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Short Communication

A preliminary evaluation of the potential of *Beauveria bassiana* for bed bug control

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ABSTRACT

Residual biopesticide treatments of *Beauveria bassiana* were tested against the bed bug *Cimex lectularius*. An oil formulation of conidia was applied to different substrates. Bed bugs were exposed for 1 h, transferred to an unsprayed environment and monitored for mortality. Separate bioassays evaluated the effect of bed bug strain, sex, life stage, and exposure substrate on mortality. Rapid mortality was observed in all bioassays, with bed bugs exposed to treated jersey knit cotton dying most rapidly. A further assay demonstrated efficient autodissemination of conidia from exposed bed bugs to unexposed bed bugs within artificial harborages.

1. Introduction

The human bed bug *Cimex lectularius* is a hematophagous insect that requires blood meals for growth and development throughout its life cycle. Over the past decade, bed bug infestations have grown virtually exponentially in both North America and Europe (Hwang et al., 2005). This resurgence in bed bug infestations has been linked to increased international travel, changes in pest management practices (including increased regulatory constraints removing certain chemical insecticides from operational use (Boase, 2007)) and the wide scale spread of insecticide resistance (Moore and Miller, 2006; Romero et al., 2007; Seong et al., 2010). Insecticide resistance, together with concerns over extensive use of chemicals in the domestic environment (Sanborn et al., 2002), create a need for safe alternative methods of bed bug control. One candidate approach is the formulation of fungal entomopathogens as novel biopesticides.

Entomopathogenic fungi lend themselves to development as biopesticides because, like many conventional chemical insecticide active ingredients, they act through contact. Fungal species such as *Beauveria bassiana* and *Metarhizium anisopliae* are capable of infecting a broad range of insect hosts and several biopesticide products have been developed for use in horticulture and agriculture (Lacey et al., 2008). Recently, research has extended to blood feeding insects and disease vectors including mosquitoes (Scholte et al., 2005; Blanford et al., 2005, 2011; Darbro et al., 2011), ticks (Fernandes et al., 2011), tsetse flies (Maniania and Odulaja, 1998) and triatomid bugs (Pedrini et al., 2009).

To date, there are no published studies on the efficacy of entomopathogenic fungi against bed bugs. In this study, we evaluated the efficacy of one candidate isolate of *B. bassiana* as a residual biopesticide against the common bed bug in laboratory conditions, considering effects of feeding status, sex, bed bug strain, life history stage, and exposure substrate. Additionally, we evaluated autodissemination of conidia as a means to spread infection among bed bug populations in untreated, inaccessible areas.

2. Materials and methods

2.1. Bed bugs

A pyrethroid-susceptible laboratory strain (Harlan; cultured without introductions nor pesticide exposure since 1973) of bed bugs (designated HS) was obtained from Virginia Polytechnic Institute and State University. A second 'field' strain (an amalgam of several populations collected from cities across the US in 2005; designated FS) was obtained from University of Minnesota. Both strains were reared in our lab under standard conditions of 27 °C, 50% relative humidity (RH), and 14:10 (L:D) in glass rearing jars containing folded filter paper (Whatman No. 1, 90 mm) for a harborage and offered a blood meal weekly via an artificial feeding system (Montes et al., 2002).

2.2. Fungal isolate

B. bassiana I93-825 was maintained in long-term storage at −80 °C on microporous beads (Pro-Lab Diagnostics, Austin, TX, USA). Conidia were mass-produced using our standard 2-stage production system on barley flake (Jenkins et al., 1998; Anderson et al.,

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2011). Conidia were harvested using a Mycoharvester (Acis Manufacturing, Devon, UK), dried to 5% moisture content over silica gel, sealed in foil laminated sachets and stored at 5 °C until use.

Conidia were formulated in oil containing 80% Isopar M (Exxon Mobil) and 20% Ondina 22 (Shell) and adjusted to a concentration of 1.6×10^9 conidia/mL (viability 94–98%).

