Some Observations on the Small Hive Beetle, *Aethina tumida* Murray in Russian Honey Bee Colonies

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ABSTRACT

The response of Russian honey bees to adult small hive beetles (SHB) and their effect on colony survival were compared with Italian honey bees. In a study conducted near Titusville, FL using observation hives, both stocks removed significantly more dead beetles (Russian = 67%, Italian = 57%) than live beetles (Russian = 13%, Italian = 0). Russian honey bees also removed live beetles (4.01 ± 1.96 min) as fast as the Italian bees removed dead beetles (4.30 ± 1.11 min) suggesting heightened aggressiveness toward SHB adults by Russian bees. This behavior may have played an important role in the survival of field colonies monitored for five months near Lula, Mississippi. Results from this experiment showed that Italian bees were more susceptible to small hive beetle infestation, having significantly higher colony mortality (41%) observed in October (five months after colony establishment). Russian colonies suffered lower mortality (10%) during this time, which was similar to the colony loss recorded for Italian bees in June, one month after the colonies were made. It is possible that aggression to invading SHB by Russian bees may have prevented oviposition of beetles in the colonies. Studies should be done to further assess beetle removal or aggression to beetles as a potential mechanism of resistance to SHB by this mite-resistant stock.

Key words: Russian honey bees, resistance, small hive beetle, colony survival

INTRODUCTION

The small hive beetle (SHB) is native to sub-Saharan Africa and was first found in the United States in 1998 (Elzen et al. 1999). It has since spread to 29 states and is especially prevalent within the southeastern region of the U.S. (Neumann and Elzen 2004). SHB can be abundant and very damaging to honey bee colonies (Hood 2000, Neumann and Elzen 2004). Often, slimy conditions brought by the beetles' feeding activity and fecal deposits lead to the putrefaction of brood and honey causing eventual loss of the honey crop and beekeeping equipment. Furthermore, its presence in European honey bee (EHB) colonies significantly reduces brood production, honey production, and flight activities of bees (Ellis et al., 2003).

Chemicals are commonly used to control SHB; Check Mite+® as a colony treatment and Gardstar® for drenching the soil in front of the hives, but both produce negative effects. Therefore, complementary strategies such as the use of resistant stock will be helpful to manage this pest. Resistance to SHB has been widely known in Cape honey bees (*Apis mellifera capensis*) of South Africa. Cape bees regulate SHB populations within colonies by a myriad of defensive mechanisms such as corralling, social encapsulation, and active aggression by the bees [see review by Neumann and Elzen (2004) for more examples]. EHBs also defend their colonies against SHB, but at a lower frequency (Elzen et al. 2001). Recently, Ellis et al. (2004) compared the hygienic behavior of Cape bees and EHB toward egg-infested brood. The authors found that EHB removed infested brood as well as did the SHB-resistant Cape bees. Their results are very promising. Thus, further study on the response to SHB by different stocks of EHB should be studied. This study was conducted to determine if mite-resistant Russian honey bees exhibit some potential resistance or tolerance toward the small hive beetles.

MATERIALS AND METHODS

a) Response of honey bees to adult beetles - The behavior of Russian honey bees, which are known to be resistant to both varroa (Rinderer et al. 2001a and b) and tracheal mites (de Guzman et al. 2001a and b, 2002), toward adult SHB was compared to that of colonies headed by Italian queens purchased from California. Fifteen Russian and 15 Italian uninfested honey bee colonies were established in Louisiana in June 2002 and taken to Florida for this study. Behavioral responses of both stocks were observed using 10 observation hives. On the day prior to observations being made, a frame of honey and a frame of brood together with the queen, and about 3,000 adult bees from each colony were transferred into an observation hive. Five colonies for each bee type were evaluated at a time. In each observation hive, one dead beetle (control) and one living adult beetle were introduced sequentially through access windows. All live beetles were collected from a highly infested colony. Dead beetles were collected from the bottom board of a dead colony. Both dead and living beetles were painted with acrylic paint to facilitate observing the beetles. The response of the bees towards the adult beetles was monitored for 15 minutes by two observers. The incidence of worker bees removing, stinging or biting, and inspecting beetles (bees antennated beetles and then moved away) was noted. Beetles were considered removed when the bees physically removed the beetles from the hive or when bees continuously carried the beetle around the observation hive for a minimum of one minute in a manner that we interpreted to be a search for the hive entrance. The length of time it took for the bees to remove beetles was recorded. Two trials were conducted with different observers for each trial.

