Laboratory Evaluations to Determine the Ability of the Averzion Slick Barrier and Nanofilm Tape to Inhibit Bed Bug (*Cimex lectularius)* Access to a Blood Meal

Final Report For:

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Overall Objective: To determine the efficacy of the Averzion Slick Barrier and Nanofilm tape as barriers against bed bugs under laboratory conditions in bioassay arenas.

Start: August 2023
Complete: September 2023

Materials and Methods

Test Facilities:

All laboratory bioassays were conducted at the Dodson Urban Pest Management Laboratory (DUPML), on the Virginia Tech campus in Blacksburg, VA.

Bed Bug Strain and Rearing Conditions:

Mixed stage bed bugs from the Richmond strain were used in all laboratory assays. The Richmond strain bed bugs were collected from a senior living facility in Richmond, VA in 2008 and have been maintained at the DUPML ever since.

All bed bug strains at the DUPML are maintained in plastic rearing containers (pill jars), each containing intersected pieces of cut cardboard for harborage. The rearing containers are inverted (the screw-top lid serves as the floor of the rearing jar), and the former bottom of the jar has been removed and replaced with mesh fabric. The fabric allows for bed bugs to feed through the mesh from an artificial feeding system. All bed bugs are fed once a week on defibrinated rabbit blood (Hemostat, Dixon, CA). Prior to feeding, the blood is heated to an optimal range between 37°C and 38°C. Between feedings, all bed bug rearing jars are held in an environmental chamber at 25°C, 60% relative humidity, and on a 12:12 L:D photoperiod.

Bioassay Design:

Averzion Slick Barrier and 1" Nanofilm Barrier tape

Prior to testing, all bed bugs were starved for at least one week. Two test groups of five replications each (plus 2 control replications) were removed from rearing containers and allowed to acclimate in Petri dishes lined with filter paper for 24 hours. Each replication consisted of 20 mixed stage bed bugs (10:10 adult males and mixed-stage nymphs). After the acclimation period, each filter paper holding the bed bug replicate was removed from the Petri dish and placed on one side of a plastic bioassay arena (30.48 cm x 30.48 cm x 15.24 cm). all bioassay arenas had been previously lined at the bottom with brown parchment paper, and had the interior walls coated with a 1:2 mixture of petroleum jelly and mineral oil to prevent bed bug escape. Note that each arena also had a removable plastic lid that had two circular holes cut in the top that were covered in mesh fabric (Figure 1).

In order to evaluate the barrier products, each bioassay arena also contained a single upright cardboard tube (10 cm x 5 cm). This cardboard tube had been covered in fabric typically used on the bottom of a box spring mattress. The tube was glued into place within the arena so that it stood directly underneath one of the mesh covered holes in the arena lid.

In the Group 1 bioassay arenas, each covered tube had the top 5 cm coated twice (two layers) with the Averzion Slick Barrier. The double coating was used because preliminary trials had indicated that bed bugs could immediately climb the “slick barrier” if the fabric had only been coated once.

In the Group 2 bioassay arenas, each tube had its center wrapped in Nanofilm barrier tape (tape width 1“ (2.5 cm)). This left the bottom (and the top) of the tube accessible to the bed bugs.

The two control arenas for each group (four total) also contained a cardboard tube that was lined with box spring fabric. But these tubes did not have any Slick Barrier or Nanofilm placed on top of the fabric. All bioassay and control arenas were held at a room temperature of 25°C, on a 12:12 L:D cycle.

At the start of the bioassay, each arena then was placed under a multi-station feeding device where individual feeding stations had been filled with heated defibrinated rabbit blood (Fig. 1; Hemostat). The fabric covered tube in each arena was oriented directly under the feeding station so that the heated blood would lure the bed bugs to the tube and entice them to climb the tube to reach the blood source. The feeding stations were lowered onto the mesh fabric of the arena lid, and held in place on top of the tube (Figure 1). Bed bugs that could climb the tubes (with a barrier or without) and feed from the station were allowed to do so for 30 minutes. After 30 minutes, the number of bed bugs that climbed the tube and fed were recorded for each bioassay arena.

Addition of the 2” Nanofilm Barrier

When conducting the initial Group 2 bioassays, it was immediately observed that the 1” Nanofilm barrier tape had a very specific compromising feature. This feature was the horizontal edge of the tape that had been cut to complete the barrier around the tube. This cut edge allowed adult bed bugs to scale the tape edge where the ends overlapped. It was determined that if this barrier tape were to be applied in the field, any adult bed bug could follow that edge of the cut tape across the barrier and still gain access to the food source. To address this issue, a third bioassay that was run to test the efficacy of a wider Nanofilm (2”) barrier, with the tape cut in a diagonal curve.

