EFFICACY OF *BEAUVERIA BASSIANA* FOR CONTROL OF LYGUS BUGS IN ALFALFA SEED FIELDS

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Lygus bugs (primarily *Lygus hesperus*) are the most important insect pests of alfalfa grown for seeds in Idaho. They extensively feed on developing flower buds and seeds, reducing viable seed yields. Feeding by older nymphs and adults is responsible for most economic damage. Current lygus bug management relies principally on chemical pesticides. However, this management strategy is becoming increasingly difficult due to resistance development. Furthermore, currently registered pesticides do not effectively control the adult stage. Thus, alternative lygus bug control strategies should be investigated for reliable resistance management. Any lygus management strategy must minimize impact on the alfalfa pollinator *Megachile rotundata* and native lygus bug predators such as *Geocoris*, and *Nabis* species.

Our previous bioassays suggested that the entomopathogenic fungus *Beauveria bassiana* effectively kills lygus fifth instar and adults in laboratory conditions. The use of this biopesticide may be advantageous because 1) unlike other microbial agents (i.e., bacteria, virus), the fungus is able to penetrate the exoskeleton of host insects, 2) resistance development to fungi has not yet been reported, and 3) the fungus is more host specific than most pesticides and appears to be safe to vertebrates including humans. This paper reports efficacy of *B. bassiana* for control of lygus bugs in southern Idaho alfalfa fields.

Objectives

1) To investigate impact of *B. bassiana* on lygus bug and its predator populations during the alfalfa growth season in southern Idaho.
2) To examine the longevity of *B. bassiana* conidia (spores) sprayed on alfalfa foliage in southern Idaho.
3) To study temperature effects on virulence of *B. bassiana* on lygus bug adults.

Materials and methods

1) **Objective 1**. Small alfalfa plots (8 x 10m, n = 45) were used to investigate the efficacy of *B. bassiana* to control lygus populations in Parma, ID from June to August 1996. 1.5m-wide corridors were created between plots by mowing to minimize lygus adult movements across plots. A completely randomized block design was used in which the field was divided into 5 blocks to control for apparent differences in plant density at the beginning of the season. Each block contained 9 plots consisting of every combination of three prebloom and three bloom treatments. Treatments consisted of either *B. bassiana*, chemical pesticide, or water (control). *B. bassiana* conidia formulated in oil-water emulsion (Mycontrol, GHA strain, Mycotech Corp., Butte, MT) were sprayed at the rate of 1.16 L / ha (2.47×10^{13} conidia / ha) using a CO_2 (40 PSI) hand sprayer (R & D Sprayers Inc., Opelousas, LA). *B. bassiana* treatments consisted of three prebloom sprays, with 5-7 d intervals between the applications, and/or three bloom sprays with the same intervals. The chemical pesticide treatments consisted of one pre-bloom spray with Capture at the rate of 468ml / ha and one bloom spray with MS-R at 2.25×10^3 ml / ha. All spray applications were conducted between 7 and 10pm. Lygus bugs and their predators (*Geocoris* and *Nabis* spp.) were sampled weekly by three 180° sweeps / plot. Analysis of variance followed by Tukey's studentized range tests were used to detect significant block, time and treatment effects for overall season and each sampling date.

2) **Objective 2**. After each of three bloom spray applications [see Materials and Methods (1)] in July 1996, 10 leaves were randomly collected daily from top-middle alfalfa foliage in *B. bassiana* sprayed plots. The conidia on the sampled leaves were washed off in 10ml 0.01M phosphate buffer with 0.01% Silwet which was placed on a rotary shaker for 2 h. 0.1ml of the wash solutions was spread on oatmeal-dodine medium, selective for *Beauveria* [17.5g oatmeal agar, 2.5g agar, 0.45g Syllit (dodine), 2.5mg crystal violet, 0.2 g penicillin G, and 0.5g streptomycin in 500 ml of deionized water]. The cultures were incubated at 25°C for a wk and the number of colony-forming units were counted on each plate to determine relative viability of conidia at each sampling date.

