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NEW ISOLATES OF THE ENTOMOPATHOGENIC FUNGUS, BEAUVERIA BASSIANA, FOR CONTROL OF TARNISHED PLANT BUG, LYGUS LINEOLARIS IN WILD HOST PLANTS AND COTTON

Jarrod E. Leland
USDA-ARS-SIMRU
Stoneville, MS
Michael R. McGuire
USDA-ARS
Shafter, CA
Jeff Gore
Stoneville, MS
Stefan T. Jaronski
Sydney, MT

Abstract

New isolates of the entomopathogenic fungus, *Beauveria bassiana* (Balsamo), are being tested for control of *Lygus* spp. Previous studies showed that specific isolates from the tarnished plant bug *L. lineolaris* (Palisot de Beauvois) in MS and the western tarnished plant bug *L. hesperus* (Knight) in CA are prolific conidia producers, highly pathogenic, low mycotoxin producers, and more tolerant to high temperature and solar radiation. One isolate each from MS and CA and the commercial isolate (GHA) were evaluated in field trials against *L. lineolaris* on wild host plants (MS) and cotton (MS), and *L. hesperus* on alfalfa (CA). Incidence of infection in Lygus was high in wild host plant trials (40-60%) and alfalfa trials (60-90%), but much lower in cotton trials (20-30%), which was likely related to coverage problems. Infection in beneficial insects was negligible in all trials. Although infection was high in wild host plants and alfalfa, in most cases *Lygus* populations were not significantly reduced by *B. bassiana* application. The lack of significant population reduction may in part be attributed to the heterogeneity of populations in experimental plots and migration between small plots. However, it may also be an indicator that infection is not progressing to mortality at the high temperatures present during the field tests. Studies using caged adult *L. lineolaris* in cotton indicated similar mortality of insects sprayed directly and those exposed to treated plant surfaces, however, efficacy of the *B. bassiana* was greatly reduced after 24 hr indicating a need for formulations to improve persistence.

Introduction

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

By obtaining isolates from *Lygus* spp. in warmer regions where *Lygus* spp. are major pests, 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* populations of the San Joaquin Valley (SJV), CA demonstrated relatively high natural infection levels ranging from 0 to 50% with a trend toward increasing infection levels throughout the season (May through November). Surprisingly, some populations demonstrated relatively high infection levels (>20%) in July, when temperatures exceed 40 °C (McGuire, 2002). A similar survey was conducted in *L. lineolaris* populations of the Mississippi delta and hill regions determined natural infection levels in *L. lineolaris* to be approximately 30 times lower than those observed in *L. hesperus* (Leland and Snodgrass, submitted).

Isolates from *L .hesperus* and *L. lineolaris* were independently screened for pathogenicity to the host from which they were obtained, spore production, and high temperature tolerance (McGuire et al., submitted; Leland, submitted). Based on the initial screening seven isolates were selected from the two collections for further characterization (Leland and McGuire, 2004; Leland et al., submitted). When compared with GHA, specific isolates were over 10 times more pathogenic, better able to grow at 32 °C, more tolerant to artificial sunlight, and all isolates produced similar or lower beauvericin concentrations (Leland et al., submitted).

The objectives of this study were to determine infection levels and population change for *Lygus* spp. and beneficial insects following field application of GHA, one isolate from CA (WTPB2), and one isolate from MS (TPB3) to populations in wild host plants, cotton, and alfalfa.

Materials and Methods

Fungal Cultures

Conidia production for wild host plant and alfalfa field experiments was described previously (Leland et al., submitted). Briefly, isolates were initially obtained by transferring sporulating cadavers to selective media (Doberski & Tribe 1980). Once a colony was established, the fungus was transferred to Sabouraud's dextrose agar with 1% yeast extract (SDAY). Conidia were harvested from culture plates, enumerated by hemacytomer counting, and their viability determined based on percentage germination. Conidia were stored in aliquots in 20% glycerol at –80°C. These aliquots were used to inoculate SDAY plates, which were subsequently used as inocula for spore production in a biphasic culture system that simulates industrial-scale production systems (Bradley, et al., 1992; 2002). Flasks (1L) of liquid CSYE medium (40 g L^{-1} glucose, 10 g L^{-1} KNO_3, 5 g L^{-1} KH_2PO_4, 1 g L^{-1} $MgSO_4$, 0.05 g L^{-1} $CaCl_2$, and 2 g L^{-1} yeast extract), were inoculated with conidia from SDAY media and incubated for 3-4 d at 25-26 ºC and 150 rpm. These liquid cultures were then used to inoculate autoclaved, pearled barley (Minnesota Grain, Eagan MN) in sterilized, plastic, vented mushroom spawn bags (Unicorn Implement and Manufacturing Co., Commerce TX). Whole cultures were then transferred to plastic chambers where they were dried for 7 d at 28° C at an air exchange rate of 0.08 vvm for the first three days then 0.04 vvm for another 5 days. Conidia from each replicate batch were individually harvested by mechanical sieving through 20 and 100 mesh sieves in an ultrasonic sieve shaker (AS200, Retsch Corp., Newton PA). Spores from the 100-mesh fraction were dried at 15% R.H. and 25 ºC to less than 10% moisture for storage.

Field experiments in cotton did not include the CA isolate (WTPB2). TPB3 for these experiments was produced in 60, 250 mL Erlenmyer flasks each containing 50 mL of SDAY media. Conidia were harvested by adding 20 mL of 0.05% Silwet-L77 plus 6 mm glass beads and shaking at 400 RPM for 5 min. Resulting conidia suspensions were filtered through two layers of cheese cloth to remove mycelia and beads and then enumerated by hemacytometer. Technical grade powder GHA (Emerald BioAgriculture) was used for comparison in cotton field trials.

