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Body size variation, abundance and control techniques of *Pseudohypocera kerteszi*, a plague of stingless bee keeping

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Abstract

A common technique used to control phorid fly infestations on stingless bee's nests is the use of traps with commercial vinegars, especially the red wine vinegar, which is stated to be more efficient than the white wine vinegar. However, the claims on its greater efficacy are only circumstantial, not based on experiments. Thus, the aim of this work was to test the efficacy of traps composed of red wine or white wine commercial vinegars on the attractiveness of adult *Pseudohypocera kerteszi* Enderlein (Diptera Phoridae) females that infested colonies of *Melipona fasciculata* Smith, and *Melipona seminigra* Friese. The comparisons were made by placing one trap of red wine vinegar and other of white wine vinegar in infested colonies of the studied species for a week to check for possible differences in their attractiveness on phorids. We also tested the possibility that traps could attract phorid flies from outside the nests, thus potentially increasing an infestation. This was tested by placing traps of red wine vinegar in empty hives in the meliponary, thus eliminating bee's odours and food stores as factors that could attract phorid flies. There was no difference on the attractiveness of traps composed of red wine or white wine vinegars (Wilcoxon matched-pairs test), and traps did not any attract phorid flies from the outside to the interior of hives. Thus, the red wine and white wine vinegars can be equally used to capture phorid flies that invade nests of stingless bees, and they do not attract flies from outside the nests, thus not increasing infestations. The other objective of this work was to correlate the body size, particularly the maximum head width of the captured phorids and their abundance, with the monthly rainfall levels during the experimental period. We noticed an increase of abundance of phorid flies on the rainy periods, but there was no correlation with body size, which we suggest to be a consequence of the weakening of bee colonies that normally occur during the rainy season, which ease the invasion of them by phorid flies from the environment.

Key words: scuttle flies, Diptera Phoridae, meliponiculture, acetic acid, maximum head width, rainfall, kleptoparasitism.

Introduction

Stingless bees (Hymenoptera Apidae Meliponini) are a diverse group of insects with a Pan-Tropical distribution, comprising several hundred species (Michener, 2007), and like honeybees, they can supply beekeepers with their honey and act as pollinators of commercial crops (Heard, 1999; Venturieri *et al*., 2012). This activity is known as meliponiculture or stingless bee keeping, and it is an old tradition in the New World, being practiced by the native Kayapó in the Brazilian Cerrado and by the Mayans in Central America, among other cultures (Camargo and Posey, 1990; Villanueva-G *et al*., 2005; González-Acereto *et al*., 2006). Nowadays in Brazil, there is a new flourishing of stingless beekeeping, especially rearing of colonies of *Melipona* spp. (Contrera *et al*., 2011), which is becoming more advanced and profitable, especially for low-income and familiar farmers (Venturieri *et al*., 2003; Magalhães and Venturieri, 2010).

Stingless bees have several pests and predators that look for the resources they store inside the nest, especially honey and pollen. Among the more serious pests that threaten stingless bees and meliponiculture, we can include the infestations and colonies losses caused by the phorid flies (Diptera Phoridae). Phorid flies (or scuttle flies) comprises at least 10,000 species in the Neotropical region alone (Brown, 2005), and are particularly important in the forensic area (Disney, 2008). They parasite not only stingless bees (Brown, 1997) and other bee taxa, but can infect other groups as well (e.g. ants, Seid and Brown, 2009; mushrooms, Lewandowsky *et al*., 2012).

The most common scuttle fly that attack nests of stingless bees (and also honeybees; Robinson, 1981; Wolff and Nava, 2007) in Neotropical areas is *Pseudohypocera kerteszi* Enderlein (Diptera Phoridae), whose females try to invade stingless bee's nests to lay batched eggs inside the pollen pots, on the involucrum, in crevices and narrow spaces, and even inside the brood comb (Roubik, 1989; Nogueira-Neto, 1997). Its larval development is fast (Chaud-Netto, 1980; Robroek *et al*., 2003), and in large infestations the larvae start to feed from the pollen stores (figure 1) and even the bee's larvae and pupae. Thus, infestations can cause the death of the colony and also spread to other colonies when they are not controlled.

