2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

# STRAIN SELECTION OF A FUNGAL ENTOMOPATHOGEN, *BEAUVERIA BASSIANA*, FOR CONTROL OF PLANT BUGS (LYGUS SPP.)

**Jarrod E. Leland**
**USDA-ARS**
**Stoneville, MS**
**Michael R. Mcguire**
**USDA-ARS**
**Shafter, CA**

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned

## Abstract

Surveys of natural *Beauveria bassiana* infection levels in tarnished plant bug (*Lygus lineolaris*) populations from the delta and hill country regions of Mississippi were conducted. Natural infection levels in *L. lineolaris* were significantly lower than those observed in *L. hesperus* populations from the San Joaquin Valley (SJV), CA in previous surveys. Infection levels in *L. hesperus* ranged from 0 to50% with an overall average of approximately 10%, whereas overall percentage infection in natural *L. lineolaris* populations was less than 0.3% with the highest infection level from a site being 8%. There was a trend toward higher infection levels occurring later in the both surveys, which were conducted from May through November, with nearly all isolates from *L. lineolaris* being collected in mid August through mid October. Isolates from *L. hesperus* were highly variable with regards to pathogenicity to *L. hesperus* and growth temperature optima. Seven isolates from *L. hesperus* and *L. lineolaris* that were more pathogenic than the commercial *B. bassiana* isolate (GHA) and able to grow at 35°C were selected for further evaluation of characteristics relevant to mycoinsecticide development. These characteristics included spore production, growth at 35°C, pathogenicity at 35°C, pathogenicity to select beneficial insects, and survival under solar radiation. Six of the seven isolates were prolific sporulaters producing spore concentrations equal to or greater than the GHA isolate. Although the seven isolates were able to grow at 35°C at a reduced rate, they had very low pathogenicity to *L. lineolaris* at this temperature. Thus far, bioassays against beneficial insects have only been conducted with isolates from *L. lineolaris* and the GHA isolate. Pathogenicity was low to ladybugs (*Hippodamia convergens*), lacewing larvae (*Chrysopa carnea*), and pirate bug (*Orius insidiosis*) nymphs. However, only approximately 30% of lacewing larvae treated with high spore concentrations that formed pupae emerged as adults and sporulation was observed in pupa (vs. 90% control emergence). Pathogenicity to *O. insidiosus* adults was high, similar to that observed in *L. lineolaris* adult bioassays. The isolates selected from *L. hesperus* survived longer under exposure to simulated solar radiation than the GHA isolate, whereas survival of isolates from *L. lineolaris* was similar to the GHA isolate. Two to four isolates will be selected from these characteristics for scaled-up spore production sufficient to conduct field trials against *L. lineolaris* in wild host plants and *L. hesperus* in alfalfa, and further bioassays against beneficial insects.

## Introduction

Two commercial mycoinsecticides have been evaluated against *L. lineolaris* and *L. hesperus* with mixed success. Mycotrol® (Emerald Bioagriculture) demonstrated moderate success against *L. lineolaris* in cotton, particularly when applied with low rates of imidacloprid (Steinkraus and Tugwell, 1997). However, Mycotrol® control of *L. hesperus* in alfalfa was relatively poor, which may in part be attributed to poor coverage, particularly at bloom (Noma and Strickler, 1999). Naturalis-L® (Fermone Corp.) demonstrated relatively poor control against *L. lineolaris* in cotton (Snodgrass and Elzen, 1994).

The first entomopathogenic fungal isolate from an indigenous Lygus spp. (*B. bassiana* ARSEF 3769) was isolate from *L. lineolaris* in Arkansas. This isolate caused high *L. lineolaris* mortality in caged field trials (Steinkraus and Tugwell, 1997). In a study comparing the pathogenicity of 32 fungal isolates from six genera to *L. lineolaris*, 21 isolates were statistically as pathogenic as the GHA isolate and 15 caused higher, although not statistically significant, mortality (Liu et al., 2002). Four *B. bassiana* isolates, including *B. bassiana* ARSEF 3769, and a *Metarhizium anisopliae* isolate were selected based on pathogenicity in single dose screenings and spore production for multiple dose assays, and three of these (*B. bassiana* ARSEF, 1394, 5665, and *M. anisopliae* 3540) were significantly more pathogenic than *B. bassiana* (GHA). It is worth noting that the two *B. bassiana* isolates were non-indigenous isolates which may cause complications for mycoinsecticide development in the U.S. and bioassays were conducted at 20°C which may select for isolates with temperature growth optima outside the desired range for control conditions of Lygus spp. in cotton growing regions.

By obtaining isolates from Lygus spp. in cotton growing regions it may be possible to select isolates better suited to infecting Lygus spp. in these areas. A survey of natural *B. bassiana* infection levels in *L. hesperus* population of SJV, CA over a three year period has demonstrated relatively high natural infection levels ranging from 0 to 50% with a trend toward increasing

infection levels throughout the season (May through November) and some populations demonstrating relatively high infection levels (>20%) in July when temperatures are very high (ca. 35°C) (McGuire, 2002).

The following objectives were investigated in this study. 1) evaluate natural infection levels in the delta and hill country of the Mississippi delta for comparison to previous work with *L. hesperus* populations of SJV, CA, which would provide isolates for mycoinsecticide development and establish information on background infection levels for later field trials with mycoinsecticides. 2) compare characteristics relevant to mycoinsecticide development (spore production, pathogenicity, growth at 35°C) for a large pool of *B. bassiana* isolates from *L. hesperus* and *L. lineolaris* for demonstrating the natural diversity of entomopathogenic fungi in natural Lygus populations and selecting isolates for further testing. 3) evaluate additional characteristics for seven selected isolates including: pathogenicity to both *L. lineolaris* and *L. hesperus*, growth at 35°C, pathogenicity to *L. lineolaris* at 35°C, pathogenicity to beneficial insects, and spore survival under simulated solar radiation for further isolates selection.

Based partly on the results of the studies presented herein, two to four isolates will be selected for scaled-up production, field trials, and continued bioassays against beneficial insects. Ultimately it is the aim of this project to provide a mycoinsecticide that may be used for controlling Lygus species in multiple crops and wild host plants. Given a large enough market, it should be possible to produce a viable commercial product.

