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CONTROL OF BED BUGS (HETEROPTERA: CIMICIDAE) WITH DIATOMACEOUS EARTH LOADED TAPE

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Abstract Bed bug infestations are most likely to establish close to human resting and sleeping area. We took advantage of the stereotyped feeding behaviour of bed bugs and developed a diatomaceous earth (DE) loaded tape, Nattaro Safe (NS), to be installed between bed bug harbourage and feeding place. DE absorbs the waxy epicuticle leading to desiccation and death of the insect. We show that bed bugs continuously exposed to DE die within 2 to 4 days. In a bed room situation bed bugs only get exposed to DE each time they pass through the DE loaded tape. We tested this in laboratory simulations where adults and nymphs passed through DE tape, were fed, and passed through the tape again and control tests without DE were performed. After 7 days 97% of adults and nymphs died. Seven and 10 days later the remaining adults and nymphs died. A few eggs were laid and hatched in the boxes with DE treated adults, but the nymphs only survived shortly. We conclude that repeated brief exposures to the DE loaded tape is able to shorten the bed bug life cycle by killing adults before they lay eggs or killing nymphs soon after they hatch.

Key words Residual effect, proactive treatment, bed bug tape, dispersal

INTRODUCTION

Bed bugs seek contact with conspecifics forming aggregates or harbourages where all life stages can be found together. Bed bugs are in contact with their substrate hiding in cracks and crevices and other dark places out of reach of the host and potential predators. Marking studies have shown that bed bugs move extensively but movement or dispersal seems to differ between small and large populations (Potter et al 2013a; Cooper et al. 2015). New introductions or small infestations are most likely to establish close to the food, i. e., in the proximity of the human resting and sleeping area. When infestations increase in size bed bugs are, for unknown reasons, more prone to move and may be found further and further away from the human resting areas. Thus, proactive work should concentrate on securing these areas to avoid dispersal and that an introduction of bed bugs becomes a large and difficult to eradicate infestation. We took advantage of the stereotyped behaviour of bed bugs and developed a diatomaceous earth (DE) loaded tape, Nattaro Safe (NS), to be installed between a bedbug's harbourage and its feeding place, usually the human resting area. DE has, as long as it is dry and non-contaminated a long-lasting residual effect that, when an insect moves through it, abrades and absorbs its waxy epicuticle leading to its desiccation and death (Korunic, 2013). Following a successful pilot study with the bed bug tape installed on beds in refugee accommodations we wanted to verify these findings in the lab. Our expectations were that the bed bug's life cycle would be cut short at one or more life stages, hence preventing an increase in the population's size and eventually eradicate the infestations as such.

First, we tested the efficacy of DE to kill adult bed bugs during 1) continuous exposure and 2) one hour's exposure followed by choice exposure (container tests). Second, we simulated a bedroom situation in which we tested the efficacy of the bed bug tape, to disrupt the life cycle of bed bugs by preventing 1) 1st instar nymphs from further development (nymph test) and 2) adult male and female from founding an infestation (life cycle test).

MATERIALS AND METHODS

Adult bed bugs were from a London field strain of the Common bed bug, *Cimex lectularius* L., (CimexStore, UK) and first instar nymphs were first generation progeny of females obtained from CimexStore. The bed bugs were kept on filter paper in 60 ml containers with bed bug proof netting in the lid at room temperature and RH, 19-24°C and 36-77 %, respectively and a normal night/day rhythm. Bed bugs were fed weekly with defibrinated sheep's blood. Fed

females were used in the container tests because prior tests had shown that of adults, fed females are most resistant to desiccation by DE. The DE, InsectoSec® (Biofa, Germany), was used in all experiments.

Container tests: DE was applied at a surface coverage of 13 g/m^2 (corresponding to the lower label amount in NS) on a circle of filter paper fitting the bottom of the experimental containers. Similar containers with filter paper but no DE were used as controls. Bed bugs were fed to satiation on sheep blood approx. 72 hours before experimental start and were not fed further during the experiment. For each replicate 12 female bed bugs were picked randomly six of which were added to an experimental and six to a control container. In the short exposure test a piece of filter paper was added after an hour allowing the bed bugs to leave the DE at the bottom and crawl onto the paper. In the continuous exposure test no such choice was available. Treatment and control containers were placed randomly and were inspected daily until all bed bugs in the treatment containers had died.

