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FORMULATION OF THE ENTOMOPATHOGENIC FUNGUS, *BEAUVERIA BASSIANA*, WITH RESISTANCE TO UV DEGRADATION FOR CONTROL OF TARNISHED PLANT BUG, *LYGUS LINEOLARIS*

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*The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Abstract

Formulations are being developed for protecting entomopathogenic fungi from solar radiation to improve mycoinsecticide efficacy. *Beauveria bassiana* (GHA) spores were coated by spray drying with either water-soluble lignin or water-insoluble Ca^{2+}-cross-linked lignin. These coated spores were suspended in either water (0.04% Silwet L77) or oil (Orchex 692) and compared with non-coated spores in water or oil to demonstrate the impact of the coating on spore survival under simulated solar radiation and pathogenicity to tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). The rate of spore mortality under simulated solar radiation was approximately ten times lower for the three formulations in which spores in suspension remained coated with lignin (cross-linked lignin in water, lignin in oil, and cross-linked lignin in oil). The pathogenicity of the six formulation strategies did not differ significantly on the basis of LC_{50} values for direct spray applications to *L lineolaris* adults. However, the three lignin coated formulations that provided the greatest UV protection were slower to kill *L. lineolaris* (higher LT_{50} values). Adult *L. lineolaris* mortality was approximately 20 times lower when exposed to broccoli florets treated with *B. bassiana* formulations than when *L. lineolaris* was sprayed directly. In bioassays with treated broccoli florets, non-coated spores in 0.04% Silwet L77 were the most pathogenic. Under field conditions where solar radiation significantly impacts mycoinsecticide efficacy, the improved persistence of lignin coating formulations may outweigh the negative effects of reduced pathogenicity.

Introduction

Economic losses from tarnished plant bugs in reduced yield and control costs were approximately $70 million for U.S. cotton in 2002, and most of the chemical insecticides applied to cotton were intended to control this insect pest (Williams, 2003a, b). Options for controlling *Lygus lineolaris* (Palisot de Beauvois) are limited to a relatively narrow range of chemical classes; for example only three insecticide classes (organophosphates, carbamates, and chloronicotinyls) were recommended for *L. lineolaris* control in 2003 in Mississippi, with organophosphates being the most used (Layton, 2003). Pyrethroid resistance in *L. lineolaris* was first reported in the delta region of Mississippi in 1993 and later found to be widespread throughout Arkansas, Louisiana, and Mississippi; it included multiple resistance to certain organophosphates, carbamates, and cyclodienes and a seasonal increase from spring to fall (Snodgrass & Elzen, 1995; Snodgrass, 1996; Pankey *et al*., 1996; Hollingsworth *et al*., 1997; Snodgrass & Scott, 2002). Control options not based on insecticides are needed for controlling *L. lineolaris* and these will become increasingly important if further resistance develops to insecticides. Cultural controls (e.g., wild host management, host plant resistance, and trap cropping), sterile male releases, and biological control with predators, parasitoids, and pathogens are being investigated as potential contributors to IPM systems (Ruberson & Williams, 2000; Smith & Nordlund, 2000; Snodgrass *et al*., 2000; Stewart & Layton, 2000). These technologies may be used to target dense *L. lineolaris* populations developing on wild host plants before they migrate to cotton, which may be particularly effective in intensively agricultural regions where wild host plant areas represent a small percentage of the total land area (Robbins *et al*., 2000; Snodgrass *et al*., 1991; Snodgrass *et al*., 2000). Of the microbial biocontrol agents (viruses, bacteria, fungi, and protozoa), entomopathogenic fungi have the greatest potential for controlling sucking insect pests because they have the contact mode of action, infecting through the cuticle rather than requiring ingestion (Tanada & Kaya, 1993).

Two commercial *B. bassiana*-based mycoinsecticides have been evaluated against *L. lineolaris*, with Mycotrol (Emerald Bioagriculture) causing greater mortality than Naturalis-L (Fermone Corp.) in field trials on cotton and in laboratory bioassays (Snodgrass & Elzen, 1994; Steinkraus & Tugwell, 1997; Leland, unpublished data). In caged insect field trials, *B. bassiana* (ARSEF 3769) caused similar mortality when *L. lineolaris* was directly sprayed or exposed indirectly to sprayed cotton plants, but mortality through indirect exposure was reduced by approximately 50% after 24h indicating the potential for improving efficacy by increasing persistence (Steinkraus & Tugwell; 1997).

