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ORIGINAL RESEARCH ARTICLE

Seasonal population dynamics of small hive beetles, *Aethina tumida* Murray, in the south-eastern USA

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Summary

The population of small hive beetles, *Aethina tumida* (SHB), was monitored from 2005 to 2008 in colonies of Italian and Russian honey bees located near St. Gabriel, Louisiana, USA. SHB populations differed between honey bee strains (only in one site out of two), with Italian colonies supporting more beetles (7.45 ± 0.98 SHB per colony) than the Russian colonies (4.48 ± 0.51 SHB per colony). No difference between the two strains was observed at site 1 where the SHB population was generally low (Italian = 2.73 ± 0.36 SHB; Russian = 2.69 ± 0.57 SHB per colony). Our results also revealed that SHB populations varied throughout the year, with peak infestations observed in the autumn (September and November). SHB abundance was significantly correlated with the proportion of hot days, but not with the proportions of cool, dry, or humid days, or the percentage of days with rainfall. Our results suggest that in-hive autumn trapping of SHB in the south eastern USA may reduce springtime numbers of SHB.

Dinámica poblacional estacional del pequeño escarabajo de las colmenas, *Aethina tumida* Murray, en el sureste de los Estados Unidos de América

Resumen

Se monitorizó la población del pequeño escarabajo de las colmenas *Aethina tumida* (SHB) desde 2005 hasta 2008 en colonias de abejas rusas e italianas localizadas cerca de San Gabriel, Louisiana, USA. Las poblaciones de SHB fueron diferentes entre los sotcks de abejas (sólo en un sitio de los dos) soportando más escarabajos las colonias de abeja italiana (7,45 ± 0,98 SHB por colonia) que las rusas (4,48 ± 0,51 SHB por colonia). No se observaron diferencias entre los dos stocks en la localización 1 donde la población de SHB fue baja (Italianas = 2,73 ± 0,36 SHB; Rusas = 2,69 ± 0,57 SHB por colonia). Nuestros resultados también revelaron que la poblaciones de SHB varían a lo largo del año, con picos de infestación en el otoño (Septiembre y Noviembre). La abundancia de SHB se correlacionó significativamente con la proporción de días calurosos pero no con las proporciones de días fríos, secos o húmedos, o con el porcentaje de días con lluvia. Nuestros resultados sugieren que las trampas de otoño en el interior de las colmenas para SHB en el sureste de USA pueden reducir el número de SHB en primavera.

Keywords: Russian honey bees, Italian honey bees, resistance to *A. tumida*, hot days

Introduction

The small hive beetle (SHB: *Aethina tumida*, Coleoptera: Nitidulidae) is an invasive species in the USA (Neumann and Ellis, 2008). An indigenous pest of honey bee (*Apis mellifera*) subspecies residing in sub-Saharan Africa, SHB were first detected in the USA in Florida in 1998 (Elzen *et al.*, 1999), or perhaps as early as 1996 in South Carolina as suggested by Hood (2004). By 2004, SHB were established in 30 states (Hood, 2004) and have now spread to a few more. This rapid spread of SHB is partly attributed to their ability to fly (Schmolke, 1974) but probably results mostly from migratory beekeeping. A SHB population is now established in Australia (Gillespie *et al.*, 2003), and SHB have also been detected in: Egypt (Mostafa and Williams, 2002); Portugal (Ritter, 2004); Canada (Clay, 2006); and Sudan (El-Niweiri *et al.*, 2008), but no established SHB populations have been reported in these latter countries.

De Guzman and Frake (2007) hypothesized that the establishment of a SHB population and its impact on honey bee colonies may be influenced by varied rates of development in different thermic regimes. With mass-reared SHB larvae, a wide variation in developmental periods (24-81 days) has been recorded, partially due to differences in rearing temperature (Lundie, 1940; Schmolke, 1974; Neumann *et al.,* 2001; Mürrle and Neumann, 2004; and Haque and Levot, 2005). When larvae were reared individually, exposure to lower temperature (24-28ºC) resulted in a 15-day extension of total developmental time with a mean of 36 days as compared to 21 days at higher temperature (34ºC) (de Guzman and Frake, 2007). In Louisiana, SHB populations may reach extremely high numbers (i.e. hundreds of adult beetles per colony) where winter is mild and short, and summer is hot (pers. obs.). The SHB is also more prevalent in the warmer regions of South Africa where five complete generations have been recorded despite the lack of reproduction during the cool months of April to August (Lundie, 1940).

