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SUSCEPTIBILITY OF *LATRODECTUS GEOMETRICUS* (ARANEAE: THERIDIIDAE) TO A *MUCOR* STRAIN DISCOVERED IN NORTH CENTRAL FLORIDA, USA

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Abstract

A population of adult reproductive female brown widow spiders, *Latrodectus geometricus* Koch (Araneae: Theridiidae) was collected in North Central Florida. Within weeks, the spiders displayed reduced foraging behavior and began to die. A white fungal growth was observed on deceased individuals and others who showed signs of decline. Fungal samples were collected from living spiders as well as from recently deceased specimens. Samples were excised from the spiders and isolated on acidified potato dextrose agar. Morphological characterization was cross referenced with the most recent phenotypic variability studies to confirm probable matches. Molecular identification was rendered using ITS1/5.8S/ITS2 and *Mucor fragilis* Bainier (Mucorales: Mucoraceae) was indicated with 99% match. Little is documented regarding *M. fragilis*, and no prior records of pathogenic occurrence are known by the authors. Subsequent bioassay trials supported with 83% mortality that this novel strain of *M. fragilis* does infect and kill *L. geometricus.*

Key Words: *Latrodectus geometricus*, brown widow, *Mucor fragilis*, pathogen, fungus, biocontrol

Resumen

Se recolectó una población de adultos hembras reproductores de la araña viuda marrón, *Latrodectus geometricus* Koch (Araneae: Theridiidae) en el norte de la Florida Central. En pocas semanas, las arañas muestran el comportamiento de alimentación reducida y comenzaron a morir. Se observó un crecimiento de hongos blancos sobre los individuos muertos y otros que mostraban se ales de deterioro. Se obtuvieron muestras de hongos de las arañas vivas, así como de especímenes que murieron recientemente. Las muestras fueron extirpados de las arañas y aislados en agar de dextrosa de papa acidificada. La caracterización morfológica fue comparada con las referencias de estudios más recientes de la variabilidad fenotípica para confirmar las coincidencias probables. Se hizo la identificación molecular utilizando ITS1/5.8S/ITS2 y *Mucor fragilis* Bainier (Mucorales: Mucoraceae) fue el designado con el 99% de similitud. Poco se ha documentado sobre *M. fragilis*, y no hay registros anteriores de la ocurrencia de patógenicad conocida por los autores. Pruebas de bioensayo posteriores apoyaron los resultados con una mortalidad del 83% que esta nueva cepa de *M. fragilis* infecta y mata a *L. geometricus*.

Palabras Clave: *Latrodectus geometricus*, viuda marrón, *Mucor fragilis*, patógenos, hongos, biocontrol

The brown widow, *Latrodectus geometricus* C. L. Koch (Araneae: Theridiidae), is an invasive species that was confirmed to be in the United States in 1935 (Pearson 1936). Since its establishment, *L. geometricus* progressed into northern Florida in the 1980's and, by 2008, had continued its expansion into Texas, Georgia, Mississippi, Louisiana, and California (Brown et al. 2008; Vincent *et al*., 2008). This active spread is attributed to human assisted movement that has ultimately led to this species becoming a cosmopolitan invasive (Garb et al. 2004).

L. geometricus is more likely than the black widow, *L. mactans* Fabricius, to invade peridomestic environments. The readiness with which *L. geometricus* inhabits artificial structures contributes to its establishment within or near regularly inhabited urban areas. This habitat choice increases potential for human contact with this venomous spider, creates medical concern for envenomation and drives people to seek management strategies in a variety of urban settings, including residential property, schools, and nurseries.

Ecological information about *L. geometricus* as an invasive species is limited. There is currently no knowledge base to establish competitive effects of *L. geometricus* against other species, particularly other members of *Latrodectus*. Although egg sac parasitoids have been documented, there is no confirmation that the existing parasitoids significantly suppress brown widow populations (Brown et al. 2008; Pemberton & Rosa 1940). *Latrodectus geometricus* also seems to prefer urban habitats and has a microhabitat preference that allows great prevalence in areas lacking suitable protection for other *Latrodectus* spp. reported from urban areas (Vetter et al. 2012). The anxiety generated by *L. geometricus* may incite increased chemical usage in populated areas (Vetter et al. 2012).

