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The Effects of UV Radiation on *Metarhizium anisopliae*

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Abstract

The development of fungal insecticides as biological control agents provides a safer, more natural approach to pest control than chemical insecticides. *Metarhizium anisopliae*, one of the most widely used mycoinsecticides, is one fungal species of high economic potential in current use. However, a rapid decrease in pathogen activity in the field due to ultraviolet radiation presents a problem for efficacy and further adoption by farmers. The present study seeks to determine if certain strains of *M. anisopliae* are more tolerant of UV radiation, thus providing the potential for higher effectiveness in the field. Utilizing a novel method for examining fungal persistence that is more conducive to real-world scenarios of fungicide application to crops, fungi were applied on leaf disks rather than agar plates. Leaf disks were then exposed to UV-A and UV-B from an artificial source at UV intensity equivalent to mid-day June or July. After being exposed to UV irradiation, the conidia were removed and subjected to a germination test as a measure of the lethality of the UV exposure. This study examined 15 strains of *M. anisopliae* obtained from the Sidney, Montana, USDA-ARS collection of entomopathogenic fungal cultures.

Introduction

Insects are major pests to global agriculture systems, from the gypsy moth in the United States, to the locusts and grasshoppers in sub-Saharan Africa, to the spittlebugs in Brazil (Vega and Kaya, 2011). Chemical insecticide application, a common solution, can have adverse effects such as groundwater contamination leading to environmental concerns as well as harmful effects on the natural enemies of pests (Niassy et al., 2011). Furthermore, the development of chemical resistance to insecticides by agricultural pests leads to ineffective control and has posed the need for substituting chemical insecticides with biological control agents such as fungi (Moon San Aw, 2017).

Fungal insecticides provide a safer response to pest problems than chemical insecticides, as fungicides are considered safe with minimal risks to vertebrates, humans, and the environment (Zimmerman, 2010). In 2006, 129 mycoinsecticide products were available commercially worldwide (Faria and Wraight, 2007). The entomopathogenic hyphomycete, *Metarhizium anisopliae*, is one of the most widely used mycoinsecticides (Zimmerman, 2007; Faria and Wraight, 2007) in programs of control of agricultural pests and disease vectors in mainly Asia, Latin America, and Eastern Europe (Faria and Wraight, 2007). Nine mycoinsecticides are currently registered by the U.S. Environmental Protection Agency (Jaronski, 2014). Termites, locusts, grasshoppers, and cockroaches are common pests among the 204 insect species from seven orders currently being targeted by this generalist fungi species (Zimmerman, 2007).

However, the rapid decrease in pathogen activity following application observed in the field is a serious challenge to the more extensive use of fungal insecticides (Braga et al., 2001a,b). Some attempts to increase fungal persistence, such as with night application, incorporation of photoprotectors to conidial formulations, and the selection of more tolerant strains of fungi, have been partially successful (Moore, 1993 et al., 1993; Braga et al., 2000c). However, multiple applications of fungi can be inefficient and thus costly for farmers. A more effective commercial alternative to chemical insecticides could lead to safer and more environmentally friendly pest control around the globe.

Conidia are the primary infective form of most fungal entomopathogens (Faria and Wraight, 2007). Fungi are applied as spores to crop foliage in a similar manner as chemical pesticides (Jaronski, 2010). While some of the spores land directly on the target insect (*spp.*), the insect acquires many more spores as it moves through the crops and feeds on treated foliage. A number of spores, tens to thousands of conidia for a median effective dose (LC_{50}/LD_{50}), are required to fatally infect an insect (Jaronski, 2010). Spore germination from penetration of the insect cuticle initiates infection, whereby the fungal hyphae enters into the hemocoel of the insect (Jaronski, 2010). After 24 hours the fungus proliferates internally in the insect (Jaronski, 2010), consuming the insect's energy sources and releasing metabolites that kill the insect within a matter of days (Jaronski, 2010).

