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EVALUATION OF *CIMEX LECTULARIUS* PREFERENCE TO HARBOURING MATERIAL

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Abstract Traps need to be highly attractive to the bed bug, *Cimex lectularius,* to catch them at low population numbers. Typically, traps are manufactured in plastic or cardboard and have an aggregation pheromone incorporated into the design for attraction. Yet, the material of the trap will also influence how attractive the trap is to the bed bug,but is usually not considered in the design. This is relevant as a pheromone lure within the trap will have to compete with the natural pheromones secreted by bed bugs in the room, from more favourable harbouring locations. A glue board may not be an effective way of detecting bed bugs even though they are widely used. In this study, four test materials were evaluated in a choice bioassay to determine which harbouring material was the most attractive to bed bugs. Further testing evaluated the effectiveness of the preferred materials as a monitoring device for detecting bed bugs. Bed bugs had a preference for harbouring in corrugated cardboard and wood compared to cardboard and plastic. Placing a glue board within a custom corrugated cardboard trap did not lead to a high likelihood of bed bugs being caught on the glue board.

Key words. Bed bug, monitor, attractive

INTRODUCTION

Detecting a bed bug (*Cimex lectularius*) infestation as soon as possible is key to limiting the spread of bed bugs to new locations and will lead to a higher success rate at managing the infestation (Wang et al., 2009). Additionally, with resistance to pyrethroids already high in bed bug populations (Romero et al., 2007), treating bed bugs at low numbers leads to smaller amounts of insecticide being applied and safeguards them for the future management of the pest. Early detection of an infestation relies on well trained staff to visually inspect sites with the aid of traps that will catch bed bugs between inspections. Bed bug traps are manufactured both as ‘active’ and ‘passive’, with an active being a lure that attracts bed bugs to the trap such as carbon dioxide, heat or bed bug pheromones (Vaidyanathan and Feldlaufer, 2013). A passive trap contains no attractant but their shape, size, material and colour will affect how attractive they are to the bed bug. Bed bugs are known to prefer rough materials (Hottel et al., 2015), are thigmotactic and negative phototaxic (Usinger, 1966) but this is not reflected in the traps that are made of smooth surface materials such as plastic or cardboard and made with large voids to help the pest technician inspect the traps. The efficacy of these traps is debatable (Cooper, 2006; Harlan, 2007) and studies have been conducted to find a trap that is attractive (Crawley and Borden., 2021; Hottel et al., 2019) and able to compete with the natural bed bug aggregation pheromone, humans scent and harbouring locations in bed bug habitats. A good monitoring trap is vital to ensure a survey can be carried out quickly and with minimal disruption to a client’s premises, though many surveys are laborious and lengthy (Gangloff-Kaufmann et al., 2006; Harlan, 2007).

In this study, different materials will be evaluated to identify which was preferred by the bed bug. Plastic and cardboard were chosen as they are used in monitoring traps. These were in competition with wood, a rough textured material often found in domestic and commercial habitats of the bed bug and corrugated cardboard, proposed because of its rough texture and affordability. A choice test evaluated how the most attractive material performed as a without a glue board, a trap with a plain glue board and a trap with a glue board with an aggregation pheromone.

METHODS AND MATERIALS

Insects. *Cimex lectularius* were obtained from the field through *Cimex* store ltd and kept at a stable temperature and humidity (18 ± 2ºC, 55 ± 5% RH) on a 12:12 light/dark cycle (08:00-20:00 light). All bed bugs were fed through a blood membrane feeder at Cimex and only bed bugs that had been fed within 10 days were used in experiments.

Harbourages and test arena. Four harbourages of different materials were tested, corrugated cardboard, soft wood (balsa), plastic (acrylic Perspex) and smooth card. Each harbourage was constructed of four pieces of the test material, two pieces for a base and top (60mm x 60mm) and two pieces for the sides (5 mm x 60 mm). When constructed the harbourage would have a gap in the middle for bed bugs to harbor in (Figure 1). Two types of glue board were used, plain with no lure and bed bug aggregation pheromone, these glue boards stuck to the base of a corrugated harbourage. A black plastic tray (area = 0.22 m^2, perimeter = 1.92 m) was lined with paper and the perimeter walls were coated in fluon (PTFE).

