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Effects of surface type and distance traveled on the efficacy of a *Beauveria bassiana* (Hypocreales:Cordycipitaceae)-based biopesticide (Aprehend) for bed bug (Hemiptera: Cimicidae) control

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The cryptic behavior that characterizes bed bugs (*Cimex lectularius* L.) makes them one of the most difficult indoor pests to control. Thus, the use of products with long residual efficacy is a key component of successful bed bug management. Aprehend is a biopesticide for bed bug control, whose active ingredient is the entomopathogenic fungus, *Beauveria bassiana*. This product shows promise for extended bed bug control against pyrethroid-resistant bed bugs, causing mortality days after contact with the treated surface. However, it is unclear how surface type and distance traveled by bed bugs over a treated surface impact efficacy. Therefore, we evaluated the efficacy of different Aprehend band widths applied to different substrates commonly encountered by bed bugs, looking specifically at average time to mortality and overall mortality. Our findings indicate that exposure to fresh applications of Aprehend resulted in high mortality when bed bugs traveled very short distances (1 cm) over some Aprehend-treated surfaces (vinyl tile and cotton jersey fabric), while exposure to other treated surfaces (unfinished pinewood and painted drywall) resulted in comparatively lower mortality even over longer distances (5 cm). Furthermore, we found that bed bugs crawling on cotton jersey fabric picked up a significantly higher number of spores compared to unfinished pinewood. These results indicate that applications of Aprehend as 5 cm (2 inches) bands according to label recommendations can be effective, although surface type is an important determinator of efficacy. This information will help guide pest management professionals in their use of *B. bassiana* for bed bug control.

Key words: bed bug, *Cimex lectularius*, biopesticide, Aprehend, *Beauveria bassiana*

Introduction

Cimex lectularius L., the common bed bug, is an obligate hematophagous ectoparasite that primarily feeds on humans, chickens, and bats (Usinger 1966). Since the early 2000s, bed bugs have resurged with outbreaks reported worldwide in homes, hospitals, trains, hotels, and other public facilities (Doggett et al. 2018). Bed bugs pose a significant threat to humans, primarily through their bites (Goddard and deShazo 2009, Hwang et al. 2018) and their often-associated psychological burden (Goddard and de Shazo 2012) that often leads people to self-harm in the desperate need of controlling bed bugs (Doggett 2018). Recently, this threat has expanded to include the introduction of microbes and novel environmental contaminants indoors (DeVries et al. 2018, Kakumanu et al. 2020, Gordon et al. 2023, Principato et al. 2023). In addition to these risks, bed bugs remain one of the most challenging indoor pests to manage.

Bed bug management is particularly challenging due to their cryptic behavior, unique biological features, and the increase in insecticide resistance that they have developed (Romero et al. 2007). Specifically, bed bugs have the ability to survive prolonged periods of time without feeding, persisting in locations even if the host is not present. They can passively be dispersed through clothes, suitcases, mattresses, and other belongings, but starvation can also induce bed bugs to actively travel in search of the host, facilitating the spread of the infestation to adjacent locations, such as rooms or apartments (Hentley et al. 2017). Detecting early-stage infestations can pose a challenge due to the nocturnal and cryptic nature of bed bugs. They often conceal themselves in cracks and crevices, making it arduous to locate them, especially when their numbers are limited (Romero et al. 2010, Reis and Miller 2011). For this reason, an integrated pest management approach that includes the use of insecticides with

residual efficacy is a pillar for the successful management of bed bugs. However, many available products, such as pyrethroid-based sprays, have reduced efficacy on eggs and can fail to provide residual efficacy, which makes the control of bed bug infestations challenging (Potter 2011).

Failure of insecticide treatments to control bed bugs can be largely attributed to widespread insecticide resistance (Romero et al. 2007, Zhu et al. 2013, Dang et al. 2017, Holleman et al. 2019, Lewis et al. 2023), mainly reported for pyrethroids (Zhu et al. 2013) and neonicotinoids (Romero and Anderson 2016). Worse, a population collected from the United States of America was recently documented to be resistant to fipronil (phenylpyrazole), despite no fipronil-based products currently labeled for bed bug control in the United States of America (González-Morales et al. 2021). Bed bug resistance to many of the active ingredients most commonly used for their control presents a major challenge to those attempting to eradicate them from homes. Therefore, there is a need to explore residual insecticides with novel modes of action.

Beauveria bassiana (Bals.–Criv.) Vuill. (Hypocreales: Cordycipitaceae) is an entomopathogenic fungus known to cause mortality in a number of different arthropods (Blanford et al. 2005, 2011, Scholte et al. 2005, Lacey et al. 2008, Darbro et al. 2011, Fernandes et al. 2011). This fungus was explored for bed bug control (Barbarin et al. 2012), with reports showing it can be effective even when bed bugs are resistant to pyrethroids (Barbarin et al. 2017) and retain efficacy for several weeks following application (Shikano et al. 2021). On contact with bed bugs, the conidia (spores) attach to the host cuticle and then germinate, penetrating the cuticular layer, a mechanism that is helped by enzymes and mechanical pressure (Al-Ani 2019). Once the hyphae reach the insect's hemolymph, the fungal growth causes the death of the host in 3–10 d (Barbarin et al. 2017). *B. bassiana* efficacy is also enhanced by the horizontal transfer of the conidia from exposed to unexposed individuals, allowing the spread of the fungal infection even to bed bugs that do not contact the original application site (Barbarin et al. 2012, Aak et al. 2018).

Although *B. bassiana* has been used extensively to control agricultural pests (Zimmermann 2007, Arthurs and Dara 2019), it was only registered in the United States of America as an indoor residual product for bed bug control (Aprehend, ConidioTec, State College, PA) in 2017. Like many insecticides, the microbial biopesticide Aprehend requires bed bugs to contact the treated surface for it to work. While initial feedback on the efficacy of Aprehend appears encouraging, it is essential that we better understand how factors such as exposure time, distance traveled across treated surfaces, and the type of surface itself affect efficacy. This information will benefit pest management professionals looking to incorporate entomopathogenic fungi into their pest management programs.

Materials and Methods

Bed Bug Populations and Rearing

A field-collected, laboratory-maintained colony of bed bugs (Fuller Mill Road [FMR]) was used for all experiments. The FMR population was collected from an infested home in 2017 from High Point, North Carolina, and has previously been shown to be resistant to insecticides (fipronil and pyrethroids) (González-Morales et al. 2021, Hayes and Schal 2022). In addition, the Harold Harlan (HH) laboratory population was used as a reference (susceptible) population to determine the resistance ratio of the FMR strain. The HH population was originally collected at Fort Dix, New Jersey, in 1973, and has been maintained in the laboratory without insecticide selection since that time. Both populations were reared under standard laboratory conditions (25 °C, 50% RH, and a photoperiod of 12:12 [L:D] h) and maintained in plastic jars, with folded cardstock paper (Office Depot, Lexington, KY) as harborage. Bed bugs were fed on human blood (Kentucky Blood Center, Lexington, KY), containing the anticoagulant citrate phosphate dextrose, using an artificial feeding system (Gaire et al. 2022).

Pyrethroid (Deltamethrin) Resistance

To determine the resistance of the FMR population to pyrethroids, bed bugs (both HH and FMR populations) were topically treated (ventral side) with 0.5 µl of acetone (Sigma Aldrich, St. Louis, MO, USA) containing various doses of deltamethrin ([(S)-cyano-(3-phenoxyphenyl)methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-imethylcyclopropane-1-carboxylate); CAS 52918-63-5; Chem Service Inc., West Chester, PA, USA) ranging from 0 (acetone control) to 160,000 ng using a 25 µl syringe in a repeating dispenser (Hamilton Company, Reno, NV). It should be noted that the higher doses (80,000 and 160,000 ng/bug) required multiple applications of 40,000 ng per 0.5 µl of acetone to prevent crystallization of deltamethrin on the syringe. Mortality was assessed 48 h after treatment and defined as an inability to make coordinate movements away (escaping) when touched with a probe.