While observing field-collected specimens of *L. geometricus,* behavioral changes and mortality were documented. Upon closer examination, it was found that the spiders exhibiting reduced foraging behavior were infected with a fungal contaminant. A non-obligate pathogen was investigated as the cause of mortality. Results indicated a novel strain of *Mucor* that may be functional for use against *L. geometricus*. Exploration of this strain could reveal insight into causes of mortality of *L. geometricus* in its invasive range and build a case for researching alternative management strategies for this species. Since there is little information regarding what environmental opposition this species encounters, determining the cause of mortality may indicate that the spider still meets immunological barriers in its invasive range and lead to a valuable biological control for this venomous spider.

Materials and Methods

Pathogen Isolation

Brown widow spiders were field-collected from urban areas within a 3-mile radius of the University of Florida campus in Gainesville, Florida. Areas such as crevices of brick benches, buildings, signs, mailboxes, refuse containers, storage containers, and storage sheds were investigated from ground level to a height of 6 feet around the structures and in close proximity to potential food sources such as ant and termite colonies. Spiders were removed from their webs with forceps and were contained in individual screw-top vials for transport to the lab. Thirty-four specimens were collected from their webs from 1 Dec 2010 to 31 Jan 2011 (Suppl. Table 1).

After transport to the lab, specimens were placed individually into ventilated 16 oz. plastic deli cups and held at 24 °C ± 0.5 °C and 12:12 h L:D in a Percival environmental chamber (Percival Scientific, Perry, Iowa) for behavioral observation. Spiders were fed laboratory-reared adult female crickets, *Acheta domesticus* (L.) (Orthoptera: Gryllidae), as needed. Containers were cleaned within 6 h of cricket consumption in a biological safety hood with HEPA filtration and using aseptic technique with forceps used for debris removal. No fungal growth or sign of decline was observed on the feeder crickets or in the cricket colony. A fungal growth was noted on spiders that were in decline and those that had recently died. Once a change in behavior or a fungal growth was observed, individuals were placed on a water agar plate for 16 days in order to leech non-target fungi away from the host and starve out fungi not using the spider for nutrition. Spiders were not surface sterilized before admission to water agar so as not to risk the loss of the target fungus. Mycelial growth for DNA extraction was collected from the ventral surface of 3 coxae and from the dorsal side of the prosoma of each spider. After mortality, cadavers were transferred to McCoy agar plates (McCoy Health Science Supply, Maryland Heights, Missouri) to inhibit the growth of bacteria. The entire cadaver was transferred to the agar plate because it was uncertain if the fungus had an obligatory relationship with the host. After 5 days, dense fungal growth was apparent on the cadavers and on the surrounding agar. Samples of mycelia were taken from the spider body and from the surrounding agar for subculturing to obtain pure isolates, followed by DNA extraction of the pure colony.

Samples excised from spiders were cultured on acidified potato dextrose agar (APDA) to minimize contamination and maintained at 24 °C ± 0.5 °C and 12:12 h L:D in an environmental chamber separate from spider containment. Pure isolates were obtained and morphological and molecular characterizations were conducted. Q Imaging digital and differential interference contrast microscopy images were taken of phenotypically significant structures to facilitate morphological identifications. Microscopy results were cross-referenced with phenotypic variability studies for probable matches to Zygomycota.