## Materials and Methods

### Natural *B. bassiana* Infection Survey in Mississippi

Native *L. lineolaris* populations were sampled on wild host plants from May through November, 2003. This survey included 55 locations across 20 counties in the delta and hill regions of Mississippi (Fig. 1). An initial survey was conducted by choosing a single location from 20 counties in the delta and hill regions during May-June, 2003 (Fig. 1a). In this survey, 100 *L. lineolaris* adults and nymphs were collected from a single site within each county, transferred to individual 20mL scintillation vials containing a broccoli floret and incubated for 10d at 26.6°C and 14h light : 10h dark. Broccoli was changed at 2 to 3intervals. After 10d incubation, all of the insects in the bioassay were surface sterilized in 10% ethanol and 0.525% sodium hypochlorite (10% household bleach) and incubated on water agar (15 g/L agar) for 72h for sporulation (Noma & Strickler 2000). The survey continued through November, collecting *L. lineolaris* adults and nymphs from wild host stands in Washington, Bolivar, and Sunflower counties. Insects (120 per location) were held individually in 30 mL medicine cups containing broccoli capped with 45mm foam plugs (Scimart) and incubated as described above. Sites were georeferenced, wild host species recorded, and number of insects per 50 sweeps estimated for each site. Wild host plants included Erigeron spp, curly dock (*Rumex crispus*), horseweed (*Conyza canadensis*), pigweed (*Amaranthus* spp.), iron weed ( ), giant ragweed (Ambrosia trifida), goldenrod (*Solidago altissima*), and smartweed (*Polygonum pensylvanicum* ), reflecting the changing consortium of wild host plants in the region through the season. The survey was terminated at the end of November just prior to the first hard freeze.

### Fungal Cultures

*B. bassiana* isolates GHA and ARSEF 3769 were obtained from the Agricultural Research Service Entomopathogenic Fungi Culture Collection (Ithaca, NY, Richard Humber Curator). Starter cultures were grown on Sabouraud dextrose agar with 2g/L yeast extract (SDAY, Goettel and Inglis, 1997) for 2 weeks at 25°C. Spore suspensions (5 x $10^6$ spores/mL) in 0.01% Tween 80 and 15% glycerol were made from these cultures and frozen in 1.5mL aliquots at -80 C to serve as inocula for future experiments. Sporulating insects from the *L. lineolaris* natural infection survey were suspended in 1.5mL of 0.01% Tween 80, vortexed for 30 sec and then spore suspensions were spread on *Beauveria* selective media (Goettel and Inglis, 1997). Isolates from *L. lineolaris* and *L. hesperus* that grew on *Beauveria* selective media were harvested after 2 weeks in 0.01% Tween 80 and 15% glycerol, diluted to 5 x $10^6$ spores/mL, and stored in 1 mL aliquots at -80 °C to be used as inocula for future experiments. Fungal cultures to be used in bioassays were grown for 10 to 14d at 25°C on either SDAY in 9 cm diameter Petri dishes for *L. hesperus* bioassays or barley agar (30g/L pulverized hulled organic barley (Organic Kingdom), 1g/L yeast extract (Sigma), and 15g/L agar (Sigma)) in 250mL Erlenmyer flasks for *L. lineolaris* bioassays. Spores grown on barley agar were harvested in 20mL of 0.01% Tween 80 using 6mm glass beads and shaking at 400RPM for 5min (New Brunswick Gyratory G-76 Orbital Shaker), then passed through two layers of cheese cloth to remove mycelia. Estimates of total spore production from each isolate were based on barley agar cultures. For simplicity, isolates are designated as TPB1 through 16 for isolates from *L. lineolaris* and WTPB 1 through 3 for isolates from *L. hesperus*. These correspond to longer description identifying the location and date of collection (e.g. WTPB 1, 2, and 3 correspond to S44-03-, 17-41, and 54-43 of the *L. hesperus* isolate collection). Collections of *L. hesperus* isolates were made in 2001as described previously (McGuire, 2002). Collections of *L. lineolaris* isolates TPB 1 through 4 were made from wild host plants in MS during 2002, and TPB 5 through 16 from wild host plants in MS during 2003.

### Bioassays with *L. hesperus*

Lab reared adult *L. hesperus* (2-4 days old) were used in all bioassays. Isolates were applied to *L. hesperus* in 5mL of 0.01% Silwet in a spray tower equipped with a TG 0.4 solid cone nozzle (Spraying Systems) to produce $5.2 \times 10^2, 5.2 \times 10^3, 5.2 \times$

$10^4$, and $5.2 \times 10^5$ spores/cm$^2$. Twenty insects were anesthetized with $CO_2$ and transferred to a 9 cm diameter petri dish lined with dry filter paper. The dish was then transferred to the tower, insects were sprayed and then transferred to small plastic vials (5 dram) containing a piece of green bean and capped with a ventilated lid. Beans were changed every 2 – 3 days and insects were held for 7 d at 28°C, 16 L:8D, or until dead. As insects died, they were transferred to 1.5% water agar and held for at least three days for sporulation to occur. Controls consisted of L. hesperus treated with $CO_2$ only and adults anesthetized and sprayed with 0.01% Silwet.

