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DEVELOPMENT OF MYCOINSECTICIDES FOR CONTROLLING TARNISHED PLANT BUGS ON WILD HOST PLANTS

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Abstract

The efficacies of entomopathogenic fungal isolates are being evaluated for microbial biocontrol of tarnished plant bugs (Lygus lineolaris). Eight isolates of entomopathogenic fungi were compared for relative virulence to L. lineolaris adults. These isolates included the following: 1) two commercial Beauveria bassiana isolates used in the mycoinsecticides Mycotrol® (Emerald Bio-Agriculture) and Naturalis-L® (Troy Biosciences) (B. bassiana ARSEF 6444 and ARSEF 3097, respectively); 2) an isolate of Metarhizium anisopliae (ARSEF 3540) selected for its demonstrated virulence to L. lineolaris; and 3) five isolates of B. bassiana from L. lineolaris. One of the isolates from L. lineolaris (B. bassiana ARSEF 3769) was obtained in Arkansas and had previously been tested against L. lineolaris in field trials and the remaining four are new isolations from L. lineolaris in Mississippi. Those isolates that were obtained from L. lineolaris were more virulent to L. lineolaris adults than the two commercial B. bassiana isolates and the M. anisopliae isolate on the basis of LT_{50} and LT_{90} values. In addition, L. lineolaris cadavers killed by isolates that were obtained from L. lineolaris sporulated more rapidly than cadavers killed by B. bassiana (ARSEF 6444). We evaluated survival of conidia from B. bassiana (ARSEF 6444) and this five isolates from L. lineolaris following exposure to simulated solar radiation. Although there were differences among these isolates regarding their tolerance to solar radiation, each of the isolates was rapidly inactivated by solar radiation. Novel formulation strategies were tested that used water soluble lignin derivatives as sunscreens for protecting spores from solar radiation. Beauveria bassiana spores (ARSEF 6444) were coated with water soluble lignin derivatives using spray drying techniques. Lignins were either cross-linked with calcium ions to reduce the water solubility of the coating or not cross-linked to produce a highly water soluble spore coating. Spores coated with cross-linked or non-cross linked lignin were suspended in either a water (0.04% Silwet L77) or oil (70% Shellsol OMS : 30% cotton seed oil) carrier. Non coated spores suspended in water and oil carriers were used as controls. A greater percentage of spores survived exposure to solar radiation in those treatments where the spores remained coated when suspended in the carrier. The most infective formulation against L. lineolaris adults was non-coated spores suspended in water. Non-coated spores in oil, cross-linked lignin coated spores in water, non-cross linked lignin coated spores in water, and non-cross linked lignin coated spores in oil all demonstrated similar efficacy. The least infective formulation was cross-linked lignin coated spores in oil. These results indicate a potential for improving the efficacy of mycoinsecticides against L. lineolaris through isolate selection and formulations that improve their environmental persistence.

Introduction

In the southeastern cotton belt, L. lineolaris usually completes one or more generations on spring wild hosts before moving into cotton fields. (Robbins et al., 2000). In many cotton growing regions, wild host plants of L. lineolaris are restricted to a small percentage of the total land area due to the dominance of agricultural land in the region (Robbins et al., 2000). Studies have been conducted on the area-wide management of L. lineolaris through the destruction of wild host plants with herbicides and mowing with a goal of reducing the numbers of L. lineolaris moving from wild host plants into cotton (Snodgrass et al., 2000). Another potential approach would be to manage L. lineolaris populations in wild host plants with a microbial biocontrol agent. These control agents may be particularly well-suited to an area-wide management strategy in non-crop areas because of the potential to minimize environmental impacts, resistance development and preserve beneficial insects.

Entomopathogenic fungi have contact infectivity providing them with an advantage over other microorganisms that require ingestion for infectivity, especially for controlling insects with piercing-sucking mouthparts, such as L. lineolaris. Several studies have evaluated the entomopathogenic fungus, B. bassiana as a microbial control agent of L. lineolaris and found mixed success. The majority of field trials against L. lineolaris have been conducted using the commercial isolate of B. bassiana (ARSEF 6444) formulated as Mycotrol® (Emerald BioAgriculture) (Brown et al., 1997; Noma and Strickler, 1999; 2000; Steinkraus, 1996; Steinkraus and Tugwell, 1997), and one used B. bassiana (ARSEF 3097) formulated as Naturalis L® (Snodgrass and Elzen, 1994). Mycotrol® was effective in causing high mortality in L. lineolaris on cotton, but the time to kill was slow (Steinkraus, 1996; Brown et al., 1997). This slow kill time may be particularly significant since L. lineolaris has been shown to continue feeding and ovipositing following infection (Noma and Strickler, 2000). Imidacloprid has been

shown to act synergistically with B. bassiana reducing the time to kill and increasing mortality (Brown et al., 1997; Steinkraus, 1996). For the purposes of controlling L. lineolaris populations in wild host plants, feeding damage prior to mortality may not be important, but female fecundity will be a critical factor in managing nondiapausing populations. In another study, Mycotrol® failed to consistently control L. hesperus in alfalfa seed plots, which may have been related to poor spray coverage as the plant canopy became more dense during the growing season (Noma and Strickler, 1999). Naturalis L® reduced populations of L. lineolaris adults and nymphs by 20 and 54%, respectively, but control was not sufficient to effectively manage populations within the cotton fields (Snodgrass and Elzen, 1994).

