

ORIGINAL RESEARCH ARTICLE



# Detection and removal of brood infested with eggs and larvae of small hive beetles (*Aethina tumida* Murray) by Russian honey bees.

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Received 30 October 2007, revised manuscript received 4 January 2008, accepted for publication 4 January 2008.

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## Summary

The response of Russian honey bees to brood infested with small hive beetle (SHB) eggs and larvae was compared to that of a commercial stock (predominantly of *A. m. ligustica*). Test brood was grouped as follows: a) NoP = no perforation either of capping or cell wall; b) PWall = perforation of cell wall only; c) PCap = perforation of capping only; and d) PBoth = capping and cell wall perforations. All perforations were made by SHB. Our results showed that brood cells with perforations of the cell wall (PWall and PBoth) had the highest infestation ( $76.53 \pm 2.10\%$ ) and number of eggs ( $58.46 \pm 2.85$  eggs). Because PCap showed low levels of infestation ( $29.17 \pm 3.31\%$  and  $15.60 \pm 1.31$  eggs per infested cell), we calculated brood removal based on data from PWall and PBoth groups. Within 6 h, both stocks removed the contents of  $39.24 \pm 4.94\%$  of PWall cells. A higher removal rate of  $50.51 \pm 5.80\%$  was observed in PBoth cells. These two groups of brood had the highest numbers of eggs per infested cell (50-70 eggs). Eggs hatched after the 6 h observation and subsequent hygienic removal was of brood infested with larvae. At 20 h, additional  $56.41 \pm 4.62\%$  (PWall) and  $42.04 \pm 4.91\%$  (PBoth) removal rates were observed. Overall, the cumulative removal rates for both stocks were similar with means of  $85.11 \pm 2.98\%$  and  $84.32 \pm 4.29\%$  for the commercial and Russian honey bees, respectively. In conclusion, we observed that both honey bee stocks were able to detect eggs inside the sealed brood cells and remove them with the infested brood.

## Detección y eliminación de cría infestada con huevos y larvas del pequeño escarabajo de las colmenas (*Aethina tumida* Murray) por abejas rusas

### Resumen

La respuesta de las abejas rusas a la infestación de la cría con huevos y larvas del pequeño escarabajo de las colmenas (PEC) se comparó con la de un stock comercial (predominantemente de *A. m. ligustica*). La cría examinada se agrupó como sigue: a) NoP = opérculo o pared de la celda no perforada; b) PPared = sólo pared de la celda perforada; c) POpe = perforación sólo en el opérculo; y d) PAmbos = perforaciones en la pared y en el opérculo. Todas las perforaciones fueron hechas por PEC. Nuestros resultados mostraron que las celdas de cría con perforaciones de la pared (PPared y PAmbos) tuvieron la infestación más alta ( $76,53 \pm 2,10\%$ ) y el mayor número de huevos ( $58,46 \pm 2,85$  huevos). Como POpe mostró niveles de infestación bajos ( $29,17 \pm 3,31\%$  y  $15,60 \pm 1,31$  huevos por celda infestada), calculamos la eliminación de la cría basándonos en datos de los grupos PPared y PAmbos. A las 6h, ambos grupos eliminaron el contenido de  $39,24 \pm 4,94\%$  celdas PPared. Una mayor tasa de  $50,51 \pm 5,80\%$  se observó en celdas PAmbos. Estos dos grupos de cría mostraron los valores más altos de huevos por celda infestada (50-70 huevos). Los huevos nacieron después de seis h de observación y la consecuente eliminación higiénica fue de cría infestada con larvas. A las 20 h, se observaron tasas de eliminación adicionales de  $56,41 \pm 4,62\%$  (PPared) y  $42,04 \pm 4,91\%$  (PAmbos). En total la tasa acumulativa de eliminación para ambos stocks fue

similar con valores medios de  $85,11 \pm 2,98\%$  y  $84,32 \pm 4,29\%$  para las abejas comerciales y rusas respectivamente. En conclusión hemos observado que ambos stocks de abejas fueron capaces de detectar huevos dentro de las celdas con cría operculada y de eliminarlos de la cría infestada.

