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Evaluating *Beauveria bassiana* Mycoinsecticides Against Spotted Lanternfly (*Lycorma delicatula*) Adults, Nymphs, and Eggs in Semi-Urban Areas

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Abstract. Background: The spotted lanternfly (*Lycorma delicatula*)(SLF) is an invasive planthopper that is spreading in the United States over recent years. Not only is SLF a nuisance pest in urban areas, but its feeding causes millions of dollars in damage to the forestry, ornamental plant, and viticulture industries annually, necessitating research on effective and sustainable control methods. The fungal pathogen *Beauveria bassiana* was previously found to infect and kill SLF. Our study sought to evaluate commercially available *B. bassiana* mycoinsecticides against various SLF life stages. Methods: In field trials, SLF were either directly sprayed with mycoinsecticides or exposed to 24-hour old mycoinsecticide residues on trees. We documented mortality over time, as well as the percentage of *B. bassiana*-infected individuals. Results: Direct contact sprays of *B. bassiana* strain GHA killed 95% of SLF adults after 2 weeks. Approximately 34% of early instar nymphs were killed after 9 days. However, laboratory testing confirmed that > 70% of nymphs were infected with the *B. bassiana* fungus. Late instar nymphs and adults exposed to residuals were infected at averages of 20% and 7% respectively, suggesting that mycoinsecticide tree bark sprays are not effective and quickly deteriorate within 24 hours. There was no effect of *B. bassiana* applications on egg hatch rate and only 16% of emerging hatchlings were infected. Conclusions: Our results indicate that direct contact spray applications of *B. bassiana* strain GHA can effectively kill SLF adults and nymphs under field conditions.

Keywords. Biological Control; Entomology; Entomopathogen; Invasive Insect; Pest Management.

INTRODUCTION

The spotted lanternfly, *Lycorma delicatula* (White) (SLF), is an invasive Fulgorid planthopper first detected in Berks County, Pennsylvania, USA, in 2014 (Barringer et al. 2015). Since then, SLF has spread to 18 other states and the District of Columbia (USDA-APHIS 2025). This rapid expansion can be attributed to several factors: its capacity for multiple short-duration, short-distance flights, averaging 30 m every 13-second flight bout; its tendency to lay eggs on vehicles or other structures, which facilitates transport to new locations; and its ability to feed on over 70 plant species commonly found in agricultural and urbanized areas (Dara et al. 2015; Wolfin et al. 2020; Urban and Leach 2023; Elsensohn et al. 2024). In its adult stage, SLF's preferred host is the widespread and invasive tree of heaven (*Ailanthus altissima*) (Sapindales: Simaroubaceae). Beyond this, SLF nymphs and adults also utilize a diverse range of host plants. These include crop plants like grapes (*Vitis* spp.) (Rhamnales: Vitaceae) and hops (*Humulus* spp.) (Rosales: Cannabaceae), ornamental plants such as maples (*Acer* spp.)(Sapindales: Simaroubaceae) and walnuts (*Juglans* spp.)(Fagales: Juglandaceae), and various fruit trees, including apples (*Malus* spp.) (Rosales: Rosaceae), peaches, plums, and cherries (*Prunus* spp.)(Rosales: Rosaceae)(Barringer and Ciafre 2020; Murman et al. 2020). Tree of heaven and cherry trees are particularly known as preferred hosts for oviposition (Liu 2019a).

SLF uses its piercing-sucking mouthparts to feed on the plant's phloem (Harner et al. 2022). During feeding, SLF depletes plant nutrients and excretes copious amounts of sugary, sticky waste known as

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honeydew. Since SLF populations on a single plant or tree can reach extremely high densities (i.e., thousands per tree), honeydew accumulation can increase the activity of stinging insects and promote sooty mold growth (Urban and Leach 2023). Furthermore, SLF feeding can reduce photosynthesis, cause canopy or branch dieback, and result in millions of dollars' worth of economic damages (Harper et al. 2019; Urban 2020; Harner et al. 2022; Taleb 2022; Acevedo 2024). As a result of this diverse host range and voracious feeding, SLF is both a nuisance pest in urban areas and a serious threat to the viticulture, ornamental plant, and forestry industries across the United States (Urban 2020; Urban and Leach 2023).

The economic impact caused by the SLF invasion has prompted extensive research into effective pest management options. Currently, chemical insecticides, specifically neonicotinoids, organophosphates, and pyrethroids, are heavily relied upon for controlling this pest (Leach et al. 2019; Leach et al. 2021; Leach et al. 2023). In the United States, these insecticides often require repeated applications because spotted lanternflies can rapidly repopulate treated areas (Urban and Leach 2023). However, insecticide use carries several risks: it can threaten pollinators and human health and may also lead to outbreaks of secondary pests like mites and scales (Leach et al. 2019; Elmquist et al. 2023). Furthermore, there is significant potential for the development of insecticide resistance in SLF populations. Consistent, widespread control efforts across the United States can exert strong selective pressure, favoring individual SLF with resistance to these insecticides. The mobility of SLF then facilitates the rapid spread of these resistance alleles across broad geographical areas, a phenomenon observed in other mobile generalist insects such as *Helicoverpa armigera* and *H. zea* (Walsh et al. 2022). Consequently, strategies to minimize reliance on chemical insecticide applications, such as attract-and-kill traps, are actively under development (Lewis et al. 2023; Urban and Leach 2023).

To sustainably manage SLF outbreaks, it is essential to investigate alternative measures such as biological control. Many commonly occurring North American arthropods and several vertebrates have been reported to feed on SLF (Barringer and Smyers 2016; Liu 2019b; Johnson et al. 2023). Vertebrate predators include amphibians, reptiles, birds, and mammals, while arthropod natural enemies include spiders (Araneae: Agelenidae, Salticidae, and Araneidae), mantids (Mantodea: Mantidae), predatory stink bugs (Hemiptera: Pentatomidae), and parasitoids (Hymenoptera: Encyrtidae). The generalist predators *Podisus maculiventris* (L.)(Hemiptera: Pentatomidae) and *Arilus cristatus* (L.)(Hemiptera: Reduviidae) were previously shown to consume groups of 25 nymphal SLF within a week, suggesting their potential utility for conservation or augmentative biological control strategies (Johnson et al. 2025).