In a laboratory study comparing the pathogenicity of 32 fungal isolates from the genera Beauveria, Verticillium, Paecilomyces, Metarhizium, Mariannaea, and Hirsutella, only three isolates were significantly more virulent than the commercial isolate used in Mycotrol® (B. bassiana ARSEF 6444), which included two B. bassiana isolates from foreign countries and one M. anisopliae isolate indigenous to the US (Liu, 2002). The first, and only reported, entomopathogenic fungus isolated from naturally infected L. lineolaris was B. bassiana (ARSEF 3769) from Arkansas (Steinkraus, 1996). It has demonstrated high virulence towards L. lineolaris in laboratory bioassays and caged field trials (Steinkraus, 1996; Steinkraus and Tugwell, 1997).

For a mycoinsecticide to have practical application, it must be sufficiently virulent and have sufficient persistence under realistic environmental conditions. Formulations can greatly enhance the both the efficacy and persistence of mycoinsecticides under adverse environmental conditions. The most critical environmental factor related to virulence is availability of sufficient moisture for germination and infection. Application of mycoinsecticides in oil rather than water carriers enhances efficacy in arid climates (Bateman et al., 1993; Burgess, 1998). However, it remains to be seen if this enhanced efficacy extends to relatively moderate humidity conditions common in the eastern cotton growing regions of the US.

The most important environmental stress factors that affect spore survival in the field are thermohydric stress and solar radiation. The effects of temperature on spore survival are greatly correlated with spore moisture, with drying generally improving thermal-stress tolerance and shelf-life (Hedgecock et al., 1995; McClatchie et al., 1994; Moore et al., 1997; Hong et al., 1997; 1998; 2000). Solar radiation (particularly UVB, 280-320 nm) may be the most damaging environmental factor for entompathogenic fungi (Ignoffo, 1992) and several formulation strategies have been employed over the years to protect entomopathogenic fungi from solar radiation with mixed success (Burgess, 1998). These strategies may be classified in the following generalized approaches: 1) oil-soluble sunscreens with oil-carriers (Hunt et al., 1994; Moore et al., 1993; Shah et al., 1998); 2) oil-water emulsions (Alves et al., 1998); 3) water-soluble or suspendable sunscreens with water carriers (Cohen et al., 1991, 2001; Ignoffo et al., 1991; Shapiro 1989, 1992; Shapiro et al., 1992; Shapiro and Robertson, 1990; Shasha et al., 1998), 4) encapsulation in a sunscreen with low water solubility and suspension in water carriers (Behle, et al., 1996; Ignoffo et al., 1991; McGuire and Shasha, 1995; 1996; McGuire et al., 1990, 1994, 1996; Shasha and Dunkle, 1989; Shasha and McGuire, 1989; Shasha et al., 1998; Tamez-Guerra, et al., 2000 a,b), and 5) encapsulation in a water soluble sunscreen and suspension in oil (Leland, 2001). Oil carriers sprayed at ultra-low volume (ULV) are generally more appropriate than water carriers applied at high volume (HV) for controlling insects over large area natural areas (Bateman et al., 1993; Burgess, 1998). New approaches to formulating entomopathogenic fungi for application in oil carriers are needed because oil soluble sunscreens, although promising in lab trials, have generally failed to protect spores under realistic environmental conditions (Burgess, 1998; Inglis et al., 1995)

Materials and Methods

Obtaining New Isolates

New isolates were obtained from geographically distinct areas within Washington County, MS in 2002 by placing sporulating cadavers from field collected insects on Beauveria selective media (Chase et al., 1986). Colonies that grew on Beauveria selective media were transferred to Sabouraud dextrose agar plus yeast (SDAY) for production of inocula (Goettel and Inglis, 1997). Colonies were scraped from the surface of SDAY media, suspended in 0.04% Tween 80, filtered through cheese cloth to separate spores from mycelia, and stored at -80 °C in 15% glycerol. Multiple, 1 mL aliquots were made to serve as consistent sources of inocula for future experiments.

Relative Virulence of Isolates

Two bioassays were used to evaluate the relative virulence of fungal isolates. The first bioassay compared the following isolates of B. bassiana ARSEF 6444 (Mycotrol®), ARSEF 3097 (Naturalis-L®), ARSEF 3769, New Isolate 1, and New Isolate 2, and M. anisopliae (ARSEF 3540). Each of these isolates was applied at four concentrations (1×10^8, 1×10^7, 1×10^6 and 1×10^5 spores/mL) to three replicates of 15 insects per replicate. The second bioassay compared the following isolates of B. bassiana ARSEF 6444 (Mycotrol®), ARSEF 3769, and New Isolates 1, 2, 3, and 4 at a single concentration of 1×10^7 spores/mL to three replicates of 20 insects per replicate. Adult L. lineolaris were collected as adults from wild host plants in Washington

