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Short Communication

Persistence and Lethality of a Fungal Biopesticide (Aprehend) Applied to Insecticide-impregnated and Encasement-type Box Spring Covers for Bed Bug Management

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Abstract

The newly developed fungal biopesticide Aprehend, containing spores of *Beauveria bassiana*, is the first biological control agent to be incorporated into management programs to control the common bed bug (*Cimex lectularius* L.) (Hemiptera: Cimicidae). Aprehend is sprayed as barriers where bed bugs are likely to walk and pick up spores as they search for a bloodmeal. A key application target for Aprehend is the box spring, which may be covered by encasement-type or insecticide-impregnated covers. Since some insecticides can reduce the persistence of fungal spores, we tested the efficacy and spore germination percentages of Aprehend when applied to the two types of box spring covers. We found that spore germination was about 11% lower on the permethrin-impregnated *Active*Guard cover than on the encasement-type AllerEase cover. However, bed bugs exposed for 15 min to Aprehend on the two box spring covers suffered similarly high levels of mortality irrespective of the cover material. Thus, there was no inhibitory or additive effect of the *Active*Guard cover on bed bug mortality. Lastly, overall mortality was higher if bed bugs were exposed to Aprehend-treated *Active*Guard than the *Active*Guard cover alone. Our findings indicate that if pest managers are using *Active*Guard covers in combination with Aprehend, best practice would be to use *Active*Guard on mattresses and apply Aprehend directly to the box spring or to a box spring covered by an encasement-type cover.

Key words: biological control, *Cimex lectularius*, fungal entomopathogen, integrated pest management, mattress liner

Bed bug infestations have increased throughout most of world during the last decade (Potter et al. 2015). Heavy reliance on a few groups of insecticides has resulted in the development of numerous insecticide-resistant bed bug populations (Romero et al. 2007, Davies et al. 2012, Dang et al. 2017). Thus, current efforts to control bed bug infestations involve an integrated pest management (IPM) approach, with the goal to prevent and eliminate bed bug infestations through education and use of effective, safe, and economic management methods (Bennett et al. 2016).

Aprehend (ConidioTec LLC, Centre Hall, PA) is a recently developed biopesticide that contains spores of the entomopathogenic fungus, *Beauveria bassiana* (GHA). The spores act through contact by attaching to the bed bug's cuticle, where they germinate and penetrate, eventually colonizing the body and causing death in 4 to 10 d (Barbarin et al. 2012). Only brief exposure is required for bed bugs to pick up *B. bassiana* spores on their tarsi and other body parts (Barbarin et al. 2017). Thus, Aprehend is sprayed as narrow 5-cm-wide barriers around bed frames, box springs (not mattresses), and items of furniture where bed bugs are likely to walk as they search for a bloodmeal. After application, Aprehend remains effective for 3 mo, which reduces the requirement for re-application and provides long-term protection from bed bug introductions (US EPA, Pesticide Product Label, Aprehend, 27 Mar. 2017).

Aprehend is the first registered product that employs a biological control agent for bed bugs. Biological control agents are critical components of IPM in other systems, such as agriculture and forestry. In these IPM systems, considerable attention is paid to protecting biological control agents, especially the toxic effects of pesticides on predators and parasitoids (Croft 1990).

As chemical insecticides are still the most widely used bed bug control tool among professional pest managers (Potter and Haynes 2014), the successful integration of Aprehend into a bed bug IPM program requires assessments of its compatibility with a wide range of chemical control products. Insecticide-impregnated mattress/box spring covers are targeted chemical insecticide tools that are likely to interact with Aprehend. Insecticide-impregnated covers are used to cover mattresses and box springs and are designed to kill bed bugs that come in contact with the treated material. They serve as an alternative to encasement-type covers, which do not kill bed bugs but completely envelop mattresses and box springs to block bed bugs from hiding inside or escaping once encased. While surveys of pest managers revealed that encasement-type covers were used by 84–86% of pest managers, the use of insecticide-impregnated covers increased from 4% of pest managers in 2011 and 2013 to 9% in 2015 (Potter et al. 2011, 2013, 2015). *Active*Guard is a polyester-fitted cover impregnated with permethrin (1.64%). In addition to killing bed bugs that are continuously exposed (Jones et al. 2013), fecundity and feeding rate can be reduced following sublethal exposure to the *Active*Guard cover (Jones et al. 2015). *Active*Guard covers can be used to cover the mattress and/or the box spring. When used as a mattress cover, the product is placed over the top (sleeping surface) of the mattress just like a fitted sheet. If used as a box spring cover, *Active*Guard is fitted upside down, so that the flat surface completely covers the bottom of the box spring and the elasticated sides fit just over the top surface of the box spring to hold the cover in place. Unlike encasement-type covers, *Active*Guard covers do not envelop the entire mattress or box spring and their efficacy is not compromised by rips or holes in the fabric.