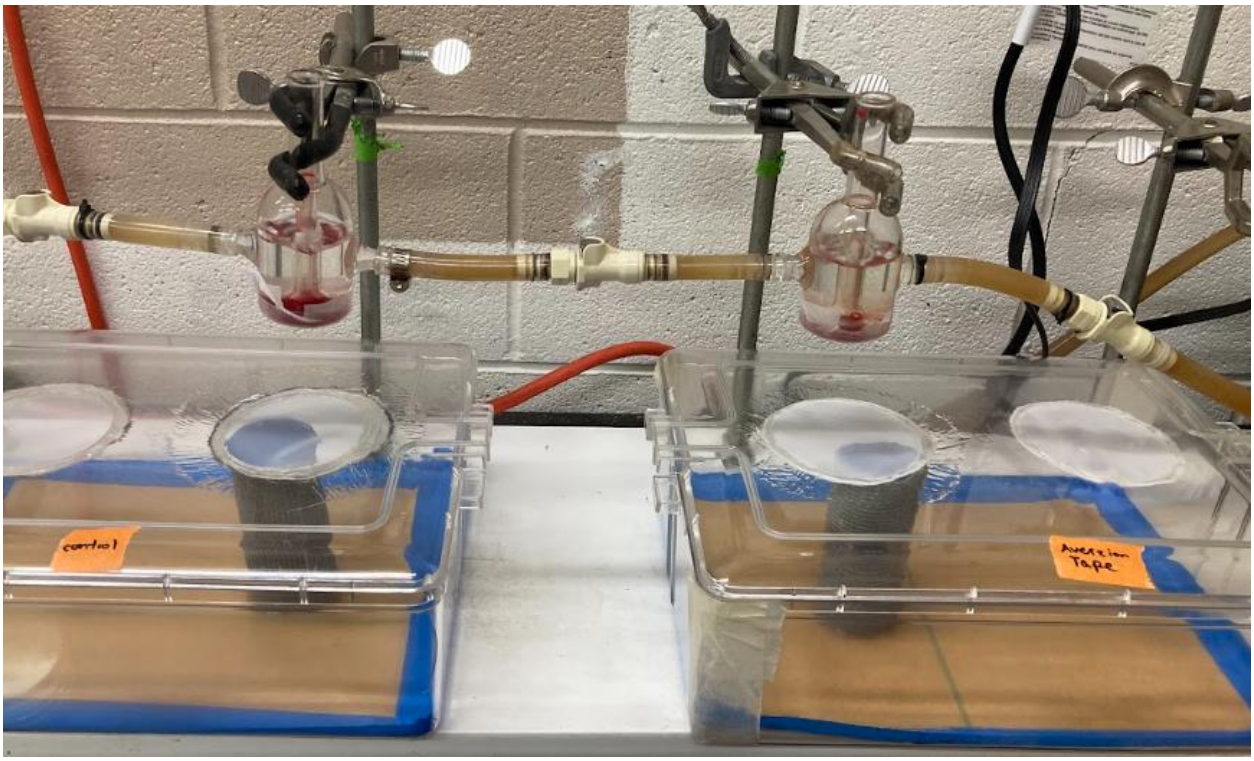

Figure 1. Bioassay set up: arenas were placed under a heated multi-station feeding device. Individual feeding stations were lowered onto the mesh fabric that covered the top of over the treated tubes inside the bioassay arenas. The heated blood was used as lure to entice bed bugs to climb the tube.

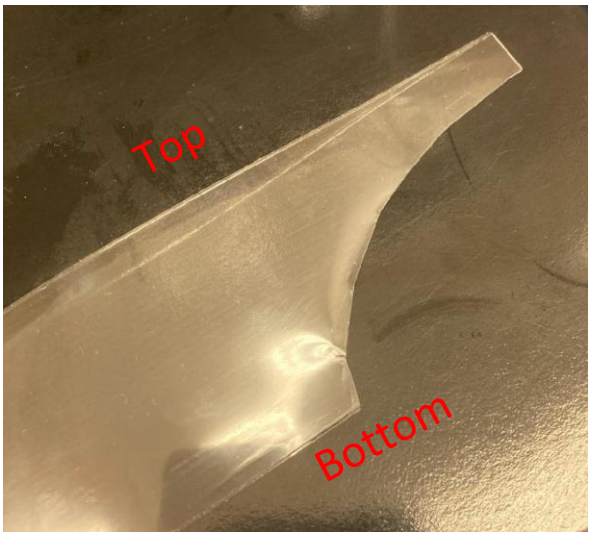

Figure 2. The 2" nanofilm tape that was cut at a curved diagonal prior to placement on the box spring fabric tube.

