Efficacy of selected *Beauveria bassiana* isolates against adult *Lygus hesperus* at low temperatures

D.W. Spurgeon
USDA-ARS, Western Integrated Cropping systems Research Unit, Shafter, CA
Dale.Spurgeon@ars.usda.gov
661-746-8001

Background and Justification

The western tarnished plant bug (*Lygus hesperus*) is a key pest of cotton and other crops in the San Joaquin Valley. While the primary control tactic for lygus is conventional pesticides, a major objective of the Western Integrated Cropping Systems Research Unit is to develop more ecologically benign management strategies for lygus. *Beauveria bassiana* is a fungal pathogen of lygus that is naturally-occurring in the San Joaquin Valley (McGuire 2002). Previous research at Shafter sought to select strains of this fungus that were effective under the high temperature conditions typical of the cotton production season (Leland et al. 2005, McGuire et al. 2005). However, field tests of these strains indicated less impact on lygus population levels than was expected (McGuire et al. 2006).

As an alternative to chemical pesticides, *Beauveria* exhibits the undesirable traits of being sensitive to high temperatures and ultraviolet (UV) radiation, as well as being relatively slow acting. Therefore, application of *Beauveria* as a rescue treatment in lygus-infested cotton may not be the most appropriate use of this pathogen. However, winter weather conditions in the San Joaquin Valley feature relatively cool temperatures and extended periods of fog that may enhance the activity of *Beauveria*. In addition, application of *Beauveria* to non-crop hosts, or to crop hosts that are not highly susceptible to damage such as alfalfa, would eliminate the need for rapid mortality of treated lygus. Assessment of the potential of a lygus control tactic aimed at reducing overwintering populations to the extent that subsequent colonization of summertime crops would be reduced will be dependent on two additional pieces of information. First, the overwintering ecology and host use patterns of lygus must be better understood so that applications of *Beauveria* can be made to the proper hosts at the proper time. And second, studies must be conducted to determine whether *Beauveria* is capable of producing substantial mortality to lygus under low temperature conditions.

Objectives

The objective of the project was to examine the efficacy of selected *Beauveria bassiana* isolates against *Lygus hesperus* adults under low temperature conditions.

Procedures

Lygus hesperus adults of known age were obtained from our laboratory colony. Evaluated *Beauveria* strains included s44 (referred to as WTPB2 by Leland et al. 2005, and previously selected for high-temperature activity), GHA (derived from a commercial formulation produced by Mycotrol, Emerald BioAgriculture, Lansing, MI), and strains 1-25, 3-3, and 4-11. The latter three strains were derived from infected lygus collected in the San Joaquin Valley during the winter. The assays were conducted in three environmental chambers, one each maintained at a temperature of 55, 65, and 75°F with a 14-hour daylength. A control treatment consisting of

lygus not treated with *Beauveria* was also included at each temperature. Each repetition of the experiment included all five strains of *Beauveria* and the control at each of the three temperatures. The experiment was repeated four times.

Adult lygus were knocked down with CO_2 and placed in a Petri plate lined with filter paper for application of the *Beauveria* treatments. Twenty-five bugs were treated at a single time. Spores of each strain of *Beauveria* were applied at a rate of 1×10^6 spores per ml in a spray chamber. Immediately after treatments bugs were transferred to individual plastic vials, each vial containing a section of green bean pod. Vials were closed with an air-permeable foam plug. In each repetition of the experiment a total of 20 treated bugs was assigned to each strain of *Beauveria* and the control at each temperature.

Following treatment application the lygus were examined daily for mortality, and live bugs were provided new bean pods three times weekly. Mortality data were recorded for 21 days following treatment. When a dead bug was found, the foam plug closing the vial was replaced with a plastic cap, and the vial was transferred to the chamber set at 75°F. Vials of dead lygus were held for five days to detect *Beauveria* hyphal growth or production of spores. Survival patterns of treated lygus corresponding to the various *Beauveria* strains were compared at each temperature using survival analyses. In addition, the proportions of dead lygus that exhibited signs of *Beauveria* infection were compared among *Beauveria* strains within temperatures, as were the temporal patterns of appearance of *Beauveria* hyphae or spores on dead bugs.

