A Novel bioassay using a non-autoclaved solid Lygus diet to evaluate the effect of *Beauveria Bassiana* and the insect growth regulator novaluron on tarnished plant bug, *Lygus lineolaris*

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Summary

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) is an important economic pest for a range of crop hosts; including cotton, alfalfa, safflower, and beet. Infection and growth inhibition of *L. lineolaris* after treatment with *Beauveria bassiana* or novaluron (Diamond) were study using a non-autoclaved solid Lygus diet. The effects of the treatments on growth, survival, feeding, and infection are presented and discussed.

Introduction

The tarnished plant bug, TPB *Lygus lineolaris* (Palisot de Beauvois), attacks a wide variety of economically important herbaceous plants, vegetable crops, commercial flower plants, fruit trees, and nursery stock (Kelton 1975). Half of the cultivated plant species grown in the United States are listed as host plants for tarnished plant bugs (Capinera 2001). Effective management of TPB in cotton is complicated due to its mobility. Its control has been solely based on insecticides, and insecticide-resistant populations of tarnish plant bug have been reported in the Delta region (Snodgrass, 1996). Utilization of the entomopathogenic fungal, *Beauveria bassiana* to control TPB in cotton is being study. This study was conducted in order to develop a method for determining the effect of the NI8 strain of *B. bassiana* and the insect growth regulator novaluron on fecundity and growth inhibition of the TPB using artificial diet.

Results

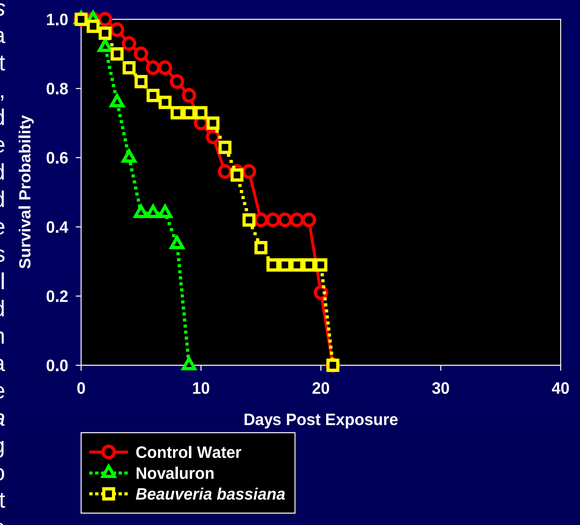

Figure 1.Survival of second instar of *Lygus lineolaris* exposed to *Beauveria bassiana* and the Insect growth regulator Diamond fed with artificial diet.

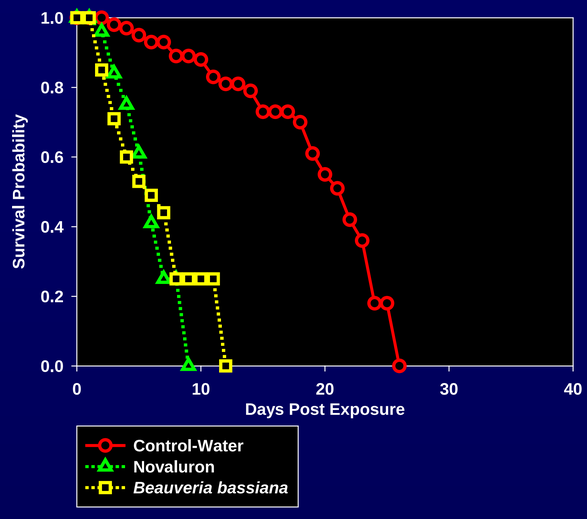

Figure 2.Survival of third instar of *Lygus lineolaris* exposed to *Beauveria bassiana* and the Insect growth regulator Diamond fed with artificial diet .