Surprisingly, considering the extensive damages phorid flies can cause to bee colonies, published studies that focused on control techniques against phorid fly infestations are scarce (exceptions: Moretto, 2000; Ramos *et al*., 2003; Freire *et al*., 2006; Wolff and Nava, 2007). Most known control techniques in meliponiculture use vinegar traps inside the colonies (Nogueira-Neto, 1997; Ramos *et al*., 2003), but most of the reports on their efficacy are only circumstantial, not based on experiments. An exception is the study by Ramos *et al*. (2003), where the authors showed that *P. kerteszi* adult females are more attracted to apple vinegars and acetic acids with acidity around 5%.

Figure 1. A dead colony of *M. marginata* infested with larvae of *P. kerteszi*, most of them walking and feeding on the honey and pollen pots. (photo by Cristiano Menezes)
(In colour at www.bulletinofinsectology.org)

In Brazil, many beekeepers use traps based on commercial vinegars of red wine, which is thought to be better than traps based on white wine vinegars, but again, there are no published studies evaluating the efficacy of these vinegars on attracting phorid flies. There are also some criticisms to these vinegars traps. The criticisms are based on the possibility the traps would attract phorid flies that are outside the nest, because of their acid odour, thus increasing infestations (Souza *et al*., 2009).

Therefore, based on the lack on knowledge about phorid fly control techniques and aspects of morphometry and seasonal abundance, the goals of this study were to test the efficacy of traps composed by two different types of vinegars commonly used in stingless bee keeping (red wine and white wine), to test whether the traps would attract phorid flies from outside the colonies, thus increasing infestations and verify if there is any relationship between rainfall and the size and abundance of individuals.

Materials and methods

Phorid fly traps

To test the efficacy of phorid fly traps, we performed two series of experiments, the first to test the attractiveness of traps composed of red wine vinegar and white wine vinegar on phorid fly species that infested nests of *Melipona fasciculata* Smith and *Melipona seminigra* Friese, and the second to test whether the traps would attract external phorid flies to the colonies. Both experiments were performed in the meliponary of Embrapa Amazônia Oriental, in Belém, Pará State, Brazil (1°26'11.52"S, 48°26'35.50"W), from August 2010 until July 2011. The climate of the region is type *Af* (tropical rainforest climate, according to the Köppen-Geiger classification), which is characterized by dry seasons with a precipitation no less than 60mm on the dry months of the year and high temperatures (Peel *et al*., 2007). The experiments were performed sequentially, to avoid interference of one experiment on another.

In order to perform the first experiment, the colonies of the studied species present in the meliponary (around 200 colonies) were constantly checked (at least twice a week) to detect possible infestations by phorid flies. When phorid flies were observed, two phorid fly traps, composed of rounded-shaped plastic vials (40 mL full capacity, figure 2), were put on the colonies; one was filled with 30 mL of red wine vinegar (Minhoto™, 4.0% acidity) and the other with 30 mL of white wine vinegar (Minhoto™ 4.0% acidity). The vials had a small orifice (0.4 cm) in their top, enough to allow the entrance of phorid flies but not enough to allow the entrance of bees (see model in Ramos *et al*., 2003). Vials were put inside the colonies at randomly positions, always on the top corners of the colonies, and each vial was put diametrically opposed to the other, in order to avoid site preferences and interferences of one trap on another. The distance of the traps to the lid of the colonies was around 2 centimetres, sufficient to avoid the traps to be obstructed by the lid and to allow the entrance of the phorids (figure 2).

Both traps were left in the colony for seven consecutive days. This period was determined because we observed (in previous observations) that after this period

Figure 2. Phorid traps composed of red wine (bottom right) and white wine vinegars (upper left), being removed from a colony of *M. flavolineata*, after a week inside the colonies. Notice the holes on the top of the vials are closed with cerumen. (photo by Cristiano Menezes)
(In colour at www.bulletinofinsectology.org)

of time the workers inside the colony normally close the trap hole with cerumen, thus not allowing more captures of phorid flies. After the seven-day period, the traps were removed and the identification of the phorid flies captured in each trap was done. Twenty-six trials were performed for *M. fasciculata* colonies and 13 for *M. seminigra*. The comparisons of the amount of phorid flies attracted on each trap, per bee species, were performed by using a Wilcoxon matched-pair test (Zar, 1999), with a significance level of 5%.

