ORIGINAL RESEARCH ARTICLE

Alternative food sources of *Aethina tumida* (Coleoptera: Nitidulidae).

Sven Buchholz[1], Marc O Schäfer[2], Sebastian Spiewok[1], Jeffery S Pettis[3], Michael Duncan[4], Wolfgang Ritter[2], Robert Spooner-Hart[4] and Peter Neumann [5,6,7*].

[1]Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 4, 06099 Halle (Saale), Germany.
[2]Chemisches und Veterinäruntersuchungsamt Freiburg (CVUA), Fachgebiet Bienen, Am Moosweiher 2, 79108 Freiburg, Germany.
[3]USDA-ARS Bee Research Laboratory, Bldg. 476 BARC-E, Beltsville, MD 20705, USA.
[4]Centre for Plant and Food Science, University of Western Sydney, Richmond, NSW, Australia.
[5]Swiss Bee Research Centre, Agroscope Liebefeld-Posieux Research Station ALP, CH-3033 Bern, Switzerland.
[6]Eastern Bee Research Institute of Yunnan Agricultural University, Kunming, Yunnan Province, China.
[7]Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa.

*Corresponding author. Email: peter.neumann@alp.admin.ch

Summary

The small hive beetle (SHB) is a parasite and scavenger of honey bee colonies, but may also be able to exploit alternative food sources. We conducted experiments to shed further light on the role of alternative foods for SHB. 1) Laboratory choice experiments showed that adult SHB oviposit on fruit and even on decaying meat and that SHB larvae feed on it despite the presence of bee products. 2) In the laboratory, SHB reproduced on mango, banana and grapes at lower rates than on a pollen and honey mixture. 3) Adult SHB were rarely observed on fruit buckets in the field. They reproduced only when caged and in much smaller numbers than Drosophilidae and other Nitidulidae. 4) While *Aethina concolor* was repeatedly observed during a field survey, no adult SHB were found on any flowers. 5) Less than 2% of adult SHB survived on blooming pot plants and no reproduction was recorded, suggesting that flowers are unlikely to serve as an alternative food and breeding substrate. Nevertheless, the high degree of opportunism displayed, supports the view that honey bee nests are not essential for SHB survival and reproduction. Despite the observed high degree of SHB opportunism, it appears as if alternative food sources play a minor role only for reproduction in the field when host colonies are available. Even though SHB may use alternative food sources in the absence of bee hives (e.g. after migratory beekeeping), it is unclear whether this is likely to contribute to SHB population build up.

Fuentes alternativas de alimentación de *Aethina tumida* (Coleoptera: Nitidulidae)

Resumen

El pequeño escarabajo de las colmenas (PEC) es un parásito carroñero de las colmenas de abejas, pero también puede explotar fuentes de alimento alternativas. Hemos realizado experimentos para dilucidar el papel de alimentos alternativos para el PEC. 1) Experimentos de elección en el laboratorio mostraron que PEC adultos ponen huevos en fruta e incluso en carne en estado de descomposición, y que larvas de PEC se alimentan de ello a pesar de la presencia de productos de abejas. 2) En el laboratorio, PEC se reproduce en mango, plátano y uvas en una tasa menor que en una mezcla de polen y miel. 3) PEC adultos fueron raramente observados en cajas de frutas en el campo. Se reprodujeron únicamente cuando estuvieron encerradas en cajas pero en menor número que Drosophilidae y otros Nitidulidae. 4) Mientras que *Aethina concolor* se observó repetidamente durante un viaje de muestreo al campo, no se encontró ningún adulto PEC en ninguna flor. 5) Menos del 2% de PEC adultos sobrevivieron en plantas floreciendo y no se observó reproducción, lo cual sugiere que las flores no sirven como alimento alternativo ni como sustrato para la reproducción. En cualquier caso, el alto nivel de oportunismo desarrollado, apoya la observación de que los nidos de abejas

nos son esenciales para la supervivencia ni para la reproducción de PEC. A pesar del alto nivel de oportunismo observado en PEC, parece que las fuentes de alimento alternativas juegan un papel menor únicamente para la reproducción en el campo cuando hay disponibles colmenas hospedadoras. Aunque PEC puede usar fuentes de alimento alternativas en ausencia de colmenas de abejas (p. ej. después de movimientos migratorios de las colmenas), no está claro si este hecho contribuiría al crecimiento de las poblaciones de PEC.

Keywords: *Aethina tumida, Apis mellifera*, flower blossoms, fruit, honey bee, nutrition, small hive beetle

Introduction

The beetle taxon Nitidulidae consists of species occupying a large variety of different ecological niches, including scavengers, predators and herbivores. Thus, feeding on different food sources such as decaying fruit, fermenting plant juices, fungi, flowers, stores of social insects, insect larvae or dead vertebrates have all been reported (Lundie, 1940; Borror *et al.*, 1989; Nadel and Pena, 1994; DiZinno *et al.*, 2002; Habeck, 2002; Ellis *et al.*, 2002; 2008). In light of the wide variety of accepted food sources, some nitidulid beetles may be relatively opportunistic. With regard to insect pests like the small hive beetle (SHB), *Aethina tumida* Murray, knowledge about the level of opportunism is essential for their control, as alternative feeding or reproduction sites probably undermine the efficacy of treatments in apiaries. Since SHB naturally infest and also reproduce in commercial bumble bee colonies in the field (Spiewok and Neumann, 2006) and in greenhouses (Hoffmann *et al.*, 2008), SHB seem to be rather opportunistic.

SHB is endemic to sub-Saharan Africa (El-Niweiri *et al.*, 2008) but has become an invasive species (Neumann and Elzen, 2004; Hassan and Neumann, 2008; Neumann and Ellis, 2008). It is known as a parasite and scavenger of honey bee, *Apis mellifera* L., colonies (Lundie, 1940; Schmolke, 1974; Neumann and Elzen, 2004), but laboratory studies have shown that SHB can feed and reproduce on Kei apples (Ellis *et al.*, 2002). In an abandoned apiary, a large number of adult SHB were caught in traps baited with different fruit (Eischen *et al.*, 1999), suggesting that at least some types of fruit may serve as alternative food sources in the absence of honey bee colonies. These experiments did not, however, show whether alternative substrates would also be accepted in the presence of bee products.