**Keywords:** small hive beetles, *Aethina tumida*, hygienic, removal, Russian honey bees, *Apis mellifera*

## Introduction

The ability of honey bees to detect, uncap and remove brood that is inflicted with diseases, pests or parasites before the infection causes serious colony damage has been well-recognized. Honey bees that demonstrate this behaviour are termed 'hygienic'. Hygienic honey bees can resist the prevalence of American foulbrood (AFB) (Rothenbuhler, 1964), chalkbrood (Gilliam *et al.*, 1983) and varroa mites (Peng *et al.*, 1987; Boecking and Drescher, 1991; Spivak, 1996; Ibrahim and Spivak, 2006; Ibrahim *et al.*, 2007). Two sub-Saharan African honey bee subspecies (*Apis mellifera capensis* and *A. m. scutellata*) are reported to have well-developed removal behaviour toward eggs, larvae and adults of small hive beetles (SHB) *Aethina tumida* (Spiewok and Neumann, 2006). A high removal of SHB infested brood (about 91%) was observed in *A. m. capensis* (Ellis *et al.*, 2003). However, results of a subsequent study using the same honey bee subspecies showed a lower brood removal of 67%, which was comparable to the 57% removable rate observed in the same experiment in unknown stocks of European honey bees (EHB) (Ellis *et al.*, 2004). Using colonies of mixed race EHB, a wide range (10.6 – 77.2 %) of removal rates was observed (Ellis and Delaplane, 2008).

The two African honey bee subspecies are also known to remove eggs and larvae of SHB as part of nest cleaning. Eggs laid at the edges (unprotected) of Apidea (polystyrene mating nucleus hives with a transparent cover for the top) lids and larvae on Petri dishes were easily removed by *A. m. scutellata*, but these bees had difficulty removing eggs laid on the inner lids (protected) of the Apidea boxes (Neumann and Härtel, 2004). *A. m. capensis* quickly removed larvae on Petri dishes and also SHB eggs that were deliberately placed between two glass slides (Spiewok and Neumann, 2006). This increased nest cleaning behaviour by African honey bees may have been the selective force resulting in SHB laying eggs in crevices and cracks (Lundie, 1940).

The removal response to SHB infested brood by different stocks of EHB has not been studied. Russian honey bees are known for their resistance to varroa and tracheal mites (Rinderer *et al.*, 2001; de Guzman *et al.* 2002a; 2006; 2007) and also found to be hygienic toward dead brood (de Guzman *et al.*, 2002b). In an effort to fully establish the value of this mite resistant stock, their potential resistance to SHB was explored in this study by assessing the bees' response toward SHB infested brood in comparison to that of a commercial stock. The effect of time on brood removal was also investigated to determine the ability of both stocks to detect SHB eggs inside the sealed brood.

## Materials and Methods

### Colony set-up

Twenty four colonies were used in this study. Russian queens ( $n = 12$ ) were obtained from the Russian honey bee program. The commercial colonies (*A. m. ligustica*,  $n = 12$ ) were headed by queens purchased from a queen breeder in California who advertises Italian queens. All colonies had three 16.5 cm deep Langstroth hive bodies with equal amounts of bees, brood and food stores. All colonies had low infestations of SHB (~2-3 adults per colony). In order to obtain brood that was infested with SHB, the method described by Ellis *et al.*, (2003; 2004) was adopted with modifications. A brood frame with about 70-90% capped brood was removed from each colony. In each brood frame, three sections were established. Two sections each received 20 adult beetles and one section had no beetle, and served as the control. The beetles used were a mixture of laboratory reared and wild specimens collected from infested colonies. The beetles were allowed to lay eggs in the brood cells. They were confined to specific brood areas using push-in cages (7.62 cm x 7.62 cm) made of hardware cloth (8 mesh) which was covered with nylon screen with smaller mesh (~20 mesh). At the middle of each cage, an access hole (diameter = 1 cm) plugged with a cork facilitated the introduction of beetles.