A promising development in SLF biological control is the discovery of microbial entomopathogens capable of infecting SLF. In 2018, the fungi *Beauveria bassiana* (Hypocreales: Cordycipitaceae) and *Batkoa major* (Entomopthorales: Entomophthoraceae) caused localized population collapses of SLF near Pennsylvania apple orchards (Clifton et al. 2019). The results from this discovery suggested that mycoinsecticides containing *B. bassiana* spores could be an effective and sustainable control tactic. *Beauveria bassiana* is known to infect many insect pests, including other Hemipteran species (Lecuona et al. 2001; Leland et al. 2005; Islam et al. 2009; Mascarin and Jaronski 2016), while typically having only a minor and ephemeral impact on natural enemies and pollinators (Thungrabeab and Tongma 2007; Goettel et al. 2021). The pathogenic life cycle of *Beauveria* begins with the attachment of spores (also called conidia) to the insect's cuticle. The fungus then germinates and grows, penetrating the insect's body, suppressing its immune system, and depleting nutrients, eventually leading to host death. Following the insect's demise, the fungus grows externally, producing characteristic white spores that can then be disseminated by wind, rain, or other factors to infect subsequent hosts (Mascarin and Jaronski 2016).

For mycoinsecticides to be effectively integrated into SLF control strategies, it is crucial to understand which product types perform best under field conditions, which life stages are most susceptible to infection, and what application techniques are most effective. The United States currently has an array of commercially available mycoinsecticides registered for use, containing various *B. bassiana* strains and formulations such as wettable powders (WP), emulsifiable suspensions (ES), and water dispersible granules (WDG)(Arthurs and Dara 2019). Some of these products have shown promising results against SLF in laboratory bioassays. For instance, direct contact

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sprays of *B. bassiana* strains GHA, ANT-03, and ATCC 74040 achieved between 90 to 93% nymph mortality and 96 to 98% adult mortality after 2 weeks (Clifton and Hajek 2022). Field tests have also been conducted: *Beauveria bassiana* strain GHA has been applied in commercial vineyards against early instar nymphs using helicopters for aerial applications (Keller et al. 2023), and in managed forest plots against late instar nymphs and adults using orchard sprayers and tractors (Clifton et al. 2020).

However, key knowledge gaps remain. It is less understood whether SLF egg masses are susceptible to *B. bassiana* in the field (Clifton et al. 2025) and if mycoinsecticide residues (i.e., remaining spores on surfaces after application) on trees are effective against SLF. Addressing these gaps, particularly through treatments that kill SLF before hatching or residues that prevent population buildup, could significantly reduce the number of sprays, treatment time, and labor needed for successful SLF control (Park et al. 2009). Therefore, the main goals of our study were to quantify the efficacy of mycoinsecticides against all SLF life stages in the field and to assess management options for arborists, foresters, and homeowners using readily available, relatively inexpensive tools like backpack or handheld pump sprayers. In the current study, we: (1) determined the efficacy of 3 different mycoinsecticides with different fungal strains and formulations against adult SLF; (2) quantified the effect of direct contact applications on early instar nymphs (2nd and 3rd) under field conditions; (3) assessed adult and late instar nymph (4th) mortality after exposure to mycoinsecticide residues; and (4) established whether *B. bassiana* has ovicidal activity against SLF.

MATERIALS AND METHODS

Efficacy of Direct Contact Mycoinsecticide Sprays on Adult Spotted Lanternflies

To assess the efficacy of mycoinsecticides, SLF adults were collected and sprayed with mycoinsecticides, then placed in field cages to document mortality and infection. Two distinct adult phases were evaluated in our study, since adult physiology and behavior are known to change over time. The early adult phase—which is typically described as the period when adults emerge, aggregate on trees in a 50:50 sex ratio, and intensively feed on host trees—began in August of 2022. The mid adult phase—which is typically described as when more females are found feeding on trees, SLF disperse, and mate—began in September of 2022 (Cooperband and Murman 2022).

This experiment was conducted at field sites in Sharpsburg, Washington County, MD, USA, and Blandon, Berks County, PA, USA, during August and September 2022. These locations contained common SLF host plants, including tree of heaven, black walnut (*Juglans nigra*), and black cherry (*Prunus serotina*), and have supported high SLF densities since at least 2021. The Sharpsburg area experiences a humid climate, with average temperatures of 24.3 °C in August and 18.1 °C in September, and total precipitation for both months was 166.37 mm (NOAA 2024). The Blandon area experiences a humid continental climate, with average temperatures of 24.3 °C in August and 18.8 °C in September, and total precipitation for both months was 144.78 mm (NOAA 2024). The sites were surrounded by woodlots, unmanaged grassy fields, and roads. Adults were collected from city parks in Hagerstown, MD, or Blandon, PA.

A total of 3 mycoinsecticides at their recommended high label rates were used (Table 1). To dilute *B. bassiana* strain ANT-03 WP (formulated as BioCeres WP)(BioSafe Systems, East Hartford, CT, USA) for application, we added 3.6 g of product per L of water, with a resulting concentration of approximately 3.59×10^7 spores/mL. For *B. bassiana* strain GHA WP (formulated as BotaniGard WP)(Certis USA LLC, Columbia, MD, USA), 1.2 g of product per L of water was mixed, with a resulting concentration of approximately 2.64×10^7 spores/mL. To dilute *B. bassiana* strain GHA ES (formulated as BotaniGard ES)(Certis USA LLC, Columbia, MD, USA), 7.8 mL of product per L of water was mixed for a final concentration of approximately 1.65×10^8 spores/mL. A 700-mL handheld bottle sprayer was used to apply treatments, with approximately 3.5 mL of product applied to each group of insects.

For each trial, groups of 10 early or mid-phase adults were placed in 350 mL plastic cups. During the early adult phase trial, 5 females and 5 males were added to each cup. However, during the mid adult phase, some replicates contained all females, due to the limited number of males available. Cups were covered with a piece of mesh and held shut with a rubber band. Groups of SLF were randomly assigned to 4 treatments: (1) *B. bassiana* strain GHA formulated as an ES; (2) *B. bassiana* strain GHA formulated as a

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Table 1. Products tested for efficacy against spotted lanternfly adults, nymphs, or eggs.

Product/manufacturer	Formulation	Fungal strain	Conidia in undiluted product	Reccommended high label rate	Tested against
BioCeres (BioSafe Systems)	Wettable powder	ANT-03	1×10^{10} conidia/g	3 kg/ha	Adults
BotaniGard (Certis)	Wettable powder	GHA	2×10^{10} conidia/g	1 kg/ha	Adults
BotaniGard (Certis)	Emulsifiable suspension	GHA	2×10^{10} conidia/mL	9 L/ha	Adults, nymphs, eggs
Omni Supreme Spray (Helena Agri-Enterprises, LLC)	Oil concentrate	–	–	37 L/ha	Eggs

WP; (3) *B. bassiana* strain ANT-03 formulated as a WP; and (4) water control. Treatments were replicated 10 times. After treatments were applied, cups were turned upside down to drain excess liquid.