Persistence may be improved by mitigating the impact of environmental stress through formulations. Two of the most important environmental factors effecting mycoinsecticide efficacy are adequate moisture for infection and degradation of spores by exposure to solar radiation, both of which may be addressed through formulation (Burgess, 1998; Ignoffo, 1992). Oil-based formulations may be used to improve mycoinsectide efficacy in drier climates (Bateman *et al*., 1993; Prior *et al*., 1988), provide formulations compatible with ultralow volume (ULV) application technology for large control areas (Burgess, 1998), improve thermal stress tolerance (McClatchie *et al*., 1994; Hedgecock *et al*., 1995; Hong *et al*., 1997, 1998, 2000), and survival under simulated solar radiation (Alves *et al*., 1998; Moore *et al*., 1993). Most of the research on protecting spores from solar radiation in oil formulations has focused on oil soluble sunscreens, which generally demonstrated promise in laboratory bioassays on glass under simulated solar radiation (Hunt *et al*., 1994; Moore *et al*., 1993), but failed to protect spores on natural hydrophobic surfaces or improve efficacy in field trials (Burgess, 1998; Inglis *et al*., 1995; Shah *et al*., 1998). Encapsulation techniques used to protect baculovirus in cross-linked lignin coatings (Shasha *et al*., 1998; Tamez-Guerra *et al*. 2000; Behle *et al*. 2003) may be adapted for mycoinsectides. Coating spores with a water-soluble spore coatings, such as non-cross-linked lignin derivates, could be used in oil carriers to provide protection from solar radiation and potentially not interfere with infectivity (Leland, 2001).

This study investigated formulations using *B. bassiana* (GHA) spores coated with lignin or cross-linked lignin, suspended in aqueous or oil carriers. Spore survival under simulated solar radiation, and pathogenicity of these formulations to adult *L. lineolaris* in direct spray bioassays, and in bioassays of adult *L. lineolaris* placed on sprayed broccoli florets were evaluated. Comparisons were made to non-coated spores in aqueous and oil carriers.

Materials and Methods

Coating *B. bassiana* Spores By Spray Drying

Two *B. bassiana* formulations were prepared with the ingredients at concentrations described in Table 1: *Beauveria bassiana*, strain GHA was provided as technical grade spore powder at 1.49 x 10^{11}spores/g (Lot 99-03-2, Emerald BioAgriculture Corp., Butte, MT formerly Mycotech Corp.). One formulation received $CaCl_2$ which cross linked the lignin thus rendering the formulation water insoluble. The other formulation received NaCl, which did not form cross-linking bonds; thus the formulation was water soluble.

Formulations were spray dried using a Niro Atomizer Spray Dryer (Niro Atomizer, Inc., Columbia, MD). Drying conditions included 93-122°C inlet temperature, 52-64°C outlet temperature, 20 mL/min feed rate, and 4.4-5.0 kg/cm^{2} air pressure. The formulation powders were collected in glass jars and stored at room temperature until sampled for spore germination and insecticidal activity assays. The two formulations were prepared seven times and spray dried to powders for use among the following experiments.

Spore Germination and Concentration

Two techniques were used to evaluate germination. Prior to shipment, viablility of spores was determined based on percent germination after shaking spores at 1 x 10^{7} spores/mL for 14h at 28º C in 50mL nutrient broth (2g/L yeast extract and 2g/L sucrose). After shipment to USDA-ARS, SIMRU, Stoneville, MS spore germination was determined by rehydrating spores for 1h at 100% relative humidity, suspending in 0.04% Silwet L77 to 1 x 10^{6} spores/mL, and incubating for 24h on germination agar (20g/L malt extract, 0.02g/L Benlate® 50 WP, 0.2g/L chloramphenicol, 15g/L agar) (Moore *et al*., 1997; Milner, 1991;Goettel & Inglis, 1997). Glass cover slips were pressed into the agar and percent spore germination was determined by observing 200 spores per treatment at 1000 × magnification. Dry spore formulations were stored at 4°C and germination was determined using the same methods prior to each experiment.

To evaluate the rate at which cross-linked lignin released spores in 0.04% Silwet L77, the concentration of visible spores in the cross-linked lignin formulation was determined by hemacytometer after soaking for 3min, 5h, 16.5h, and 23h. Then 10 M, NaOH was added to formulation subsamples to increase the pH of the suspension to 14 and dissolve remaining particulate lignin and the spore concentrations determined. The concentrations of spores were calculated on a liquid volume basis using a hemacytometer and then the total number of spores was divided by the mass of the subsample prior to rehydration to determine spores/g in each formulation.