Wild Host Plant Trials (MS)

One acre of redroot pigweed (*Amaranthus retroflexus* L.) was planted at an experimental site in Washington Co., MS. at 20 lb/acre. The 1-acre plot was divided into 16, 55' square plots, which were bordered by four rows of corn to reduce migration between plots. A 12' x 24' x 5' cage was placed in the center of each plot (screening was added 1 day post application). Plots were sampled weekly for one month prior to application and at 1 day pre-treatment and 2, 7, and 14 days post treatment with 50 sweeps outside of the cages and 25 sweeps inside the cages. Sweepnet samples were stored at -20 °C in mesh bags for later counting. Treatments consisted of four replicates each of control, GHA, TPB3, and WTPB2 in a randomized complete block design. Conidia were applied at a rate of 1 x 10^{13} conidia/acre in 0.05% Silwet L77 using a CO_2 backpack sprayer equipped with 4, 8004 flat fan T-jet nozzles at 34 PSI. Applications were made at 6 pm over a two hour period on 6 July 6 2004. The air temperature maximum and minimum on this day were 92 °F and 72 °F, respectively. Adults and nymphs (25 each) of *L. lineolaris* were collected from inside and outside of the cages 2 days post treatment and returned to the laboratory. The first 30 beneficial insect predators were collected from each plot at 3 days post treatment regardless of their developmental stage or taxonomy and returned to the laboratory. All insects were held individually in 30 mL medicine cups with polyurethane foam lides and incubated at 25 °C, 80 ± 5 % relative humidity, and a 14:10 L:D photoperiod. *Lygus lin*eolaris were provided broccoli florets changed at 2 d intervals and beneficial insects were provided moist filter paper and *Helicoverpa zea* eggs. Mortality was determined daily. After 10 d incubation, all of the insects in the bioassay were surface sterilized in 10% ethanol and 0.525% sodium hypochlorite and incubated in 96 well plates (0.4 mL wells, Steriline) containing 170µL of water agar (15 g L^{-1} agar) in each well for 72 h for sporulation (Noma & Strickler, 2000). Estimates of LT_{50} values for mortality and sporulation at each conidia concentration were made using survivorship analysis (Proc Lifereg, SAS) using right censored data for insects surviving beyond the 10 d incubation period for each bioassay and combined data from both bioassays. Standard errors were generated independently for each isolate at each spore concentration by Proc Lifereg, SAS, and therefore LT_{50} values were compared based on pairwise T-tests. The interaction of treatment and time with population was estimated using a mixed model and a repeated measures ANOVA for $Log_{10}+1$ transformed data (proc mixed, SAS).

Alfalfa Trials (CA)

Alfalfa trials consisted of five treatments, Control, GHA, TPB3, WTPB2, and Warrior T (Cyhalothrin, Lambda). Warrior T was applied at 0.02 lb AI/A, *B. bassiana* treatments were applied at $1x10^{13}$ conidia/A in 0.01%Silwet-L77; and control plots received 0.01% Silwet-L77. Each treatment plot (60 ft x 60 ft) was replicated four times and plots were laid out in a randomized complete block design. All treatments were applied in 20 gal water/acre with a 20 ft boom at 6 PM, 15 June 2004. Temperature at application was approximately 40C. A second field trial was conducted on 31 August 2004 using the same protocol with the exception that conidia application rates were reduced to 1×10^{13} conidia/Ha. Plots were sampled at 1 day pre-treatment, and 3, 7, 10, and 14 days post-treatment. At each sample time, ten sweeps were taken per plot and the samples frozen for future analysis. Twenty live adults per plot were returned to the laboratory and individually transferred to small plastic vials (5 dram) containing a piece of green bean, and each vial capped with a ventilated lid. Beans were changed every 2 – 3 d and insects were held for 10 d on a laboratory bench 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.

Cotton Trials (MS)

Cotton trials consisted of three treatments, control, GHA, and TPB3. Two field trials were conducted. Plots in the first field trial were 4 rows x 40 ft and in the second trial they were 6 rows by 40 ft. Each treatment was replicated four times in a randomized complete block design. Conidia were applied at a rate of 1×10^{13} conidia/acre in 12 gal. of 0.05% Silwet L77 using a CO_2 backpack sprayer equipped with 4, 8002 flat fan T-jet nozzles at 34 PSI. Applications were made at 6 PM on 31 July 31 2004 and 9 August 2004. The air temperature max./min. for these dates were 92 / 73 °F and 92/65 °F, respectivly. Six nylon tulle sleeve cages (1 mm mesh), 12 cm x 20 cm, were placed in each plot at the terminal, middle, and low in the cotton canopy over squares and bolls. Five adult *L. lineolaris* were placed in two sleeve cages at each canopy height just prior to field application, within 1 hr after field application, and then 24 hr after field application. Adults were left in these cages for 48 hr at which time they were returned to the laboratory and held individually to determined mortality and sporulation as described in wild host plant experiments. Eight 3 m beat sheet samples were taken from each plot at 3, 7, and 10 d post treatment. Immature *L. lineolaris* and beneficial insect predators were returned to the lab and held individually to determine mortality and sporulation as described previously.

Results

Wild Host Plant Trials

Total infection of *L. lineolaris* adults and nymphs ranged from approximately 40 to 60% in treated plots and was negligible in control plots (Fig. 1). Sporulation data for nymphs was combined for samples taken within and outside of cages since it is less likely that they would migrate between plots than adults. The low sporulation observed in adults collected from control plots was evidence that migration between plots was uncommon at least within the first 2 days after application. Sporulation was generally highest in TPB3 for adults. Estimates of LT_{50} values (± std. err.) for all *L. lineolaris* from Control, GHA, TPB3, and WTPB2 plots were 16.6 (1.1), 8.3 (0.3), 7.6 (0.2), and 8.3 (0.3). All treatments were significantly different than controls but there was not difference among treatments.

Beneficial insect predators collected by sweepnet from wild host plants in order of there predominance were *Geocoris* spp., Nabidae, ladybird beetles, Reduviidae, and lacewings (Fig. 2). The only sporulations observed in beneficial insects were one Geocoris individual each from the control and the TPB3 plots (Fig. 2).

Wild host pants were beginning to senesce during the time of this field trial and *L. lineolaris* adults populations were decreasing from all plots including controls over the course of the experiment (Fig. 3). Abnormally heavy rains during June and July caused flooding in these plots and likely damaged the roots of the pigweed allowing for the invasion of a variety of other weed hosts and early senescence of the pigweed. As a result, *L. lineoalris* populations were also lower than what we typically observe on pigweed at this time of year and were very heterogeneous. In part due to this heterogeneity, treatment ($F=0.51$; d.f.=3, 12 $P=0.6$) and treatment*time ($F=1.0$; d.f.=9, 22.9 $P=0.5$), were not significant for *L. lineolaris* nymphs. Nor were treatment ($F=0.4$, d.f. = 3, 11.6, $P = 0.8$) and treatment*time ($F = 1.1$, d.f. = 9, 23.7, $P = 0.9$) significant for *L. lineolaris* adults.