In order to further clarify the utilization of alternative food sources by SHB we conducted the following experiments:

1) In the laboratory, we performed oviposition and feeding choice trials between fruit, meat, honey bee pollen and brood to investigate the usage of alternative food sources in the presence of bee products. We expected SHB to accept alternative substrates even when bee combs were available.

2) We evaluated the reproductive success and mortality of adult SHB on mango, grapes, banana and a honey and pollen paste (1:1) in the laboratory. We expected differential reproductive success and mortality according to the protein and fat content of the offered food stuffs.

3) Fruit were offered at various distances from honey bee colonies, to test whether they (a) attract adult SHB, (b) enable reproduction outside of honey bee colonies, and

(c) to assess the influence of ant predation on SHB reproduction in the field.

4) We determined the rate of survival of SHB on blooming plants as a possible alternative food source in gauze cages in the field. We expected SHB to survive on pollen and nectar provided by the flowers.

5) A botanical survey was conducted to investigate the abundance and distribution of adult SHB on a range of blooming flowers in the vicinity of naturally infested colonies. If SHB were a facultative parasite, we hypothesized that they may also visit flowers.

These experiments will allow a better assessment of the degree of opportunism in SHB concerning alternative breeding and feeding substrates.

Materials and Methods

Experiments were performed in 2004/5 at Rhodes University, Grahamstown (South Africa), in 2006 at the Bee Research Laboratory, Beltsville, Maryland (USA) and at the University of Western Sydney (Australia). At all research facilities, laboratory rearing was established using field sampled adult SHB and standard protocols (Muerrle and Neumann, 2004). The first generation of emerging adults was sexed (Schmolke, 1974) and males and females were kept separately prior to the experiments.

Laboratory trials with fruit

Oviposition and larval feeding choice experiments
To investigate the oviposition preference of SHB, newly emerged adults were fed with a pollen and honey paste (2:1) for four days and introduced in 2.8 l plastic boxes (16 × 22 × 8 cm; n = 35 SHB each). Per trial, four substrates were placed in different corners of the boxes: A) Nine boxes were each provided with: 30 g banana (Musa sp.) slices, 15 g cut strawberries (*Fragraria ananassa*), pollen comb and honey comb [2 × 4 cm]. B) Ten boxes were each provided with 15 g beef schnitzel, 30 g banana slices, pollen comb and brood comb [2 × 4 cm]. After 30 h, the numbers of eggs were counted on all substrates.

Feeding preferences of hatching SHB larvae were investigated by introducing 200-500 eggs (< 24 h old) in the middle of the 2.8 l boxes. C) Fourteen boxes were equipped like A); D) 12 boxes were equipped like B). After 40 h, the numbers of larvae were counted on all substrates. All trials were conducted at +30°C and 60% RH in constant darkness.

The proportions of eggs and larvae were analyzed using Friedman ANOVAs and Wilcoxon matched pairs tests as post hoc tests (Bonferroni-Holm adjusted $\alpha = 0.008$). The medians

of the transformed data were used in goodness-of-fit analyses to compare the distribution of eggs and larvae on the same substrates. All analyses in this study were conducted using STATISTICA©.

Reproductive success and mortality of adult SHB
In Maryland (USA), N = 7 Ziploc™ containers (946 ml) were equipped with 180 g of cut mango (*Mangifera indica*), banana or grapes (*Vitis vinifera*). Water was provided in 5 ml glass vials. Three newly emerged male and female SHB were transferred into each of the containers, which were provided with an opening (Ø = 4.8 cm) in the lid covered by nylon gauze [mesh width < 0.05 cm] to provide ventilation and prevent SHB escape. As a positive control, we used pollen and honey paste (1:1; 30 g) on plastic comb (5 × 6 cm Permacomb®, J Steed; Catonsville, MD, USA). Water without any nutrition served as a negative control. All containers were kept in an incubator at +30°C and 60% RH in constant darkness. After 13 days, surviving adults were transferred into new containers with fresh diets and water, following the suggestion of Ellis *et al.* (2002), to separate adults from feeding larvae and to prevent a potential negative influence due to space limitation. After 26 days, adult mortality was determined. The offspring were provided with their respective food ad libitum until all larvae had reached the wandering stage (Schmolke, 1974). Then, their numbers were recorded. For the evaluation of their pupation success, 50 randomly chosen wandering larvae each were set on moist (multi purpose) sand in similar Ziploc™ containers (N = 7 per trial; for grapes: N = 3) and with identical environmental conditions as described above. The emerging adults were sampled on a daily basis.

To detect potential variations in reproductive success on different diets in the laboratory, the absolute numbers of wandering larvae (n) were log-transformed and a one-way ANOVA was performed with Newman-Keuls as post hoc tests (α = 0.05). Kruskal-Wallis tests and multiple comparisons as post hoc tests (α = 0.05) were used to test for differences in adult beetle mortality and rate of successful pupation between diets in the laboratory, both expressed in [%].

Field trials with fruit
In Richmond (NSW, Australia), field experiments were conducted to evaluate the acceptance of fruit as a food source and breeding site during local springtime (Nov. – Dec. 2006).

Four different trials with N = 6 replicates each were performed as follows:

(I) New plastic buckets (10 l) were filled ~3/4 with sieved, moist local soil which was covered with insect screen (Cyclone®, mesh size = 1.0 mm²) to allow any SHB wandering larvae to enter the soil and to prevent emerging adults from escaping. One mango, one banana and ~200 g grapes were placed on top of the screened soil. Only mangos and bananas were slit to facilitate beetle access, as we observed in our laboratory experiments (2.1.2.) that adults can easily penetrate the grape peels. The buckets were covered with a plastic mesh (mesh width = 0.49 cm²) to prevent access by larger animals. Two timber logs (5 × 6 × 25cm) were used as spacers between the rim and lid of the buckets to enable SHB access and to cover the trials from direct weather exposure.

