Population of small hive beetles (*Aethina tumida* Murray) in two apiaries having different soil textures in Mississippi

Lilia I. de Guzman[1], Jacquelyn A. Prudente[2], Thomas E. Rinderer[1], Amanda M. Frake[1], Hubert Tubbs[3]

[1]USDA-ARS, Honey Bee Breeding, Genetics and Physiology Laboratory, 1157 Ben Hur Rd., Baton Rouge, LA 70820-5502,
[2]311 MB Sturgis Hall, Dept. of Agronomy & Environmental Management, LA State Univ. Agr. Center, Baton Rouge, LA 70803
[3]Tubbs' Apiaries, P.O, Box 274, Mize, Mississippi 39116

Abstract

Soil samples collected at 0-10, 11-20 and 21-30 cm from two apiaries in Lula, Mississippi were separately analyzed for soil texture. Populations of small hive beetles (SHB) in the soil and inside the hives were also counted. Our results showed that the two apiaries had different soil textures with different levels of infestation both in the soil and inside the hives. We recorded significantly more adult beetles in apiary 1 (May = 109.35 ± 24.42, June = 607.6 ± 136.25 beetles per colony) where the soil was predominantly silty clay and silty clay loam than in apiary 2 (May = 37.08 ± 5.40, June = 260.4 ± 54.97 beetles per colony), which had mostly sandy loam and loam soil. Regardless of soil type, the density of SHB per 1,200 cm^3 soil varied with soil depth. The density of SHB was greatest (8.54 ± 1.92 beetles) in the first 10 cm of soil in which most of the larvae and pupae were observed close to the surface of the soil. A few (0.48 ± 0.12 beetles) SHB were also found at 11-20 cm, but no beetle was found at 21-30 cm. This preference for the first 10 cm was not influenced by soil type since no consistent soil type was recorded for a particular soil depth.

Our results showed that SHB populations successfully developed in various types of soil and that vertical movement of larvae in the soil was not influenced by soil type. Nevertheless, it is possible that the discrepancy in SHB populations between the two locations may have been due to the amount of available soil moisture. In a field setting, the difference in water retention ability of different soil types and field slope (drainage) potentially affected the amount of moisture in the soil. The presence of nematodes also may have contributed to the death of developing SHB in apiary 2. This is the first report on the natural infestation of nematodes in teneral adults of SHB in the soil.

Key Words: soil type/soil depth/nematodes/honey bees/IPM/small hive beetle/*Aethina tumida*

Introduction

The small hive beetle (SHB, *Aethina tumida* Murray) is one of the most economically important pests of honey bees (*Apis mellifera* L.) because of its ability to kill honey bee colonies (Elzen *et al*. 1999, Hood 2004). Likewise, SHB significantly reduces brood production, honey production, and flight activities of bees (Ellis *et al*. 2003). Adult females usually lay eggs in protected areas to avoid predation (Lundie 1940) or hygienic behavior by the bees as observed in both African (Ellis *et al.* 2004b, Neumann and Härtel 2004,) and European honey bees (EHB) (de Guzman *et al.* 2008). Adult honey bees also can remove live beetles from observation hives (de Guzman *et al*. 2006). Nevertheless, the ability of EHB to deter adult SHB invasion depends on honey bee stocks. In colonies deliberately freed from SHB and in those with freely colonizing

beetles, Italian colonies had more beetles than the Russian bees (Frake *et. al*. 2008). The authors also observed more Italian colonies that supported SHB reproduction than Russian colonies.

Young larvae are active feeders and thus they are responsible for most of the damage in the hives. Upon maturity or when the wandering phase is reached, larvae stop feeding, leave the hive and pupate in the soil. Therefore, soil conditions greatly influence the completion of their development or survival. Lundie (1940) speculated that soil type can be an important limiting factor in the abundance of SHB. SHB's preference for light sandy soil for pupation was reported by Pettis and Shimanuki (2000). This observation agreed well with the claim that severe damage to honey bee colonies caused by SHB has been concentrated along the coastal regions of the United States having light sandy soil. In heavy clay soil in Georgia, no serious infestations were found (Delaplane as cited by Wenning 2001). Hence, Wenning (2001) recommended placing colonies in clay-based soil to prevent significant infestations.