In Louisiana, SHB were first detected around the greater New Orleans area in 2000 (J Dunkley, pers. com.) and subsequently at Baton Rouge in 2003. Although adult beetles can be found in honey bee colonies throughout the year, especially in warm places like Louisiana (pers. obs.) and Rhodesia (Zimbabwe) (Schmolke, 1974), seasonal population fluctuations have not been established. This study was conducted to monitor SHB population build up and to determine seasonal population dynamics in the southern-eastern USA. Such information is needed to decide when is the best time to take action, and also may be useful in the development of future SHB management strategies.

Materials and methods

Colony set up

Honey bee colony divisions were made in July 2004, with each new colony having at least three frames of brood, two frames of honey and five empty frames. A total of 80 colonies were located in two apiaries near St. Gabriel, Louisiana, with 40 (20 Italian and 20 Russian) colonies per apiary. The two apiary sites were about 4.28 km apart. Colonies in one apiary (Site 1) were exposed to full sunlight, while those in the other site (Site 2) were under large evergreen oak trees which provided full shade throughout the year. All colonies had one medium-sized (depth = 17 cm) hive body, and were set on pallets containing four hives; colonies on each pallet had queens of only one strain. Each spring, queens that had superseded were replaced with the correct queen strain. Colonies that had died the previous year were replaced with new colony divisions having the appropriate queen strain. Colony divisions used to replace dead colonies were obtained from existing colonies of the appropriate bee strain. Site 1 received colony divisions from site 2 and *vice versa*. Throughout the experiment, Russian queens were produced using island-mating while the Italian queens were purchased from a queen breeder in California who advertises Italian queens. All colonies were treated with two Apistan strips in 2005 to suppress Varroa mite infestations throughout the experimental apiaries, and thereafter, no chemical treatment was applied in any of the test colonies.

SHB, colony and weather parameters

Since two colony inspections conducted in August and December 2004 showed no SHB in nearly all colonies (~98%), the first full colony examination was conducted in February 2005 when beetles were found in more colonies. Colony populations of SHB were monitored visually by examining all individual frames, hive covers and bottom boards for each colony (de Guzman *et al.*, 2006; Frake *et al.*, 2009). Empty frames were also bumped onto a white plastic tray in order to knock beetles out of the combs. Frames containing brood or honey were not bumped, to avoid injury to developing bees or the honey combs. Beetles were not removed from the colonies at any time. A total of 12 observations were conducted between February 2005 and April 2008. Weather data were obtained from a weather station at Site 1 and downloaded through the Louisiana Agriclimatic Information website (http://www.lsuagcenter.com/weather).

Data analysis

Data of the numbers of beetles in the colonies were analyzed using Proc Mixed (SAS version 9.2; SAS Institute, 2008) with stock, apiary, and sampling period as fixed effects. While there was no three-way interaction, there were two-way interactions (apiary x sampling period, apiary x stock, sampling period x stock) detected so the data were analyzed separately for each apiary. Since no interaction between stock and sampling period was found and the numbers of SHB were not normally distributed in either apiary, non-parametric analyses were conducted. A Wilcoxon two-sample test was used to compare the two honey bee stocks and a Kruskal-Wallis test was used to determine overall sampling period differences. For the Proc Mixed procedure, the number of the beetles was transformed using the square-root transformation, but the non-parametric tests were done on the original data.

The influence of weather on the number of beetles was assessed using the Pearson correlation analysis. Since beetles can complete development in about two months or less, depending on the temperature, weather data between two observation periods were considered to determine whether weather within those two periods influenced SHB abundance during the subsequent observation period. The climatic variables were: the proportion of hot, cool, dry, and humid days and also percentage of days with rainfall. The calculation of the proportion of hot, cool, dry and humid days was as described by Pitts-Singer and James (2008). A day was considered as: a. "hot" when maximum temperature was ≥27°C; b. "cool" when maximum temperature was ≤6°C; c. "humid" when relative humidity (RH) was ≥80%; and d. "dry" when RH was ≤40%. The percentage of days with rainfall was calculated as the number of days with precipitation out of the total number of days in the month prior to the observation. A multiple linear regression with stepwise selection was performed to determine the effect of these variables on the average number of SHB (SAS version 9.2; SAS Institute, 2008).

