Inhibition of an Entomopathogenic Fungus by Volatile Aldehydes Associated with Bed Bug Exuviae

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Introduction

The bed bug (*Cimex lectularius*) is a parasite of humans that exclusively feeds on blood [1]. After the widespread use of synthetic pesticides in the 20th century, bed bugs had largely diminished in industrialized countries [2]. However, in recent years, bed bugs have made a worldwide resurgence [2].

Two aldehydes, (*E*)-2-hexenal and (*E*)-2-octenal, are known to function as an alarm pheromone when released in high concentrations. These compounds may also function as an aggregation pheromone when released in lower concentrations. Two other aldehydes, 4-oxo-(*E*)-2-hexenal and 4-oxo-(*E*)-2-octenal, have also been found to be associated with bed bugs. In addition to their functions as pheromones, these aldehydes may also function as antimicrobial defensive compounds.

This study investigates the effect of the fungus *Beauveria bassiana* on bed bug (*C. lectularius*) mortality when the aldehydes (exuviae or synthetic blend) of bed bug are present. As the exuviae are known to contain and release these aldehydes over time, the presence of exuviae in the microhabitat of bed bugs may reduce the effectiveness of pathogenic fungi as a control method. The ability of synthetic aldehydes to inhibit fungal growth was also examined. This poster reports some preliminary results of this ongoing research.

Materials and Methods

Filter paper disks (≈50 mm diameter) were placed into a plastic culture dish (60 mm diameter). Aprehend (ConidioTec LLC, Centre Hall, PA) and BotaniGard 22WP (Laverlam International, Butte, MT) were used as commercial formulations of *B. bassiana* GHA. Equivalent concentration of one fungal source was applied to the filter paper disk and was kept uncovered in a fume hood overnight until dry. Four groups of ten adult bed bugs (mixed age and sex, ≈10 days post-blood meal) were collected. Three groups were placed into separate fungus treated dishes, sealed, and remained at room temperature (25-26 C°) for one hour. Each group of 10 bed bugs were transferred into a 20-mL scintillation vial containing a folded piece of clean filter paper (50 mm x 10 mm). One vial contained bed bug exuviae (≈50) of mixed age and stage. Another vial contained a blend of the four synthetic aldehydes equivalent to 50 freshly shed exuviae. The final group of ten bed bugs were used as a control and were placed onto paper to which clean water was applied. All vials remained sealed for the duration of the study. Mortality was monitored for 15 days. This experiment was replicated a total of nine times using either BotaniGard or Aprehend as the fungal source. Survivorship curves were generated with Statistix 9.0 via Kaplan-Meier survival analyses with the pooled data (n = 90 bed bugs) and the mortality curves were compared using the log-rank test.

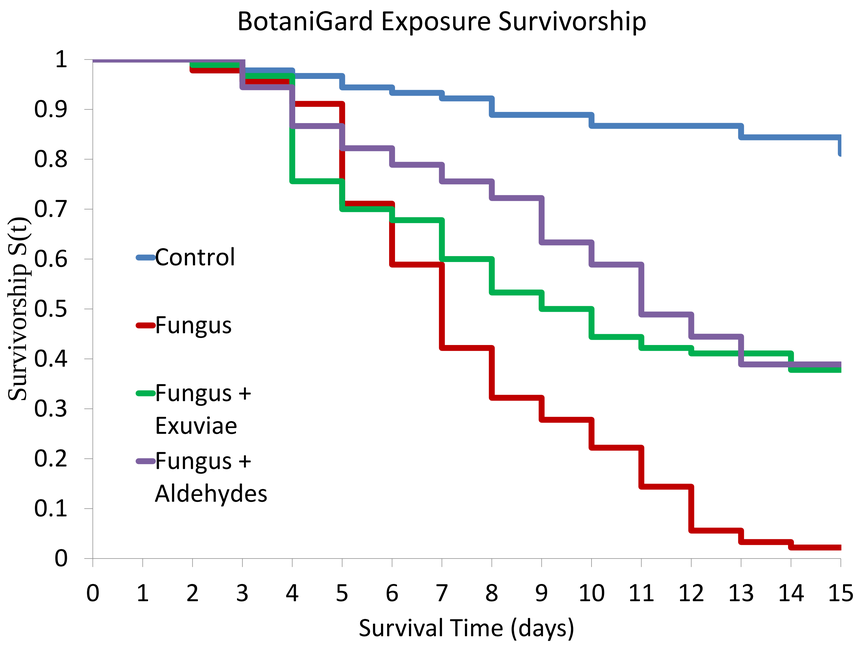

Fig. 1 Survivorship of bed bugs following BotaniGard exposure. Blue = Control (n = 90), Red = Fungus (n = 90), Green = Exuviae + Fungus (n = 90), Purple = Exuviae + Synthetic Aldehydes (n = 90).