2.3. Application of conidia to exposure substrate

Conidial formulations were applied to substrates (paper or cotton jersey) at a rate of 3×10^6 conidia/cm^2 using an airbrush sprayer (Anderson et al., 2011), to give an equivalent volume application rate of 20 mL/m^2. Spore formulations were applied to HP™ Color-Laser Paper or jersey knit cotton, which were then cut into 9 cm circles. Control substrates were sprayed with blank oil formulation only. After spraying, substrates were allowed to dry at room temperature overnight. Coverage of conidia was verified by extracting the conidia from three replicate, 2 cm^2 swatches in Isopar M and counting the resulting conidial suspension using an improved Neubauer hemocytometer.

2.4. Exposing bed bugs to fungal spores

FS bed bugs were used in all experiments except that which compared the susceptibility of the two strains. Most experiments used adult bed bugs of mixed sex, which were removed from the colony one day prior to exposure and fed 12 h prior to experimentation. Variations to this procedure are described in the specific methods for each study.

In all studies, three replicates of 10 bed bugs were exposed to each treatment by placing them on pre-sprayed, dry substrate in a Petri dish for 1 h. After exposure, bed bugs were placed on clean filter paper in a Petri dish. All bed bugs were fed on day 7, 14, and 21 following exposure and mortality recorded daily for 21 d. Cadavers were incubated under high humidity to confirm mycosis. Survival data were analyzed using Kaplan–Meier survival analysis (SPSS, software version 18). Differences in median survival time between treatments were compared using the log-rank test.

2.5. Impact of feeding status, sex, and strain

All bioassays to evaluate the effect of feeding status, sex and strain of bed bugs were conducted on HP™ Color-Laser Paper as the test substrate. To evaluate the effect of feeding status, 60 adult bed bugs of mixed sex were randomly selected from the FS colony prior to feeding. Thirty bed bugs were left unfed (no blood meal for 14 d), while the remaining 30 were blood fed 12 h prior to exposure and the bioassay conducted on three replicates of 10 bed bugs as per the standard bioassay procedure.

To evaluate sex and strain differences, males versus females or mixed sex populations from the HS and FS colonies were used, respectively.

2.6. Impact of sprayed substrate on conidial transfer

Adults of mixed sex from the FS colony were fed 12 h prior to exposure and placed on either sprayed HP™ Color-Laser Paper or jersey knit cotton for 1 h.

2.7. Impact of life history stage

FS bed bugs were grouped according to instar. Adult, first and fifth instar bed bugs were selected as these life stages were most easily distinguishable. All bed bugs were fed 12 h prior to exposure. Three replicate groups of 10 bed bugs from each instar were placed on treated jersey knit cotton for 1 h.

2.8. Autodissemination of conidia

Bed bugs were removed from the FS colony, fed, and placed into 30 mL diet cups in six groups of 20 and left overnight. The following day, 10 bed bugs were removed at random from each group and exposed to either treated or unsprayed jersey cotton (three replicates) and allowed to remain in contact with the substrate for 1 h. After exposure, bed bugs were returned to their respective diet cups to comingle with the 10 unexposed bed bugs. A sterile filter paper harborage was provided. Mortality was assessed daily as above.

3. Results

There was no difference in mean survival times (MST) of treated bed bugs regardless of feeding status (MST fed 4.30 ± 0.160 days, unfed 4.17 ± 0.230 days, chi-square = 0.714, d.f. = 1, $p = 0.398$), sex (MST males 4.60 ± 0.214 days, females 5.60 ± 1.070 days, chi-square = 0.328, d.f. = 1, $p = 0.567$), or strain (MST HS 5.03 ± 0.559 days, FS 5.10 ± 0.552 days, chi-square = 0.259, d.f. = 1, $p = 0.611$) (Fig. 1A–C). Mycosis was confirmed in 100% of cadavers.

Mean survival times of bed bugs exposed to sprayed jersey knit cotton were significantly shorter than those exposed to sprayed paper (MST jersey 3.03 ± 0.580 days, paper 4.30 ± 0.160 days chi-square = 43.382, d.f. = 1, $p \leqslant 0.001$) (Fig. 1D).

All bed bug instars tested were susceptible to infection following exposure to sprayed jersey material (MST 1st instar 3.00 ± 0.00 days, 5th instar 4.00 ± 0.048 days, adults 3.03 ± 0.033 days) (Fig. 1E).

