Strategies to reduce the infestation of *Pseudohypocera kerteszi* (Diptera: Phoridae) in colonies of *Scaptotrigona mexicana* (Hymenoptera: Apidae)

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ABSTRACT

Objective: We intend to describe the strategies used for the control *Pseudohypocera kerteszi* in colonies of *Scaptotrigona mexicana* which help to reduce infestation during transfer, division, and harvest.

Design/methodology/approach: In this research, bibliographic sources of information on the control of *P. kerteszi* during the management of *S. mexicana* were used. The selected strategies were implemented by the authors of this manuscript, so they provided the experiences that were had in the control of *P. kerteszi* in the colonies of *S. mexicana*.

Results: We report six strategies applied to reduce the attack of *P. kerteszi*: 1) perform the transfer in a closed place, 2) minimize the damage to the offspring, 3) do not introduce food, 4) close the entrance to the nest for at least two days, 5) place vinegar traps, when necessary and in the initial stages of the infestation and 6) feed and clean bees the following days after the transfer or division.

Limitations on study/implications: Any limitation was involved in this study.

Findings/conclusions: The attack of *P. kerteszi* on *S. mexicana* colonies takes place during the transfer of nests, artificial division and the harvest of honey. Strategies to avoid infestation consist of using the appropriate box design, harvesting in an enclosed place, and avoiding breaking honey pots. During the critical stages of infestation, the revision must keep daily to clean the box, place vinegar traps, and make a manual control.

Keywords: Stingless bees, kleptoparasite, management, scuttle flies, meliponiculture.

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INTRODUCTION

Scaptotrigona mexicana (Guérin-Méneville, 1845) (Orden: Hymenoptera, Familia: Apidae) is an ecologically, economically and culturally important stingless bee (Quezada-Euán, May-Itzá & González-Acereto, 2001). In recent years, the destruction

of its habitats and attacks by natural predators have placed its populations at risk, thus the importance of their protection and conservation (Martínez-Fortún, Ruiz, Acosta & Vit, 2018). These bees naturally live in tree trunks, where natural enemies find it difficult to destroy their nest, yet may reduce its population. However, the commercial management of these bees in artificial housing (pots and poxes) present a high risk of their enemies completely destroying the colony if the necessary control measures are not taken.

One of the most relevant natural enemies of *Scaptotrigona mexicana* is the phorid *Pseudohypocera kerteszi* (Enderlein, 1912) (Order: Diptera, Family: Phoridae). An infestation of a bee colony by this pest is very difficult to control, since rescue interventions are arduous and not very efficient. In general terms, the elimination or burning of the colony is recommended to avoid the infestation of other colonies (Chaud-Netto, 1980; Moo-Valle, 2018).

P. kerteszi is also known as the phorid fly or "nenem" (in Mayan) (Pat, Anguebes, Hernández & Ramos, 2018). Its distribution in the Americas ranges from Mexico to Central America, and even South America (Robroek, De Jong, Arce & Sommeijer, 2003). In general, phorids are considered scavengers and saprophagous (Brown, 1992), making it a pest, particularly in the rainy season (Pat *et al.*, 2018).

The anatomy and life cycle of *P. kerteszi* is very characteristic. Its eggs are white and measure 1 mm. Larvae measure 1.6 mm in the first stage and 6 mm y the final stage and they are a dull white colour. Pupae measure 5 mm and they are light to dark maroon. Adults measure between 2.25 and 3 mm. Their development from eggs to adulthood takes, on average, between 12.8 and 16 days, whereas the egg stage lasts between 12.3 and 19.5 hours, the larval stage, between 6.9 and 7.2 days, and the pupa, 5.4 to 8.0 days. Adults present a prominent pronotum and a short abdomen that points downward, giving it a curve-shaped appearance. Females can lay anywhere from 31 to 102 eggs, with a viability of 72 to 82%, with a laying period of 35 to 45 days (Robroek, De Jong & Sommeijer, 2003; Wolff & Nava, 2007). The *P. kerteszi* female presents a very prolonged egg-laying apparatus, which lets it lay eggs in the cracks or fissures of boxes or certain structures of bees' nests, making it difficult for worker bees to remove the phorid's larvae from the colony.

There are three critical points in which *P. kerteszi* can infest the nest of a colony of *S. mexicana* under commercial management: 1) during the transfer of nests, 2) during the artificial division of the colony, and 3) during the harvest of the colony's products. The female phorids enter the colony through the entrance to the nest and past the guardian bees, since they are attracted by the acidic smell of the pollen, honey and larval food. Generally, on the female's first day inside the colony, it lays eggs, preferably in the pollen stored by the bees, in the involucre, in the cells of the honeycombs of the offspring destroyed by inadequate management, in broken pots with pollen and in garbage deposits (Moretto, 2000; Robinson, 1981; Robroek *et al.*, 2003; Tolsá & Ballesta, 2017; Wolff & Nava, 2007).

