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Original article

Alternative control of *Aethina tumida* Murray (Coleoptera: Nitidulidae) with lime and diatomaceous earth*

Sven BUCHHOLZ[1], Katharina MERKEL[2], Sebastian SPIEWOK[1], Jeff S. PETTIS[3], Michael DUNCAN[4], Robert SPOONER-HART[4], Christian ULRICHS[5], Wolfgang RITTER[6], P. NEUMANN[7–9]

[1] Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 4, 06099 Halle (Saale), Germany
[2] Institut für Ökologie und Evolutionsbiologie, FB 2, Universität Bremen, 28359 Bremen, Germany
[3] USDA-ARS Bee Research Laboratory, Bldg. 476, Beltsville, MD 20705, USA
[4] Centre for Plant and Food Science, College of Science Technology and Environment, University of Western Sydney, Richmond, Australia
[5] Humboldt-Universität zu Berlin, Institut für Gartenbauwissenschaften, Urban Horticulture, 14195 Berlin, Germany
[6] Chemisches und Veterinäruntersuchungsamt Freiburg (CVUA), Fachgebiet Bienen, Am Moosweiher 2, 79108 Freiburg, Germany
[7] Swiss Bee Research Centre, Agroscope Liebefeld-Posieux Research Station ALP, 3033 Bern, Switzerland
[8] Eastern Bee Research Institute of Yunnan Agricultural University, Kunming, Yunnan Province, China
[9] Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

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Abstract – Aiming at alternative small hive beetle control, slaked lime, powdered limestone and diatomaceous earth (Fossil Shield® FS 95, FS 90.0 and FS 90.0s) were evaluated for their effects on pupation and adult emergence in the laboratory. Limestone, FS 90.0 and FS 95 showed no significant effect. Slaked lime in autoclaved soil prevented pupation, but was lethal only in high dosages of 10 and 15 g per 100 g soil. In non-autoclaved soil, low slaked lime dosages of 0.5 and 5 g resulted each in >90% mortality, possibly due to enhanced pathogen activity. However, with FS 90s (also using non-autoclaved soil) it's the reverse. Larvae penetrated a slaked lime layer and pupated in untreated soil below. Slaked lime and FS 90.0s were also tested in traps (diagnostic trays) in the laboratory and in honeybee field colonies. In the field, 30.5 ± 29.3% of the adults were caught in the traps with slaked lime. FS 90.0s caused 100% adult mortality in field traps, where 57.9 ± 8.3% of the hives' adult SHB infestation died within 48 h. Our data showed a good potential for the use of FS 90.0s as inhive treatment and suggest further research with slaked lime as alternative control of SHB.

amorphous silica / integrated pest management / powdered limestone / slaked lime / small hive beetle

1. INTRODUCTION

The small hive beetle (= SHB), *Aethina tumida* Murray is a pest of honeybee colonies, *Apis mellifera* L. (Lundie, 1940; Neumann and Elzen, 2004) and is endemic to sub-Saharan Africa (Hepburn and Radloff, 1998; El-Niweiri et al., 2008). SHB is usually considered to be a minor pest in its native distribution areas (Lundie, 1940; Schmolke, 1974; Neumann and Elzen, 2004). However, it can cause considerable damage to apiculture in populations of European-derived honeybees in North America (Elzen et al., 1999; Hood,

Corresponding author: S. Buchholz,
buchholz.sven.berlin@googlemail.com
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2004; Neumann and Elzen, 2004) and Australia (Spiewok et al., 2007).

The life cycle of *A. tumida* in association with honeybee colonies can be divided into two parts, which offer different possibilities for pest control:

(1) Within the hive, adult beetles reproduce and feed on brood, pollen and honey (Lundie, 1940). Although adults can have a negative impact on colonies of European subspecies (Ellis et al., 2003), the larvae are usually the most destructive life stage (Lundie, 1940). However, cryptic low reproduction levels of SHB have been observed without obvious damage to colonies (Spiewok and Neumann, 2006).

(2) The wandering larvae leave the honeybee colony and their majority pupates in the ground about 10 cm deep within a radius of ca. 90 cm around the hive, depending on soil conditions (Pettis and Shimanuki, 2000). In the USA, SHB have been controlled by drenching the soil with insecticides containing permethrin (Baxter et al., 1999) and beetle-infested colonies have been treated with coumaphos traps on bottom boards (Elzen et al., 1999). Although effective, conventional chemical control carries the risks of pest resistance as in case of *Varroa destructor* Anderson & Trueman (Spreafico et al., 2001; Pettis, 2003) and of chemical residues in the bee products (Wallner, 1999; Kochansky et al., 2001).

Thus, alternative approaches to control SHB in the hive and in the soil are desirable within an integrated pest management program for SHB. Hood (2004) and Ellis (2005a, b) reviewed different treatments and strategies for controlling SHB. A major impediment to using traps equipped with liquid agents within the hive (Hood and Miller, 2003; West, 2004), is that the beekeeper should not move colonies without first removing the traps. In our study, we tested the potential of dry substances (slaked lime and diatomaceous earth) as possible alternative in-hive treatments to achieve effective control, and to enable beekeepers to relocate hives even during treatment. Furthermore, we evaluated the potential of these substances and powdered limestone to control SHB larvae in the ground.