Groups of 10 treated SLF were randomly assigned to a cage and placed inside. Non-infested trees of heaven (diameter at breast height [DBH] 2.54 to 7.62 cm, > 2 m apart) were selected and a single cage was constructed per tree. Two pieces of batting material were placed approximately 0.6 m apart and a 112 cm × 64 cm piece of mesh was wrapped around the trunk. To secure the mesh to the trunk, zip ties were secured around the batting material edges. Alligator clips were attached to the edge of the mesh to fully close the cage. After the cages were set up, the number of dead and alive insects were counted 4 days later. Since we did not remove SLF from cages at 4 days, we could not record the sex of dead or alive individuals. Fourteen days later, cages were opened and the sex, mortality status, and whether there was sporulation of live and dead SLF were recorded.

Impact of Direct Contact Mycoinsecticide Sprays on Early Instar Spotted Lanternflies

To quantify the effect of direct contact mycoinsecticide sprays on early instar (2nd and 3rd) spotted lanternflies, infested tree saplings were sprayed and caged with insects, and the number of dead and infected SLF were recorded. This experiment was conducted at the Sharpsburg, Washington County, MD, USA site (described above) and a second site in Wrightsville, York County, PA, USA, during June and July 2023. The Wrightsville site is a 0.3-ha woodlot surrounded by agricultural fields and roads, predominantly containing tree of heaven, black cherry, and boxelder maple (*Acer negundo*), which have been infested with SLF since 2019. This region of Pennsylvania has a humid continental climate, with the average temperatures of 19.9 °C in June and 25.1 °C in July, and precipitation of 92.46 mm and 124.71 mm, respectively (NOAA 2024).

For this experiment, *B. bassiana* strain GHA ES (formulated as BotaniGard ES)(Certis USA LLC, Columbia, MD, USA) was used at its recommended high label rate (Table 1). It was diluted by mixing 7.8 mL of product per L of water for a final concentration of approximately 1.65×10^8 spores/mL. Treatments were applied until runoff (30 PSI at 0.5 liters per min) using 7.5 L manual pump sprayers (DeWalt Industrial Tool Company, New Britain, CT, USA) (Clifton et al. 2020).

Individual tree of heaven saplings (0.3 to 0.6 m tall, < 3 cm DBH, > 3 m apart) infested with at least 20 early instar SLF (a mixture of both 2nd and 3rd instars) were selected and randomly assigned to 2 treatments: (1) *B. bassiana* strain GHA ES spray or (2) water control. Visual counts were used to estimate the number of SLF nymphs naturally feeding on each sapling before and after sprays. If fewer than 5 nymphs were present after the spray application, a new sapling was selected and treated. Immediately following spray applications, a 57 × 63 cm nylon mesh bag (Uline Inc, Pleasant Prairie, WI, USA) was placed over a single stem and tied shut. There was one mesh bag per sapling. Each treatment was replicated 5 times in PA and 13 times in MD. After 9 days, the number of dead and live nymphs inside the bags were counted.

Sapling stems were cut and the bagged nymphs were transported to University of Maryland and East Stroudsburg University laboratories to assess for

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infection. Any living nymphs were euthanized in a −17 °C freezer for 25 min and dead nymphs were also placed in the freezer to maintain uniformity in treatment. In a biosafety cabinet, nymphs were immersed in a 1% NaOCl solution for 1 min to sterilize the external surface of the cadavers. Nymphs were rinsed twice in water for 30 s. Insects were placed on paper towels to remove excess liquid and transferred to water agar petri dishes (5 cm diameter). Nymphs were placed at least 5 mm apart in the dish. Once sealed shut, petri dishes were placed inside of 3-L plastic containers (Araven, Miami, FL, USA) and remained at room temperature (25 °C) for a week to confirm mycosis from *B. bassiana* infections. *Beauveria bassiana* infection has a distinct appearance, with dense white spores covering the insect cadavers (Clifton et al. 2019).

Residual Activity of Mycoinsecticides on Late Instar Nymphs and Adult Spotted Lanternflies

Since it can be difficult to directly spray SLF, we tested the effect of residual mycoinsecticides on SLF late instar (4th) and early phase adults. Twenty-four hours post-spray on trees, insects were caged on treated surfaces for a week to maximize exposure and determine whether residuals were sufficient to infect lanternflies. Mortality and infection of the insects were recorded.

Trials were conducted at the Sharpsburg, Washington County, MD, USA site (described above), the Wrightsville, York County, PA, USA site (described above), and a third site in Fairless Hills, Bucks County, PA, USA, during July and August 2023. The Fairless Hills site (5 ha) contains mostly tree of heaven, black cherry, and gray birch (*Betula populifolia*)(Fagales: Betulaceae) and is surrounded by wood lots and roads and has hosted SLF populations since 2019. The Fairless Hills site also has a humid continental climate, with average temperatures of 24.8 °C in July and 22.3 °C in August, and precipitation totals of 164.59 mm and 104.9 mm, respectively (NOAA 2024).

For this experiment, *B. bassiana* strain GHA ES (formulated as BotaniGard ES)(Certis USA LLC, Columbia, MD, USA) was used at its recommended high label rate (Table 1). It was diluted by mixing 7.8 mL of product per L of water for a final concentration of approximately 1.65×10^8 spores/mL. Treatments were applied until runoff (30 PSI at 0.5 liters per min) using 7.5-L manual pump sprayers (DeWalt Industrial Tool Company, New Britain, CT, USA)(Clifton et al. 2020). Non-infested trees of heaven (7.6 to 12.7 cm DBH, > 5 m apart) were selected and randomly assigned 2 treatments: (1) *B. bassiana* strain GHA ES tree trunk spray or (2) water control tree trunk spray. Twenty-four hours after treatment, mesh cages were placed around tree trunks and insects were added.

SLF were collected from other, untreated locations (Western Maryland Research and Education Center, Keedysville, MD, USA, and a field site in East Stroudsburg, PA, USA). For each trial, groups of 10 late instar or adult SLF were randomly assigned a cage and placed inside. Treatments were replicated 10 times at the Fairless Hills PA site, 5 times at the Wrightsville PA site, and 15 times at Sharpsburg MD (n = 300 SLF per treatment). After one week, the mortality of late instar nymphs and early phase adults was recorded. As described above, live and dead insects were brought back to the laboratory and plated on water agar to determine the number of sporulated live and dead SLF.