Alfalfa Trials

In the June trial, populations of L. hesperus adults were not significantly different on any sample day except 10 days after application when all treated plots had lower populations than the untreated plot. In the September field trial in alfalfa, only Warrior T significantly reduced populations when populations were sampled three days after application. At 7 days after application, plots treated with the MS and CA isolates also had significantly fewer L. hesperus adults. (Fig. 5). Infection levels were high at 3 days post treatment in all fungal treated plots in both the June (80 to 90%, Fig. 6) and September (approx. 70%, Fig. 7) field trials. Increases in infection levels in the control and Warrior T treatments after application to *B. bassiana*-treated plots was likely the result of migration of adults. Molecular markers are currently being used to identify the isolate (GHA, WTPB2, TPB3 or wild) associated with all sporulating cadavers from the study. The only significant difference in infection levels among treatments was the higher level of infection in WTPB2 treated plots at 7 days post treatment during the June field trial (Fig. 6) This effect was not observed in the September study and further work is needed to evaluate the horizontal transmission potential of isolates.

Cotton Field Trials

Infection levels for *L. lineolaris* nymphs treated with TPB3 and GHA in cotton were much lower (20-30%, Fig. 8) than those observed in wild host plant (Fig. 1) and alfalfa (Figs. 6 and 7) field trials. Infection levels were not significantly different among the *B. bassiana* treatments and TPB2 was not significantly different than controls in the second trial.

Beneficial insect predators collected from cotton in order of their predominance in sweep net samples were *Geocoris* spp., ladybird beetles, spiders, Nabidae, lacewings, and Reduviidae (Fig. 9). Very little sporulation was observed in beneficial insects; one *Geocoris* individual sporulated from the control treatment and a few ladybird beetle larvae sporulated from each of the treatments including controls (Fig. 9). Molecular markers specific to GHA and TPB3 will be used to determine if sporulating beneficial insects were infected with GHA, TPB3, or were natural background infection.

Cotton was beginning to cut out during the course of this experiment and *L. lineolaris* populations were leaving the cotton during both the first and second field trial as evidence by the population reduction in control plots (Fig. 10). Treatment effects (Trial 1: $F = 1.47$; d.f. = 2, 10.1 $P = 0.28$; Trial 2 : $F = 0.59$; d.f.= 2, 7.8 $P = 0.58$) and treatment*time effects (Trial 1: $F = 0.1$; d.f. = 4, 12.5 $P = 0.98$; Trial 2 : $F = 1.6$; d.f. = 4, 10.9 $P = 0.25$), were not significant in either field trial.

Results from caged insects are combined in Fig. 11 from both cotton field trials. Infection levels for adults caged on cotton squares and bolls prior to application were higher (50 to 70%, Fig. 11 – sprayed) than those observed in non-caged nymphs. Caged adults may have been less protected than non-caged nymphs since five adults were caged on one to two bolls and squares, whereas nymphs may have been feeding within bolls and squares making application to the target more difficult. Coverage difficulties are also reflected in generally lower infection observed low in the canopy for caged adults (Fig. 11). Adults caged on cotton immediately following application had high levels of infection indicating that uptake from plant surfaces can significantly contribute to infection (Fig. 11 - contact). However, much of this activity was lost after 24 hr demonstrating the low persistence of these conidia on plant surfaces (Fig. 11 – residual). A similar loss in activity was observed for *L. lineolaris* caged on cotton and treated with *B. bassiana* ARSEF 3769, however these studies showed that some infection still occurred following exposure to 96 hr old residues (Steinkraus and Tugwell, 1997).

Discussion

Field application of *B. bassiana* to *L. lineolaris* on wild host plants and *L. hesperus* in alfalfa resulted in infection levels ranging from approximately 40 to 90%. However, in most cases the high infection observed in insects treated in the field then returned to the laboratory did not consistently correspond to significant population reduction in the field. This lack of significance may have been in part related to the heterogeneity of populations in field plots (particularly wild host plants) or migration of insects among small plots (particularly alfalfa). However, further experiments are needed to determine if disease progression is slowed by the high temperature observed in the field relative to incubation at 25 to 28 ºC in the laboratory. Additional field trials are planned for spring and fall wild host plants of *L. lineolaris* at which time temperatures may be more amenable to disease progression. The low infection levels observed in *L. lineolaris* nymphs during cotton field trials is likely related to difficulties in reaching nymphs in protected bolls and squares. Additional trials are planned earlier in the season in cotton and application

technology and formulations will be investigated as means for improving coverage. Coverage difficulties may also be circumvented by improving the persistence of spores in the field. Caged insect trials demonstrated that conidia on plant surfaces can significantly contribute to infection, however their persistence is limited. Formulations currently being developed to protect spores from solar radiation and thereby improve field persistence may improve infection of *Lygus* as they move among relatively protective fruiting structures (Leland and Behle, 2004; 2005 in press). Production scale-up is being initiated to provide sufficient spore product to evaluate control over a range of alternate hosts and cotton that support *Lygus* populations throughout the season. Formulation and application technology is being to developed improve the efficacy of these new isolates through increased persistence and coverage. *Beavueria bassiana* may provide an effective tool for managing *Lygus* populations and reducing the non-target impact of management practices, particularly if used in an IPM system targeting populations on alternate hosts to reduce populations on an area-wide basis.

