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3 December 2012

Structural Pest Control Board
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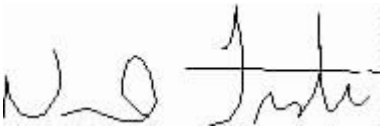
Dear Board Members,

Please find attached my final report for the project, "Bedbug detection using airborne pheromone cues". During the course of this research, we have fulfilled the proposed objectives, as described below. We also presented our research at the Entomological Society of America meeting in November, 2011 and November 2012. We have summarized our research in a multi-author article that has been submitted to the journal *California Agriculture*, and we are preparing to submit a second manuscript that focuses in more detail on the results of this research. I hope you will agree that the development of this new technology for bedbug detection holds great promise and I look forward to continuing to build on these finding in future studies.

Thank you, again, for your support of our research.

Sincerely,



Neil Tsutsui
Associate Professor
Vice Chair for Instruction

Final Report

Objective 1. To determine the lower limits of bed bug detection using A) SPME fibers and B) charcoal adsorbant.

Progress: We have completed this objective. We found that, in the laboratory setting, we can detect bed bug-specific pheromones from a single adult bed bug. We have also added to these data by analyzing the background chemical signatures from wooden refugia, to ensure that such naturally-occuring chemicals from the wood substrate do not interfere with or mask the bed bug-emitted chemicals.

Objective 2. To quantify the bed bug:air volume ratio necessary for accurate bed bug detection using A) SPME and B) charcoal adsorbant.

Progress: We have completed this objective. In principle, detection of airborne pheromones might be hampered if the half-life of the sample retention on the detection medium is short. For example, in a field setting, a pest control operator might sample air containing the pheromones, but then continue to sample pheromone-free air afterward. If the passage of this "clean" (pheromone-free) air acts to remove bound pheromone from the sampling device, small numbers of bed bugs might be overlooked.

Using precise quantities of synthetic 2-hexenal and 2-octenal, we tested the ability of two different sampling techniques (charcoal cartridges and SPME fibers) to detect the pheromones when various volumes of clean air were passed across the sampling device.

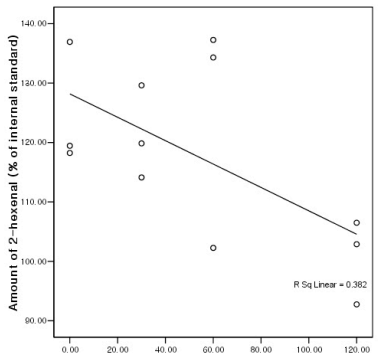
We found that when SPME fibers were used, there was no detectable decrease in the amount of pheromone sample recovered, regardless of the volume of air or sampling time (up to 432 L of air; 2 hours). In contrast, when the charcoal filters were used as the sampling device, we observed a significant decrease in the amount of 2-hexenal recovered with increasing air volume (= sampling time)(Figure 1A). We did not see a similar pattern when sampling 2-octenal (Figure 1B).

When large concentrations (20 insect equivalents) of pheromone were used, we saw a decline in chemical concentration for both pheromones (not shown), but the amounts retained were still well above our sample detection threshold.

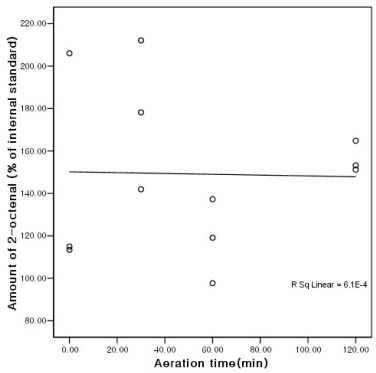
These data suggest that, when possible, SPME fibers should be used as the pheromone sampling device. When charcoal cartridges are used, care should be taken when using the absence of 2-hexenal as an indicator of no bed bugs present.

Figure 1. Sensitivity of bed bug detection using charcoal cartidges to collect airborne A) 2-hexenal and B) 2-octenal. When SPME fibers were used to sample these pheromones (not shown), there was no noticable decrease in pheromone concentration across different sampling times.

A.



B.



Objective 3. To determine the amount of time that dead bed bugs continue producing characteristic pheromones.

Progress: We have completed this objective. Using gas chromatography-mass spectroscopy, we have collected head-space volatile chemicals emitted by bed bugs at a series of time points post-mortem (1, 3, 6 and 22 hours post-mortem). We were able to detect bed bug pheromones 1 hour after freeze killing insects (dry ice), but not at 3, 6 or 22 hours. When the insects were killed by heat or insecticide, no pheromones were detect at any post-mortem time point (Figure 2).

Our findings represent an important step forward in the development of this method for bedbug detection. In theory, dead insects (from either natural causes or various control methods) could potentially emit pheromones despite the absence of a living insect infestation. However, our data indicate that our method of detecting active bed bug infestations will not be hampered in the field by erroneous "false-positives" from previously killed bed bugs.

Figure 2. Neither 2-hexenal nor 2-octenal are emitted in significant quantities by dead bed bugs (head-killed, frozen, or insecticide-killed).