Co., MS, fed green beans, Phaseolus vulgaris L. and incubated at 26.6 °C, with a photoperiod of 14/10 (light/dark) for 48 hr prior to use. For the first isolate bioassay, L. lineolaris were collect from pigweed (Amaranthus spp.)at a single site on October 9, 2002. For the second isolate bioassay, L. lineolaris adults were collected from pigweed, smart weed (Polygonum pensylvanicum) and a mix of pigweed and golden rod (Solidago altissima L.) at multiple sites on November 15, 2002. The effect of collection site was blocked within each replicate. Following 48 hr incubation, adults were transferred to 100 mm Petri dishes containing moist Whatman #1 filter paper for treatment. Insects were treated by applying 1 mL of spore suspensions to replicate groups of insects contained in Petri dishes using a Potter spray tower. The Potter spray tower was calibrated to ensure consistent treatment to each replicate by spraying 1 mL spore suspensions at 1 x 10^7 spores/mL onto 4.8 cm^2 plastic cover slips, then folding these cover slips into 1.5 mL Eppendorf microcentifuge tubes, resuspending the spores in 0.04% Silwet L77 and determining spore concentrations by hemacytomer. Using this calibration method 1 mL of 1 x 10^7 spores per mL was equal to 6.9 (± 1.8) x 10^4 spores/cm^2. After treatment, insects were delivered to individual 30 mL medicine cups with screened lids containing broccoli florets. Insects were incubated at 26.6 °C, 80% relative humidity, with a photoperiod of 14/10 light/dark; mortality was determined daily and broccoli was changed at 48 hr intervals.

Solar Simulation

Two solar simulation experiments were conducted to evaluate the survival of B. bassiana following exposure to simulated solar radation. The first compared the survival of spores from B. bassiana isolates ARSEF 6444, ARSEF 3769, and New Isolates 1, 2, 3, and 4. The second compared survival of B. bassiana (ARSEF 6444) spores in the following six formulations: 1) non-coated spores in 0.04% Silwet L77; 2) non-coated spores in oil (70% Shellsol OMS:30% cotton seed oil); 3) non-cross linked lignin coated spores in 0.04% Silwet L77; 4) non-cross linked lignin coated spores in oil; 5) cross-linked lignin coated spores in 0.04% Silwet L77; and 6) cross-linked lignin coated spores in oil. Spore suspensions (10 mL of 1x10^7 spores/mL) were deposited onto nylon membrane filters (Magna, 0.45 µm pore size, 47 mm diameter) by vacuum filtration (Advantec MFS, Inc.). In experiments evaluating relative tolerance of fungal isolates, membrane filters were exposed to 0, 0.5, 1.0, 2.0, and 4.0 hr of simulated sunlight (13.8 ± 0.2 mW/cm^2) (Thermo Oriel Model 91193 Solar Simulator with Air Mass 1.5 Global Filter) and three subsamples per exposure time. In experiments evaluating relative tolerance of formulated spores, membrane filters were exposed to 1.0, 2.0, 4.0, 8.0 hr and 16 hr of simulated sunlight at a power density of (14.1 ± 0.1 mW/cm^2) and three subsamples per exposure time. After exposure, spores were suspended in 2 mL of 0.04% Tween 80 and 100 mL aliquots were spread on two 60 mm Petri dishes containing germination agar (20 g/L malt extract; 15 g/L agar; 0.02 g/L Benlate 50WP; 0.2 g/L chloramphenicol; 200,000 units penicillin/L; 0.2 g/L streptomycin). Spores on the first plate were killed after 24 hr incubation and after 72 hr incubation on the second plate with lactophenol acid fuschin mounting media. Glass cover slips were pressed into the agar and percent spore germination was determined by observing 200 spores per treatment at 1000 x magnification.

Relative Infectivity of Spores in Six Formulations

A bioassay was conducted to evaluate the relative efficacy of the six formulations of B. bassiana (ARSEF 6444, Emerald BioAgriculture) described in the above subsection "Solar Simulation". Spore were applied in 1 mL of water at concentrations of 1 x 10^8, 1 x 10^7, 1 x 10^6 and 1 x 10^5 viable spores/mL or in 250 µL of oil at concentrations of 4 x 10^8, 4 x 10^7, 4 x 10^6 and 4 x 10^5 viable spores/mL. The lower volume of oil (250 µL) was used to reduce potential toxic effects of the oil carrier and better simulate ULV application. For the formulation bioassay, L. lineolaris adults were collected from marestail (Conyza canadensis) at a single site on September 10, 2002. Adults were treated and incubated using the conditions described above for bioassays on fungal isolates.

Results

Relative Virulence of New Isolates

The six fungal isolates tested in the first bioassay caused a wide variety of L. lineolaris mortality (Figure 1). LC_{50} values were difficult to estimate accurately from this data and therefore LT_{50} values, LT_{90} values, and final percent mortality at the two highest concentrations (1 x 10^7 and 1x10^8) spores/mL were used to compare to the relative virulence of the six isolates (Table 1). Two of the isolates from L. lineolaris (B. bassiana ARSEF 3769 and New Isolate 1) were the most virulent isolates on the bases of LT_{50} values at 1 x 10^7 spores/mL and LT_{90} values at 1 x 10^7 and 1 x 10^8 spores/mL. The commercial isolate B. bassiana (ARSEF 3097) was the least virulent isolate at the highest concentration (1 x 10^8 spores/mL) based on LT_{50} values. The only significant differences in percent mortality at 8 d post inoculation were that B. bassiana (ARSEF 3769) caused greater mortality than both B. bassiana (ARSEF 3097) and M. anisopliae (ARSEF 3540) at 1 x 10^7 spores/mL.