Aprehend Application

The ready-to-use oil formulation of Aprehend (*B. bassiana*, Strain GHA 2%, minimum of 2.2×10^9 viable conidia per milliliter of product) was applied using a low-volume, low-pressure spray gun applicator (ConidioTec LLC, State College, PA), held approximately 10 cm from the surface, moving the gun horizontally across the surface, at an approximate speed of 3 s per 60.6 cm. The following materials were used for the experiments and sprayed with Aprehend: cotton jersey fabric (Joann Fabric and Crafts, Hudson, OH, USA), unfinished pinewood (Lowes, Mooresville, NC, USA), vinyl tile (Lowes, Mooresville, NC, USA), painted drywall (Lowes, Mooresville, NC, USA), unfinished oak (Lowes, Lexington, KY, USA), unfinished maple (Lowes, Mooresville, NC, USA), finished pinewood (Lowes, Mooresville, NC, USA), and red oak wood veneer (Edgebanding Supplies, San Dimas, CA). Painted drywall was obtained by painting the drywall with primer white paint flat and allowed to dry for 24 h (Beher Process Corporation, Santa Ana, CA, USA). After applying Aprehend, treated materials were placed in a dark environment and allowed to dry for 24 h at room temperature (22–24 °C, 30–50% RH) before initiating bioassays. Controls for each material were represented by the same substrate, and the same number of replicates as for the treatments but received no application of Aprehend (untreated).

Surface Conidia Count and Viability

The resulting concentration of conidia per cm^2 was verified for each application by placing a 15 × 15 mm glass cover slide (Machino Corp, Chicago, IL) on the surface of the material to be sprayed. After spraying, the glass cover slide was removed with forceps and placed in a 20 ml plastic vial (Thermo Fisher Scientific, Waltham, MA, USA) containing 1 ml Isopar M (ExxonMobil Chemical Company, Houston, TX). The vial was then vortexed (DLAB Scientific Inc, Beijing, China) for 1 min. The vial was then placed in a bath sonicator (VEVOR, Rancho Cucamonga, CA, USA) for 2 min to allow the conidia to detach from the glass cover slide. The concentration of conidia was determined by placing 0.1 µl of conidia extract into an Improved Neubauer hemocytometer (Sigma Aldrich,

St. Louis, MO, USA) and counted at 400× magnification (Zeiss, Oberkochen, Germany). Three coverslips, per Aprehend application, were evaluated for each of the treated surfaces.

Each spray application was also verified for conidia viability either by removing a 2 cm^2 piece of fabric or by swabbing a 2 cm^2 area from solid surface treatments immediately after the spray application. Swabs were collected from spots adjacent to the areas used for bed bug exposure, using a sterile cotton swab (Dynarex, Corporation, Orangeburg, NY, USA) dipped in Isopar M (ExxonMobil, Spring, TX, USA). The cut fabrics or swabs were placed in a 2 ml Eppendorf (2 ml, USA Scientific, Ocala, FL) containing 1 ml of Isopar M in which the conidia could be suspended. The Eppendorf was vortexed for 1 min and then sonicated for 2 min. A 10 µl droplet of the conidia suspension was plated on a Sabouraud Dextrose Agar medium (Sigma Aldrich, St. Louis, MO, USA) in 9 cm diameter Petri dishes. After gently tilting the plates in a circular motion to spread the droplets, the plates were incubated for 21 h at 25 °C to allow germination. Three-hundred conidia were evaluated and recorded as germinated and non-germinated for each Petri dish using a microscope at 400× magnification. Three replications, per Aprehend application, were evaluated for each of the treated surfaces.

Brief Exposure Bioassays—1, 2, and 5 cm

After allowing treated materials to dry for 24 h, bioassays were carried out in arenas with a 1, 2, or 5 cm radius, drawn with a compass on each of the substrates prior to spray application (Supplementary Fig. S1). The first bioassay was carried out at a distance of 2 cm using cotton jersey fabric, unfinished pinewood, vinyl tile, and painted drywall. Based on the mortality results from the 2 cm Aprehend barrier width bioassays, a second bioassay was carried out by either reducing (to 1 cm) or increasing (to 5 cm) the radius. This allowed us to further evaluate the distance over which a bed bug must travel over a treated surface for the product to work.

Thirty replicates were conducted for each distance, using multiple arenas and a single bed bug per replicate. All substrates were tested at the same time. Bed bugs were placed in the center of the arena and allowed to crawl to the edge while time was recorded. Those that did not crawl in a straight line toward the edge were discarded from the experiment, and trials were continued until 30 successful replicates were obtained. Once reaching the edge of the marked arena, bed bugs were gently collected using soft-tip forceps (Ecology supplies, Glen Cove, NY) and placed individually into plastic cups (30 ml, Comfy package, Brooklyn, NY) with a clean paper harborage (2 cm^2, Office Depot, Boca Raton, FL, USA). Forceps were cleaned with ethanol in between every bioassay to avoid cross-contamination. Mortality was monitored daily for 14 d, with bed bugs considered dead if they did not respond when prodded. To verify that bed bugs died due to fungal infection, cadavers were placed onto a wet piece of filter paper (Thermo Fisher Scientific, Waltham, MA, USA) in a sealed Petri dish (Thermo Fisher Scientific, Waltham, MA, USA) and incubated at room temperature (23 °C) to verify mycosis. Replicates where external sporulation was not observed within 7 d were not considered to have been killed by *B. bassiana*.

Prolonged Exposure Bioassays

Reduced overall mortality was observed in populations of bed bugs exposed to treated unfinished pinewood at both 2 and 5 cm compared to the other substrates. Therefore, in an effort to increase exposure in terms of both time and distance traveled, additional bioassays were conducted. Instead of a set distance, a set time of 15 min was used. Time, rather than distance was used due to the difficulty in accurately measuring distances longer than 5 cm in a reproducible manner. In this evaluation, we added 4 additional types of woods that are normally found in homes as part of furniture where bed bugs could be encountered (unfinished oak, unfinished maple, finished pinewood, and red oak wood veneer). Aprehend applications, conidia counts, and germination tests were conducted as previously described. Three replicate surfaces were treated for each material, using 10 bed bugs per replicate for treatments and controls. Bed bugs were placed on the treated surface and permitted to move freely within a 5 cm diameter Petri dish (Thermo Fisher Scientific, Waltham, MA, USA) for 15 min. Bed bugs were then removed and placed in Petri dishes with clean harborage and mortality was monitored daily for 14 d. Fungal infection was verified in dead bugs as described above.

Conidia Acquisition by Bed Bugs

To investigate the differences in mortality observed between different surface types, we evaluated the number of conidia acquired by bed bugs after crossing a 2 cm band of Aprehend applied to cotton jersey fabric in comparison to unfinished pinewood. These 2 materials were chosen because the observed mortality of bed bugs, following 2 cm exposure, was significantly different. Thirty bed bugs were exposed per treatment using the technique described previously. After exposure, bed bugs were placed in microcentrifuge tubes (2 ml, USA Scientific) containing 0.1 ml ethanol which was vortexed for 1 min and sonicated for 2 min. The conidia suspension (0.1 µl) was placed in the hemocytometer, and conidia were counted. The approximate number of conidia extracted from the legs and body of each bed bug was then calculated to estimate the number of conidia picked up by each bed bug when crossing the treated surface.