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b) Effects of SHB on colony survival - A total of 76 colony divisions were made in May 2003 and established in two apiaries near Lula, MS. Each colony consisted of about three deep frames of brood, two honey frames, enough bees to cover five frames and five empty frames. In one apiary, 16 and 24 colonies received Italian and Russian queens, respectively. In another apiary, there were 12 Italian and 24 Russian colonies. Italian queens were obtained from the same queen breeder who supplied queens used in the observation hives. The colonies were set on 4-way pallets; each pallet had only one type of queen. The number of beetles per colony was determined by examining individual frame on top of a white table and counting beetles. Bee population was determined by counting the number of frames of brood and the number of frames covered by bees. Varroa infestation was estimated by sampling about 300-500 bees and washing using soapy water (Rinderer et al. 2003). Colonies were monitored in June and October 2003.

c) Data analyses - For the response of honey bees to adult beetles, data for each behavioral response were analyzed using a one-way analysis of variance (ANOVA) using Proc Mixed (SAS Institute 2001, Version 8.2). Since there was no bee type by month interaction, a one-way ANOVA was also used to determine differences in the number of adult SHB, number of frames of bees, number of frames of brood, and varroa infestation among the different groups (June Italian, June Russian, October Italian, and October Russian). Colony mortality was examined using Fisher's exact test.

RESULTS AND DISCUSSION

a) Response of honey bees to adult beetles - Table 1 shows the proportion and number of different responses of Russian and Italian honey bees towards the introduced adult SHB. Both stocks removed significantly ($P = 0.001$) more dead beetles (20/30 = 67% [Russian], 17/30 = 57% [Italian]) than live beetles (4/30 = 13% [Russian], 0/30 = 0 [Italian]). No differences in the proportion of biting/stinging ($P = 0.662$), and inspecting ($P = 0.454$) activities were observed. Among the three responses, both honey bee stocks performed significantly more inspecting than biting/stinging or removing of either dead or living beetles. The low percentages obtained for the biting/stinging behavior in both stocks may be due to the difficulty of accurately quantifying these responses, especially when bees were extremely agitated (multiple biting and stinging) by the presence of beetles. Further, the lower frequency of this behavior in the Russian colonies was probably a result of their quick capture and removal of beetles. Despite fast movement of adult beetles, Russian honey bees removed live beetles as fast as the Italian bees removed dead beetles ($P = 0.299$) suggesting heightened aggressiveness

Table 1. Percentage and number of different activities (mean ± SE) by Russian and Italian honey bees in response to introduced adult small hive beetles.

Bee Type	Behavioral Responses			Total Responses	Removal Time (min)*
	R	BS*	I*		
Russian					
Dead beetle	3.94 (20) [a]	24.21 (123)	71.85 (365)	508	3.50 ± 0.81
Live beetle	0.63 (4) [b]	25.99 (164)	73.38 (463)	631	4.01 ± 1.96
Italian					
Dead beetle	3.57 (17) [a]	27.73 (132)	68.70 (327)	476	4.30 ± 1.11
Live beetle	0 [b]	37.73 (203)	62.27 (335)	538	0

Means within a column followed by the same letters were not significantly different (P > 0.05).
*** Not significant**
R=Removal, BS=Biting and Stinging, I=Inspection
Numbers of activities are in ( ).

Table 2. Small hive beetle population, bee population, varroa infestation and colony mortality (mean ± SE) in Russian and Italian colonies.

Treatments	No. of SHB	Number of deep frames		% Varroa infestation on adult bees	Colony Mortality (%)
		Adult bees	Brood		
Italian, June 2003	6.28 ± 1.10[b]	4.92 ± 0.59[b]	3.17 ± 0.38[b]	0.6 ± 0.3	10.34[b]
Russian, June 2003	5.32 ± 1.24[b]	6.24 ± 0.48[b]	4.14 ± 0.32[b]	1.4 ± 0.4	0[b]
Italian, October 2003	141. 82 ± 35.69[a]	14.09 ± 1.7[a]	5.79 ± 0.97[a]	1.0 ± 0.4	41.38[a]
Russian, October 2003	97.08 ± 10.19[a]	11.25 ± 0.84[a]	4.12 ± 0.23[b]	1.1 ± 0.3	9.52[b]

Means followed by the same letters in columns were not significantly different (P > 0.05).

towards SHB adults by Russian bees. Italian bees bit and stung beetles, yet they failed to remove any live beetles. Stinging also can be an indicator of aggressiveness. However, we did not consider it as such because no live beetle was killed as a result of the bees' stinging attempts. Adult SHB has elytra or hard exoskeleton, which may have prevented sting penetration.