The six isolates compared in the second isolate bioassay caused a variety of mortality in L. lineolaris adults (Figure 2) and could be discerned on the basis of LT50 values and final percent mortality (Table 2). Those isolates from L. lineolaris were significantly more virulent than the commercial isolate (B. bassiana ARSEF 6444) on the bases of LT_{50} and LT_{90} values, and final percent mortality (New Isolate 1 was only more virulent based on LT_{90} values). Isolates from L. lineolaris had LT_{50} and LT_{90} values that were as much as 2 and 3 days faster, respectively, than the commericial isolate (B. bassiana ARSEF 6444). In addition, a greater percentage of cadavers killed by three of the five isolates from L. lineolaris, B. bassiana (ARSEF 3769,

NI 1, and NI 4,) sporulated within 24 hr than cadavers killed by the commercial isolate B. bassiana (ARSEF 6444), which may effect the potential of isolates to spread by horizontal transmission following initial application of a mycoinsecticide.

Solar Simulation

Exposure to solar radiation rapidly reduced spore germination in all six isolates that were tested (Table 3). In addition to killing spores (72 hr germination rate), exposure to solar radiation delayed the germination of spores as demonstrated by the percentage of viable spores that germinated within 24 hr. Isolates from L. lineolaris were more tolerant of exposure to solar radiation than the commercial isolate (B. bassiana ARSEF 6444) based on spore viability (72 hr germination) following 2 hr of exposure to solar radiation.

Initial values for percent of viable spores for spore formulations prior to the solar simulation experiment were as follows; non-coated spores in water = 92 ± 1%, non-coated spores in oil = 95 ± 0.3%, non-cross linked lignin coated spores in water = 52 ± 4%, non-cross linked lignin coated spores in oil = 51 ± 4%, cross linked lignin coated spores in water = 78 ± 3%, and cross linked lignin coated spores in oil = 75 ± 4%. When evaluating survival of spores in the six formulations following exposure to simulated solar radiation, spore viability based on 48 hr spore germination was corrected for these initial spore viability values. The formulations that demonstrated the greatest potential for protecting spores based on 48 hr spore germination (corrected for initial viability) were those in which the spores remained coated by lignin in the carriers; cross-linked lignin coating in water, cross-linked lignin coating in oil, and non-cross linked lignin coating in oil (Figure 3).

Relative Infectivity of Spores in Six Formulations

The six formulations of B. bassiana ARSEF 6444 caused a variety of mortality in L. lineolaris adults (Figure 4) and could be discerned on the basis of LT_{50} and LT_{90} values and final percent mortality at the two highest spore concentrations (Table 4). The most infective formulation was non-coated spores in water and the least was spores coated with cross-linked lignin in oil. All other formulations demonstrated a similar infectivity to L. lineolaris.a All other formulations demonstrated a similar infectivity to L. lineolaris causing mortalities of greater than 67% at 1 x 10^7 spores/mL and greater than 80% at 1 x 10^8 spores/mL at 10 d post treatment. In contrast spores coated with cross-linked lignin in oil only caused All other formulations demonstrated a similar infectivity to L. lineolaris causing mortalities of greater than 67% at 1 x 10^7 spores / mL and greater than 80% at 1 x 10^8 spores / mL at 10 d post treatment. In contrast, spores coated with cross-linked lignin in oil only caused 44% mortality even at the highest spore concentration (1 x 10^8 spores / mL).

Discussion

Those isolates from L. lineolaris were generally the most virulent to L. lineolaris and had a greater potential to sporulate from L. lineolaris cadavers. These qualities would be important in developing a mycoinsecticide, but further information is need in evaluating these isolates as potential Lygus-specific mycoinsecticides. Two important characteristics that need to be investigated are the impact of these isolates on beneficial insects (Ruberson and Williams, 2000) and their potential to produce spores under industrial production conditions. All of the B. bassiana isolates were prolific sporulators, and produced spore concentrations on SDAY medium similar to that of commercial isolates (data not presented). Whereas, M. anisopliae (ARSEF 3540) was not a prolific sporulator on SDAY medium and did not produce spores in sufficient concentrations to easily produce a spore concentration of 1 x 10^8 spores/mL for bioassays. Further evaluation under conditions that more closely simulate industrial production conditions will be the next step in the evaluation process (e.g. Bradley et al., 2002). One important obstacle to the efficacy of each of these isolates is their inability to survive exposure to solar radiation. Although those isolates from L. lineolaris were more tolerant to solar radiation than the commercial isolate (B. bassiana ARSEF 6444), all isolates were highly susceptible with the majority of spores dying within 2 hr of exposure to simulated solar radiation. In addition to killing spores, exposure to solar radiation delayed the germination of spores, which may reduce infection success of spores (Dillon and Charnley, 1985; Hassan and Charnely, 1983). Of the six formulation strategies that were investigated, three were very promising strategies for protecting spores from solar radiation; cross-linked lignin coating in water, cross-linked lignin coating in oil, and non-cross linked lignin coating in oil. Of these three formulation strategies, cross-linked lignin coating in water and non-cross linked lignin coating in oil, caused the highest mortality in L. lineolaris adults. However, these formulations caused less mortality than non-coated spores in water. This suggests a need for improvements in these formulation strategies related to the infection potential of formulated spores. It is worth noting that the formulation using non-cross linked lignin coating in water, in which spores became uncoated in the carrier, demonstrated similar virulence to the three formulations where the spores remained coated. This suggests that for the non-cross linked lignin coating formulation, it is not the lignin coating that is interfering with the infection process, rather it may be related to spore physiology. A percentage of the spores die during the formulation process and it may be that the remaining spores are adversely effected resulting in reduced infection potential. Improvements in the formulation process that reduce the negative impacts on spore physiology may improve the efficacy of these formulations and shelf life. The cross-linked lignin in oil formulation caused the least mortality in L. lineolaris. It may be these cross-linked lignin coated spores in oil are not able to obtain sufficient moisture to allow for spores to become uncoated inhibiting infection processes. Approaches need to be investigated for improving the shelf life of the formulations that were tested, which may involve spore anabiosis, alterations in formulation components, reducing available oxygen, and regulating moisture (e.g. Crowe et al., 1984; Jin et al., 1999).