Statistical Analysis

Probit analysis was used to determine deltamethrin LD_{50} values. Kaplan–Meier with log-rank test was conducted to investigate survival. Paired comparisons were done using Student's *t*-tests to evaluate conidia count, and conidia viability for the 1 and 5 cm bioassay, and crawling time for the 2 cm (treated vs. untreated), 1 and 5 cm bioassays (treated vs. untreated—treated vs. treated—untreated vs. untreated), as well as for the conidia acquisition (cotton jersey fabric vs. unfinished pinewood). ANOVA was used to evaluate conidia count, spore viability for the 2 cm and 15 min bioassays as well as the crawling time among the treatments and controls for the bioassay of 2 cm. Analyses were performed using R Studio (Vienna, Austria, Core Team R, 2021) and JMP Pro 17 (JMP Statistical Discovery LLC, Cary, NC, USA). Data analyzed via parametric tests met the assumptions of these tests.

Results

Pyrethroid (Deltamethrin) Resistance

The HH population had a deltamethrin LD_{50} = 0.41 ng/bed bug (95% CI: 0.31–0.54, n = 192, slope ± SE = 1.83 ± 0.22, X^2 [df] = 3.23 [4]). In contrast, we were unable to determine the deltamethrin LD_{50} for the FMR population, as the highest dose we were able to test (160,000 ng/bed bug) only resulted in 35% mortality. Based on this, we can reasonably assume that the LD_{50} resistance ratio between FMR and HH (LD_{50} of FMR divided by the LD_{50} of HH) is greater than 390,000-fold, indicating the FMR population is highly resistant to pyrethroids, and to our knowledge, one of the most pyrethroid-resistant bed bug population documented to date.

Surface Conidia Count and Viability

The number of conidia applied was not significantly different across substrates at all distances/times tested (1 cm: t = –0.92, df = 2.49,

Table 1. Surface conidia count and viability

Bioassay (Distance/time)	Substrate	Conidia count per cm² (± SEM[1]) × 10⁶	% Viability
1 cm	Cotton jersey fabric	7.1 ± 0.4	89 ± 2.8
	Vinyl tile	7.5 ± 0.1	92 ± 1.9
2 cm	Cotton jersey fabric	6.9 ± 0.1	91 ± 0.4
	Vinyl tile	7.5 ± 0.1	88 ± 1.5
	Painted drywall	7.4 ± 1.0	88 ± 1.2
	Unfinished pinewood	7.7 ± 0.6	88 ± 0.4
5 cm	Painted drywall	7.8 ± 0.1	92 ± 0.3
	Unfinished pinewood	7.0 ± 0.4	89 ± 1.5
15 min	Cotton jersey fabric	6.8 ± 0.6	88 ± 1.8
	Vinyl tile	7.8 ± 0.2	89 ± 2.2
	Painted drywall	7.8 ± 0.3	90 ± 0.8
	Finished pinewood	6.9 ± 0.3	91 ± 0.3
	Unfinished pinewood	6.5 ± 0.7	85 ± 1.7
	Unfinished maple	7.4 ± 0.2	90 ± 1.9
	Unfinished oak	6.4 ± 0.6	85 ± 5.7
	Wood veneer	6.8 ± 0.4	90 ± 1.6

[1]SEM represents the standard error of the mean.

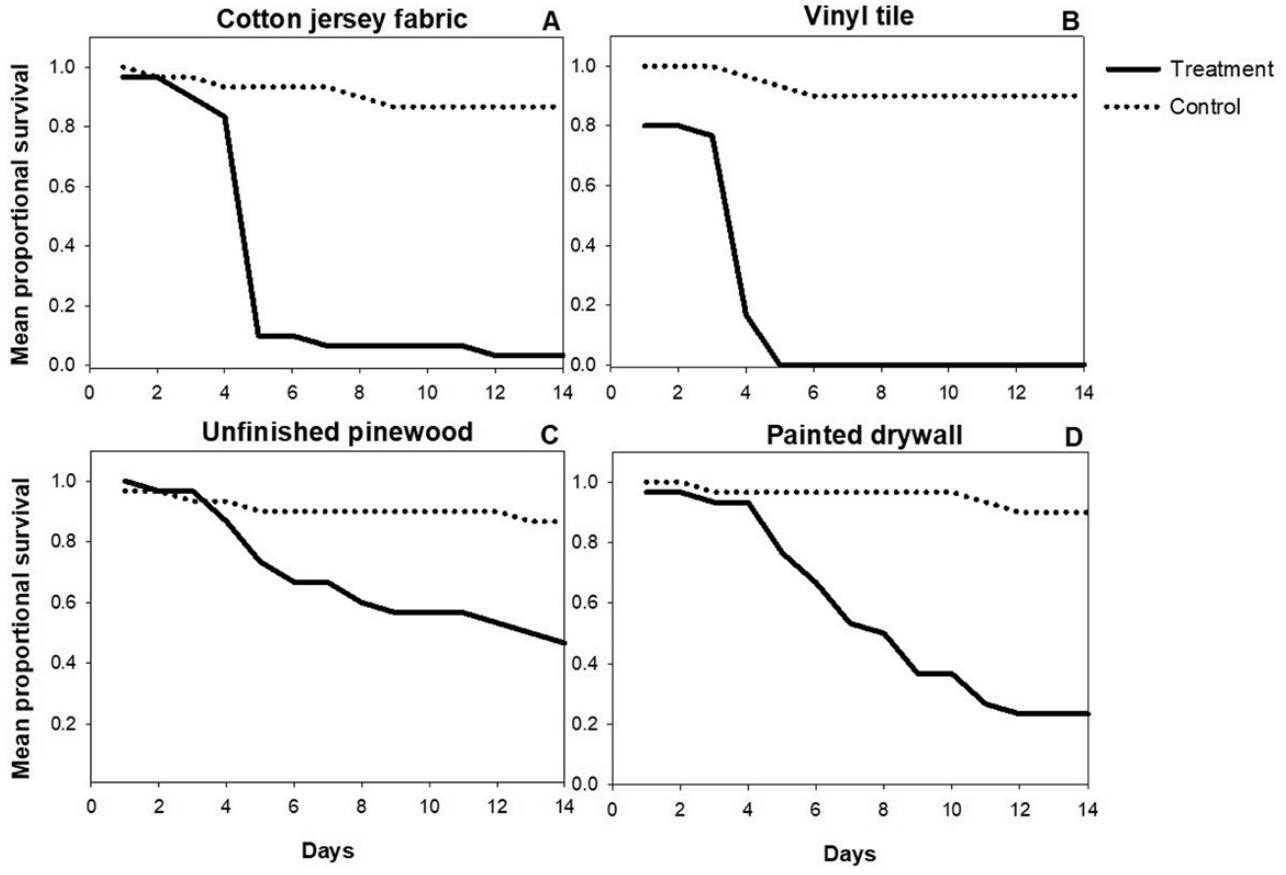

Fig. 1. Bed bug survival after crawling across a 2 cm Aprehend barrier on the following 4 types of substrates: A) cotton jersey fabric, B) vinyl tile, C) unfinished pinewood, and D) painted drywall. Comparison of Kaplan–Meier survival curves for each substrate treated with Aprehend relative to their controls, using the log-rank test, showed significant differences for all substrates ($P < 0.001$).

$P = 0.43$; 2 cm: $f = 0.28$, $df = 3.8$, $P = 0.83$; 5 cm: $t = -1.95$, $df = 2.37$, $P = 0.16$; 15 min: $f = 1.5$, $df = 7.16$, $P = 0.23$; Table 1). Furthermore, the percentage of viable conidia was also not significantly different across substrates at all distances/times tested (1 cm: $t = -0.82$, $df = 3.5$, $P = 0.46$; 2 cm: $f = 1.93$, $df = 3.8$, $P = 0.20$; 5 cm: $t = -1.84$, $df = 2.11$, $P = 0.19$; 15 min: $f = 0.50$, $df = 7$, $P = 0.81$; Table 1).

Bed Bug Mortality Following Exposure to 2 cm Aprehend Barrier

Treated surfaces resulted in significantly lower survivorship (greater mortality) for all surfaces tested than untreated surfaces (cotton jersey fabric: $\chi^2 = 42.1$, $df = 1$, $P < 0.001$; vinyl tile: $\chi^2 = 59.1$, $df = 1$, $P < 0.001$; unfinished pinewood: $\chi^2 = 8.6$, $df = 1$, $P = 0.002$; and painted drywall: $\chi^2 = 25.7$, $df = 1$, $P < 0.001$; Fig. 1, Table 2).