Other activities showed no significant differences between the two stocks, but also suggested trends of differential aggressiveness towards SHB. About 87% (13 out of 15) of Russian colonies removed at least one beetle compared to 67% (10 out of 15) of Italian colonies. Feeding of beetles by worker bees was also recorded in three Italian colonies. This activity was first observed between guard bees of *A. capensis* and imprisoned beetles by Ellis et al. (2002). Grooming behavior, such as removal of paint from the beetles, was also observed in five Italian colonies. Only one Russian colony appeared to groom beetles and no Russian bees displayed trophallactic activity toward beetles. Since some colonies were able to remove live beetles, field studies should be done to further assess live beetle removal as a potential mechanism of resistance to SHB.

b) Effects of SHB on colony survival - Since there was no interaction between bee type and month of observation, data for experiment 2 were analyzed using a one-way analysis of variance (ANOVA). Results showed that the number of beetles significantly differed among the different groups ($F = 27.32$, df = 3, 132, $P < 0.0001$) (Table 2). In June, both stocks had similarly low numbers of SHB. Initially, some colonies were not infested, but the highest counts of 25 and 55 adult beetles were recorded in the Italian and Russian honey bee colonies, respectively. The number of SHB significantly increased after five months (October), but no difference was detected between the two stocks. At this time, all colonies were infested with SHB ranging from 16-630 beetles for the Italian and 20-546 beetles for the Russian bees. A similar trend was also observed in the number of frames with adult bees ($F = 23.33$, df = 3, 132, $P < 0.0001$). In June, adult bee population was similarly low in both stocks, but increased significantly in October. Likewise, the two stocks had similar colony strength during this time. Analysis of the number of brood frames also revealed significant differences among the different groups ($F = 4.89$, df = 3, 132, $P = 0.003$). The amount of brood in June for both stocks, and in October for Russian bees was significantly lower than that of Italian bees observed in October.

Reports showed that even strong colonies of European honey bees can be overwhelmed by SHB infestation (Standford 1998). This circumstance occurs because adult females usually lay eggs in protected areas (Lundie 1940); eggs laid in unprotected areas are immediately removed as is also the case with African bees (Neumann and Härtel 2004). In Florida, EHB colonies harboring more than a thousand adult beetles and hundreds of larvae can cause colony deaths (Elzen et al. 1999, Hood 2000). However, EHB colonies can also host thousands of adult beetles without visible symptoms (Wenning 2001). In our second experiment, we did not observe thousands of beetles. Yet, colony deaths occurred and differed significantly ($P < 0.0001$) among the different groups. About 10% of the Italian colonies died in June, which was similar to the colony loss incurred by Russian bees in June and October. After five months, there were significantly (41%) more dead Italian colonies, despite their having similar numbers of beetles. All of the dead colonies had SHB adults and larvae crawling on slimy combs. SHB has caused considerable colony losses, particularly in the southeastern region of the United States (Hood 2000). Since infestation of varroa mites on adult bees was similarly low ($F = 0.79$, df = 3, 132, $P = 0.499$) (Table 2) in both stocks at both sampling periods, SHB infestation was likely the cause of colony death. European bees are less aggressive toward SHB than the resistant African honey bees (Elzen et al. 2001). However, this study suggests that variation in the aggressiveness toward SHB among EHB exists. Our first experiment showed that Italian honey bees failed to remove any live beetles. Perhaps, the early death of the Italian colonies may be explained by their inability to successfully defend their colonies from invading adult beetles. In contrast, the good survival of infested Russian colonies may be brought about by the aggressiveness of Russian bees toward adult beetles, as suggested by the successful removal of four live beetles in the first experiment.

Deaths of colonies may also be attributable to several other factors such as starvation, prolonged queenlessness, or varroa and tracheal mite infestations. Starvation of colonies is unlikely to have caused colony deaths since all dead colonies had ample honey present at the time of death. Honey was not harvested to avoid introduction of small hive beetles into our collaborator's honey house and surrounding apiaries, which were free of beetles at that time. It is also unlikely that queenlessness may have contributed to the eventual loss of the colonies. All colonies in June had large brood nests, so bees could have raised and replaced their own queens. Colony mortality may not be due to parasitic mites. Infestation of varroa mites on adult bees was only about 1% in all colonies. We did not monitor tracheal mite infestation in our colonies because several studies consistently showed that tracheal mite populations never grew to devastating levels in Webb, MS (de Guzman et al. 2001 a, b) which is about 41 miles away from Lula, MS.