Results

Differences in lygus survival were detected among the treatments at each temperature ($p < 0.01$; Figure 1). Pairwise comparisons among the treatments indicated that, with the exception of s44 at 55°F, all of the *Beauveria* treatments resulted in lower survival than in the controls. Survival of lygus treated with s44 at 55°F was not statistically different from the controls ($p = 0.056$). No differences in lygus survival were demonstrated among the tested strains of *Beauveria*. Most of the mortality from *Beauveria* occurred after about 4 days at 75°F and 7 days at 65°F. Substantial mortality from *Beauveria* at 55°F did not occur until after about day 10 after treatment.

None of the dead lygus in the control treatments exhibited any sign of infection by *Beauveria*, thus, the controls were excluded from subsequent analyses. Analyses based on the frequencies of occurrence of dead lygus that exhibited either hyphae or spores did not indicate differences among *Beauveria* strains in the proportions of dead lygus that were infected (55°F, $p = 0.17$; 65°F, $p = 0.17$; 75°F, $p = 0.99$). Over all *Beauveria* strains and experimental repetitions, the proportions of dead lygus showing signs of infection were 82, 82, and 80% for 55, 65, and 75°F, respectively. The global test comparing fungal strains while controlling for temperature also indicated no differences among strains in the frequencies of occurrence of dead bugs that exhibited hyphae or spores ($p = 0.24$). Therefore, estimates of survival corresponding to individual *Beauveria* strains were not confounded by differences in background mortality.

Fungal hyphae appeared soon after the death of most infected bugs. However, production of spores occurred more slowly at 55°F than at the two higher temperatures ($p < 0.01$, Figure 2). Comparisons among *Beauveria* strains within temperatures in the timing of appearance of hyphae and spores did not indicate differences at 55°F ($p = 0.67$). However, differences were

indicated at 65 ($p < 0.01$) and 75°F ($p = 0.0496$). For lygus held at 65°F, examination of the counts of dead lygus that exhibited no symptoms, hyphae, or spores revealed that on day 2 after death more bugs than expected exhibited hyphae, and fewer than expected exhibited spores, for *Beauveria* strains 1-25 and s44, compared with the other strains (Figure 3a). The opposite trend was observed for strain 4-11. Although similar trends were observed for lygus treated at 75°F, when pairwise comparisons were corrected for multiplicity no statistically significant differences were detected ($p = 0.08$, Figure 3b).

Discussion

These results demonstrate that *Beauveria bassiana* can inflict considerable mortality on adults of *Lygus hesperus* at low temperatures typical of daytime highs through much of the non-cotton season of the San Joaquin Valley of California. Results of the study also suggest there is no particular advantage to working with native isolates in trials of lygus population suppression in overwintering habitat, as mortality caused by the commercial *Beauveria* isolate (GHA) was comparable to that caused by any of the native strains.

Although mortality realized from *Beauveria* treatment at the lowest temperature was lower and occurred later than at higher temperatures, the speed with which lygus are killed is not of paramount importance in the context of overwintering treatments. In fact, it is possible that slower mortality may be advantageous if it results in infected lygus disseminating the pathogen in the environment. Of perhaps greater importance is whether *Beauveria* will also infect and kill other insects in the overwintering habitat, such as larvae of the alfalfa weevil. If so, pest insects besides lygus may assist in disseminating the fungus, and alfalfa producers who do not grow cotton may have incentive to use *Beauveria* treatments to the advantage of producers who grow crops that are more susceptible to lygus damage.

References

Leland, J.E., M.R. McGuire, J.A. Grace, S.T. Jaronski, M. Ulloa, Y.-H. Park and R.D. Plattner. 2005. Strain selection of a fungal entomopathogen, *Beauveria bassiana*, for control of plant bugs (*Lygus* spp.) (Heteroptera: Miridae). Biol. Control 35: 104-114.

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: 237-246.