Results

Number of adult beetles

For Site 1, no significant interaction between stock and sampling period ($P = 0.284$) nor stock effect ($P = 0.082$) was detected for the number of adult beetles in the colonies. Overall, both stocks supported low numbers of beetles with means of 2.73 ± 0.36 and 2.69 ± 0.57 beetles per colony for Italian and Russian bee colonies, respectively. A significant effect of sampling period was observed ($\chi^2 = 233$, $P < 0.0001$) (Fig. 1). The smallest SHB populations were observed from February to June 2005, 11 months from the time the colony divisions were made. Although beetle counts increased significantly thereafter, populations were still small until June 2006, 23 months after making the divisions. An increase in SHB number was observed in September 2006 followed by a decrease in May and July 2007. Another small peak was observed in September 2007 followed by a decrease in April 2008.

While the SHB population grew faster in Site 2, the same trends were observed. No two-way interaction ($P = 0.096$) was recorded for the number of beetles, but significant stock ($P < 0.0001$) and sampling period ($P < 0.0001$) effects were observed. On average, there were more beetles recorded in the Italian colonies (7.45 ± 0.98 beetles per colony) than in the Russian colonies (4.48 ± 0.51 beetles per colony) (W = 33282, $P = 0.043$). The lowest beetle counts were recorded in February to May 2005 (Fig. 2). By June (11 months after making the divisions), the beetle population started to gradually increase with a significant increase in November (about 15 beetles per colony). Although the beetle population declined significantly in June 2006 to about 5 beetles per colony, the highest beetle population of about 25

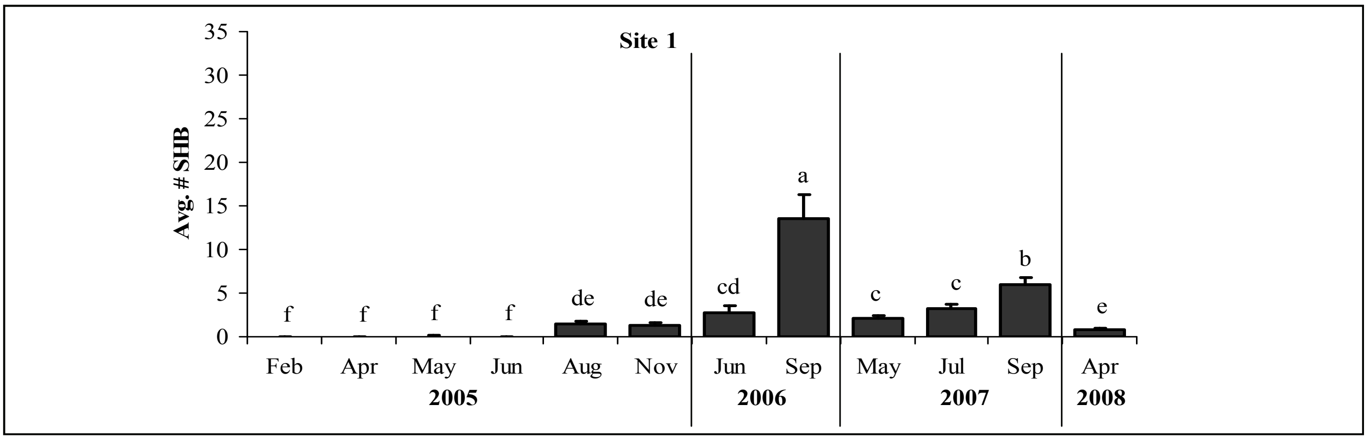

Fig. 1. Average number of adult SHB per colony regardless of honey bee strain from 2005 to 2008 for Site 1. Bars with different letters are significantly different ($P < 0.05$). Colony divisions were made in July 2004, but beetle counts began in February 2005.