The importance of soil type, soil moisture and soil density on the successful pupation of SHB was studied under laboratory conditions (Ellis *et al.* 2004a, Schmolke 1974). In contrast to Lundie's (1940) claim, both studies showed that soil type did not influence SHB pupation. Schmolke (1974) showed that both soil density and soil moisture are important in the successful emergence of adult beetles. However, Schmolke claimed that soil density has the most effect especially in the burrowing of larvae into the soil. Recently, an experiment assessing the importance of these three factors showed that pupation is influenced by a combination of them (Ellis *et al.* 2004a). In 2000, Pettis and Shimanuki examined soil samples in front of six colonies from three apiaries (two colonies/apiary) in central Florida. The authors showed that about 80% and 20% of the SHB recovered were found in the first 10 cm and 20 cm of Florida's (St. Lucie) light sandy soil, respectively. The ability of SHB to pupate beyond 20 cm was not examined and no analysis of soil sample taken from every depth was conducted by the authors. Therefore, this study was conducted to determine if soil texture varies according to soil depth, and to determine if such differences affect the vertical movement of SHB.

Materials and Methods

Number of adult SHB in the colony - Two established apiaries with colonies of Italian and Russian ancestry were used in this study. A total of 44 queenright colonies (apiary 1 = 20, apiary 2 = 24) were analyzed in May 2004; only 19 (apiary 1 = 10, apiary 2 = 9) survived in June. These colonies were set on 4-colony pallets located near Lula, Mississippi. Populations of beetles were determined by inspecting individual frames, one hive body at a time, on top of a white table (de Guzman *et al.* 2006).

Density and distribution of SHB in the soil - A first set of soil samples was collected in front of 34 colonies (apiary 1 = 16, apiary 2 = 18). Soil samples were collected by digging a hole measuring 30 cm in diameter and 30 cm deep in front of a colony; one hole for each of the two sides of a pallet where hive entrances were located. Thereafter, 102 samples consisting of three slices of soil measuring about 8 x 15 x 10 cm (width x length x depth) collected with a knife from 0-10, 11-20, and 21-30 cm depths were placed in separate Ziploc® bags and stored in a walk-in freezer in the laboratory until processing. Large aggregates of soil in each sample were broken up, and were inspected both visually and under a dissecting microscope for the presence of SHB. All stages of SHB were collected and counted to determine the density of SHB per soil sample. All beetles were individually examined and dissected under a dissecting microscope for the presence of diseases, pests and parasites.

Particle soil analysis - All soil samples were analyzed at the Soil Testing laboratory of the Department of Agronomy, Louisiana State University. The proportions of sand, silt, and clay in each soil sample were determined using the Bouyoucos hydrometer method (Day 1965, Soil Survey Laboratory Methods Manual 1996).

Data Analyses - Data on the number of SHB were analyzed using Proc Mixed to determine if there was an interaction. Soil type and sampling depth were modeled as fixed effects. When no interaction was found, a Kruskal-Wallis test was performed (due to the non-normality of the data) to determine the effect of soil type and sampling depth. A paired t-test was performed to compare the beetles at the 0-10 and 11-20 cm sampling depths (where beetles were found) for each colony. Data on the number of SHB per colony were analyzed using a Wilcoxon two-sample test. A correlation was performed using Proc Reg to determine the relationship between the number of beetles in the colony and the number of beetles in the infested soil. Bee population and varroa infestation was compared using a *t*-test. (SAS Institute 2001, Version 8.2).

Results

Bee population, adult SHB population and varroa infestation - In May 2004, adult bee populations of colonies in both apiaries (apiary 1 =29,469 ± 3,779 bees; apiary 2 = 27,507 ± 2,737 bees) were very comparable (t = 0.43, $P = 0.67$). Similar trends (t = 1.04, $P = 0.311$) were observed in June (apiary 1 = 28,522 ± 5,018 bees; apiary 2 = 20,671 ± 5,628 bees). All colonies had abundant stored honey.