To test whether the traps would attract phorid flies from the surroundings to the interior of colonies, we placed five empty hives used for nesting *Melipona flavolineata* Friese colonies (Venturieri, 2004) in the meliponary, which received two vials containing 30 mL of red wine vinegar. The traps were offered seven days consecutively, like the previous experiment, and the position of the hives in the meliponary were randomly determined in each trial (14 trials). We used empty and unused hives in order to avoid the effect of bee and food stores odours in the experiment. If phorid flies were attracted to these traps, they should therefore have been exclusively to the odour of the vinegars, not to bees or food stores.

Body size, abundance, and rainfall

To evaluate if there was a relationship between local rainfall on the body size and abundance of phorid flies throughout the study period (August-2010 to July-2011) we used the individuals captured in the traps on the phorid traps experiment (phorid flies from nests of *M. fasciculata* and *M. seminigra*). We measured (by using a Zeiss-V9 dissecting microscope) their maximum head width (MHW) and verified its relation with the monthly rainfall data (mm^3) with a Mann-Whitney test (since the data were not normal nor had homogenous variances) by categorizing the months in dry (rainfall levels lower than 200 mm) and rainy (rainfall levels greater than 200 mm).

To verify the relationship between the abundance of phorids and monthly precipitation, we also performed a Mann-Whitney test. All tests were performed in Statistica® 7.0 with a significance level of 5%.

Results

Phorid fly traps

In the first experiment, the only species of phorid fly captured inside the nests was *P. kerteszi*. The majority of flies captured were females (99.3%, N = 617), and thus only females were used in the statistics of vinegar attractiveness. There was no significant difference in the number of *P. kerteszi* females attracted on the red wine vinegar and in the white wine vinegar nor in *M. fasciculata* (red vinegar: N = 297, white vinegar: N = 227, Wilcoxon-test: Z = 0.77, p = 0.44, 26 trials; figure 3a) nor in *M. seminigra* (red vinegar: N = 57, white vinegar: N = 36, Wilcoxon-test: Z = 0.45, p = 0.65, 13 trials; figure 3b). In the hives that contained only vinegar traps (14 trials), no phorid fly was captured, but instead we only found a small number of drosophilids (N = 16).

Body size, abundance and rainfall

P. kerteszi females were more abundant in the rainy season (dry season: August-December 2010, N = 65; rainy period: January-July 2011, N = 552; Mann-Whitney test: U = 3.50, Z = −2.27, p = 0.02; figure 4),