McGuire, M.R., J.E. Leland, S. Dara, Y.-H. Park and M. Ulloa. 2006. Effect of different isolates of *Beauveria bassiana* on field populations of *Lygus hesperus*. Biol. Control 38: 390-396.

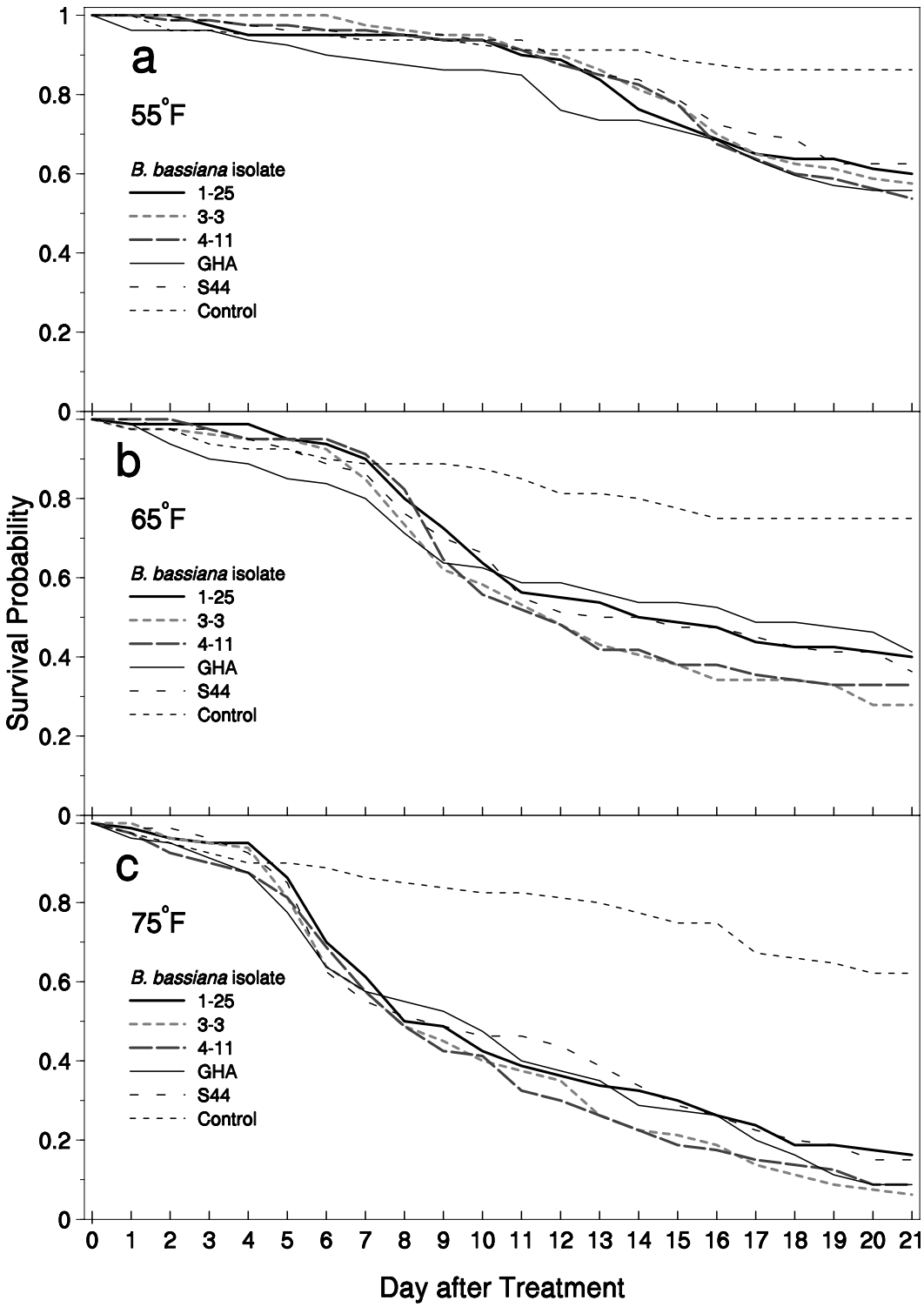

Fig. 1. Survival curves of adult *Lygus hesperus* treated with five strains of the fungal pathogen, *Beauveria bassiana*, at three temperatues.