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Table 1. Relative Virulence of Six Fungal Isolates to Lygus lineolaris Adults at 1 x 10^7 spores/mL and 1 x 10^8 spores/mL (Isolate Bioassay 1).

	1 x 10^7 spores/mL			**1 x 10^8 spores/mL**		
Fungal Isolate	**% Mortality (Abbott's) at 8 days**	**LT 50 (95% C.I.)**	**LT 90 (95% C.I.)**	**% Mortality (Abbott's) at 8 days**	**LT 50 (95% C.I.)**	**LT 90 (95% C.I.)**
Not Isolated From Lygus						
B. bassiana (ARSEF 6444)	55 ± 18 ab[1]	6.8 a (6.3 – 7.3)	11.9 a (11.0 –13.2)	73 ± 25 a	5.7 a (5.2-6.2)	11.1 ab (10.3-12.2)
B. bassiana (ARSEF 3097)	44 ± 20 b	7.6 a (7.1 – 8.2)	13.2 a (12.2-14.7)	56 ± 24 a	7.1 b (6.7-7.6)	11.8 a (11.0-12.8)
M. anisopliae (ARSEF 3540)	50 ± 21 b	7.1 ab (6.5 – 7.7)	13.4 a (12.3-15.0)	N/A[2]	N/A	N/A
Isolated From Lygus						
B. bassiana (ARSEF 3769)	83 ± 9 a	5.0 c (4.6 – 5.5)	9.1 b 8.5-9.9	89 ± 28 a	5.1 a (4.5-5.7)	8.4 c (7.6-8.5)
B. bassiana (New Isolate 1)	77 ± 4 ab	5.4 c (5.0 – 5.8)	8.8 b 8.4-9.5	88 ± 15 a	5.1 a (4.2-5.8)	8.5 c (7.6-10.0)
B. bassiana (New Isolate 2)	76 ± 3 ab	6.2 bc (5.4 – 7.0)	10.8 ab 9.7-12.5	91 ± 10 a	5.5 a (4.5-6.4)	8.9 bc (7.8-10.8)

[1] Means in column followed by the same letter are not significantly different at the $\alpha = 0.05\%$ level. Proc GLM (SAS) for % Mortality and Probit Analysis (SAS) for LT values.

[2] Bioassays were not conducted with M. anisopliae (ARSEF 3540) at the 1 x 10^8 spores/mL concentrations because colonies on SDAY media did not produce spores in sufficient numbers to provide a high spore concentration.

Table 2. Relative Virulence of Six Fungal Isolates to Lygus lineolaris Adults at 1 x 10^7 spores/mL (Isolate Bioassay 2).

Fungal Isolate	% Mortality (Mean ± S.D.) (Abbott's) at 8 days	LT 50 (95% C.I.) Days	LT 90 (95% C.I.) Days	% Cadaver Sporulation in 24 hr	% Cadaver Sporulation in 48 hr	Spores (x 10^3) /Insect After 48 hr Incubation
B. bassiana (ARSEF 6444)	70 ± 14 a	6.7 a (6.2 – 7.4)	9.2 a (8.3 – 10.8)	5 ± 8 a	83 ± 14 a	3.3 ± 3.8 a
B. bassiana (ARSEF 3769)	97 ± 6 b	5.4 b (5.0 – 5.8)	6.7 cd (6.2 – 7.4)	52 ± 9 c	89 ± 10 a	3.6 ± 4.1 a
B. bassiana (New Isolate 1)	89 ± 11 ab	6.0 ab (5.7 – 6.2)	8.0 b (7.6 – 8.5)	36 ± 13 bc	95 ± 5 a	339 ± 771 a
B. bassiana (New Isolate 2)	94 ± 5 b	5.5 b (5.3 – 5.7)	7.1 c (6.8 – 7.4)	12 ± 9ab	87 ± 16 a	8.7 ± 9.1 a
B. bassiana (New Isolate 3)	96 ± 4 b	4.8 c (4.6 – 5.0)	6.7 cd (6.3 – 7.1)	30 ± 3 b	96 ± 3 a	6.9 ± 10.0 a
B. bassiana (New Isolate 4)	100 ± 0 b	4.8 c (4.6 – 5.0)	6.2 d (5.9 – 6.5)	39 ± 12 bc	84 ± 9 a	3.3 ± 2.7 a

[1] Means in column followed by the same letter are not significantly different at the $\alpha = 0.05\%$ level. Proc GLM (Tukey's HSD) for % Mortality, % Sporulation, and # of pores/insect and Probit Analysis (SAS) for LT values.