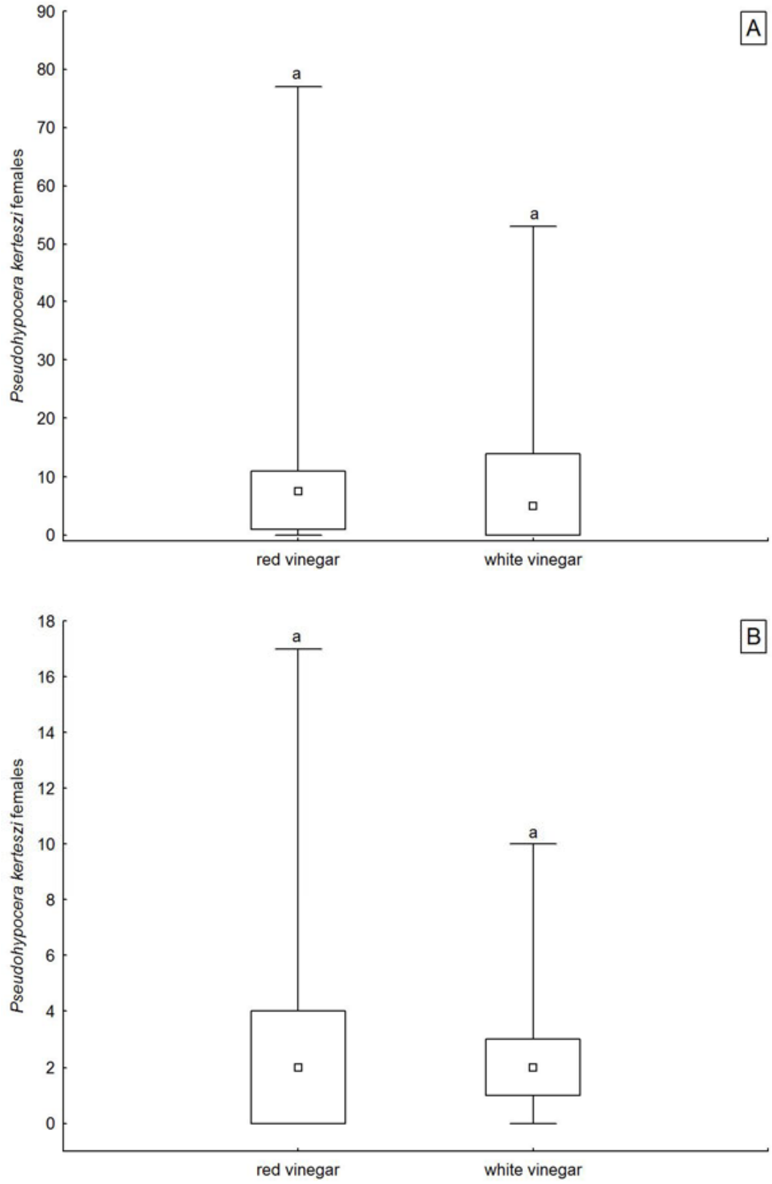

Figure 3. Median, 25-75%, and maximum and minimum *P. kerteszi* females captured in traps composed of red wine vinegar and white wine vinegar placed in nests of *M. fasciculata* (A), and *M. seminigra* (B) in the meliponary of Embrapa Amazonia Oriental, from August-2010 to September-2011. The same letters above the box-plots denote no significant differences ($p > 0.05$) between treatments (Wilcoxon-signed ranks test).

but there was no significant difference ($U = 14{,}244.5$, $Z = -0.82$, $p = 0.41$) between the MHW of *P. kerteszi* females in the rainy (0.87 ± 0.003 mm) and dry (0.86 ± 0.01 mm) seasons.

Discussion

Effectiveness of vinegar traps

In forums on stingless bee keeping, and in conversations with beekeepers, it can be observed that there several solutions for phorid fly infestations are used, most using different kinds of vinegars and other artisanal products. Unfortunately, there were few attempts to compare their efficiency. Most data on treatments are only circumstantial, because they do not use control and experimental treatments: thus their efficacy cannot be confirmed.

One of the most common treatments against phorid flies, and the one tested in our experiments, is the use of red wine vinegar, which was thought to be more attractive than white wine vinegar to flies. However, our results show that there is no significant difference on the attractiveness of red wine and white wine vinegars on *P. kerteszi* females. A similar result was found by Peruquetti *et al*. (2012), comparing red wine vinegars and apple cider vinegars, which did not have any difference on their attractiveness, but the authors placed the traps outside the nests, thus they did not actually compared the effect of the vinegars on phorid flies that had invaded the bee colonies. In our study, the two tested vinegars (derived from grape wine) had the same acidity level (4%).

