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Original article

Removal of small hive beetle (*Aethina tumida*) eggs and larvae by African honeybee colonies (*Apis mellifera scutellata*)

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Abstract – The removal of small hive beetle [SHB] eggs and larvae was studied in seven *Apis mellifera scutellata* colonies. Because female beetles can protect their eggs by oviposition in small cracks we introduced unprotected eggs and protected eggs into these colonies. Whereas all unprotected eggs were removed within 24 hours, 66 ± 12% of the protected eggs remained, showing that SHB eggs are likely to hatch in infested colonies. However, all larvae introduced into the same seven colonies were rejected within 24 hours. Workers responded quickly to the presence of SHB offspring in the colonies because 72 ± 27% of the unprotected eggs and 49 ± 37% of the larvae were removed within the first hour after introduction. The removal of SHB eggs and larvae was not correlated with colony phenotypes (size, amount of open and sealed brood, pollen and honey stores). Our data show that African colonies remove both SHB unprotected eggs and larvae within short periods of time. Therefore, we conclude that this removal behavior plays an important role for the apparent resistance of African honeybees towards SHB infestations.

***Aethina tumida* / *Apis mellifera* / honeybee / host-parasite relationship / small hive beetle**

1. INTRODUCTION

The small hive beetle, *Aethina tumida* Murray [SHB], is a honeybee parasite endemic to Africa, where it is considered only a minor pest (Lundie, 1940; Schmolke, 1974). In contrast, SHB can be harmful parasites in populations of European honeybees (Elzen et al., 1999). One possible explanation for differences in pest severity might be that honeybee subspecies sympatric with the SHB have evolved efficient resistance mechanisms. In particular, African honeybee colonies should remove efficiently SHB eggs and larvae.

It has been reported that African honeybee workers remove SHB eggs (Swart et al., 2001), but not a single study has quantified this behavior yet. Likewise, little is known of the removal of SHB larvae. Lundie (1940) and Schmolke (1974) describe the "jetting" behavior of the host bees (Fig. 1). Workers that get hold of a larva can carry it out of the colony at some distance (~20 meters; Lundie, 1940; Schmolke, 1974). Field observations indicate that larvae are efficiently rejected by such jettisoning workers (Lundie, 1940; Swart et al., 2001). This is supported by the observation hive study of Schmolke (1974), who reported that all introduced larvae are rejected within 24 hours. However, this jettisoning behavior has never been rigorously quantified in field colonies yet. Moreover, the potential impact of colony phenotypes on the removal of SHB eggs and larvae has also never been quantified.

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Figure 1. A jettisoning worker is carrying a small hive beetle larva.

Here we investigate the removal of SHB eggs and larvae by African honeybee colonies (*A. m. scutellata* Lepeletier).

2. MATERIALS AND METHODS

2.1. Experimental colonies and sampling of beetles

Seven unrelated colonies of *A. m. scutellata* were placed in 10-frame standard Langstroth hives with two boxes in a test apiary in Pretoria, South Africa. The bottom box contained honey, pollen and brood frames while the top box was empty. The colonies were given four days to settle down to prevent absconding before they were used as test colonies in the experiments. Adult SHB (N = 491) were collected from the bottom board, outer frames and from closed prisons (Neumann et al., 2001b) of a single infested *A. m. scutellata* colony. Then, beetles were reared in the laboratory following standard protocols (Neumann et al., 2001a) with modifications as described below for each experiment.

2.2. Egg removal

Freshly collected beetles (N = 371) were introduced into eight Apidea©-boxes containing pieces of comb with honey, pollen and brood of all stages. After 24 hours, the boxes were opened and the inner lids were removed. Because female beetles oviposit in small cracks (Lundie, 1940) we were able to obtain two kinds of eggs on these lids (Fig. 2): (a) unprotected eggs at the edges and (b) protected eggs around the inner circles. These lids were introduced into the test colonies (one lid into each colony) on top of the bottom box frames. After one, two, three, five, ten and 24 hours, the lids were briefly removed and remaining eggs were counted in the field using magnifying glasses [10×] before they were reintroduced into their respective test colony at the same within-hive location.