Powdered limestone and slaked lime ($Ca(OH)_2$, also known as hydrated lime) have already been tested for beetle pest control (Vittum, 1984, 1985; Abdalla, 1991; Watson et al., 2003). As slaked lime is characterized as hydrophilic and both substances increase pH-level, they may affect SHB pupation or serve as an alternative control agent for beetle traps in the hive.

Diatomaceous earth (DE) consists of unicellular or colonial silicified skeletons of algae (Bacillariophyceae). The inert dusts have been reported to be effective for the control of various pests (e.g. McLaughlin, 1994; Golob, 1997 among others). The lethal effect of a DE product is primarily based on the sorptive properties of DE (Mewis and Ulrichs, 1999, 2001): The nano-structured DE-particles absorb the lipids from the epicuticle and become partially absorbed from the integument. Through the destruction of the waxy epicuticle layer, which acts as water barrier, arthropods dehydrate. However, the efficacy of DE is influenced by the specific morphology and physiology of different species as well as by the lipid binding capacity of the DE and the water pressure deficiency of the surrounding atmosphere (relative humidity = RH; Mewis and Ulrichs, 1999). At low RH, regular DE formulations absorb water and become easily saturated and therefore ineffective (Völk et al., 2004). New formulations like the tested Fossil Shield® products overcome this problem since they are altered to become hydrophobic (Ulrichs et al., 2006) and should therefore not be affected by the humidity within the bee hive.

In this study, dry agents were evaluated for two purposes:

(1) Aiming at an alternative control of SHB in the ground, we investigated the pupation success of *A. tumida* in soil treated with different dosages of powdered limestone, slaked lime and DE in the laboratory;

(2) Field trials with slaked lime and DE were performed by placing a diagnostic tray on the bottom board of the hive box (modified according to Charrière et al., 1998), to establish a monitoring and/or control system for adult SHB within the hive.

Since the mode of action is physicosorption, it is very unlikely that pest organisms can develop any resistance against DE products. We assume the same to be true for any potential effects of slaked lime or powdered limestone. Given these properties, the described substances could serve as suitable substitutes for conventional chemical treatment of SHB.

2. MATERIALS AND METHODS

Experiments were performed in 2004/5 at Rhodes University (South Africa), in 2005 at the Bee Research Laboratory, Beltsville (USA) and in 2006 at the University of Western Sydney (Australia). At all research facilities, laboratory rearing was established using field collected adults and standard protocols (Muerrle and Neumann, 2004). Wandering SHB larvae used in the experiments were 11 ± 3 days old.

2.1. Laboratory trials with slaked lime

At Rhodes University, the effect of slaked lime (CLC Lhoist group, Limelette, Belgium) on pupation success of SHB larvae was evaluated for concentrations of 5, 10 and 15 g $Ca(OH)_2$/100 g soil, untreated soil served as control. Prior to the experiment, the soil (very fine, quartz- and organic-rich, clay-poor sand, Muerrle and Neumann, 2004) was autoclaved to eliminate possible effects of biological antagonists, e.g. entomopathogenic fungi (Ellis et al., 2004b; Richards et al., 2005; Muerrle et al., 2006) or nematodes (Cabanillas and Elzen, 2006). Then water was added to obtain adequate conditions for SHB pupation (Muerrle and Neumann, 2004). Wandering larvae were transferred into plastic cups (100 mL) containing 85 g of $Ca(OH)_2$/soil mixture (N = 10 replicates with n = 10 wandering larvae for each concentration). Lids were provided to prevent SHB escape and to avoid dehydration of the soil. The samples were kept in an environmental chamber in permanent darkness at 30 ± 1 °C and 65 ± 5% RH. Emerging adults were counted after 16 days, as preliminary tests suggested this to be a suitable time window to complete development in the given environment. Finally, all samples were sieved (mesh width: <0.3 cm^2) to collect all beetles (dead or alive) that did not emerge.

The experiment was repeated in the USA with some modifications, using the same dosages of slaked lime (Aldrich Chemical Co., Inc., USA) plus a concentration of 0.5 g (N = 12 replicates per dosage, n = 10 wandering larvae per cup). In contrast to the trials above, the soil (all purpose sand, Kolorscape, Oldcastle Retail Atlanta, Georgia, USA) was not autoclaved, to evaluate the effect of the substances under more natural conditions, including possible presence of biotic agents. In contrast to South Africa, emerging adults in the US were counted on a daily basis for 35 days, as it became evident that duration of pupation was longer in these experiments (Ellis and Delaplane (2007) observed 22.7 ± 0.1 days for pupation at 25.6 ± 0.3 °C, ~38% RH and in Ellis et al., 2004a, the duration was about 23 days at 24.6 ± 1.3 °C). After termination the soil was sieved for remaining live stages. Experimental conditions were 30 ± 1 °C and 65 ± 5% RH in permanent darkness.

At Rhodes University, we also evaluated the effect of a mixed $Ca(OH)_2$/soil-layer placed on top of untreated soil on pupation success. We wanted to investigate whether or not slaked lime would prevent pupation even though larvae could penetrate the limed layer of soil and pupate beneath it. Wandering larvae (n = 20 each) were transferred into plastic cups (600 mL, N = 6) containing ~3 cm layer of 15 g $Ca(OH)_2$/100 g soil-mixture placed upon ~ 6 cm of untreated soil. After 16 days, all life stages were recorded (see above).