DNA was extracted using DNeasy Plant Mini Kit™ (Qiagen catalog no. 69106 Valencia, California). The ribosomal ITS1/5.8S/ITS2 region was amplified using Schwartz's V9D and LS266 primers (Schwarz et al. 2006). The amplification mixture (40 uL) consisted of 20.0 uL REDExtract-n-Amp™ PCR ReadyMix™ (Sigma catalog no. R4775, St. Louis, Missouri), 20 uM concentration of each primer and 2 uL of extracted DNA. The parameters for the Bio-Rad T3000 (Bio-Rad, Hercules, California) thermocycler were as follows: initial denaturation at 94 °C for 10 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 30 s; with a final extension cycle at 72 °C for 10 min. The PCR products were sequenced using Sanger Sequencing (UF-ICBR, Gainesville, Florida) and compared to sequences in NCBI GenBank.

Koch's Postulates

Twenty-four hours prior to testing, spiders were field-collected and placed into individual containers (Suppl. Table S2). Fungal cultures were prepared by rinsing spores from the agar cultures with 0.01% Triton-X 100 solution to create a spore suspension at 10^6 CFU/mL. Spiders were rinsed topically with 5 mL 0.01% Triton-X 100 to mitigate pathogen introduction from the field, and then topically inoculated with 5 mL of the spore suspension. Controls received a treatment of 5 mL 0.01% Triton-X 100. Inoculated and control specimens were contained individually in UV-sterilized 16 ounce (473 mL) plastic deli containers and held at 24 °C ± 0.5 °C, 65% ± 5% RH and 12:12 h L:D in an environmental chamber. Sixty spiders were tested over 4 replicates of 12 treated spiders and 3 controls. The spiders were monitored daily over 10 days for behavioral changes, evidence of fungal growth, and mortality.

After mortality and evidence of external fungal growth was observed, spiders were moved to UV sterilized moist chambers modified from Doberski et al. (1981) (Fig. 1) to provide approximately 100% humidity. To avoid contamination, all procedures were carried out in a biological safety hood with HEPA filtration. Fungal samples from the spiders were collected and transferred to APDA media and cultured for 5 days. DNA extraction, ribosomal ITS1/5.8S/ITS2 amplification, and Sanger Sequencing (UF-ICBR) were repeated and compared to those derived from the cultures used for inoculation.

Results

The PCR products sequenced using Sanger Sequencing (UF-ICBR) and compared to sequences in NCBI GenBank via nucleotide BLAST resulted in a 99% identity match with *Mucor fragilis* Bainier, comparison strain CBS 236.35, GenBank accession FN650655.1, V9D with 991 base pairs, LS266 with 998 base pairs. Identification using columella (Fig. 2), sporangia and spores (Fig. 3), chlamydospores (Fig. 4), and branching patterns (Fig. 5) indicated a match with *Mucor circinelloides f. lusitanicus* (Schipper 1978), which is based on *M. fragilis* being a synonym of *Mucor circinelloides* Tieghem (Schipper 1973, 1978). Molecular re-evaluation of *Mucor* has not been published to date, so confirmation of species will need revision with continued work in the Zygomycota. Cultures are in the care of USDA-ARSEF, Ithaca, New York (storage number: ARSEF 11762).

Spiders began to show reduced foraging activity and heightened aggression at day 2 and began to die at day 3. Once individuals showed visible signs of fungal growth, foraging behavior ceased. Spiders responded to disturbances of the web by rapid retreat and threatening displays with forelegs and fangs. Heightened aggression in infected individuals was displayed by increased strikes and strikes without the threat display seen in healthy spiders. Infected individuals suffered from rapid health decline, noted in the decreased size of the opisthosoma and the wrinkling of the cuticle. These symptoms were consistent among the spiders from which the fungus was acquired and among those inoculated. The initial collection of *L. geometricus* infected with *M. fragilis* from the field resulted in 82% mortality. Inoculative bioassays yielded 83% mortality of the treated spiders within 3 to 5 days (Fig. 6) with no control mortality. Within 3 days of inoculation with *M. fragilis*, there was visible fungal growth on the exterior of the spider cuticle, beginning around the cervical grooves on the dorsal surface of the prosoma, moving to the peripheral eyes, and in some instances spreading to the dorsal peak of the opisthosoma immediately after the pedicel adjoining the body regions (Figs. 7 and 8). Post-bioassay comparisons of sequenced DNA showed 100% match with the DNA extracted from pure isolates used for inoculum.

