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Effect of different isolates of *Beauveria bassiana* on field populations of *Lygus hesperus*

Michael R. McGuire [a,*], Jarrod E. Leland [b], Surendra Dara [c], Young-Hoon Park [c,1], Mauricio Ulloa [a]

[a] *USDA-ARS, Western Integrated Cropping Systems Research Unit, Shafter, CA 93263, USA*
[b] *USDA-ARS, Southern Insect Management Research Unit, Stoneville, MS 38776, USA*
[c] *University of California, Davis, Shafter Research and Extension Center Shafter, CA 93263, USA*

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Abstract

Lygus hesperus (Knight) (Hemiptera: Miridae) is a particularly damaging pest of many crops in the Western United States. Current control tactics are chemically based and there is some concern over resistance building up in populations. Based on previous laboratory studies conducted in California and Mississippi, USA, two new isolates of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) were selected for field-testing against *L. hesperus* in California. Alfalfa plots were treated with one of three isolates of *B. bassiana* (a commercial isolate, an isolate from CA (WTPB2) or an isolate from MS (TPB3)) or the chemical pesticide Warrior T. More than 75% of the adults collected from plots 3 days after application with *B. bassiana* were infected but no differences in percentage infection occurred among fungal treatments. In addition, approximately 30% of the insects collected from control plots or plots treated with Warrior T were also infected. PCR analysis using SSR markers revealed that the isolate causing most of the infections in fungus treated plots was the isolate applied. A mix of infections was found in control plots and plots treated with Warrior T. Despite high levels of infection, no significant reductions of adult populations occurred until 10–14 days after application when plots treated with Warrior T or *B. bassiana* had about half the numbers of adult *L. hesperus* as the control plots.

Keywords: *Beauveria bassiana*; *Lygus hesperus*; Alfalfa; SSR markers

1. Introduction

Lygus species (Hemiptera: Miridae) across the world impart significant economic damage to a wide range of crops including cotton, seed alfalfa, strawberries, and others. Currently, these pests are controlled through the use of chemical pesticides that also may reduce populations of beneficial arthropods. Coupled with concerns over the development of resistance to pesticides (Snodgrass, 1996; Snodgrass and Scott, 2002), research to discover specific and effective controls for *Lygus* spp. has increased.

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) strain GHA is sold as commercial products such as Mycotrol (Laverlam S.A. Cali, Colombia) in a large number of crops but has not been widely adopted. Laboratory tests have identified several isolates of *B. bassiana* and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes), some from *Lygus* spp. but most from other insects, that performed better than GHA (e.g., Leland, in press; Leland et al., 2005; Liu et al., 2002, 2003; McGuire et al., 2005). Field tests with *B. bassiana* (GHA) against *Lygus hesperus* in seed alfalfa resulted in relatively high levels of infection but little population reduction (Noma and Strickler, 1999). Additional tests in Arkansas (Steinkraus and Tugwell, 1997) demonstrated high infection rates from

* Corresponding author. Fax: +1 661 746 1619.
E-mail address: mmcguire@pw.ars.usda.gov (M.R. McGuire).
[1] Present address: Sakata Seed America, Inc., Salinas, CA, USA.

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either GHA or an isolate from *L. lineolaris* (ARSEF 3769), however, side by side field studies have not been conducted to compare GHA to any of the isolates found to be more pathogenic to *Lygus* spp. in the laboratory. More recently, McGuire (2002) recovered a large number of *B. bassiana* isolates from *L. hesperus* in California and further tests to characterize these isolates in the laboratory resulted in the identification of an isolate that was more pathogenic against *L. hesperus* than GHA (McGuire et al., 2005). Similarly, Leland and Snodgrass (2004) reported on the recovery of isolates of *B. bassiana* from *L. lineolaris* in Mississippi and demonstrated higher pathogenicity to *L. lineolaris* under laboratory conditions (Leland, in press). An exchange of isolates between the California and Mississippi groups was made to determine if one or more isolates had better activity against both *Lygus* species, could grow at higher temperatures, and were more tolerant of solar radiation in hopes of identifying a viable strain that could potentially be used under field conditions to control both pests. This work (Leland et al., 2005) resulted in the identification of two isolates, one from California (WTPB2) and one from Mississippi (TPB3) that showed some promise for better activity than GHA.