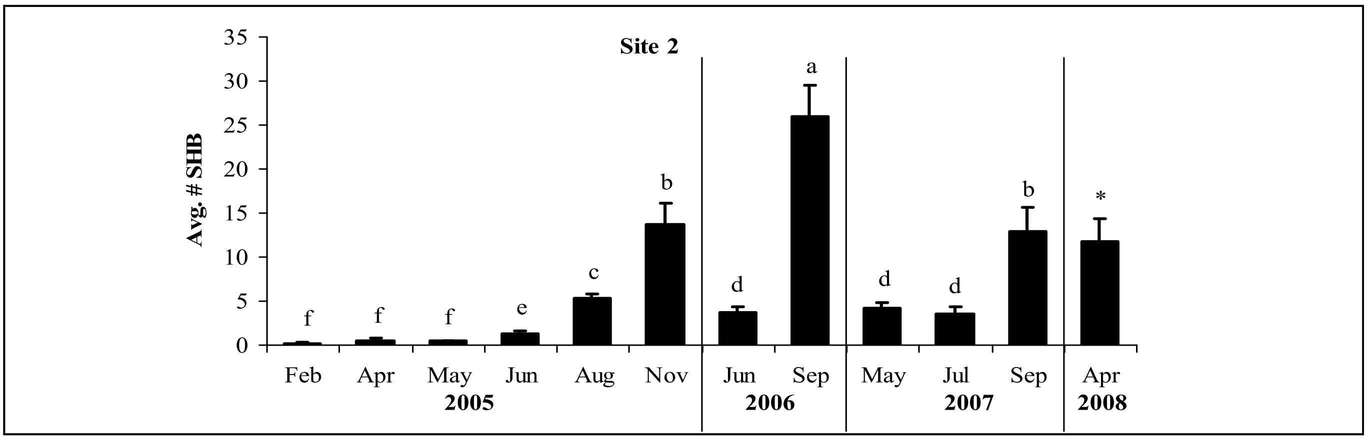

Fig. 2. Average number of adult SHB per colony regardless of honey bee strain from 2005 to 2008 for Site 2. Bars with different letters are significantly different ($P < 0.05$). Colony divisions were made in July 2004, but beetle counts began in February 2005. * By April 2008 the majority of the queens in the colonies had been superseded and were not included in the analysis thereby making the data too small to accurately compare with the others.

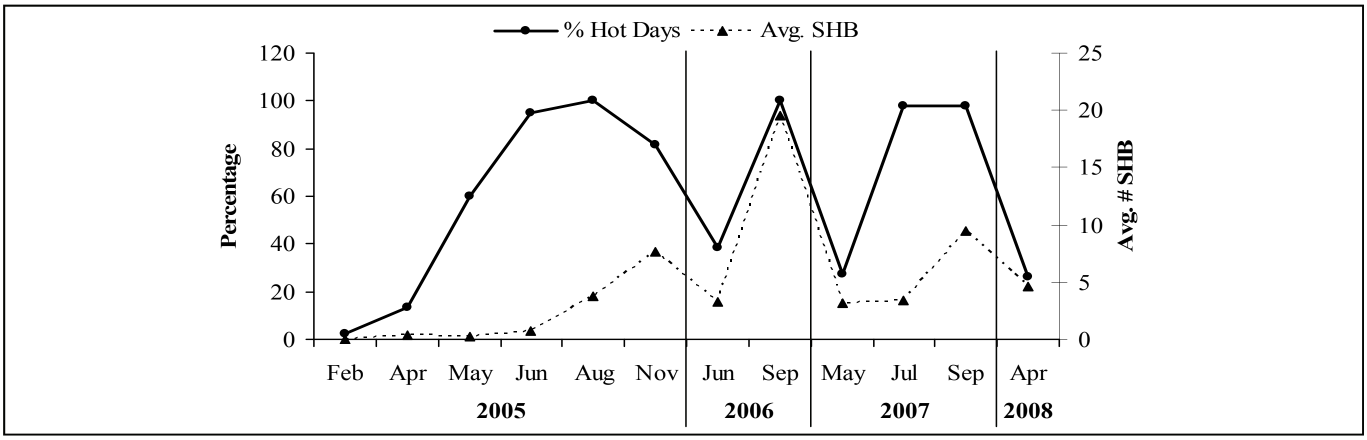

Fig. 3. Average number of adult SHB per colony as affected by the proportion of hot days over time.

beetles per colony was observed three months later (September 2006). This was the highest average count observed throughout the experimental period. In 2007, only a few beetles were observed in May and July but the number rose again in September. A slight decrease in beetle count was recorded in April 2008.