(II) The same design was used as in (I) but newly emerged adult SHB (3 males, 3 females) were transferred from the aboratory rearing onto the fruit. Another sheet of insect screen was applied on top of the buckets to prevent SHB escape.

(III) One trial identical to (II) was additionally equipped with a circle of anti-ant glue, Tac-gel (Rentokil Initial Pty. Ltd; Chatsworth, Australia), on the outside of each bucket to ward off possible ant predation.

(IV) The negative control was similar to (I) but without any fruit.

To compare the influence of the immediate presence or absence of honey bee colonies on the acceptance of fruit as alternative food source, two locations on the University campus were chosen. One set with all four trials (N = 24 buckets in total) was placed within an apiary with an average natural infestation level of 21 [13; 30] SHB per colony based on visual colony surveys (Spiewok *et al.*, 2007; Neumann and Hoffmann, 2008; N = 48 colonies, ~50 m from a honey extraction facility and ~10 m distance to the nearest hives). A second set of trials was located in a meadow with at least ~600 m distance to the nearest hives.

The buckets were observed on a daily basis for immigrant SHB. Water (150 ml) was supplied to all trials whenever required to prevent soil dehydration. After three weeks, the fruit and soil of all buckets were screened for SHB.

Morphometric characteristics of nitidulid larvae found in the trials were compared to local SHB reference larvae from the laboratory rearing (Lundie, 1940; Schmolke, 1974; Hayashi, 1978). For comparisons of the frequency of reproduction (number of buckets with offspring) between recorded taxa (*A. tumida*, other Nitidulidae, Drosophilidae) a Friedman ANOVA and Wilcoxon matched pairs tests as post hoc tests were conducted. Comparisons of the magnitude of reproduction (numbers of larvae) between the recorded taxa were analyzed with Wilcoxon matched pairs tests. Mann-Whitney U-tests (α = 0.05) were conducted to compare magnitude and frequency of species' reproduction between fruit trials (II and III).

Field trials with flowers
In Beltsville (USA), three different flower species of the taxon Asteraceae were tested as a potential food source of SHB in August 2006. The plants were obtained from a local nursery, where they had been visited by a variety of insects. The plants were arranged in the following trials each with N = 6 replicates: a) *Rudbeckia hirta* L var. "Goldilocks" (blooming); b) *R. hirta* (cut off flowers and butts); c) *Coreopsis verticillata* L, var. "Zagreb" (blooming); d) Pots with pollen and honey paste (30 g; 1:1) in wet pulp paper on a Petri dish [Ø = 5.5 cm] were used as positive control; e) Pots with soil and wet pulp paper were used as negative control.

Each potted plant was enclosed in a cylindrical wire fence and then covered in gauze sacks (insect screen, mesh width = 1.0 mm²). Plants without flowers were used to test whether SHB can survive on a phytophagous diet in the absence of pollen or nectar. Per cage, n = 20 newly emerged adults were transferred from the laboratory rearing into the sacks. All trials were placed on wood pallets in the USDA apiary under a pavilion to protect them from rain and direct sunlight. The trials were sprayed with

water on a daily basis and the plants were watered whenever required. Surveys were conducted on days 10 and 14 to assess the rate of adult survival, as most of the individuals would have been dead by this time without feeding (Schmolke, 1974; Pettis and Shimanuki, 2000). Kruskal-Wallis tests and Mann-Whitney U tests as post hoc tests (Bonferroni-Fischer adjusted α = 0.006) were performed to evaluate differences in the survival rate of adult SHB caged on flowers in the field. Wilcoxon matched pairs test (α = 0.05) was conducted to compare the number of live adult SHB after 10 and 14 days.

Botanical survey for SHB

A variety of five flowering plants, all located on the campus of the University of Western Sydney, Australia, were surveyed in local springtime 2006, once on 2 December (6:30 pm), 5 December (11:30 am) and 11 December (6:30 am) as well as three times on 14 December (9:30 am; 2:30 pm; 8:30 pm). Per survey, 20 blossoms were randomly chosen for detection of SHB on: a) *Rosa canina* L.; b) *Romneya coulteri* Harv.; c) *Chrysanthemum leucanthemum* (L.) Merr.; d) *Gardenia augusta* (L.) Merr.; e) *Rosa* sp. L. The plants were selected because prior to the experiment

they had been visited by other coleopteran species. Their blossoms were manually searched with a bucket underneath, and all insects were sampled with an aspirator

Results

The results are given as medians [1st; 3rd quartile]. If not mentioned in the text, statistical details are shown in the corresponding tables.