Bed bugs sharing harborages with conidia-exposed individuals experienced significantly more mortality than in the control harborages (chi-square = 124.04, d.f. = 1, $p \leqslant 0.000$). Mean survival times for adult bed bugs in the exposed treatment was 5.42 ± 0.532 days. There was no control mortality in this experiment. Overall mortality in the treated group was 95%, demonstrating that practically all of the unexposed beg bugs became infected when sharing the harborage with recently exposed individuals (Fig. 1F).

4. Discussion

B. bassiana (I93-825) was highly virulent to bed bugs, causing rapid mortality (3–5 days) following short-term exposure to spray residues. Infection levels were generally 100% indicating complete susceptibility to fungal infection under these exposure conditions. In a couple of assays 5–8% of individuals did not die, but re-exposure of these few survivors resulted in infection and mortality (results not shown), suggesting sub-optimal pick up of spores (especially from the paper substrate) rather than any physiological resistance. Results were robust across six separate assays.

There were no striking differences in susceptibility due to bed bug feeding status, sex, strain, or life stage. With respect to test substrates, jersey knit cotton was a better substrate for conidial transfer than paper, probably due to the relatively contoured surface resulting in more conidia coming into contact with the insect cuticle. These results demonstrate that choice of substrate is important in both bioassay design and end product development. Studies exploring transfer of conidia to mosquitoes following short-term residual exposure also show substrate type to effect infection levels and spore persistence (Farenhorst et al., 2011).

The current study focused on the lethal effects of infection, not least because our bioassay system resulted in such rapid and extensive mortality. In ‘field’ settings (i.e. in domestic environments where the fungus would be deployed) it is possible that bed bugs might experience lower doses via transient exposures, or when fungal spray residues begin to decay, resulting in slower