Ovicidal Activity of Mycoinsecticide and Horticultural Oil Applications

Here, we evaluated the efficacy of *B. bassiana* strain GHA ES spray and horticultural oil on SLF egg mass hatch, mortality, and infection. Two different spray timing periods were also tested. Given that SLF egg hatch typically occurs around 360 degree days (DD) (Coop and Barker 2024), we applied a "pre-hatch" spray at 214 DD (approximately 2 weeks before hatch) and an "at-hatch" spray at 310 DD (about 5 days before hatch).

Sharpsburg, MD, USA Trial

For this experiment, *B. bassiana* strain GHA ES (formulated as BotaniGard ES)(Certis USA LLC, Columbia, MD, USA) was diluted by mixing 7.8 mL of product per L of water for a final concentration of approximately 1.65×10^8 spores/mL. The horticultural oil (formulated as Omni Supreme Spray)(Helena Chemical Company, Collierville, TN, USA) was diluted by adding 30.1 mL product per L of water for a 3% concentration. Both were used at their recommended high label rates (Table 1). Treatments were applied until runoff (30 PSI at 0.5 liters per min) using 7.5-L manual pump sprayers (DeWalt Industrial Tool Company, New Britain, CT, USA)(Clifton et al. 2020).

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A total of 60 individual trees (7.6 to 12.7 cm DBH, > 5 m apart) with at least 2 SLF egg masses (1 to 3 m up from the ground) with an intact oothecum or waxy covering (Malek et al. 2019) were selected. Trees were randomly assigned to a treatment: (1) *B. bassiana* strain GHA (ES) at "pre-hatch;" (2) 3% horticultural oil at "pre-hatch;" (3) water control at "pre-hatch;" (4) *B. bassiana* strain GHA (ES) at "at-hatch;" (5) 3% horticultural oil at "at-hatch;" and (6) water control at "at-hatch." Each treatment and timing combination was applied to 10 trees.

After spray applications dried (approximately 30 minutes), 2 egg masses per tree were removed with a chisel (1.27 cm) and placed in petri dishes (13 cm diameter) with damp filter paper. Because SLF prefer to lay eggs near previous oviposition sites (Liu 2022), egg masses were inspected under a stereoscope for old eggs, which could be identified by a lack of oothecum or open operculum. Old eggs were marked with red ink and excluded from future counts. Petri dishes were incubated (Percival Scientific Inc., Perry, IA, USA) at 25 °C, 40% relative humidity, and 12:12 light:dark cycle. Egg masses were checked daily for hatch or fungal outgrowth. To ensure all nymphs emerged, egg masses remained in incubators for 5 weeks after the first hatch was recorded. The total number of unhatched and hatched eggs were recorded with hatched eggs identified by their open opercula.

To quantify emerging nymph mortality and infection, we reared hatchlings on plants since *B. bassiana* conidia can take between 24 to 48 hours to germinate (Mwamburi et al. 2015). We reared hatchlings on squash plants (*Cucurbita pepo* L., Dixie hybrid), as recent reports state that SLF can feed on cucurbit plants (Avanesyan and Lamp 2020). Additionally, our preliminary experiments found that survival of early instar nymphs fed squash or tree of heaven branch clippings was the same after a 2-week period. Two weeks before eggs were collected, two squash seeds (Ferry-Morse Home Gardening, Norton, MA, USA) were planted per pot (10.16 cm diameter × 10.16 cm depth) in growing medium (Sun Gro Horticulture, Agawam, MA, USA). Squash plants were grown in greenhouses at 21 to 25 °C and no supplemental light was provided. Plants were watered *ad libitum*.

Upon emergence, 20 nymphs were transferred to each potted squash plant. If fewer than 20 nymphs emerged simultaneously from an egg mass, additional nymphs from the same egg mass were assigned to a separate plant to accurately track their age. Nylon paint strainer bags (Trimaco, Morrisville, NC, USA) were secured over the plants with rubber bands. Five days later, the number of alive and dead nymphs in each cage were counted. All insects were frozen and plated on water agar to observe and confirm *B. bassiana* outgrowth on cadavers.

Paint Branch Turfgrass Facility Trial

Since there were not enough eggs at the Sharpsburg, MD, USA site to conduct a second trial, egg masses were collected at a site in Royersford, Montgomery County, PA, USA, and treated at the University of Maryland Paint Branch Turfgrass Facility in College Park, Prince George's County, MD, USA. The Royersford site (0.4 ha) is a woodlot surrounded by roads and commercial buildings. Tree of heaven, black walnut, and amur honeysuckle (*Lonicera maackii*)(Dipsacales: Caprifoliaceae) were the predominant tree and shrub species in the woodlot and has been infested since 2019. The Paint Branch Turfgrass facility is a 14-ha research farm that has plots of turfgrass, ornamental and native grasses, wildflowers, and trees. In April 2024, the average temperatures were 14.4 °C and 12.3 °C at the Paint Branch Turfgrass Facility and Royersford, respectively. The total precipitation in April 2024 was 64.26 mm at the Turfgrass Facility and 119.13 mm in Royersford (NOAA 2024).

For this experiment, *B. bassiana* strain GHA ES (formulated as BotaniGard ES) and the horticultural oil (formulated as Omni Supreme Spray) were prepared at the same dilutions and applied using the same method (7.5-L manual pump sprayers until runoff) as described for the Sharpsburg ovicidal trial.

Thirty egg masses were collected from Royersford using a chisel. At the Paint Branch Turfgrass Facility, 30 linden trees (*Tilia* spp.)(DBH 35 to 40 cm, 5 m apart) were randomly assigned to a treatment: (1) *B. bassiana* strain GHA (ES); (2) horticultural oil; or (3) water. A single egg mass was attached to each tree with quilting pins, 1.5 m high on the trunk. After sprays, egg masses were removed and incubated under the same conditions for eggs described above. The number of hatched and unhatched eggs were recorded, and the nymphs were subsequently transferred to squash plants to track mortality and infection. All nymphs were plated on water agar, and the number of sporulated cadavers was counted.