**Bioassays with *L. lineolaris***

Insects used in bioassays of the nine isolates (TPB1, TPB2, TPB3, TPB4, WTPB1, WTPB2, WTPB3, GHA, and ARSEF 3769) () were collected from a mixed stand of horseweed (*C. canadensis*) and iron weed ( ) on July 11, 2003 as adults of unknown age and held 48h prior to the bioassay at 25°C, 80 ± 5 % relative humidity, with a photoperiod of 14h light : 10h dark. Insects used in bioassays of these same nine isolates following single spore isolation were collected from pigweed (*Amaranthus* spp.) on October, 3, 2003 as adults of unknown age and held 48h prior to the bioassay at 25°C, 80 ± 5 % relative humidity, with a photoperiod of 14h light : 10h dark. Insects used in bioassays on 12 of the 18 isolates collected during the 2003 natural infection survey (TPB5 through TPB21) and *B. bassiana* (GHA) were collected from pigweed (*Amaranthus* spp.) on Nov, 21, 2003 as adults of unknown age and held 13d prior to the bioassay at 10°C, 80 ± 5 % relative humidity, with a photoperiod of 10h light : 14h dark. Isolates were applied to *L. lineolaris* in 1mL of 0.01% Tween 80 using a Potter spray tower to produce $5.4 \times 10^2$, $5.4 \times 10^3$, $5.4 \times 10^4$, and $5.4 \times 10^5$ spores/cm$^2$. Three replicates of 10 insects held in 10cm Petri dishes moistened with 700µL water were exposed to each concentration of each isolate for the two bioassays of isolates TPB1, TPB2, TPB3, TPB4, WTPB1, WTPB2, WTPB3 GHA and ARSEF 3769. Two replicates of ten insects were used in bioassays from the 2003 natural infection survey which included TBP5 through TPB16 and GHA. Sixteen replicates of ten *L. lineolaris* were exposed to 0.01% Tween 80 in the spray tower to serve as controls in each the first two bioassays and twelve replicates of ten insects for the 2003 natural infection survey isolates. After being sprayed, insects were delivered to individual 30mL medicine cups capped with 45mm foam plugs (Scimart) each containing a single broccoli floret. Insects were incubated at 25°C, 80 ± 5 % relative humidity, with a photoperiod of 14h light : 10h dark. When conducting bioassays of single spore isolates (TPB1, TPB2, TPB3, TPB4, WTPB1, WTPB2, WTPB3 plus GHA and ARSEF 3769) three replicates of ten insects were exposed to the highest spore concentration ($5.4 \times 10^5$ spores/cm$^2$) and incubated at 35°C under the same conditions with 160 sprayed controls. Mortality was determined daily and broccoli was changed at 2d intervals. After 10d incubation, all of the insects in the bioassay were surface sterilized in 10% ethanol and 0.525% sodium hypochlorite (10% household bleach) and incubated in 96 well plates (0.4mL wells, Steriline) containing 170µL of water agar (15 g/L agar) in each well for 72h for sporulation (Noma & Strickler 2000).

**Bioassays with Beneficial Insects**

Adult ladybugs (*Hippodamia convergens*), lacewing larvae (*Chrysopa carnea*), and pirate bug (*Orius insidiosis*) adults and nymphs were obtained from Extremely Green Garden, Co. Ladybugs (30 insects/isolate) and lacewing larvae (30 insects/isolate) were exposed to the highest spore concentration used in *L. lineolaris* bioassays, $5.4 \times 10^5$ spores/cm$^2$ using a Potter spray tower as described above. Pirate bug adults and nymphs (30 insects / concentration / isolate) were exposed to the same four spore concentrations described in *L. lineolaris* bioassays using a Potter spray tower. Insects were held individually in 30 mL medicine cups with moist filter paper. Ladybugs were provided Eliminade™ (Entopath Inc.) and lacewings and pirate bugs were provided tobacco budworm eggs. Insects were incubated at 25°C, 80 ± 5 % relative humidity, with a photoperiod of 14h light : 10h dark. Mortality was determined daily and food added at 2d intervals. After 10d incubation, all of the insects in the bioassay were surface sterilized in 10% ethanol and 0.525% sodium hypochlorite (10% household bleach) and incubated on water agar (15 g/L agar) for 72h for sporulation.

**Exposure to Solar Radiation**

Solar simulation experiments were conducted to evaluate the survival of *B. bassiana* isolates using an Oriel Solar Simulator (Model 91193) equipped with a 1000W Xenon Arc Lamp corrected with air mass 0 and air mass 1 filters to simulate direct noon sunlight. Three replicate suspensions (10mL of $1x10^7$ spores/mL) of each isolate from individual barley agar flasks were deposited onto nylon membrane filters (Magna, 0.45µm pore size, 47mm diameter) and exposed to 1, 2, 4, and 8h of simulated solar radiation 19.1 ± 0.5 mW/cm$^2$. Filters were held at 4°C prior to exposure and appeared dry at the time of exposure to simulated solar radiation. After exposure, spores were rehydrated for 1h at 100% r.h. suspended in 2mL of 0.04% Tween 80 and 100mL aliquots were spread on two 60mm Petri dishes containing germination agar (20g/L malt extract; 15g/L agar; 0.02g/L Benlate 50WP; 0.2 g/L chloramphenicol; 200,000 units penicillin/L; 0.2g/L streptomycin). Spores were killed with lactophenol acid fuschin mounting media (phenol 20g; lactic acid 20g; glycerol 40g; acid fuschin 0.1g; water 20mL) after 24h and 48h incubation periods. Glass cover slips were pressed onto the surface of the germination agar and percentage spore germination was determined for 200 spores from each replicated at 1000 × magnification.

## Results and Discussion

### Natural *B. bassiana* Infection Survey in Mississippi

No *B. bassiana* isolates were obtained from the 20 county survey conducted in May-June, 2003 (Figure 1a). The majority of *B. bassiana* isolates were obtained from August through mid October, 2003 Figure 1b. The increase in infection levels in late summer through early fall was analogous to what was observed in *L. hesperus* populations of SJV, CA (McGuire, 2002), but overall infection levels were approximately 30 times lower.

### Fungal Cultures

The majority of isolates from *L. lineolaris* and select isolates from *L. hesperus* produced spores equal to or greater than GHA on barley agar (Figure 2). This serves as a first tier screening to eliminate poor sporulaters from further evaluation. Barley was selected because it is used as a substrate in the mass production of *B. bassiana* (GHA) at an industrial scale.

### Pathogenicity to *L. hesperus* and *L. lineolaris*

Isolates from *L. hesperus* populations in the SJV, CA presented a range of LC50 values in bioassays against *L. hesperus* with several isolates exhibiting more than 10 times greater pathogenicity than the commercial *B. bassiana* (GHA) isolate (Figure 3a). Similarly several isolates from the natural infection survey of *L. lineolaris* were over 10 times more pathogenic (LC50 values) than GHA (Figure 3b). Isolates from *L. hesperus* were also highly pathogenic to *L. lineolaris* (Figure 4a) and isolates from *L. lineolaris* were highly pathogenic to *L. hesperus*. There was not a large degree of separation among the isolates from *L. lineolaris* and *L. hesperus* with regards to pathogenicity, but they were in many instances more than 10 times more pathogenic than *B. bassiana* (GHA) (Figure 4a and 4b).