Laboratory trials with fruit

Oviposition and larval feeding choice experiments
Adult SHB laid eggs (trials A and B) and SHB larvae fed on alternative substrates (trials C and D) despite the presence of bee products (Fig. 1). The numbers of eggs and larvae on the different substrates differed significantly (Friedman ANOVAS: A: χ^2_3 = 19.2, p < 0.001; B: χ^2_3 = 25.2, p < 0.001; C: χ^2_3 = 24.3, p < 0.001; D: χ^2_3 = 30.1, p < 0.001). The proportions of eggs or larvae on bananas were higher than on strawberries or honey
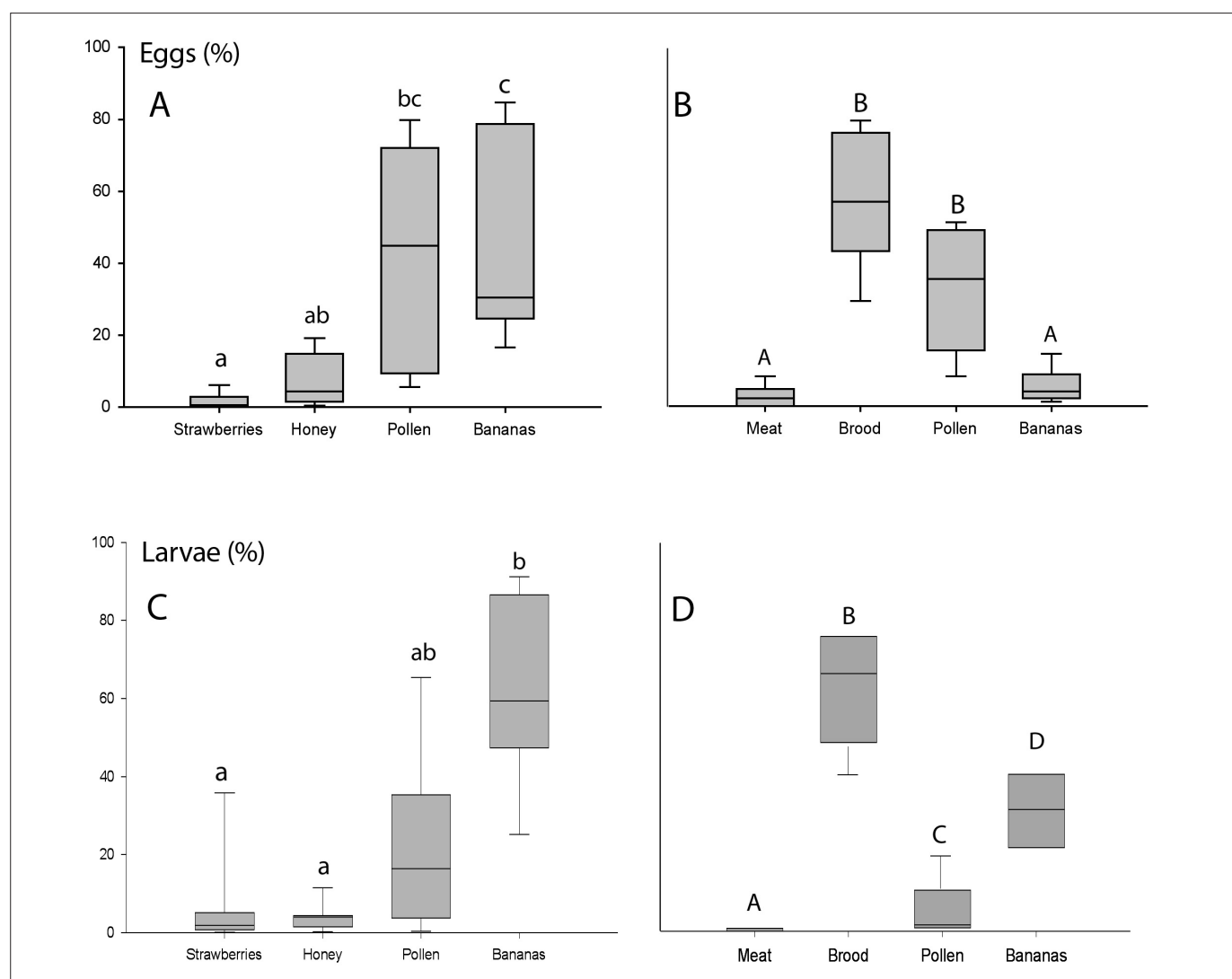
Fig 1. Choice experiments: proportions of SHB eggs (A, B) and larvae (C, D) on different substrates. Medians, quartiles, minimum and maximum values are shown. Boxes in one series marked with the same letter are not significantly different at adjusted α = 0.008 (Wilcoxon matched pairs tests).

comb but were not significantly different to the numbers on pollen, when no bee brood was available. However, the proportions of eggs and larvae on bananas were significantly smaller compared to bee brood, the most attractive feeding substrate. No significant differences in the relative distributions of eggs and larvae on the different substrates could be found between A and C ($\chi^2_3 = 0.3$; p > 0.96) or B and D ($\chi^2_3 = 2.5$; p > 0.47).

Reproductive success and mortality of adult SHB
In the negative control (water diet), nearly all adult SHB (100 [92; 100]) were dead after 26 days. More adults died each in trials with grapes and banana compared to trials with bee products and mango. Mortality of adults decreased from grapes, banana, mango to the pollen and honey diet ($H_3 = 8$; p < 0.05; Fig. 2A). The relative reproductive success, based on the numbers of wandering larvae, was pollen-honey > mango > banana > grapes. Apart from banana and mango, we found significant differences in the numbers of wandering larvae between all diets ($F_3 = 53.7$; p < 0.0001; Fig. 2B). No adults reproduced in the negative controls. No significant differences in the percentages of emerging adults were found between the diets ($H_3 = 2.3$; p < 0.52; Fig 2C) and almost all larvae (97 [87.5; 100]) developed into adults.

