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AVOIDING *PSEUDOHYPOCERA* ATTACKS (DIPTERA: PHORIDAE) DURING THE ARTIFICIAL PROPAGATION OF *MELIPONA BEECHEII* COLONIES (HYMENOPTERA: APIDAE: MELIPONINI)

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ABSTRACT. A serious problem in culturing stingless bees is the destruction of the colonies by phorid flies (*Pseudohypocera* spp.). This destruction was prevented in 12 new colonies of *M. beecheii* (six mother-restoring and six daughter colonies derived from six original-mother colonies) placing each one in a 50 x 35 x 45 cm. mosquito net cage immediately after their formation. Derived-daughter colonies were made by taking 400 to 500 brood cells, about half of both the adult bees, and stored food from one former-mother colony. The remaining brood, food, cerumen and bees from this as well as the queen were taken to a new hive and became a restoring-mother colony. The two kinds of colonies were freed from their cages when phorid flies were not observed in the neighborhood. Thus restoring mother colonies and derived-daughter colonies were freed from their cages 24 hours and 48 hours respectively after their formation. 21 days later all colonies had a queen (even the derived colonies which started without a queen), new combs, new food pots, and their involucrums were partly rebuilt. Natural foraging was reinforced with syrup supplies. 73 days after colony duplication, there were no significant differences in weight gain between the restored original colonies and new colonies derived from them. Most importantly, no phorid fly infestations occurred, which shows that the vulnerable period is in the first days of the starting of new colonies. The use of mosquito netting for the first two days is therefore a reliable method for protecting new *M. beecheii* hive colonies from phorid fly infestations.
KEY WORDS: Phoridae flies; *Melipona* stingless bees; colony duplication.

RESUMEN. Un serio obstáculo para la cria de abejas sin aguijón, es la destrucción de las colonias por el díptero *Pseudohypocera* spp. Esta destrucción se evitó en 12 colonias nuevas de *M. beecheii* (seis colonias madres en recuperación y seis colonias hijas derivadas de seis colonias madres originales) colocando cada una en una jaula de malla mosquitera de 50 x 35 x 45 cm inmediatamente después de su formación. Las colonias derivadas o "hijas" se formaron con 400 a 500 celdas de cría aproximadamente, la mitad de las abejas adultas y los alimentos almacenados de la colonia madre original. La cría, alimentos, cerumen y abejas restantes, y la reina, se llevaron a una nueva colmena para constituir una colonia madre en recuperación. Las dos clases de colonias se liberaron de sus jaulas cuando ya no se observaron dípteros *Pseudohypocera* rondando en las proximidades. Consecuentemente, las colonias madres e hijas se liberaron de sus jaulas a las 24 y 48 horas después de su formación, respectivamente. 21 días más tarde, todas las colonias tenían reina (aún las iniciadas sin ella), nuevos panales, nuevas ollas de alimento, y sus involucros

estaban reconstruidos parcialmente. El pecoreo natural se reforzó suministrando jarabe. 73 días después de la duplicación, no había diferencias significativas de ganancia de peso entre las colonias madres en recuperación y las colonias hijas. Y, más importante aún, no hubo infestación de fóridos, lo que demuestra que que el período vulnerable de las colonias ocurre durante los primeros días de su desarrollo. En consecuencia, el uso de la tela mosquitera es un método confiable para proteger de las infestaciones de fóridos las nuevas colonias de *M. beecheii*.
PALABRAS CLAVE: Fórido; *Melipona*; abejas sin aguijón; duplicación de colonias.

The eusocial stingless bee *Melipona beecheii* Bennett is still raised to produce honey and beeswax (Hendrichs, 1941; Murillo, 1984). It is distributed over most of the tropical and subtropical regions of North and Central America (Roubik, 1989). Its northern limits in Mexico are the coastal southern regions of the States of Sinaloa and Tamaulipas and it has also been reported as far south as Costa Rica (Sommeijer, 1999).

Within this geographic range *M. beecheii* has been used since pre-Hispanic times for the production of honey and beeswax because of its docility (it does not sting), and a honey harvest superior to that of other local stingless bee species. In Yucatan, Mexico, first the ancient Maya, and later their modern descendants developed honey-production based mainly on *M. beecheii* (Villanueva and Colli-Ucan, 1996).

In spite of these commercial antecedents and some later technical developments, *M. beecheii* honey production has been steeply declining since the middle of the 20th century (Calkins, 1975). At present, *Melipona* honey has lost its markets to cane sugar and *Apis mellifera* honey, to the extent that it is now used only as a connoisseurs' delicacy or as the primary ingredient of homemade folk remedies.

At present, most *M. beecheii* honey comes from domestic log or boxed colonies, themselves obtained from wild nests (Veen and Arce, 1999; González, 1992; Sommeijer, 1999), and some from wild colonies as it is still observed in Pie de Cuesta, Guerrero, Mexico (pers. obs.).