There were more (t = 3.67, $P = 0.001$) adult SHB observed in May 2004 in apiary 1 (109.35 ± 24.42, Mean ± SE, n = 20) than in apiary 2 (37.08 ± 5.40, n = 24). By June, most colonies in both sites were dead (50% mortality in apiary 1 and 63% in apiary 2). The average varroa infestations on adult bees of these colonies in May were 9.77 ± 1.6% and 4.39 ± 1.5% for apiary 1 and apiary 2, respectively. These dead colonies were probably weakened by the presence of parasitic mites and SHB took advantage thereafter. There were multiple SHB egg masses in between supers and on top of the frames, and also larvae on slimy combs in these dead

Table 1. Proportion of samples belong to each soil type at different depths for two apiaries near Lula, Mississippi

		Soil Depth (cm)		
Apiary	**Soil texture**	**0-10**	**11-20**	**21-30**
	Silt	0.00	18.75	18.75
	Silty clay	35.29	43.75	56.25
1	Silty clay loam	41.18	18.75	6.25
	Silty loam	23.53	18.75	18.75
	Clay loam	0.00	0.00	5.56
	Loam	27.78	33.33	44.44
	Loamy sand	11.11	5.56	11.11
2	Sandy loam	61.11	50.00	5.56
	Silty clay loam	0.00	0.00	11.11
	Silty loam	0.00	11.11	22.22

colonies. The average number of SHB in the surviving colonies was high with apiary 1 having significantly (t = 2.50, *P* = 0.023) more SHB (607.6 ± 136.25, n = 10) than surviving colonies in apiary 2 (260.4 ± 54.97, n = 9).

Particle soil analysis - Soil analyses showed that the two apiaries had different soil textures (Table 1). For apiary 1, there were four soil types identified, but their proportion varied at each sampling depth. At 0-10 cm, the majority of the samples were silty clay loam. Silty clay constituted most of the soil sampled at 11-20 and 21-30 cm deep. No proportionate sand was detectable in this location. Six soil types were established for apiary 2. Sandy loam comprised the highest proportion recorded at 0-10 and 11-20 cm while most 21-30 cm soil samples were of loam. Analyses also revealed that there was no specific soil type for each depth of soil. The soil sample obtained in front of a colony may have had either: a) the same soil types for all three depths, b) only two depths with the same soil types, or c) all depths with different soil types (for examples see Table 2).

Density and distribution of SHB in the soil - Among the 34 colonies selected for sampling soil in front of them, only 16 supported SHB pupation (apiary 1 = 9/16, apiary 2 = 7/18) (Table 2). Analyzing only these infested soil samples, no interaction between soil type and soil depth (*P* = 0.962), and no soil type effect (*P* = 0.536) were detected. However, soil depth significantly (*P* = 0.0001) affected SHB population in the soil. Regardless of soil type, most of the beetles were observed at the first 10 cm deep with a mean of 13.88 ± 5.37 beetles per 1,200 cm^3 soil sample. At 11-20 cm, an average of 1.0 ± 0.42 beetles was recorded. No beetle was found in soil sampled at 21-30 cm. There was no difference (*P* = 0.297) between the number of beetles pupating in apiary 1 soil (6.93 ± 3.34 beetles/1,200 cm^3) and apiary 2 soil (2.43 ± 1.38 beetles/1,200 cm^3). Further, there was no relationship (*P* = 0.766) between the number of beetles inside the colony and number of beetles in the soil in front of the colony. Those colonies with infested soil by the hive entrance had an average infestation of 133.75 ± 33.01 beetles per colony, which was similar (*P* = 0.694) to those colonies with uninfested soil (98.78 ± 17.17 beetles/colony) in front of them. Likewise, even if 0-10 and 11-20 cm soil have the same soil types, the first 10 cm supported more beetles than the 11-20 cm soil (*P* = 0.032, paired t-test). The same trend was observed in soil samples obtained from these two depths with different soil types (*P* = 0.046, paired t-test).

Nematode infestation - Unidentified species of nematodes were observed in several beetles collected from four soil samples (representing four hives) in apiary 2 (Table 3). No nematode-infested SHB was observed in apiary 1. Most of these beetles including body parts (with little tissue) had a few (≈ 20-100) nematodes with the exception of two young adult beetles; one with elytra (including hind wings) full of nematodes (Figure, a and b) and the whole body of the other was filled with nematodes (Figure, c and d).

