Bed Bugs—Down the Trap

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Bed bugs, for long a forgotten nuisance, have reappeared. The detection and eradication of them have become a million dollar industry. Bed bugs are well protected from watery solutions of pesticides by a waxy outer cuticle and they easily become resistant to pesticides. Approximately ten weeks after infestation a bed bug population change from linear to exponential growth. To detect an infestation before this point is crucial for a successful treatment. Our aim is to develop a monitoring system that detect bed bugs at low densities, partly substituting costly ocular inspections and a system that can evaluate the efficacy of a treatment.

We collected and analyzed emissions from bed bug colonies with headspace, enfleurage and GC-MS methods. Bed bug attraction in test arenas to the pheromones resulted in a solution of five chemicals, which, applied in traps, were presented together with control traps and tested in mesocosm. Pheroemission decreased with time, but was easily detectable after six days.

Ten bed bugs (4 females, 6 males) were released at start, after 24 and 72 hrs and retrieved after 24, 72 and 144 hours, respectively. Scent baited pherotraps caught 37, 38 and 37 % of the introduced bed bugs during the first, second and third period, respectively and caught significantly more bed bugs than unscented control traps ($p=2 \times 10^{-16}$). The capture of both sexes were independent of bait age for the three periods we tested, but the periods varied in length and we do not know when in each period the bed bugs went into the traps. The effect of exposure time was tested for fresh scent by releasing bed bugs at the start and retrieving them after 48 hrs. At the end of the 48 hour period there were statistically significantly more bed bugs in the pherotrap compared to the 24 hour period ($p=0.05$).

In conclusion we have a highly potent scent mixture that attracts both female and male bed bugs irrespective of bait age, at least for up to the 144 hours tested in our experiment. Our mixture is more attractive to female than to male bed bugs, which is a bonus because detection of female bed bugs, especially pregnant ones, at an early state may prevent establishment of new infestations.

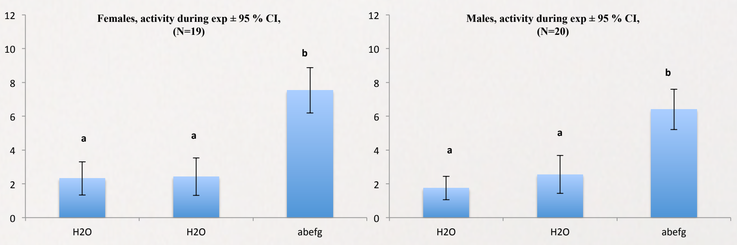

Figure 1. Arena tests, each with ten bed bugs, of the mixture abefg against water controls. The activity was video recorded for 30 minutes. Each time a bed bug went under the rim of the glass over a scent or a control vial it was scored. At the end of the 30 minute period the position of each bed bug in the arena was scored as the bugs were removed. The difference is small between the score during the 30 minute period and at the end of the period indicating that the bed bugs make a choice and then usually stay in that position the remaining time. (Of ten bed bugs, 1.3 ± 1.24 (average ± S. D.) females and 2.3 ± 2.27 males did not make any choice).

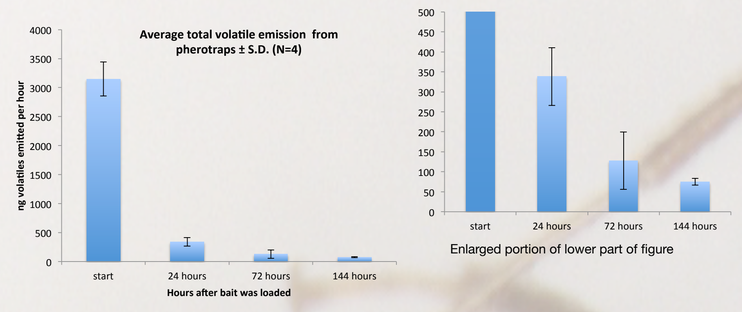

Figure 3. Longevity of the synthetic scent mixture in pherotraps. GC-MS analysis of head space from the scent mixture (ng h^{-1}) in pherotraps at the start and/or end of the three periods (0-24, 24-72 and 72-144 hrs). The scent mixture was still easily detectable after 144 hours at the end of the experiment.