Field trials with fruit
Nitidulidae (predominantly *Carpophilus* species) and Drosophilidae were the most abundant insects and occurred in all fruit trials (I-III). The same was true for the occurrence of ants (*Pheidole megacephala*, *Linepithema humile* and *Camponotus nigriceps*) in the unprotected fruit trials (I and II). Blattelidae occurred exclusively in the fruit trials without gauze (I). Adult SHB were rarely observed during the daily surveys: In the meadow, only one adult SHB was found within the banana of an open fruit bucket (I) on day 8. Within the apiary, adult SHB occurred three times on the gauze of buckets of trial III: one on day 15 and two on day 16. Another adult SHB was detected within the banana of an open fruit bucket (I) on day 17. No insects were ever found in the negative controls (IV), which were therefore excluded from further analyses. SHB larvae were only detected in fruit buckets with confined SHB (II and III). As no significant differences were detected between reproduction in trials located in the apiary or in the meadow (Mann-Whitney U-tests: p > 0.12), the respective trials were pooled for further analyses (per trial II and III, N = 12 buckets each). The frequency (number of buckets) of SHB reproduction was significantly lower compared to other nitidulid species and Drosophilidae in ant-protected trials (Friedman ANOVA: $\chi^2_2 = 20$; p < 0.0001; Wilcoxon matched pairs tests: Z = 2.80; p < 0.006) as well as those without ant-glue ($\chi^2_2 = 14$; p < 0.001; Z = 2.37; p < 0.02; for both trials adjusted $\alpha = 0.017$; Table 1A). Comparing the magnitude of reproduction (number of larvae per bucket), again SHB were outnumbered by other Nitidulidae (for both trials: Z = 3.06; p < 0.003; α = 0.05; Table 1B). No significant differences in the frequency or magnitude of reproduction were detected between trials with (III) or without (II) ant-protection (U ≥ 54; p ≥ 0.26).
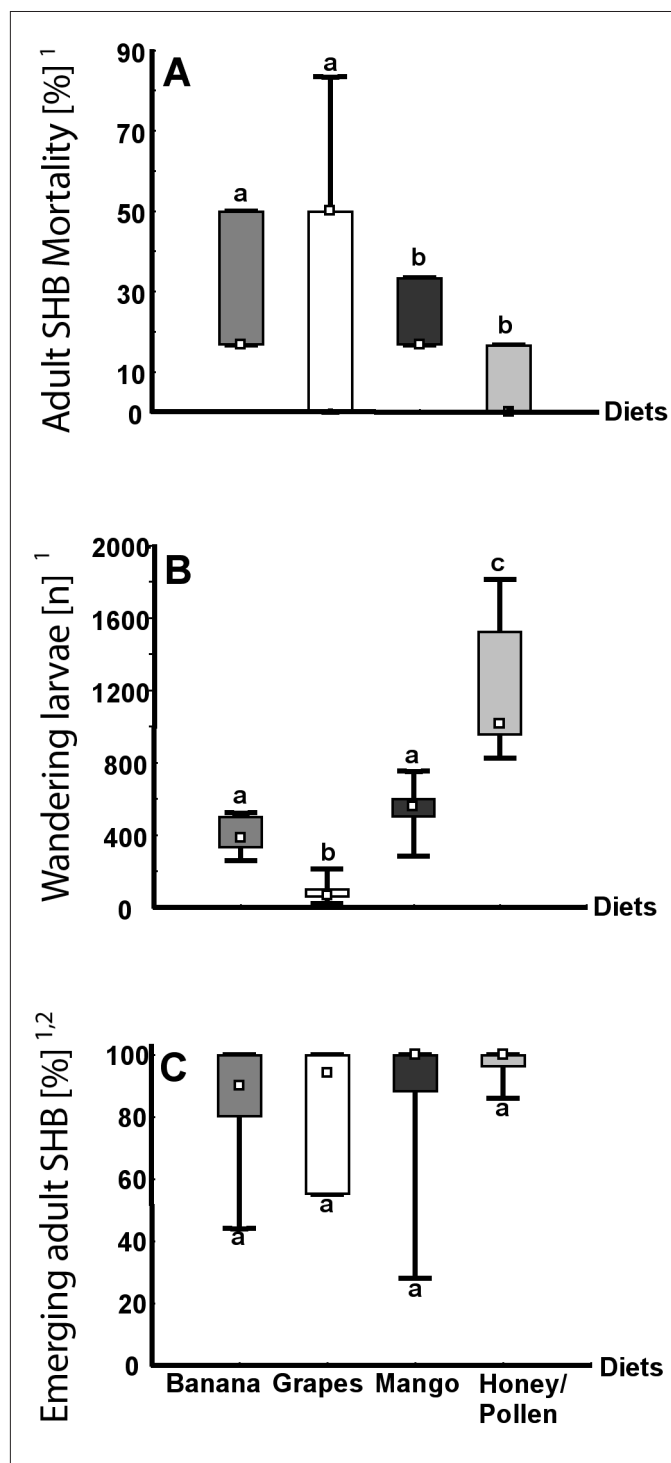
[1]Different letters indicate significant differences between diets (A, C: multiple comparison, B: Newman-Keuls test; $\alpha = 0.05$). [2]Based on a sample of 50 wandering larvae per diet

Fig 2. (A) Mortality [%], (B) reproductive success in numbers of wandering larvae [n] and (C) pupation success in [%] of emerging adults of *A. tumida* reared on different diets in the laboratory after 26 d. Medians, quartiles, minimum and maximum values are shown.

Table 1. Frequency (A) and magnitude (B) of SHB reproduction in buckets with fruit and soil after three weeks of exposure on the campus of the University of Western Sydney, Richmond. Values in A: numbers of buckets, in B: Medians [1st; 3rd quartile] of larvae.

Trials	A: Frequency of reproduction [n][1]			B: Magnitude of reproduction [n][2]	
	SHB	Other beetle larvae	Drosophilidae	SHB	Other beetle larvae
(II) Fruit SHB undergauze	2[a]	12[b]	12[b]	0 [0; 0][A]	51 [30; 78][B]
(III) Fruit SHB under gauze, anti-ant glue	5[a]	12[b]	12[b]	0 [0; 2][A]	69 [37 87][B]

[1] Different lower case letters indicate significant differences in reproduction frequency (Wilcoxon matched pairs tests, adjusted a = 0.017).