Table 3. Percent Spore Germination within 24 and 72 hr of Incubation Following 0, 1, 2 and 4 hr of Exposure to Simulated Solar Radiation for Six Isolates of B. bassiana.

	0 hr of Exposure		1 hr of Exposure		2 hr of Exposure		4 hr of Exposure
	24 hr % Germ.	72 hr % Germ.	24 hr % Germ.	72 hr % Germ.	24 hr % Germ.	72 hr % Germ.	72 hr % Germ.
B.bassiana (6444)	78 ± 9 a[1] (94%)[2]	83 ± 9 a	19 ± 14 a (40%)	48 ± 15 a	1 ± 1 a (18%)	7 ± 4 a	0.6 ± 0.3 b
B.bassiana (3769)	85 ± 5 a (89%)	95 ± 2 a	42 ± 37 a (45%)	92 ± 5 b	3 ± 4 ab (10%)	29 ± 9 b	0.6 ± 0.6 b
B.bassiana (NI 1)	88 ± 5 a (95%)	93 ± 11 a	40 ± 23 a (45%)	89 ± 6 b	5 ± 2 ab	N/A[4]	1.8 ± 1.9 b
Bbassiana (NI 2)	87 ± 8 a (91%)	96 ± 3 a	71 ± 7 a (82%)	86 ± 12 b	10 ± 1 b	N/A[4]	2.6 ± 1.7 ab
B.bassiana (NI 3)	80 ± 20 a (83%)	96 ± 1 a	54 ± 31 a (57%)	94 ± 1 b	8 ± 5 ab	N/A[4]	5.6 ± 1.1 a
B.bassiana (NI 4)	75 ± 12 a (80%)	93[3]	54 ± 25 a (58%)	93 ± 3 b	3 ± 2 ab	N/A[4]	1.6 ± 0.6 b

[1] Means in column followed by the same letter are not significantly different at the $\alpha = 0.05\%$ level. Proc GLM (SAS).

[2] Means ± St. Dev. Presented; values in parentheses represent the percent of viable spores that germinated within 24 hr (24 hr % germ. ÷ 72 hr % germ. x 100) to demonstrate that exposure to solar radiation effects spore germination rate.

[3] N=1 for B. bassiana NI 4 at 0 hr of exposure and 72 hr incubation due to contaminant fungus on 2 replicates

[4] Percent germination could not be determined for NI 1, NI 2, NI 3, or NI 4 for 2 hr of exposure and 72 hr incubation due to a contaminant fungus.

Table 4. Relative Efficacy of Six Formulations of B. bassiana (ARSEF 6444) to Lygus lineolaris Adults at 1 x 10^7 spores/mL.

	1 x 10^7 spores/mL			1 x 10^8 spores/mL		
Formulation	**% Mortality (Abbott's) at 10 days**	**LT 50 (95% C.I.)**	**LT 90 (95% C.I.)**	**% Mortality (Abbott's) at 10 days**	**LT 50 (95% C.I.)**	**LT 90 (95% C.I.)**
Formulations Suspended in Water Containing 0.04% Silwet L77						
Non-Coated Spores	93	5.4 a (4.6-6.2)	8.5 a (7.5-10.1)	100	3.89 a	4.1 a
Non-Cross Linked Lignin Coating	70	7.0 b (6.6 – 7.6)	10.7 ab (9.9-11.9)	96	5.2 b (4.9-5.6)	7.63 bc (7.13-8.28)
Cross Linked Lignin Coating	67	7.7 bc (7.1 – 8.8)	12.2 b (10.9-13.9)	81	5.9 b (5.5-6.4)	9.64 c (8.89-10.68)
Formulations Suspended in Oil Containing 70% Shellsol OMS : 30% Cotton Seed Oil						
Non-Coated Spores	71	6.8 b (6.3 – 7.3)	10.5 ab (9.7-11.7)	96	4.7 b (3.9-5.5)	7.17 b (6.28-8.71)
Non-Cross Linked Lignin Coating	74	7.3 bc (6.6 – 8.8)	12.1 b (10.8-13.9)	96	5.1 b (4.7-5.6)	8.51 bc (7.85-9.38)
Cross Linked Lignin Coating	46	9.2 c (8.6 – 10.2)	13.1 b (11.8-15.3)	44	8.8 c (8.1-9.9)	13.4 d (12.0-15.8)

[1] Values followed by different letters are significantly different at the $\alpha = 0.05\%$ level (Probit Analysis, SAS).