Numerous insecticides have been shown to reduce the germination rates of entomopathogenic fungal spores (Neves et al. 2001, Cuthbertson et al. 2008). Pyrethroids, including permethrin, have been shown to reduce or even completely inhibit the germination of *B. bassiana* spores when combined in solution (Anderson and Roberts 1983). Conversely, other authors have reported on synergistic effects from combinations of permethrin and *B. bassiana* (GHA) in pyrethroid resistant mosquitoes (Farenhorst et al. 2010), and bifenthrin and *B. bassiana* (GHA) in pyrethroid resistant populations of *Listronotus maculicollis* (Wu et al. 2017). In the present study, we compared the germination rates of *B. bassiana* spores and their efficacy in killing bed bugs when Aprehend was applied to *Active*Guard and an encasement-type cover, AllerEase, which consists of a polyester cover and polyethylene lining.

Materials and Methods

Bed Bugs

The bed bug colony was originally obtained from the University of Minnesota, and consisted of a mixture of several field populations collected from cities across the United States in 2005. They have been continuously reared on human blood meals at Pennsylvania State University as described in Barbarin et al. (2012).

Experiments

The *Active*Guard and AllerEase covers were cut into 30.5 × 30.5-cm pieces. One piece of *Active*Guard and one piece of AllerEase were hung side-by-side at the back of a reverse flow Hepa filter cabinet. Aprehend was sprayed according to the product label at one linear foot (30.5 cm) per second using a low-volume low-pressure spray applicator (ConidioTec LLC, Centre Hall, PA). The spray application was conducted to produce three equally spaced horizontal 5-cm 'spray barriers' of Aprehend on each piece of fabric. The Aprehend-treated material and untreated (unsprayed) material were stored on racks under a laboratory bench. The area under the bench was curtained off with black plastic sheet to create a realistic low-light environment. The laboratory temperature was maintained at 21°C. The effects of the two covers on the persistence of *B. bassiana* spores and on bed bug survival were assessed 1 and 5 wk after Aprehend application. In-time replications were conducted at 1 wk intervals, to produce three replicate-treated pieces of each cover type (and untreated controls).

Survival of Spores

A 2 × 1-cm piece of fabric was removed from each of the Aprehend-treated covers and placed in a glass vial with 1 ml of Isopar M (ExxonMobil Chemical Company, Houston, TX). The vials were vortexed for 1 min, followed by sonication for 1 min to release the spores from the fabric. A 10 µl droplet of the resulting spore suspension was pipetted onto three separate locations on a 10-cm-diameter Sabouraud dextrose agar plate. The plate was tilted in a circular motion to gently spread each droplet. The plate was placed in an incubator at 26.5°C for 20 h. For each droplet, the numbers of germinated and nongerminated spores were counted until a minimum of 300 spores was counted. The average numbers of germinated spores and total number of spores (germinated and nongerminated) from the three droplets were used to determine percentage germination.

Bed Bug Mortality Assay

A mixed-sex group of ten adult bed bugs that had been fed the previous day were placed on a randomly selected area of the Aprehend barrier or any part of the untreated (control) cover pieces. The bed bugs were contained by quickly placing the lid of a 10-cm-diameter glass petri dish over the group of 10 bed bugs. The bed bugs were allowed to move freely under the lid for 15 min. They were then transferred to a 10-cm-diameter plastic petri dish lined with filter paper and maintained in an incubator at 21°C, 50% relative humidity, and 14:10 (L:D) h. This process was repeated five times on different areas of the Aprehend barriers for each piece of cover fabric totaling 50 bed bugs per treatment. The assay was repeated for each treatment at 1 and 5 wk post Aprehend application and for each of the replicate mattress cover pieces. Mortality of bed bugs were monitored daily for 14 d.