2.2. Laboratory trials with powdered limestone

At the Bee Research Laboratory in Beltsville, the effects of powdered limestone (New Enterprise Stone & Lime Co., PE, USA) on pupation success were evaluated. We used the identical protocol as for slaked lime tests in the USA (2.1.) with 5, 10 and 15 g of limestone per 100 g non-autoclaved soil.

2.3. Laboratory trials with diatomaceous earth (Fossil Shield®)

In Beltsville, we also tested three types of the DE product Fossil Shield® (FS 90.0, FS 90.0s and FS 95.0, Bein GmbH, Germany) and used the same protocol as for the experiments with slaked lime (performed in the USA, 2.1.). The trials were conducted at 20 ± 1 °C and 65 ± 5% RH with dosages of 0.05, 0.5 and 5 g DE per 100 g soil.

Since soil moisture is crucial for SHB pupation (Ellis et al., 2004a), we analyzed soil mixtures

with slaked lime, powdered limestone, DE and controls using a moisture detector (MA 30 Sartorius) in Beltsville. Prior to the experiments, three soil samples per dosage (~20 g each) were weighted according to the provider's manual before and after heating at 130 °C to ascertain the average amount of moisture (%) per mixture. This method provided the total amount of moisture per weight, but it was not able to differentiate "available" moisture between particles from "stored" moisture that is absorbed by particles in the soil and therefore not accessible to the larvae.

2.4. Laboratory evaluation of slaked lime and FS 90.0s in diagnostic trays

Preliminary observations in the field suggested, that adult SHB may use a diagnostic tray as a hiding place. This device consists of a plastic tray (25×30×0.7 cm; Günther Spritzgußtechnik GmbH, Kaufbeuren-Neugablonz, Germany) with a grill, comparable to the "West SHB trap" (West, 2004), but initially equipped with formic acid for *V. destructor* treatments (Mutinelli and Baggio, 2004). At the University of Western Sydney, we evaluated the efficacy of this trap for adults and wandering larvae of *A. tumida* in a laboratory room with no direct sunshine (natural indirect light regime at 21 ± 1 °C, 50 ± 5% RH). For this purpose, the trays were equipped with a thin layer of either 15 g FS 90.0s, 45 g slaked lime (David Mitchell Ltd., N.S.W., Australia) or 100 g dry bird cage sand (Pet Pacific Pty Ltd., Australia) as control (N = 7 replicates each) and covered with the grill. After placing ten adult SHB on each device, the traps were immediately sealed in plastic bags (Ziploc™), permitting SHB individuals to leave and re-enter the trap. After 24 h, mortality and numbers of adult SHB inside and outside of the traps were recorded. The same approach was used for wandering larvae (n = 10 with N = 7 replicates per treatment and control). Here records were made after 34 h, because preliminary tests suggested that larvae appeared to be less susceptible to the tested substances.

2.5. Field evaluation of slaked lime and FS 90.0s in diagnostic trays

In Australia, the diagnostic trays used in 2.4, were also evaluated in 21 naturally infested field colonies (predominantly *A. m. ligustica*, pers.com. M Duncan), housed in single Langstroth hive boxes (10 frame box, 24.2 cm high). The colonies were placed in two rows with ~1.5 m distance between hives. For each treatment, seven hives were equipped with diagnostic trays with DE, slaked lime and sand in alternating order. The devices with FS 90.0s, slaked lime and dry sand as controls were placed in the centre of each bottom board. After 48 h, all SHB were individually collected from the hives using a mouth aspirator (see for details: Spiewok et al., 2007). Each diagnostic tray was quickly removed from the bottom board and placed into a plastic bag to assess mortality and numbers of trapped adult SHB to ascertain trap and treatment efficacy.

2.6. Data analyses

All treatment mortalities were adjusted according to Abbott's formula (Abbott, 1925). The level of significance was $\alpha = 0.05$, when not described otherwise. Kruskal-Wallis tests and multiple comparisons as post hoc tests were performed:

(1) to evaluate differences between the observed parameters of treatments and controls of the laboratory soil experiments with slaked lime, powdered limestone and DE;

(2) to compare the amounts of dead and alive larvae, pupae and adults as well as mortalities between treatments with mixtures of slaked lime and controls.

Kruskal-Wallis tests and Mann-Whitney U-tests with Bonferroni-Fischer adjusted $\alpha = 0.0167$ were performed:

(1) to detect differences in SHB mortality and "escape rate" from diagnostic trays between treatments (FS 90.0s, slaked lime) and controls (sand) in the laboratory and (2) to assess number and mortality of adult SHB in the traps on bottom boards in the field.

Mann-Whitney U-tests) were performed:

(1) to compare the numbers of larvae, pupae and adults between treatments with a layer of slaked lime and controls, (2) to investigate differences in developmental time between controls reared at 20 °C and 30 °C, (3) between controls performed in the USA und South Africa at 30 °C.

For the field trials, the total number of adult SHB per colony (hive and trap), were compared using a Friedman ANOVA between the three groups (FS 90.0s, slaked lime and sand). All analyses were conducted using STATISTICA©.

3. RESULTS

The results are given as mean ± SD. Statistical details are shown in the corresponding tables, if not mentioned in the text.