In this third bioassay group, five replications of 20 mixed stage bed bugs (10:10 adult males and nymphs) were introduced on filter paper into bioassay arenas designed as described previously. However, in these bioassay arenas, the fabric covered tube (4" long) had its entire midsection wrapped in Nanofilm tape that was 2" in width. Also, the end of the tape barrier was not cut straight across as it had been previously. Instead the tape was left long enough so that it overlapped its initial starting point on the tube, and the end of the tape was cut in a curved diagonal shape. This was so that any adventurous bed bug would be oriented upside-down when climbing the tape edge (Figure 2). Again, a control arena with no tape were set up as previously described, and all treated and control bed bug replicates were allowed thirty minutes to potentially climb the tube and feed.

Statistical Analysis

The mean percent of fed bed bugs for each replication in each test group was recorded and compared to the those in the other two treatment groups, and the controls, using Analysis of Variance (ANOVA). Values of $P \leq 0.05$ were used to indicate significance. Means were separated using the Tukey's HSD test. All data analysis was conducted using JMP Pro 16 (SAS Institute, Raleigh, NC).

Results

In Group 1 (Averzion Slick Barrier), only a mean 3% of all the bed bugs (0.6 bugs) were able to climb up the barrier and feed. Similarly, only a mean 4% (0.8) of bed bugs in Group 2 (1" Nanofilm Group) were able to climb the tape barrier and feed. However, the most successful barrier in all of these bioassays was the 2" Nanofilm barrier. The bed bugs in Group 3 were completely unable (and unwilling to attempt) to cross the 2" Nanofilm Barrier after inspecting it, so none were able to contact the feeding station (Figure 3).

The ANOVA indicated that none of the three barrier groups were significantly different from each other, although 0% of the bed bugs were able to cross the 2" Nanofilm barrier. In contrast, a mean 43% of the bed bugs (8.7) in the combined control bioassays were able to climb the fabric covered tubes to access the feeding stations and consume the blood. This mean of 43% was found to be significantly greater than the mean percentage of fed bed bugs in any of the barrier groups (Tukey's HSD, $P < 0.0001$).

Discussion

Double Coated Aversion Slick Barrier

The type of fabric (over the base of a box spring) used to cover the food access tube was arguably one of the most challenging surfaces on which to test the Averzion Slick Barrier. This fabric was very textured and provided the bed bugs with a surface that their tarsal claws could grip very easily. The fabric was also textured enough for a single

layer of the Averzion product to be absorbed into the fabric allowing some bed bugs to successfully cross the Slick Barrier surface. Therefore, when we ran the preliminary replications, it was observed that some of the bed bugs would test the surface traction, and then would climb over the barrier after discovering that they could successfully pull their weight across it. To address this issue, we added the second layer over the first barrier application and allowed it to dry.

During the two-layer bioassays, some of the bed bugs were observed climbing part way up the double coated barrier, but then they would turn around and climb back down before reaching the feeding station (Figure 4). However, three bed bugs (two from the first replication and one from the third) were able to climb the double layered Averzion Slick Barrier and access the feeding station. This observation led us to conclude that some bed bugs can, and will, climb the double coated barrier on some surface materials. However, other bed bugs may simply avoid attempting to climb a Slick Barrier that have been applied to different materials, depending on the surface texture.

Overall, the fact that only 3 bed bugs could successfully cross the Slick Barrier when it was applied to a textured fabric documented the overall the efficacy of the product. If the Slick Barrier were to be applied to a less penetrable surface (such as baseboard molding or wood) it would no doubt prevent any bed bug from climbing that surface. However, additional testing should need to be done using different vertically oriented surfaces to test this hypothesis.

It should be noted that that the application of the Averzion Slick Barrier a few inches above floor level on bed legs, furniture legs, floor wall junctions, etc. would allow for insecticide treatments (whether it's Aprehend®, Cimexa Dust®, CrossFire®, etc.) to be placed right below the barrier. In this way, the bed bugs would theoretically be exposed to these residual products for a longer period of time as they struggled to cross the Slick Barrier. This might increase the efficacy of some bed bug control efforts. However, additional testing of this methodology would be necessary.