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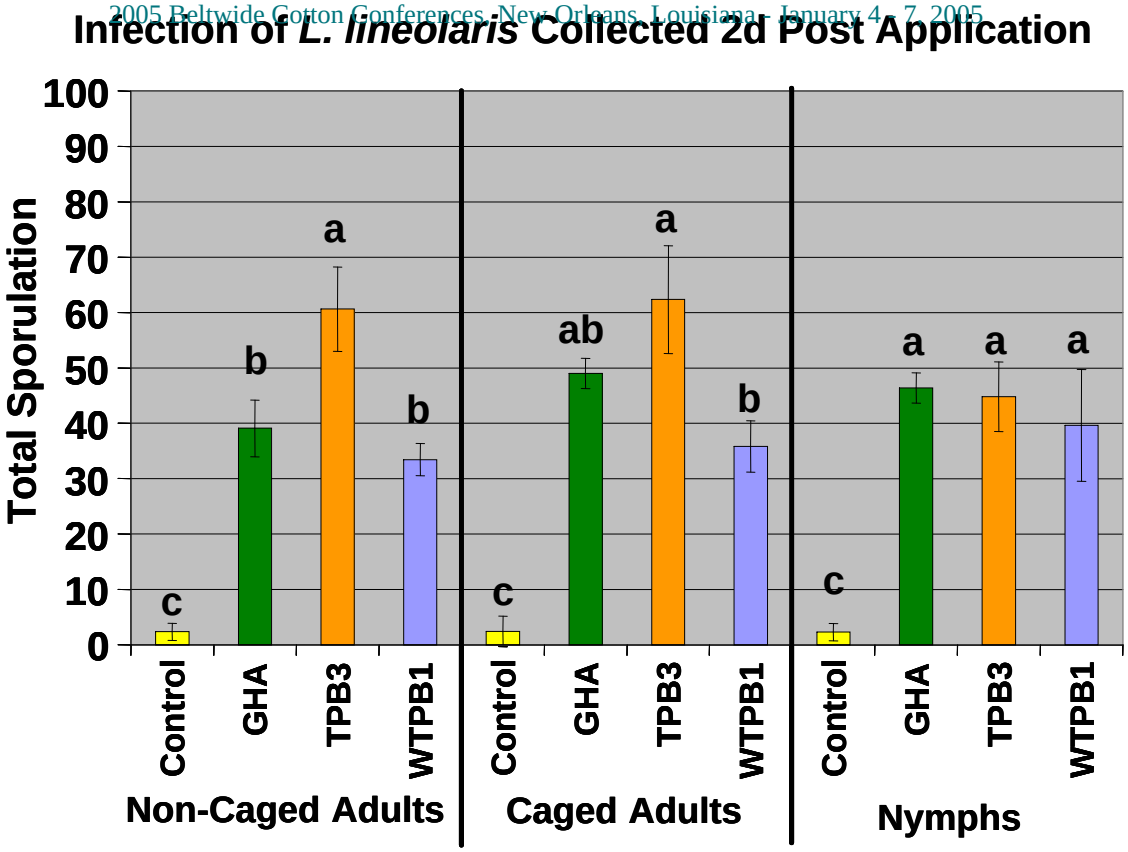

Figure 1. Percent sporulation of *L. lineolaris* collected two days post treatment on wild host plants.

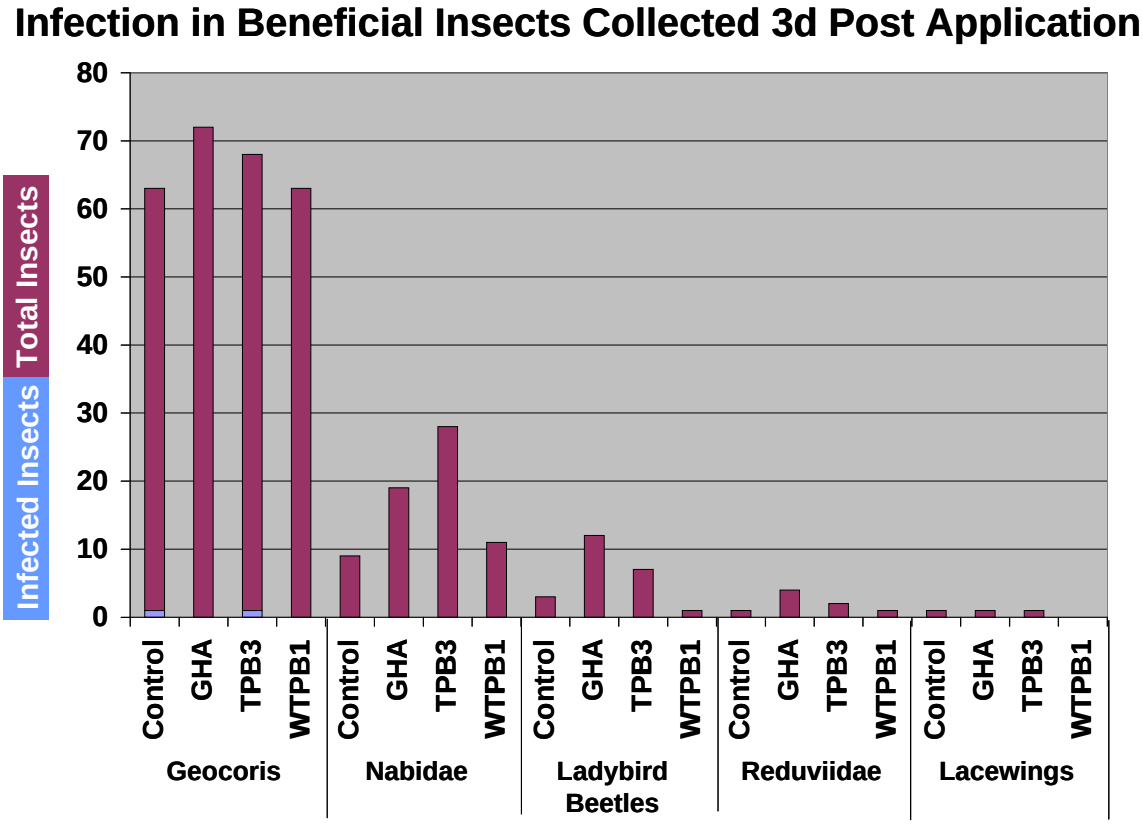

Figure 2. Total number (purple) and number infected (blue) out of 30 beneficial insect predators per plot collected three days post treatment on wild host plants.