Survivorship on Aprehend-treated surfaces was significantly affected by surface type (χ^2 = 79, *df* = 3, *P* < 0.001). Log-rank tests revealed vinyl tile resulted in faster mortality, followed by cotton jersey fabric, then painted drywall and unfinished pinewood, which were not different from one another (Table 2). Time taken to crawl 2 cm was significantly different between treatments and their controls for all substrates, except for unfinished pinewood, with treated surfaces resulting in an increase in time taken to cross compared to the untreated surfaces (cotton jersey fabric: *t* = −3.67, *df* = 58, *P* < 0.001; vinyl tile: *t* = −5.42, *df* = 58, *P* < 0.001; unfinished pinewood: *t* = −0.56, *df* = 58, *P* = 0.581; and painted drywall: *t* = −3.88, *df* = 58, *P* < 0.001; Table 2). Comparison of crawling time across surfaces found bed bugs moved fastest on cotton jersey fabric and unfinished pinewood, followed by painted drywall, and finally vinyl tile for both treated (*F* = 39.72, *df* = 3.116, *P* < 0.001) and untreated (*F* = 26.15, *df* = 3.116, *P* < 0.001) surfaces (Table 2).

Bed Bug Mortality Following Exposure to 1 and 5 cm Aprehend Barriers

Bed bug mortality following exposure to cotton jersey fabric and vinyl tile was still consistently high even after reducing the Aprehend barrier to 1 cm, with 14-d mortality being 93% and 77%, respectively (Fig. 2, Table 3). Treated surfaces resulted in significantly lower survivorship for both cotton jersey fabric (χ^2 = 55.5, *df* = 1, *P* < 0.001) and vinyl tile (χ^2 = 44.6, *df* = 1, *P* < 0.001; Fig. 2, Table 3)

Table 2. Mortality and mean survival time of bed bugs after crossing a 2 cm barrier treated with Aprehend

Substrates	Mortality (14 days)	Mean ± SEM[1] survival time (days)[2]	Median survival time (days)	Median survival time 95% CI (days)	Mean ± SEM[1] crawling time (s) on treated surfaces[3]	Mean ± SEM[1] crawling time (s) on untreated surfaces[3]
Cotton jersey fabric	97%	5.2 ± 0.4[b]	5	5-5	3.8 ± 0.3[a*]	2.6 ± 0.2[a*]
Vinyl tile	100%	3.5 ± 0.2[a]	4	4-4	8.6 ± 0.5[c*]	5.3 ± 0.3[c*]
Unfinished pinewood	53%	10.2 ± 0.7[c]	13.5	8-na	3.1 ± 0.2[a]	2.9 ± 0.2[a]
Painted drywall	77%	8.7 ± 0.6[c]	8.5	7-12	6.4 ± 0.5[b*]	4.3 ± 0.3[b*]

[1]SEM represents the standard error of the mean.
[2]Pairwise comparison of the Kaplan–Meier survival curves. Different letters show significant differences within the column. Every treatment was significantly different from its control (*P* < 0.01).
[3]Average time taken by a bed bug to crawl 2 cm on treated and untreated substrates. Comparison of crawling time among surfaces for both the treatments and the controls was performed using one-way ANOVA followed by Tukey's test, with differences among substrates indicated by different lowercase letters *P* < 0.001; comparisons made within each column. Significant differences in the time spent crawling on surfaces (treated vs. untreated) based on the Student's *t*-test (*P* < 0.001) are indicated by an asterisk (*).

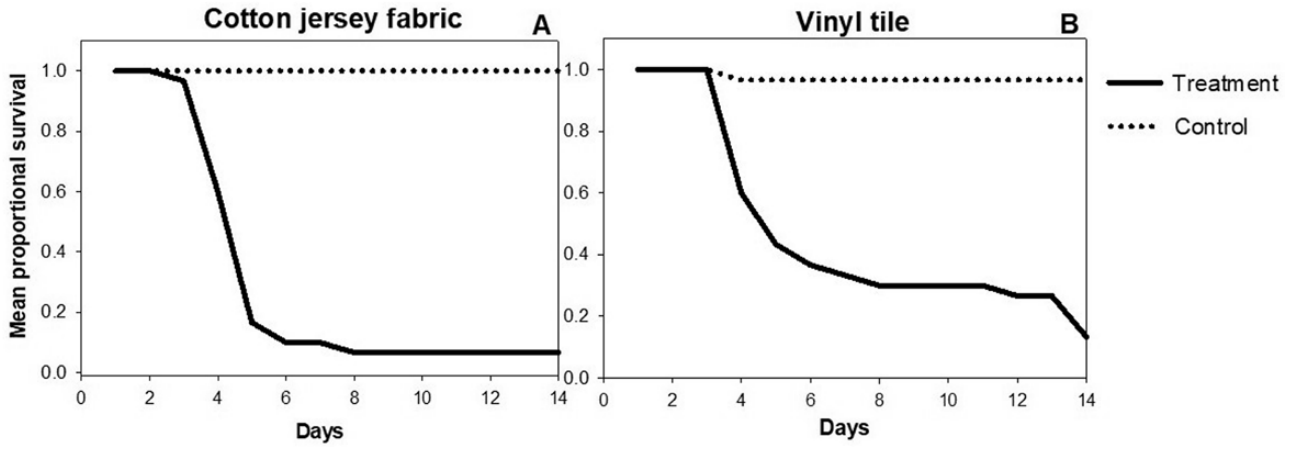

Fig. 2. Bed bug survival after crawling across a 1 cm Aprehend barrier on the following 2 types of substrates: A) cotton jersey fabric, B) vinyl tile. Pairwise comparison of Kaplan–Meier survival curves, using the log-rank test, showed significant differences between each treatment and their controls (*P* < 0.001).

Table 3. Mortality and mean survival time after crossing 1 cm of surface treated with Aprehend

Substrate	Mortality (14 day)	Mean ± SEM[1] survival time (days)[2]	Median survival time (days)	Median survival time 95% CI (days)
Cotton jersey fabric	93%	5.3 ± 0.4	5	4-5
Vinyl tile	77%	7.4 ± 0.7	5	4-12

[1]SEM represents the standard error of the mean.
[2]No significant differences were observed in survivorship between treatments (*P* = 0.14). Every treatment was significantly different from its control (*P* < 0.001).

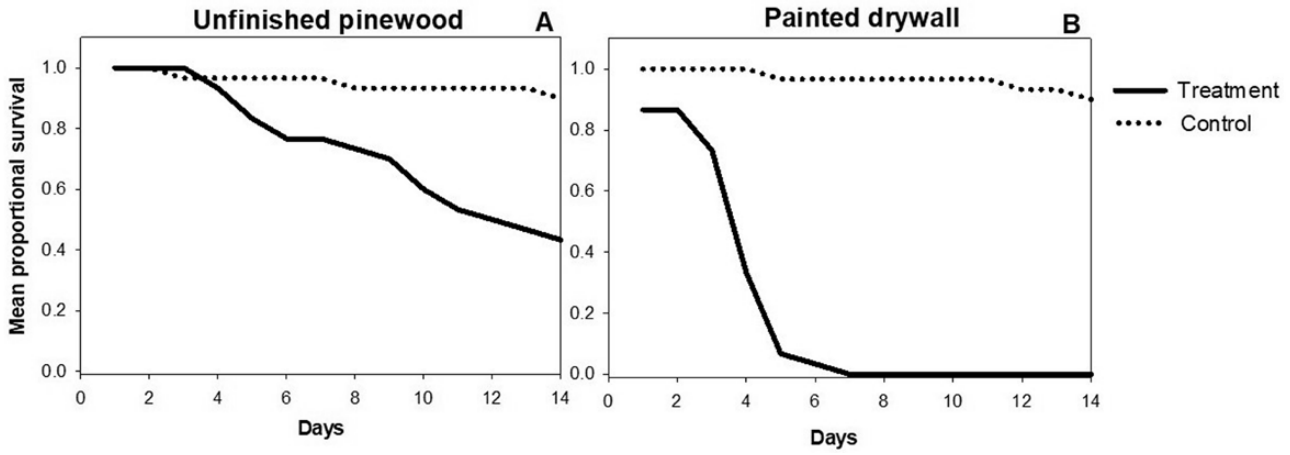

Fig. 3. Bed bug survival after crawling across a 5 cm Aprehend barrier on the following 2 types of substrates: A) unfinished pinewood and B) painted drywall. Comparison of Kaplan–Meier survival curves for each substrate treated with Aprehend relative to their controls, using the log-rank test, showed significant differences for both substrates ($P < 0.001$).