Prior to the caging of SHB, each brood section was photographed using a digital camera. Beetles were then caged on the brood overnight for about 15 h and then removed. Thereafter, each brood section was examined under a dissecting microscope for the presence of perforations either of the capping or of the cell wall, which were mapped using a print of the initial photograph. Since brood cells along the borders of each section were damaged while installing the cage, the first and last rows of brood, and the first and last cells of each row were not included in the study. Within each section, all brood that was damaged or chewed by caged SHB was also mapped, but was excluded in the calculation of brood removal. Mapping of brood sections for all colonies took about 4 h.

One treated section (hereafter referred to as treated check) was then removed from the comb, frozen and later examined for the presence or absence of SHB eggs. This section was used to estimate the level of SHB infestation in the treated section without pulling brood from it. Thereafter, the frames with treated and control sections were replaced in the middle of the brood chamber of their respective colonies.

### Estimation of brood removal

In order to determine the ability of the two stocks to detect, uncap and remove brood infested with SHB eggs only, the treated and control sections were individually photographed at 2, 4, and 6 h after the brood frames were returned to their respective

colonies. All pictures were loaded into a computer and sections were examined for the number of cells emptied and for the presence or absence of perforations of the cells that were not emptied. To estimate removal of brood infested with larvae, at 20 h all sections were again photographed and examined for the presence of perforations. At this time, all brood that had not been removed by the bees was manually removed and examined for the presence of SHB. The amount of brood removed by honey bees during a particular observation period was determined by comparing photographs. Two trials were conducted.

### Data analyses

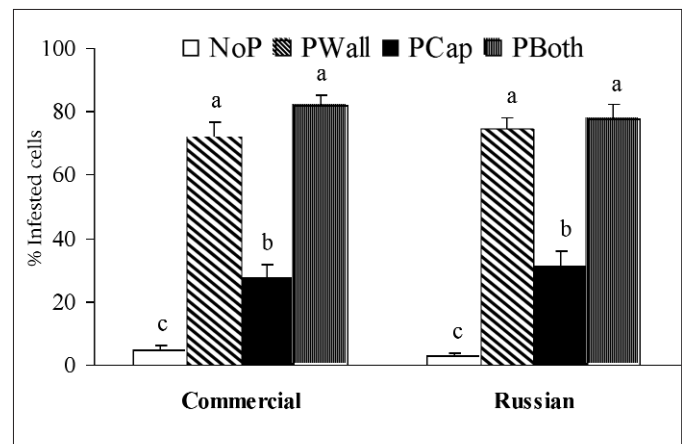
Brood cells within each of the three sections were grouped as follows: a) NoP = no perforation either of capping or cell wall; b) PWall = perforation of cell wall only; c) PCap = perforation of capping only; and d) PBoth = capping and cell wall perforations. For analyses, only those sections with perforations (PWall, PCap, PBoth) were considered. We observed that only 1/4 of the brood with perforations of the capping (PCap) were infested while PWall and PBoth had the highest infestations. Thus, we calculated brood removal only using data from these two groups. Two colonies (one from each stock) were excluded from the analysis, since nearly all brood regardless of capping type had been removed by the bees during the first 6 h of observations. To establish the bees' ability to detect the presence of eggs in the brood cells, only data during the first 6 h of observations were analyzed since after 6 h eggs were hatching. Two separate analyses were also made for the overall brood (eggs and larvae) removal; one using PWall and PBoth data only, and the other using all data from the three groups with any type of perforations (PWall, PCap and PBoth) as described by Ellis *et al.* (2004).

Data on percentage of infestation and number of eggs per infested cell (intensity) at the beginning (treated check), and at the end of the experiment (treated and control sections), and the proportion of brood removed were analyzed using Proc Mixed. A four-way factorial ANOVA was first used with honey bee type, perforation type, treatment section, and observation time modelled as fixed effects. When significant interactions were detected, further analyses were conducted using either ANOVA or t-test (SAS version 8.2, SAS Institute, 2001).