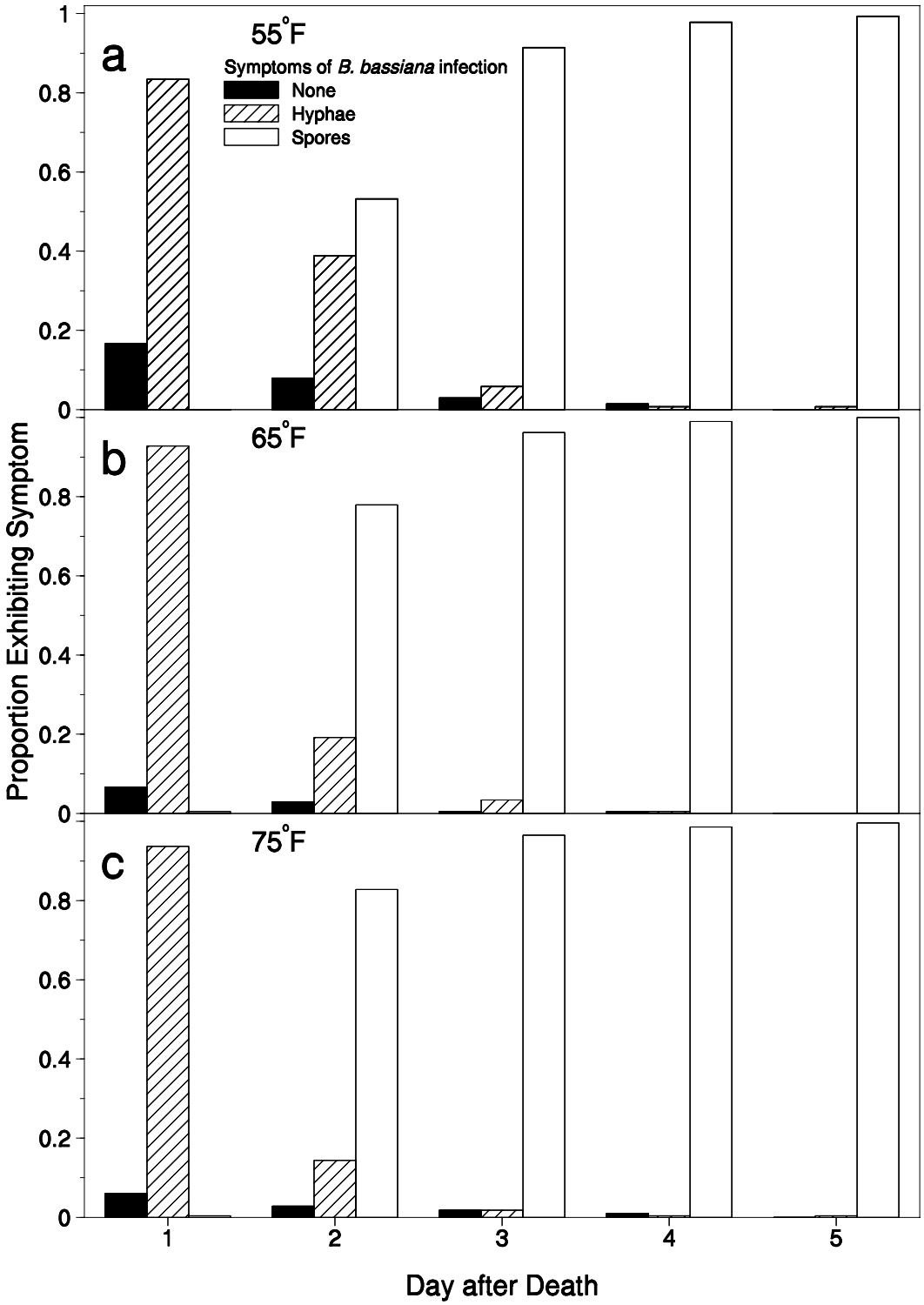

Fig. 2. Temporal patterns of occurrence of fungal hyphae and spores on dead *Lygus hesperus* adults treated with *Beauveria bassiana* at three temperatures. All dead lygus were held at 75°F for five days after death.