Table 4. Mortality, mean survival time, and crawling time after crossing 5 cm of surface treated with Aprehend

Substrate	Mortality (14 days)	Mean ± SEM[1] survival time (days)[2]	Median survival time (days)	Median survival time 95% CI (days)	Mean ± SEM[1] crawling time (s) on treated surfaces[3]	Mean ± SEM[1] crawling time (s) on untreated surfaces[3]
Unfinished pinewood	57%	10.8 ± 0.6[a]	12.5	10-na	3.2 ± 0.2[a*]	4.0 ± 0.2[a*]
Painted drywall	100%	3.9 ± 0.2[b]	4	4-5	6.5 ± 0.4[b*]	4.6 ± 0.2[b*]

[1]SEM represents the standard error of the mean.
[2]Pairwise comparison of the Kaplan–Meier survival curves. Different letters show significant differences ($P < 0.05$). Every treatment was significantly different from its control ($P < 0.001$).
[3]Average time (in seconds) taken by a bed bug to crawl 5 cm of treated and untreated substrates. Student's *t*-test was used to compare crawling time among the treatments and among the controls with differences among the substrates indicated by lowercase letters ($P < 0.001$; comparison made within each column) and time spent crawling on surfaces (treated vs. untreated) indicated by an asterisk (*).

when compared to untreated controls. Mean (± SEM) survival times were 5.3 ± 0.4 (cotton jersey fabric) and 7.4 ± 0.7 d (vinyl tile; Table 3). There were no differences in survival between cotton jersey fabric and vinyl tile ($\chi^2 = 2.5$, $df = 1$, $P = 0.1$; Fig. 2, Table 3).

Bed bug mortality following exposure to a 5 cm Aprehend barrier on painted dry wall resulted in 100% bed bug mortality by day 7, but at this time point mortality on treated unfinished pinewood only reached 57% after exposure (Fig. 3, Table 4). Treated surfaces resulted in significantly lower survivorship for both painted dry wall ($\chi^2 = 62.3$, $df = 1$, $P < 0.001$) and unfinished pinewood ($\chi^2 = 14.6$, $df = 1$, $P < 0.001$; Fig. 3, Table 4) when compared to untreated controls. Mean (± SEM) survival times were 3.9 ± 0.2 d (painted drywall) and 10.8 ± 0.6 d (unfinished pinewood). Log-rank tests revealed Aprehend painted drywall resulted in faster mortality than Aprehend-treated unfinished pinewood ($\chi^2 = 46.8$, $df = 1$, $P < 0.001$). The time taken to crawl 5 cm was significantly different between treatments and their controls, with treated painted drywall increasing the time taken to cross the surface ($t = -4.17$, $df = 58$, $P < 0.001$), and treated unfinished pinewood decreasing the time taken to cross the surface ($t = 2.98$, $df = 58$, $P = 0.004$; Table 4). Regardless of treatment, painted drywall took longer to cross than unfinished pinewood for both treated ($t = -7.45$, $df = 58$, $P < 0.001$) and untreated ($t = -2.48$, $df = 58$, $P < 0.0159$) surfaces (Table 4).

When evaluating survivorship among different distance, we found increased distance traveled significantly reduced survivorship for painted drywall (2 cm vs. 5 cm; $\chi^2 = 36.4$, $df = 1$, $P < 0.001$) and vinyl tile (1 cm vs. 2 cm; $\chi^2 = 21.9$, $df = 1$, $P < 0.001$), but not for cotton jersey fabric (1 cm vs. 2 cm; $\chi^2 = 0.5$, $df = 1$, $P = 0.49$) nor unfinished pinewood (2 cm vs. 5 cm; $\chi^2 = 0.001$, $df = 1$, $P = 0.97$). However, it should be noted that the latter 2 retained both low (cotton jersey fabric) and high (unfinished pinewood) survivorship at both distances, respectively.

Prolonged Exposure Bioassays—15 min on an Aprehend-Treated Surface

Treated surfaces resulted in significantly lower survivorship for all surfaces tested than untreated surfaces (cotton jersey fabric: $\chi^2 = 64.6$, $df = 1$, $P < 0.001$; vinyl tile: $\chi^2 = 65.9$, $df = 1$, $P < 0.001$; painted drywall: $\chi^2 = 65.0$, $df = 1$, $P < 0.001$; finished pinewood: $\chi^2 = 66.6$, $df = 1$, $P < 0.001$; unfinished maple: $\chi^2 = 63.9$, $df = 1$, $P < 0.001$; wood veneer: $\chi^2 = 63.1$, $df = 1$, $P < 0.001$; unfinished pinewood: $\chi^2 = 40.9$, $df = 1$, $P < 0.001$; and unfinished oak: $\chi^2 = 43.9$, $df = 1$, $P < 0.001$). Survivorship following a 15-min exposure on Aprehend-treated surfaces was significantly affected by surface type ($\chi^2 = 106$, $df = 7$, $P < 0.001$). Log-rank tests revealed unfinished wood surfaces generally resulted in longer survival times compared to finished pinewood and non-wooden surfaces (e.g., cotton jersey fabric, vinyl tile, and painted drywall; Fig. 4, Table 5).

Conidia Acquisition by Crawling

The estimated number of conidia attached to a bed bug that crossed 2 cm of treated cotton jersey fabric was 3.2(± 0.2) × 10^5