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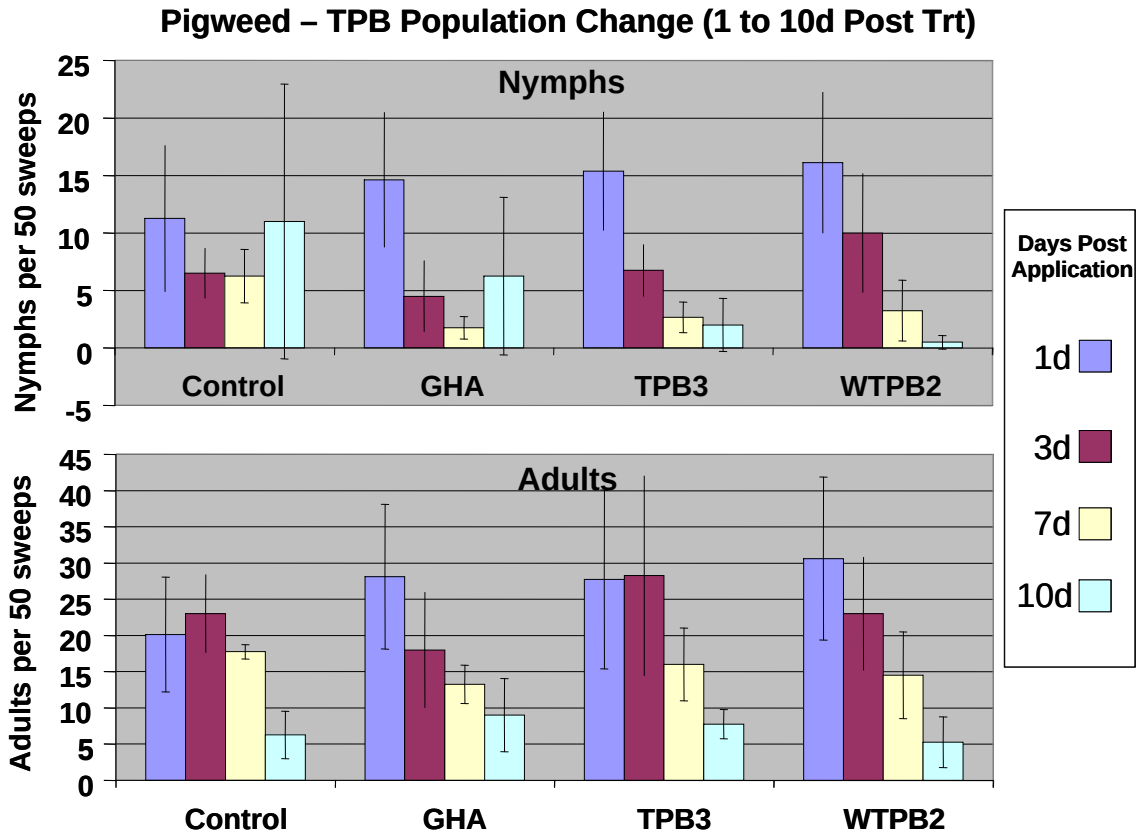

Figure 3. Population densities of adult and nymph *L. lineolaris* following application of *B. bassiana* isolate to wild host plants.

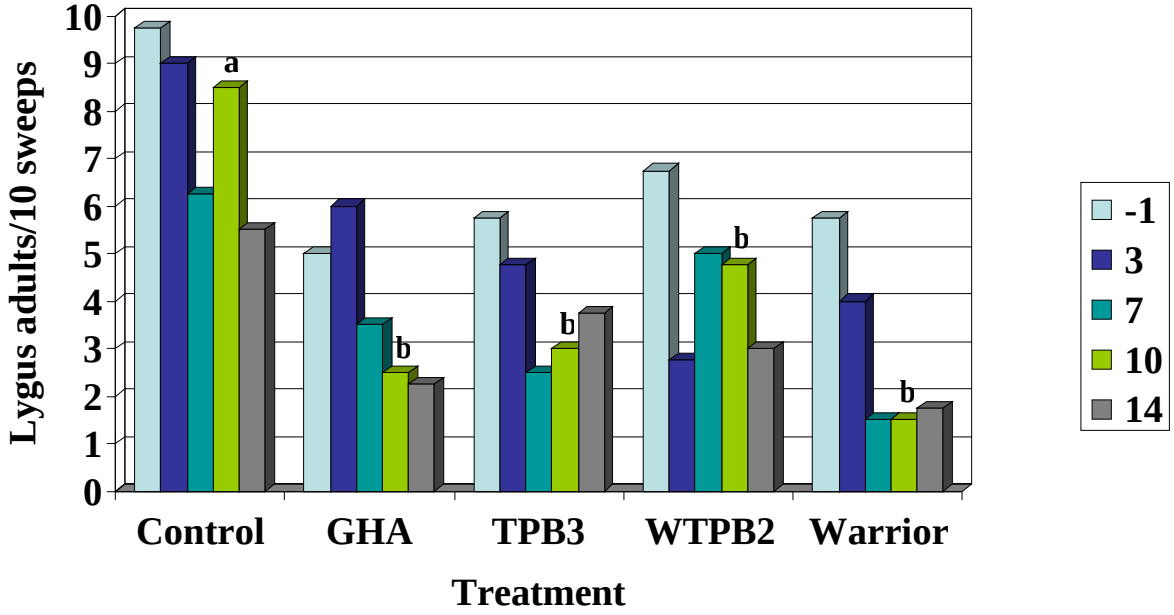

Figure 4. Population densities of adult *L. hesperus* following treatment in alfalfa (June). Treatments designated with different letters are significantly different within the same sample time period (α = 0.05; Protected Least Significant Differences). Comparisons are not provided for time periods where F was not significant.