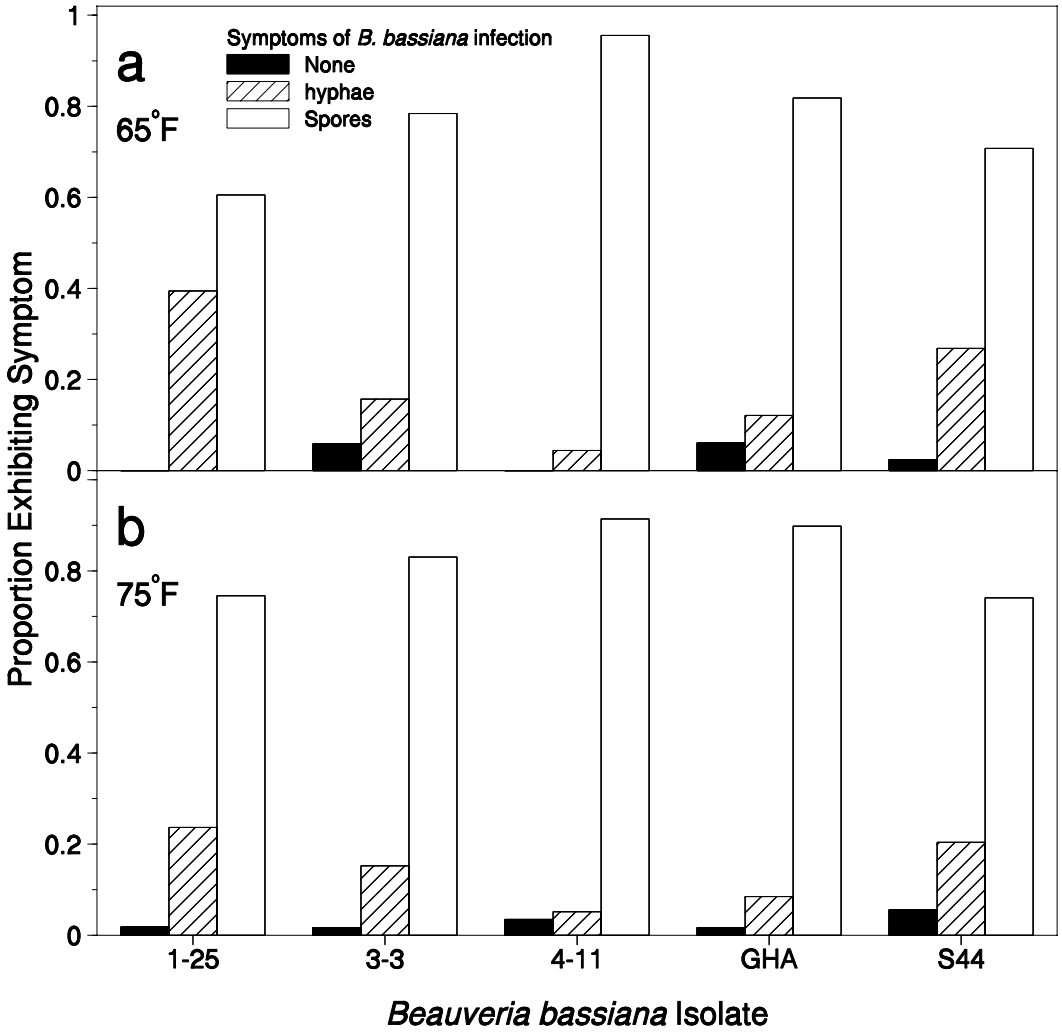

Fig. 3. Patterns of occurrence of symptoms of *Beauveria bassiana* infection on dead *Lygus hesperus* adults two days after death. Lygus adults were treated with *Beauveria bassiana* at two temperatures. All dead lygus were held at 75°F for five days after death.