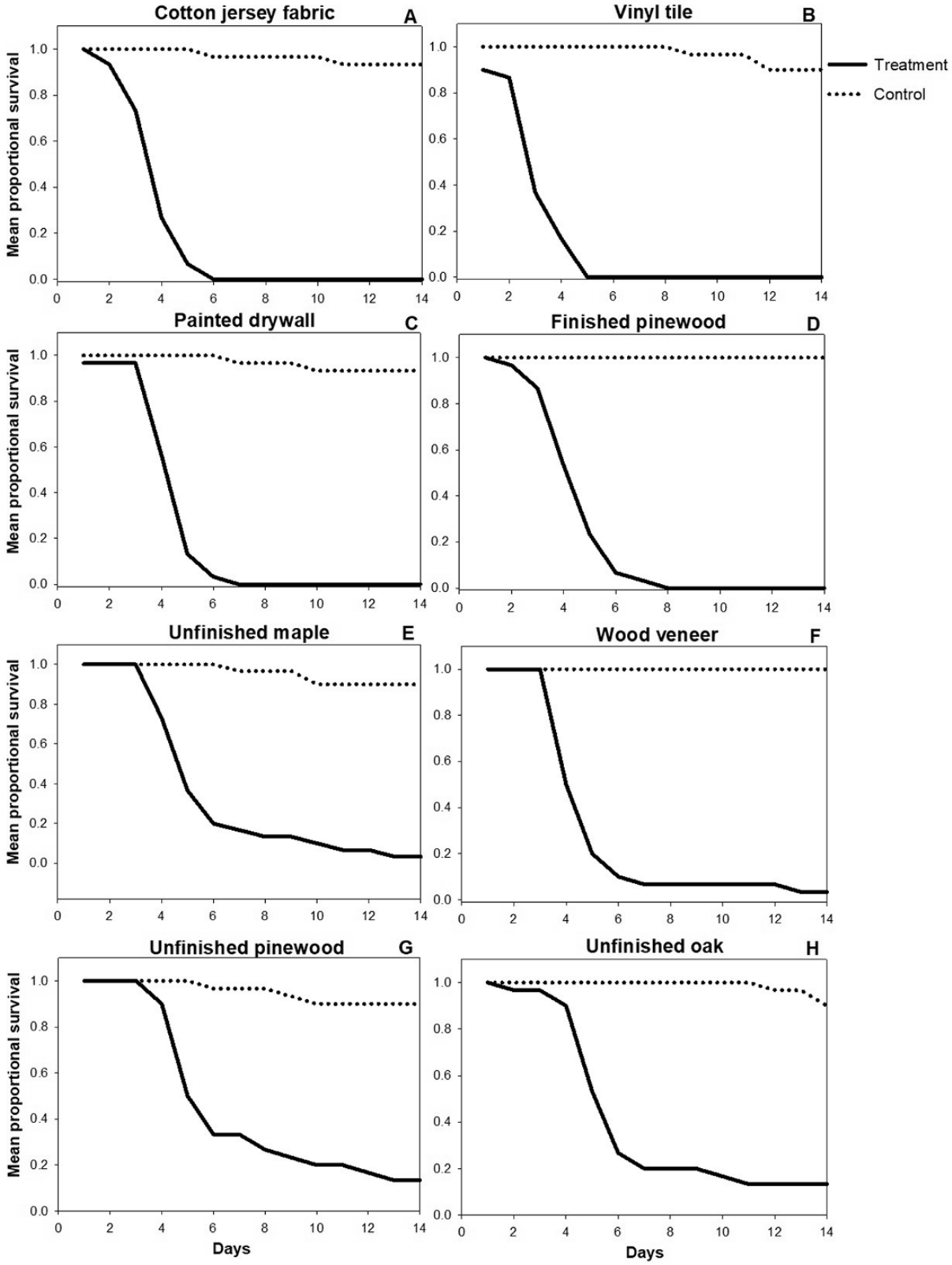

Fig. 4. Bed bug survival after crawling on an Aprehend-treated surface for 15 min on the following types of substrates: A) cotton jersey fabric, B) vinyl tile, C) painted drywall, D) finished pinewood, E) unfinished maple, F) wood veneer, G) unfinished pinewood, and H) unfinished oak. Comparison of Kaplan–Meier survival curves for each substrate treated with Aprehend relative to their controls, using the log-rank test, showed significant differences for all the substrates ($P < 0.001$).

Table 5. Mortality and mean survival time after 15 min of prolonged exposure to a surface treated with Aprehend

Substrate	Mortality (14 days)	Mean ± SEM[1] survival time (days)[2]	Median survival time (days)	Median survival time 95% CI (days)
Cotton jersey fabric	100%	4 ± 0.1[b]	4	4-4
Vinyl tile	100%	3.3 ± 0.2[a]	3	3-4
Painted drywall	100%	4.6 ± 0.1[c]	5.5	5-8
Finished pinewood	100%	4.7 ± 0.2[c]	5	4-5
Unfinished maple	97%	6.2 ± 0.5[de]	5	5-6
Wood veneer	97%	5.3 ± 0.4[cd]	5	4-5
Unfinished pinewood	87%	7.2 ± 0.6[d]	5.5	5-8
Unfinished oak	87%	6.8 ± 0.5[d]	6	5-6

[1]SEM represents the standard error of the mean.
[2]Pairwise comparison of the Kaplan–Meier survival curves. Different letters show significant differences ($P < 0.05$).

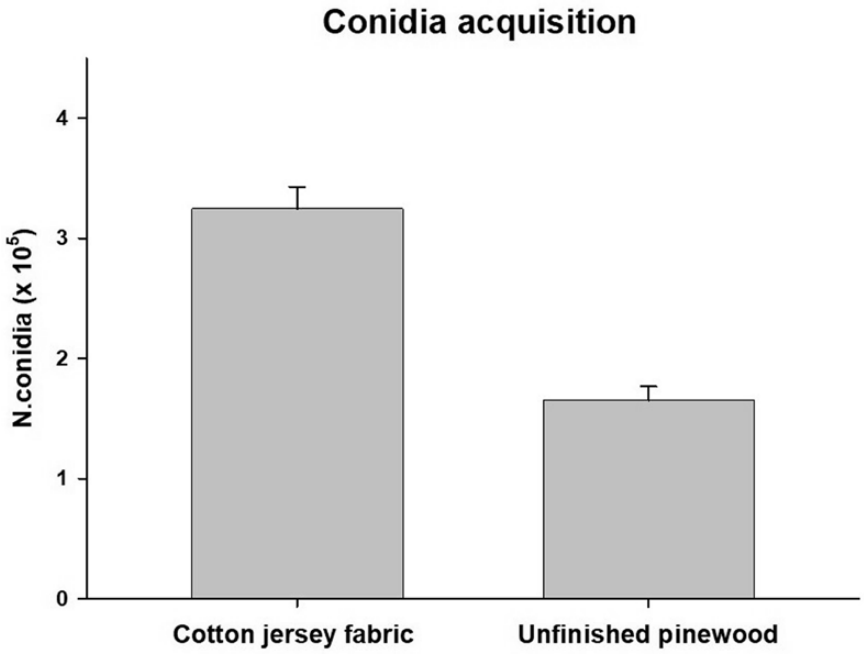

Fig. 5. Approximate number of conidia attached to bed bugs after crawling across a 2 cm Aprehend-treated surface. Significant differences in conidia acquisition between cotton jersey fabric and unfinished pinewood were detected using a Student's *t*-test ($P < 0.003$).

and $1.7(\pm 0.1) \times 10^5$ for unfinished pinewood (Fig. 5). The number of conidia attached after crawling across the 2 different treated substrates was significantly different ($t = 7.4$, $df = 4$, $P < 0.003$).

Discussion

The screening of different substrates revealed that applications of Aprehend were more effective on some surfaces than others. For both cotton jersey fabric and vinyl tile, high mortality (97–100%) was observed within 3–5 d when bugs crawled over a distance of only 2 cm. High mortality persisted on these surfaces even when the distance was dropped to 1 cm (93%: cotton jersey fabric, 77%: vinyl tile). Painted drywall had lower mortality at a 2 cm distance (77%), but this increased to 100% when the distance was increased to 5 cm (2 inches), which is the recommended application band on the Aprehend label. However, exposure to treated unfinished pinewood resulted in significantly higher survivorship at both 2 cm (47%) and 5 cm (43%) distance than the other surfaces. These results are the first to document that fresh applications of Aprehend are effective on some surfaces with only brief contact, suggesting this product has strong potential to integrate into bed bug-integrated pest management plans. However, use should be accompanied with some caution, as efficacy is reduced on some surfaces (specifically unfinished pinewood).

To better understand the impact of these surface types on efficacy, we also conducted timed, 15-min exposure bioassays to evaluate bed bug mortality using a more typical residual pesticide evaluation protocol. In these assays, bed bugs could freely crawl for 15 min over treated surfaces, maximizing the chances of exposure. Complete mortality was achieved in 14 d for all surfaces except wood veneer (97%) and the unfinished woods (maple [97%], pine [87%], and oak [87%]). While mortality was still high (> 87%) on these surfaces, it is notable that even under extended exposure mortality was less than 100%. Environmental Protection Agency (EPA) evaluation standards for residual pesticides for bed bug control stipulate exposure times of up to 4 h (EPA 2017). However, for a product that relies on the acquisition of conidia by the bed bug, efficacy will more likely be determined by the ease of conidia transfer from the surface to the bed bug. In cases where conidia transfer is less efficient, movement or distance traveled over the surface is likely more critical than the simple duration of exposure.