2.3. Larva removal

Larvae that are reared on a mixed diet including honey are often coated with a sticky film (personal observations). Preliminary tests indicated that such larvae can easily escape from open petri-dishes. However, these tests also indicated that larvae that are reared using a “dry” approach on small amounts

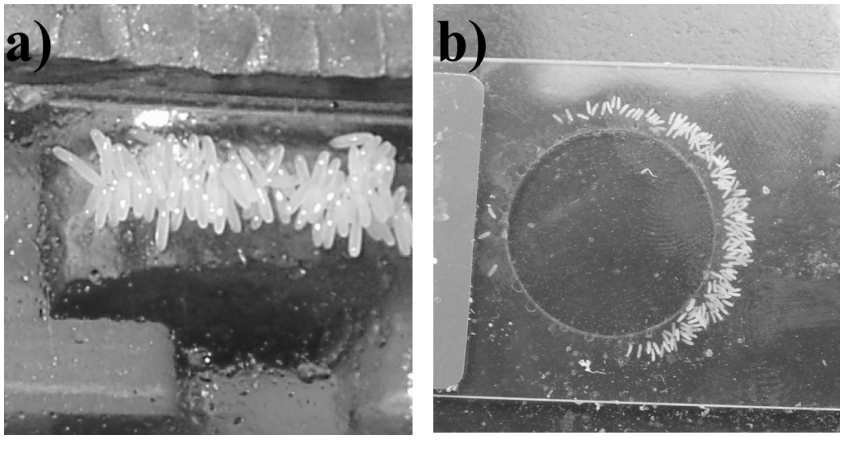

Figure 2. Unprotected (a) and protected (b) small hive beetle eggs that were laid onto the inner lids of the Apidea©-boxes.

of sealed honeybee brood, seem to have difficulties escaping from such open petri-dishes.

Freshly collected beetles (N = 120) were introduced into three containers with frames containing only small patches of sealed brood [~10 × 15 cm]. These pieces did not provide enough food for the larvae to reach maturity, so that all brood was consumed and larvae were not covered with sticky films. Larvae were collected from these containers and 100 larvae each were introduced into seven petri-dishes. Then, the dish were opened and introduced into each test colony on top of the bottom box frames. After one, two, four, seven and 24 hours, the dishes were briefly removed and remaining larvae were counted in the field before they were reintroduced into their respective test colony at the same within-hive location.

To control for the escape rate of larvae from the petri-dishes, three petri-dishes with 100 larvae each were introduced into containers and the number of remaining larvae in the open dishes was counted after one, three, five, nine and 24 hours.

2.4. Colony phenotype data

One day after the removal experiments were finished, colony phenotypes (size, amount of open and sealed brood, pollen and honey area) were evaluated for the seven test colonies using the standard Liebefelder method of colony estimation (Gerig, 1983; Imdorf et al., 1987).

2.5. Data analysis

Mann-Whitney U-tests were performed to test for differences in the removal rates of protected and unprotected eggs and to test for differences between the controls and the removal rates of larvae. Simple correlations (r-matrix) were performed between the colony phenotype data and the removal rates for SHB eggs and larvae.

3. RESULTS

3.1. Egg removal

Time to removal was determined for 9168 eggs (N = 1612 protected eggs and 7556 unprotected eggs). Because female beetles did not lay eggs around the inner circles in two rearing boxes egg removal rates for protected eggs were evaluated in five of the seven colonies (N = 7 for unprotected eggs). The number of tested unprotected eggs varied naturally between 248 and 2479 per test colony (1079.43 ± 1123.08; protected eggs: 322.4 ± 253.75, range: 74 to 716). The percentages of remaining eggs in the seven test colonies are shown in Figure 3. Significantly more protected eggs remained in the colonies than unprotected eggs (after one hour: unprotected eggs: 28.30 ± 26.47%, range 3.47 to 68.06%; protected eggs: 85.02 ± 13.67%, range: 70.53 to 99.55%; Z = –2.84, $P < 0.001$; after 24 hours: unprotected eggs: 0 ± 0%; protected eggs: 65.88 ± 11.54%, range: 47.39 to 77.97%; Z = –2.84, $P < 0.002$; Fig. 3).