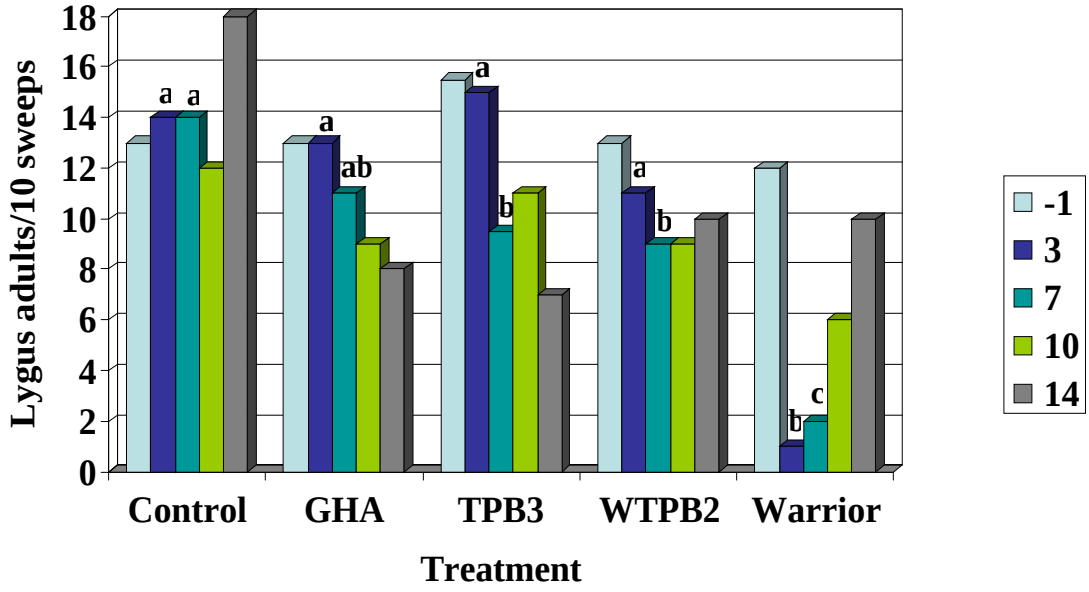

Figure 5. Population densities of adult *L. hesperus* following treatment in alfalfa (September). Treatments designated with different letters are significantly different within the same sample time period ($\alpha = 0.05$; Protected Least Significant Differences). Comparisons are not provided for time periods where F was not significant.

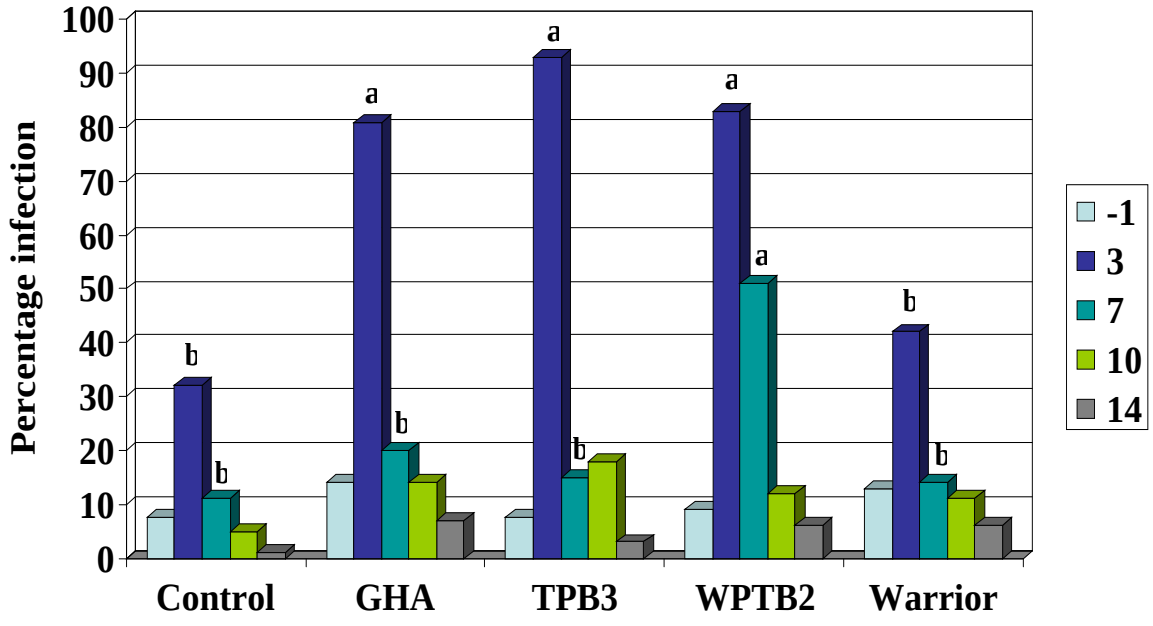

Figure 6. Infection of adult *L. hesperus* following treatment in alfalfa (June). Treatments designated with different letters are significantly different within the same sample time period ($\alpha = 0.05$; Protected Least Significant Differences). Comparisons are not provided for time periods where F was not significant.

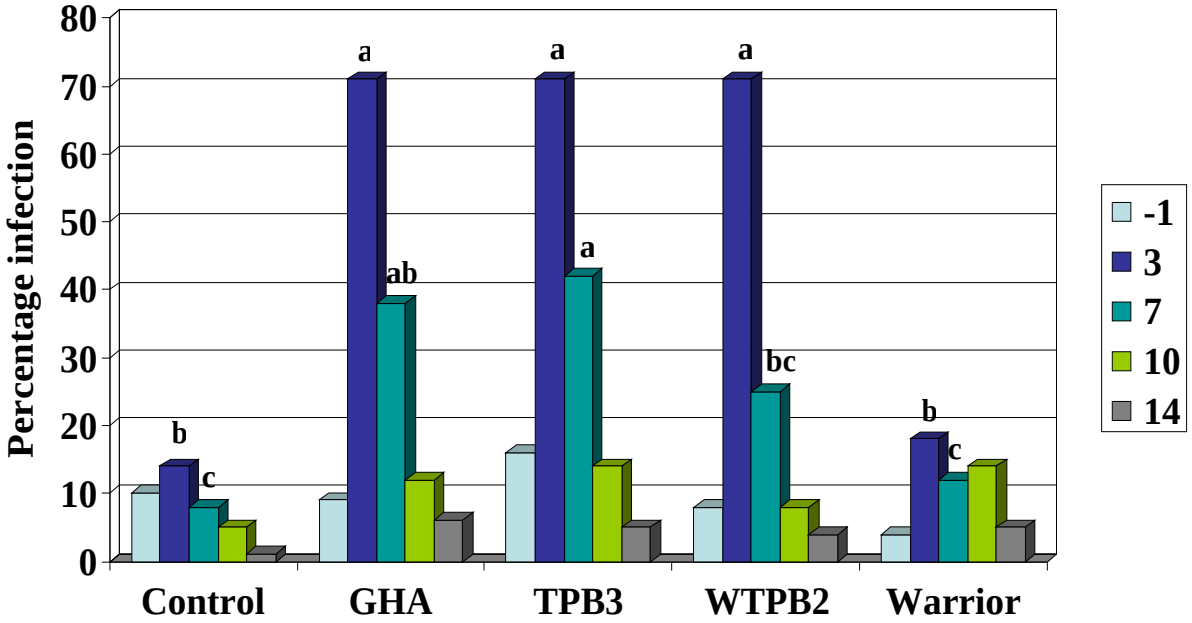

Figure 7. Infection of adult *L. hesperus* following treatment to alfalfa (September).). Treatments designated with different letters are significantly different within the same sample time period (α = 0.05; Protected Least Significant Differences). Comparisons are not provided for time periods where F was not significant.