These experiments were conducted using the FMR field population. This population was previously tested and shown to have ~1,000-fold more resistance to permethrin (Hayes and Schal 2022) and 44.4-fold resistance to fipronil (González-Morales et al. 2021) compared to the Harlan strain. Our screening for deltamethrin revealed that our FMR population was > 390,000-fold more resistant to deltamethrin when compared to the susceptible strain (HH) which, to the best of our knowledge, is one of the highest documented pyrethroid resistance described for bed bugs (Lilly et al. 2015, Gaire et al. 2020). The potential of *B. bassiana* to control pyrethroid-resistant populations of bed bugs is an important finding of our studies that aligns with that reported by Barbarin et al. (2017).

Both our brief and prolonged exposure bioassays showed a high and consistent drop in survival occurring between 3 and 7 d (Figs. 1–4), with variation in the overall mortality across the bioassays. This variation cannot be attributed to differences in the number of conidia applied or in the viability of the conidia since both were similarly comparable across all substrates (Table 1). Rather, differences in mortality across substrates appear to be due to the type of substrate that is treated, and for some surfaces, the distance traveled by the bed bug across treated surfaces. By recording the time spent crawling, it was observed that the type of surface can affect bed bug crawling speed. In addition, in most cases, treatment with Aprehend caused a significant increase in the time taken to crawl out of arenas (Tables 2 and 4). The increase in time spent crawling on treated surfaces is likely due to the oil formulation of Aprehend, and how this formulation interacts with different substrates, with some becoming more slippery (reduced traction) and harder to move across than others. The increased time and effort to cross these surfaces may have resulted in a higher number of spores being acquired by bed bugs on certain substrates than others, possibly contributing to the observed difference in efficacy across substrates. It should also be noted that, unlike the other substrates, the crawling speed on unfinished pinewood treated with Aprehend was not significantly greater than the control in both the 2 cm and the 5 cm bioassays (Tables 2 and 4). It is possible that the porosity of this type of wood may have absorbed the product, resulting in easier movements for the bed bugs.

Differences in Aprehend efficacy across different substrates have been previously observed, where slower mortality was reported on materials like paper, wood, linoleum, and polyester compared to cotton surfaces (Aak et al. 2023). However, these differences were primarily linked to a water-based formulation and not the oil-based formulation. Jersey knit cotton has also been reported to be better for conidial transfer than paper when testing the oil formulation of *B. bassiana* (Barbarin et al. 2012), although it remains unclear why this porous surface is better than others.

Given the similarities in the number of conidia counted from swabs of all surfaces, it seemed likely that differences in efficacy between unfinished pinewood and other surfaces may be due to conidia adherence to unfinished pinewood and thus limited transfer to bed bugs that crawled across this treated surface. To test this, we extracted conidia from bed bugs that crossed Aprehend-treated cotton jersey fabric and unfinished pinewood, finding that bed bugs that crawled over treated cotton jersey fabric picked up a significantly higher number of conidia compared to those that crawled the same distance on unfinished pinewood (Fig. 5). It is possible that the conidia may have fallen into the grooves of the wood which may have caused variability in the acquisition by the bed bug as they crawled for the substrate. This may have also occurred in the other unfinished woods, but the extended exposure probably gave the bed bugs more chances of acquiring the conidia, which could explain the high mortality observed (between 87% and 97%). Regardless, bed bugs exposed to Aprehend-treated unfinished pinewood acquired fewer conidia, which likely explains the lower mortality and longer mean survival time observed in the bioassays for this substrate.

Overall, the results of this study demonstrate that Aprehend is effective on insecticide-resistant populations of bed bugs and that the recommended 5 cm (~2 inches) Aprehend application bandwidth is effective on most substrates, except on unfinished pinewood. This suggests that management efforts should be focused on treating surface types like fabric and hard smooth surface or finished surfaces, as even a brief exposure to those types of surfaces can lead to 100% mortality. The reason for the reduced effectiveness of unfinished wood isn't fully understood, but in general, chemical insecticides tend to be less efficient on porous surfaces such as wood. Their effectiveness can also be influenced by the formulation used (e.g., dusts, wettable powders, suspension concentrates, microencapsulated formulations, emulsions, or oil formulations), and the concentration applied (Fletcher and Axtell 1993). The impact of different surface types on the efficacy of a range of residual insecticide products against bed bugs has been evaluated by other authors who overall showed limited efficacy of almost all the products tested when applied on unfinished wood (Fletcher and Axtell 1993, Wang et al. 2016, Shikano et al. 2021). However, under field conditions, bed bugs are likely to encounter multiple treated surfaces during their nocturnal host-seeking activity, leading to multiple exposures to the fungus-treated surfaces. Thus, as efficacy on unfinished wood is only reduced with Aprehend, it would be worthwhile to determine if applying more product (higher rate or multiple coats) could improve the efficacy of Aprehend on raw wood surfaces. It should be noted that our study was conducted only with fresh residues of Aprehend (24 h aged), and although some studies have tested Aprehend after 7 weeks from the application (Shikano et al. 2021), future studies should test Aprehend residues aged for a longer time. This will allow the evaluation of the extent of residual efficacy of this biopesticide on different surfaces when aged at different temperatures and relative humidities.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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Author contributions

Simona Principato (Conceptualization, Formal analysis, Investigation, Writing—original draft, Writing—review & editing [equal]) and Zachary DeVries (Conceptualization [equal], Funding acquisition [lead], Writing—original draft, Writing—review & editing [equal])

References

Aak A, Hage M, Rukke BA. 2018. Insect pathogenic fungi and bed bugs: behaviour, horizontal transfer and the potential contribution to IPM solutions. J. Pest Sci. 91(2):823–835. https://doi.org/10.1007/s10340-017-0943-z

Aak A, Hage M, Rukke BA. 2023. Biological control of *Cimex lectularius* with *Beauveria bassiana*: effects of substrate, dosage, application strategy, and bed bug physiology. Pest Manag. Sci. 79(11):4599–4606. https://doi.org/10.1002/ps.7659

Al-Ani LKT. 2019. Entomopathogenic fungi in IP landscape. In: Singh HB, Keswani C, Singh SP, editors. Intellectual property issues in microbiology. 1st ed. Singapore: Springer; p. 223–238.

Arthurs S, Dara SK. 2019. Microbial biopesticides for invertebrate pests and their markets in the United States. J. Invertebr. Pathol. 165(1):13–21. https://doi.org/10.1016/j.jip.2018.01.008

Barbarin AM, Jenkins NE, Rajotte EG, et al. 2012. A preliminary evaluation of the potential of *Beauveria bassiana* for bed bug control. J. Invertebr. Pathol. 111(1):82–85. https://doi.org/10.1016/j.jip.2012.04.009

Barbarin AM, Bellicanta GS, Osborne JA, et al. 2017. Susceptibility of insecticide-resistant bed bugs (*Cimex lectularius*) to infection by fungal biopesticide. Pest Manag. Sci. 73(8):1568–1573. https://doi.org/10.1002/ps.4576

Blanford S, Chan BH, Jenkins N, et al. 2005. Fungal pathogen reduces potential for malaria transmission. Science 308(5728):1638–1641. https://doi.org/10.1126/science.1108423

Blanford S, Shi W, Christian R, et al. 2011. Lethal and pre-lethal effects of a fungal biopesticide contribute to substantial and rapid control of malaria vectors. PLoS One 6(8):e23591. https://doi.org/10.1371/journal.pone.0023591

Dang K, Doggett SL, Veera Singham G, et al. 2017. Insecticide resistance and resistance mechanisms in bed bugs, *Cimex* spp.(Hemiptera: Cimicidae). Parasit. Vectors 10(1):1–31. https://doi.org/10.1186/s13071-017-2232-3

Darbro JM, Graham RI, Kay BH, et al. 2011. Evaluation of entomopathogenic fungi as potential biological control agents of the dengue mosquito, *Aedes aegypti* (Diptera: Culicidae). Biocontrol Sci. Technol. 21(9):1027–1047. https://doi.org/10.1080/09583157.2011.597913

DeVries ZC, Santangelo RG, Barbarin AM, et al. 2018. Histamine as an emergent indoor contaminant: accumulation and persistence in bed bug infested homes. PLoS One 13(2):e0192462. https://doi.org/10.1371/journal.pone.0192462

Doggett SL. 2018. Chapter miscellaneous health impacts. In: Miller DM LC, Dogget SL, editors. Advances in the biology and management of modern bed bugs. 1st ed. Hoboken (NJ): John Wiley and Sons; p. 133–138.