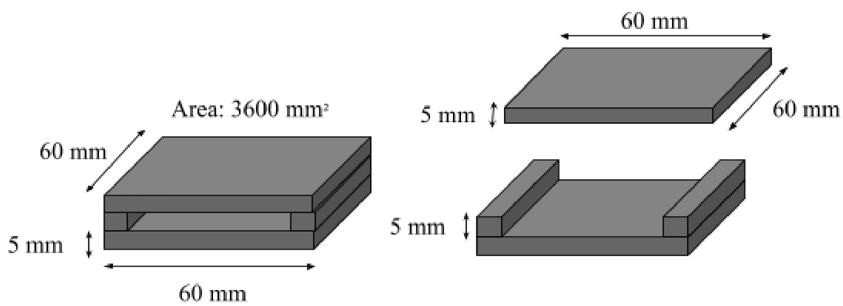

Figure 1. Harbourages created from test corrugated cardboard, wood, plastic and card.

Test Procedure. Ten bed bugs with an equal sex ratio were placed in the centre of the test arena under a plastic cup to acclimatize for 10 minutes. In each corner of the test arena a harbourage was placed to evaluate bed bug preference on harbouring material. The bed bugs were then released from the cup and allowed to explore the area. The location of the bed bugs was counted at the following time points post bed bug release: 15 and 30 minutes, 1, 1.5, 2, 4, 8 and 24 hour(s). At the end of the 24 hours the bed bugs were removed from the arena. Four replicates of the test was performed to allow each harbourage to be tested in each corner of the arena.

Secondary testing followed the same procedure but used three corrugated harbourages, one without a glue board, one with glue board and no lure and one with a glue board and a bed bug aggregating pheromone. These harbourages were placed equal distance away from the bed bug release point. Bed bugs were counted at the following time points post bed bug release: 15 and 30 minutes, 1, 1.5, 2, 2.5, 3, 8 and 24 hour(s) and three replicates were performed with the harbourages being rotated for each replicate to remove bias.

Data analysis. Location of bed bugs at each time point were analysed with ANOVA and post-hoc Tukey test.

RESULTS

No replicate of all the tests had all bed bugs within a harbourage at the end of the 24-hour test period, with the maximum number of bed bugs being within a harbourage 8 out of 10 bed bugs in the arena. If bed bugs did not choose a harbourage they would be found at the perimeter of the arena.

Harbourage choice test (Figure 2). Bed bugs showed a preference for the following harbourages in decreasing order of preference, corrugated cardboard, wood, cardboard, plastic. Over the course of the trial only two bed bugs was found in the plastic detector, on each occasion the bed bug was not found again in the plastic harbourage at the following time point. After 24 hours bed bugs had a clear preference for corrugated cardboard, with more than 7 times the number of bed bugs than the next best material, wood ($\bar{x}$ = 5.5 vs $\bar{x}$ = 0.75).

At every time point (except 0.25 hours) the mean number of bed bugs within the corrugated cardboard harbourage was statistically different from the mean number of bed bugs within the cardboard and plastic harbourages (Table 1). Additionally, the mean number of bed bugs was statistically different between the wood and corrugated cardboard at the time points 1.5, 4, 8 and 24 hours post bed bugs release. The mean number of bed bugs within the wooden harbourage was statistically different to the cardboard and plastic harbourages only at 0.5 hours post bed bug release.

Table 1. Mean number of bed bugs found at time points in harbourage
Values followed with same letter in column not significantly different ($P < 0.05$).

