Control of *Lygus hesperus* with *Beauveria bassiana*

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Justification and Problem Statement

Lygus hesperus continues to be one of the most damaging pests of cotton in the San Joaquin Valley. *L. lineolaris*, a closely related species is quickly becoming the primary pest of cotton in the Southeast US. Current controls consist of the application of broad spectrum pesticides that may also impact natural enemies. The depletion of natural enemies may lead to increases of other pests such as aphids, whiteflies and spider mites later in the season. A control that is selective for Lygus would be a benefit to the cotton industry. Both Lygus species are infected, in nature, by a fungus called *Beauveria bassiana*. *B. bassiana* occurs worldwide, and is used commercially. Tests in our laboratory and in published reports suggest that the commercial products are not very effective against either Lygus species (Noma and Stickler 2000; Steinkraus and Tugwell 1997). Surveys for *B. bassiana* done in 2000-2002 revealed widespread prevalence of the fungus (McGuire 2002). *B. bassiana* was found in all SJV counties at all times of the year. Fungal isolates have been cultured and laboratory tests demonstrate a large variation in activity against Lygus and ability of the fungus to grow at high temperatures. Parallel work in Mississippi has revealed similarities and fungal strains will be exchanged to determine which strains have activity against both species.

Objectives:

1) Determine activity of *B. bassiana* isolates against *L. hesperus* under field conditions.
2) Determine effects of formulation and application timing of *B. bassiana* on Lygus populations.
3) Determine impact of application of *B. bassiana* to Lygus populations in alfalfa on subsequent infestation of surrounding cotton fields.
4) Determine impact of application of *B. bassiana* to Lygus populations in cotton (third year).

Procedure:

Year 1: Two new isolates of *B. bassiana* will be tested against a commercial *B. bassiana* product (Mycotrol) and a chemical pesticide. Replicated plots will be established in alfalfa where Lygus populations are consistently high. Plots will be approximately 50' long by 75' wide and each treatment will be replicated 4 times. At SREC, there are two alfalfa fields each with four 75' wide checks. These fields are strip cut such that two checks are cut every two weeks. Therefore, the experiment will have to be set up in both fields, using two checks in each. Each check will be divided up into 5 plots and used as a block. Lygus populations will be estimated before spraying in each block using standard 50-sweep samples. Applications of pesticides will be made using standard techniques. Lygus populations will be estimated at 4, 7, 10, and 14 days after application using a 10 sweep sample in the middle of each plot. In addition, samples of 50 adults and 50 nymphs will be collected from each plot to estimate infection levels. To ensure *B. bassiana* infections are due to sprayed strains, not endemic strains, molecular markers will be used to identify the infections. This work is currently in progress and a specific PCR SCAR marker has already been identified for Mycotrol (Castrillo et al. 2003). In addition to Lygus, other insects will be enumerated and infections determined. This test will be repeated using the other checks in both fields. To determine if *B. bassiana* is replicating in the field, samples will be taken at 30 and 60 days after application.

Year 2: Based on results from Year 1, a single *B. bassiana* strain will be selected and extensively tested. Replicated plots will be established in alfalfa as in year one and sampling will be similar. Tests will involve different rates of spores and different formulations. Currently the commercial product suggests the use of 10^{12} spores per acre. Treatments of the new strain will include 10^{12}, 10^{11} and 10^{10} spores per acre. In addition, formulations of *B. bassiana* constructed in coordination with ARS scientists in Mississippi and Illinois, will be tested. Formulation is an essential factor in persistence and infectivity of any insect pathogen and must be tested under field conditions. In an alfalfa field separate from the rate tests, up to three new formulations will be tested at a single spore rate.

Year 3: Treatment of Lygus in alfalfa may lead to reductions of migrating populations but it is important to determine two things. First, does application of *B. bassiana* against Lygus in alfalfa impact movement of lygus into cotton and do infected individuals move as readily as uninfected individuals? Second, will *B. bassiana* be effective in controlling Lygus populations if applied to cotton? To test the first hypothesis, alfalfa plots that border cotton will established. Plots will be established based on the area available and a single high dose of *B. bassiana* will be sprayed. Transects from alfalfa into cotton will be established and Lygus will be collected at 5 points along the transect at 20 feet intervals. Prevalence of infection will be determined as in Year 1 and Lygus populations will be estimated with 10-sweep samples in between the transects to determine movement of the insects. Applications will be made approximately 21 days after alfalfa cutting. Samples will be taken along the transects 7 and 14 days after application. 8 days after application, the alfalfa will be harvested to force Lygus into moving. To test the second question, replicated plots will be established in cotton. Treatments will consist of the single *B. bassiana* strain and a chemical control. Sampling will be done at 4, 7, 10, 14 days after application as above and will include estimates of natural enemies in addition to Lygus. Prevalence of infection will be determined as above.

Previous Work and Present Outlook

The use of *B. bassiana* to control insects has been the subject of many investigations that have led to commercial products. In the early 1990's, extensive work including some at SREC was conducted to determine if *B. bassiana* could control whiteflies in cotton. With the advent of the neonicitinoids, this work was halted. However, work with *B. bassiana* against Lygus continued in Arkansas (Steinkraus and Tugwell 1997), Idaho (Noma and Strickler 1999, 2000, and Vermont (Liu et al 2002, 2003). In all situations, the commercial product seemed to be effective under laboratory conditions but failed to control Lygus in the field. However, a strain of *B. bassiana* isolated from *L. lineolaris* (Steinkraus and Tugwell 1997) did a pretty good job of controlling *L. lineolaris* in the field suggesting that isolates of the fungus collected from the host in its habitat may be more effective than other strains. Similarly Liu et al. (2002) demonstrated significant pathogenicity differences among *B. bassiana* isolates against *L. lineolaris.* During 2001 and 2002 surveys of California *L. hesperus* populations revealed the presence of relatively high numbers of *B. bassiana* infected individuals (McGuire et al. 2001, McGuire 2002). In some samples, more than half the collected bugs were infected but, typically, less than 10% of the population was affected. These data suggest that *B. bassiana* may play an important role in nature in regulating *L. hesperus* populations but, more importantly, the fungus may be used to proactively control the population before economic damage occurs. The fact that any *B. bassiana* was discovered under the hot dry conditions of the SJV is surprising and the strains have yielded interesting and important differences from most known strains of the fungus. Work in our laboratory and in the Stoneville facility has produced evidence to suggest the new strains recently isolated from California *L. hesperus* populations and Mississippi *L. lineolaris* populations may be better adapted to the target pest than the commercial strain. Some assays revealed 100 fold higher activity against *L. hesperus* than the commercial strain. In addition, local strains could grow at temperatures as high as 35°C whereas the commercial strain did not grow at temperatures above 32° C. These laboratory assays only go so far to determine potential of the new strains as effective mycoinsecticides and field testing is a necessity.