1" Nanofilm Tape

When we began the bioassays using the Nanofilm tape (1" wide) it seemed very obvious that this barrier was effective. Bed bugs were observed placing their tarsal claws on the Nanofilm surface but they were unable to cling to the tape. Those bed bugs that attempted to put their full weight on the surface lost their grip and immediately fell off the tube surface. However, as we continued to observe the bed bug interactions with the tape, we witnessed one adult bed bug clinging to the intersection of where the ends of the tape met on the tube. This adult bed bug was able to climb this edge where the tape ends met (or even overlapped) and gain access to the feeding station. Additional observations revealed that the few "smart" bed bug adults were able to locate and climb this tape edge quite quickly. Therefore, they were able to cross the 1" barrier and start feeding within the first 10 minutes of the bioassay (Figure 5). However, none of the nymphal stage bed bugs were observed climbing the edge of the tape.

These bed bug climbing observations allowed us to conclude that the edge of the cut Nanofilm Tape could potentially be exploited by the adult bed bugs, which would otherwise be incapable of climbing across the tape. In addition, these bioassays suggested that the 1" tape barrier might be too thin to prevent all bed bugs from climbing past it. It is for this reason the at the 2" Nanofilm Bioassays were conducted.

2" Nanofilm

The 2" Nanofilm bioassays not only increased the width of the barrier but also the edge of the tape was cut so that it would overlap the origin of the tape barrier, thus there was no crease between the tape edges. In addition, the tape was cut on at a curved angle so that any adventurous bed bugs would have to be oriented upside down if they attempt to climb the tape edge. These changes to the Nanofilm Tape width and cut edge had a very positive effect on the tape's efficacy as a bed bug barrier.

The results of the 2" Nanofilm bioassay documented that the bed bugs were unable to maintain a grip on the newly cut edge of the Nanofilm. In one replicate, one adult bed bug did manage to climb halfway up the modified tape edge before falling off. If this individual had been climbing the 1" tape edge, it certainly would have climbed past the barrier to reach the feeding station. However, only this one adult bed bug was observed climbing the tape even halfway, and no other bed bug (adult or nymph) could climb the newly cut edge of the Nanofilm tape. This set of bioassays supported our hypothesis that the additional width of the tape (2 inches) and the diagonal angle of the tape edge was sufficient for preventing bed bug access to the feeding station, and that the angle at which the tape was cut (and overlapped) was sufficient at preventing bed bugs from scaling the tape at any location (the cut end; Figure 6).

One observation that was seen in both Nanofilm Tape groups was that bed bugs attempting to climb the box spring fabric tube would spend a majority of their time directly beneath the tape barrier on the tube surface (Figure 7). Again, this behavior of aggregating or exploring directly underneath the tape barrier could be exploited by applying of residual insecticides just below the placement of the Nanofilm tape. In this way, bed bugs could potentially be eliminated as well as being as prevented from feeding on their hosts.

Conclusions

After testing the efficacy of the products against bed bugs, we can conclude that the 2" Nanofilm Tape, with the edge cut at a curved angle, provided a complete barrier against both life stages of bed bugs. With that being said, the 1" Nanofilm tape was also very effective, and the sleek surface would provide a complete barrier against climbing bed bugs. Operators applying the Nanofilm tape in the field should be cautious on how they cut the end pieces of the tape, due to adult bed bugs' ability to climb the cut edges. It is our recommendation that the wider tape (2") be used as a bed bug barrier when possible. When wrapping around the legs of bed frames or chairs (or even box springs), the applicators should overlap the Nanofilm ends and cut at a curved diagonal.

The Averzion Slick Barrier applied onto box spring fabric (double-coated) proved to be a difficult surface for bed bugs to climb, even though it wasn't a complete barrier. Again, fabric was a very challenging surface to apply the barrier, and if a smoother surface such as wood was tested, it is possible that no bug would have climbed it.

Finally, bed bugs were observed in all three bioassays aggregating or exploring just under or on the barrier surfaces. Further testing should be conducted on the efficacy of the Averzion Slick Barrier and Nanofilm tape applied in combination with an insecticide. If effective in increasing mortality in resistant field strains, this new methodology would be a welcome addition for any bed bug control program.

