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Efficacy of *Beauveria bassiana* formulations against the fungus gnat *Lycoriella ingenua*

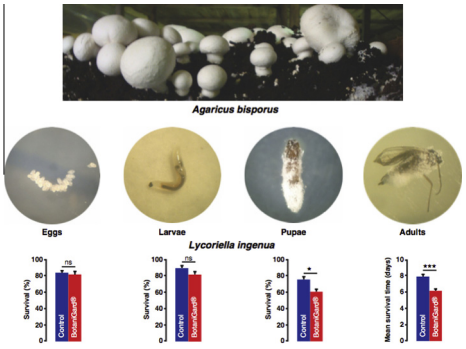
Stefanos S. Andreadis [a], Kevin R. Cloonan [a], Giovani S. Bellicanta [b], Kimberly Paley [c], John Pecchia [c], Nina E. Jenkins [b,*]

[a] Chemical Ecology Lab, Department of Entomology, Penn State University, University Park, 16802 PA, USA
[b] Merkle Lab, Department of Entomology, Penn State University, University Park, 16802 PA, USA
[c] Buckhout Lab, Department of Plant Pathology and Environmental Microbiology, Penn State University, University Park, 16802 PA, USA

HIGHLIGHTS

- Mushroom yield was not affected by the application of any *B. bassiana* formulation.
- Eggs and larvae of *L. ingenua* were unaffected by *B. bassiana* strain GHA.
- Pupae of *L. ingenua* were marginally susceptible to *B. bassiana* strain GHA.
- Adult stage of *L. ingenua* was the most susceptible to *B. bassiana* strain GHA.

GRAPHICAL ABSTRACT



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ABSTRACT

Lycoriella ingenua Dufour (Diptera: Sciaridae) is a major pest species in commercial mushroom (*Agaricus bisporus*) production throughout the world. Grower demand for alternative control measures, following the recent withdrawal of a number of chemical control options, led to a label extension for use of the fungal biopesticide BotaniGard® ES, for control of mushroom flies. Semi-field trials were conducted to evaluate the efficacy of BotaniGard® ES, and two alternative formulations of *Beauveria bassiana* strain GHA (the active ingredient in BotaniGard® ES), for control of *L. ingenua*, and their effect on crop yield when incorporated at spawning. Data collected from two replicated trials demonstrated that incorporation of *B. bassiana* was not detrimental to mushroom yield, but was also ineffective in controlling the development of *L. ingenua* larvae in artificially infested compost. Subsequent laboratory bioassays demonstrated that *L. ingenua* eggs and larvae were not susceptible to infection by *B. bassiana* strain GHA whereas pupae were somewhat susceptible (41% mortality). Bioassays conducted on adult *L. ingenua* using 1 h exposure to a surface sprayed with BotaniGard® ES resulted in 100% mortality within 8 days and a mean survival time of 6 days, which was significantly different from the control population.

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1. Introduction

Lycoriella ingenua (Dufour) (Diptera: Sciaridae) is a major pest species of commercial mushrooms throughout the world causing crop damage and reductions in yield (Park et al., 2006; Erler

* Corresponding author.
E-mail address: nej2@psu.edu (N.E. Jenkins).

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et al., 2011). *L. ingenua* damages mushrooms through direct larval feeding on developing mushroom mycelia (Cantelo, 1979; Lewandowski et al., 2004), larval competition with developing mushroom mycelia for nutrients in the compost (Binns, 1980), and negative effects of larval frass on mycelial growth (Hussey and Gurney, 1968). Furthermore, *L. ingenua* adults and larvae are associated with the vectoring of *Trichoderma aggressivum* (Samuels & W. Gams) (Hypocreales: Hypocreaceae), which causes severe epidemics of "green mold" and consequently leads to additional crop losses (Shamshad, 2010).

The developmental time for *L. ingenua* is 18–21 days at 24 °C, with the egg stage being 4 days, larval stage 12 days and the pupal stage being 4 days (Lewandowski et al., 2004). On average, adults live for about 6–12 days (Unpublished dataset). Current control efforts primarily rely on applications of conventional synthetic pesticides (Cantelo, 1979, 1983; Shamshad et al., 2008; Shamshad, 2010). However, insecticide options are limited due to removal of tolerance for many insecticides, and label restrictions on the number of applications per season and/or the total amount of active ingredient applied for those that remain in use. Targeting can also be difficult, because emerged larvae move away from the hatching site to feed in the caps and stems of mushrooms, where they are well protected. Additionally, repeated applications of conventional pesticides may produce undesirable effects, such as insecticide residues, reduced populations of natural enemies and insecticide resistance (Brewer and Keil, 1989; Bartlett and Keil, 1997). A label extension for BotaniGard® ES was recently obtained for use in Pennsylvania mushroom houses for control of *L. ingenua* and the mushroom phorid fly *Megaselia halterata* (Wood). The extension was granted based on preliminary efficacy data to control *M. halterata* from the industry (unpublished). Given the lack of information on efficacy against *L. ingenua*, and the need to evaluate the effect of BotaniGard® ES application on mushroom yield, field evaluations were conducted to determine the efficacy of BotaniGard® ES to control immature life stages of *L. ingenua* when incorporated into compost at spawning at registered label application rates. Mushroom yields were evaluated, to ensure that addition of *B. bassiana* to compost was not detrimental to mushroom growth and development as a result of mycotoxicity or competition. Additional laboratory-based 'maximum challenge' bioassays were also conducted on each life stage of *L. ingenua* with the aim of identifying an optimal application strategy based on the susceptibility of each developmental stage of the pest.