This study suggests that Italian honey bees are more susceptible to SHB infestation than Russian bees, since they died earlier than the Russian bee colonies. However, more detailed field and laboratory studies should be done to further assess beetle removal or aggression to beetles as a potential mechanism of resistance to SHB by this mite-resistant stock.

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REFERENCES

de Guzman, L. I., T. E. Rinderer, G. T. Delatte, J. A. Stelzer, G. Beaman and C. Harper. 2001a. An evaluation of Far-eastern Russian honey bees and other methods for the control of tracheal mites. *Am. Bee J.* 141: 737-741.

de Guzman, L. I., T. E. Rinderer, G. T. Delatte, J. A. Stelzer, J. L. Williams, L. D. Beaman, V. Kuznetsov, S. J. Bernard, M. Bigalk and H. Tubbs. 2001b. Multi-state field trials of ARS Russian honey bees 3. Responses to *Acarapis woodi* 1999, 2000. *Am. Bee J.* 141: 810-812.

de Guzman, L. I., T. E. Rinderer, G. T. Delatte, J. A. Stelzer, G. Beaman and V. Kuznetsov. 2002. Resistance to *Acarapis woodi* by honey bees from Far-eastern Russia. *Apidologie* 33: 411-415,

Ellis J. D., C. W. W. Pirk, H. R. Hepburn, G. Kastberger, P. J. Elzen. 2002. Small hive beetles survive in honey bee prisons by behavioral mimicry. *Naturwissenschaften* 89: 326-328.

Ellis, J. D., H. R. Hepburn, K. S. Delaplane and P. J. Elzen. 2003. The effects of adult small hive beetles, *Aethina tumida* (Coleoptera: Nitidulidae), on nests and flight activity of Cape and European honey bees (*Apis mellifera*). *Apidologie* 34: 399-408.

Ellis, J. D., K. S. Delaplane, C. S. Richards, R. Hepburn, J. A. Berry and P. J. Elzen. 2004. Hygienic behavior of Cape and European *Apis mellifera* (Hymenoptera: Apidae) toward *Aethina tumida* (Coleoptera: Nitidulidae) eggs oviposited in sealed bee brood. *Ann. Entomol. Soc. Am*. 97: 860-864.

Elzen, P.J., J.R. Baxter, D.Westervelt, C. Randall, K.S. Delaplane, L. Cutts and W.T. Wilson. 1999. Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae), attacking European honey bees in the Western Hemisphere. *Apidologie* 30: 361-366.

Elzen, P. J., J. R. Baxter, P. Neumann, A. Solbrig, C. Pirk, H. R. Hepburn, D. Westervelt and C. Randall. 2001. Behavior of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida. J. Apic. Res*. 40: 40-41.

Hood, M. 2000. Overview of the small hive beetle *Aethina tumida* in North America. *Bee World* 81: 129-137.

Lundie, A. E. 1940. The small hive beetle, *Aethina tumida*. Science Bulletin 220. Union of South Africa, Dept. of Agriculture and Forestry (Entomological Series #3). 30 pp.

Neumann P. and P. J. Elzen. 2004. The biology of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae): Gaps in our knowledge of an invasive species. *Apidologie* 35: 229-247.

Neumann P. and S.Härtel. 2004. Removal of small hive beetle (*Aethina tumida* Murray) eggs and larvae by African honeybee colonies (*Apis mellifera scutellata* Lepeletier). *Apidologie* 35: 31-36.

Rinderer, T. E., L. I. de Guzman, G. T. Delatte, J. A. Stelzer, V. Kuznetsov, L. Beaman, R. Watts and J. Harris. 2001a. Resistance to the parasitic mite *Varroa destructor* in honey bees from Far-eastern Russia. *Apidologie* 32: 381-394.

Rinderer, T. E., L. I. de Guzman, G. T. Delatte, J. A. Stelzer, J. L. Williams, L. D. Beaman, V. Kuznetsov, S. J. Bernard, M. Bigalk and H. Tubbs. 2001b. Multi-state field trials of ARS Russian honey bees 1. Responses to *Varroa destructor* 1999, 2000. *Am. Bee J*. 141: 658-661.

Rinderer, T. E, L. I. de Guzman and H. A. Sylvester. 2003. Re-examination of the efficacy of a detergent solution for varroa mite detection. *Am. Bee J*. 144:56-562.

Sanford, M. T. 1998. *Aethina tumida*: a new beehive pest in the Western Hemisphere. *Apis* 16: 1-5.

SAS Institute, Inc. 2001. SAS User's Guide, Version 8.2, SAS Institute, Cary, NC.

Wenning, C. J. 2001. Spread and threat of the small hive beetle. *Am. Bee J*. 141: 640-643.