3.1. Laboratory trials with slaked lime

In the experiments where slaked lime was mixed with autoclaved soil in South Africa, after 16 days nearly all remaining live beetles were still on the larval stage either dead or alive (Tab. I-A) This is in contrast to the controls where all individuals (99.0 ± 3.2%) but one single larva (1.0 ± 3.2%) emerged as adults. However, larval mortality increased significantly in mixtures of 10 g (28.0 ± 21.5%, $P < 0.01$) and 15 g (14.0 ± 17.8%; $P < 0.01$) when compared with the control with no dead larva. Mortality of all life stages (sum of dead individuals) was significantly higher in the 10 g slaked lime dosage (29.0 ± 22.3%, $P < 0.01$) compared to all other trials (Tab. I-A).

In the experiment with a layer of slaked lime mixture on untreated soil, significantly fewer adults (85.0 ± 8.9%, $P < 0.01$) emerged after 16 days, compared to the controls (98.3 ± 2.6%; Tab. I-B). All remaining wandering larvae in the treatment were found dead in the layer of untreated soil. Apart from one single adult beetle in a sample of a 10 g $Ca(OH)_2$/100g soil mixture, no dead adult SHB were found in any of the slaked lime treatments or controls, regardless of the application (mixture or layer).

In Beltsville, in trials with non-autoclaved soil mixed with 0.5 and 5 g slaked lime the mortality of SHB was significantly increased (0.5 g: 99.1 ± 2.9%; $P < 0.01$; 5 g: 92.9 ± 10.7%, $P < 0.01$) while the higher dosages of 10 and 15 g did not significantly differ from the controls (10 g: 27.5 ± 25.8%, $P > 0.1$; 15 g: 2.7 ± 6.8%, $P > 0.1$; Tab. II). Almost all larvae successfully pupated in the soil without slaked lime, while in the 0.5 and 5 g treatments nearly all individuals were killed in the larval stage. In the 10 and 15 g treatments about 2/3 of the larvae were found alive in the wandering phase after 35 days. With the exception of the highest dosage (15 g: 17.5 ± 10.6%, $P > 0.07$), the numbers of emerged adults were significantly lower (0.5 g: 0.8 ± 2.9%, $P < 0.01$; 5 g: 5.8 ± 7.9%, $P < 0.01$; 10 g: 10.0 ± 8.5%, $P < 0.01$) than in the controls (93.3 ± 7.8; Tab. II).

Significantly more adults (99.0 ± 3.2%) hatched in controls in South Africa within 16 days than in controls conducted in the USA (accumulated number of adults after 16 days: 0.8 ± 2.9; U = 0; $P < 0.0001$). Both trials were kept at 30 ± 1 °C and 65 ± 5% RH in permanent darkness. On day 26 the last adult hatched from the control in the USA, raising the developmental success rate to 93.3 ± 7.8%.

In the USA, initial adults emerged significantly earlier from controls kept at 30 ± 1 °C, on day 16 ± 1, compared to controls kept at 20 ± 1 °C, where the first adults appeared on day 36 ± 2 (U = 0; $P < 0.0001$).

3.2. Laboratory trials with powdered limestone

In the trials with powdered limestone (dosages of 5, 10 and 15 g/100 g soil at 30 ± 1 °C) we did not find significant differences between treatments and controls, neither for mortality nor for the number of hatched adults (for both parameter: H_3 (N = 48) = 0.48 $P > 0.92$). The percentage of emerged adults for all trials was 97.7 ± 4.7% with an overall mortality of 2.3 ± 4.7%.

3.3. Laboratory trials with diatomaceous earth (Fossil Shield®)

None of the dosages with FS 95 showed any significant effect on mortality ($H_3 = 0.20$; $P > 0.97$) or the number of emerging adults ($H_3 = 0.12$; $P > 0.98$) of SHB. The same was true for the dosages with FS 90.0 (mortality: $H_3 = 3.33$; $P > 0.34$; emerged adults: $H_3 = 2.77$; $P > 0.42$). On the other hand, trials treated with 0.5 and 5 g FS 90.0s resulted in significantly higher total mortalities compared to the controls and the 0.05 dosage treatment (Tab. III). Correspondingly, almost

Table I. Individuals and mortality of *A. tumida* (%) from laboratory trials in South Africa, 16 days after setting wandering larvae A: on autoclaved soil mixed with different quantities of slaked lime for pupation (n = 10 larvae; N = 10 replicates). Results of the Kruskal-Wallis tests (KWT) and multiple comparison as post hoc tests, $\alpha = 0.05$ are shown; column numbers followed by different letters indicate significant differences between them. B: on a layer of limed soil upon untreated soil (n = 20, N = 6). Results of Mann-Whitney U-tests (MWU, $\alpha = 0.05$) are shown. Values indicate means ± SD, * treatments significantly different to the control: ($P < 0.05$).