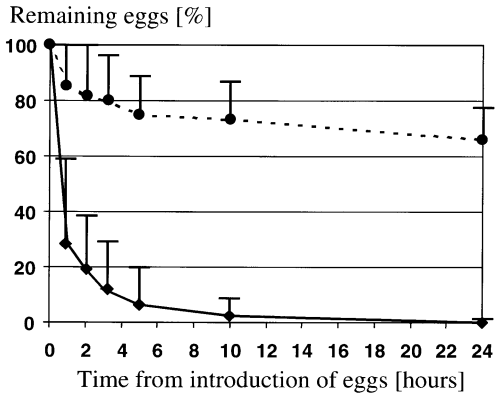

Figure 3. Removal of small hive beetle eggs (mean ± SD) after 0, 1, 2, 3, 5, 10 and 24 hours in the seven *A. m. scutellata* test colonies (triangles = unprotected eggs, circles = protected eggs).

3.2. Larva removal

As previously described (Schmolke, 1974), workers investigated larvae and carried them out of the colony (Fig. 1). Time to removal was determined for 700 larvae in the seven test colonies. The percentages of remaining larvae and in the controls are shown in Figure 4. A significantly higher proportion of larvae remained in the controls, than in the test colonies (after one hour: Z = –2.39, $P < 0.02$; after 24 hours: Z = –2.39, $P < 0.02$). After 24 hours all larvae were removed in all test colonies (Fig. 4).

3.3. Colony phenotype data

The colony phenotypes are shown in Table I and the correlation matrix for the colony phenotypes with the removal of SHB eggs and larvae in Table II. Colony sizes and honey areas were positively correlated (Tab. II). Likewise, the removal rates for unprotected

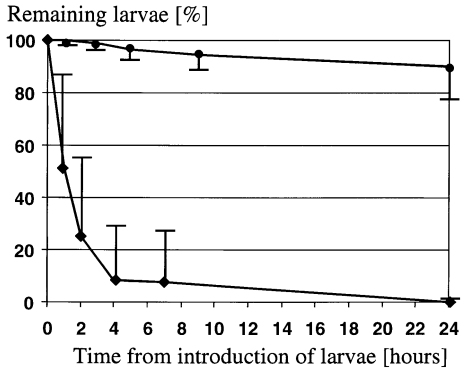

Figure 4. Removal of small hive beetle larvae (mean ± SD) after 0, 1, 2, 4, 7, 10 and 24 hours in the seven *A. m. scutellata* test colonies (triangles = treatments, circles = controls).

eggs after one and two hours were positively correlated (Tab. II). Otherwise, no significant correlations were found.

4. DISCUSSION

All adult SHB used in this study (N = 491) were obtained from a single colony neither showing SHB larvae nor any other signs of serious infestation such as damaged comb or fermented honey. This supports earlier observations that African colonies can cope with high infestation levels (Neumann et al., 2001b) and further indicates that the removal of SHB offspring by the host workers is efficient. Indeed, 72 ± 26% of all unprotected SHB eggs were removed within one hour and all of them within 24 hours. However, a significantly larger proportion of the protected eggs remained after 24 hours (66 ± 12%), indicating that eggs in such areas are likely to hatch. This shows that oviposition of female SHB in cracks is adaptive, because it significantly enhances the survival chances of eggs.

African honeybees use considerably more propolis than European subspecies (Hepburn and Radloff, 1998). It seems likely that this abundant use of propolis not only enhances prison building (Neumann et al., 2001b) but also minimizes the number of available cracks in a colony, thereby limiting the number of protected beetle eggs.

Our results for the removal of larvae confirm earlier reports that jettisoning workers efficiently remove SHB larvae from infested colonies (Lundie, 1952). Moreover, our data agree well with Schmolke (1974) who found that 50% of artificially introduced larvae were removed within 90 minutes and 100% within 24 hours. Such rapid removal rates indicate that workers react quickly to the presence of both SHB eggs and larvae in the colony. Since SHB larvae can cause substantial damage to combs (Lundie, 1940; Schmolke, 1974), rapid colony responses appear important.

Colony sizes and honey areas were positively correlated as known from routine beekeeping experience. However, there were no significant correlations of the colony phenotype data with the removal rates for SHB eggs and larvae. This suggests that all our test colonies were strong enough to remove SHB eggs and larvae and further indicates that the

Table I. Colony phenotype data for the tested *A. m. scutellata* colonies. Colony size, sealed and open brood, pollen and honey are shown.