Traditionally Maya beekeepers produced new *M. beecheii* colonies by dividing domestic colonies. They took out old brood combs and some food pots from a "mother" or donor colony and placed them in the log hive destined for the new or "daughter" colony. They also left all young brood, the laying queen, and remaining food pots in the "mother" colony. Finally, adult bees were partitioned between the mother and daughter colonies. Mud "plastering" of the new logs was used as a means to prevent attack by phorid flies. (Schwarz, 1948; Nogueira-Neto, 1997). If more brood were necessary, comb fragments from other colonies might be borrowed (Weaver and Weaver, 1981).

Another more modern method uses queens produced and mated in the laboratory. Each queen is placed with a few workers in a small box before being transferred to a normal hive (Aidar, 1995; Camargo, 1976). However this method is more difficult and requires special training.

Independently of the method used, recently set-up colonies are always vulnerable during the first few days to deadly attacks of the phorid *Pseudohypocera,* an endemic fly of the Neotropics. Attracted by pollen or larval food odors, this small fly invades stingless bees' nests and its larvae destroy the new colony by feeding on the bee's brood and pollen stores (Brown, 1993; Sommeijer, 1999).

In addition to phorid fly attacks during artificial propagation and honey harvesting, progressive regional deforestation is accelerating the reduction in the number of colonies in the stingless bee yards of most locations (Murillo, 1984).

It is therefore important to devise a simple and practical method to protect newly started *M. beecheii* colonies from phorid attacks. It is believed that the present study develops such a method.

MATERIALS AND METHODS

Six *M. beecheii* colonies were used for artificial duplication to test a method that would protect the resulting twelve colonies from phorid fly attack. The former six ones had been housed in wooden hives of eight liters (20 x 20 x 20 cm) similar to those proposed by Portugal-Araujo (1977). All of them had been lodged in the *Melipona* experimental bee yard at Cacahoatan, Chiapas, Mexico.

On February 17 1999, the colonies were moved to a stingless bee yard in Tapachula. Each hive being duplicated was enclosed in a 2.5 X 1.5 X 2.7 m mosquito net compartment inside a shaded room to confine the adult bees and allow them to be handled easily.

New clean hives of eight liters in volume lodged each of the 12 resulting colonies: six "restoring-mother" colonies and six "daughter" or "derived" colonies. Each hive was prepared by placing on the floor a mobile wooden board laid on several small cerumen (beeswax and resin mixture made by the bees) balls that would support the combs, brood, and food pots. Entrances were surrounded with cerumen strips, and the cracks and joints between brood chamber and hive floor were covered with adhesive tape. Finally, a 19-mm (3/4 in) hole was drilled at a roof corner for later use and covered with a plastic liner. This hole allows the future introduction of a plastic syrup feeder without unduly disturbing the colony.

When a colony chosen to be duplicated was opened, the worker bees escaped from the hive but remained confined within the mosquito-netting compartment. The young hive bees remained upon the hive combs. The short cerumen columns that bound the nest (combs enveloped in the involucrum) and pots that held the food (honey and pollen) to the hive walls were cut. As soon as the queen was found, she was gently captured and placed in a small, ventilated cage. If any comb surface or pot walls were soiled with spilled honey or pollen, they were washed with a domestic sprayer to avoid attracting phorids. Then, the mature combs, pot clusters and involucrum layers of cerumen were separated by means of a sharp knife into two similar portions.

Each of the derived or "daughter" colonies was formed with three or four old combs (400 to 500 cocoons), about half of the food pots, some layers of the cerumen involucrum and about half of the adult bees of the mother colony. These parts were arranged on a wooden platform supported by little balls of cerumen placed on the floor of a hive prepared as described above.

To each of previously prepared hives, the remaining components of the donor or "mother" colony were transferred: the physogastric queen (inseminated-laying queen), remaining worker bees, the whole young combs, half of the food-pot clusters, and some cerumen layers of the involucrum. Similarly, any spilled honey or pollen was carefully washed away. These colonies would serve as controls. Sealing the joints between the roof and the hive body with adhesive tape terminated the process of division.

In order to avoid entrance of phorids (*Pseudohypocera*), whose larvae eat stingless bees' brood, the resulting colony was enclosed in a mosquito net cage before being carried it to the stingless bee yard in the 'Colegio de la Frontera Sur' headquarters. It was anticipated that cage protection would save the colonies from phorid parasitism, despite the inevitable death of some bees that flew out of the hive and remained in the cage where they were killed by low temperatures and stress.