Slaked lime per 100 g soil (South Africa)		Larvae		Pupae		Adults		Mortality
		alive	dead	alive	dead	alive	dead	
A: Complete mixtures	control	1.0 ± 3.2^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	99.0 ± 3.2^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	5 g	89.0 ± 5.7^{b}	4.0 ± 5.2^{a}	2.0 ± 4.2^{a}	1.0 ± 3.2^{a}	4.0 ± 7.0^{b}	0.0 ± 0.0^{a}	5.0 ± 5.3^{a}
	10 g	70 ± 22.6^{b}	28.0 ± 21.5	1.0 ± 3.2^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{b}	1.0 ± 3.2^{a}	29.0 ± 22.3^{b}
	15 g	86.0 ± 17.8^{b}	14.0 ± 17.8^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{a}	14.0 ± 17.8^{a}
KWT: H_3 (N = 40)		25,11	17.64	3.9	3	33.67	3	17,20
B: Mixture as a layer	control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 2.6	98.3 ± 2.6	0.0 ± 0.0	1.7 ± 2.6
	15 g	1.7 ± 4.1	11.7 ± 8.2*	1.7 ± 2.6	1.7 ± 2.6	85.0 ± 8.9*	1.7 ± 4.1	13.5 ± 9.8*$^{+}$
MWU	U	15	3	12	18	0	15	3

$^{+}$ Abbott adjusted mortality.

Table II. Different life stages of *A. tumida* (%) from laboratory trials in the USA, 35 days after setting n = 10 wandering larvae (N = 12 replicates) for pupation on non-autoclaved soil mixed with different quantities of slaked lime at 30 °C. Values indicate means ± SD, results of the Kruskal-Wallis tests and multiple comparison as post hoc tests ($\alpha = 0.05$) are shown, column numbers followed by different letters indicate significant differences between them.

Slaked lime per 100 g soil (USA)		Larvae		Pupae		Adults		Mortality[+]
		alive	dead	alive	Dead	alive	dead	
Complete mixtures	(control)	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.8 ± 2.9[a]	0.0 ± 0.0[a]	93.3 ± 7.8[a]	5.0 ± 6.7[a]	6.7 ± 7.8[a]
	0.5 g	0.0 ± 0.0[a]	85.0 ± 19.3[b]	0.0 ± 0.0[a]	0.8 ± 2.9[a]	0.8 ± 2.9[b]	0.0 ± 0.0[a]	99.1 ± 2.9[b]
	5 g	0.8 ± 2.9[a]	70.0 ± 28.6[bc]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	5.8 ± 7.9[bc]	5.0 ± 6.7[a]	92.9 ± 10.7[bc]
	10 g	56.7 ± 21.9[b]	12.5 ± 14.9[ac]	1.7 ± 3.9[a]	10.8 ± 11.7[a]	10.0 ± 8.5[bc]	4.2 ± 6.7[a]	27.5 ± 25.8[ac]
	15 g	71.7 ± 15.9[c]	2.5 ± 6.2[a]	7.5 ± 6.2[a]	0.8 ± 2.9[a]	17.5 ± 10.6[ac]	0.0 ± 0.0[a]	2.7 ± 6.8[a]
KWT: H_4 (N = 60)		53.69	50.08	28.47	19.23	41.27	12,1	52.66

[+] Abbott adjusted.

Table III. Pupation success and mortality of *A. tumida* (%) in non-autoclaved soil treated with diatomaceous earth (Fossil Shield® = FS 90.0s) after 53 days in the laboratory at 20 °C (n = 10 individuals and N = 12 replicates). Values indicate means ± SD, results of the Kruskal-Wallis tests and multiple comparison as post hoc tests (α = 0.05), column numbers followed by different letters indicate significant differences between them.

Groups		Mortality+	Emerged adults	Dead larvae
FS 90.0s	0.0 g (control)	3.3 ± 6.5[a]	96.7 ± 6.5[a]	0.0 ± 0.0[a]
	0.05 g	0.8 ± 2.9[a]	97.5 ± 4.5[a]	0.8 ± 2.9[a]
	0.5 g	41.2 ± 27.3[b]	56.7 ± 26.6[b]	40.8 ± 25.0[b]
	5 g	99.2 ± 2.9[b]	0.8 ± 2.9[b]	98.3 ± 3.9[b]
H_3 (N = 48)		42.15	39.41	43.22

+ Abbott adjusted mortality.

all individuals emerged as adults in the control and 0.05 dosages, differing significantly from 0.5 g, where about half of the individuals attained adulthood, and from the highest dosage, where only a few adults emerged at all. Furthermore, mortality occurred mainly in the larval stage (Tab. III).

In the USA, the overall average soil moisture of samples of trials with slaked lime, powdered limestone, DE and controls was 6.64 ± 1.90% per weight (g) prior to the experiments.

3.4. Laboratory evaluation of slaked lime and FS 90.0s in diagnostic trays

In Australia, half of the adult SHB were found after 24 h outside of the diagnostic trays with sand (control) in the plastic bags, which differed significantly from traps with FS 90.0s and slaked lime, where no adults escaped from the devices (KW: H_2 (N = 21) = 16.7, $P < 0.01$; Tab. IV-A). The mortality of adult SHB in traps with FS 90.0s was significantly higher compared to slaked lime and the control (KW: H_2 (N = 21) = 17.1, $P < 0.01$). In traps with slaked lime, adult mortality was three times higher than in the control traps.

Larval escape from traps filled with FS 90.0s and slaked lime did not result in significant differences (KW: H_2 (N = 21) = 3.0, $P > 0.1$) compared to the control after 34 h. However, larval mortality in traps with FS 90.0s was significantly higher compared to zero larval mortality in both slaked lime and control trials (KW: H_2 (N = 21) = 12.2, $P < 0.01$; Tab. IV-A).