### Pathogenicity to Beneficial Insects

Of the three beneficial insect species exposed to high spore concentrations ($5.4 \times 10^5$ spores/cm$^2$) of *B. bassiana* isolates, *O. insidiosus* adults had the highest mortality (Figure 5c). However, there was some concern regarding potential impacts on lacewing larvae because although they did not demonstrate high mortality as larvae, only approximately 30% of those that pupated emerged as adults (vs. 90% in controls) and pupae sporulated indicating fungal infection. This effect was similar across all isolates. Further work with greater numbers of insects and multiple doses will be needed to fully discern the potential impact of these isolates on lacewings. More attention also needs to be paid to other beneficial Heteropteran predators based on results from *O. insidiosus*.

### Radial Growth Rates and Pathogenicity at 35°C

*B. bassiana* (GHA), ARSEF 3769, and select isolates from *L. hesperus* and *L. lineolaris* exhibited a range of growth rates at temperatures from 28°C to 37°C (Figure 6). The GHA isolate did not grow at 35°C, whereas all of the Lygus isolates exhibited slow growth at this temperature. However, infection levels for *L. lineolaris* from high spore concentrations ($5.4 \times 10^5$ spores/cm$^2$) of these isolates when insects were incubated at 35°C were very low (<10%) (Figure 6, top). These percentage infection values were similar to those observed for insects exposed to 100 times lower concentrations ($5.4 \times 10^3$ spores/cm$^2$) of the same spore suspensions but incubated at 25°C, which averaged 9 ± 7% infection across all isolates, suggesting that these isolates are at least 100 fold less pathogenic when held continuously at 35°C. Similar negative effects of high temperature (35°C) on pathogenicity were observed for *B. bassiana* (GHA) against *L. hesperus* (Noma and Strickler, 2000). None of the isolates grew at 37C suggesting a barrier to infection in mammalian systems (Figure 6).

### Exposure to Solar Radiation

Isolates from *L. hesperus* demonstrated the greatest survival under simulated solar radiation (Figure 7). Selecting isolates with natural resistance to solar radiation may significantly improve mycoinsecticide efficacy under situations where mycoisecticide persistence is heavily impacted by solar radiation and uptake from plant surfaces significantly contributes to Lygus mortality. Additional work is being conducted on formulations for protecting spores from solar radiation and evaluating the contribution of treated plant surfaces to Lygus mortality (Leland and Behle, 1994).

## Conclusions

Natural infection levels in *L. lineolaris* populations from wild host plants in the Mississippi delta are approximately 30 times lower than those observed in *L. hesperus* populations primarily from alfalfa fields in SJV, CA (McGuire, 2002). However, the *L. lineolaris* isolates are highly pathogenic and prolific sporulaters, therefore it may be other characteristics, such as insect behavior or environment related to plant structure that caused the relatively low natural infection levels (McGuire, 2002). Nonetheless, there is a large pool of *B. bassiana* isolates available from both wild *L. hesperus* and *L. lineolaris* populations with characteristics amenable to improving mycoinsecticide efficacy. Many isolates were more than ten times more pathogenic to the two Lygus spp. than the commercial isolate. Isolates were prolific sporulaters on simple media. Isolates had a higher temperature growth range than the GHA isolate, which may be particularly relevant to *L. hesperus* control in SJV, CA, but may not be as critical to *L. lineolaris* control on spring and fall wild hosts in the midsouth. Isolates from *L. hesperus* demonstrated some natural resistance to solar radiation relative to the GHA isolate and isolates from *L. lineolaris*. The poten-

tial effects on beneficial insects will need to be considered in a cost-benefit evaluation when comparing mycoinsecticide control with other control options for the two Lygus spp. Studies on impacts to beneficial insects will be included in small scale field trials to evaluate mycoinsecticide effects under more natural situations. This information is being used to select two to four isolates for scaled-up production and small scale field trials, which will be conducted in alfalfa in SJV, CA and on wild host plant in Mississippi.

## Acknowledgements

Collection of *L. lineolaris* from 20 counties of Mississippi in May-June, 2003 was conducted in coordination with a project of Gordon Snodgrass evaluating insecticide resistance levels in these regions; he was consulted for advice related to *L. lineolaris* biology and control. Work with *L. hesperus* is being conducted in coordination with projects by Pete Goodell and Larry Godfrey. Technical assistance was provided by Danny Ballard, Gerald Gibson, Leon Hicks, Neal Hudson, Tabatha Ramsey, and Lisa Self. Technical grade product of *B. bassiana* (GHA) was provided by Emerald BioAgriculture (Butte, MT).

## References

Finney, D. J. Probit Analysis 3rd ed. Cambridge University Press. (1971) 333 pp.

Goettel, M. S. and D. G. Inglis. 1997. Fungi: hyphomycetes. pgs 213-249. In. Lacey, L. ed. Manual of Techniques in Insect Pathology. Acedemic Press. London. 409 pp.

Leland, J. E., and R. W. Behle. 2004. Formulation of the entompathogenic fungus, Beauveria bassiana, with resistance to UV degradation for control of tarnished plant bug, Lygus lineolaris. In. Proceedings of the 2004 Beltwide Cotton Conferences, January 5-9, San Antonio, TX. National Cotton Council, Memphis, TN, (Submitted).

Liu, H., Skinner, M., Parker, B. L., and M. Brownbridge. 2002. Pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes), and other entomopathogenic fungi against *Lygus lineolaris* (Hemiptera: Miridae). J. Econ. Entomol. 95 (4): 675-681.

McGuire, M. R. 2002. Prevalence and distribution of naturally occurring Beauveria bassiana in San Joaquin Valley populations of Lygus hesperus (Heteroptera: Miridae). J. Agric. Urban Entomol. 19 (4): 237-246.

Noma, T. and K. Strickler. 1999. Factors affecting *Beauveria bassiana* for control of Lygus bug (Hemiptera: Miridae) in alfalfa seed fields. J. Agric. Urban Entomol. 16 (4): 215-233.

Noma, T. and K. Strickler. 2000. Effects of *Beauveria bassiana* on *Lygus hesperus* (Hemiptera: Miridae) feeding and oviposition. Environmental Entomology. 29 (2): 394-402.

Snodgrass, G. L., and G. W. Elzen. 1994. Efficacy of Naturalis-L® for adults and nymphs of the tarnished plant bug in cotton. Proceedings/Beltwide Cotton Conferences. v. 2: 1103-1104.