	Time (hours)							
	0.25	**0.5**	**1**	**1.5**	**2**	**4**	**8**	**24**
Cardboard	0^{A}	0^{B}	0.25^{B}	0^{B}	0.25^{B}	0^{B}	0.25^{B}	0.5^{B}
Wood	2^{A}	2.25^{A}	2$^{A, B}$	1.25^{B}	2$^{A, B}$	1.5^{B}	1.25^{B}	0.75^{B}
Plastic	0^{A}	0^{B}	0^{B}	0.25^{B}	0.25^{B}	0^{B}	0^{B}	0^{B}
Corrugated cardboard	2.75^{A}	2.75^{A}	2.5^{A}	4^{A}	3^{A}	5.25^{A}	5.25^{A}	5.5^{A}

Glue board choice (Figure 3). Over the trial only one bed bug was caught on the glue board within a harbourage, this occurred for the aggregation pheromone trap at time 2.5 hours after bed bug release. All other bed bugs recorded were found within the corrugations of the harbourage or on the underside of the harbourage roof. The most number of bed bugs at the end of the trial were found in the harbourage without any glue board, with on average 3.3 bed bugs found there, conversely with a plain glue board and a pheromone glue board only 1.3 and 1 bed bug(s) were found respectively. The mean number of bed bugs within the harbourage with no glue board ($\bar{x} = 3.33$) was statistically different to plain glue board harbourage ($\bar{x} = 0.33$) at 2 hours post bed bug release. At every other time point there was no statistical difference in the mean number of bed bugs found for either a harbourage without a glue board, a harbourage with a plain glue board or a harbourage with an aggregation pheromone on the glue board.

Table 2. Mean number of bed bugs found at the following time points in monitors without a glue board, with a plain glue board or with an aggregation pheromone glue board. Values followed with the same letter in the column were not considered significantly different from each other ($P < 0.05$).

	Time (hours)								
	0.25	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**8**	**24**
No glue board	1^{A}	1.33^{A}	2.33^{A}	2.66^{A}	3.33^{A}	3^{A}	2.66^{A}	3^{A}	2^{A}
Plain glue board	0^{A}	0.66^{A}	1^{A}	0.66^{A}	0.33^{B}	0.33^{A}	0.66^{A}	1^{A}	1.66^{A}
Pheromone glue board	0.66^{A}	1^{A}	1.66^{A}	1^{A}	1.33$^{A, B}$	2.33^{A}	1.66^{A}	2.33^{A}	2.33^{A}

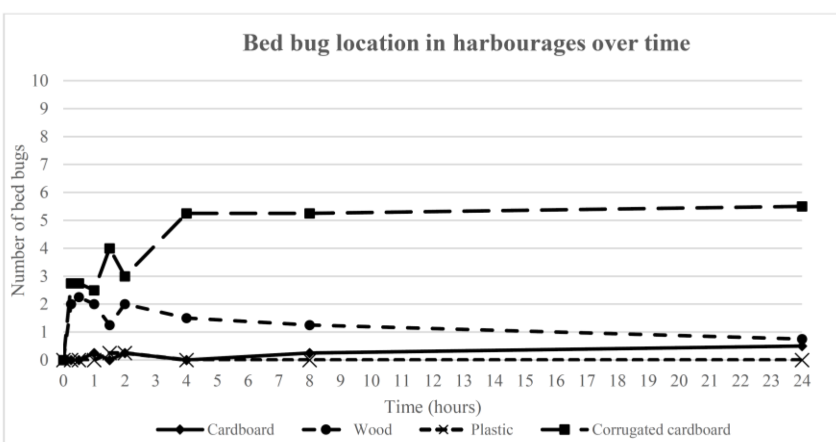

Figure 2. Location of bed bugs over time in harborages with choice test.