3) **Objective 3**. Lygus bug adults were collected from alfalfa fields in Parma, ID on 8 September and were placed in 10cm petri dishes with a filter paper on the bottom (30 bugs / petri dish). The *B. bassiana* dry conidia (GHA strain, Mycotech Corp.) were suspended in water with 0.04% Silwet (Loveland Industries Inc., Gleeley, CO). Six concentrations of conidia solutions were prepared by two-fold water dilution series. The conidia sprayer (air brush, Budger 250-5) was set 75cm above the lygus-containing petri dish. 1ml of each conidia solution was sprayed onto the petri dish. The conidia inoculated lygus bugs were caged, provided with green beans, and kept at 15, 25, or 35° C in growth chambers (L = 15 h, D = 9 h, RH $\approx$ 70%) for 20 d. There were three replicates (cages) per conidia concentration per temperature. Mortality of each replicate was recorded daily and dead bugs were removed from the cage. To verify *B. bassiana*

infection, cadavers were surface-sterilized in bleach-ethanol solution and placed on water agar plates to allow fungi to sporulate from the dead bugs. The mean infection mortality at each conidia concentration was compared among the different temperatures.

Results

1) **Effects of *B. bassiana* on lygus bugs and predators in the field.**

There were significant interactions between treatment and time effects ($\alpha = 0.05$) for both lygus bugs and their predators, so treatment effects were analyzed at each sampling date. Generally, *B. bassiana* treatment alone did not affect lygus adult or nymphal densities during either prebloom or bloom seasons, while conventional pesticide treatments often significantly lowered the densities of both lygus stages ($\alpha = 0.05$) This is illustrated for three treatment combinations in Fig. 1a,b. However, neither fungus nor pesticide prevented an outbreak of lygus adults in mid-July, and adults dropped in all fields including controls at the same time as the bloom spray (Fig. 1a). Lygus nymphs drastically increased in mid August only in pesticide plots (Fig. 1b).

The *B. bassiana* treatment did not affect any of the lygus predators during most of the alfalfa growth season, whereas pesticide applications significantly reduced big-eyed bug and damsel bug densities in July (Fig. 2a,b). Although effects of those predators on lygus densities are undetermined, the explosive lygus nymph population growth in mid-August may be a response to reduced predator densities by pesticides.

2) **Longevity of *B. bassiana* conidia on alfalfa foliage in southern Idaho.**

As expected from previous research (Inglis et al. 1993), number of viable conidia drastically decreased in the first 24 h and almost all conidia died within 3 days in southern Idaho conditions (Fig. 2). Thus, *B. bassiana* may be effective only if lygus bugs pick up conidia within a few hours after application.

3) **Temperature effects on *B. bassiana* virulence on lygus bug adults.**

B. bassiana was most effective in killing lygus bugs at 25°C, less effective at 15°C, and least effective at 35°C (Fig. 4). For example, the highest conidia concentration sprayed (10700 conidia / cm^2) caused mean infection mortalities of 70% (SE = 5.1) at 25°C, 46% (SE = 4.8) at 15°C, and 20% (SE = 3.9) at 35°C (Fig. 4).

Other than fungal growth, temperature would also affect host insect growth. High temperatures may increased immune activity and reduced molting intervals in nymphs. Lygus bugs may escape from fungal infection by molting before fungi penetrate the exoskeleton, thus reducing the effectiveness of *B. bassiana*. In Parma, average high and low daily temperatures in July were

35.6°C and 12.8°C respectively, and such daily temperature fluctuation probably influence mycosis by *B. bassiana*.

Conclusions

Beauveria bassiana alone was not effective to control lygus bugs in small alfalfa plots. Environmental conditions in southern Idaho were not appropriate. Efficacy of *B. bassiana* may improve in a UV light-protecting formulation or with a heat tolerant strain. Some synergists (e.g., low concentration of pesticides, chitin synthesis disruptors) may also interact to improve efficacy.

Information on lygus bug behavior (i.e., movement along plants during day and night) would be useful to determine if contact of conidia by the bugs can be improved. In addition, temperature should be measured in micro-habitats of lygus bugs since temperature may vary depending on location within foliage and exposure to the sun. Finally, sublethal effects of *B. bassiana* on lygus bugs (i.e., feeding rate, fecundity) should be investigated.

References

Inglis, G. D., M. S. Goettel, & D. L. Johnson. 1993. Persistence of the entomopathogenic fungus, *Beauveria bassiana*, on phylloplanes of crested wheatgrass and alfalfa. Biol. Control. 3:258-270.