Steinkraus, D. C. and N. P. Tugwell. 1997. *Beauveria bassiana* (Deuteromycotina: Moniliales) effects on *Lygus lineolaris* (Hemiptera: Miridae). J. Entomol. Sci. 32 (1): 79-90.

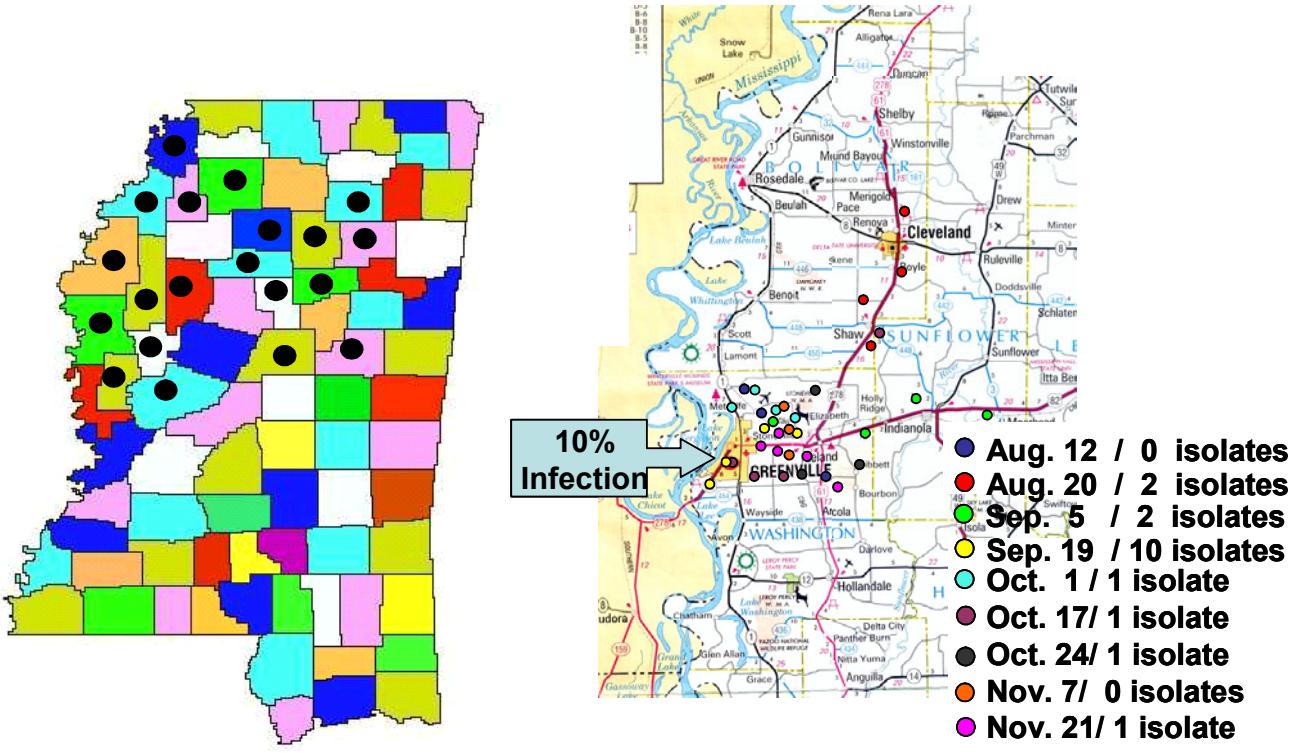


Figure 1. A) Counties surveyed for natural *B. bassiana* infection in *L. lineolaris* populations May-June, 2003; B) Survey locations August through November, 2003 in Washington, Bolivar, and Sunflower counties, MS.

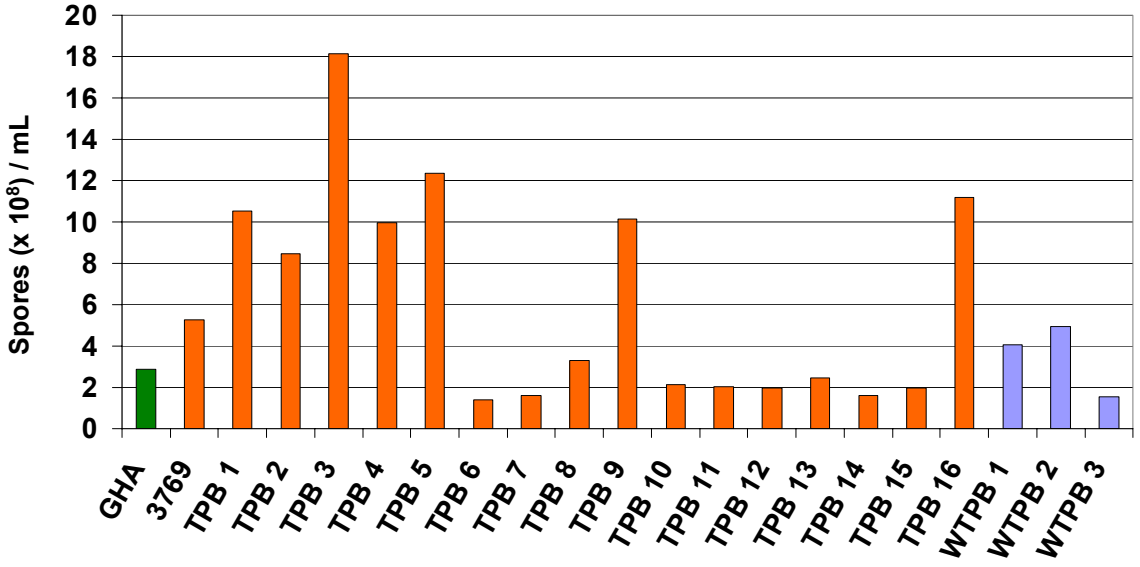


Figure 2. Spore production by *B. bassiana* (GHA), *B. bassiana* (ARSEF 3769), *B. bassiana* isolates from *L. lineolaris* (TPB), and select isolates *L. hesperus* (WTPB) *in vitro* on barley agar.