Doggett SL, Miller DM, Lee C-Y. 2018. Advances in the biology and management of modern bed bugs. Hoboken (NJ): John Wiley & Sons.

EPA. 2017. Chapter laboratory product performance testing methods for bed bug pesticide products. Environmental Protection Agency. [accessed 2024 August 13] https://www.regulations.gov/document/EPA-HQ-OPPT-2009-0150-0030.

Fernandes EK, Angelo IC, Rangel DE, et al. 2011. An intensive search for promising fungal biological control agents of ticks, particularly *Rhipicephalus microplus*. Vet. Parasitol. 182(2–4):307–318. https://doi.org/10.1016/j.vetpar.2011.05.046

Fletcher M, Axtell R. 1993. Susceptibility of the bedbug, *Cimex lectularius*, to selected insecticides and various treated surfaces. Med. Vet. Entomol. 7(1):69–72. https://doi.org/10.1111/j.1365-2915.1993.tb00654.x

Gaire S, Lewis CD, Booth W, et al. 2020. Bed bugs, *Cimex lectularius* L., exhibiting metabolic and target site deltamethrin resistance are susceptible to plant essential oils. Pestic. Biochem. Physiol. 169(169):104667. https://doi.org/10.1016/j.pestbp.2020.104667

Gaire S, Principato S, Schal C, et al. 2022. Histamine excretion by the common bed bug (Hemiptera: Cimicidae). J. Med. Entomol. 59(6):1898–1904. https://doi.org/10.1093/jme/tjac131

Goddard J, deShazo R. 2009. Bed bugs (*Cimex lectularius*) and clinical consequences of their bites. JAMA 301(13):1358–1366. https://doi.org/10.1001/jama.2009.405

Goddard J, de Shazo R. 2012. Psychological effects of bed bug attacks (*Cimex lectularius* L.). Am. J. Med. 125(1):101–103. https://doi.org/10.1016/j.amjmed.2011.08.010

González-Morales MA, DeVries Z, Sierras A, et al. 2021. Resistance to fipronil in the common bed bug (Hemiptera: Cimicidae). J. Med. Entomol. 58(4):1798–1807. https://doi.org/10.1093/jme/tjab040

Gordon JM, Santangelo RG, González-Morales MA, et al. 2023. Spatial distribution of histamine in bed bug-infested homes. Sci. Total Environ. 880(880):163180. https://doi.org/10.1016/j.scitotenv.2023.163180

Hayes CC, Schal C. 2022. Behavioral interactions of bed bugs with long-lasting pyrethroid-treated bed nets: challenges for vector control. Parasit. Vectors 15(1):1–15. https://doi.org/10.1186/s13071-022-05613-z

Hentley WT, Webster B, Evison SE, et al. 2017. Bed bug aggregation on dirty laundry: a mechanism for passive dispersal. Sci. Rep. 7(1):11668. https://doi.org/10.1038/s41598-017-11850-5

Holleman JG, Robison GA, Bellovich IJ, et al. 2019. Knockdown resistance-associated mutations dominate populations of the common bed bug (Hemiptera: Cimicidae) across the South Central United States. J. Med. Entomol. 56(6):1678–1683. https://doi.org/10.1093/jme/tjz105

Hwang SJE, Doggett SL, Fernandez-Penas P. 2018. Chapter dermatology and immunology. In: Miller DM LC, Dogget SL, editors. Advances in the biology and management of modern bed bugs. 1st ed. Hoboken (NJ): John Wiley and Sons; p. 107–116.

Kakumanu ML, DeVries ZC, Barbarin AM, et al. 2020. Bed bugs shape the indoor microbial community composition of infested homes. Sci. Total Environ. 743(743):140704. https://doi.org/10.1016/j.scitotenv.2020.140704

Lacey LA, Wraight SP, Kirk AA. 2008. Chapter entomopathogenic fungi for control of *Bemisia tabaci* biotype B: foreign exploration, research and implementation. In: Gould J HK, Goolsby J, editors. Classical biological control of *Bemisia tabaci* in the United States—a review of interagency research and implementation. 1st ed. Dordrecht (the Netherlands): Springer; p. 33–69.

Lewis CD, Levine BA, Schal C, et al. 2023. Decade long upsurge in mutations associated with pyrethroid resistance in bed bug populations in the USA. J. Pest Sci. 96(1):415–423. https://doi.org/10.1007/s10340-022-01505-4

Lilly DG, Zalucki MP, Orton CJ, et al. 2015. Confirmation of insecticide resistance in *Cimex lectularius* Linnaeus (Hemiptera: Cimicidae) in Australia. Aust. Entomol. 54(1):96–99. https://doi.org/10.1111/aen.12098

Potter MF. 2011. The history of bed bug management. Am. Entomol. 57(1):14–25. https://doi.org/10.1093/ae/57.1.14

Principato S, Romero A, Lee C-Y, et al. 2023. Histamine excretion in common indoor and hematophagous arthropods. J. Med. Entomol. 60(6):1269–1277. https://doi.org/10.1093/jme/tjad103

Reis MD, Miller DM. 2011. Host searching and aggregation activity of recently fed and unfed bed bugs (*Cimex lectularius* L.). Insects 2(2):186–194. https://doi.org/10.3390/insects2020186

Romero A, Anderson TD. 2016. High levels of resistance in the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), to neonicotinoid insecticides. J. Med. Entomol. 53(3):727–731. https://doi.org/10.1093/jme/tjv253

Romero A, Potter MF, Potter DA, et al. 2007. Insecticide resistance in the bed bug: a factor in the pest's sudden resurgence? J. Med. Entomol. 44(2):175–178. https://doi.org/10.1603/0022-2585(2007)44[175:IRITBB]2.0.CO;2

Romero A, Potter MF, Haynes KF. 2010. Circadian rhythm of spontaneous locomotor activity in the bed bug, *Cimex lectularius* L. J. Insect Physiol. 56(11):1516–1522. https://doi.org/10.1016/j.jinsphys.2010.04.025

Scholte E-J, Ng'Habi K, Kihonda J, et al. 2005. An entomopathogenic fungus for control of adult African malaria mosquitoes. Science 308(5728):1641–1642. https://doi.org/10.1126/science.1108639

Shikano I, Bellicanta GS, Principato S, et al. 2021. Effects of chemical insecticide residues and household surface type on a *Beauveria bassiana*-based biopesticide (Aprehend®) for bed bug Management. Insects 12(3):214. https://doi.org/10.3390/insects12030214

Usinger RL. 1966. Monograph of Cimicidae (Hemiptera, Heteroptera). College Park (MD): Entomological Society of America; p. 585.

Wang C, Singh N, Zha C, et al. 2016. Efficacy of selected insecticide sprays and aerosols against the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae). Insects 7(1):5. https://doi.org/10.3390/insects7010005

Zhu F, Gujar H, Gordon JR, et al. 2013. Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. Sci. Rep. 3(1):1–8. https://doi.org/10.1038/srep01456

Zimmermann G. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci. Technol. 17(6):553–596. https://doi.org/10.1080/09583150701309006