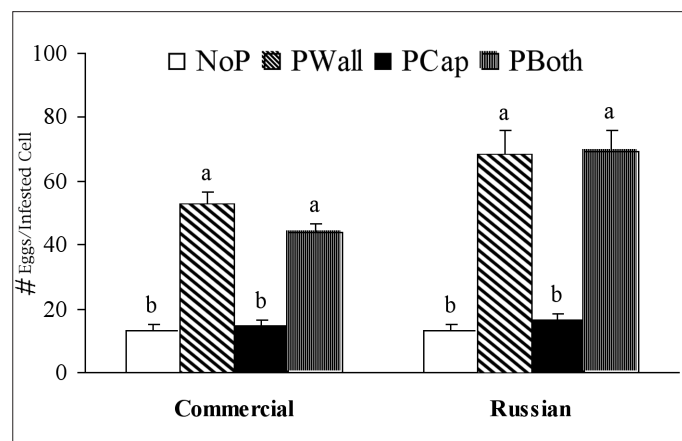
## Results

### Percentage of brood infested and intensity of SHB in the treated check sections

ANOVA revealed no interaction between honey bee stock and perforation types ( $F = 0.46$ ;  $df = 3, 182$ ;  $P = 0.707$ ) for the infestation of SHB in the treated check sections. There was a significant effect of perforation type ( $F = 179.45$ ,  $df = 3, 182$ ,  $P < .0001$ ) (Fig. 1). Both groups of cells with perforations of the walls (PWall and PBoth) had the highest levels of SHB infestation followed by PCap (perforations of the capping only). Non-perforated brood cells (NoP) had the lowest infestation level. No difference between stocks was detected ( $F = 0.00$ ;  $df = 1, 182$ ;  $P = 0.982$ ) with commercial and Russian honey bees having means of



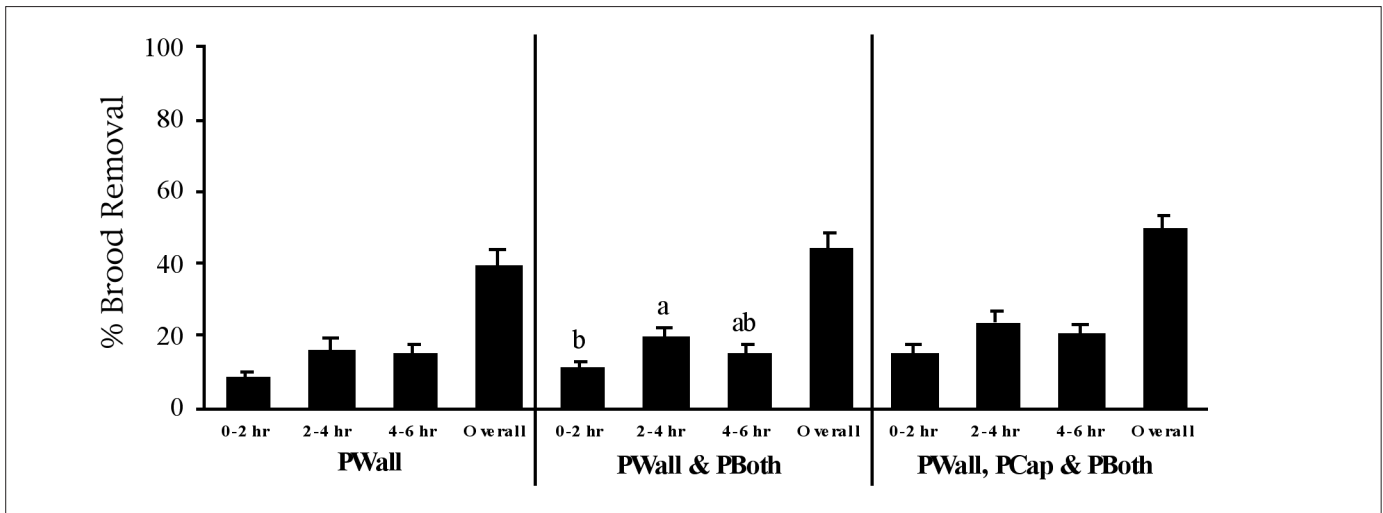
**Fig 1.** The percentage of cells infested with SHB eggs in four categories of commercial and Russian honey bee brood. NoP = no perforation either of capping or cell wall, PWall = perforation of cell wall only, PCap = perforation of capping only, PBoth = capping and cell wall perforations. The treated check section provided information on the infestation levels of the treated sections monitored for brood removal. For each stock, bars with the same letters are not significantly different ( $P > 0.05$ ).