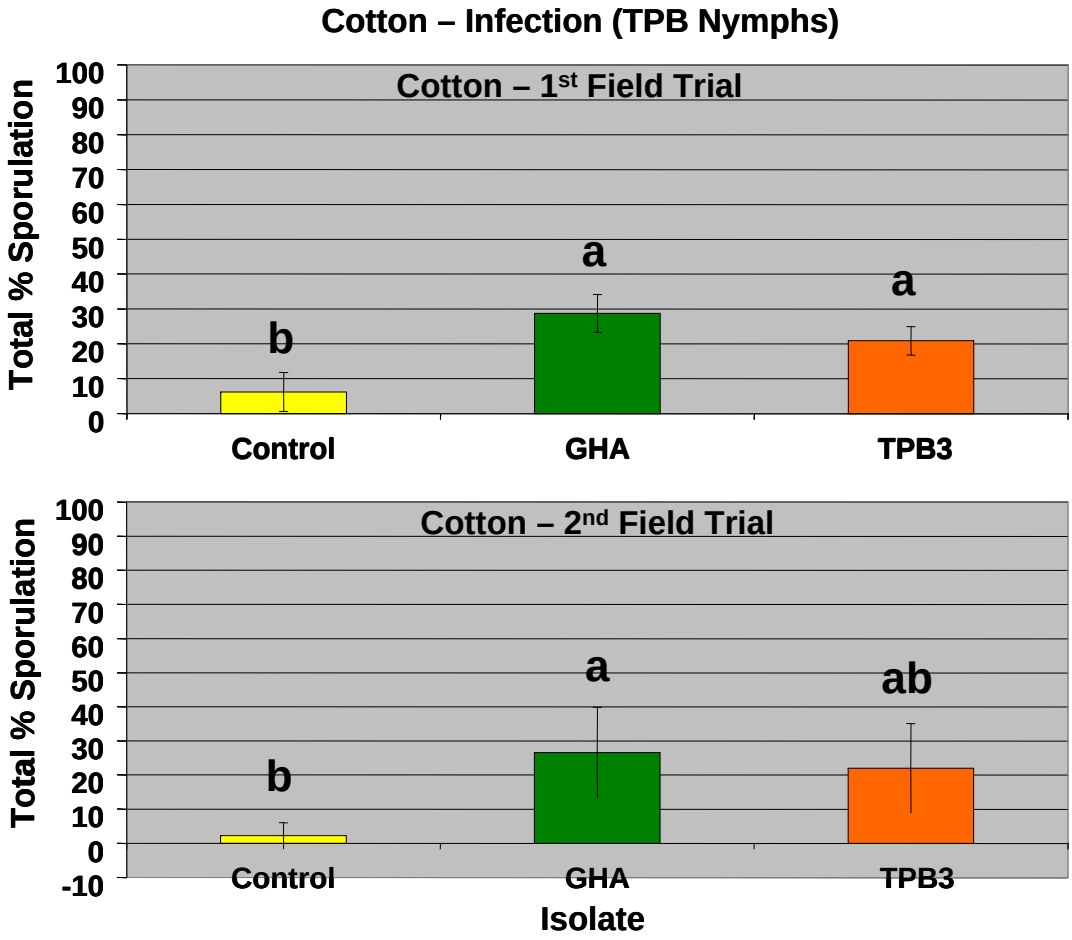

Figure 8. Infection of *L. lineolaris* following treatment to cotton (August).

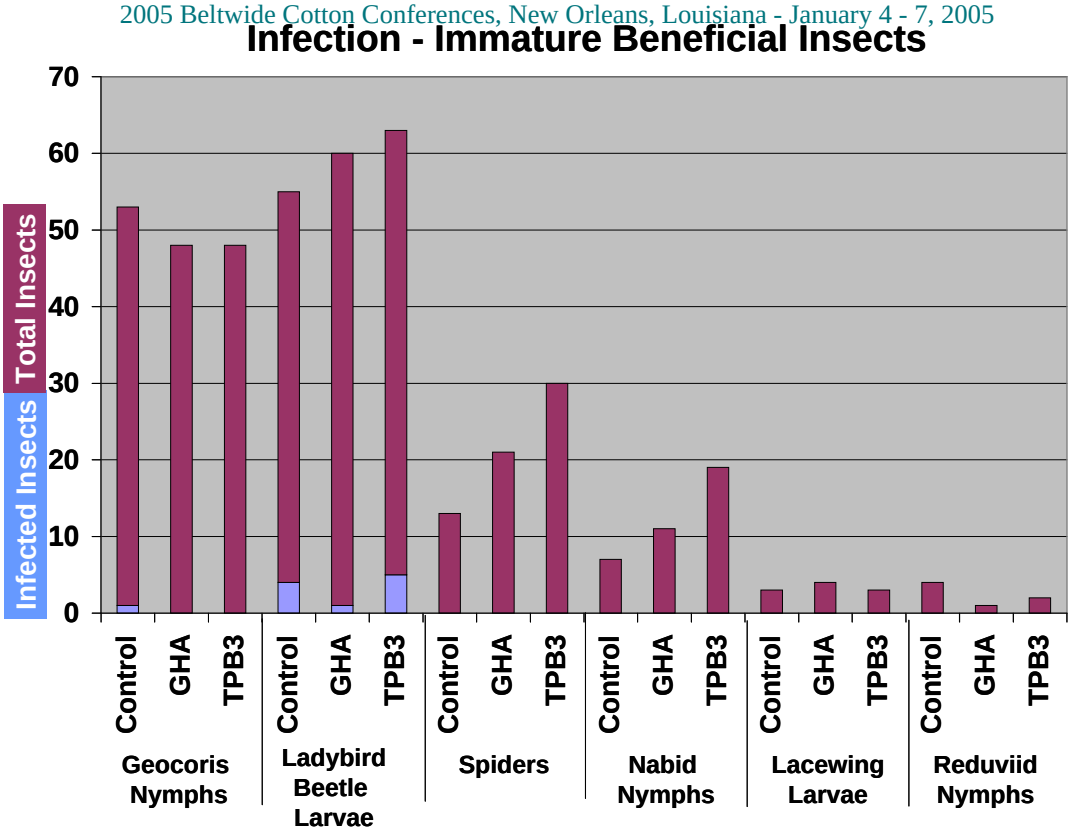

Figure 9. Total number (purple) and number infected (blue) of immature beneficial insects collected three days post treatment in cotton (August).