Discussion

Results support the hypothesis that *M. fragilis* was responsible for the deaths observed in the wild-caught population, and that this fungal species is pathogenic to *L. geometricus*. In other arthropods, entomopathogenic fungi such as *Beauveria bassiana* (Bals.) Vuillemin (Hypocreales: Cordycipitaceae) and *Isaria fumosorosea* Wize (Eurotiales: Trichocomaceae) invade the host through direct penetration of the cuticle during spore germination (Lei et al. 2011; Vineet et al. 1999). Subsequent invasion of the hemocoel and draining of bodily fluids, nutrients, or occupancy of all available space results in the host's death (Lei et al. 2011; Vineet et al. 1999). Mucorales typically infect the host through injury, or microscopic lesions in the host cuticle (Heitor 1962; Nentwig & Prillinger 1990). Such a route would lead to mucormycosis, i.e., an invasive necrotic disease caused by one of the Mucorales and typically affecting respiratory tissue, but also capable of exhibiting symptoms in the central nervous system, gastrointestinal tissue and skin (Sugar 1992). This condition is applicable to several types of organisms, including humans, various other mammals, and invertebrate arthropods (Camalt 2004; Heitor 1962; Stewart et al. 1999; Sugar 1992; Sychev 1993). This is the first record of *M. fragilis* as a pathogen on *L. geometricus*, so mechanisms of infection have not been defined.

In our study of symptomatic spiders, without exception, we observed that once the fungus was visible on the prosoma, the spider would no longer accept food and the opisthosoma had become noticeably reduced in size. Because of the cessation of foraging activity, dehydration and starvation are also possible causes of mortality. In rearing trials, healthy brown widows can remain robust for approximately 30 days without feeding (Bibbs, unpublished). Despite the ability to survive prolonged