Exposure to Solar Radiation

Solar simulation experiments were conducted to evaluate the survival of formulated *B. bassiana* following exposure to simulated solar radiation using an Oriel Solar Simulator (Model 91193) equipped with a 1000W Xenon Arc Lamp corrected with air mass 0 and air mass 1 filters to simulate direct noon sunlight. The following six formulations were evaluated: 1) non-coated spores in 0.04% Silwet L77; 2) non-coated spores in oil (Orchex 692); 3) lignin-coated spores in 0.04% Silwet L77; 4) lignin-coated spores in oil; 5) cross-linked lignin coated spores in 0.04% Silwet L77; and 6) cross-linked lignin coated spores in oil. Spore suspensions (10mL of 1x10^{7} spores/mL) were deposited onto nylon membrane filters (Magna, 0.45µm pore size, 47mm diameter) by vacuum filtration (Advantec MFS, Inc.). Subsamples were exposed to different time intervals of simu-

lated solar radiation at 19.1 ± 0.5 mW/cm^2 based on expected protection provided by each formulation. Non-coated spores in 0.04% Silwet L77, non-coated spores in oil, and lignin-coated spores in 0.04% Silwet L77; were exposed to 1, 2, 3, 4, 5, 6, 7, and 8.5h of simulated solar radiation. Lignin-coated spores in oil, cross-linked lignin coated spores in 0.04% Silwet L77, and cross-linked lignin coated spores in oil were exposed to 4, 8.5, 12, 17, 20, 24, 36, and 48h of simulated solar radiation. After exposure, spores were rehydrated for 1h at 100% r.h. suspended in 2mL of 0.04% Tween 80 and 100mL aliquots were spread on two 60mm Petri dishes containing germination agar (20g/L malt extract; 15g/L agar; 0.02g/L Benlate 50WP; 0.2 g/L chloramphenicol; 200,000 units penicillin/L; 0.2g/L streptomycin). Spores were killed with lactophenol acid fuschin mounting media (phenol 20g; lactic acid 20g; glycerol 40g; acid fuschin 0.1g; water 20mL) after a 48h incubation period and spore germination was evaluated as described previously.

Direct Spray Bioassays with *L. lineolaris*

Adult *L. lineolaris* of unknown age were collected from a native stand of horseweed [*Conyza canadensis* (L.) Cronquist] at a single location on August 23, 2003. The six spore formulations of *B. bassiana* described in the solar simulation experiments were applied to *L. lineolaris* in 1mL of water or oil using a Potter spray tower to produce 1.7×10^3, 5.4×10^3, 1.7×10^4, 5.4×10^4, and 1.7×10^5 viable spores/cm^2. Three replicates of 10 insects held in 10cm Petri dishes moistened with 700µL water were exposed to each concentration of each formulation. Nine replicates of ten *L. lineolaris* were exposed to either 0.04% Silwet L77 or Orchex 692 to serve as water and oil controls, respectively. After being sprayed, insects were placed individual into 30mL medicine cups capped with 45mm foam plugs (Scimart) each containing a single broccoli floret. Insects were incubated at 25°C, 80 ± 5 % relative humidity, with a photoperiod of 14h light : 10h dark. Mortality was determined daily and broccoli was changed at 2-d intervals. After a 10d incubation period, all of the insects in the bioassay were surface sterilized in 10% ethanol and 0.525% sodium hypochlorite (10% household bleach) and incubated in 96 well plates (0.4mL wells, Steriline) containing 170µL of water agar (15 g/L agar) in each well for 72h for sporulation (Noma & Strickler 2000).

Broccoli Residue Bioassays with *L. lineolaris*

Methods for exposing *L. lineolaris* to broccoli florets treated with formulated *B. bassiana* were similar to those described in the direct spray bioassay above. Adult *L. lineolaris* of unknown age were collected from the same native stand of horeseweed (*C. canadensis*) as those used for the direct spray bioassay on August 8, 2003. Petri dishes (10cm) containing 10 broccoli florets (1.5 ± 0.4 g) were exposed to the six formulations described in the second solar simulation experiment and direct spray bioassay. Spore concentrations were calibrated to deliver 5.4×10^3, 1.7×10^4, 5.4×10^4, 1.7×10^5, and 5.4×10^5 viable spores/cm^2 using the Potter spray tower as described in direct spray bioassays. Florets were transferred to individual 30mL medicine cups with 45mm foam plugs (Scimart) and insects were held (1 per cup) on these treated florets for 48h and then for 8d on untreated florets under conditions describe above. Mortality was determined daily and, at the end of the 12d incubation time, all of the insects were surface sterilized and evaluated for sporulation after 72h on water agar as described in the direct spray bioassay.

Results and Discussion

Spore Germination and Concentration

Dry formulations of non-coated spores, lignin-coated spores, and cross-linked lignin-coated spores contained 1.2 (± 0.1) x 10^{11}spores/g, 3.9 (± 0.6) x 10^{10}spores/g, and 4.6 (± 0.6) x 10^{10}spores/g, respectively. Viability of non-coated spores, lignin-coated spores, and cross-linked lignin coated spores after spray drying based on 14-h shake flask germination tests were 91.7, 56.4, and 59.6%, respectively. Reduced spore concentrations and germination percentages were comparable with many previous preparations using this formulation technique (Behle, unpublished data). Viability of *B. bassiana* spores in lignin-coated and cross-linked lignin coated formulations after spray drying and shipment based on 24h on germination agar were 51 ± 1 and 77 ± 3%, respectively. This viability was stable for 5 months storage at 4°C, at which time spore viability in non-coated spores, lignin-coated spores, and cross-linked lignin coated spores were 98 ± 1, 52 ± 6, and 64 ± 7%, respectively.