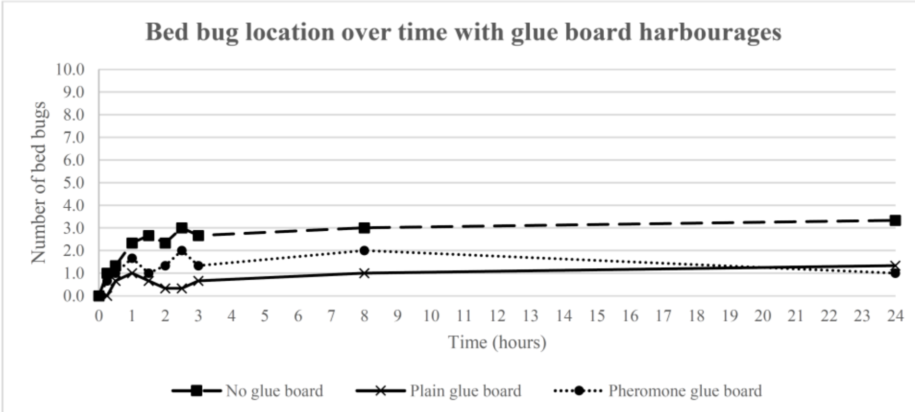

Figure 3. Location of bed bugs over time with use of glue boards in harborage

DISCUSSION

Commercial bed bug traps are typically produced either in plastic or in cardboard due to the cheapness and convenience of these materials. However, as shown in this study, these materials do not compete well against corrugated cardboard or wood in attracting bed bugs to harbour within them, likely a result from bed bugs preferring rougher materials (Hottel et al., 2015). There was a preference for corrugated cardboard compared to other test materials, this could be due to the greater surface area of the material from the flutes creating numerous places for the bed bugs to harbour. This is supported by bed bugs being highly thigmotactic and the bed bug choosing harbourages based thigmotaxis rather than negative phototaxis (Rivnay, 1932). Bed bugs were rarely found within the inner gap created between the four pieces of material but were found in the flutes of the corrugated cardboard where owing the size of the flute (height - 3mm, width - 5mm) a bed bug could contact the surface at multiple points round the body.

The majority of bed bug traps utilise a glue board to catch the bed bugs on for detection but this study highlighted that a glue board whether using an aggregation pheromone or no lure did not effectively trap bed bugs with only one bed bug caught over the course of three trials with 10 bed bugs per trial. This outcome leads to the question of whether the bulk of bed bug glue board traps in the field are effective tools at detecting populations of bed bugs. Without an effective monitor a more intensive and time consuming survey must be performed (Gangloff-Kaufmann et al., 2006) as bed bugs will harbour deep within cracks and crevices (Vaidyanathan and Feldlaufer, 2013) making them hard to detect and less likely to be detected by visual inspection alone (Wang et al., 2009).Monitoring traps are designed to retain the bed bug as proof of evidence of an active infestation, however, instead of retaining a bed bug as proof could traps evolve into highly attractive monitors that allow bed bugs to come and go freely? This idea is based on the fact that bed bugs will aggregate in locations nearby a blood meal (Harlan, 2007) (Usinger, 1966) and will return to a harbourage after feeding (Reis and Miller, 2011). Traps are already placed optimally in a room where there is greatest likelihood of bed bugs being found with 70% of bed bugs will be found on beds (Potter et al., 2006), so placing a highly attractive monitor there could also lead to bed bugs harbouring in that monitor. Furthermore, factoring in that surveys are usually conducted during the day where bed bugs will seek out their harbourages (Reis and Miller, 2011) and that other signs of the infestation (eggs, faecal spotting and nymphal skins) will also be found in the harbourage there is a high probability that an infestation will still be discovered even if it does not have a glue board to retain a bed bug. Of course, the use of an interceptor trap would be considered a suitable alternative if not using a glue board as they are highly successful at detecting bed bugs (Wang et al., 2009). Yet, interceptor traps do not have the versatility to be placed in as many locations around a room (behind a head board, under furniture) and are not suitable for commercial sites (hotels, cruise ships and aeroplanes) where customers may discover them and become concerned about bed bugs . A monitor without a glue board may likely be viewed poorly by consumers and technicians alike, but if properly designed to incorporate cues bed bugs seek in a harbourage, there would be a high chance of detecting bed bugs at an early point in the infestation and being a valuable tool for technicians.

CONCLUSION

A pest management technician needs tools that allow easy, quick and effective surveys to be performed. However, relying on bed bug traps that have a low chance of catch leads to a more arduous survey in the search of bed bugs. By using a monitor that removes the glue board but makes the monitor more attractive could lead to a higher success rate of detecting bed bugs and improving survey times and management of the pest.

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