Effect of climatic variables on the number of adult beetles

The results of a multiple linear regression revealed that the proportions of cool (r = -0.3338, n = 24, P = 0.111), dry (r = 0.1067, n = 24, P = 0.6196), and humid (r = 0.1144, n = 22, P = 0.612) days, and also the percentage of days with rainfall (r = 0.1064, n = 24, P = 0.6196) did not influence the number of beetles observed in the colonies. Only the proportion of hot days significantly correlated (r = 0.5613, n = 24, P = 0.0043) with the number of beetles. About 80% hot (≥27°C) days resulted in an increase in the number of adult beetles (Fig. 3).

Discussion

In this study, we consistently observed that the largest population of beetles was during the autumn months. This observation agrees well with the findings of Frake *et al.* (2009) who documented the increase in the number of invading beetles in colonies deliberately freed from beetles in November 2005 and September 2006. This increase in the number of beetles may be due to the influx of beetles emerging from the soil. Most of the damage (indicated by slimy conditions and hundreds or thousands of larvae) inflicted by SHB usually occurs from June to August in Louisiana (pers. obs.). The peak natural reproduction of SHB also occurs during this time when the most pupating larvae were trapped in front of colonies (Frake *et al.*, 2009). In addition, high temperatures (≥27°C) during these summer months in Louisiana may have accelerated beetle development. De Guzman and Frake (2007) observed that beetles can develop from eggs to adults in about 23 days when exposed to hot temperatures (34°C), or two weeks shorter than when beetles are reared at room temperature. This observation was supported by our results showing an increase in beetle population during periods of more hot (≥27°C) days. In the south-eastern USA, therefore, where winter is mild and short, and summer is warm, more generations of beetles are completed, thereby increasing the annual population growth rate. This increase in adult population during the autumn months may be a result of massive infestations and reproduction from honey bee colonies destroyed by SHB during the summer months. There was one dead colony that had at least one hundred larvae in Site 2, but no dead colonies caused by SHB were observed at Site 1. Dead colonies from nearby apiaries may be sources of invading beetles, and another possible contribution to this within colony population increase may be due to "free living" beetles (i.e. those that feed on other food sources such as fruit) entering colonies as an adaptive preparation for winter. Pettis and Shimanuki (2000) documented that SHB overwinter within the honey bee cluster. Nevertheless, the decrease in adult SHB population in June 2006, and May and July 2007 may suggest that either not all beetles observed in the autumn actually overwinter within the cluster, that the majority of the beetles died during the winter or that most of them had left the colonies or apiary to find other hosts early in the spring.

Honey bee resistance to SHB has been clearly illustrated (Ellis *et al.*, 2003; 2004b) in African bees (*Apis mellifera capensis*). Similarly, Frake *et al.* (2009) documented the importance of honey bee stock in regulating SHB invasion in honey bee colonies when they showed that Russian honey bees support fewer adult beetles than Italian colonies. Our results showed the same trend for colonies in Site 2 where higher counts were recorded in the Italian colonies than in the Russian colonies. On average, Site 1 colonies had comparatively fewer beetles making it difficult to detect differences, but a trend can be observed. Numerically, Russian bees had fewer SHB than Italian bees on each of the observation dates, except in April 2008 when only 6 Italian colonies and 20 Russian colonies remained in this apiary. Further, more Italian colonies harboured SHB reproduction than Russian colonies, based on counts of eggs, larvae and pupae using bottom board traps and traps placed in front of each test colony (Frake *et al.*, 2009).

It is noteworthy that population growth of beetles varied between the two sites despite their close proximity. Fewer beetles were observed in Site 1 colonies, which were exposed to direct sunshine, than in Site 2 colonies which were shaded by trees. A preference of SHB for shady locations has already been suggested by a study conducted by Arbogast *et al.* (2007) where the authors trapped more beetles in traps located in the shady part of an apiary than in traps placed in full sunshine. Negative effects of apiary exposure to full sunshine on infestation levels of other honey bee parasites have been documented, for Varroa mites by Rinderer *et al.* (2004) and for tracheal mites (Harbo, 1993). Although beetles are known to pupate in a wide range of soil textures (Ellis *et al.*, 2004a; de Guzman *et al.*, 2009), it is possible that soil directly exposed to sunshine will desiccate quickly and thus not support the successful pupation of beetles.

Our results established that adult SHB are most abundant during autumn. The autumn trapping of beetles may significantly reduce overwintering populations of beetles which will infest colonies in the spring and thereby help regulate SHB populations throughout the year.

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