One approach to managing *Lygus* spp. in cotton is to identify areas where *Lygus* spp. over winter and/or build to large populations before they move to a crop where they impart economic damage. In the Western US, *L. hesperus* typically build to large numbers in hay alfalfa, where they do not cause economic damage, and then move to cotton when the alfalfa is harvested (Cave and Gutierrez, 1983). In the Midsouthern US, *L. lineolaris* populations develop on a wide range of wild host plants, which senesce and become unsuitable hosts during hot and dry summer months at which time *L. lineolaris* move to early squaring cotton (Snodgrass et al., 1984). As a result, these wild host plants have been targeted for *L. lineolaris* area wide management programs (Snodgrass et al., 2000, 2005).

This manuscript reports the results of the field tests conducted in California with the two new isolates of *B. bassiana* in comparison with GHA. Tests were done on alfalfa to determine if isolates from different sources and localities would differentially impact *Lygus* spp. populations. In a companion paper (Leland and McGuire, submitted), the same isolates were tested against *L. lineolaris* on pigweed.

2. Materials and methods

Five treatments included three isolates of *B. bassiana*, a sprayed control, and a chemical control. The isolates of *B. bassiana* included GHA as a commercial standard, one isolate of *B. bassiana* from California *L. hesperus*, and one from Mississippi *L. lineolaris* (WTPB2 and TPB3, respectively in Leland et al., 2005). Conidia were grown using a two-stage mass production process as described by Leland et al. (2005) and provided by Stefan Jaronski and Julie Grace, USDA-ARS Sidney MT. The full amount of conidia to be used in the field was added to tap water containing 1% Silwet L-77 (Loveland Industries, Greeley, Colorado) to provide a concentrated slurry. Final dilutions were made by adding the concentrate through a 80 mesh stainless steel sieve to the spray tank and mixing thoroughly such that the concentration of Silwet L-77 was 0.05% and the concentration of conidia resulted in an application rate of 2.5×10^{13}/ha for the June application and 1×10^{13} conidia/ha for the August application. Additional treatments included a control consisting of just 0.05% Silwet L-77 and a chemical control, Warrior T (lamda-cyhalothrin Syngenta, Greensboro, NC) applied at label rate in 0.02% Silwet L-77.

Alfalfa, *Medicago sativa* L., was cultivated at two sites on the University of California Research and Extension Center, Shafter, California. Alfalfa was chosen because *L. hesperus* populations are typically large and consistent. One field was planted in August 2003 and the other field, located approximately 2 km away, was planted in April 2000. Both fields were divided into four strips; 18 m wide × 82 m long, and managed such that every other strip was harvested at biweekly intervals. Two applications were made, one in June and one in August. For each application, two strips were used in each field for a total of four blocks. Blocks were separated by the 18 m wide alfalfa strips not used in the study. Each block was divided into five plots, each 15 m long with 2–3 m between plots and the experimental design was in a randomized complete block.

The first application of test materials was made June 15, 2004, starting at approximately 18:00. A tow behind trailer contained a pressurized CO_2 tank and other stainless steel tanks that had been loaded with the material. A 6 m wide boom with 13 flat fan nozzles (8002, Spraying Systems Wheaton, IL) with 50 mesh screens delivered the material at 2.8 kg/cm^2 pressure for an application volume of 225 L/ha. The spray line was flushed with water between treatments. Alfalfa was flood irrigated the following day. The second application was made on August 31, 2004, and the entire experiment was repeated. Temperatures were monitored via an on site weather station maintained by the California Irrigation Management Information System.

Sweep samples were made in the center of each plot to determine relative abundance of insect populations. Ten 180° sweeps using a 38 cm diameter sweep net were made at 1 day before application and 3, 7, 10, and 14 days after application. Insects were transferred from the sweep net bags into plastic bags and frozen for later enumeration. To reduce variability related to sweep net efficiency, the same individual swept all plots in two assigned blocks on each sample date. In the laboratory, insects were sorted by species, counted, and refrozen in glass vials. Insects enumerated included *L. hesperus* adults and nymphs, big eyed bug (*Geocoris* spp.) adults and nymphs (Lygaeidae), damsel bug adults and nymphs (Nabidae), lady beetle adults and larvae (Coccinellidae), green lacewing adults and larvae (Chrysopidae), and minute pirate bug (*Orius* spp.) adults and larvae (Anthocridae).

To determine if *L. hesperus* adults were infected with *B. bassiana*, up to 20 live adults were collected from each plot