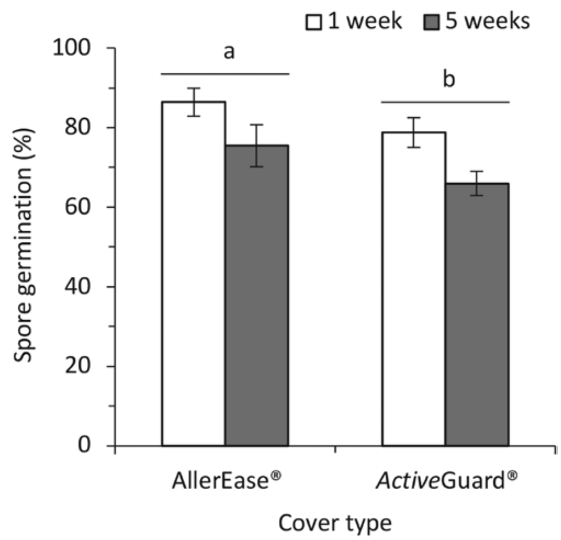

Fig. 1. The average germination percentages of *Beauveria bassiana* spores (Aprehend) 1 and 5 wk after applying Aprehend on *Active*Guard and AllerEase box spring covers. Different letters above bars indicate significantly lower germination on *Active*Guard covers compared with AllerEase covers (main effect of cover type, $P < 0.0001$). Germination was also significantly lower at 5 wk than 1 wk post Aprehend application ($P < 0.0001$).

Statistical Analysis

The germination of *B. bassiana* spores were analyzed by generalized linear model using a binomial distribution. The type of cover (*Active*Guard or AllerEase), time post Aprehend application (1 or 5 wk), and their interaction were included as factors. The survival time (time-to-event) of bed bugs was analyzed by Cox proportional hazards model to assess differences in bed bug survival time on the four treatments and the influence of time post Aprehend application. Bed bugs that survived beyond the 14-d observation period were coded as right censored. Since an interaction between treatment and time post Aprehend application significantly influenced survival times, we conducted pairwise comparisons among combinations of treatments and time post Aprehend application using Kaplan–Meier survival estimator with Bonferroni-adjusted *P* values. Mean and median survival times were also obtained from the Kaplan–Meier estimator. As censoring of 30% or more of the data creates bias in the estimated mean survival time (Zhong and Hess 2009), mean survival time was only estimated for treatments that produced more than 70% mortality. The Kaplan–Meier estimator of the median survival time is minimally biased by censoring (Zhong and Hess 2009). All analyses were performed on JMP Pro 14 (SAS Institute, Cary, NC).

Results

Spore Persistence

The percentage germination of *B. bassiana* spores on *Active*Guard was significantly lower by approximately 11% than spores applied to the AllerEase cover (Fig. 1; cover type: $X^2_1 = 40.23$, $P < 0.0001$). The viability of spores declined to a similar degree (approximately 14%) on the *Active*Guard and AllerEase covers from 1 to 5 wk post application (weeks post Aprehend application: $X^2_1 = 77.41$, $P < 0.0001$; cover type and weeks post Aprehend application: $X^2_1 = 0.28$, $P = 0.59$).

Table 1. Kaplan–Meier estimates of mean survival times (not given in cases where bias was caused by mortality below 70%) and median survival times.

Time post aprehend application	Treatment	Grouping[a]	Mortality[b]	Mean survival time (± SE)[c]	Median survival time
1 wk	AllerEase	A	3%	–	>14
	*Active*Guard	C	30%	–	>14
	AllerEase + Aprehend	F	94%	7.23 ± 0.21	6
	*Active*Guard + Aprehend	F	87%	7.02 ± 0.30	6
5 wk	AllerEase	B	15%	–	>14
	*Active*Guard	D	51%	–	13
	AllerEase + Aprehend	E	82%	8.91 ± 0.28	8
	*Active*Guard + Aprehend	DE	72%	9.16 ± 0.32	9

[a]Pairwise comparisons for all combinations of treatment and time point post Aprehend application using Bonferroni-adjusted *P* values (α = 0.05/8). Treatments with the same letter do not differ significantly.

[b]Number of bed bugs tested per treatment = 150–151.

[c]A dash denotes that estimation could not be performed.