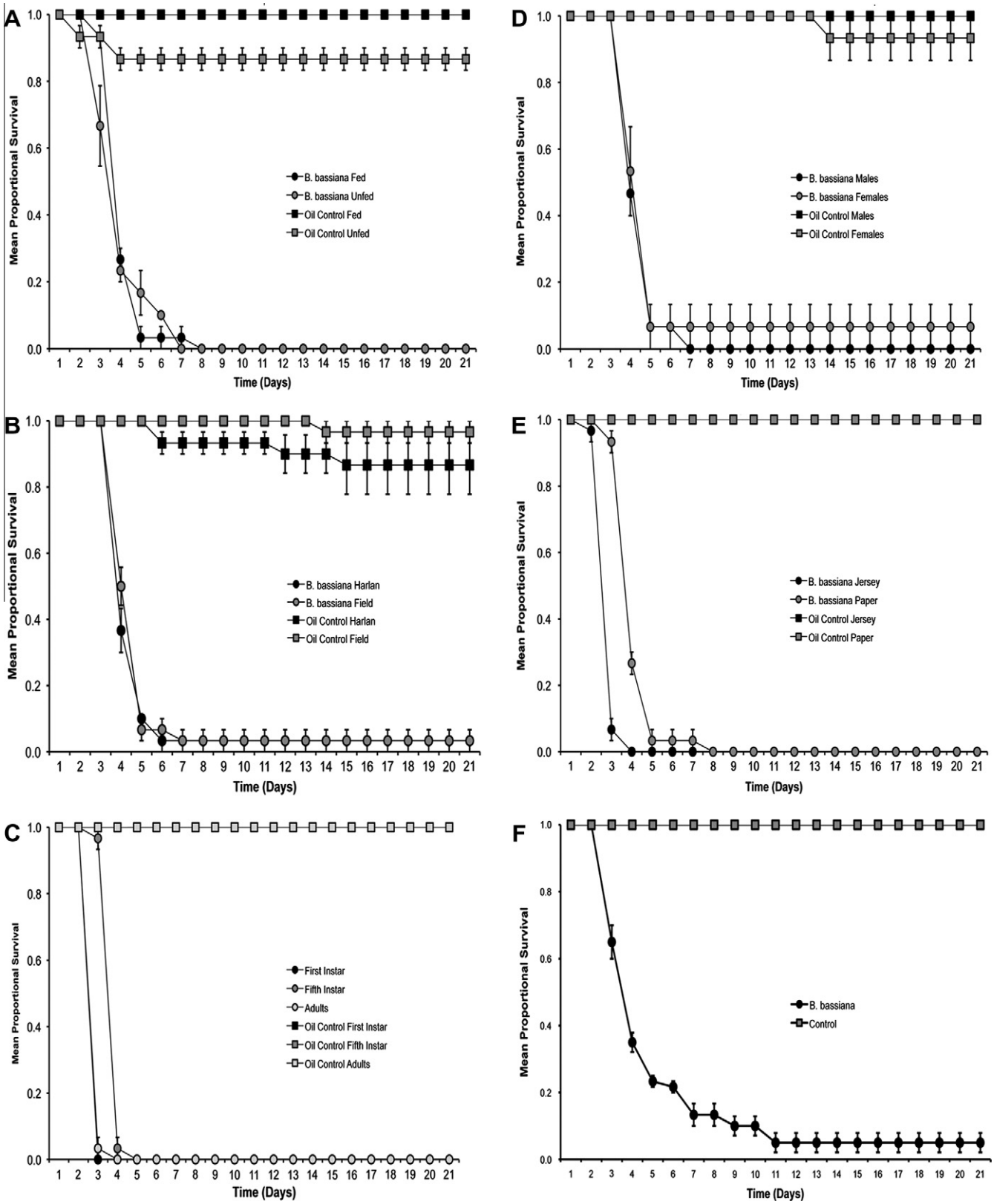

Fig. 1. (A–C) Mean proportional survival of bed bugs exposed to paper sprayed with oil formulation of *B. bassiana* conidia at 3×10^6 conidia/cm^2 (circles), or blank oil formulation (squares) for 1 h. (A) Bed bugs blood-fed 12 h prior to exposure (black circles), or left unfed prior to exposure (grey circles). (B) Male (black circles) and female (grey circles) bed bugs. (C) Harlan strain (black circles) and field strain (grey circles). (D) Mean proportional survival of adult field strain bed bugs exposed to sprayed paper (grey circles) or cotton jersey (black circles) for 1 h. (E) Mean proportional survival of first instar (black circles), fifth instar (grey circles) and adult (open circles) bed bugs, exposed to treated and untreated cotton jersey. (F) Mean proportional survival of adult bed bugs where only 50% of the population was exposed to fungus-treated cotton jersey (black circles) or blank formulated control (squares), and the remaining bed bugs sharing the harborages were unexposed. All data points represent the mean (± SE) of three replicates of 10 bed bugs except (F) where three replicates of twenty bed bugs were used.

mortality. However, slower speed of kill might be of relatively little consequence with respect to population suppression and ultimate elimination from a residence. Bed bug nymphs typically take 4–5 weeks to complete development and reach sexual maturity (Omori, 1941). This relatively slow development provides many days for a fungus to act while still preventing reproduction. Furthermore, sub- or pre-lethal effects of fungal infection, which include reduced feeding, mobility, and fecundity, are well documented in other systems (Blanford and Thomas, 2001; George et al., 2011; Howard et al., 2010) and have the potential to supplement lethal effects substantially.

Elimination of established bed bug infestations is challenging because it is difficult to identify and target all concealed harborages. However, bed bugs make nightly excursions in search of a blood meal (Mellanby, 1939; Usinger, 1966). Therefore, development of delivery systems based on barrier treatments, such as a 'bed skirt', positioned between the harborages and the human host show potential for effective control. In addition, our results suggest the potential for efficient autodissemination of conidia via contact with contaminated individuals. Our assay demonstrated that when only 50% of a bed bug population was directly exposed to fungus, total mortality exceeded 95%. Other studies have demonstrated autodissemination of conidia (Scholte et al., 2004) and potential for disease cycling following biopesticide spray applications (Arthurs and Thomas, 1999; Thomas et al., 1995). Since bed bugs are highly gregarious with all life stages aggregating in confined harborages with humid microclimates (Usinger, 1966), horizontal transmission could greatly increase the impact of fungal treatments relative to conventional chemicals.

Overall, this study represents an important first step in developing *B. bassiana* as a biopesticide for use against bed bugs within novel strategies of integrated pest management. Further research is now required to develop appropriate formulations and delivery systems to investigate population level impact under more realistic 'semi-field' and 'field' settings.

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