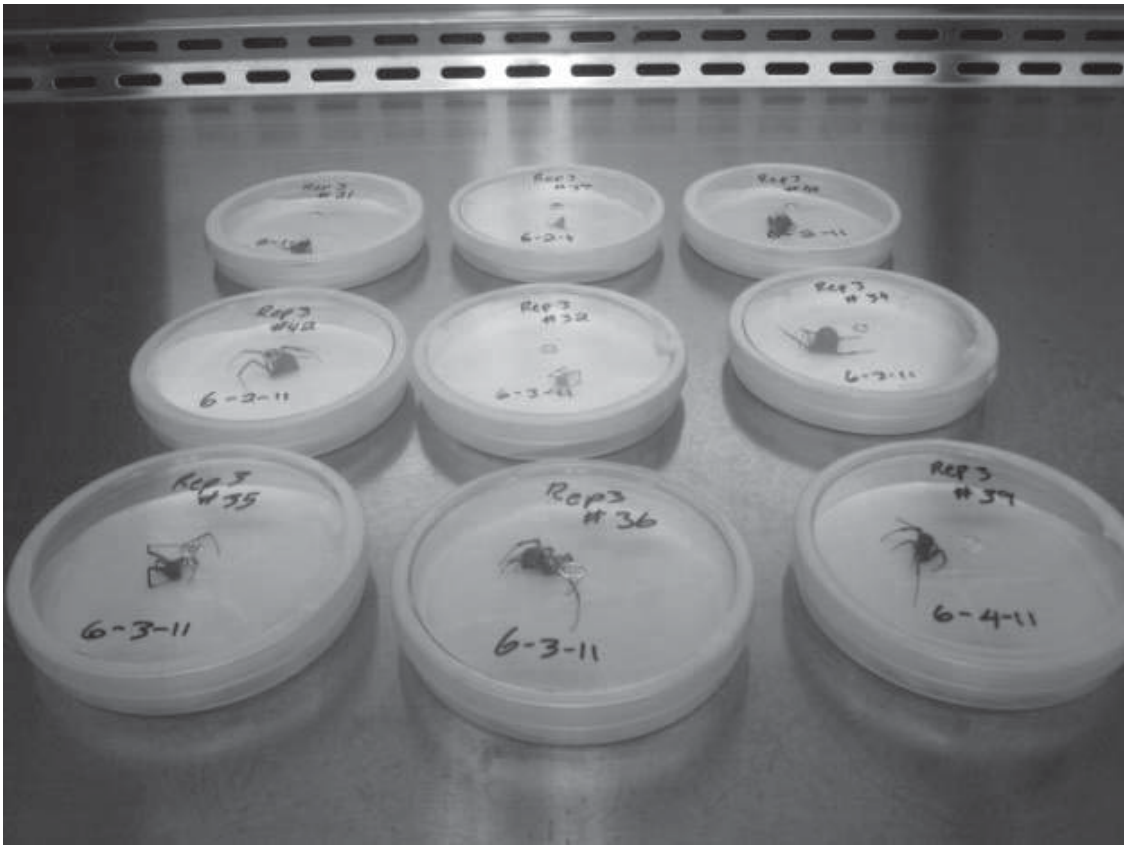

Fig. 1. *Latrodectus geometricus* with fungal growth in moist chambers assembled from a standard petri dish and Whatman No. 1 filter paper; wetted using sterile water and UV-sterilized prior to admission of cadavers.

intervals without nourishment, mortality peaked at 3 to 4 days after appearance of the fungus, so it is unlikely that starvation is the principal cause of mortality. Yoder et al. (2006) noted the inability of tick test subjects to maintain water balance when infected with *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavipitaceae); water loss may be an indicator of pathogenicity.

Spiders may be become infected by fungal spores in several ways. In urban environments and within structures ants have been found with *Mucor* spp. present on the cuticle (Pantoja et al. 2009), so. *L.*

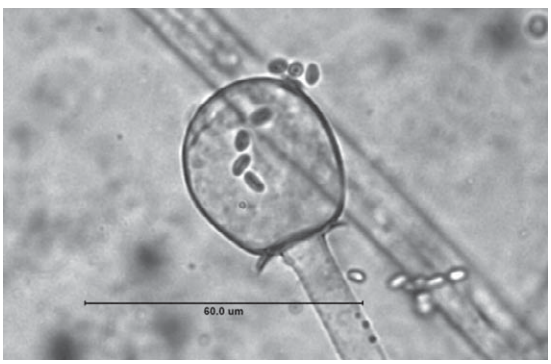

Fig. 2. *Mucor fragilis* columella; taken with Q Imaging digital microscopy and recorded through Image Pro Express.

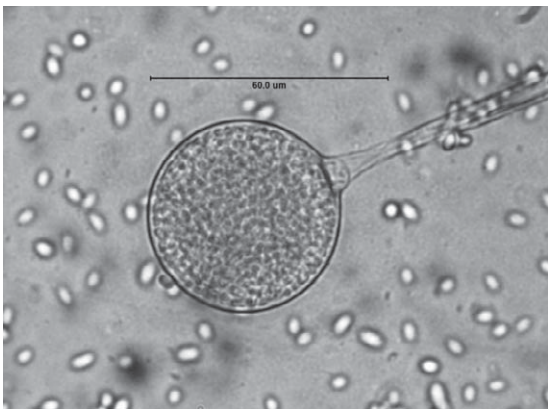

Fig. 3. *Mucor fragilis* sporangium with spores in the background; taken with Q Imaging digital microscopy and recorded through Image Pro Express.`