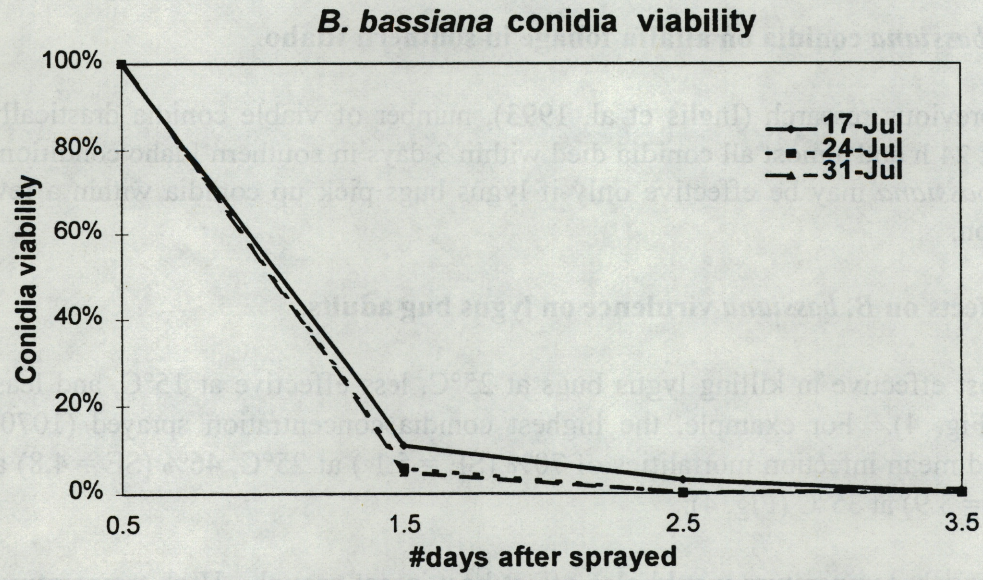

Fig. 3. Longevity of *B. bassiana* conidia sprayed onto alfalfa foliage in July in Parma, ID. Sprays were applied in the evening and the first leaf samples were collected the following morning (0.5 d after the application).