**Fig 2.** The number of SHB eggs per infested cell of commercial and Russian honey bees for four categories of brood cells. NoP = no perforation either of capping or cell wall, PWall = perforation of cell wall only, PCap = perforation of capping only, PBoth = capping and cell wall perforations. For each stock, bars with the same letters are not significantly different ( $P > 0.05$ ).

46.48 ± 3.74% and 45.79 ± 3.79% of their infested brood, respectively.

For the number of eggs per infested cell, a significant interaction between stock and perforation type ( $F = 3.40$ ;  $df = 3, 157$ ;  $P = 0.019$ ) was observed (Fig. 2). For both stocks, PWall and PBoth groups had the highest egg intensity while the lowest egg load was recorded in the PCap and NoP groups. The interaction resulted from both PWall and PBoth categories having numerically more eggs in Russian colonies.



**Fig 3.** Brood removal at 0-2 h, 2-4 and 4-6 h in the PWall group only, PWall and PBoth combination and when PWall, PCap and PBoth groups were combined. PWall = perforation of cell wall only, PCap = perforation of capping only, PBoth = capping and cell wall perforations. For PWall and PBoth combination, bars with the same letters are not significantly different ( $P > 0.05$ ).

### Detection and removal of egg-infested brood

Analysis of the PWall group only showed no interaction between time and stock ( $F = 1.12$ ;  $df = 2, 113$ ;  $P = 0.329$ ), and no time of observation ( $F = 2.40$ ;  $df = 2, 113$ ;  $P = 0.096$ ) or stock ( $F = 1.57$ ;  $df = 1, 113$ ;  $P = 0.214$ ) effects for the detection and removal of egg-infested brood (Fig 3). When considering the two groups with sidewall perforations (PWall and PBoth), no time by stock interaction ( $F = 1.17$ ;  $df = 2, 229$ ;  $P = 0.312$ ) or stock effect ( $F = 3.26$ ;  $df = 1, 229$ ;  $P = 0.072$ ) were detected. However, the time of observation significantly ( $F = 3.36$ ;  $df = 2, 229$ ;  $P = 0.036$ ) influenced removal of egg-infested brood with the highest removal observed at 4 h. No stock ( $F = 0.96$ ;  $df = 1, 339$ ;  $P = 0.328$ ), observation time ( $F = 2.72$ ;  $df = 2, 339$ ;  $P = 0.067$ ) or two-way interaction ( $F = 0.98$ ;  $df = 2, 339$ ;  $P = 0.375$ ) effects were observed when all perforated cells (PWall, PCap and PBoth) were analyzed.

During the first 6 h of observation, detection and removal of egg-infested brood in the PBoth group ( $50.51 \pm 5.80\%$ ) was similar to the removal observed in the PWall group ( $39.24 \pm 4.94\%$ ) (t-test,  $P = 0.143$ ). At 20 h, brood removal in the PWall group was significantly (t-test,  $P = 0.036$ ) higher than in the PBoth group with a mean of  $56.41 \pm 4.62\%$  and  $42.04 \pm 4.91\%$ , respectively.

Analysis of the proportion of brood removed showed that detection of eggs and removal of brood was significantly ( $F = 8.85$ ;  $df = 1, 352$ ;  $P = 0.003$ ) higher in the treated group ( $30.17 \pm 2.13\%$ ) than the control group ( $3.05 \pm 0.93\%$ ) during the first 6 h of observations.