The aim of this work is to describe the strategies used to control *P. kerteszi* in *S. mexicana* colonies to reduce infestation and implement them in the transfer, division and harvest.

MATERIALS AND METHODS

The information on the strategies for the control of *P. kerteszi* was obtained by a bibliographical revision based on manuals, books and on the search of scientific articles in the referential databases Scopus, Web of Science Group, Google Scholar, Elsevier and Springer Link, using the following keywords: *P. kerteszi*, control, management, stingless bees, phorid and meliponiculture. On the other hand, the strategies chosen were implemented by the authors of the present manuscript, therefore contributing the experiences in the control of *P. kerteszi* in the colonies of *S. mexicana*, thus reducing the risk of infection by the phorid.

RESULTS AND DISCUSSION

Strategies to control *P. kerteszi* applied to transferred colonies. The transfer of the *S. mexicana* colony nests from clay pots to wooden boxes, complete with its food reserves, causes a high infestation of *P. kerteszi*, since this process destroys parts of the structure of the nest, which includes honey pots. This causes a disorganization of the members of the bee colonies, facilitating the access for *P. kerteszi* in the time taken to carry out the transfer.

The first strategy to avoid infestation by *P. kerteszi* is to carry out the transfer inside a small room made of mosquito nets, commonly called a "pavilion". A second strategy is to avoid introducing broken honey or pollen pots in the boxes in which the honeycombs are placed when performing the transfer (Moo-Valle, 2018). The third strategy consists in closing the entrance tube with wax for the first two days after the division, that is, blocking the entrance with the wax and resin that bees use to build their nests, since this time helps bees rebuild their nests and reorganize in their jobs inside the nest (Gennari, 2019). Generally, bees open the entrance themselves when the recovery of the nest is complete.

Strategies to control *P. kerteszi* during artificial division. It is recommended to carry out the division of the *S. mexicana* colonies when the colony no longer has enough space to develop. Once again, it is recommended to carry out the division inside a pavilion and avoid breaking the pots containing pollen or honey, since this may attract flies (Moo-Valle, 2018) (Figure 1).

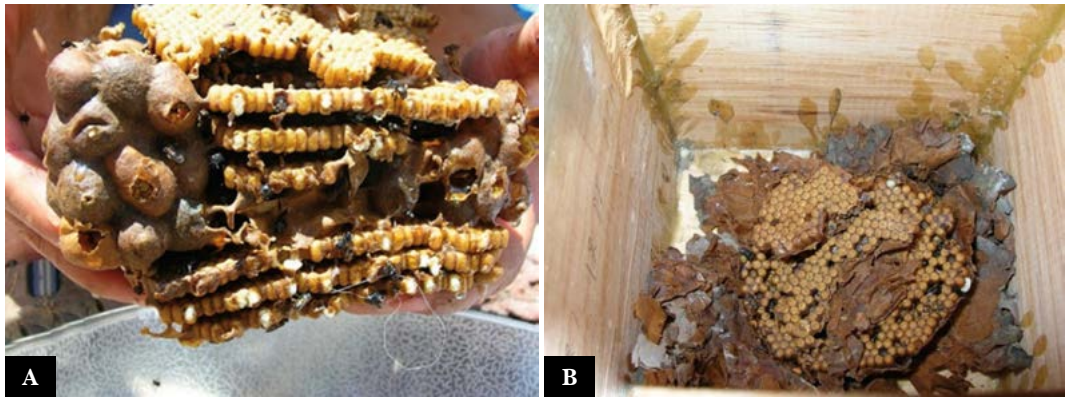

Figure 1. Artificial division of a nest of *S. mexicana*. A) Part of the offspring nest to be transferred to a wooden box, B) *S. mexicana* nest with involucre and without food pots. Photos: Juan Antonio Pérez Sato.

If a honeycomb with the offspring is damaged in any of the first stages, it is recommended against placing these inside the box, since the larval food may be released and this attracts the phorid (Shanahan & Guzmán, 2017). In addition, panels must be places with young offspring and in the pupal stage, in order to guarantee the emergence of new bees to strengthen the new colony in a short period (Moo-Valle, 2018). Likewise, worker bees, a queen bee and/or a queen cell must be placed for the colony to be quickly established and strengthened, and the bees must be fed 24 hours after the division is carried out. In addition, the box must be inspected every 3 days, and if a phorid is found, internal traps must be placed containing apple vinegar, and depending on their condition, clean the inside of the box, feed the bees and control the phorid by hand (Medina, Hart & Ratnieks, 2014).