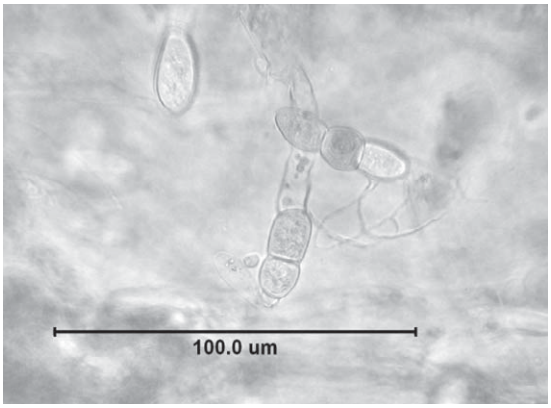

Fig. 4. Chlamydospores of *Mucor fragilis*. Taken with differential interference contrast microscopy.

geometricus could be exposed to the fungus from their arthropod food source. Another possibility is that as a web maker, *L. geometricus* is more exposed to airborne spores. In an evaluation of fungal mortality in warmer climates, it was noted that web makers expose soft areas on the body, such as the epigynal opening or lung entry, during normal postures, which may lead to increased rates of infection (Nentwig 1985). In the case of *L. geometricus*, this seems less likely because of the dense conical design of the web protecting much of the exposed parts of the body as well as a thickly-lined retreat in the depth of the web; however it is of interest to note that juveniles may lack this web design (Eberhard 2008), and so by consequence may represent a vulnerable stage for infection.

Mortality due to fungal infection increases with temperature in some fungal species, and declines with rise of temperature in others. This difference is because of heat neutralization of infection (Houle et al. 1987). In the case of the *Mucor* genus, several assessments indicate operational growth from 5-38

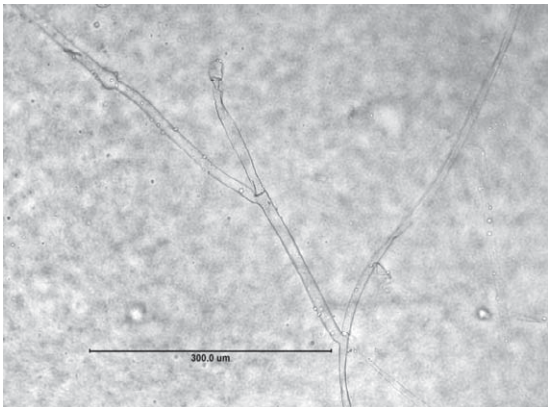

Fig. 5. *Mucor fragilis* sympodial branching pattern; taken with Q Imaging digital microscopy and recorded through Image Pro Express.

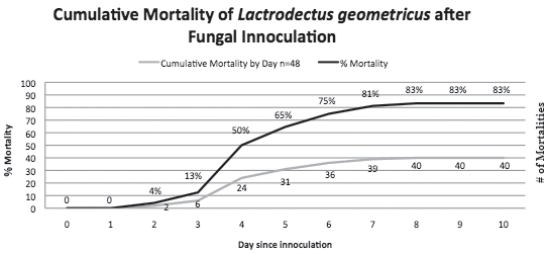

Fig 6. Mortality of *Latrodectus geometricus* from *Mucor fragilis* over inoculation trials. Control mortality was zero.

°C depending on biogeography and tolerances, but trends within the genus display enhanced metabolic function at the lower temperature range of its growth tolerance, i.e., from 5-24 °C (Carvalho et al. 1999; Cheng et al. 2011; Laoteng et al. 1999; Stewart et al. 1999; Yazdi et al. 2006). This lends support to the observation that in the winter season with the mean winter temperature recorded at 9.7 °C, infection accelerated, and the laboratory containment conditions at 24 °C were not hot enough to deter continued infection.