The cross-linked lignin coating dissolved slowly in 0.04% Silwet L77. The concentration of visible spores increased from 1.3 (± 0.3) x 10^{10} spores/g 3 min after suspension in 0.04% Silwet L77 to 4.0 (± 0.3) x 10^{10} spores/g after 23h of soaking at a 'release' rate of 1.2 x 10^9 spores/g/h (R^2 = 0.998). After the addition of NaOH to dissolve residual lignin, spore concentrations were 4.6 (± 0.6) x 10^{10} spores/g. This indicated that approximately 28% of the spores in the cross-linked lignin formulation became uncoated almost immediately in the presence of water and 87% become uncoated slowly over a 23h period.

Exposure to Solar Radiation

Germination of *B. bassiana* spores following exposure to simulated solar radiation was greatest for the three formulations where spores remained coated in suspension, which included cross-linked lignin in water, cross-linked lignin in oil, and lignin in oil (Table 2). The rates of spore mortality in these formulations were approximately 10 times lower and LT_{50} estimates up to 15 times higher than for the three formulations where spores were not coated in suspension; non-coated spores in water, non-coated spores in oil, lignin-coated spores in oil. The physical appearance of the cross-linked lignin in water formulation differed from formulations with cross-linked lignin in oil or lignin in oil. The cross-linked lignin formulation did not remain as distinct spheres of lignin-coated spores on the filter membrane's surface and in places formed a crust of lignin.

Bioassays with *L. lineolaris*

The highest LC_{50} and LC_{75} values were observed in the three formulations in which spores remained coated in suspension; cross-linked lignin in water, cross-linked lignin in oil, and lignin in oil. However, these LC estimates were not significantly different because of large confidence limits (Table 3). The slopes for concentration mortality regression lines were shallow relative to those generally observed for chemical insecticides; a characteristic of mycoinsecticides that often results in large 95% confidence intervals around LC estimates. The lower pathogenicity of these three formulations could be statistically discerned on the basis of LT_{50} values particularly at 5.4×10^3 and 1.7×10^4 spores/cm^2 (Table 4). The percentage of Abbott's corrected mortality that sporulated was 93% across all formulations which indicated that Abbott's mortality accurately reflected mortality caused by *B. bassiana*. Sporulation was observed in insects that died up to the end of the 10d incubation period and in some insects surviving 10d after exposure to *B. bassiana*, but not in controls. Sporulation was also observed in insects that were alive at the end of the experiment after they were killed and placed on water agar, but not in controls. Mortality caused at later days post inoculation may not be relevant for controlling insect pests in crops if crop damage occurs prior to mortality (Noma & Strickler, 2000), and synergists may be needed to reduce LT values from mycoinsecticides to acceptable levels in these crop situations (Steinkraus & Tugwell, 1997). However, when controlling populations on wild host plants, before emigration to crops, these high LT values may be acceptable, particularly if oviposition rates are reduced by infection as demonstrated in *L. hesperus* (Noma & Strickler, 2000).

The pathogenicity of formulations to *L. lineolaris* when exposed to residues on broccoli florets was approximately 20 times lower than when the formulations were sprayed directly onto insects (Table 3). Spores applied to broccoli florets in 0.04% Silwet L77 generally had the lowest LC_{25} and LC_{50} values among the formulations, and other formulations were difficult to separate on the basis of LC values. The higher pathogenicity of spores in 0.04% Silwet L77 was also reflected in LT_{50} values (Table 4). There was not a consistent difference among the LT_{50} values of the remaining formulations, particularly at the highest two concentrations where LT_{50} values were within the time frame of the bioassay (12d). If the potential for uptake from treated plant surfaces is similarly low under field conditions as in laboratory assays on broccoli, it will be important to ensure adequate coverage through application technology to hit insects directly with sprayed spores. In this case the benefits of longer spore persistence provided by protective coatings will be less important. However, other studies have indicated that indirect uptake of *B. bassiana* from cotton plants may contribute significantly to Lygus control (Steinkraus & Tugwell, 1997). The tightly packed florets on broccoli may not allow for sufficient contact between *L. lineolaris* and the plant surface or allelochemicals in broccoli may be adversely affecting *B. bassiana* activity. Further work is needed to evaluate the pathogenicity of formulations on treated wild host plants.