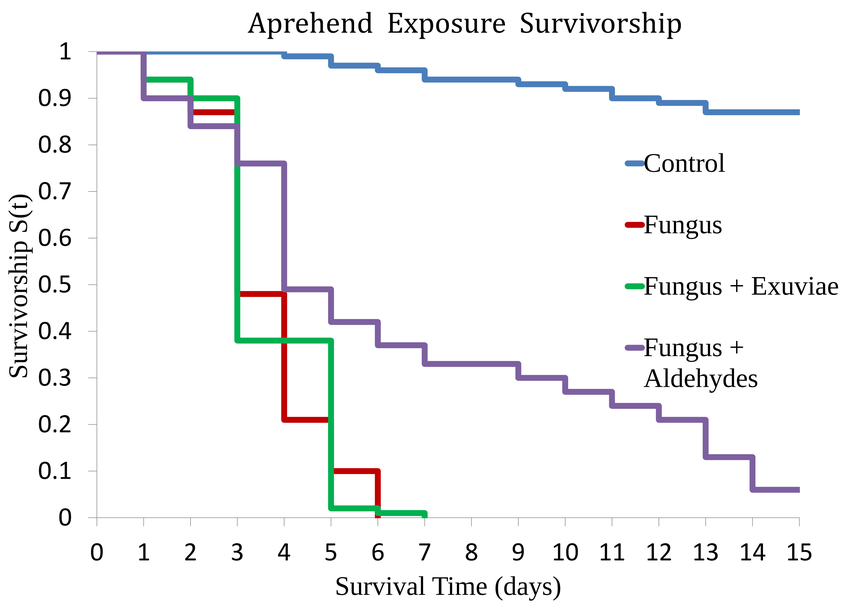

Fig. 2 Survivorship of bed bugs following Aprehend exposure. Blue = Control (n = 90), Red = Fungus (n = 90), Green = Exuviae + Fungus (n = 90), Purple = Exuviae + Synthetic Aldehydes (n = 90).

Results

When exposed to BotaniGard, the final mortality of bed bugs when exposed to fungus alone, fungus + exuviae, and fungus + synthetic aldehydes was 97.7, 62.2, and 61.2%, respectively. (**Fig. 1**). All treatment survival curves were significantly different from the control (log-rank tests; $\boldsymbol{P} < 0.001$). The survival curves of fungus exposed bed bugs with exuviae or synthetic aldehydes were both significantly different than bed bugs without an aldehyde source (log-rank tests; $\boldsymbol{P} < 0.001$). There was not a significant difference in survival between fungal exposed bed bug with exuviae or synthetic aldehydes log-rank test; $\boldsymbol{P} > 0.05$).

In the experiment with Aprehend, the final mortality of bed bugs when exposed to fungus alone, fungus + exuviae, and fungus + synthetic aldehydes was 100, 100, and 94%, respectively. (**Fig. 2**). The survival curves from the fungal treatments were significantly different from that of untreated control (log-rank tests; $\boldsymbol{P} < 0.001$). While the survival curves of the exposed bed bugs were significantly different when the bed bugs were exposed to volatiles from the exuviae (log-rank test; $\boldsymbol{P} < 0.05$), both "fungus" and "fungus + exuviae" experienced 100% mortality within a day of each other. The survival curve from "fungus + synthetic aldehyde blend" was significantly different from "fungus" (log-rank test; $\boldsymbol{P} < 0.001$), suggesting the bed bugs exposed to the synthetic aldehyde blend took longer to succumb to the fungus than those without.

Conclusion / Future Directions

The continuous presence of exuviae or synthetic aldehydes with fungal exposed bed bugs resulted in a reduction or delay in mortality. Preliminary results show that a blend of (*E*)-2-hexenal, (*E*)-2-octenal, 4-oxo-(*E*)-2-hexenal, and 4-oxo-(*E*)-2-octenal is capable of inhibiting the growth of this fungus in culture. In addition to the role these aldehydes play in intraspecific communication, these aldehydes may function to prevent the growth of pathogenic fungi in the bed bugs' harborage sites.

Further work will be needed to investigate the ability of bed bug aldehydes to inhibit this fungus. We will continue this work by quantifying the inhibition of *B. bassiana* when exposed to a blend of these four aldehydes in culture.

References

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