[2] Different capital letters indicate significant differences between the magnitudes of reproduction (Wilcoxon matched pairs tests, a = 0.05).
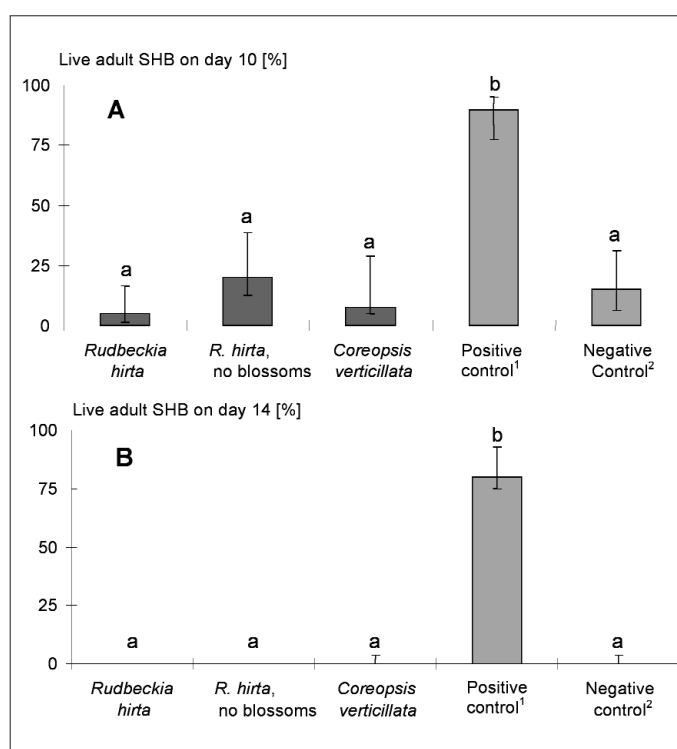
[1] pollen-honey diet, no plant or soil.

[2] soil but no plant or food.

Fig 3. Live adult SHB [%] on different plants in gauze cages after (A) 10 days and (B) 14 days in the field. Columns indicate medians, bars 1st and 3rd quartiles. Columns marked with different letters indicate significant differences (Mann-Whitney U tests, adjusted α = 0.006).

Field trials with flowers

After ten (Fig. 3A) and 14 days (Fig. 3B), the numbers of live adult SHB on flowers were significantly lower in comparison to the positive controls with bee products, and not significantly different from the negative (water only) controls (Kruskal-Wallis tests: after ten days H_4 = 16.1; p < 0.003; after 14 days H_4 = 21.6 p < 0.001). However, the decrease in live adults on pollen and honey

was not significantly different between days 10 and 14 (Wilcoxon matched pairs test: Z = 1.60; p > 0.10). SHB larvae were only found in the positive controls after 10 and 14 days.

Botanical survey for SHB

No SHB were found during the six surveys on any of the flowers examined. However, 13 individuals of the hibiscus beetle, *Aethina concolor* Macleay, which is closely related to *A. tumida* and endemic to Australia (Gough and Hamacek, 1989), were observed on seven blossoms of *Romneya coulteri* and on six of *G. augusta*. *Rosa canina* and *R. coulteri* were regularly visited by honey bees.

Discussion

Our data support former findings that SHB readily exploit alternative food sources in the laboratory (Ellis *et al.*, 2002) and further suggest that such nutrition is also used despite the presence of bee products. While confined SHB reproduced at lower numbers compared to other insects, no SHB reproduction was detected in any of the open fruit trials. This suggests that SHB rarely use other foods as alternatives to hive products. While we could show that SHB visit fruit in the field, our observations did not show that adult SHB visit flowers or use them as an alternative food or breeding substrate.

Laboratory trials with fruit

Oviposition and larval feeding choice experiments
We were able to confirm the strong preference of female SHB for oviposition into capped brood cells (Spiewok, 2007; de Guzman *et al.*, 2008; Ellis and Delaplane, 2008). Elzen *et al.* (2000) also showed a preference of larvae for bee brood over other bee products. Nevertheless, our data also show that adult SHB readily laid eggs and that SHB larvae fed on fruit despite the presence of honey bee products. In the absence of bee brood, bananas constitute the favourite oviposition or feeding substrate. This might be explained by the high protein content and the moisture provided by bananas (Franke, 1997). Furthermore, a characteristic

odour component of bananas is isopentyl acetate (Mayr *et al*., 2003), which is an integral part of the alarm pheromone blend of honey bees (Boch and Shearer, 1966) and appears to be attractive to adult SHB (Torto *et al*., 2005; 2007). The results underline the strong connection of SHB with honey bees but also demonstrate their opportunistic acceptance of other breeding and feeding substrates. The relative distribution of the eggs was not significantly different to that of the larvae, indicating that oviposition preference matches larval feeding preference. However, it is unclear why larvae favour bee brood over pollen combs since pollen seems to be more efficient than brood for successful reproduction (Ellis *et al*., 2002). Although resource quality is important for larval development, correlations between oviposition site preference and optimal larval performance on chosen substrates are seldom found in other insects (Bernays and Graham, 1988; Thompson, 1988; Valladares and Lawton, 1991).

Reproductive success and mortality of adult small hive beetle. The number of wandering larvae produced on mango, banana, and grapes was smaller compared to a pollen and honey diet, probably due to the lower protein content of the fruit, (Franke, 1997; Roulston *et al*., 2000). Alternatively, but not mutually exclusively, decay of the fruit and / or fungal infections (Muerrle *et al*., 2006) may also play a role. As expected, no adults reproduced on water only. However, three out of 42 adults survived for 26 days on a water only diet. The severed body parts of the dead adults showed signs of cadaver consumption. Hence we deduce that cannibalism occurred, as described from the laboratory (Schmolke, 1974) and observation hive studies (Neumann *et al*., 2001). According to other reports, adult SHB kept in groups of 5 on water only diets survived for 8 d (Schmolke, 1974); when maintained singly with neither food nor water they survived for 5 d (Pettis and Shimanuki, 2000). Half of the SHB adults reared on grapes and ~17% on either banana or mango died, probably because they drowned in the liquids produced by the rotting fruit and / or died as a result of fungal infections (Lezama-Gutiérrez *et al*., 2000; Muerrle *et al*., 2006).