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Statistical Analysis

All statistical analyses were conducted in RStudio (RStudio Team 2020). Mortality counts were corrected using Abbott's formula (Abbott 1925). To determine the effect of various *B. bassiana* mycoinsecticides on adult SLF, a generalized linear mixed model (GLMM) (glmer function from the lme4 package) was used (Bates et al. 2015; R Core Team 2022). Mortality, recorded as a binary outcome (live/dead), was the response variable. Fixed predictor variables included mycoinsecticide treatment, adult phase, and time after spray application, while site was included as a random effect. A separate GLMM was used to analyze the day 14 mortality data to determine the effect of sex on adult mortality. The proportion of infected individuals per cage was calculated by dividing the number of sporulated individuals by the total number in each cage. A 2-way ANOVA was used to determine if the proportion of infected individuals differed amongst treatments and adult phases. Post-hoc comparisons were conducted using the emmeans package (Lenth 2024).

To analyze the effect of direct mycoinsecticide sprays on early nymph mortality, a GLMM was used. Mortality, modeled as a binary outcome, was the response variable. Treatment was the fixed predictor and site was included as a random effect. Differences in the proportion of infected individuals were analyzed using a linear mixed model (LMM)(lmer function from the lme4 package). The proportion of infected individuals was the response variable, treatment was the predictor, and location was a random effect. Mean separation tests for both mortality and infection data were conducted using emmeans.

In the residual activity trials, control mortality exceeded 50%; therefore, these data are not shown. However, the proportion of sporulated (infected) individuals per cage was calculated for both late instar nymphs and adults. Differences in infection between treatments were analyzed using a LMM, with the proportion of infected individuals as the response, treatment as the predictor, and site as a random effect. In our ovicidal activity trials, hatchling mortality was calculated as the number of dead nymphs on squash plants after 5 days divided by the total number of hatched nymphs. Control hatchling mortality exceeded 50%, which was determined to be due to high temperatures in our greenhouses; therefore, we do not present that data here. The proportion of hatched eggs per mass was calculated by dividing the number of newly opened eggs by the total number of eggs in a mass. The proportions of hatched eggs and sporulated individuals were compared across treatments using two-way ANOVAs for the Sharpsburg, MD trial. The response variables were the proportions, with treatment and spray timing as the predictor variables. In the Paint Branch Turfgrass Facility trial, a one-way ANOVA was used, and the response variables were the proportions, with treatment as a predictor. Post-hoc comparisons were conducted using Tukey's HSD tests.

RESULTS

Beauveria bassiana Strain GHA as an ES Formulation Kills Majority of Adults After 2 Weeks

Adult mortality differed across treatments ($\chi2 = 149.31$, $p < 0.001$) and *B. bassiana* strain GHA ES spray treatments had the highest mortality overall (data pooled across both sampling dates and adult phases; $p < 0.05$)(Figure 1A). Days after treatment also affected mortality ($\chi2 = 145.51$, $p < 0.001$), with the highest adult SLF mortality after 2 weeks (Figure 1B) ($p<0.001$). *Beauveria bassiana* strain GHA ES killed 95% of adults and strain GHA WP killed 67% of adults after the 2-week period (Figure 1C). No significant interaction between treatment and time was observed, however ($\chi2 = 5.22$, $p = 0.07$). Adult phase affected mortality ($\chi2 = 24.05$, $p < 0.001$) and there was a significant interaction between adult phase and treatment ($\chi2 = 9.96$, $p < 0.01$)(Figure 1D). *Beauveria bassiana* strain GHA ES spray killed 22% more early-phase than mid-phase adults, whereas ANT-03 WP killed 21% more early-phase than mid-phase adults ($p < 0.05$). There was no difference in mortality between male and female SLF ($p > 0.05$). Sporulated cadavers were observed in all *B. bassiana*-treated cages, but not control cages ($F_{3,16} = 8.37$, $p = 0.001$) (Figure 1E). For the GHA strain ES spray, 58% of cadavers had *B. bassiana* outgrowth, although this was not different than the GHA strain WP (36%) and ANT-03 WP (41%)($p > 0.05$). More sporulated cadavers were observed from early adult trials than mid-adult trials ($F_{1,16} = 20.34$, $p < 0.001$). There was a significant interaction between treatment and adult phase ($F_{3,16} = 3.65$, $p = 0.03$), with more infected

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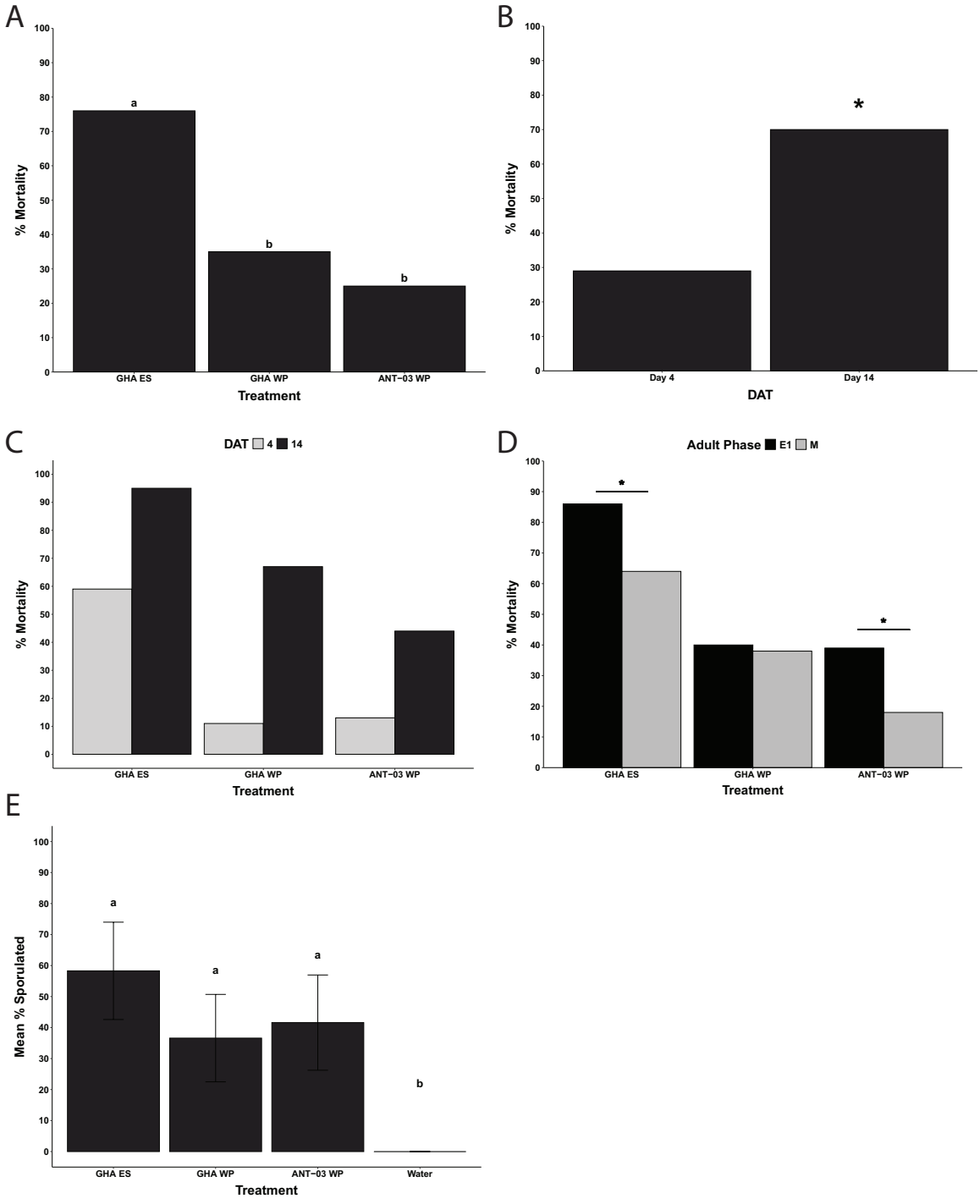