Three main environmental factors influence the stability and thus effectiveness of fungal entomopathogens: ultraviolet light, temperature, and humidity (Vega & Kaya, 2011). Ultraviolet radiation from the sun may be most

detrimental to the viability of fungi for pest control applications (Moore et al. 1993). Ultraviolet light occurs at three different spectra: UV-C (100-280 nm), UV-B (280-315 nm), and UV-A (315-400 nm) (Vega & Kaya, 2011). The visible range occurs at 380-780 nm. Only UV-A and B are a concern because UV-C is blocked by ozone and does not reach the Earth (Blumthaler, 1993). Conidia of hyphomycetes are very susceptible to UV-B, as well as the more abundant UV-A (Vega & Kaya, 2011). This is because exposure to UV-B irradiation may cause damage to cellular macromolecules such as DNA, proteins, biomembranes, RNA, and ribosomes (Griffiths et al. 1998).

A deeper understanding of the effects of solar radiation on *M. anisopliae* conidia would bridge an important gap in our understanding of the relationship between ecological factors and fungal persistence and provide direction for pest control programs to improve UV radiation tolerance in this entomopathogenic species of high economic potential. The present study used a UV-irradiation chamber to measure both UV-A and UV-B spectra to ensure accurate UV irradiation on *M. anisopliae* spores to specifically examine the effects of ultraviolet light on spore germination on various strains of *M. anisopliae* and consequently to determine the most tolerant strains among a group being examined by USDA-ARS in Sidney, Montana. Current growth in commercial interest has stimulated the development of various cost-effective production methods and efficient, shelf-stable conidial formulations (Jaronski and Mascarin, 2016). Thus, some strains were expected to perform better than others. Unlike previous studies that assessed germination on agar plates, the present study seeks to create a more natural exposure situation by plating spores on plant cuticles to simulate foliar application

and exposing them to UV-A and UV-B from an artificial source at UV intensity equivalent to mid-day June or July. After being exposed to UV irradiation, the conidia were moved and subjected to a germination test as a measure of the lethality of the UV exposure.

Materials and Methods

Fungal isolates

Fifteen strains of *M. anisopliae*, as well as one strain of *B. bassiana*, obtained from the Sidney, Montana, USDA-ARS collection of entomopathogenic fungal cultures were used in this study. All fungal isolates were stored at -20°C as technical grade conidial powders that had been produced by the USDA-ARS of Sidney, Montana, using biphasic solid substrate fermentation. Spore concentrations for all fungi were determined prior to the start of the experiment with a hemocytometer to ensure enough spores were present in the sample to count. Similarly, the viability of the fungal spores was assessed on the day prior to each UV exposure to ensure that spore samples were more than 50% viable.

Preparation of spore suspensions

All tools were sterilized using 70% ethanol to ensure there was no cross-contamination of strains. One mL of 0.1% Silwet L-77 (a commercial wetting agent, Momentive LLC) was dispensed into a 2 mL microcentrifuge tube. Using a sterile cotton applicator, spores were dipped into the dry conidial powder so that the cotton applicator was completely covered in spores. The spores were then prehydrated at 100% relative humidity for at least 30 minutes before suspended in the dilute Silwet. The spores were then dislodged from the cotton swab within the centrifuge tube, shaken vigorously with the capped tube, and then sonicated in a bath sonicator for three minutes to obtain completely disperse suspension of spores. Six clear Petri plates were each set up with three to five pieces of Whatman No. 3 filter paper and wetted with reverse osmosis water until a sheen of liquid was

visible on the paper. Leaf disks six millimeters in diameter were cut from flat spinach leaves with a metal cork borer. Six leaf disks were used per fungal sample. Spores were gently added to the leaf disks using a fresh, sterile cotton applicator dipped in the spore suspension. After all disks were inoculated, the excess fluid was dried off of the leaf disks using the air flow of a laminar air hood. After all surface liquid seemed to have evaporated from the leaf disks each Petri disk was covered with a transparent lid.