Conclusions

Formulations in which spores remain coated with lignin in suspension greatly improved spore survival under solar radiation but, these formulations were less pathogenic to *L. lineolaris*. The small reduction in pathogenicity would likely be outweighed by the large improvements in persistence in control situations where solar radiation significantly reduced mycoinsecticide efficacy. However, if the contribution to *L. lineolaris* mortality by uptake from contaminated wild host plants is similar to uptake from broccoli in laboratory bioassays, then improvements in persistence may not greatly improve efficacy. Rather, strategies for improving pathogenicity and coverage to ensure kill through direct contact should be considered. Further work is needed to determine if the contribution of uptake from wild host plant surfaces is more significant and evaluate the impact of solar radiation on formulated spores applied to these plants. The use of water-soluble lignin coatings in oil rather than cross-linked lignin coatings did not greatly improve pathogenicity. Until spore survival during spray drying can be improved for the lignin-coated formulation to at least the levels observed for cross-linked lignin formulations it will not be a practical option. Improvements in spore survival during spray drying would make coating strategies more practical. In insect control situations were solar radiation significantly reduces mycoinsecticide efficacy and application with high volume water carriers are appropriate, cross-linked lignin coatings in water show the greatest promise. In insect control situations where solar radiation is a significant factor and ultralow volume applications in oil carries are appropriate, either cross-linked lignin coatings or lignin coatings could be used, particularly if spore survival during spray drying were improved for the non-cross-linked lignin formulations.

Although the advantages of these formulations for *L. lineolaris* control still needs to be demonstrated in field trials, this study demonstrates the potential of coated-spore formulation strategies for greatly improving spore survival under solar radiation exposure.

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References

Alves, R. T., Bateman, R. P., Prior, C., and S. R. Leather. 1998. Effects of simulated solar radiation on conidial germination of *Metarhizium anisopliae* in different formulations. Crop Protection. 17 (8): 675-679.

Bateman, R. P., Carey, M., Moore, D., and C. Prior. 1993. The Enhanced Infectivity of *Metarhizium flavoviride* in oil formulations to Desert Locusts at Low Humidities. Ann. Appl. Biol. 122. 145-152.

Behle, R. W., Tamez-Guerra, P., and M. R. McGuire. 2003. Field activity and storage stability of *Anagrapha falcifera* nucleiopolyhedorvirus (*Af*MNPV) in spray-dried lignin-based formulations. J. Economic Entomol. **96**, 1066-1075.

Burgess, H. D. 1998. Formulation of microbial biopesticides. Kluwer Academic Publishing, Dordrecht, The Netherlands.

Finney, D. J. Probit Analysis 3rd ed. Cambridge University Press. (1971) 333 pp.

Goettel, M. S. and D. G. Inglis. 1997. Fungi: hyphomycetes. pgs 213-249. In. Lacey, L. ed. Manual of Techniques in Insect Pathology. Acedemic Press. London. 409 pp.

Hedgecock, S., Moore, D., Higgins, P. M., and C. Prior. 1995. Influence of moisture content on temperature tolerance and storage of *Metarhizium flavoviride* conidia in an oil formulation. Biocontrol Sci. and Technol. 5: 371-377.

Hollingsworth, R. G., Steinkraus, D. C., and N. P. Tugwell. 1997. Response of Arkansas populations of tarnished plant bugs (Heteroptera: Miridae) to insecticides, and tolerance differences between nymphs and adults. J. Econ. Entomol. **90**, 21-26.

Hong, T. D., Ellis, R. H., and D. Moore. 1997. Development of a model to predict the effect of temperature and moisture on fungal spore longevity. Ann. Botany. 79: 121-128.

Hong, T. D., Jenkins, N. E., and R. H. Ellis. 2000. The effects of duration of development and drying regime on the longevity of conidia of *Metarhizium flavoviride*. Mycol. Res. 104 (6): 662-665.

Hong, T. D., Jenkins, N. E., Ellis, R. H., and D. Moore. 1998. Limits to the negative logarithmic relationship between moisture content and longevity in conidia of *Metarhizium flavoviride*. Ann.Botany. 81: 625-630.

Hunt, T. R., Moore, D., Higgins, P. M., and C. Prior. 1994. Effect of sunscreens, irradiance and resting periods on the germination of *Metarhizium flavoviride* conidia. Entomophaga. 39 (3/4): 313-322.

Ignoffo, C. M. 1992. Environmental factors affecting persistence of entomopathogens. Florida Entomol. 75 (4): 516-524.

Inglis, G. D., Goettel, M. S., and D. L. Johnson. 1995. Influence of ultraviolet light protectants on persistence of the entomopathogenic fungus, *Beauveria bassiana*. Biol. Control. 5: 581-590.

Layton, B. 2003. 2003 Cotton Insect Control Guide. Publication 343 of the Extension Service of Mississippi State University.