Figure 1. Corrected percent mortality and percent infection of adult SLF. Following direct sprays to insects in containers, SLF were caged on trees for a 2-week period. Letters above bars indicate significant differences among treatments, whereas asterisks indicate pairwise differences ($p < 0.05$). Days after treatment (DAT) and adult phase (E1 for early-phase and M for mid-phase) are abbreviated. (A) *Beauveria bassiana* strain GHA ES caused the highest mortality of adults overall ($p < 0.001$). Data were pooled across sampling dates and adult phases. (B) Mortality was highest overall after 2 weeks ($p < 0.001$). Data were pooled across treatments and adult phases. (C) Strain GHA ES had the highest mortality (95%) after 2 weeks ($p < 0.05$). Data were pooled across adult phases. (D) *Beauveria bassiana* strain GHA ES and ANT-03 WP applications caused higher mortality in early compared to mid-phase SLF ($p < 0.001$). Data were pooled across DAT. (E) Of the strain GHA ES-exposed cadavers, 58% were sporulated, 36% of strain GHA WP-exposed cadavers were sporulated, and 41% of strain ANT-03 WP-exposed cadavers were sporulated. Data are from 14 DAT and were pooled across both adult phases.

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cadavers found in early-phase *B. bassiana* strain GHA WP and ES-treatments than mid-phase individuals treated with the same products or water ($p < 0.05$).

Direct Contact Applications Lead to Infection in Early Instar Nymphs

Using the best-performing mycoinsecticide from the previous experiment, we directly sprayed early instar nymphs in the field and tracked mortality and infection. *Beauveria bassiana* strain GHA ES applications killed 34% of nymphs after 9 days, which was higher than water-treated nymphs ($\chi 2 = 14.94$, $p < 0.01$). Overall, 73% of treated insects were confirmed to be infected by *B. bassiana*. Interestingly, 74% of live nymphs and 73% of dead nymphs exhibited *B. bassiana* infection. None of the water-treated control insects showed any signs of infection.

Residual Activity of *B. bassiana* Does Not Affect Late Instar Nymphs and Adults

The residual efficacy of *B. bassiana* strain GHA ES spray was tested on late instars and adults. Control mortality was extremely high in both studies (> 50%), which was likely due to high temperatures. However, confirmation of infection was investigated for all insects, live or dead. *Beauveria bassiana* caused increased infection of late instar nymphs ($\chi 2 = 28.23$, $p < 0.0001$) and adults ($\chi 2 = 17.19$, $p < 0.001$). On average, 20% of late instar SLF were infected with *B. bassiana* and 7% of adults were infected.

Beauveria bassiana Does Not Have Strong Ovicidal Activity

To assess the ovicidal activity of *B. bassiana* strain GHA ES spray, SLF egg masses were treated at the Sharpsburg site, and the number of hatched eggs and infected nymphs were counted in the laboratory. Treatment of eggs with *B. bassiana* or an industry-standard pesticide (3% horticultural oil) did not affect egg hatch ($F_{2,56} = 1.57$, $p = 0.21$)(Figure 2A). There was a significant effect of spray timing ($F_{1,56} = 8.73$, $p < 0.01$), with more eggs hatching from the "at-hatch" spray group compared to the "pre-hatch" spray group ($p < 0.05$). The interaction between treatment and spray timing, however, was not significant ($F_{2,56} = 0.35$, $p = 0.71$). On average, 16% of hatchlings were infected with *B. bassiana* (Figure 2B), but there was no effect of spray timing on infection ($F_{1,20} = 1.34$, $p = 0.26$) and the interaction between treatment and spray timing was not significant ($F_{2,20} = 1.59$, $p = 0.22$). In the second field trial, we removed eggs from a Pennsylvania site and treated them at the Paint Branch Turfgrass Facility in Maryland, as there were not enough eggs locally available for testing. The proportion of eggs that hatched from an egg mass were the same across treatments ($F_{2,27} = 0.27$, $p = 0.76$)(Figure 2C). Additionally, the proportion of infected hatchlings did not differ amongst treatments ($F_{2,18} = 3.2$, $p = 0.06$)(Figure 2D).

DISCUSSION

This study highlights the potential of the fungal entomopathogen *B. bassiana* as a method for controlling SLF. Findings demonstrate that direct contact applications of *B. bassiana* strain GHA formulated as an emulsifiable suspension (ES) were highly effective, killing 95% of adult SLF. While direct contact applications resulted in lower observed mortality (approximately 30%) for early nymphs, a significant proportion of these nymphs were found to be infected with *B. bassiana*. In contrast, residual activity of the *B. bassiana* GHA ES spray 24 hours after application proved less effective, showing minimal infection in late instars and adults. Similarly, direct contact sprays applied prior to egg hatch had limited impact on reducing egg hatch or infecting newly emerged nymphs. Overall, results suggest that direct contact applications of *B. bassiana* can be an effective tool for managing SLF nymphs and adults, particularly those in the early phase. However, this work also indicates that further research is needed to optimize application techniques for broader efficacy.