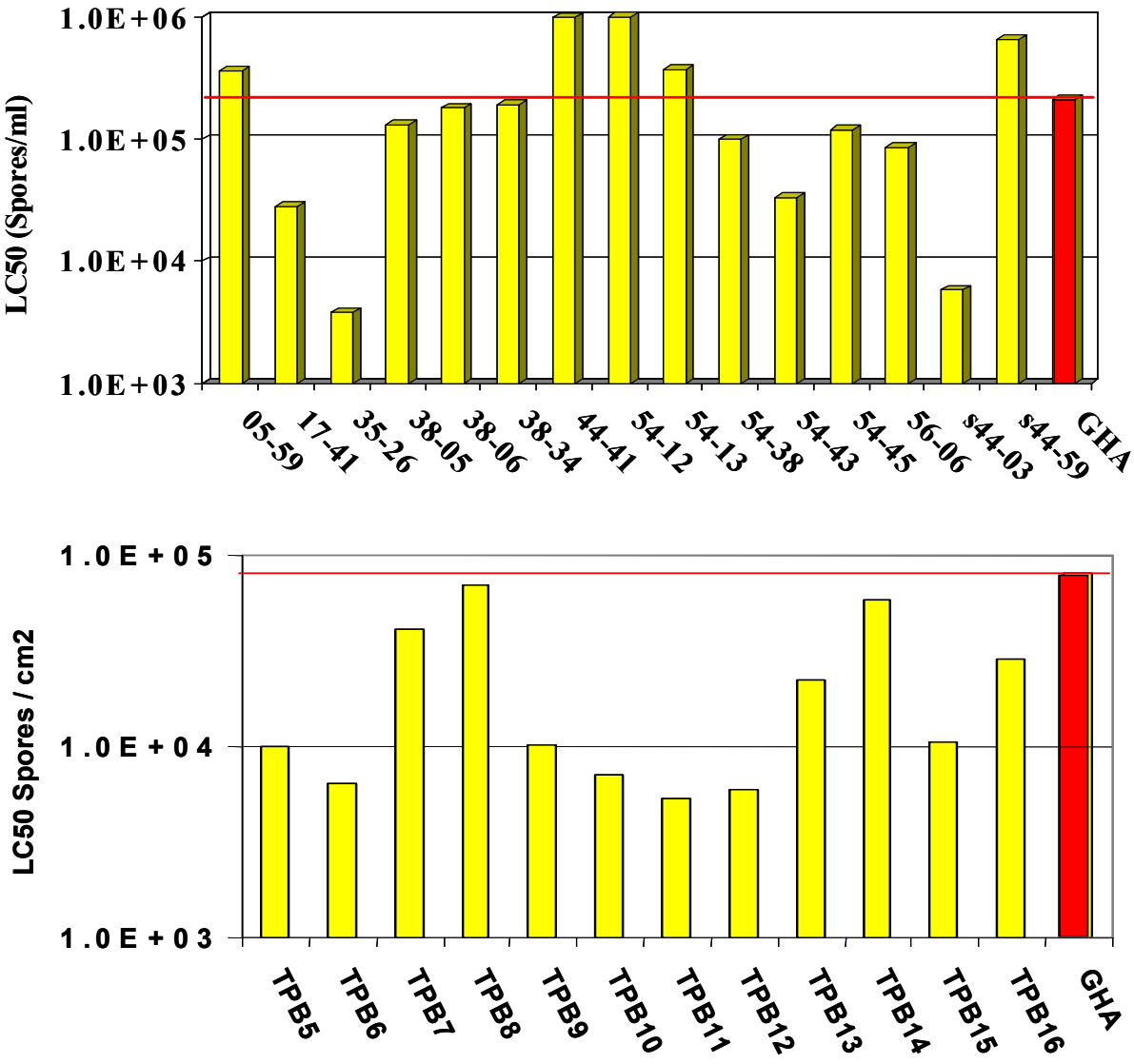


Figure 3. Pathogenicity (LC50 values) of *Beauveria bassiana* (GHA) compared to new isolates from A) *L. hesperus* to *L. hesperus* from a survey of populations in SJV, CA (McGuire, 2002) and B) *L. lineolaris* to *L. lineolaris* from a survey of population in the MS delta, 2003.