UV exposure

The UV exposure chamber was set for an interior temperature of 18-20°C. The chamber contained three banks of Fiji sunlamps (KBD Inc., Crescent Springs, KY), each with 4 FR20T12UVB-BP fluorescent bulbs. The UV lamps inside the chamber were arranged to deliver 0.22-0.225 mW UV-B/cm² and 1.2-1.3 mW UV-A/cm² to the level of the leaf disks. Lamps were turned on prior to the start of the experiments, resulting in a stable radiation range. The Petri dish covers were removed and dishes with leaf disks were placed beneath the sunlamps and exposed to UV light for irradiances of 0, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 Joules UV-B/cm².

Control plates were wrapped with aluminum foil and thus physically protected from any radiation. UV-A and UV-B radiation was monitored using Solar Designs sensors connected to an integrating logger. After each designated exposure had passed, the dish assigned to that exposure was taken out of the UV chamber and kept in the dark until the remainder of the dishes had received the selected UV exposures.

Processing exposed leaf disks

When the UV exposures were finished, leaf disks were taken out of the UV chamber and returned to the lab. For each UV exposure, the six leaf disks containing a fungus strain were transferred into a 10 mL tube with 0.5 mL of 0.1% Silwet L-77, vortexed briefly, and sonicated in a bath sonicator for 1-2 minutes to wash the spores from the leaf disk surface and disperse them in suspension for a more accurate viability reading.

Viability test

A cotton-tipped applicator was dipped into a suspension and the suspension was streaked onto Petri plates of potato dextrose yeast agar (PDAY) or half-strength Sabouraud dextrose yeast agar (SDAY). Agar plates were incubated at 27-28°C for 18-20 hours, depending on how fast germination occurred for each fungal isolate. After appropriate incubation, agar squares (~18x18mm) were cut out and transferred to a microscope slide. A drop of lactophenol cotton blue stain added to the agar surface to fix and stain the spores and germination hyphae. Spores stained blue with visible hyphae longer than the diameter of the conidia were considered to have germinated, while clear spores without hyphae were counted as ungerminated. Spores stained blue that were starting to germinate but did not have visible hyphae were counted as ungerminated. At least 200 spores per sample were examined with 650X brightfield or phase contrast microscopy and scored as germinated or ungerminated. Percent germination was calculated based upon germinated spores over the total spores counted in each viability determination.

Statistical analysis

The percent viability data were transformed by angular transformation for graphing against the Joules of UV-B radiation received by the spores for visual examination of the data. The LD₅₀ (UV-B dose in Joules necessary to kill 50% of the spores), slope of the regression and associated statistics was calculated using probit analysis.

Statistical analysis was performed using Probit V. 5.5 (A.M.M Ebieda,

<https://www.freewebs.com/ebieda/agriculturalprograms.htm>).