In this evaluation of 3 mycoinsecticides, direct applications of the emulsifiable suspension (ES) formulation of *B. bassiana* strain GHA demonstrated high efficacy, killing 95% of adult SLF after 2 weeks under field conditions. Nearly 60% of these cadavers also exhibited visible outgrowth of *B. bassiana*, confirming infection from our treatments. While some insects died without showing fungal outgrowth, sporulation (i.e., the production of spores) is not always guaranteed, even if *B. bassiana* was the cause of mortality (Clifton et al. 2020). This phenomenon can be influenced by field conditions, as factors like temperature and humidity can hinder or prevent sporulation (Fargues and Luz 2010). Overall, these results align with other studies; for example, over 90% mortality of SLF adults was observed 9 days after

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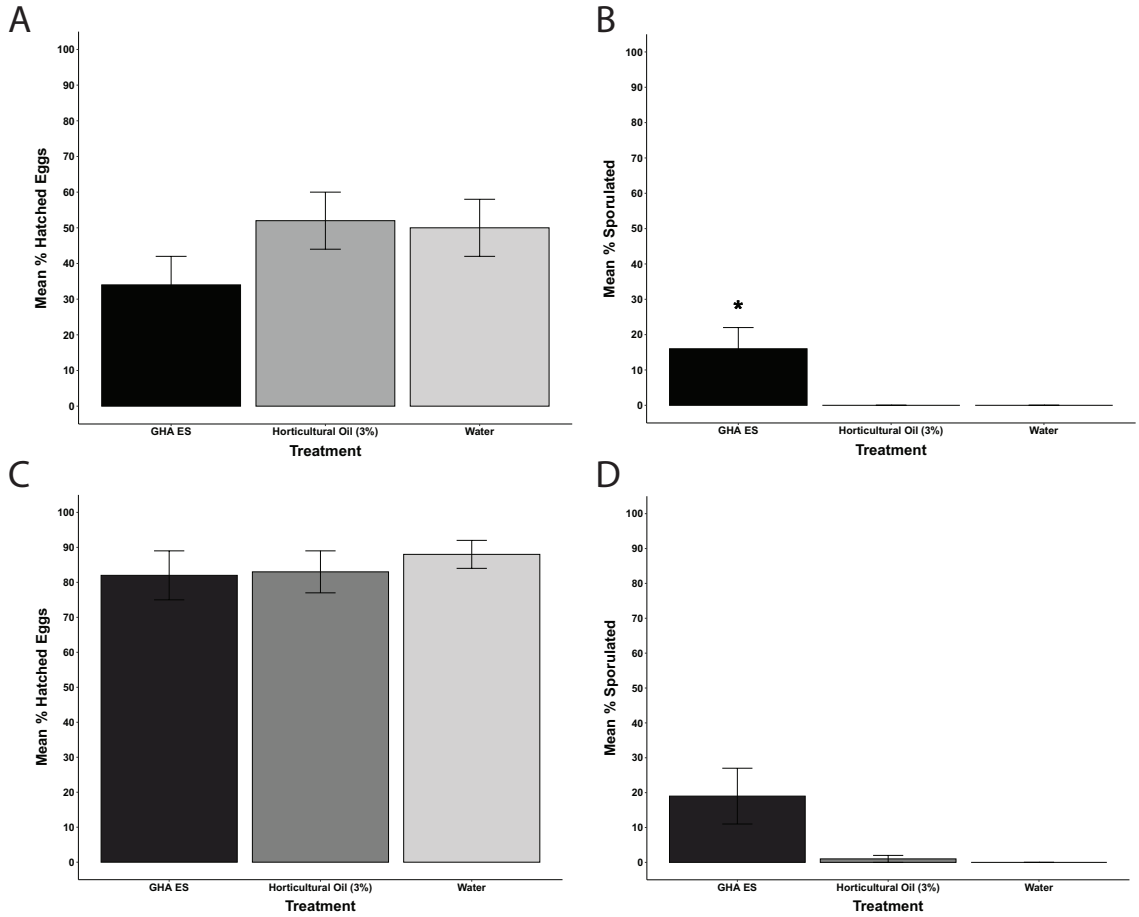

Figure 2. SLF hatch rate and proportion of infected hatchlings. Asterisks indicate significant differences among treatments ($p < 0.05$). (A) and (B) depict data from the Sharpsburg, Maryland, USA field site. Egg masses on trees were directly sprayed, removed, incubated under laboratory conditions, and the number of hatched eggs and sporulated hatchlings were recorded. (C) and (D) depict data from the trials the at Paint Branch Turfgrass Facility in Maryland. Eggs were collected from Pennsylvania and pinned to trees in Maryland to apply treatments. Egg hatch and infection were recorded using the same methods as the Sharpsburg trial. (A) The percentage of hatched eggs within an egg mass did not differ among treatments ($p = 0.21$). (B) Sixteen percent of hatchlings were infected with *B. bassiana*, and no infection was detected in the other groups ($p < 0.01$). (C) The proportion of hatched eggs did not differ amongst treatments. (D) The proportion of infected nymphs did not differ amongst treatments.

treatment with *B. bassiana* strain GHA ES on potted grape plants in the field (Clifton et al. 2020). This study was the first to test *B. bassiana* strain GHA formulated as a wettable powder (WP) against SLF. While the formulation of mycoinsecticides can significantly influence effectiveness (Wraight et al. 2016), findings showed that the WP formulation of the GHA strain provided some control, resulting in 67% adult mortality after 2 weeks. Collectively, these results indicate that *B. bassiana* strain GHA is an effective agent for killing SLF under field conditions. In contrast, the ANT-03 strain, also formulated as a WP, caused less than 45% mortality of adults after 2 weeks. This lower field efficacy for ANT-03 WP contrasts with laboratory bioassays, which reported 98% mortality (Clifton and Hajek 2022), suggesting that the ANT-03 strain and its WP formulation may be less effective under field conditions.

Interestingly, this study found that mortality differed significantly between early- and mid-phase adults. Specifically, more early-phase adult SLF treated with *B. bassiana* strain GHA ES and ANT-03 WP were killed compared to mid-phase adults. This observation aligns with previous research indicating that younger or recently molted insects are generally more susceptible to *B. bassiana* infection than mature

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insects (Boyle and Cutler 2012; Maehara and Kanzaki 2013; White et al. 2021). These findings suggest that direct contact sprays of *B. bassiana* strain GHA ES hold significant potential for adult SLF control. This approach is particularly beneficial as it could target and kill adults before they transition into the mid-phase, when they typically disperse across the landscape to mate and lay eggs. Furthermore, the recent development of kairomone or pheromone-based lures (Cooperband et al. 2019; Cooperband and Murman 2024) could facilitate the integration of these mycoinsecticides into effective and sustainable attract-and-kill technologies for early-phase adults (Brandl et al. 2017). Additionally, field results revealed that total mortality after 2 weeks did not differ between male and female adult SLF. This outcome was unexpected, given that previous laboratory work had shown male SLF adults exposed to *B. bassiana* treatments died faster than females (Clifton and Hajek 2022).