Feeding frequency and quality of food also affect the immune responses of organisms. Engorged ticks showed great degrees of resistance to infection by fungal pathogens during experimentation with ticks in varying life stages and conditions; conversely, unfed ticks showed accelerated rates of infection and quicker mortality (Samsinakova et al. 1974; Gindin et al. 2002; Yoder et al. 2006). A *Mucor* sp. was able to infect and kill ticks during a winter study, especially after oviposition (Samsinakova et al. 1974). Although unable to engorge like ticks, *L. gemoetricus* and several other araneaemorphs have exceptional capacity for opithosomal distention, allowing for food reserves to last long intervals. Unfed captive groups waited as long as 15-20 days during rearing and colony maintenance without noticeable decline in opisthosomal size. It is probable that

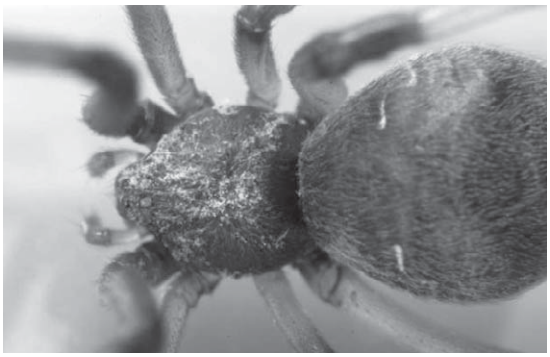

Fig. 7. *Mucor fragilis* on *Latrodectus geometricus*' prosomal cuticle around cervical grooves and eyes; subject imaged 3 days after inoculation.

Fig 8. *Mucor fragilis* progressing to opisthosoma; subject imaged 4 days after inoculation.

with cooler temperatures, the feeding frequency and quality was less than adequate to maintain appropriate immune response. Reduced feeding combined with reduced temperatures likely increased the susceptibility of the population to fungal infection.

There are no significant findings to support the notions that true entomopathogens of insects infect spiders and that true entomopathogens of spiders infect insects (Evans & Samson 1987). Thus even though *Isaria* sp. *(= Paecilomyces)* and *Beauveria* sp. can be found externally as normal mycoflora on some araneaeids, dissection of these spiders revealed no infections or internal spores despite the known entomopathogenicity of these 2 groups (Yoder et al. 2009). A limited number of the *Mucor* genus has shown contrary evidence, but they are not obligatory parasites and so are harder to define as true entomopathogens, but they have facultative characteristics. In another example, *Mucor hiemalis* (Wehimer), a known pathogen of insects and plants, was observed infecting spiders in a laboratory setting (Nentwig & Prillinger, 1990). This example is of a recognized saprophytic fungus (Zycha et al. 1969) and situational pathogen of plants and insects (Burnside 1935; Heitor 1962; Zycha et al. 1969) infecting an arachnid species. Given the potential of *M. fragilis* to be a pathogen, and with undetermined mechanisms of infection and mortality, it would be prudent to consider this species as facultative. However, there is disagreement as to the true tendencies of *Mucor* spp. They are generally classified as saprophytic (Samisinakova et al. 1974; Verma & Krishnan 1985; Pai et al. 2004; Pantoja et al. 2009; Yoder et al. 2009; Cheng et al. 2011) but seem to have facultative potential comparable to recognized pathogens (Heitor 1962; Nentwig 1985; Sugar 1992; Sychev 1993; Camalt 2004).

Although the authors feel their containment methods and sterilization of equipment between uses would limit infection from lab materials, histological examination may have identified the method of infection in the spider hosts. Due to the limited scope of this project, the authors were not able to employ histopathology to conclusively identify the mechanism of infection. This would be an area for future research. The act of forcefully removing the spiders from the wild and placing them in containers would have caused considerable stress for the animal. This stress could have compromised the immune response of the organisms long enough for infection to occur, also resulting in the 2-3 day pattern of mortality immediately following collection.