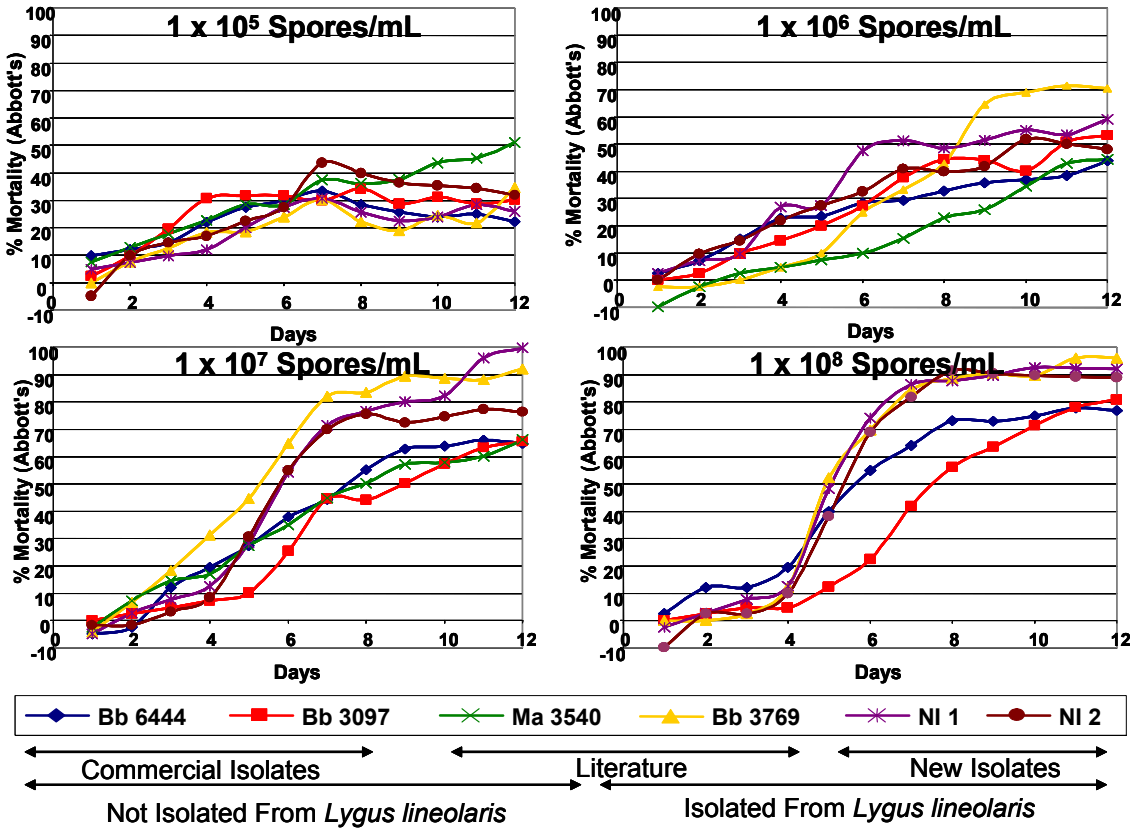

Figure 1. Mortality (Abbott's) over time Lygus lineolaris adults exposed to four concentrations of six fungal isolates in the first isolate bioassay. Bb = Beauveria bassiana; Ma = Metarhizium anisopliae; NI = New Isolate of B. bassiana.

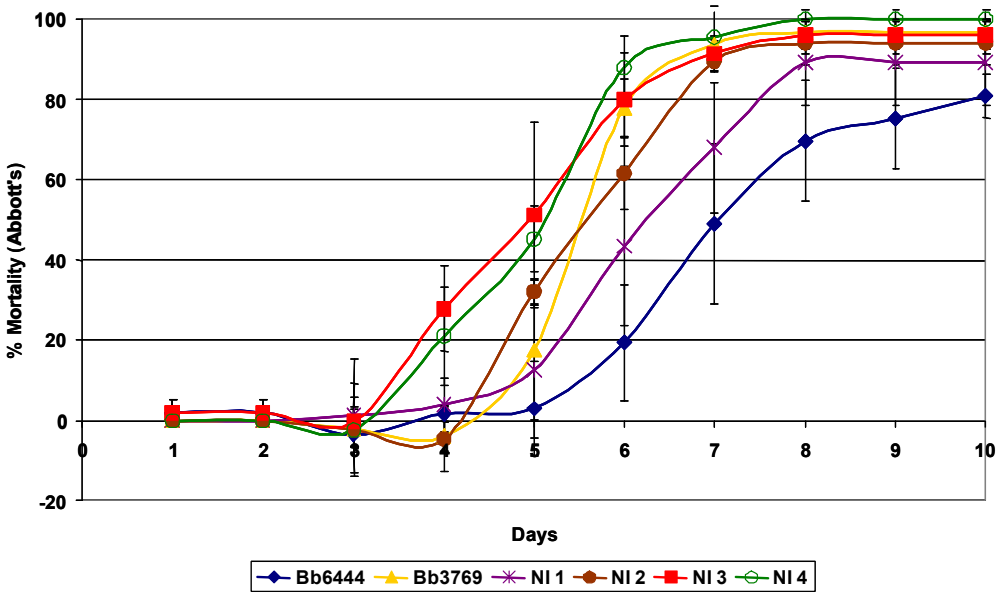

Figure 2. Mortality (Abbott's) (Mean ± S.D.) over time of Lygus lineolaris adults exposed to 1 x 10^7 spores/mL of six fungal isolates in the second isolate bioassay. Bb = Beauveria bassiana; NI = New Isolate of B. bassiana.