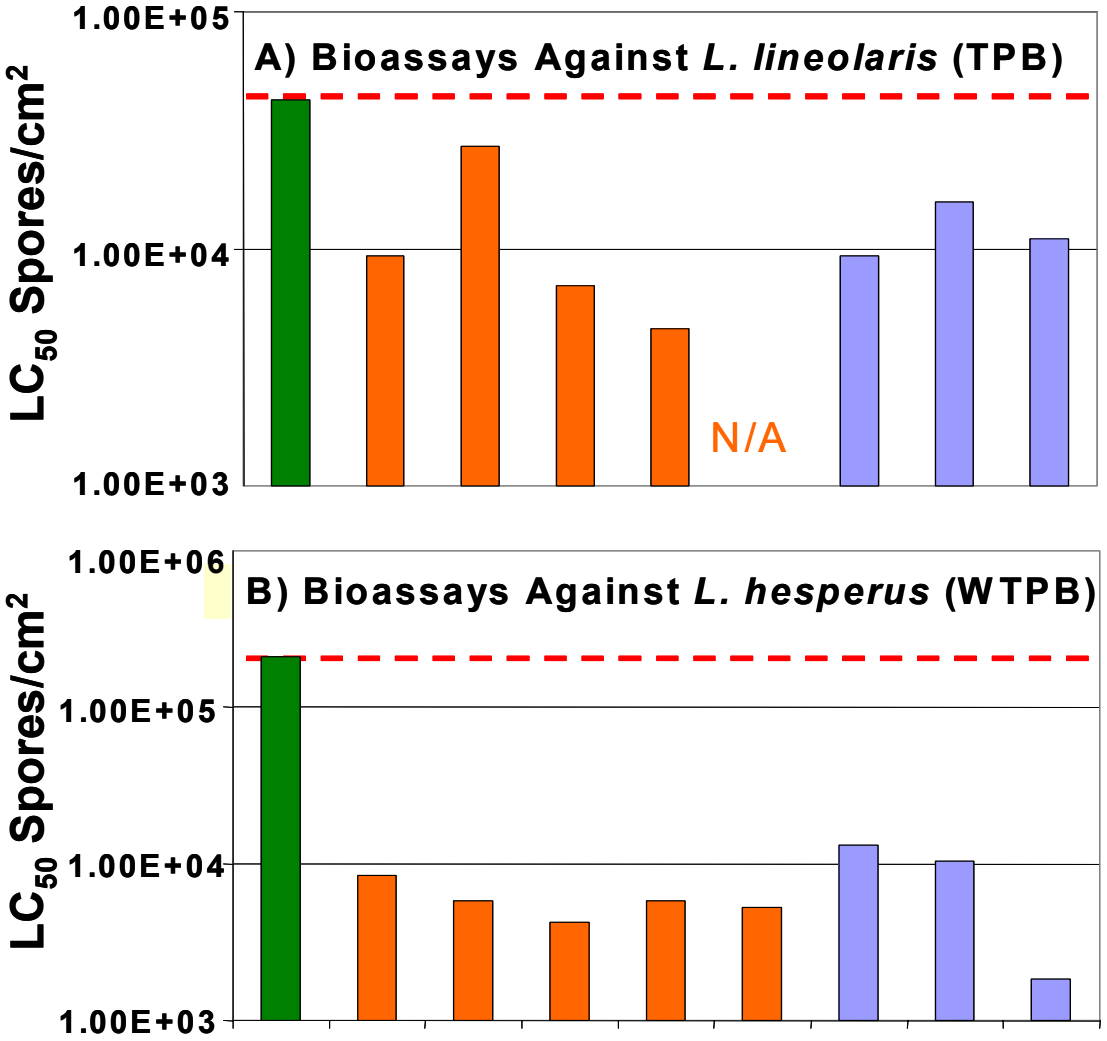


Figure 4. Pathogenicity (LC50 values) of *Beauveria bassiana* (GHA) compared *B. bassiana* (ARSEF 3769) and select *B. bassiana* isolates from *L. lineolaris* (TPB) and *L. hesperus* (WTPB) to A) *L. lineo-laris* and B) *L. hesperus*.

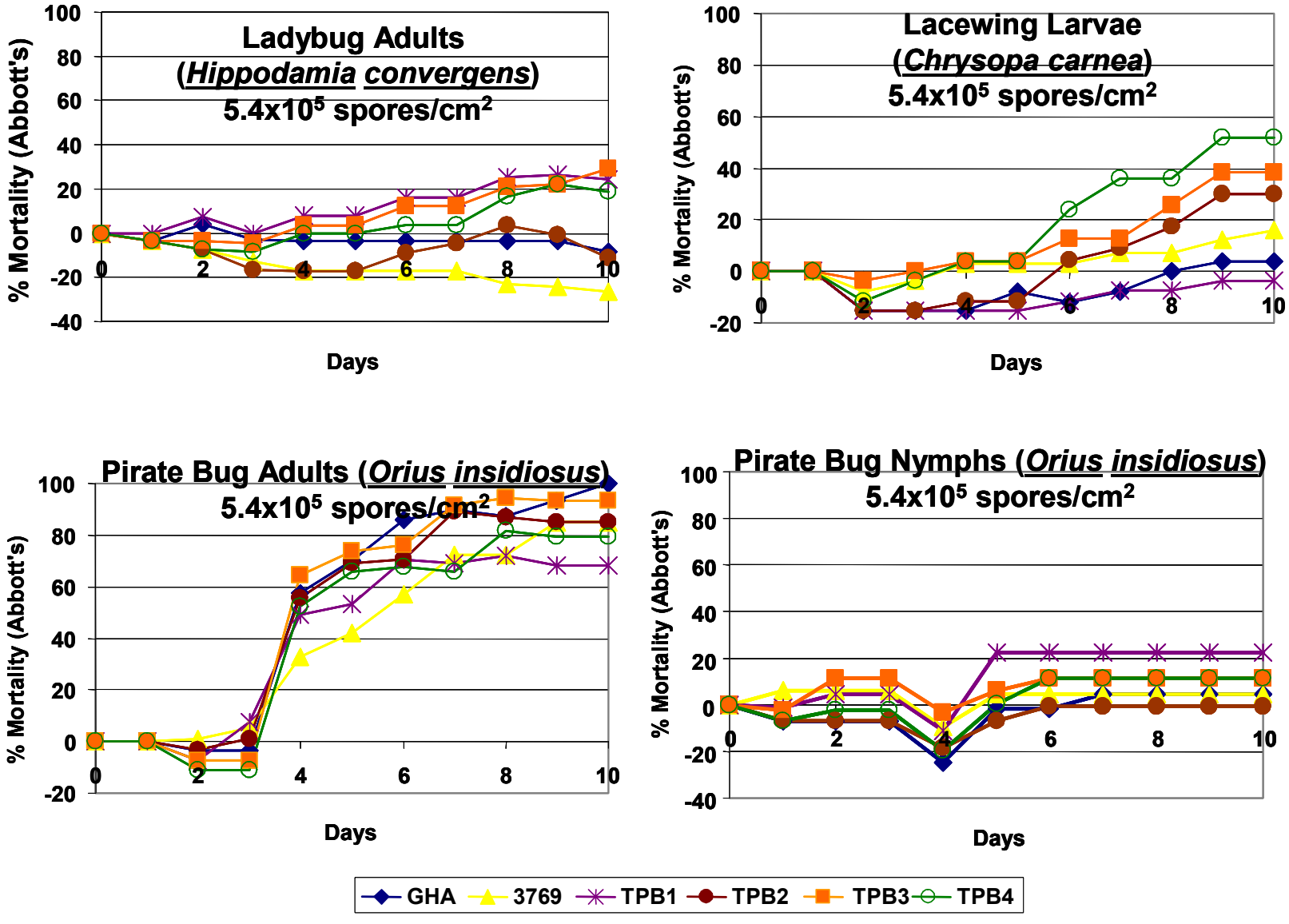


Figure 5. Mortality (Abbott's) over time for select beneficial insects exposed to the highest spore concentration tested against *L. lineolaris* 5.4 x $10^5$ spores/$cm^2$.