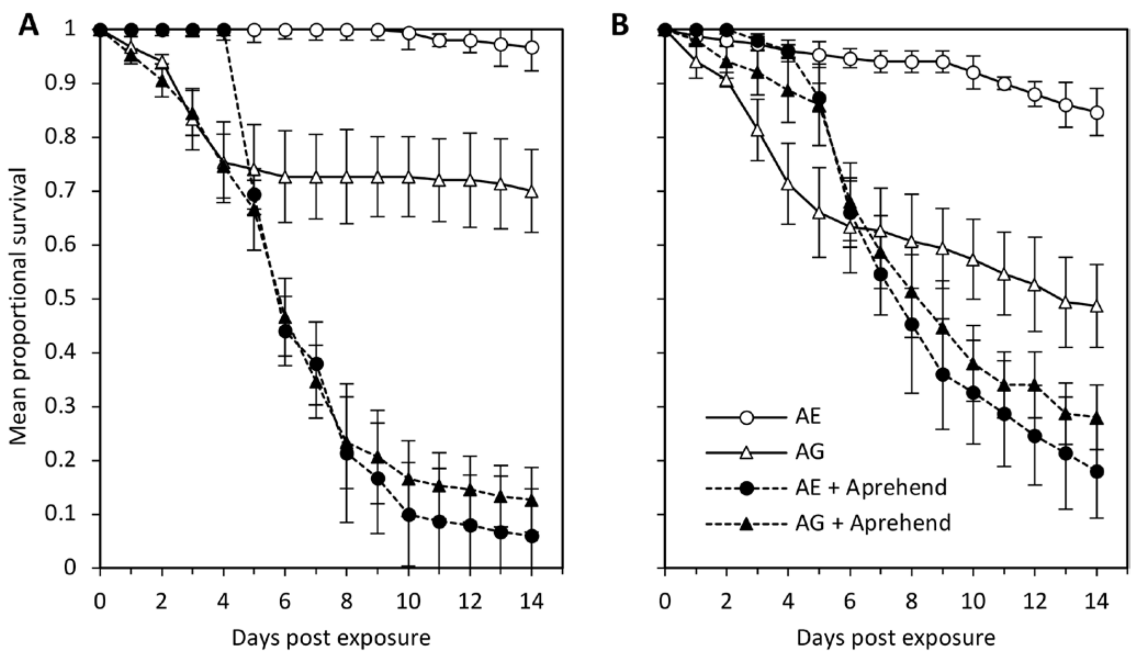

Fig. 2. The mean proportional survival of bed bugs that were exposed for 15 min to *Active*Guard (AG) and AllerEase (AE) box spring covers, treated or not treated with Aprehend. Each cover was tested 1 wk (A) and 5 wk (B) after Aprehend application. Data points and SE represent data from three separate bioassays, each using 5 sets of 10 bed bugs per treatment.

Bed Bug Mortality

The survival times of bed bugs exposed to treatment covers for 15 min were influenced by an interaction between the treatment and time post Aprehend application ($X^2_1 = 50.60$, $P < 0.0001$). At both 1 and 5 wk post Aprehend application, exposure to untreated *Active*Guard covers resulted in higher mortality than untreated AllerEase covers, but when the two cover types were treated with Aprehend, mortality did not differ significantly between the two cover types (Table 1; Fig. 2). Mean survival time was significantly shorter when bed bugs were exposed to Aprehend-treated AllerEase covers than untreated *Active*Guard. Mean survival time was also significantly shorter when *Active*Guard covers were treated with Aprehend compared with untreated *Active*Guard at 1 wk, but not 5 wk, post Aprehend application. Lastly, mortality on *Active*Guard and AllerEase covers without Aprehend was higher at 5 wk than 1 wk post Aprehend application.

Discussion

Bed bugs exposed to Aprehend, on either cover type, suffered high levels of mortality. Under the conditions of this study, there was no synergistic effect of *Active*Guard and Aprehend on bed bug mortality despite the fact *Active*Guard alone induced 30–50% mortality after just 15 min of exposure. The viability of *B. bassiana* spores on *Active*Guard was lower than on the AllerEase cover at both 1 and 5 wk post Aprehend application, but this did not appear to impact the efficacy of Aprehend as measured by bed bug mortality. Our experiments were designed to compare the efficacy of Aprehend on insecticide-impregnated and encasement-type mattress/box spring covers. Thus, we did not examine whether permethrin directly decreased the viability of the spores. Assessing the compatibility of Aprehend with active ingredients in insecticide formulations currently used for bed bug control is a key focus of our ongoing research.

Our results indicate that there is no advantage to combining *Active*Guard covers and Aprehend in the same location. However, the use of *Active*Guard covers on mattresses, where Aprehend cannot be applied, and Aprehend on box springs and other key bed bug harborage areas, could provide pest management professionals with complementary tools that both provide long-term residual efficacy. Importantly, Aprehend is equally effective against insecticide-susceptible and insecticide-resistant bed bugs (Barbarin et al. 2017). Thus, with the numbers of insecticide-resistant bed bug populations increasing worldwide (Dang et al. 2017), the use of Aprehend and *Active*Guard liners for pro-active treatments in hotels and other situations where there is a high risk of bed bug introductions, could provide protection from the establishment of infestations.

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