3.5. Field evaluation of slaked lime and FS 90.0s in diagnostic trays

After 48 hours, 495 SHB were collected from 21 Australian beehives and diagnostic trays. No significant differences were detected between the average total numbers of adult SHB found in hives with FS 90.0s, slaked lime and sand traps (ANOVA χ^2(N = 7, df = 2) = 1,5, $P > 0.1$; Tab. IV-B).

The percentages of adults collected from traps with FS 90.0s and slaked lime were both significantly higher than in the control traps, where only a few adults were found (KW: H_2 (N= 21) = 13.9, $P < 0.01$). No significant difference was detected between FS 90.0s and slaked lime (Tab. IV-B).

4. DISCUSSION

Our data imply that slaked lime and the diatomaceous earth product FS 90.0s are suitable alternatives to conventional chemical control of SHB. While slaked lime hindered wandering larvae from pupation, FS 90.0s was lethal for both adults when applied in traps within the bee colony and for larvae in laboratory soil trials.

Table IV. A: Mortality and proportion of individuals (%) of *A. tumida* escaping from diagnostic trays (N = 7 replicates) in the laboratory with diatomaceous earth (FS 90.0s), slaked lime [$Ca(OH)_2$] and sand as control after 24 hours (adults: n = 10) and 34 hours (wandering larvae: n = 10). B: Number of adults in naturally infested field colonies in diagnostic trays with DE, slaked lime or sand (control). Values indicate means ± SD. Results of the Kruskal-Wallis tests H_2 (N = 21) and Mann-Whitney U as post hoc tests ($\alpha = 0.0167$) are shown. The comparison between numbers of adult SHB (n) in the field were performed with a Friedman ANOVA χ^2 (N = 7) and $\alpha = 0.05$.

Groups	A: Laboratory experiments				B: Field experiments		
	24 hours		34 hours		48 hours		
	Adults outside of trap (%)	Adult mortality (%)[+]	Larvae outside of trap (%)	Larval mortality (%)	Sum of adults in hive and trap (n)	Adults in traps (%)	Adult mortality as (%) of all adults in hive and trap
Sand (control)	50.0 ± 23.9[a]	8.57 ± 9.0[a]	35.7 ± 22.3[a]	0.0 ± 0.0[a]	18.1 ± 9.17[a]	1.8 ± 3.50[a]	0.0 ± 0.0[a]
$Ca(OH)_2$	5.71 ± 9.76[b]	24.2 ± 17.6[a]	15.7 ± 11.3[a]	0.0 ± 0.0[a]	25.1 ± 16.8[a]	40.0 ± 31.6[b]	30.5 ± 29.3[b]
FS 90.0s	0.0 ± 0.0[b]	82.2 ± 15.9[b]	34.3 ± 27.6[a]	27.1 ± 19.8[b]	27.4 ± 14.0[a]	57.9 ± 8.3[b]	57.9 ± 8.3[b]
Tests	H = 16.33	H = 16.27	H = 2.96	H = 12.24	$\chi^2_{(2)} = 1.46$	H = 13.87	H = 15.51

[+] Abbott adjusted mortality.

4.1. Slaked lime

Trials with slaked lime/soil mixtures in South Africa resulted in lower mortality compared to trials performed in the USA. In the controls significant differences in developmental time, despite the same temperature, were found between USA and South Africa. This may have been caused by different types of soil used in the experiments and thereby in divergences of available soil moisture for pupation, the sandy soil used in South Africa was not as course as the all purpose sand in the US.

Since *A. tumida* is an invasive species within Northern America, divergences between populations of the two continents may be due to genetic bottlenecks, which are well known for other species (Nentwig, 2007). We assume that the effect of the lavae's age was negligible, as we used the same range in all experiments.

Since the soil used in the USA was not autoclaved, we suppose that the higher mortality may be due to pathogen activity in the soil. However, we cannot tell whether the infection occurred while the larvae were dead or alive, although fungi are known to kill SHB (Richards et al., 2005; Muerrle et al., 2006) and dead larvae were found with obvious signs of fungal infection.

We would have expected mortality to increase with the quantity of slaked lime in the USA trials but could not observe any positive correlation. Surprisingly, the lowest concentration resulted in the highest mortality. As only few individuals died in the controls and in the higher dosages of 10 and 15 g, we assume that lower concentrations of slaked lime may modify soil conditions to enhance pathogen activity in comparison to untreated soil. Maybe with small dosages of slaked lime the alteration of the pH Level enhances pathogen activity and at the same time the effect of dehydration has little impact on these pathogens. Higher dosages of slaked lime may counteract this effect, because of its dehydrating properties and/or by enhancing the pH-level (Watson et al., 2003).

On the other hand, we observed a cessation of SHB development in South Africa; almost all larvae remained alive in the wandering stage for up to 16 days in the treatment. We assume that the soil was probably unsuitable for pupation due to the properties of slaked lime (high pH-Level, dehydration). According to our findings in the USA, wandering larvae can survive for at least 35 days on soil unsuitable for pupation. This high longevity of wandering SHB larvae is in line with observations that they can remain alive even without soil on humidity only, for up to 48 days (Cuthbertson et al., 2008).

We found a discrepancy between the results and our expectations concerning a dose depending mortality. With autoclaved soil, higher mortality was observed in 10 g (29.0 ± 23.3) than in 5 g (5.0 ± 5.3) and 15 g (14.0 ± 17.8). As all larvae used for experimental trials in South Africa were of the same cohort, we can rule out a different age of the larvae as reason for this unexpected result.