Materials and Methods

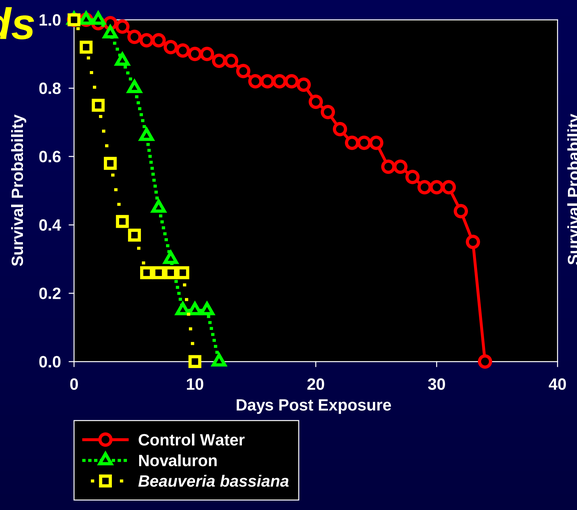

Figure 3.Survival of fourth instar of *Lygus lineolaris* exposed to *Beauveria bassiana* and the Insect growth regulator Diamond fed with artificial diet.

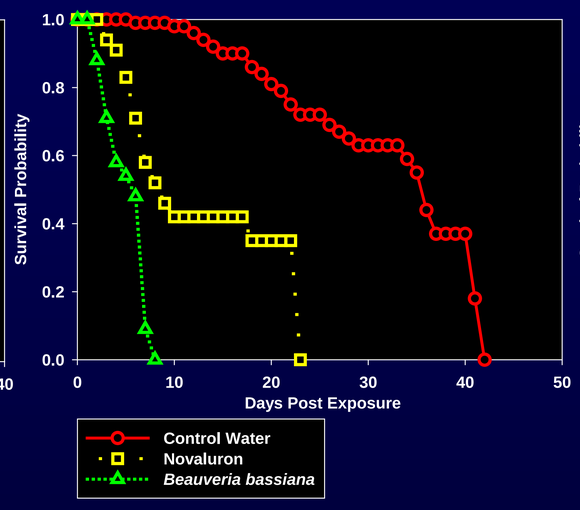

Figure 4.Survival of fifth instar of *Lygus lineolaris* exposed to *Beauveria bassiana* and the Insect growth regulator Diamond fed with artificial diet.

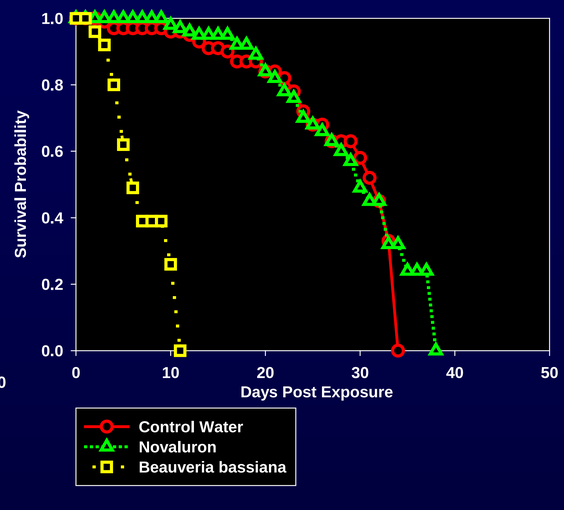

Figure 5.Survival of adults of *Lygus lineolaris* exposed to *Beauveria bassiana* and the Insect growth regulator Diamond fed with artificial diet.

Fungal Isolate

The NI8 isolate of *B. bassiana* was obtained from the Collection of USDA-ARS-SIMRU (Stoneville, MS) and was produced in a biphasic culture system that simulated industrial scale production according to the method described for solid substrate fermentation of *B. bassiana.* Aliquots of 6 ml of a solution of 0.5 g of harvested spore powder in 50 ml of 0.04% Sylwet L77 water solution ($6x10^9$ conidia suspension) were used to inoculate the TPB

Bioassay Procedure

A single concentration screening assay was carried out to evaluate longevity, mortality, infection, and growth inhibition. Twenty groups of ten insects (4 groups (replicate) / *L. lineolaris* stage) 2-I, 3-I, 4-I, and 5-I 1-2 d-old, and mixed-sex 2 days old T *L. lineolaris* were sprayed with 6 ml of water control, 6 ml of *B. bassiana* strain NI8 solution ($6x10^9$ conidia), and 6 ml of the growth regulator novaluron solution (1.44 ml of novaluron solution in 50 ml of 0.04% Sylwet L77 water solution). The application for all treatments used a specially-designed spray tower. After application, adult and nymphs were released in a insect observation cage and knocked down individually into a solo cup with solid diet. Adults and nymphs were examined daily for mortality and for molting in nymphs. Insects sprayed with *B. bassiana* that molted were transferred to a new cup to avoid contact with the infected exuviae. Dead insects were kept in the same cup and were daily checked for sporulation. Adults and nymphs of *L. lineolaris* were held in an environmental room at 27ºC, 65% RH, and 12: 12 (L:D) h photoperiod. Insects were kept until all were dead.