### Cumulative brood removal after 20 h

Using data from PWall, PCap and PBoth, ANOVA revealed no interaction between honey bee stock and perforation types ( $F = 0.57$ ;  $df = 2, 90$ ;  $P = 0.566$ ) and no perforation type ( $F = 1.69$ ;  $df = 2, 90$ ;  $P = 0.191$ ) effect for the overall brood removal in the treated sections. Also no stock effect ( $F = 0.02$ ;  $df = 1, 90$ ;  $P = 0.896$ ) was detected with commercial and Russian honey bees removing about  $81.87 \pm 3.41\%$  and  $82.0 \pm 3.73\%$  of infested

brood, respectively. Similarly, analysis of data from PWall and PBoth showed no interaction between honey bee and perforation types ( $F = 0.47$ ;  $df = 1, 55$ ;  $P = 0.495$ ) and no perforation type effect ( $F = 0.19$ ;  $df = 1, 55$ ;  $P = 0.663$ ). High rates of brood removal were obtained with the commercial honey bees having a mean of  $85.11 \pm 2.98\%$  and Russian honey bees having a mean of  $84.32 \pm 4.29\%$ . No difference between the two stocks was detected ( $F = 0.14$ ;  $df = 1, 55$ ;  $P = 0.708$ ).

### Percentage of brood infested and intensity of larvae in the treated sections after 20 h

After 20 h, all brood cells that were not emptied by the bees were examined for the presence of perforations and SHB. Analysis showed no significant interaction between stock and perforation type ( $F = 0.44$ ;  $df = 1, 140$ ;  $P = 0.727$ ) and no stock effect ( $F = 0.19$ ;  $df = 1, 140$ ;  $P = 0.666$ ). However, infestation was significantly influenced by perforation type ( $F = 15.67$ ;  $df = 1, 140$ ;  $P < 0.0001$ ). Of the cells not emptied by the honey bees, PBoth had the highest infestation ( $47.57 \pm 8.11\%$ ), followed by PWall ( $31.48 \pm 4.42\%$ ) and PCap ( $24.41 \pm 3.17\%$ ). The lowest infestation was observed in the NoP group with a mean of  $7.25 \pm 1.19\%$ . The rates of infestation were lower but follow the same trends on the rates of infestation observed in the treated check sections.

The same trend was observed for the intensity of larvae. There was no significant interaction between stock and perforation type ( $F = 0.03$ ;  $df = 1, 103$ ;  $P = 0.992$ ) and no influence of stock ( $F = 0.81$ ;  $df = 1, 103$ ;  $P = 0.370$ ). A significant effect of perforation type ( $F = 5.94$ ;  $df = 1, 103$ ;  $P = 0.0009$ ) was detected. Both PWall ( $17.54 \pm 2.04$  larvae) and PBoth ( $16.93 \pm 3.86$  larvae) had the highest number of larvae per infested cell. NoP and PCap showed the lowest intensity with a mean of  $9.88 \pm 1.71$  and  $7.99 \pm 0.90$  larvae, respectively.

## Discussion

Our results showed that both Russian and the commercial honey bees tested in this study recognized and removed brood that was infested with SHB eggs to the same extent. Both bee stocks removed the contents of about 40% of the PWall (with perforation of the cell wall only) group, and 50% of the PBoth (perforations of both capping and cell wall) group within 6 h of introduction. According to Gramacho and Spivak (2003), hygienic bees initiate uncapping, and removal of dead or diseased brood more quickly than non-hygienic bees. Hygienic behaviour bioassay normally uses dead brood, which is observed after 24 h. Thus, ~40% removal of the PWall group can be considered very quick since the SHB eggs inside the live sealed brood were removed within only 6 h.

The African honey bees (*A. m. scutellata*), which are resistant to SHB had difficulty in removing well-concealed eggs (Neumann and Härtel, 2004). This difficulty of removing concealed eggs may explain the lower removal rate observed in the PWall than in the PBoth group since the eggs were more concealed.