Nymph and life cycle tests: "Minicosms" (39x28x14 cm SAMLA box, IKEA Sweden) were prepared with four 12 cm x 3 cm strips of Nattaro Safe (NS) forming a rectangle with 0.5 cm openings between the strips. In the DE treatment boxes the inner bottom surface of NS was covered with DE at 13g/m^2 and in the control boxes with 13g/m^2 baking powder to cancel out the stickiness of the adhesive on the inside of the tape. Outside of the NS rectangle two potential harbourages made from folded paper (4.5 cm x 8 cm) were placed in each minicosm (Figure 1). Either unfed 1st instar nymphs (31 to 80 individuals in each replicate, nymph tests) or unfed adult bed bugs (10 females and 10 males in each replicate, life cycle tests) were released in the middle of the NS rectangle. The bed bugs were allowed to walk around in the minicosm for approximately one hour, where after they were collected using soft tweezers, fed for 10 minutes on a human volunteer and released again in the middle of the rectangle. This simulates the situation bed bugs encounter in a bed were NS has been installed: passing the bed bug tape on their way to the food and again after they have fed on their way back to their harbourages (aggregations). Alive adult bed bugs and nymphs were fed weekly following the same procedure as at start. First instar nymphs are very brittle and to ensure sufficient numbers in each test an excess of nymphs was introduced at start of the experiment. Various proportions of these died before they left the open space in the middle of the rectangle. These are excluded from the experiment which results in a variable number of individuals in each replicate. The minicosms were inspected daily except during weekends (nymph tests) or weekly (life cycle tests with adults). Dead adult bed bugs were removed, their sex identified, and the numbers counted. Dead nymphs were removed and counted. Similarly, the control boxes were inspected, and dead insects removed and counted. The number of insects alive in three control boxes were followed and counted one week after all DE exposed insects had died. This gave us insight into how fast a population of bed bugs can increase when left untreated.

Data Analysis. The 95 % confidence intervals of survival day by day for exposed nymphs and their respective controls were calculated. Because adult bed bugs both in container and life cycle tests died off very quickly compared to their controls no statistics were calculated.

RESULTS

Container Tests. The time to death of bed bugs in both continuous and short DE treatment was much shorter than their respective controls (Table 1). Continuously exposed bed bugs died within 4 days while no bed bugs died in their controls. The shortly exposed bed bugs died within an average of 12.4 days (range 5-24d) while 3 bed bugs died in the short exposure controls, two 19 days and one 24 days after start (Table 1).

Nymph Tests. After 8 days 99 % of all nymphs in treatment and 34 % in control boxes had died (Table 2). Three of the 9 replicate boxes still contained a few live nymphs, which after 17 days all had died. The mortality of the DE treated nymphs was significantly higher compared to nymphs in the control boxes (Table 2, 95 % confidence interval).

Life cycle Test. After one 96.7 % and after two weeks all DE exposed adults had died as had 2 nymphs that hatched during the test, and only 1 bed bug in the control treatments had died (Table 3). After 3 weeks the adults in three control boxes were accompanied by an average of 18 living nymphs per box and with more to be expected from an average of 70 eggs counted per control box (including the already hatched ones).

Table 1. Container tests. Average time to death of first, half and all bed bugs in continuous and short DE exposure tests. Six fed female bed bugs were used per replicate in each experiment (N=8)

	Continuous exposure		**Short exposure**	
	Average ± S.D. (days)			
Average days to:	**DE**	**Control**	**DE**	**Control**
First death (days)	1.6 ± 0.52	0	1.6 ± 0.92	19
(range)	(1-2 days)		(1-3 days)	
50 % had died (days)	1.9 ± 0.35	0	3.3 ± 1.98	NA
(range)	(1-2 days)		(1-6 days)	
All dead (days)	2.9 ± 0.83	0	12.4 ± 6.37	NA
(range)	(2-4 days)		(5-25 days)	

