defense was when young bees emerged from their cells exposing larvae that were developing in the capped cells. Worker bees captured larvae as the cells were cleaned and repaired. Captured larvae ruptured and were eaten by the bees.

The cuticle of large larvae was tough and resistant to bee stings when larvae were captured by bees. During the attack, the larva twisted from side to side as numerous bees tried to sting it on either end, or tried to drag it from the hive. All 2000 late-stage larvae placed in the tops of 20 large colonies were removed by the bees less

than 4 min after they were placed in the hives. Every larva (100) removed from one colony contained at least one sting, and one larva contained 7 stings. From the other 19 colonies a total of 23 larvae contained stings, and they were removed from 5 colonies. Thus 14 colonies did not sting any larvae. Of the 1000 larvae (50 from each colony) observed for mortality after being removed from colonies by bees, 6 survived to adulthood. Three of the 6 that survived had been removed from the same colony, and the other 3 larvae each came from a different hive. All that survived came from colonies that did not sting larvae.

Wax Moth Strain Difference

The laboratory strain has been reared for ca. 230 generations on artificial diet, and it survived equally well on all diets tested (Table 1).

The resident strain survived well on crushed dark comb pollen and honey (Table 1). The number that developed on artificial medium and honey was nonsignificantly less than the number that developed from the natural diet. A diet of crushed dark comb and pollen resulted in significantly fewer adults than a diet that contained honey, and more females developed than males. Few adults developed on artificial medium, and the number of females was ca. twice that of males (Table 1).

When 2000 larvae from each strain were allowed to burrow into brood combs, 32% of laboratory-strain larvae started tunnels in pollen cells compared to 9% of resident strain larvae.

Strain differences to temperature were found by using the device of 2 vials connected by a 10-m tube. When one vial was placed at 22° and the other at 32°C, the 475 larvae placed in either vial moved into both vials and within a day they selected the desired temperature for development. Resident larvae always chose 32°C to start development but in 10 replications, 2.1–3.9% ($\bar{x}$ 3.1%) of laboratory-strain larvae chose 22°C.

Discussion

Our studies confirmed many of the observations made by Paddock (1918), but there were some exceptions. He stated that the 1st meal consisted of wax as larva tried to gain entrance to the comb, but we found that the 1st meal was honey and that survival of resident population larvae depended greatly on honey. A general statement was made that larvae avoided light, but we found that even direct sunlight did not inhibit movement of 1st instars. Paddock also reported that larvae reached the midrib in 4–8 days compared with our observations of 6–8 days, but this is a minor difference that could be accounted for by differences in combs.

Because bees in strong colonies destroy nearly all wax moth larvae in the hives, any mechanism that would cause selective infestation of weak colonies or that would insure infestation of all bee colonies would be of considerable importance to the population level and survival of the moth. Adults did not locate weak colonies or randomly visit colonies, but instead 7–12 ♀ visited a few strong colonies each night, and other colonies seemed to be avoided (Nielsen and Brister 1977). If larvae did not disperse, adult moths could develop in only a few colonies. Dispersal of larvae makes adult development possible in every colony.

Beekeepers have reported heavy loss of colonies to the greater wax moth during long periods of rain. We found that rain stopped dispersal of larvae and may result in so many larvae in colonies where eggs were deposited that they overwhelm the colonies.

It is not known how dispersing larvae find the colonies or how much mortality occurs in the process. If dispersal were random, it would seem that mortality would be considerable. However, Paddock (1918) observed that larvae removed from a comb seem lost for a time, but then invariably proceed in the direction of the comb.

Dispersal of larvae and preference of larvae for warm comb to start their tunnels gives new insight on how comb becomes infested. Paddock (1918) theorized that strong bee colonies would drive moths out, causing them to infest comb left unprotected by bees, but decoy boxes left in an apiary for 3 months did not become infested with larvae. We now know that moths would not be expected to visit decoy combs (Nielsen and Brister 1977) and that even if moths were confined to decoy combs, infestation by larvae would not have occurred unless temperatures were sufficiently high to make the combs suitable for 1st instars to become established. As long as bee colonies were close, 1st instars would migrate to colonies where they could find suitable temperatures in the brood combs.

Knowledge of larval movement and selection of warm temperatures for development may have usefulness in protecting stored comb from reinfestation by larvae after combs were fumigated. A bee colony having an outside

Table 1.—Mean number of greater wax moth adults reared from 15-mg eggs placed on various diets, 10 replications.

	Larval Strain							
	Resident				Laboratory			
Larval food	♂	SD	♀	SD	♂	SD	♀	SD
Crushed dark comb, pollen, and honey[a]	196.2	14.8	190.5	15.2	195.3	7.4	191.4	7.4
Crushed dark comb and pollen	67.1[b]	16.1	82.9[b]	17.5	192.4	8.7	191.6	8.4
Artificial medium	3.1[c]	1.0	6.3[c]	1.5	194.5	6.8	193.6	4.9
Artificial medium and honey[a]	178.7	15.1	184.0	10.0	197.0	3.7	195.4	5.1

[a] Honey was provided only as a single drop for newly emerged larvae.
[b] Significant differences ($P < 0.05$) from diets containing honey.
[c] Significant differences ($P < 0.05$) from all other diets.

entrance would do very well in a stored-comb room that was kept at ca. 22°C. The 1st instars that may develop would likely find the stored comb unsuitable and migrate to the colony. As larvae developed, bees would destroy nearly all larvae.

REFERENCES CITED

Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1962. A technique for mass rearing the greater wax moth (Lepidoptera: Galleridae). Proc. Entomol. Soc. Wash. 64: 56–8.

Nielsen, R. A., and D. Brister. 1977. The greater wax moth: Adult behavior. Ann. Entomol. Soc. Am. 70: 101–3.

Nielsen, R. A., and E. N. Lambremont. 1976. Radiation biology of the greater wax moth: Inherited sterility and potential for pest control. USDA Tech. Bull. 1539, 31 pp.

Paddock, F. B. 1918. The beemoth or waxworm. Texas Agric. Exp. Stn. Bull. 231, 38 pp.