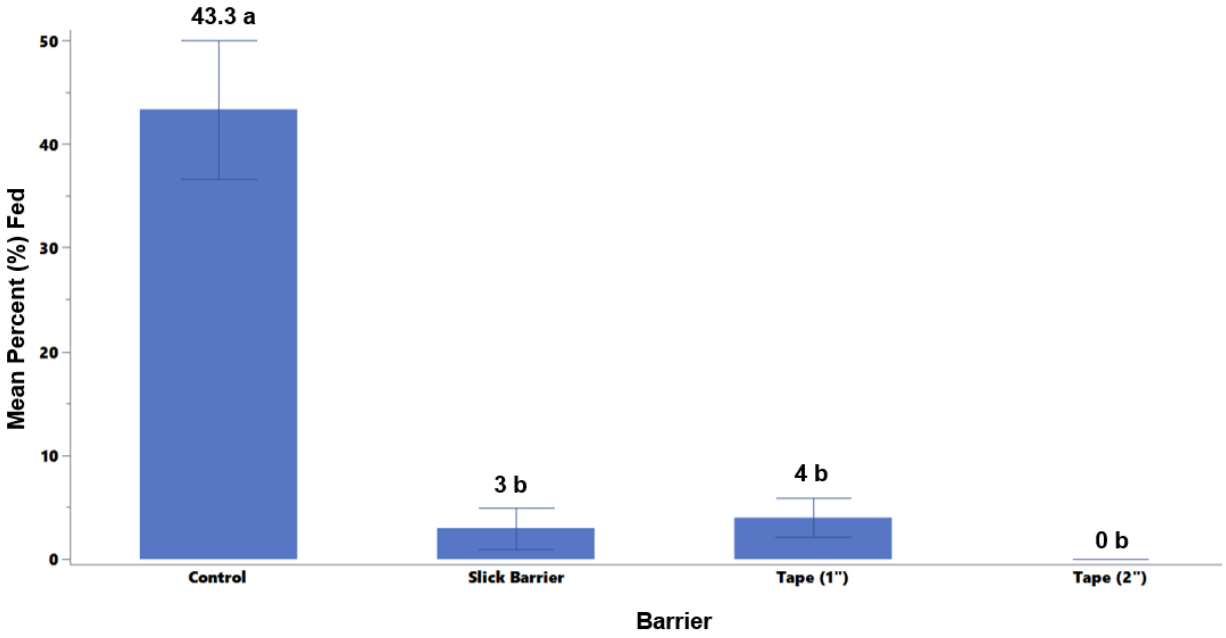

Figure 3. The mean percent (%) of fed bed bugs after the thirty-minute bioassays. Means followed by different letters are statistically different from each other (Tukey's HSD, DF = 3; *F*-value = 47.6; $P < 0.0001$).