Discussion

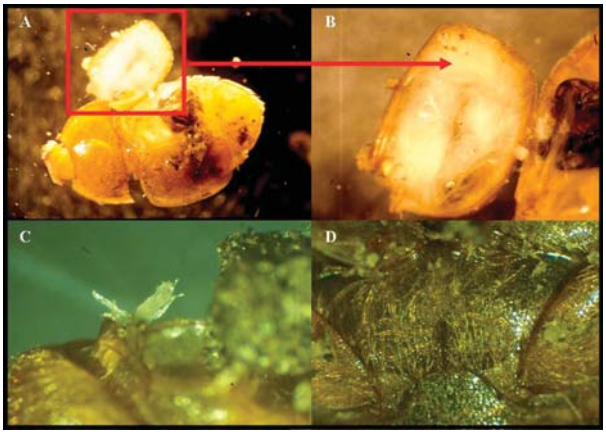
Small hive beetles spend >75% of their developmental time in the soil (de Guzman and Frake, 2007). Therefore, edaphic environmental factors such as soil type, soil moisture, soil density, field slope, drainage, rainfall, temperature and others greatly affect their biology. Our results showed that the two apiaries had different soil textures with different rates of SHB infestation both in the soil and inside the hives. We recorded more adult beetles in apiary 1 where the soil was predominantly silty clay and silty clay loam than in apiary 2, which was mostly sandy loam and loam soil. Nevertheless, our results showed that SHB pupation occurred in any type of soil. This field observation agreed with laboratory studies conducted by Ellis *et al*. (2004a) and Schmolke (1974). Ellis *et al.* (2004a) reported comparably high emergence of adult SHB (72-98%) in moist silty clay, silty clay loam and sandy loam and loamy sand soils. Schmolke (1974) also found high emergence (74-95%) in sand, loam and clay loam soil types. De Guzman and Frake (2007) observed a significant effect of temperature in the developmental periods of SHB. Thus, differences in climatic and

Table 2. Soil profile and the distribution of SHB in infested soil samples collected in front of colonies in two apiaries. Number in parenthesis indicates the total number of beetles found in the soil.

Apiary	Colony number	Soil depth 0-10 (1,200 cm3)	11-20 (1,200 cm3)	1-30 (1,200 cm3)
	1	Silty clay (1)	Silt loam (0)	Silt (0)
	2	Silty clay (10)	Silty clay (0)	Silt loam (0)
	3	Silt loam (6)	Silt clay loam (3)	Silt clay loam (0)
	4	Silt clay loam (2)	Silty clay (0)	Silty clay (0)
1	5	Silt clay loam (3)	Silt clay loam (0)	Silty clay (0)
	6	Silt clay loam (71)	Silty clay (4)	Silty clay (0)
	7	Silty clay (61)	Silt (4)	Silt (0)
	8	Silty clay (8)	Silty clay (0)	Silty clay (0)
	9	Silt clay loam (10)	Silt loam (4)	Silt loam (0)
	1	Loam (5)	Silt loam (0)	Silt clay loam (0)
	2	Sandy loam (28)	Loam (0)	Silt loam (0)
2	3	Loamy sand (10)	Sandy loam (0)	Loam (0)
	4	Sandy loam (3)	Sandy loam (0)	Silt loam (0)
	5	Sandy loam (1)	Sandy loam (0)	Loam (0)
	6	Loam (2)	Loam (1)	Loam (0)
	7	Sandy loam (1)	Sandy loam (0)	Loam (0)

Table 3. SHB population inside the colonies and nematode infestations of the adult SHB collected at 0-10cm soil in apiary 2. No nematode-infested SHB were found in apiary 1. (Note: Nematode-infested beetles were sent to an expert but identification was impossible because samples were preserved in alcohol for transport which ruined the key characteristics).

Colony #	# adult SHB in the hive	# SHB per 1,200 cm3 of soil	
		Infested	Uninfested
557	344	1 adult	0
559	603	11 adults, 12 body parts	5 adults
563	13	4 adults, 1 elytra	5 adults
575	117	1 thorax and 1 abdomen	0



Natural infestation of nematodes in teneral adults of small hive beetles collected from apiary 2. (A) an elytron full of nematodes, (B) close-up view of the nematode-infected elytron, (C) nematodes exiting between abdominal segments, (D) silhouettes of nematodes inside a beetle's body.

also soil conditions used by Ellis *et al.* (2004a) and Schmolke (1974) as compared to this study may have contributed to the differences in SHB emergence and ultimately in the number of SHB invading our colonies. Nevertheless, the populations of beetles caused deaths of colonies in our apiaries.