The effects of liming we recorded are probably induced by a change of soil moisture, especially of the available water content of the treated soil, rather than by modifying pH-levels (Vittum, 1984; Abdalla, 1991). The drying effect of slaked lime was observed in poultry houses (Watson et al., 2003). According to Ellis et al. (2004a), soil moisture seems to be one of the most important parameters for pupation. Due to its hydrophilic properties, we conclude that slaked lime absorbs water from the soil and thereby disturbs the ability of SHB larvae to pupate. Abdalla (1991) described symptoms such as shrinking and desiccation of grubs (*Tropinota squalida* Scop.) after contact with the water-absorbent lime. As the grubs were still feeding in the ground they also ingested the lime, in contrast to the post-feeding SHB wandering larvae which showed no signs of shrinkage.

From our results we conclude that slaked lime in high dosages and thereby in the absence of soil-borne pathogens prevent pupation of SHB. On the other hand, low doses of slaked lime also resulted in high mortality when used in non-autoclaved soil, which may be due to possible activity of fungal pathogens. Thus, low concentrations of slaked lime in combination with spore suspensions of entomopathogenic fungi (Ellis et al., 2004b; Richards et al., 2005; Muerrle et al., 2006)

could be tested for control of SHB in the ground.

When a slaked lime/soil mixture was applied as a layer, a considerable proportion (> 80%) of the wandering larvae completed their development in the untreated soil beneath. For field experiments we suggest to test the impact of slaked lime on all soil dwelling life stages of SHB and a practical application of the agent.

An important aspect for the pest management of SHB is the knowledge of its developmental duration. In the USA, we found the first adult emerged after 15 days from soil samples kept at 30 °C which was comparable to former findings (Schmolke, 1974: ~16 days at 30 °C), while at 20 °C the first adult emerged almost three weeks later on day 34. This was not surprising as the rate of most physiological and biochemical processes are positively correlated with temperature (Nylin and Gotthard, 1998).

4.2. Powdered limestone

No dosages of pulverized limestone had any controlling effect on SHB. This is in line with findings that limestone had no effect on larvae of the Japanese beetle (*Popillia japonica* Newman; Vittum, 1984). In contrast, the number of emerging adult SHB in each powdered limestone/soil mixture was slightly higher compared to the controls. We assume that the treatment of the soil with powdered limestone may have transformed the soil's properties into a more suitable environment for the larvae to pupate, in contrast to the effects of slaked lime (Watson et al., 2003).

4.3. Diatomaceous earth

Even in high dosages, the lower hydrophobic formulations of Fossil Shield® (90.0 and 95) showed no effect on pupation of SHB. On the other hand, the most hydrophobic formulation FS 90.0s resulted in almost 100% mortality among the larval stage in the USA laboratory. We assume that *A. tumida* dehydrated due to the sorptive effect of Fossil Shield®, destroying the outer wax layer of the cuticle and thereby the waterproof barrier of the insect. The abrasive properties of DE are rather inferior (Ulrichs et al., 2006). This is supported by findings that fungal infection of SHB larvae and pupae with *Aspergillus niger* and *A. flavus* did not increase when an unspecified type of DE was added (Richards et al., 2005). However, in our study high dosages were required to increase mortality, which might be too costly for a soil treatment in apiaries. Furthermore, as the DE products are unspecific, non-target organisms in the ground might be affected, too.

4.4. Evaluation of slaked lime and FS 90.0s in diagnostic trays

In the laboratory, we were able to demonstrate the efficiency of FS 90.0s as a dry agent to kill adult SHB. In contrast to sand and slaked lime, dead larvae were only found in devices filled with FS 90.0s; but no matter which substance was applied, larvae managed to leave the traps.

In the field, we were able to confirm the results from the laboratory experiments. The principle of diagnostic trays as a trap showed efficiency within the bee hive: More than half of the adult beetles in naturally infested hives were captured and died within 48 hours in the trays filled with FS 90.0s. However, the dusty FS 90.0s was ventilated during thermoregulation by the bees and may thereby endanger the bees themselves and reduce the quality of their products.

Although we did not count the initial number of SHB infestation of the tested hives we assume that the control treatment with sand neither acts as an attractant nor as a repellent to SHB. An initial sampling might have enhanced the aggression potential of the bees against introduced adult SHB. The beetles might have left the hive which we could have misjudged as an effect of the treatment.

Since we found no significant differences in the total numbers of beetles in the three cohorts of beehives with traps, we suggest that the treatments did not lead to significant beetle migrations.

4.5. Conclusions – Outlook

From the tested DE products FS 90.0s was the most promising formulation that killed all adult SHB in the used traps. However, the potential non-target effects of DE on honeybees and possible residues in the bee products need to be investigated. In addition, future in-hive traps should be conceived in a way that the dusty substances remain completely in the device. Given these properties, traps equipped with DE would appear to be a very effective method to monitor and control SHB within the bee colony as part of an integrated management program for this pest (Ellis, 2005b).

Although soil treatments with slaked lime inhibited SHB development, the results were partially inconsistent. Further studies would be essential to evaluate the use of slaked lime as a practicable and cost-effective substitute for conventional pesticides, for both preventive and decontamination treatment of the soil-dwelling stages of *A. tumida*.

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La chaux et la diatomite comme moyens de lutte alternatifs contre *Aethina tumida* Murray (Coleoptera : Nitidulidae).