Some important additional recommendations to avoid phorid infestations are: a) to provide fresh food with no fermented odours; b) feed with sugar syrup, which must be previously heated; c) do not provide excess food, to avoid fermentation and the attraction of phorids; d) provide wax from the same colony so it rebuilds its nest faster, particularly the tunnel that stretches between the entrance and the offspring chamber, so the guardian bees at the entrance have greater control over the phorids and e) keep the box sealed, avoiding cracks which *P. kerteszi* can use to enter it (Shanahan & Guzmán, 2017).

Other general recommendations for the process of artificial division of a colony are the following: a) the two colonies obtained must each have an adequate population of bees so that strong colonies with worker bees of different ages are quickly formed, in order to have the ability to defend their colony. In case one of the colonies is found to be weak, it is to be moved to a place occupied by a strong one, and in this way, worker bees will enter that will strengthen the cleaning of the colony (González-Acereto, 2008); b) place honeycombs with mature offspring (pupae) about to emerge, from strong colonies, and c) carry out an early detection of *P. kerteszi*, thus the recommendation of carefully reviewing and observing the inside of the box, since phorids move quickly between reserves, honeycombs and the structure of the nest. In case adults are found inside the box, the different structures (offspring panels, reserves, etc.) that have been affected by the phorids must be removed immediately, and when the garbage containers of the infested honeycombs are cleaned, they must not be left near to the colonies, since this attracts more phorids (Figure 2), therefore they must be placed in a bag and discarded or buried in another, remote place.

Other actions recommended when infestation levels are low are a) to capture the adults, larvae and pupae of the phorids in the box manually; b) to blow between the structures in order to remove the adults and eliminate them; c) trap and eliminate the adults using a tulle fabric bag and d) place an adhesive glue in the entrance of the nest so the phorids are trapped when they try to enter (Guzmán, Balboa, Vandame, Albores & González-Acereto, 2011; Shanahan & Guzmán, 2017).

Recommendations when infestation levels are high are: a) to remove the colony from the meliponary so it does not contaminate the other colonies and b) clean the box and burn the infested honeycombs, since it will be difficult to eliminate the flies, which may wipe out the entire colony in a matter of a few days (Moo-Valle, 2018).

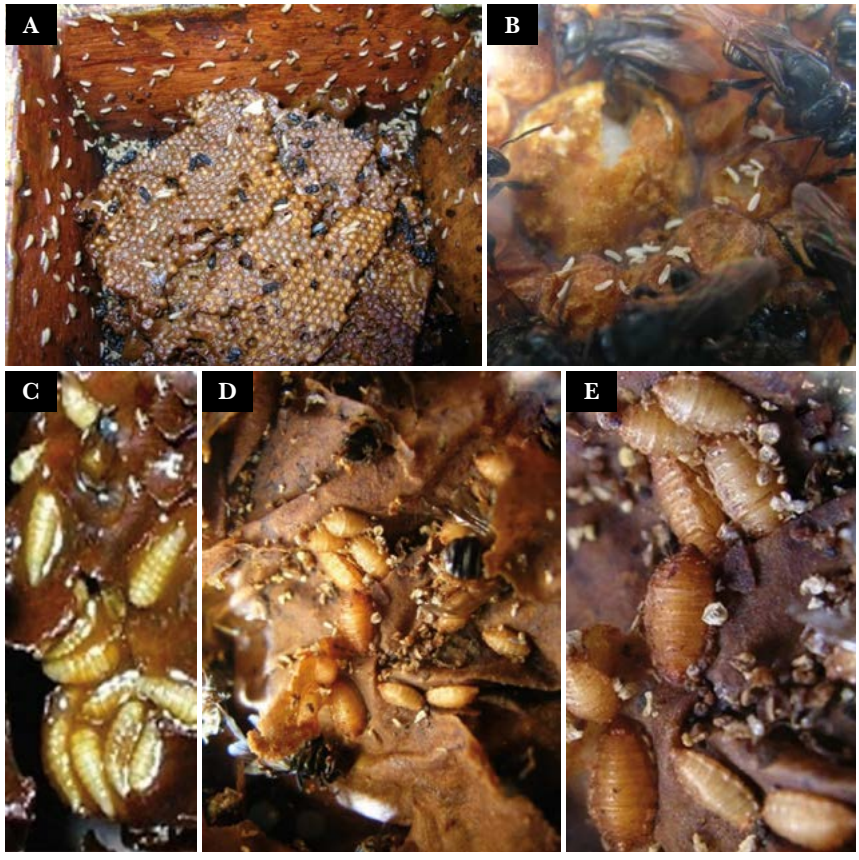

Figure 2. Infestation of *Pseudohypocera kerteszi*. A) *Scaptotrigona mexicana* nest with phorid larvae, B) *P. kerteszi* eggs on honey pots, C) Larvae of the phorid feeding off honey, D) Phorid prepupae, E) Phorid pupae. Photos: Juan Antonio Pérez Sato.