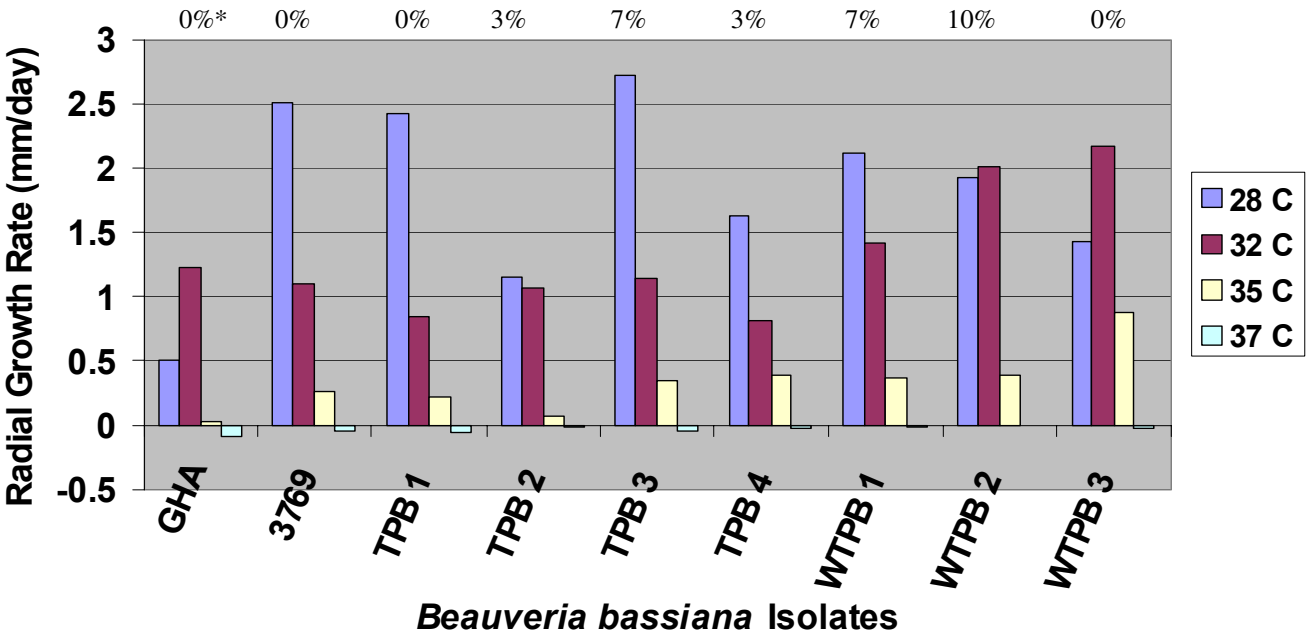


Figure 6. Radial growth rates of *B. bassiana* (GHA), *B. bassiana* (ARSEF 3769), and select *B. bassiana* isolates from *L. lineolaris* (TPB) and *L. hesperus* (WTPB) at various temperatures. *Percent infection levels for *L. lineolaris* exposed to a single high spore concentration (5.4 x $10^5$ spores/$cm^2$) at 35°C are give in parenthesis above each isolate.

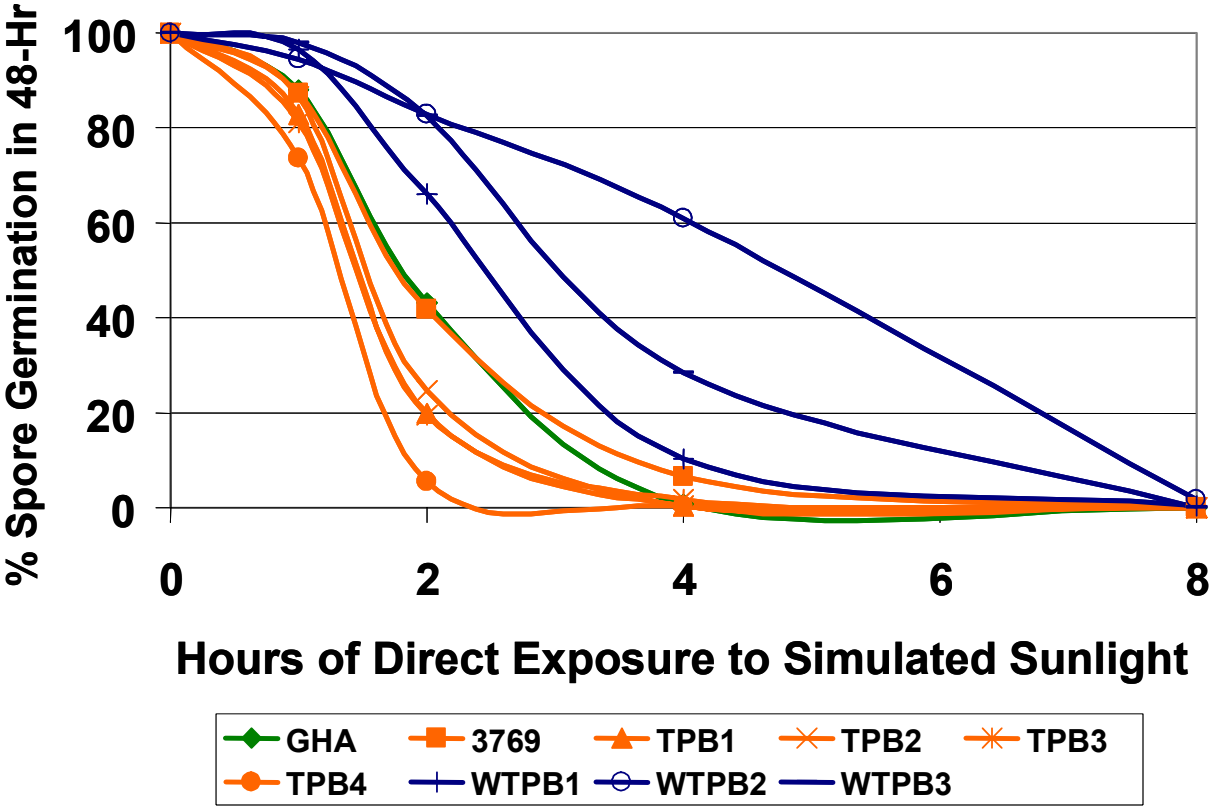


Figure 7. Spore germination within 48 for *B. bassiana* (GHA) and *B. bassiana* isolates from *L. lineolaris* (TPB) and *L. hesperus* (WTPB) following increasing exposure times to simulated solar radiation.