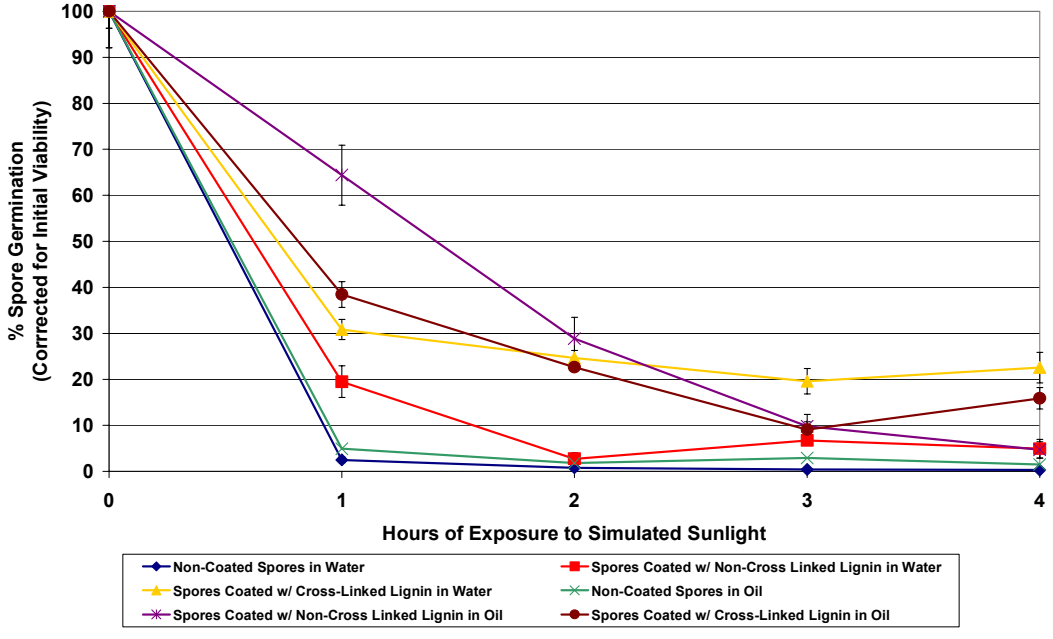

Figure 3. Percent spore germination (means ± standard deviation) over exposure time to simulated solar radiation for six formulations of B. bassiana (ARSEF 6444).

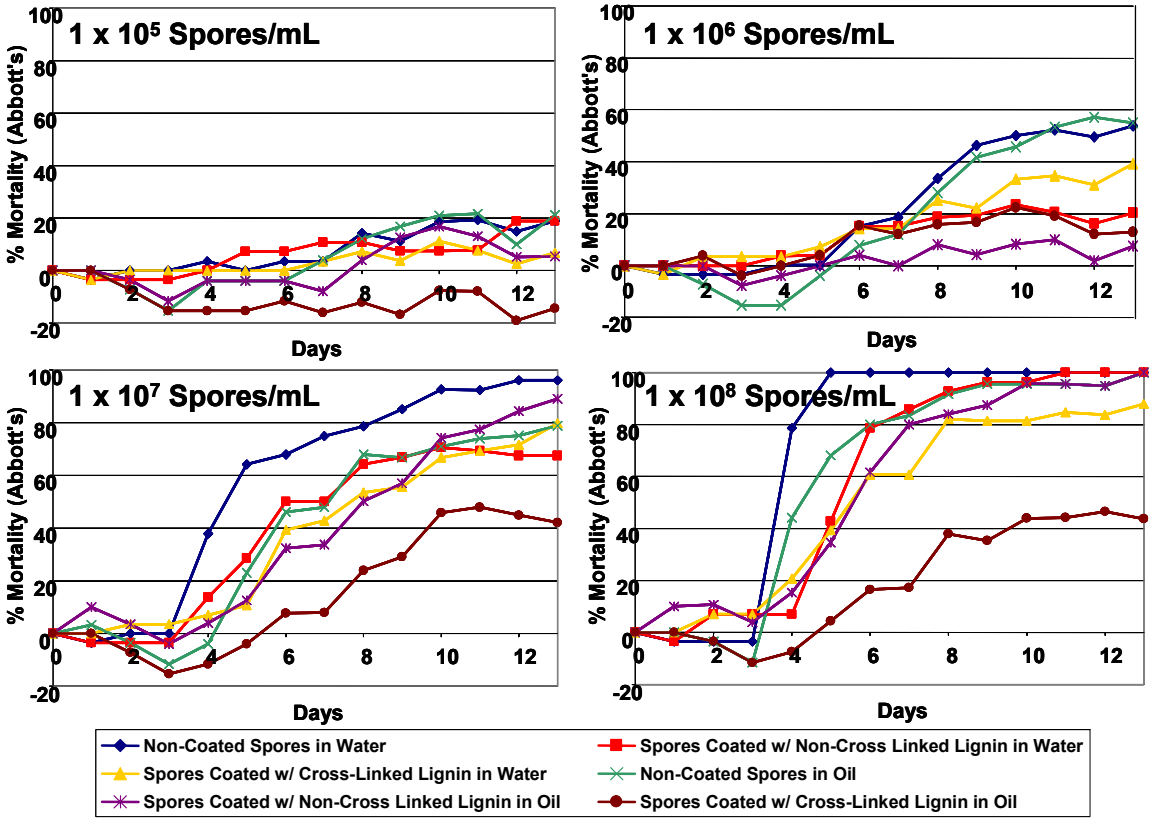

Figure 4. Mortality over time for Lygus lineolaris adults exposed to four concentrations of six formulations of Beauveria bassiana (ARSEF 6444). Water = 0.04% Silwet L77; Oil = 70% Shellsol OMS : 30% cotton seed oil.