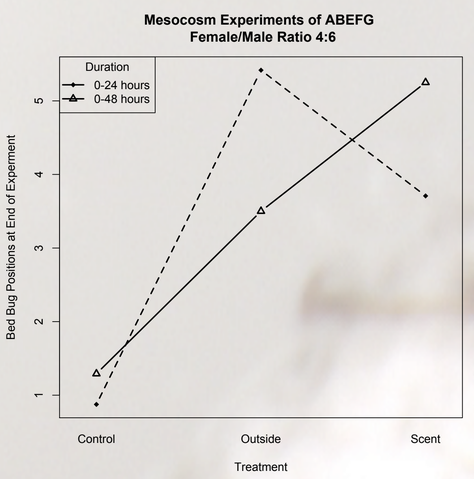

Figure 4. Interaction plot between 24 and 48 hour exposure. Mean number of bed bugs in the phero-trap, control trap or outside during the 24 (0-24) and 48 (0-48) hour exposure experiment. There were significantly more bed bugs in the pherotrap in the 48 hour exposure group compared to the 24 hour group. The female/male ratio in the mesocosm was 4:6, N=24 for both exposures.

Bed bugs spend most time between blood meals in harborages, aggregations consisting of a relative constant number of adult bed bugs and nymphs. We predicted bed bugs to use volatiles to locate their harborages and expected that we could use the volatiles to lure bed bugs into a trapping device. To pursuit this idea we 1) initiated a study of emissions from bed bug aggregations and identified a number of volatiles that we 2) tested in arena tests in the lab. We then 3) tested the most promising mixture in traps in mesocosm under circumstances that approached natural conditions.

In the lab, head-space and enfleurage samples were collected from vials containing equal numbers of nymphs, female and male bed bugs that had aggregated into harborages on filter paper. Samples were eluted with hexane, added an internal standard and analyzed by GC-MS and most compounds were identified with authentic standards.

We tested a number of compounds and combination of these in test arenas resulting in a highly attractive blend composed of five compounds that was statistically significantly more attractive to both female and male bed bugs than control blends Fig. 1.

The attraction of bed bugs to this synthetic mixture was then tested in a semi-natural environment established in mesocosm that contained wooden skirting boards and clothing which are favourite materials for bed bugs to hide in or under. Ten bed bugs, four females and six males, were released into a mesocosm with two traps one with the synthetic scent bait (pherotrap) and the other with control bait. The proportion of females to males reflects that found in natural populations. The bed bugs were scored at three positions: pherotrap, control trap and outside of either trap at the end of each period. The pherotrap caught statistically significant more bed bugs than the control trap ($p=2 \times 10^{-16}$) (Fig 2). There were no differences between the sexes caught in the trap but significantly more males were left in the mesocosm compared to either sex in the pherotrap ($p<0.05$).

The life length of the scented bait lasted the whole test period. Although the emission decreased about 40 fold from when the bait was loaded into the traps (3147 ng/hr) and until the experiment ended 144 hours later (75 ng/hr, Fig. 3) it still attracted bed bugs during the third period.

The effect of exposure time was tested for the first period by extending the duration to 48 hrs (0-48 hrs). The pherotrap caught significantly more bed bugs during the longer exposure ($p=0.05$) (Table 1 and Fig. 4). This highlights the traps efficiency as the bed bugs aggregate in the mesocosm but are then attracted from their initial aggregation towards the pherotrap after the first 24 hrs.

The scented pherotraps displayed a strong hit-rate for all three periods as well as the extended first period (Fig 5). Few control traps caught more than one or two bed bugs, whereas the probability of capturing 2 to 4 bed bugs was higher in the scented traps.

CONCLUSIONS

We have composed a synthetic scent mixture based on bed bugs own aggregation scent, which is active and attracts both male and female bed bugs. Female bed bugs appear to be more attracted to the scent compared to males. Our results show that the pherotrap has the potential to continue to attract and trap bed bugs in an infested area for up to 144 hours. The next step is to test the pherotrap under field conditions and hopefully be able to confirm the results in the bed bugs natural environment. The pherotrap has the potential to become a useful tool to check if a treatment has been successful, as a monitor device in hotels and other public places that are at risk of bed bugs or to control luggage after travelling.

Table 1. Distribution of bed bugs in relation to the length of exposure of pherotraps. The presence of bed bugs was scored at three different positions: in pherotraps, in control traps and in the mesocosm outside traps. The distribution was scored for two periods.

Period/mean ± S. D. in mesocosm	in traps with		
	scented bait	control bait	outside traps
1 (0-24 hrs old)	3.71 ± 3.11[a]	0.88 ± 1.30[c]	5.42 ± 3.71[a,b]
1.2 (0-48 hrs old)	5.25 ± 2.27[b]	1.29 ± 1.37[c]	3.50 ± 2.00[a,b]

[abc]Tukey HSD post hoc test for 3-way ANOVA of distribution of bed bugs during periods 1 and 1.2. N=24 and the female/male ratio in the mesocosm were 4:6. Different letters in each period indicates a statistically significant difference between the positions of bed bugs at the end of the period ($p<0.001$ for all except between the two scented baits which was only significant at $p=0.05$).