Colony	Colony size [bees]	Brood [dm^2]		Pollen [dm^2]	Honey [dm^2]
		Open	Sealed		
1	9035	13.25	22	8	44.25
2	8645	16	14.25	5.5	83.5
3	8450	14.75	13.25	9.5	32.5
4	5623	0	1.75	2.75	34
5	4290	0.5	0.5	8	29.25
6	7540	15.5	12	6.5	40
7	8125	15.25	11.75	7	39.25
Mean	7387 ± 1765	10.8 ± 7.23	10.8 ± 7.5	6.8 ± 2.2	43.3 ± 18.5

Table II. Correlation r-matrix for the colony phenotype data and removal data for the tested *A. m. scutellata* colonies. Colony size, sealed and open brood, pollen, honey, removal of small hive beetle protected and unprotected eggs after one and two hours and removal of small hive beetle larvae after one and two hours were considered. The Bonferroni adjusted significance level is $\alpha = 0.0041$. Significant correlations are indicated with * for $P < 0.0041$.

			Colony size	Brood		Pollen	Honey	Egg removal				Larva removal	
				Open	Sealed			Unprotected		Protected			
								1 hour	2 hours	1 hour	2 hours	1 hour	2 hours
Colony size			1										
Brood	Open		0.89	1									
	Sealed		0.95	0.82	1								
Pollen			0.28	0.44	0.47	1							
Honey			0.98*	0.85	0.96	0.23	1						
Egg removal	Unprotected	1 hour	–0.37	0.59	0.15	0.19	0.22	1					
		2 hours	–0.30	0.54	0.07	0.14	0.15	1*	1				
	Protected	1 hour	–0.53	–0.34	–0.60	0.14	–0.67	0.48	0.51	1			
		2 hours	–0.18	–0.35	–0.28	–0.14	–0.30	0.30	0.30	0.68	1		
Larva removal		1 hour	–0.73	–0.58	–0.75	–0.56	–0.64	–0.40	–0.32	–0.01	–0.38	1	
		2 hours	–0.48	–0.35	–0.53	–0.62	–0.37	–0.36	–0.29	–0.27	–0.55	0.95	1

removal of SHB offspring is probably not triggered by the amount of brood and/or food storage.

Because protected eggs are likely to hatch, the removal of larvae is a key element for resistance. Nevertheless, the removal of eggs is also relevant because it reduces the number of hatching larvae. It seems likely that the removal behaviour of eggs and larvae is also present in colonies of European subspecies. However, there might be quantitative differences between African and European subspecies similar to the aggression behaviour towards adult SHB (Elzen et al., 2001), e.g. African honeybees may remove faster and/or more efficient. We conclude that removal behavior plays an essential part for the apparent resistance of African honeybees. Future control efforts for SHB infestations might consider the role of cracks for successful beetle reproduction.

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Résumé – Élimination des œufs et des larves du Petit Coléoptère des ruches (*Aethina tumida*) par les colonies d'abeilles mellifères africaines (*Apis mellifera scutellata*). Le Petit Coléoptère des ruches (SHB), *Aethina tumida* Murray est un parasite d'*Apis mellifera* endémique en Afrique, où il est considéré comme un ennemi mineur. Il peut par contre causer des dégâts dans les populations d'abeilles européennes. Le comportement d'élimination des œufs et des larves du SHB par les abeilles africaines, *A. m. scutellata* Lepeletier, a été étudié sur 7 colonies (Fig. 1). Puisque les femelles du SHB peuvent protéger leurs œufs en pondant dans des fentes, nous avons testé deux groupes d'œufs (Fig. 2) : (i) des œufs non protégés (N = 7556 dans 7 colonies) et (ii) des œufs protégés (N = 1612 dans 5 colonies). Tous les œufs non protégés ont été éliminés en 24 h, alors qu'il restait de 47,39 à 77,97 % d'œufs protégés (moyenne : 65,88 ± 11,54 ; Z = –2,84, $P < 0{,}002$, Fig. 3). Mais au bout d'une heure, il restait un nombre significativement plus grand d'œufs protégés, de 70,53 à 99,95 % (moyenne 85,02 ± 13,67 %), contre 3,47 à 68,06 % (moyenne 28,30 ± 26,47 %) pour les œufs non protégés (Z = –2,84, $P < 0{,}001$). Ceci suggère que l'œuf du Petit Coléoptère des ruches parvient à éclore dans des colonies infestées. Pourtant toutes les larves introduites (N = 700) dans ces colonies ont été éliminées en 24 h (Fig. 4). Les ouvrières