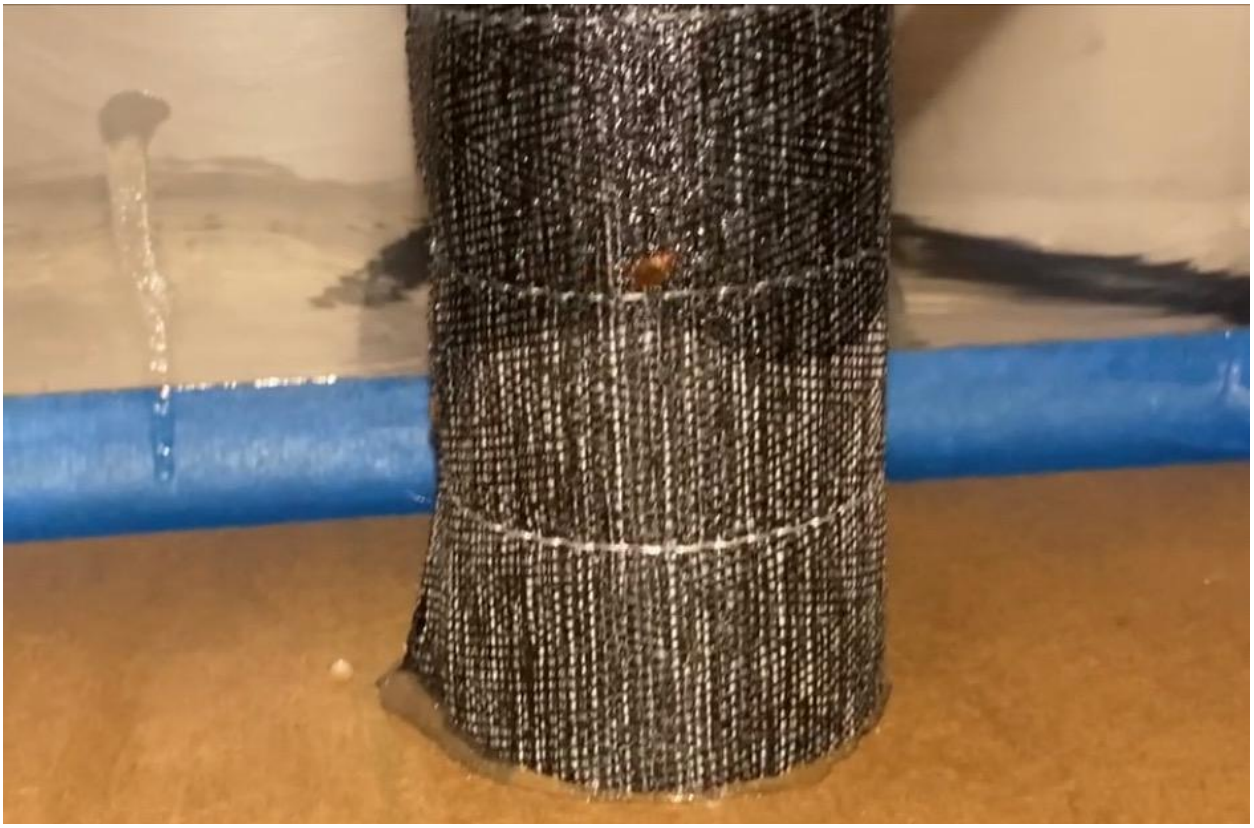

Figure 4. One adult bed bug attempting to climb up the double-coated Averzion Slick barrier.