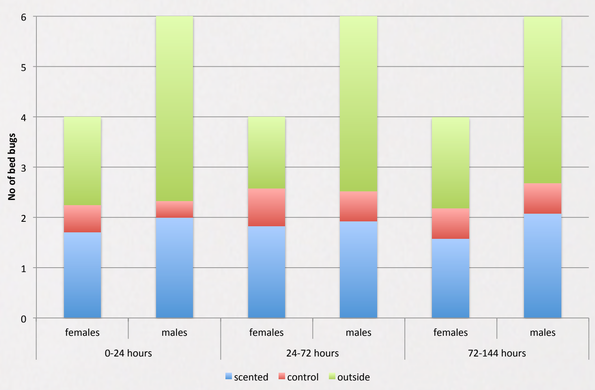

Figure 2. Distribution of bed bugs in mesocosm in relation to bait age. Bed bug attraction to bait exposed for up to 144 hours. Bed bugs were scored at three different positions: pherotraps (blue), control traps (red) and outside in mesocosm (green). Ten bed bugs (4 female and 6 males) were used in each replicate. Pherotraps attracted equal numbers of female and male bed bugs and significantly more bed bugs than the control traps ($p<0.001$). Significantly fewer female than male bed bugs were still located outside in the mesocosm at the end of each period ($p<0.05$), (Tukey HSD post hoc test for 3-way ANOVA)

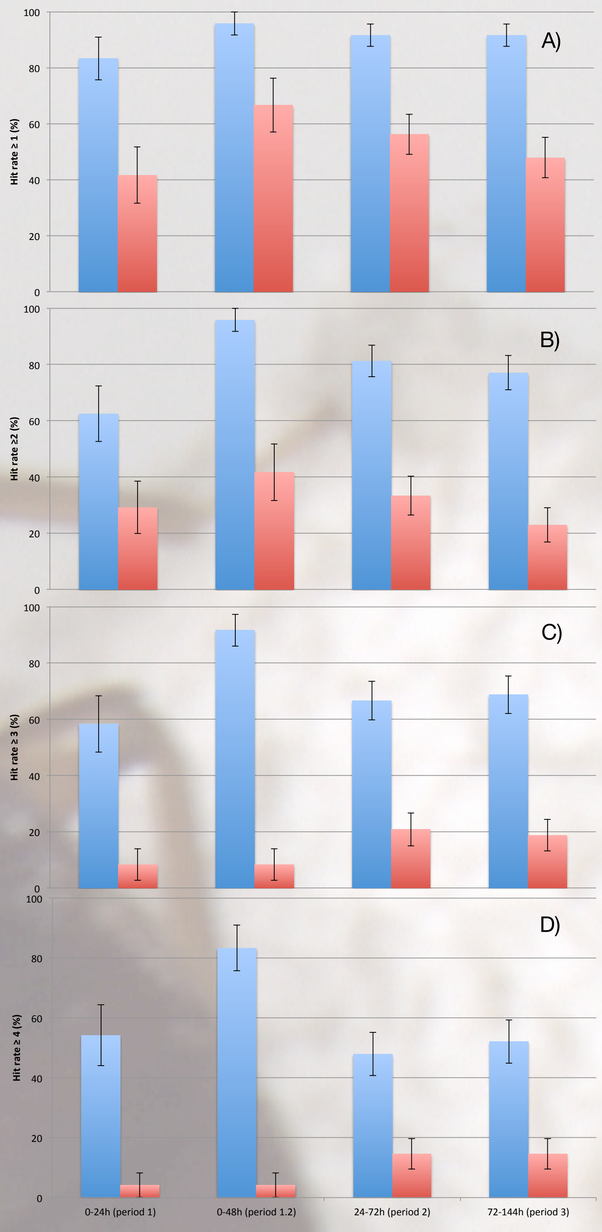

Figure 5. Hit-rate. Scent age, mean hit-rate ± S.D., the rate at which at least A) one, B) two, C) three and D) four of the ten bed bugs was/were found in each trap. Hit rate is calculated: $n_{\text{At least one bed bug found in scented or control trap}}/N$. The hit-rate should not be interpreted alone but compared between scented and control traps. The female/male ratio in the mesocosm was 4:6, $N_{\text{period 1 \& 1.2}}=24$, $N_{\text{period 2 \& 3}}=48$.