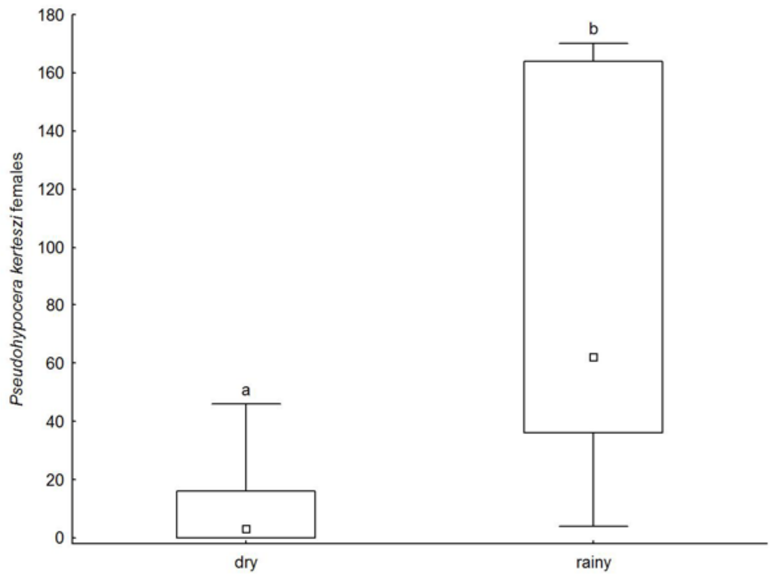

Figure 4. Median, 25-75%, and maximum and minimum *P. kerteszi* females captured in the rainy and dry seasons in nests of *M. fasciculata* and *M. seminigra* in the meliponary of Embrapa Amazonia Oriental, from August-2010 to September-2011. Different letters above the box-plots denote significant differences ($p < 0.05$) between seasons (Mann-Whitney U-test).

The work of Ramos *et al*. (2003) showed that *P. kerteszi* adult females were more attracted to apple vinegars with acidity around 5%. Thus, it might be possible that the attractiveness of vinegars is primarily related to its acidity level, a factor that should be investigated in future studies.

In this study, we aimed to test a cheap and common procedure used against phorid flies, because stingless bee keeping is yet an activity mostly performed by low-income and familiar farmers (Magalhães and Venturieri, 2010), which do not have access to more expensive chemicals, like glacial acetic acid. In other contexts, like in scientific meliponaries, with better infra-structure and availability of chemicals, the use of more advanced procedures might be considered, although it should always be a solution that avoids the contamination of food pots and larvae. For example, in the study of Moretto (2000), the author used a solution of oktrine (fly poison) plus vinegar or honey inside the colony, which was efficient on killing *P. kerteszi* adults and larvae, but it is unclear whether the treatment could contaminate and thus hinder the colonies.

Regarding the possibility that vinegar traps could attract phorid flies to the nests, thus increasing an infestation (Souza *et al*., 2009), our data show that this is not true. Since the hives used in the experiment were new, the only possible attraction for phorid flies was the vinegar traps placed inside them. In 14 trials, none of them attracted phorids. We can therefore state the traps are safe to use, and can be used preventively, since they do not attract extra phorid to the nests.

However, the use of phorid traps should not be the only procedure against infestations. The vinegar traps only attract adult phorid flies that entered the nest or were born in it, but not the larvae. For the elimination of larvae, manual cleanings of the colony and removal of damaged pollen pots is recommended. Also, the most important procedure against phorid fly infestations is to keep the nests in good conditions, without damaging the food pots and internal structures during handling and during the transportation of colonies to other locations, when eggs can be destroyed if the handling is not careful. In cases of extreme infestations is recommended to discard the contaminated structures of the nest, like pollen pots with fly's larvae and even the brood comb, if necessary, to avoid they spread to other colonies (Nogueira-Neto, 1997).

Body size, abundance and rainfall

Studies relating body size measures, phorid abundance and rainfall data are still rare. However, the work of Pereira (2006) compared the body size of females and males of *Megaselia scalaris* Loew (Diptera Phoridae) and *P. kerteszi* in relation to rainfall in the Western Amazon and found that females of both species were bigger than males and males were bigger in the rainy season, although the females did not show this trend. In our work, we did not find significant differences on the maximum head width of *P. kerteszi* females on the rainy and dry seasons, like the work of Pereira (2006).

However, we found that *P. kerteszi* females were more abundant in months with higher rainfall levels than in drier periods. Since the captured phorids were inside the colonies, we suggest their abundance variation a result of the weaker state of the colonies during the rainy season as well as the conditions of the environment, which may provide better breeding rates for *P. kerteszi* females. Regarding the colonial conditions, other studies (e.g. Roubik, 1982; Veiga *et al*., 2013) showed that in the Amazonian rainy season stingless bee colonies experience a decrease in their populations, food stores, and general conditions, thus being more susceptible to be invaded by parasites and natural enemies, like ants and phorid flies. In these conditions, the phorid flies from the environment may have more facilities to invade and parasite the bee colonies, compared with the dry season, when colonies are more populated and healthier.

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