Leland, J. E. 2001. Environmental-Stress Tolerant Formulations of *Metarhizium anisopliae* var. *acridum* for Control of African Desert Locust (*Schistocerca gregaria*). Ph.D. Dissertation. Virginia Polytechnic Institute and State University.

McClatchie, G., Moore, D., Bateman, R. P., and C. Prior. 1994. Effects of temperature on the viability of the conidia of *Metarhizium flavoviride* in oil formulations. Mycol. Res. 98: 749-756.

Milner, R. J., Huppatz, R. J., and S. C. Swaris. 1991. A new method for assessment of germination of Metarhizium conidia. J. Invert. Pathol. 57, 121-123.

Moore, D., Bridge, P. D., Higgins, P. M., Bateman, R. P., and C. Prior. 1993. Ultra-violet radiation damage to *Metarhizium flavoviride* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. Ann. Appl. Biol. 122. 605-616.

Moore, D., Langewald, J., and F. Obogon. 1997. Effects of rehydration on the conidial viability of *Metarhizium flavoviride* mycopesticide formulations. Biocontrol Sci. Technol. 7: 87-94.

Noma, T. and K. Strickler. 2000. Effects of *Beauveria bassiana* on *Lygus hesperus* (Hemiptera: Miridae) feeding and oviposition. Environmental Entomology. 29 (2): 394-402.

Pankey, J. H., B. R. Leonard, J. B. Graves, and E. Burris. 1996. Toxicity of acephate, cypermethrin, and oxamyl to tarnished plant bugs inn vial bioassays and cage studies on cotton, pp. 882-887. *In* Proceedings Beltwide Cotton Production Research Conference, Nashville, TN. National Cotton Council, Memphis TN

Prior, C., Jollands, P., and G. le Patourel. 1988. Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). J. Invert. Pathol. 52: 66-72.

Robbins, J. T., Snodgrass, G. L., and F. A. Harris. 2000. A review of wild host plants and their management for control of the tarnished plant bug in cotton in the southern U.S. Southwest. Entomol. Suppl. 23: 21-26.

Ruberson, J. R. and L. H. Williams. 2000. Biological control of Lygus spp. – a component of area wide management. Southwest. Entomol. Suppl. 23: 96-110.

SAS Institute. 1989. SAS/STAT user's guide, version 6, 4th ed., vol. 2. SAS Institute, Cary, N.C.

Shah, P. A., Douro-Kpindou, O.-K., Sidibe, A., Daffe, C. O., Van Der Pauuw, H., and C. J. Lomer. 1998. Effects of the sunscreen oxybenzone on field efficacy and persistence of *Metarhizium flavoviride* conidia against *Kraussella amabile* (Orthoptera: Acrididae) in Mali, West Africa. Biocontrol Sci. Technol. **8**, 357-364.

Shasha, B. S., McGuire, M. R., and R. W. Behle. 1998. Lignin-based pest control formulations. US Patent 5750467

Smith, R. A. and D. A. Nordlund. 2000. Mass rearing technology for biological control agents of *Lygus* spp. Southwest. Entomol. Suppl. 23: 121-127.

Snodgrass, G. L. 1996. Pyrethroid resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. J. Econ. Entomol. 89: 783-790.

Snodgrass, G. L. and G. W. Elzen. 1995. Insecticide resistance in a tarnished plant bug population in the Mississippi Delta. Southwest. Entomol. 20: 317-323.

Snodgrass, G. L. and W. P. Scott. 2002. Tolerance to acephate in tarnished plant bug (Heteroptera: Miridae) populations in the Mississippi river delta. Southwest. Entomol. 27 (2): 191-199.

Snodgrass, G. L., and G. W. Elzen. 1994. Efficacy of Naturalis-L for adults and nymphs of the tarnished plant bug in cotton. Proceedings/Beltwide Cotton Conferences. v. 2: 1103-1104.

Snodgrass, G. L., Scott, W. P., Robbins, J. T., and D. D. Hardee. 2000. Area wide management of the tarnished plant bug by reduction of early-season wild host plant density. Southwest. Entomol. Suppl. 23: 59-67.

Snodgrass, G. L., Stadelbacher, E. A., and J. W. Smith. 1991. Distribution and abundance of early season wild host plant and bollworm and tobacco budworm populations (Lepidoptera: Nuctuidae) in an intensively cropped area of the mid-delta of Mississippi. J. Entomol. Sci. 26: 9-16.

Steinkraus, D. C. and N. P. Tugwell. 1997. *Beauveria bassiana* (Deuteromycotina: Moniliales) effects on *Lygus lineolaris* (Hemiptera: Miridae). J. Entomol. Sci. 32 (1): 79-90.