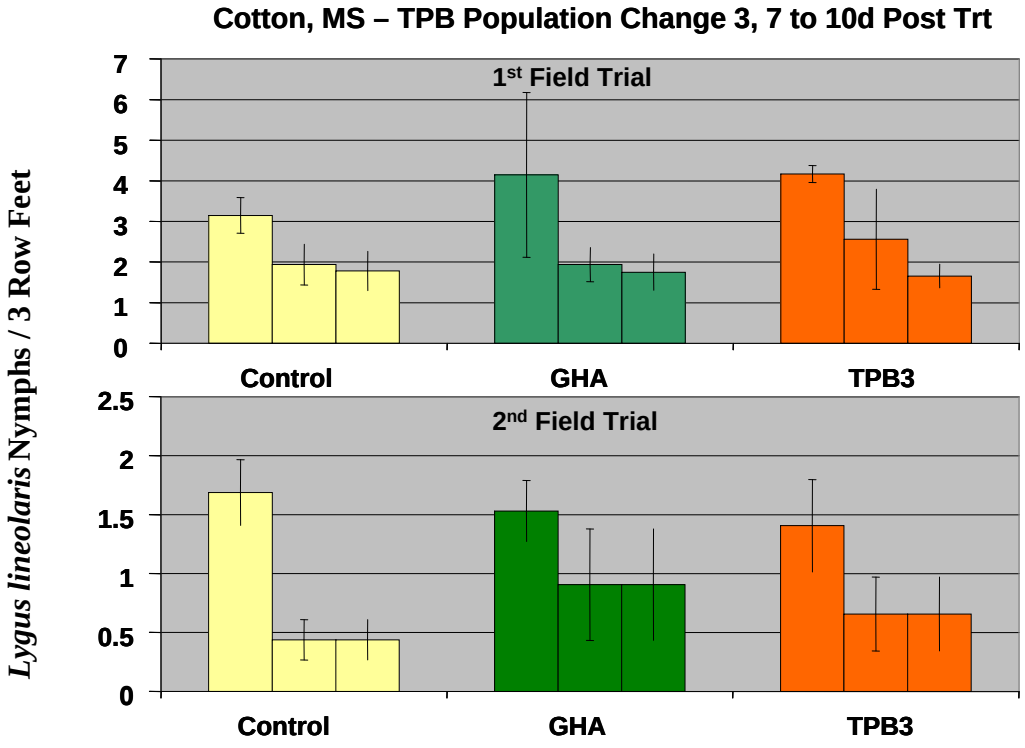

Figure 10. Population densities of *L. lineolaris* nymphs following application of *B. bassiana* isolates in cotton (August).

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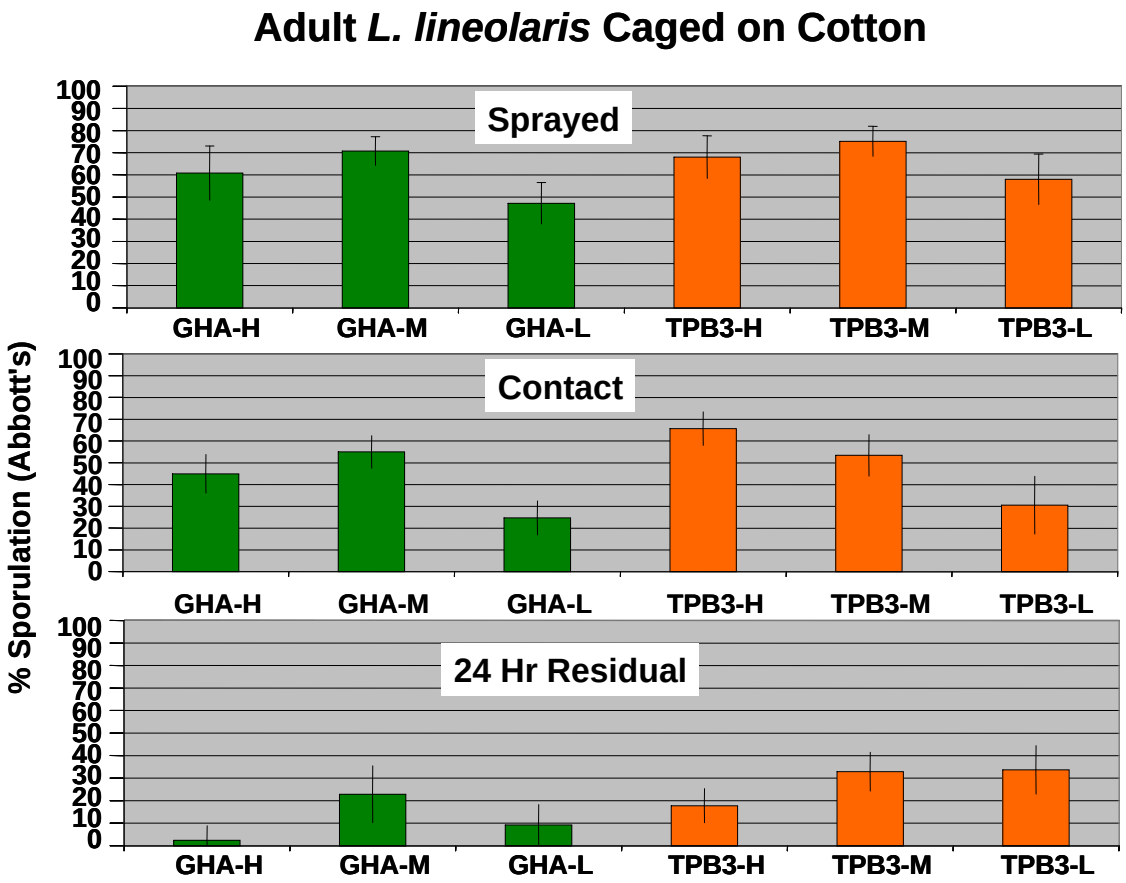

Figure 11. Infection of caged *L. lineolaris* adults high (H), mid (M), and low (L) in the canopy prior to treatment (sprayed), immediately after treatment (contact), and 24 hr after treatment (residual) in cotton (August).