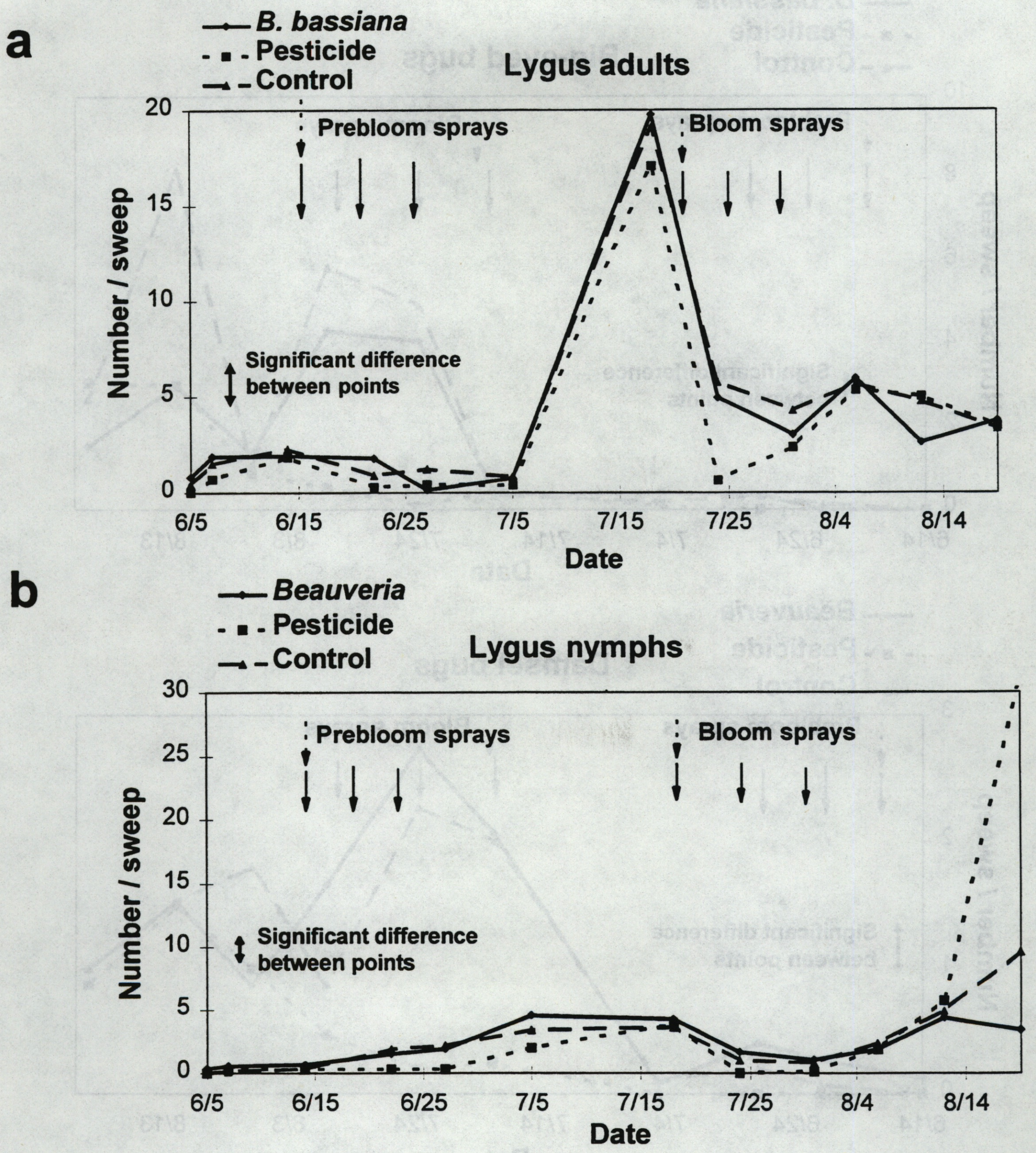

Fig. 1. Population dynamics of lygus a) adults and b) nymphs in plots treated with *B. bassiana* (prebloom and bloom sprays) and conventional pesticides (prebloom spray = Capture, bloom spray = MS-R) in Parma, ID. Control plots were sprayed with water. Two treatment effects are significantly different when the vertical distance between two points are greater than the arrow length (Tukey's studentized test, $\alpha = 0.05$).