Stewart, S. D. and M. B. Layton. 2000. Cultural controls for the management of *Lygus* populations in cotton. Southwest. Entomol. Suppl. 23: 83-95.

Tamez-Guerra, P., McGuire, M. R., Behle, R. W., Hamm, J. J., Sumner, R. H., and S. S. Shasha. 2000. Sunlight persistence and rainfastness of spray-dried formulations of baculoviruses isolated from *Anagrapha falcifera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 93, 210-218.

Tanada, Y. and H. K. Kaya. 1993. Insect Pathology. Academic Press, San Diego.

Williams, Michael, R. 2003a. Cotton Insect Losses – 2002. pp. 1208-1216. *In* Proceedings Beltwide Cotton Production Research Conference January 6-10, Nashville, TN. National Cotton Council, Memphis TN

Williams, Michael, R. 2003b. Cotton Insect Loss Estimates – 2002. pp. 1217-1273. *In* Proceedings Beltwide Cotton Production Research Conference January 6-10, Nashville, TN. National Cotton Council, Memphis TN.

Table 1. Ingredients for Spray-Dried (Cross-Linked and Non-cross Linked) Lignin-Based Formulations of *Beauveria bassiana* (strain GHA).

	Formulation Components	
Ingredients	**Lignin Coating**	**Cross-Linked Lignin Coating**
Technical spore powder	10g	10g
Water	87mL	87g
Corn oil	2g	2g
Nixtamalized corn flour	3.4g	3.4g
PC-1307 Lignin solution, 100 g/L	67mL	67g
KH_2PO_4 solution, 100 g/L	2mL	2g
$CaCl_2$ solution, 52.5 g/L	0	26.8mL
NaCl solution, 41.6 g/L	26.6mL	0

Table 2. Germination of *Beauveria bassiana* (strain GHA) in Six Formulations within 48h of Incubation Following Exposure to Increasing Time Intervals of Simulated Solar Radiation.

Spore Coating	Slope ± S. E.[3]	RT_{50} (h)[4]	95% CL	χ^2	$P > \chi^2$
Water Suspensions					
Non Coated	0.55 ± 0.04	3.6	3.3 - 3.9	201.8	< 0.0001
Lignin	0.51 ± 0.05	4.5	3.8 - 5.0	87.3	< 0.0001
Cross-Linked Lignin	0.05 ± 0.01	39.8	30.8-54.8	25.0	< 0.0001
Oil Suspensions					
Non Coated	0.43 ± 0.05	5.5	5.0 - 6.1	80.1	< 0.0001
Lignin	0.06 ± 0.03	11.5	-340 - 41.4	5.4	0.02
Cross-Linked Lignin	0.04 ± 0.01	25.4	11.2 - 69.5	7.6	0.006

[1, 2] Water = 0.04% Silwet L77 solution and oil = Orchex 692.

[3] Slope represents regression of proportion of non-germinating spores versus exposure time (h).

[4] RT_{50} values representing exposure time (h) required to reduce spore germination to 50% of spore germination prior to exposure to simulated solar radiation (Proc Probit, SAS Institute).

Table 3. Abbott's Corrected Mortality for Adult Tarnished Plant Bugs (*Lygus lineolaris*) Seven Days After Exposure to Six Formulations of *Beauveria bassiana* (strain GHA) Through Direct Spray Contact and Uptake from Contaminated Broccoli Florets.

Direct Exposure to Sprayed Formulation							
Spore Coating	**Slope ± S. E.**[3]	**LC_{50}**[4]	**95% CL**	**LC_{75}**	**95% CL**	**χ^2**	**P > χ^2**
Non-coated spores in water[1]	0.35 ± 0.12	1.9	-0.29 – 3.6	7.1	5.1-11.1	21.1	<0.0001
Lignin-coated spores in water	0.42 ± 0.15	1.7	N/A[5]	6.3	N/A	4.5	0.03
Cross-linked lignin coated spores in water	0.38 ± 0.07	4.6	-9.0 – 27	11.4	5.9-162	11.2	0.0008
Non-coated spores in oil[2]	0.80 ± 0.10	2.4	NA	5.4	N/A	0.78	0.37
Lignin-coated spores in oil	0.70 ± 0.18	4.6	3.6 – 6.4	7.0	5.5-10.2	23.5	<0.0001
Cross-linked lignin coated spores in oil	0.41 ± 0.15	5.1	3.6 - 7.0	9.2	7.2-12.8	33.6	<0.0001
Indirect Exposure to Formulation Sprayed on Broccoli Florets							
Spore Coating	Slope ± S. E.	LC_{25}	95% CL	LC_{50}	95% CL	χ^2	P > χ^2
Non-coated spores in water	0.43 ± 0.13	19.7	11.5-28.0	41.1	32.2 – 57.7	27.2	<0.0001
Lignin-coated spores in water	0.37 ± 0.10	36.5	25.3-58.3	64.4	47.5 – 113.7	19.9	<0.0001
Cross-linked lignin coated spores in water	0.45 ± 0.12	40.1	NA	71.3	NA	2.5	0.1
Non-coated spores in oil	0.61 ± 0.16	40.5	NA	62.8	NA	3.7	0.05
Lignin-coated spores in oil	0.49 ± 0.13	39.0	29.3-54.8	61.0	47.5 – 91.6	14.2	0.0002
Cross-linked lignin coated spores in oil	0.17 ± 0.08	44.3	NA	100.1	NA	3.8	0.05