Strategies to control *P. kerteszi* applied during the harvest of honey. During the harvest of honey, the risk of infestation of the colonies by *P. kerteszi* has been observed to be low, since the honey-producing colonies have a high adult population. In addition, the design of the boxes in which the bee colonies are housed allows for a harvest of honey without affecting the structures that protect the offspring area, reducing infestation. However, in order to reduce the risk of infestation during the harvest, the following is recommended: a) to carry out this activity inside a pavilion, b) avoid breaking offspring honeycombs or honey and pollen pots during the revision and harvest of honey in the colony, c) performing the harvest as quickly as possible, trying to leave the colonies open the least time possible, mainly in the more humid seasons (the time of year in which the *S. mexicana* colonies are the most affected by *P. kerteszi* is in the months of June, July and August, which are part of the rainy season) and d) keeping the meliponary clean of organic matter, making sure to remove any pollen, honey or wax residues, since they may attract phorids (Medina *et al.*, 2014).

Other recommendations that can apply to any of the three critical stages by the infestation of flies in a colony are:

a) Use of natural repellents, using *Pluchea carolinensis* (Jacq.) D. Don, *Bursera simaruba* (L.) Sarg., *B. graveolens* (Kunth) Triana & Planch., *Croton humilis* L., *Psidium guajava* L., *Ruta chalepensis* L., *Euphorbia milii* Des Moul., *Ricinus communis* L., *Dieffenbachia picta* Schott y *Melia azedarach* L. plant leaf extracts. These are to be placed outside or on the inside corners of the box (González-Acereto, Quezada-Euán & Medina-Medina, 2006; González-Acereto & De Araujo, 2005).
b) Use of vinegar traps, since the *P. kerteszi* females are attracted to fermented pollen by its smell, and since one of the components of pollen is acetic acid, apple vinegar has been used as an attraction agent in traps placed inside the bee colony. Traps are made with small, plastic containers with airtight lids with holes punched in them, approximately 4 cm in height and 2 mm in diameter, in such a way that bees cannot fit through them; the container is filled with apple vinegar, to approximately two-thirds of its capacity (Guzmán *et al.*, 2011; Wolff & Nava, 2007) (Figure 3 A).

Other mixtures used for traps are: a) a mixture of white vinegar and water (30-50% vinegar) or apple vinegar (5% of acetic acid). These mixtures are placed in a small container without a lid, which is to be covered with a thin piece of fabric with small holes punched into it, and held onto the container with a rubber band. The flies will be attracted to the smell and will fall inside and die from drowning (De Oliveira, Venturieri & Contrera, 2013).

A third type of vinegar trap is made with a plastic funnel, which is placed upside down in the opening of a small jar containing vinegar and leaving a small orifice at the end of the funnel, making sure that bees cannot fit through it; the funnel is to be attached to the jar using scotch tape (Gennari, 2019) (Figure 3 B).

Figure 3. Traps with apple vinegar to control *P. kerteszi*, A) Trap with holes, B) Trap with a plastic funnel. Photos: Natalia Real Luna.

Depending on the degree of infestation, one or several containers may be placed inside the box, since the flies are attracted, they enter the trap and die from drowning. It is important to emphasize that the holes must allow flies to enter, but not bees. For a better control of the *P. kerteszi* flies, the vinegar traps must be replaced every 2 or 3 days and the adults that have previously entered the trap and died there must be removed. The holes in the lid must also be reopened, since bees can seal them with propolis or wax (De Oliveira *et al*., 2013; Ramos, Medina & May-Itzá, 2003). It is recommended to use the traps only when infestations are severe, and when flies are no longer found, the traps must be disposed of. If the number of adult flies is not reduced, the colony must be cleaned or transferred to another box. The vinegar trap method allows bees, without any adult flies, to efficiently clean the areas in which flies have laid eggs and where larvae are found. Vinegar reduces the adult populations, which favours bees, since they use large amounts of energy chasing adult flies.

CONCLUSIONS

The attack of *P. kerteszi* on *S. mexicana* colonies takes place during the transfer of nests, artificial division and the harvest of honey. The strategies to avoid infestation consist of: a) performing management procedures in a closed space, b) transferring the entire offspring area without damaging the structures that cover the offspring honeycombs, c) transferring mature and young offspring honeycombs without any damage, d) adding enough involucre to allow for the quick covering of the offspring area, e) closing the spaces of the box so as to leave it airtight, along with the entrance of the colony with wax for at least two days, d) placing vinegar traps inside the nest and e) avoid transferring in the rainy months, as well as to use the suitable box design. During the critical infestation stages, revision must be carried out on a daily basis in order to clean the box, place vinegar traps and perform a manual control.

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