Field trials with fruit

There were no insects in the buckets with soil alone. Although two adult SHB were observed on exposed bananas, SHB offspring were only found in treatments II and III, where adult SHB were kept on the fruit with gauze. Here the reproductive success was extremely low in comparison to the laboratory fruit trials. We propose that the other insects may have out-competed SHB on fruit. Indeed, both adults and offspring of Drosophilidae and other Nitidulidae occurred in great abundance in all fruit treatments. Since an identification key for Australian nitidulid beetle larvae does not yet exist, nitidulid beetle larvae other than those of SHB could not be further determined. However, due to the abundance of adults of the fruit pest *Carpophilus* spec. in the buckets, the other beetle larvae are most likely *Carpophilus* larvae. Since *A. concolor* could not have passed the fine mesh screens on the buckets of trials II and III due to its size, we can exclude the occurrence of larvae of other *Aethina* species on the fruit. When not repelled by glue, foraging ants were also present in most buckets (21 out of 24) but had no impact on the overall reproduction, probably due to the abundance of fruit as a feeding

substrate. We conclude that SHB reproduction on fruit may also occur in the field but at orders of magnitude less than on bee products in hives, possibly due to competitors and / or predators. Thus, we assume that SHB prefers to reproduce on bee products whenever possible. Furthermore, SHB has not yet been reported to be a fruit pest, and in our study adult SHB were rarely found on fruit buckets.

Small hive beetles on flowers

In the field trials with flowers, almost all adult SHB died within 14 days, despite the provision of floral nectar and pollen, water and shelter. Furthermore, no SHB larvae were found in any of the flower trials. However, in the positive controls (pollen and honey diet) almost all adults survived and larvae were also observed. Obviously, SHB were not able to receive adequate nutrition from the nectar and pollen in the flowers provided in our field trial. Thus, successful reproduction of SHB in association with flowers alone seems to be very unlikely.

Botanical survey for small hive beetles

While no SHB were observed on any of the blossoms during the botanical surveys in Australia, the closely related species *A. concolor* was repeatedly found in the flowers of some species. The flowering plants we surveyed were unattractive to SHB, probably because successful reproduction of SHB in association with flowers alone seems to be very unlikely (see above). In combination with the results of the field trials, it appears unlikely that flowers serve as an alternative food for SHB.

Final conclusions

For an invasive species, the use of a wide range of food sources enhances the possibility of colonization and establishment in introduced areas (Ehrlich, 1989; Lodge, 1993). Our data show that SHB reproduces readily on a variety of fruit in the laboratory despite the presence of bee products, but clearly prefers bee brood. However, despite occasional visits on fruit, SHB did not reproduce voluntarily on alternative substrates in the field in the presence of bee colonies. Moreover, when adults were caged in field fruit buckets, their breeding success was orders of magnitude lower than on bee products. Finally, flowers are also very unlikely to serve as alternative food sources. In conclusion, it appears as if alternative food sources play only a minor role for SHB reproduction in the field when host colonies are available. The opportunism of SHB demonstrated in the laboratory experiments and the report of Eischen *et al*. (1999), however, indicates that SHB may use alternative food sources in the absence of bee hives (e.g. after migratory beekeeping). Fruits might help to maintain SHB populations during the temporary absence of honey bee colonies but due to the lower reproductive success in the laboratory and the field this is unlikely to result in high population numbers.

Acknowledgements

References

BERNAYS, E A; GRAHAM, M (1988) On the evolution of host specificity in phytophagous arthropods. *Ecology* 69: 886–892.

BOCH, R; SHEARER, D A (1966) Iso-pentyl acetate in stings of honey bees of different ages. *Journal of Apicultural Research* 5: 65–70.

BORROR, D J; TRIPLEHORN, C A; JOHNSON, N F (1989) *An introduction to the study of insects* (6th edition). Saunders; Orlando, FL, USA.

DE GUZMAN, L I; FRAKE, A M; RINDERER, T E (2008) Detection and removal of brood infested with eggs and larvae of small hive beetles (*Aethina tumida* Murray) by Russian honey bees. *Journal of Apicultural Research* 47(3): 216–221.

DIZINNO, J A; LORD, W D; COLLINS-MORTON, M B; WILSON, M R; GOFF, M L (2002) Mitochondrial DNA sequencing of beetle larvae (Nitidulidae: Omosita) recovered from human bone. *Journal of Forensic Science* 47: 1337–1339.

EHRLICH, P R (1989) Attributes of invaders and invading processes: vertebrates. *In* Drake, J A *et al.* (eds.) *Biological invasions: A global perspective.* John Wiley; Chichester, UK. pp. 315–328.

EISCHEN, F A; WESTERVELT, D; RANDALL, C (1999) Does the small hive beetle have alternative food sources? *American Bee Journal* 139: 125.

EL-NIWEIRI M A A; EL-SARRAG, M S; NEUMANN, P (2008) Filling the Sudan gap: the northernmost natural distribution limit of small hive beetles. *Journal of Apicultural Research* 47(3): 184–185.

ELLIS, J D; NEUMANN, P; HEPBURN, H R; ELZEN, P J (2002) Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural diets. *Journal of Economic Entomology* 95: 902–907.

ELLIS, J D; DELAPLANE, K (2008) Small hive beetle (*Aethina tumida*) oviposition behaviour in sealed brood cells with notes on the removal of the cell contents by European honey bees (*Apis mellifera*) *Journal of Apicultural Research* 47(3): 210–215.

ELLIS, J D; DELAPLANE, K; CLINE, A; JOSEPH, V; Mc HUGH, J V (2008) The association of multiple sap beetle species (Coleoptera: Nitidulidae) with western honey bee (*Apis mellifera*) colonies in North America. *Journal of Apicultural Research* 47(3): 188–189.

ELZEN, P J; BAXTER, J R; WESTERVELT, D; RANDALL, C; WILSON, W T (2000) A scientific note on observations of the small hive beetle, *Aethina tumida* Murray (Coleoptera, Nitidulidae), in Florida, USA. *Apidologie* 31: 593–594.