Table 2. Nymph tests. Average percentage dead nymphs exposed to DE in Nattaro Safe and their respective controls (± 95 % confidence interval). 31 to 80 first instar nymphs/replicate, (N=9)

	Average percentage dead	
	nymphs ± 95 % CI	
Day no.	**DE**	**Control**
1	81.6 ± 13.25	10.8 ± 6.18
2	92.1 ± 8.81	15.9 ± 10.32
3	95.6 ± 5.85	20.3 ± 11.91
4	95.9 ± 5.24	20.9 ± 11.64
6	96.4 ± 4.90	21.1 ± 11.54
7	97.8 ± 2.73	30.3 ± 12.01
8	99.0 ± 1.29	34.4 ± 13.22
9	99.0 ± 1.29	37.6 ± 14.76
10	99.7 ± 0.46	39.8 ± 13.59
13	99.7 ± 0.46	40.5 ± 13.86
14	99.9 ± 0.22	42.2 ± 14.58
15	99.9 ± 0.22	42.3 ± 14.65
17	100.0	42.6 ± 14.81

Table 3. Life cycle test of adult bed bugs exposed to DE in Nattaro Safe and their corresponding c ontrols. Ten females and 10 males per replicate, (N=6).

	Percentage dead adults		Number of eggs		Number of nymphs	
Day no	**DE**	**Control**	**DE**	**Control**	**DE**	**Control**
0	0	0	0	0	0	0
7	96,7	0	0,2	8,0	0	0
14	100	3,3	0,7	64,3	0,3	1,0
20	100	6,7	1,0	69,7	0,7	18,7

DISCUSSION

The rough experimental simulation of a feeding situation with NS installed between bed bug harbourages and the host in the bed that forced most bed bugs to pass through the DE tape on their way to and from a blood meal, showed that the bed bug tape efficiently interrupted the life cycle of bed bugs (Table 2 and 3). All adult bed bugs died within 14 and all 1st instar nymphs within 17 days of first exposure. Alive adults and nymphs were fed one week after experimental start, and were most likely exposed to DE again, though our experimental setup allowed the bed bugs to pass through DE free strips between the four pieces of DE-loaded tape. Agnew and Romero (2017) showed that bed bugs have behavioural mechanisms that reduce their exposure to some insecticide dusts including DE dust. This may also account in our study, though we observed adult bed bugs move in and out of the triangular funnel created by the DE tape and some even rested inside the tape in direct contact with the DE, admittedly less than in the controls. It took between 5 and 24 days for all shortly DE exposed bed bugs in a container to die (Table 1). The large variation in time to death indicates an uneven exposure to DE probably because bed bugs to varying degrees went into the DE at the bottom of the container. The continuously DE exposed fully fed, adult females died within four days (Table 1) and we are confident that this is the maximum survival at these conditions, because in prior DE exposure tests fed females survived significantly longer than either fed males, unfed females and males.

Bed bugs are mainly active at night where they, dependent on their physiological state, search for food, harbourages or a mate. During early stages of an infestation bed bugs gather in harbourages near the host's resting area (Potter et al, 2013a), most likely the bed, and regularly leave the harbourage to engorge themselves on the host. With a DE loaded bed bug tape installed between the harbourages and the host, bed bugs will be contaminated with DE during their passage and our study shows that a few passages will be sufficient to kill bed bugs irrespective of feeding status. Singh et al. (2016) and Potter et al. (2013b) found that DE performed poorly in brief exposure bioassays, however, with two enforced exposures per blood meal, our experiments shows that the DE bed bug tape is able to cut the bed bug life cycle short and stop an introduction from becoming an infestation. The advantages of the DE tape compared to loose DE is that the DE stays in place within the paper triangle and becomes less mixed with household dust, and bed bugs seems to like the paper construction in itself. The efficacy of the DE tape is long lasting, as we got highly similar results when we tested the efficacy of DE tape after it had been installed for 15 months in a private home.

CONCLUSION

The DE in paper with double adhesive works proactively by killing new introductions of bed bugs and preventing an introduction becomes an infestation.

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