réagissent vite à la présence d'œufs ou de larves du SHB puisque 72 ± 27 % des œufs non protégés et 49 ± 37 % des larves sont éliminés en 1 h. Il n'y a pas de corrélation entre le comportement d'élimination des œufs et des larves et les performances des colonies testées (force de la colonie, couvain operculé et non operculé, réserves de pollen et de miel ; données évaluées selon la méthode de Liebefeld). Nos résultats montrent que les colonies africaines éliminent les œufs et les larves du SHB dans un laps de temps court, avant que ne surviennent des dégâts aux rayons. Nous en concluons que ce comportement efficace peut jouer un rôle important dans la résistance évidente des abeilles africaines aux infestations par le SHB.

***Aethina tumida* / *Apis mellifera scutellata* / relation hôte-parasite**

Zusammenfassung – Ausräumverhalten von Eiern und Larven des kleinen Beutenkäfers (*Aethina tumida*) durch afrikanische Bienenvölker (*Apis mellifera scutellata*). Der kleine Beutenkäfer, *Aethina tumida* [SHB], ist ein in Afrika endemischer Parasit der dortigen Honigbiene. Dort macht er nur geringe Schäden. In den Populationen der Europäischen Bienen dagegen kann der SHB zu großen Schäden führen. Afrikanische Bienen entfernen sowohl Eier als auch Larven (Abb. 1) des kleinen Beutenkäfers. Dieses Ausräumverhalten wurde in sieben *A. m. scutellata* Völkern untersucht. Da Käferweibchen ihre Eier durch Oviposition in kleine Spalten schützen können, wurden zwei verschiedene Gruppen von Eiern getestet (Abb. 2): (a) ungeschützte Eier (N = 7556 in sieben Völkern) und (b) geschützte Eier (N = 1612 in fünf Völkern). Alle ungeschützten Eier wurden innerhalb von 24 Stunden entfernt (Abb. 3). Es verblieben jedoch signifikant mehr geschützte Eier in den Völkern (nach einer Stunde: ungeschützte Eier: 28,30 ± 26,47 % (3,47 bis zu 68,06 %); geschützte Eier: 85,02 ± 13,67 % (70,53 bis zu 99,55 %); Z = –2,84, $P < 0{,}001$; nach 24 Stunden: ungeschützte Eier: 0 ± 0 %; geschützte Eier: 65,88 ± 11,54 % (47,39 bis zu 77,97 %); Z = –2,84, $P < 0{,}002$; Abb. 3). Dies lässt vermuten, dass Eier des kleinen Beutenkäfers in infizierten Völkern zum Schlupf gelangen. Jedoch wurden alle Larven (N = 700), die in diese Völker eingesetzt wurden, innerhalb von 24 Stunden entfernt (Abb. 4). Die Arbeiterinnen reagierten schnell auf die Präsenz von Käfereiern und Larven, da 72 ± 27 % der ungeschützten Eier und 49 ± 37 % der Larven innerhalb einer Stunde entfernt wurden. Das Ausräumverhalten von Eiern und Larven korrelierte nicht mit den Leistungsdaten der getesteten Völker (Volksstärke, offene und verdeckelte Brut, Pollen- und Honigvorräte; nach Liebefelder Methode evaluiert). Unsere Daten zeigen, dass afrikanische Völker sowohl Eier als auch Larven des kleinen Beutenkäfers innerhalb kurzer Zeit ausräumen, bevor es zur Schädigung der Waben kommt. Wir schlussfolgern, dass dieses effiziente Ausräumverhalten eine wichtige Rolle für die offensichtliche Resistenz afrikanischer Bienen gegenüber Infektionen mit dem kleinen Beutenkäfer spielen kann.

***Aethina tumida* / *Apis mellifera* / Honigbiene / kleiner Beutenkäfer / Wirt-Parasit interaktion**

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