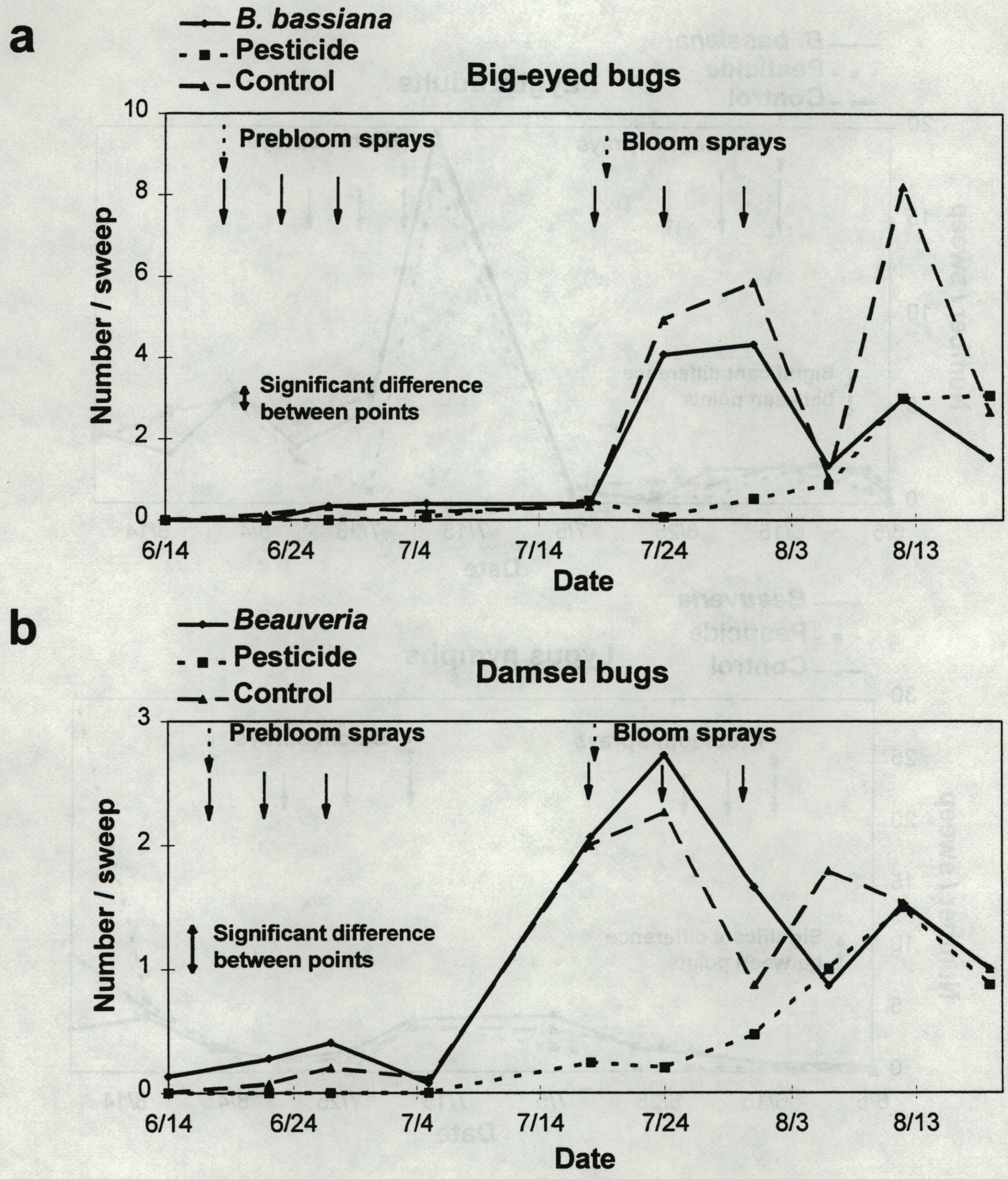

Fig. 2. Population dynamics of lygus predators, a) big-eyed bugs and b) damsel bugs, in plots treated with *B. bassiana* (prebloom and bloom sprays) and conventional pesticides (prebloom spray = Capture, bloom spray = MS-R) in Parma, ID. Control plots were sprayed with water. Two treatment effects are significantly different when the vertical distance between two points are greater than the arrow length (Tukey's studentized test, $\alpha = 0.05$).

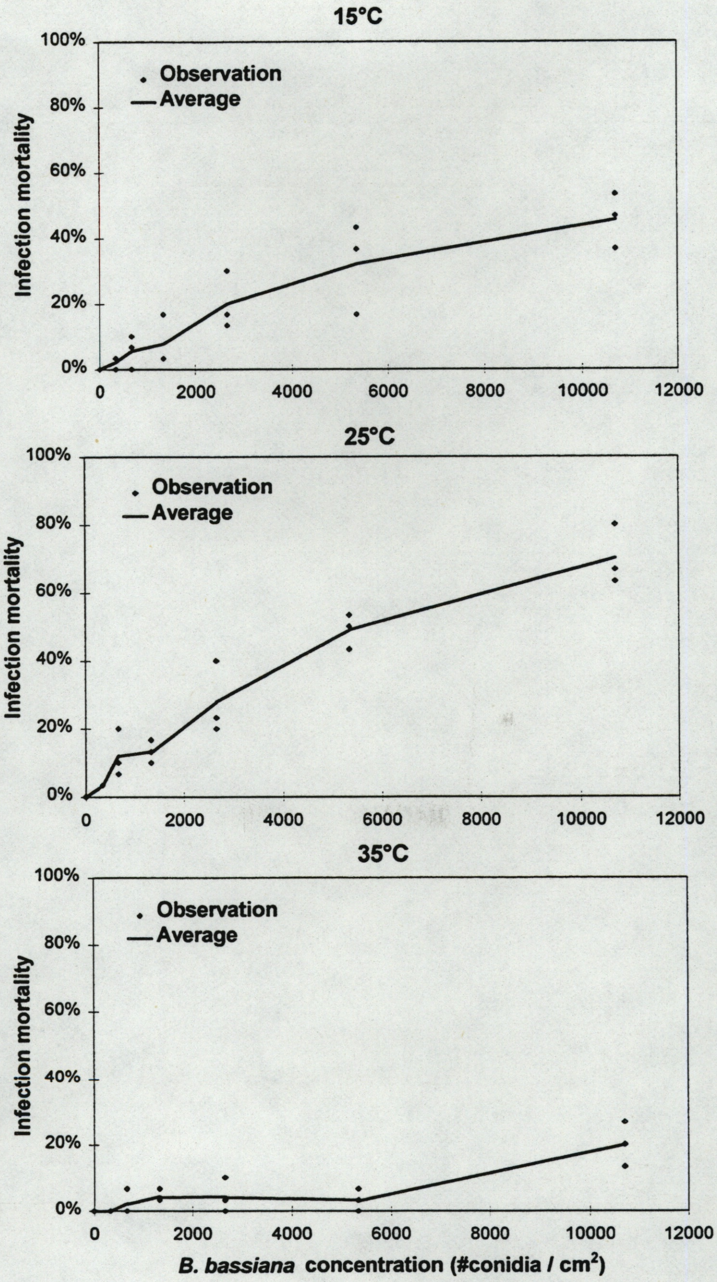

Fig. 4. Effect of temperature on lygus adult mortality caused by *B. bassiana* infection. Mortalities were recorded 20 d after conidia inoculation.