Petit coléoptère des ruches / lutte intégrée / silice amorphe / calcaire en poudre / chaux éteinte

Zusammenfassung – Alternative Kontrolle von *Aethina tumida* Murray (Coleoptera: Nitidulidae) mit Kalk und Diatomeenerde. Der Kleine Beutenkäfer, *Aethina tumida*, ist eine Bedrohung für Honigbienen europäischen Ursprungs. In seinen neuen Verbreitungsgebieten in Nordamerika und Australien bekämpfen die Imker Larven und Puppen des Kleinen Beutenkäfers im Boden durch den Einsatz von Pestiziden. In den USA werden Adulte und Larven mit Coumaphos-haltigen Fallen abgetötet, die man auf dem Bodenbrett des Bienenstockes platziert. Mögliche Resistenzen beim Schädling und die Rückstandsproblematik in den Bienenprodukten und im Boden verlangen nach alternativen Methoden, um diesen Bienenschädling unter Kontrolle zu bringen.
In unseren Laboruntersuchungen haben wir gelöschten Kalk, Kalksteinpulver und drei Formulierungen eines Diatomeenerde-Produktes (Fossil Shield® = FS) als Mittel zur Bodenbehandlung gegen den Kleinen Beutenkäfer untersucht. In den USA wurden Wanderlaven von *A. tumida* auf nicht-autoklavierte Erde gesetzt, die mit verschiedenen Dosen der oben genannten Substanzen gemischt wurde. Die Versuche wurden bei 20 und 30 °C in permanenter Dunkelheit durchgeführt. Jeweils 100 g Erde wurden mit verschiedenen Mengen der Substanzen gemischt. Der Verpuppungserfolg wurde durch das Zählen der erwachsenen Käfer evaluiert, und die Entwicklungsstadien der verbliebenen Individuen sowie deren Mortalität wurden erfasst. In Südafrika wurden der gelöschte Kalk mit autoklavierter Erde gemischt und Testreihen mit Wanderlarven bei 30 °C durchgeführt. Außerdem untersuchten wir den Effekt einer dünnen Schicht aus Kalk-Erde-Gemisch auf unbehandelter Erde. In Australien wurden gelöschter Kalk, FS 90.0s und trockener Sand (Kontrolle) in Diagnoserahmen (Vorrichtung zur *Varroa*-Kontrolle) ausgebracht. Ihre Effekte wurden im Labor gegen Larven und Adulte sowie im Freiland auf Bodenbrettern von Bienenstöcken gegen erwachsene Käfer evaluiert.
In Südafrika stoppte gelöschter Kalk gemischt mit autoklavierter Erde die Entwicklung des Kleinen Beutenkäfers, kaum eine Larve begann mit der Verpuppung im Gegensatz zu einer vollständigen Entwicklung in der Kontrolle (Tab. I-A). Dosen von 10 und 15 g gelöschten Kalks/100 g Erde töteten signifikant mehr Laven als die Kontrolle und die 5 g Dosis. Wenn jedoch eine 15 g Dosis gelöschten Kalks nur als Schicht appliziert wurde, konnten sich > 80 % der Larven erfolgreich in der darunter liegenden unbehandelten Erde verpuppen (Tab. I-B). In den Testreihen, die in den USA mit Dosen von 0,5 und 5 g gelöschten Kalks gemischt mit nicht-autoklavierter Erde durchgeführt wurden, starben fast alle Individuen. Die Mortalität erfolgte hauptsächlich im Wanderlarvenstadium, vermutlich durch die Aktivität von Pathogenen in der nicht-autoklavierten Erde verursacht. Dosen von 10 und 15 g verhinderten bei 2/3 der Individuen die Verpuppung, während hier die Mortalität signifikant niedriger war verglichen mit der 0,5 g Dosis (Tab. II).
Kalksteinpulver zeigte keinen Effekt auf die Entwicklung der Käfer. Das Gleiche galt für FS 90, FS 95 und die 0,05 g Dosis von FS 90.0s. Dagegen tötete FS 90.0s als 5 g Dosis fast alle und als 0,5 g Dosis etwa 2/5 der Individuen in der larvalen Wanderphase (Tab. III). In den Laborversuchen

in Australien starben signifikant mehr adulte Käfer in Diagnoserahmen mit FS 90.0s als in solchen mit gelöschtem Kalk oder trockenem Sand. Keine adulten Käfer entkamen aus den Fallen, während Larven diese verlassen konnten, unabhängig von der applizierten Substanz (Tab. IV-A). Im Freiland wurden in Fallen mit gelöschtem Kalk ca. 16% und in Fallen mit FS 90.0s mehr als die Hälfte der adulten Käferpopulation in den Bienenstöcken innerhalb von 48 h abgetötet (Tab. IV-B).

Unsere Daten lassen vermuten, dass gelöschter Kalk und das Diatomeenerde-Produkt FS 90.0s als Alternative zur konventionellen chemischen Kontrolle des Kleinen Beutenkäfers geeignet sind. Es besteht jedoch weiterer Forschungsbedarf hinsichtlich der praktischen Anwendung dieser Substanzen unter verschiedenen Umweltbedingungen.

Amorphe Silikate / Integrierte Schädlingsbekämpfung / Kalksteinpulver / Gelöschter Kalk / Kleiner Beutenkäfer

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