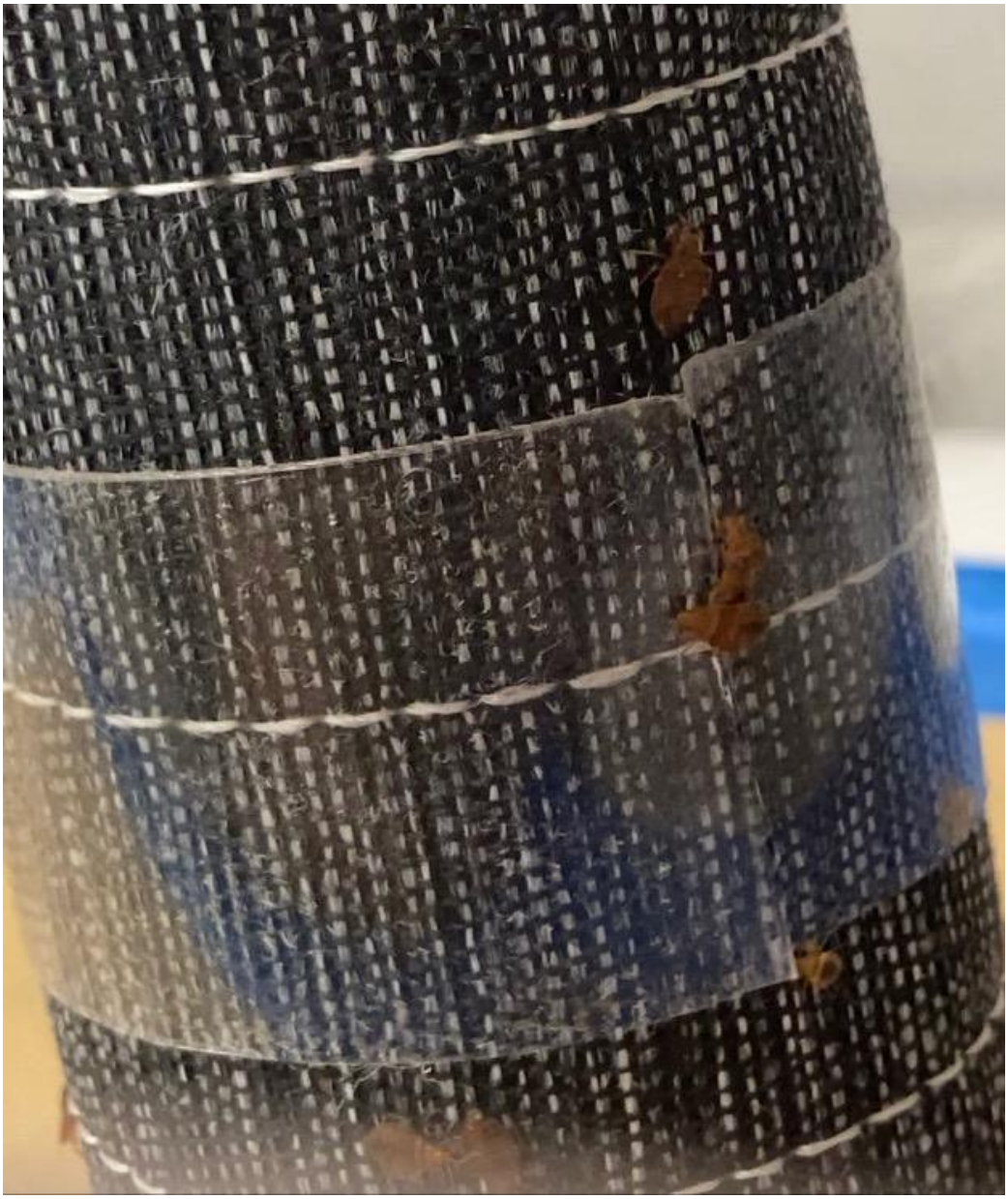

Figure 5. Adult bed bugs scaling the cut crease where the two 1” Nanofilm ends meet.

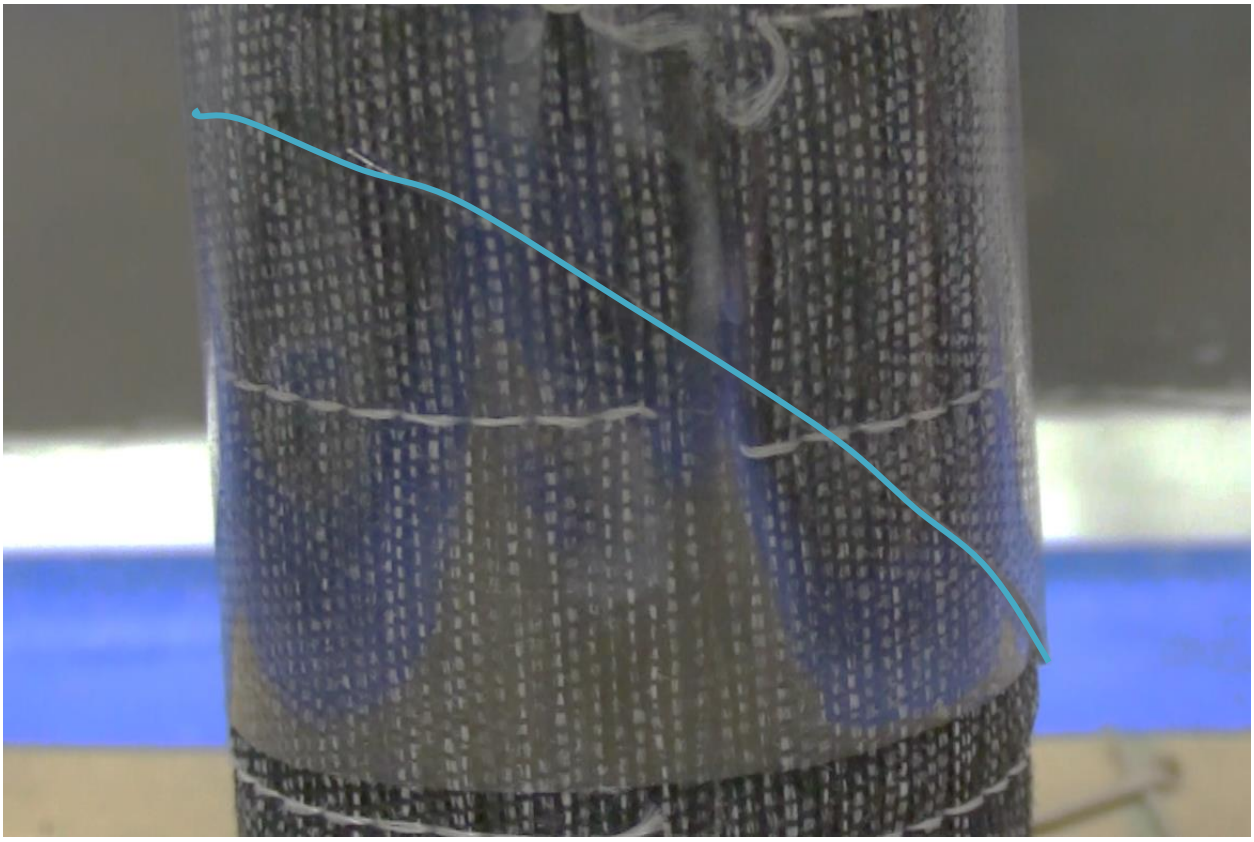

Figure 6. The 2” Nanofilm Tape cut at a curved angle and overlapped to prevent bed bugs from successfully climbing the cut edge.