2. Materials and methods

2.1. Insect rearing

Lycoriella ingenua eggs, first instars, pupae and adults were obtained from a 2-year-old laboratory colony maintained at the University Park Campus of Penn State University. This colony was initiated in 2012 using gravid adult female flies that had been aspirated from the mushroom production beds of spawned *Agaricus bisporus* compost in Berks County (PA, USA). Flies were reared in white popup cages (30 × 30 × 30 cm) with a single vinyl window (Raising Butterflies, UT, USA) containing plastic cups (500 ml) (Solo, MI, USA) filled with phase II mushroom compost with an added commercial nitrogen supplement (100:1 w/w, compost:supplement). Cages were maintained in an environmental growth chamber at 21 ± 1 °C, 70 ± 5% RH, and a 12:12 (light:dark) photoperiod regime to allow the females to oviposit in the compost mixture in the cups. After 2 days, the cages were covered with plastic autoclave bags (VWR International, Atlanta, GA, USA; 61 × 76.2 cm) to prevent the compost from drying out. Adult flies emerged approximately 21 days later, at which point fresh compost cups were provided for egg collection from the emerging adults. New cages were then utilized until a continuously emerging colony was established as described in Andreadis et al. (2015).

2.2. Fungal production

Beauveria bassiana strain GHA (the active ingredient in BotaniGard® ES), was regenerated from −80 °C storage on Mirobank® microporous beads, placed onto potato dextrose agar (PDA) and incubated at 25 °C for 10 days until fully sporulated. Conidia from this culture were suspended in sterile 0.05% Tween 80 in de-ionized water to a concentration of approximately 1×10^8 conidia ml^{-1}. This suspension was used to inoculate 75 ml CSYE liquid media (4% glucose, 1% KNO3, 0.5% KH2PO4, 0.1% MgSO4, 0.005% CaCl, 0.2% yeast extract in de-ionized water) in 250 ml capacity Erlenmeyer flasks, 1 ml per flask. Flasks were incubated on a rotary shaker (160 rpm) at 24 °C for 3 days.

One kg of Barley flakes (Grain Millers, Iowa, USA) was added into a mushroom spawn bag (Unicorn, Garland, Texas, USA) along with 600 ml tap water, and the contents mixed by hand to ensure even absorption of the water. Each spawn bag was then placed inside an autoclave bag for protection and autoclaved for 30 min at 121 °C. Once cool, the bags were inoculated under aseptic conditions with 75 ml of the liquid culture plus 75 ml of sterile water to achieve a final moisture content of approximately 48%. The inoculated bags were carefully massaged to ensure even distribution of the inoculum, then sealed and incubated on shelves for 10 days at 25 °C. Following incubation, the bags were opened in a reverse flow cabinet (Labconco, USA) and the contents transferred to brown paper bags for drying. The paper bags were placed in a dehumidified room for 4 days (24–30 °C), until the sporulated substrate reached <20% moisture content. The conidia were then harvested from the barley flakes using a Mycoharvester (Acis, Devon, UK).

The harvested conidia were placed in glass dishes and further dried in a desiccator over dry silica gel at room temperature (approx. 20 °C). Once the conidia powder reached 5% moisture content, it was sealed in foil laminated sachets and stored at 5 °C until use. Spent grain was transferred to autoclave bags and placed in the freezer until use. Prior to use, spent grains were ground in a commercial coffee grinder to obtain a homogenous granular powder similar in size to ground coffee.

2.3. Field evaluations

Two separate mycotoxicity cropping and efficacy trials were conducted in May and August 2014 respectively. The first trial included one *B. bassiana* treatment and a control to evaluate the potential utility of the spent grain waste product, following production and extraction of the active ingredient (pure conidia powder of GHA) of BotaniGard® ES. This preliminary trial also provided useful information on evaluation methodology, which was subsequently incorporated into the following field trial. In the August trial, BotaniGard® ES (Laverlam International Corp., Buttle, MT, USA) was included at the label recommended application rate and compared with the spent grain waste product and pure conidia powder formulated in 0.05% Tween 80, all with equivalent conidia per kg compost. Both trials included evaluation of crop yield and effect on larval development and adult emergence of *L. ingenua* in artificially infested compost. Viability of all conidia suspensions was verified by plating on Sabouraud Dextrose Agar (SDA). Plates were incubated for 20 h at 25 °C and conidia evaluation under a microscope at 400× magnification. Conidia with a visible germ tube were counted as germinated and a total of 300 conidia were counted for each plate. All suspensions used in these experiments had >85% germination.

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