Although no complete or 100% removal was observed in this study, the removal of 40–50% of the most infested brood within 6 h will certainly result in a significant reduction in the number of larvae inside the colony. This early defensive response against eggs of SHB is advantageous in preventing further damage by larval feeding. The larval stage is the most damaging period of the beetles' life because they feed on brood, honey and pollen causing slimy conditions of combs (Lundie, 1940).

If we determine cumulative brood removal (after 20 h) using data from all perforated brood cells (PWall, PCap and PBoth) as described by Ellis *et al.* (2004), our results (commercial =  $81.87 \pm 3.41\%$ ; Russian =  $82.0 \pm 3.73\%$ ) were lower than the removal rate (91%) observed by Ellis *et al.* (2003) in Cape honey bees. However, rates of brood removal for both stocks were higher than those reported by Ellis *et al.* (2004) for Cape honey bees (67%) and unknown stocks of European honey bees (57%). A subsequent study using mixed-race EHB colonies showed a wide variation (10.6 – 77.2 %) in the removal of beetle-perforated brood cells (Ellis and Delaplane, 2008).

When PWall and PBoth groups were analyzed, commercial ( $85.11 \pm 2.98\%$ ) and Russian honey bees ( $84.32 \pm 4.29\%$ ) displayed higher removal rates than the Cape and European honey bees (Ellis *et al.* 2004), but lower than the Cape honey bee observation reported by Ellis *et al.* in 2003. These discrepancies may be due to the differences in the length of observation time (caging of beetles to brood removal determination). Ellis *et al.* (2004) determined brood removal after 72 h (excluding mapping time) while we used 40 h (including mapping time) for this study. High temperature (34°C, similar to the temperature inside the brood chamber) significantly accelerates SHB development (de Guzman and Frake, 2007). Thus, high brood removal observed by Ellis *et al.* (2003; 2004) was probably enhanced by the presence of older larvae inside the brood cells. SHB larvae have strong mandibles and can chew out of one sealed brood cell into another (personal observation). An infestation spreading because of SHB larval movement within brood may increase the apparent rate of brood removal by honey bees.

The detection of inconspicuous eggs inside the brood cells during the first 6 h of observations was probably enhanced primarily by olfactory cues. It is possible that SHB egg deposition induces the production of volatiles when eggs come in contact with the pupa. Likewise, the detection of brood infested with larvae was probably accelerated by the release of honey bee volatiles in response to SHB larval feeding. It is also possible that larval movement inside the brood cells may have assisted detection of infested brood. Insect frass and its volatile components can also provide cues in habitat location (Weiss, 2006). Thus, the presence of faeces of SHB larvae inside the brood cells may have assisted worker bees in locating the infested cells. While feeding of SHB larvae may induce pupae to synthesize and release volatile compounds, the increase in food intake will also result in increased faecal depositions as the SHB larvae moult (personal observation). Thus, it is possible that a complex blend of volatiles were emitted by SHB faecal deposits and by the damaged honey bee pupae.

We also observed that infestation of brood that was not removed by bees after 20 h was still high. High infestation may be due to the invasion of larvae freed from opened cells or in the process of being removed. New SHB infestation after test combs were returned to colonies was also observed as indicated by the presence of eggs in brood cells that showed no perforation on the original map. Further, decreased intensity was due to larvae that left from opened brood cells. A few larvae were seen crawling on the comb, but were excluded from the analyses since their original pupal host could not be traced.

Overall, our results show that both Russian and commercial honey bees can detect, uncap and remove brood infested with eggs and larvae of SHB, and that neither stock removes SHB eggs and larvae better than the other. This observation suggests that both stocks have good olfactory sensitivity and hygienic responses to the presence of SHB eggs and larvae. This behaviour may help minimize damage to colonies, since a large proportion of infested brood is removed before eggs have a chance to hatch into larvae.

## Acknowledgments

We thank T. Stelzer, G. Delatte, J. Wales and R. Spencer for their technical assistance. This research was completed in cooperation with the Louisiana Agricultural Experiment Station.



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