Table 1. Molt and Mortality percentage *L. lineolaris* exposed *to Beauveria bassiana* and the growth regulator Diamond fed with solidified Lygus diet

Treatment	Immature Development							
	Second Instar		Third Instar		Fourth Instar		Fifth Instar	
	Molt (%)	Mortality Day 10 (%)	Molt (%)	Mortality Day 10 (%)	Molt (%)	Mortality Day 10 (%)	Molt (%)	Mortality Day 10 (%)
Water -Control	89.7 0.49 a	51.3 0.49 c	92.5 0.42 a	47.5 0.57 c	100 a	20.0 0.64 b	100 a	5.0 0.34 b
Diamond	30.0 0.73 b	87.5 0.52 a	10.0 0.48 c	100 a	12.5 0.52 c	97.5 0.25 a	22.5 0.66 c	92.5 0.42 a
Beauveria bassiana	92 .7 0.42 a	53.7 0.72 b	45.0 0.79 b	62.5 0.77 b	35.0 0.48 b	90.0 0.48 a	80.0 0.64 b	90..0 0.48 a

Means within a column followed by the same letter were not significantly different at P = 0.005, Tukey's test

Statistical Analysis

The experiment was set up as a completely randomized design with a factorial arrangement 3 x 5 x 3 for mortality and 3 x 5 for longevity and molt (three treatments: water (control), *B. bassiana*, and novaluron; five stages of TPB; and three evaluation times: Day-3 (D-3), Day-5 (D-5), and Day-10 (D-10)). Each treatment combination was repeated four times. Statistics were performed using SAS system software (SAS Institute, 2008). Non-parametrics estimates of the survival functions of *L. lineolaris* stages were compared among treatments using the LIFETEST procedure of SAS. The analyses controlled for repetitions of the experiment using the strata statement and insect development was included as a covariate in the test statement (Allison 1995). Statistical differences in the TPB stages survival among the treatment were declared based on the Log-rank statistic. Mortality, longevity, fungal infection, sporulation, and molt was analyzed using the PROC GLM procedure to detect differences between treatments.

Discussion

A non-autoclaved solid diet was used to evaluate the entomopathogenic fungus *Beauveria bassiana* strain NI8 and the growth regulator novaluron (Diamond ®) for control of the tarnished plant bug (TPB) *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae). The diet was composed of toasted wheat germ, ground lima bean meal, soy flour, yolk chicken eggs, inhibitor and agar. It was prepared in one step by blending the ingredients in heated boiling water. The diet was used to bioassay TPB from the second instar to the adult stage. Fourth instar (97.5 SE 0.02), fifth instar (95.0 SE 0.03) and adults (95 SE 0.03) of TPB were more susceptible (infection %) than second (52.5 SE 0.07) and third instar (85.0 SE 0.05) to *B. bassiana*; while, second instar (100), third instar (100) and forth instar (97.5 SE 0.02) had higher mortality than fifth instar (92.5 SE 0.04) after ten days of novaluron exposure. No effects on longevity (days) were observed in adults (21.57 SE 0.9) treated with novaluron when compared with the control (20.47 SE 1.2), but longevity was significantly different when compared with adults exposed to *B. bassiana* (5.2 SE 0.2). Adults of TPB were maintained for over a month without changing the diet. The non-autoclaved diet is semi-liquid before it cools which facilitate the mechanics of diet packaging similar to food packaging or lepidopteran diet preparation. The solid artificial diet for Lygus bugs provides improved research capacity for studying the ecology and susceptibility of insects to a number of different control agents including beneficial organisms, insect pathogens and insecticidal toxins being developed for transgenic technologies.

Acknowledgements

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