FRANKE, W (1997) *Nutzpflanzenkunde.* Thieme; Stuttgart, Germany. pp. 490.

GOUGH, N; HAMACEK, E L (1989) Insect induced bud fall in cultivated hibiscus and aspects of the biology of *Macroura concolor* (Macleay) (Coleoptera: Nitidulidae). *Journal of the Australian Entomology Society* 28(4): 267–277.

HABECK, D H (2002) Nitidulidae. *In* Arnett, R H; Thomas, M C; Skelly, P E; Frank, J H *American Beetles. Vol. 2.* Polyphaga: Scarabaeidea through Curculionidea. CRC Press; Boca Raton, FL, USA. pp. 311–315.

HASSAN A R, NEUMANN, P (2008) A survey for the small hive beetle in Egypt. *Journal of Apicultural Research* 47(3): 186–187.

HAYASHI, N (1978) A contribution to the knowledge of the larvae of Nitidulidae occurring in Japan (Coleoptera: Cucujoidea). *Insecta Matsum* N.S. 14: 1–97.

HOFFMANN, D; PETTIS, J S; NEUMANN, P (2008) Potential host shift of the small hive beetle (*Aethina tumida*) to bumble bee colonies (*Bombus impatiens*). *Insectes Sociaux*, 55: 153–162.

LEZAMA-GUTIÉRREZ, R; TRUJILLO-DE LA LUZ, A; MOLINA-OCHOA, J; REBOLLEDO-DOMINGUEZ, O; PESCADOR, A R; LÓPEZ-EDWARDS, M; ALUJA, M (2000) Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on *Anastrepha ludens* (Diptera: Tephritidae): Laboratory and Field Trials. *Journal of Economic Entomology* 93(4): 1080–1084.

LODGE, D M (1993) Biological invasions: lessons for ecology. *Trends of Ecological Evolution* 8: 133–137.

LUNDIE, A E (1940) The small hive beetle *Aethina tumida*. *Science Bulletin 220*, Dep. Agr. Forestry, Government Printer; Pretoria, South Africa. 30 pp.

MAYR, D; MÄRK, T; LINDINGER, W; BREVARD, H; YERETZIAN, C (2003) Breath-by-breath analysis of banana aroma by proton transfer reaction mass spectrometry. *International Journal of Mass Spectrometry* 224: 743–756.

MUERRLE, T M; NEUMANN, P (2004) Mass production of small hive beetles (*Aethina tumida* Murray, Coleoptera: Nitidulidae). *Journal of Apicultural Research* 43: 144–145.

MUERRLE, T M; NEUMANN, P; DAMES, J F; HEPBURN, H R; HILL, M P (2006) Susceptibility of adult *Aethina tumida* (Coleoptera: Nitidulidae) to entomopathogenic fungi. *Journal of Economic Entomology* 99: 1–6.

NADEL, H; PENA, J E (1994) Identity, behaviour, and efficacy of nitidulid beetles (Coleoptera: Nitidulidae) pollinating commercial Annona species in Florida. *Environmental Entomology* 23 (4): 878–886.

NEUMANN, P; ELZEN, P J (2004) The biology of the small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): Gaps in our knowledge of an invasive species. *Apidologie* 35: 229–247.

NEUMANN, P; HOFFMANN, D (2008) Small hive beetle diagnosis and control in naturally infested honey bee colonies using bottom board traps and CheckMite+ strips. *Journal of Pest Science* 81: 43–48.

NEUMANN, P; ELLIS, J D (2008) The small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): distribution, biology and control of an invasive species. *Journal of Apicultural Research* 47(3): 181–183.

NEUMANN, P; PIRK, C W W; HEPBURN, H R; SOLBRIG, A J; RATNIEKS, F L W; ELZEN, P J; BAXTER, J R (2001) Social encapsulation of beetle parasites by Cape honey bee colonies (*Apis mellifera* capensis Esch.). *Naturwissenschaften* 88: 214–216.

PETTIS, J S; SHIMANUKI, H (2000) Observations on the small hive beetle, *Aethina tumida*, Murray, in the United States, *American Bee Journal* 140: 152–155.

ROULSTON, T H; CANE, J H; BUCHMANN, S L (2000) What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecological Monographs* 70(4): 617–643.

SCHMOLKE, M D (1974) A study of *Aethina tumida*: the small hive beetle, Project Report, University of Rhodesia. 178 pp.

SPIEWOK, S (2007) Reproduction and population biology of the small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae). PhD thesis, Martin-Luther-Universität Halle-Wittenberg, Germany.

SPIEWOK, S; NEUMANN, P (2006) Infestation of commercial bumblebee (*Bombus impatiens*) field colonies by small hive beetles (*Aethina tumida*). *Ecological Entomology* 31(6): 623–628.

SPIEWOK, S; PETTIS, J; DUNCAN, M; SPOONER-HART, R; WESTERVELT, D; NEUMANN, P (2007) Small hive beetle, *Aethina tumida*, populations I: Infestation levels of honey bee colonies, apiaries and regions. *Apidologie* 38: 595–605.

THOMPSON, J N (1988). Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata* 47: 3–14.

TORTO, B; SUAZO, A; ALBORN, H; TUMLINSON, J H; TEAL, P E A (2005) Response of the small hive beetle (*Aethina tumida*) to a blend of chemicals identified from honey bee (*Apis mellifera*) volatiles. *Apidologie* 36: 523–532.

TORTO, B; BOUCIAS, D G; ARBOGAST, R T; TUMLINSON, J H; TEAL, P E A (2007) Multitrophic interaction facilitates parasite-host relationship between an invasive beetle and the honey bee. *Proceedings of the National Academy of Science* 104: 8374–8378.

VALLADARES, G; LAWTON, J H (1991) Host-plant selection in the holly leaf-miner: does mother know best? *Journal of Animal Ecology* 60: 227–240.