In the second direct contact study, only 34% of early nymph SLF were killed by *B. bassiana* strain GHA ES sprays after 9 days. However, a significant finding was that 74% of the live nymphs collected in the laboratory were infected with *B. bassiana*. It is hypothesized that if mortality counts had been taken after longer intervals (e.g., 2 weeks, similar to our adult study), the observed nymph mortality caused by *B. bassiana* may have increased (Figure 1B). It is also important to consider the experimental setup: early instar nymphs on saplings were sprayed and immediately caged on the same sapling. This design potentially prolonged their exposure to *B. bassiana* spores on the plant's surface. In less controlled field conditions, SLF are highly mobile and could easily move away from mycoinsecticide-treated areas, thereby reducing contact and subsequent *B. bassiana* infection (Keller et al. 2023). Furthermore, nymphs commonly feed on a diverse range of hosts, such as multiflora rose (*Rosa multiflora*), sassafras (*Sassafras albidum*), and tree of heaven saplings (Liu 2019a), leading to patchy dispersal patterns within a landscape (Calvin et al. 2021). This complex nymphal behavior and movement significantly complicate efforts to directly spray nymphs and achieve high levels of coverage and spore exposure. Despite these challenges, results emphasize that prolonged mycoinsecticide contact ultimately results in mycosis and death in nymphs. Therefore, future work should focus on developing passive spore dissemination techniques that specifically target nymphs and maximize their exposure to mycoinsecticides.

Recognizing the challenges of directly spraying spotted lanternflies, the efficacy of 24-hour old mycoinsecticide residues (*B. bassiana* strain GHA as an ES) was investigated. Results showed minimal effects on infection of late instar and adult SLF. While spore densities on surfaces were not measured in this study, previous research by Keller et al. (2023) demonstrated that *B. bassiana* spore densities on tree foliage can decline within as little as 5 days, subsequently failing to affect SLF nymphal populations. In contrast to our findings, *B. bassiana* mycoinsecticide residues on potted grape plants were reported to kill 57% of adults 9 days after treatment (Clifton et al. 2020). The lower level of efficacy observed in our present study may stem from differences in the timing of insect exposure to residues. In the potted grape study, SLF were exposed to plants just 30 minutes after application (Clifton et al. 2020), whereas this study introduced SLF to trees 24 hours post-application. Moreover, while specific weather data was not collected, it is well-understood that abiotic factors such as UV radiation, rainfall, and heat can degrade mycoinsecticides before a host even comes into contact with spores (Ortiz-Urquiza et al. 2015). Humidity is another critical factor, as it facilitates *Beauveria* infection and mortality in insect hosts (Mascarin and Jaronski 2016). Therefore, unfavorable environmental conditions could have significantly affected the activity of mycoinsecticides and contributed to the reduced infection observed in these trials. Additionally, relatively high levels of control mortality were observed in these residual activity trials. Spotted lanternfly adults and nymphs are known to move up and down tree trunks to avoid direct sunlight (PMS and MJR, pers. comm.). The caging likely constrained this natural movement, potentially contributing to the elevated control mortality.

Regarding ovicidal activity, our study found that mycoinsecticide and horticultural oil sprays on SLF eggs did not significantly reduce the proportion of hatched nymphs or increase infection in newly emerged nymphs. While *B. bassiana* has demonstrated ovicidal activity against various other insect pests and mites, such as *Lasioderma serricorne* (Coleoptera: Anobiidae)(Saeed et al. 2017), *Tetranychus cinnabarinus* (Acari: Tetranychidae)(Shi and Feng 2004),

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and *Clavigralla tomentosicollis* (Hemiptera: Coreidae) (Ekesi et al. 2002), no fungal growth on any SLF eggs was observed, and hatch rates remained unaffected. Similarly, other tests using *B. bassiana* strain GHA (both ES and WP formulations) reported high hatch rates (> 90%) from SLF eggs on tree surfaces (Bielski 2024). However, Bielski (2024) did note that 31% of emerging nymphs were infected with *B. bassiana*, a higher rate compared to the 16% infection observed in these tests. In this study, treatments with 3% horticultural oil also did not affect SLF emergence. This contrasts with other research where mineral oils at the same concentration killed 71% of eggs (Leach et al. 2019). Furthermore, Leach et al. (2019) found that paraffinic oil decreased egg hatch by 45%, and unpublished data (PAL) indicated 74% mortality of eggs with a 50% mix of soybean oil. These discrepancies suggest that future research should explore a wider range of OMRI-listed (Organic Materials Review Institute) oil and mycoinsecticide products, potentially at higher concentrations, to evaluate their effectiveness against SLF eggs. Additionally, given that egg masses collected from the same tree can hatch over a period of up to 2 weeks (EMR, pers. observation), further work is needed to determine if multiple applications would be necessary to ensure that all hatchlings are treated effectively.

CONCLUSIONS

This work demonstrates that *B. bassiana* strain GHA, formulated as both emulsifiable suspension (ES) and wettable powder (WP), is a promising tool for managing SLF adults and nymphs, particularly when direct contact can be achieved. However, the practical implementation of mycoinsecticides by arborists, foresters, and other practitioners requires special considerations. For instance, exposure to high temperatures (above 29 °C, especially during peak summer activity) or rainfall can significantly impact the viability or concentration of *B. bassiana* spores on plant surfaces. Therefore, proper product storage and strategic application timing are crucial to ensure efficacy. It is also important to note that *B. bassiana* infection and subsequent insect mortality are not immediate; this research indicated that it could take approximately one week for SLF mortality to be observed. Additionally, as is common with mycoinsecticides, multiple applications may be necessary to achieve desired control, especially before population densities become high (Poprawski et al. 1997; Ugine et al. 2007). To forecast optimal application dates, practitioners can utilize online degree-day models (Barker et al. 2025). These resources provide state-specific information regarding when adult SLF are expected to be active in a given region. Given these challenges associated with applying direct contact treatments, future research should prioritize innovative spore dispersal methods. Such advancements would enhance both the efficacy and practicality of mycoinsecticides in the field.

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