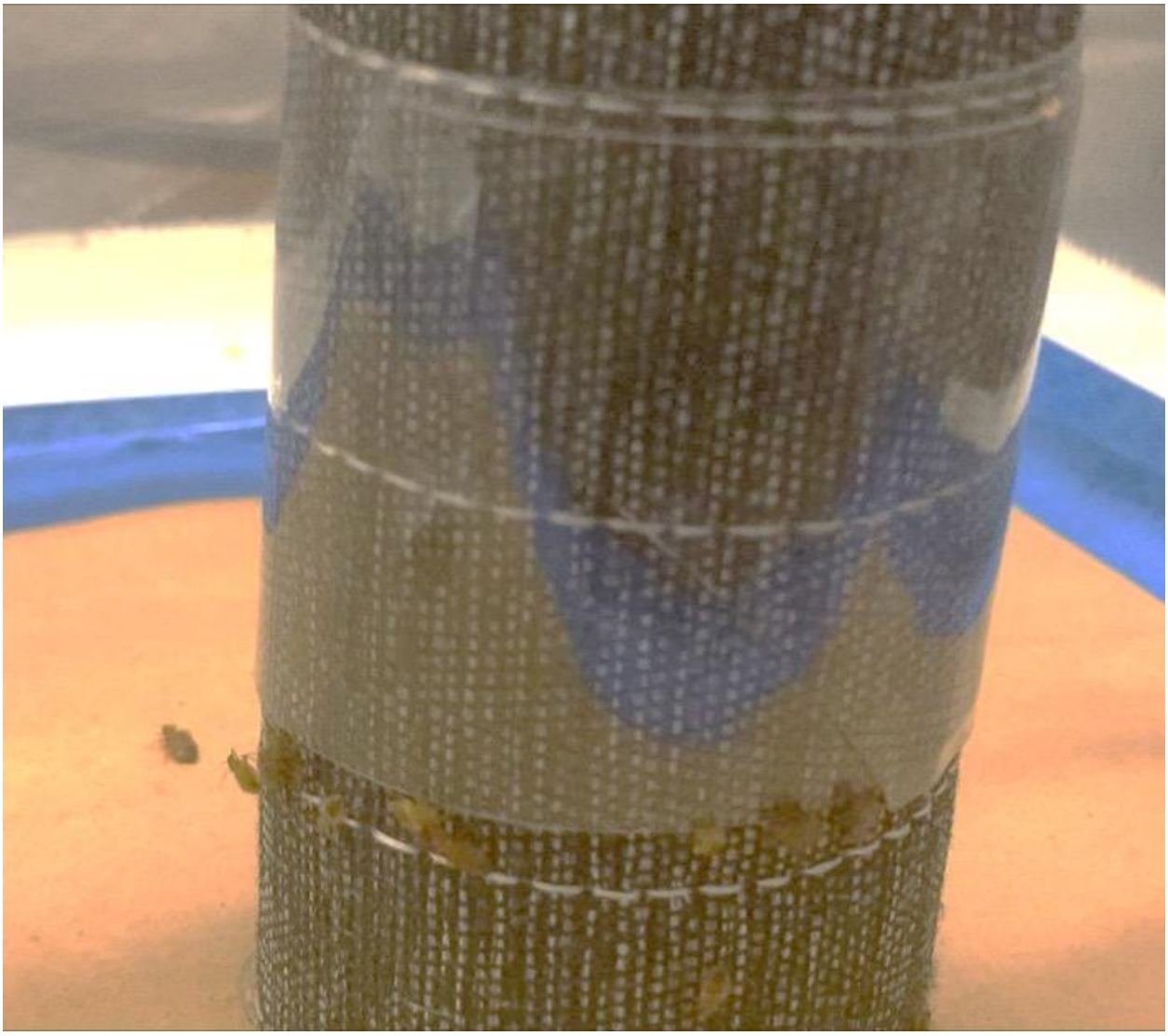

Figure 7. Bed bug adults and nymphs resting or exploring around the bottom of the 2” Nanofilm barrier.