We also observed that there was no consistent type of soil for a particular depth of soil. Nevertheless, the density of SHB varied with soil depth. Although Spiewok and Neumann (2006) claimed that SHB constantly reproduce in all colonies but at low levels, not all our soil samples were infested with beetles. Considering only those infested soil samples, there were more SHB recorded in the first 10 cm soil (mostly just below the surface), only a few at 20 cm, and no beetle at 30 cm .These observations on soil depth agree with the findings of Pettis and Shimanuki (2000) and Schmolke (1974) indicating that most beetles pupate at <10 cm or below the soil surface. This preference of the uppermost layer for SHB pupation was probably due to the presence of decaying litter or loose organic materials for easy burrowing of larvae as well as emergence of adult beetles.

Based on laboratory studies, Schmolke (1974) showed that SHB pupation was not influenced by soil type. Although Schmolke (1974) observed that both soil moisture and soil density affected adult emergence, Schmolke claimed that soil density has the most profound effect especially in the burrowing of larvae into the soil. Recently, Ellis *et al.* (2004a) observed that a combination of soil moisture, soil density and soil type significantly affected pupation success in the laboratory. In a field setting, however, the amount of precipitation, field slope and others can potentially affect soil moisture levels. Our apiaries were about 2.88 km apart. It is likely that both locations had received similar amounts of rain. From May to early June, it rained at least once a week (www.weatherunderground.com). However, different soil textures have different soil moisture retention curves. Macdonald and Ellis (1990) demonstrated that soil samples held at a bulk density of 1.5 g/cc with a moisture level of -0.38 bar is equivalent to 29% moisture for silty clay, 26% for loam and 11% for loamy sand. Although apiary 1 was predominantly silty/clay, the colonies were positioned at the edge of a creek allowing rain water to drain into the creek while possibly supplying enough soil moisture for successful SHB pupation. Several trees were also present along the creek which provided some shade. On the other hand, apiary 2 was on a slope allowing fast drainage and drying of its sandy loam soil. However, the presence of trees in apiary 2 may have provided enough shade to maintain soil moisture favorable for the growth of biotic agents.

The presence of biotic agents such as pathogens and parasites can regulate populations of soil-dwelling insects. Two species of entomopathogenic nematodes have been shown to be infective against prepupal stage of SHB under laboratory conditions (Cabanillas and Elzen 2006). In this study, we found teneral adult beetles infested with unknown species of nematodes in apiary 2 only. No nematode-infested beetle was found in apiary 1. These infested beetles appeared to be intact but when dissected the inside tissues were devoured by nematodes. Several nematode-infested body parts of adult SHB such as thoraces and elytra were also recovered. No larva or pupa was found infested with nematodes. This is the first report of a natural infestation of nematodes in teneral adults of SHB in the soil. The extent of nematode infestation in the soil was not determined. Nevertheless, the level may have been enough to significantly decrease the number of adult beetles emerging, which can potentially become the scavengers of established colonies.

Conclusion and Recommendations

SHB population successfully developed in various types of soil. They also preferred to pupate just below the surface of the soil; larvae did not move deeper into the soil to pupate even in soil having the same soil types at different depths. Thus, selection of apiary sites is very important. By avoiding putting colonies under trees where decaying litter or loose organic materials are abundant, pupation success may be reduced. Dissipation of soil moisture necessary for SHB pupation is expected in exposed apiaries. However, the use of parasitic nematodes in the soil may also help reduce SHB populations in shady apiaries. Their use can be an essential component of an IPM (Integrated Pest Management) program designed to control SHB.

Acknowledgments

Tony Stelzer, Gary Delatte and Chris Tubbs were very instrumental in the field while Rachel Watts helped in counting beetles in the laboratory. Dr. Khoung B. Nguyen of the Entomology and Nematology Department, University of Florida attempted to identify the nematodes. This research was completed in cooperation with the Louisiana Agricultural Experiment Station.

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