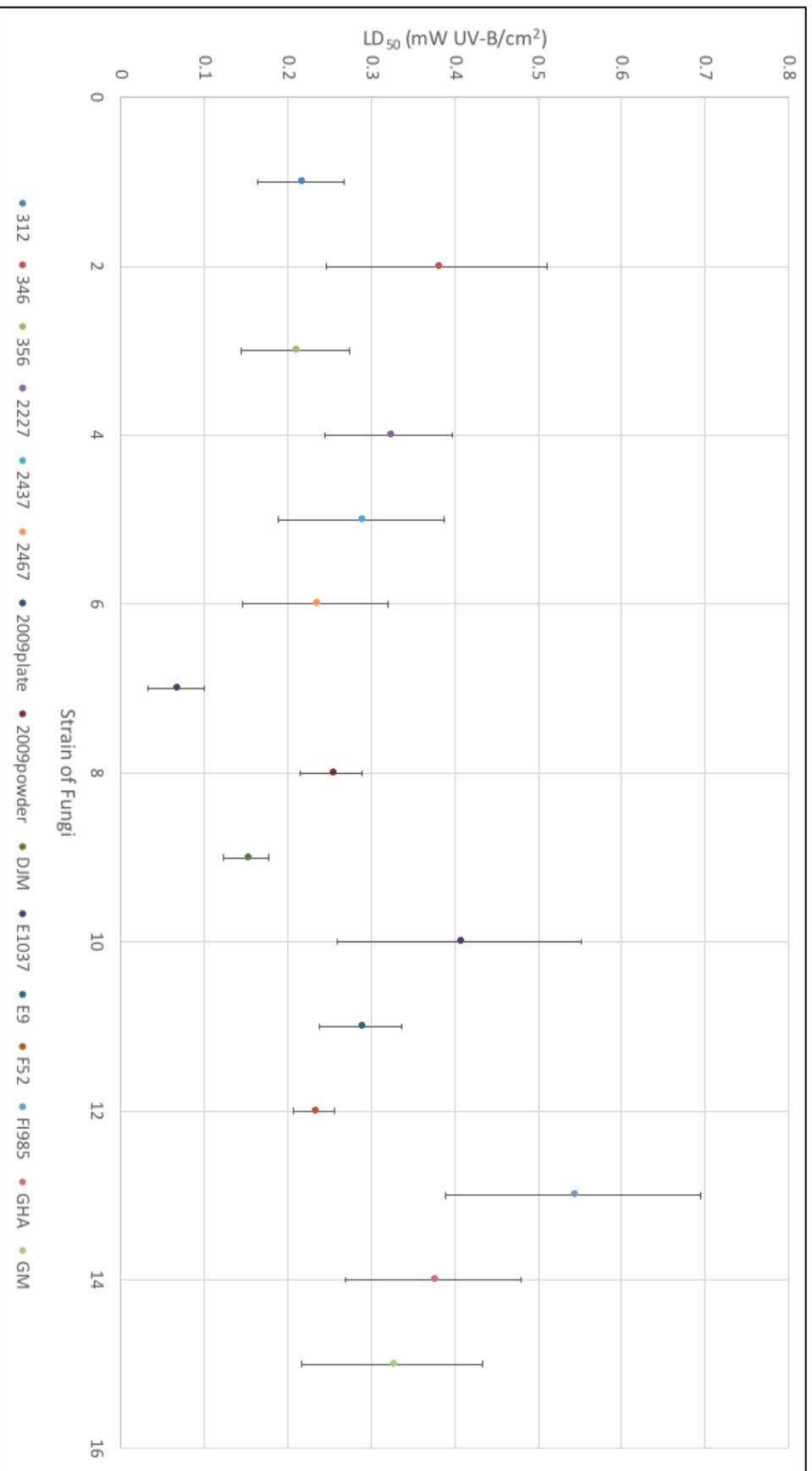


Figure 1. Mean LD₅₀ (in mW UV-B/cm²) per fungal strain (on right).

In the mean LD₅₀ plot (Figure 1) it appears that the 2009 plate has the lowest LD₅₀, while F1985 has the highest.

The graph seems to indicate a statistically significant difference in LD₅₀ between strain F1985 and strains 312, 2467, DJM, E9, F52, and 2009 plate and powder. However, there does not appear to be a statistically significant difference between strain F1985 and strains 346, 2227, E1037, GHA, and GM.

Discussion

The results of this study suggest the FI985 *M. anisopliae* strain should be prioritized in field trials. Moreover, some strains, such as the 2009 strain, could be eliminated from field trials. The 2009 strain was stored on an agar plate rather than in a technical grade plastic container, which may provide insight into fungal isolate storage. Furthermore, these results may suggest that the technique utilized in the present study of applying fungal spores to leaf disks rather than agar plates may be useful for future trials attempting to replicate a more “realistic” scenario of fungicide application to crops. The results of the present study confirm the findings of intraspecies variability in susceptibility of UV radiation (Braga et al., 2001; Fernandes et al., 2015). The present study also furthers the Braga et. al 2001 study that measured only the UV-A component by measuring both UV-A and UV-B radiation components. While there are a number of laboratory studies using artificial UV sources, there are far fewer studies using outdoor UV light (Jaronski, 2010). Further research into why some fungal strains lose viability faster than others and how multiple ecological factors such as UV light, temperature, and humidity interact in more real-world field experiments by treating actual natural leaves may provide more compelling evidence of which strains of *M. anisopliae* and other fungicides are likely to be most effective in the field, to the benefit of both farmers and consumers.

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