[1, 2] Water = 0.04% Silwet L77 solution and oil = Orchex 692.

[3] Slope represents regression of proportion of Abbott's corrected mortality (Finney, 1971) versus spore concentration (natural log).

[4,5] LC values expressed as spores ($x10^4$) per cm^2, 95% confidence limits (CL) not presented for P > χ^2 values > 0.02.

Table 4. Abbott's Corrected Mortality (LT_{50} Values) for Adult Tarnished Plant Bugs (*Lygus lineolaris*) After Exposure to Six Formulations of *Beauveria bassiana* (strain GHA) Through Direct Spray Contact and Uptake from Contaminated Broccoli Florets.

Formulation	LT_{50} Values (95% C.I.)[3,4] at Four Spore Concentrations (spores cm-2)					
	1.7×10^3	5.4×10^3	1.7×10^4	5.4×10^4	1.7×10^5	5.4×10^5
Direct Exposure to Sprayed Formulated Spores						
Non-coated spores in water[1]	8.5 (7.7 – 9.4)	8.7 (7.7 – 9.4)	6.8 (6.3 – 7.2)	5.9 (5.5 – 6.3)	4.2 (3.3 – 5.1)	N/A
Lignin-coated spores in water	9.1 (8.6 – 9.7)	7.7 (7.2 – 8.2)	5.8 (5.4 – 6.2)	6.7 (6.3-7.1)	4.2 (3.7 – 4.7)	N/A
Cross-linked lignin coated spores in water	10.2 (9.4 – 11.5)	8.3 (7.8 – 9.0)	8.6 (8.1 – 9.3)	6.3 (6.0 – 6.7)	5.2 (4.3 – 6.1)	N/A
Non-coated spores in oil[2]	19.6 (14.0 –50.1)	9.4 (8.6 – 10.6)	7.3 (6.9 – 7.7)	4.9 (4.6 – 5.1)	4.3 (0.9 – 6.7)	N/A
Lignin-coated spores in oil	31.0 ($Pr>Xi^2$=0.2)	12.7 (10.6 –17.1)	9.5 (8.9 – 10.3)	6.3 (6.0 – 6.8)	4.1 (3.8 – 4.4)	N/A
Cross-linked lignin coated spores in oil	12.6 (10.3– 18.1)	12.6 (10.8-16.5)	8.8 (8.3 – 9.4)	7.0 (6.6 – 7.5)	4.6 (4.2 – 4.9)	N/A
Uptake From Broccoli Florets Sprayed with Formulated Spores						
Non-coated spores in water	N/A	13.1 (12.1-15.2)	10.2 (9.6-11.0)	10.9 (10.2-11.9)	9.7 (9.2-10.2)	6.9 (5.6-8.0)
Lignin-coated spores in water	N/A	14.1 (12.7-17.9)	12.0 (11.2-13.2)	12.2 (11.3-13.6)	10.3 (9.7-11.1)	8.8 (8.3-9.4)
Cross-linked lignin coated spores in water	N/A	12.4 ($Pr>Xi^2$=1)	11.7 (11.2-12.5)	11.4 (10.5-12.8)	9.1 (8.4-10.0)	8.5 (8.1-9.0)
Non-coated spores in oil	N/A	15.7 (13.4-23.6)	27.9 (18.4-160.5)	20.8 (15.4-82.2)	9.7 (9.1-10.4)	8.6 (8.0-9.2)
Lignin-coated spores in oil	N/A	15.8 (13.5-25.8)	14.4 (12.7-18.0)	14.4 (12.7-17.8)	12.0 (11.1-13.5)	8.9 (8.4-9.4)
Cross-linked lignin coated spores in oil	N/A	15.6 (13.0-21.5)	14.6 (12.8-18.3)	13.2 (11.8-15.5)	10.5 (9.9-11.4)	9.8 (9.2-10.4)

[1, 2] Water = 0.04% Silwet L77 solution and oil = Orchex 692.

[3] LT50 Values for Abbott's corrected mortality (Proc Probit, SAS Institute).

[4] Unless otherwise noted $Pr.>Xi^2$ slopes of time mortality regression lines < 0.05