Bees from experimental colonies were foraging over pockets of trees and weeds interspersed in the surrounding urban environment. However, five days after duplication, all colonies began to be supplied with 10 ml of syrup (50% water-diluted *Apis mellifera* honey) provided in a 10 ml plastic feeder introduced twice a week through a hole made in the hive roof for a 28-day period. During a similar following period, the syrup was offered *ad libitum*; any syrup not consumed within four-days was discarded.

All colonies were left undisturbed until the 25th day, when they were checked for the presence of the queen, degree of comb-development, involucrum construction, strength of colony, and presence or absence of the phorid *Pseudohypocera*.

Weight increments of the colonies were determined, after 25 days, after 50 days, and at the end of the experiment. This was accomplished by weighing the whole colony and subtracting from this the weight of the empty hive, which had been determined before use.

In order to test the efficiency of this protection against phorids in daughter colonies, and the success of the multiplication method, the weight increases of both daughter and mother colonies were recorded. Mother colonies that had kept their laying queen were considered more viable and were then used as a control group. This decision was also taken because their protection period would be so short (one or two days) and so simple and inexpensive, that it was decided to save an additional formal control group of this endangered species in Chiapas, Mexico.

An analysis of variance was performed with the data of both groups and their mean weight differences examined by an F test (Bonnier & Tedin, 1966).

RESULTS

An absence of phorids was observed one or two days later in the area next to caged colonies. Therefore, mother colonies were taken out of their cages 24 hours after being placed there, and daughter colonies were freed 48 hours later. No phorid flies appeared later in these hives.

A survey made 21 days after duplication found queens, new brood (young combs), partly restored involucrum, and new food pots, in all of the twelve colonies.

Weight variations measured for two 25-day periods showed a general average gain of 140 ± 65 g (n = 12) during the first period, and of 32 ± 7 g (n = 12) for the second one. These values, showed no significant difference between weight increments ($F_{1/22}$ = 2.93, P = 0.10).

Daughter colonies showed a whole average weight increment of 154 g (n = 6, s.e. = 58) and the control or mother colonies 190 g (n = 6, s.e. = 108). However, these values (see Table 1) showed no significant differences ($F_{1/10}$ = 0.086, P = 0.775). Similarly, no statistically significant differences were found between increments occurring in hives within each mother or daughter colony groups.

Table 1

Average weight gain of mother and daughter groups of *M. beechii* colonies. Figures correspond to two 25-day periods following corroboration (21-days after duplication) that colonies had laying queen, new brood cells and new food pots

Colony type	Quantity (n)	1st period (g)	2nd period (g)	Total increase (g)	m ± s e (g*)
Mother (ctrol)	6	981	160	1141	190 ± 118 a
Daughter	6	699	226	925	154 ± 64 a
All colonies	12	1680	386	2066	172 ± 94

* Means with the same letter are not significantly different using the ratio of means and error variances (*F* = 0.086, df = 1/10, P= 0.77).

DISCUSSION

The range of brood cells used for making each daughter colony (from 400 to 500) provides enough hive bees and forager bees for daughter colonies to start. Perhaps these initial quantities were too large because colonies have been developed from smaller amounts of brood. Thus full colonies were developed from 90 to 140 cells, 30 to 60 workers and a virgin queen (Veen *et al.*, 1992), or from 100 cells, 100 adult bees and a queen emerged and mated in the laboratory (Aidar, 1995; Camargo, 1972, 1976). In any case, the colony size used in this study furnished a good possibility of survival and success appropriate to test a simple method of preventing phorid fly attacks.

Pseudohypocera phorids were neither observed upon the cages that protected the colonies since the second day after multiplication, nor seen on the hives after the cages were removed. It seems then that the mosquito net cage was able to prevent entrance of these parasites, and allowed the worker bees time to develop defenses against them. Careful colony manipulations and well-removed smears and spills of larval food, pollen or honey may prevent phorid attacks (Nogueira-Neto 1997), but this is usually a very difficult task to accomplish thoroughly. However, cage protection appeared to limit the attractiveness of duplicate colonies to phorids to two days or less.

Average weight increments measured for two 25-day periods did fall from 140 to 62 g on the second period, that is, 72 days from the beginning of the experiment. But this decrease was more likely a normal consequence of the nectar and pollen shortage in the suburban environment and the arrival of the rainy season, than a cessation of colony growth.

It seems clear that random fluctuations of colony weights explain satisfactorily the observed differences of weight increments between mother and daughter colonies; within groups of mothers and daughters; and between the weight gains of the ensembles in the two periods studied. Moreover, it seems reasonable to suggest that the mother and daughter colonies showed a similar capacity for recuperation, and both were well protected against phorid attacks and consequently equally able to survive and develop into full colonies.

CONCLUSION

This new procedure in which bee colonies are duplicated inside mosquito net cages appears to protect the stingless bee colonies from phorid flies and so provide a safe simple method to increase the number of colonies of *Melipona beecheii* bees.

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