There are suggestions that *L. geometricus* may have originated from either South America or Africa (Levy 1959; Garb et al. 2004). The authors conjecture that *Mucor* spp., although present worldwide, might suffer from considerably more competition in these warm climates due to high species richness (Evans & Samson 1987) and as a consequence be less abundant, limiting the pressure for the widow spider to develop immunity. Regarding pathogenic efficacy, Posada & Vega (2005) assembled criteria for testing fungal pathogens for biocontrol potential. Subjects would be totally immersed in a spore solution and then assessed for mortality rate and distribution, percent spore germination, fungal life cycle duration, and spore production on the host. Because *L. geometricus* also falls within the range of *L. mactans*, future experimentation should involve both species to compare resistance and susceptibility of a native, temperate species with that of the invasive, tropical species. Potential for horizontal transmission would be another desirable investigation for the purposes of cost-effective autodissemination of the fungal pathogen. *Mucor fragilis* would need to be evaluated for survival in various environmental regimes and application techniques evaluated for efficacy as a biological control.

Acknowledgments

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SUPPLEMENTAL MATERIALS

Supplemental collection data for all *Latrodectus geometricus* C. L. Koch used during pathogen acquisition and bioassays. Contained values are the date of collection, coordinates of location, and nearest facility, structure, or crossroads for location detailed for each individual spider. Table S1 reports information for spiders collected during discovery of the *Mucor fragilis* Bainier strain. Table S2 reports information for spiders collected for use in inoculatory bioassays.

SUPPLEMENTAL TABLE S1:

Specimen Label	Latitude	Longitude	Date	Nearest Building
1	29°39.122’	82°20.487’	3-XII-2010	U.F. Anderson Hall
2	29°39.122’	82°20.487’	3-XII-2010	U.F. Anderson Hall
3	29°39.122’	82°20.487’	3-XII-2010	U.F. Anderson Hall
4	29°39.122’	82°20.487’	3-XII-2010	U.F. Anderson Hall
5	29°39.122’	82°20.487’	21-XII-2010	U.F. Anderson Hall
6	29°39.122’	82°20.487’	21-XII-2010	U.F. Anderson Hall
7	29°39.122’	82°20.487’	21-XII-2010	U.F. Anderson Hall
8	29°39.122’	82°20.487’	21-XII-2010	U.F. Anderson Hall
9	29°37.849’	82°19.589’	5-I-2011	Treehouse Village Condominiums
10	29°37.835’	82°19.609’	5-I-2011	Treehouse Village Condominiums
11	29°37.835’	82°19.609’	5-I-2011	Treehouse Village Condominiums
12	29°37.778’	82°19.605’	5-I-2011	Treehouse Village Condominiums
13	29°37.829’	82°19.567’	5-I-2011	Treehouse Village Condominiums
14	29°39.122’	82°20.487’	16-I-2011	U.F. Anderson Hall
15	29°39.114’	82°20.441’	16-I-2011	U.F. Matherly Hall
16	29°39.114’	82°20.441’	16-I-2011	U.F. Matherly Hall
17	29°39.114’	82°20.441’	16-I-2011	U.F. Matherly Hall
18	29°39.114’	82°20.441’	16-I-2011	U.F. Matherly Hall
19	29°39.114’	82°20.441’	16-I-2011	U.F. Matherly Hall
20	29°39.105’	82°20.550’	31-I-2011	U.F. Keene-Flint Hall
21	29°39.105’	82°20.550’	31-I-2011	U.F. Keene-Flint Hall
22	29°39.105’	82°20.550’	31-I-2011	U.F. Keene-Flint Hall
23	29°39.105’	82°20.550’	31-I-2011	U.F. Keene-Flint Hall
24	29°39.105’	82°20.550’	31-I-2011	U.F. Keene-Flint Hall
25	29°39.105’	82°20.550’	31-I-2011	U.F. Keene-Flint Hall
26	29°39.105’	82°20.550’	31-I-2011	U.F. Keene-Flint Hall
27	29°39.049’	82°20.547’	31-I-2011	U.F. Library West
28	29°39.049’	82°20.547’	31-I-2011	U.F. Library West
29	29°39.049’	82°20.547’	31-I-2011	U.F. Library West
30	29°39.049’	82°20.547’	31-I-2011	U.F. Library West
31	29°39.049’	82°20.547’	31-I-2011	U.F. Library West
32	29°39.049’	82°20.547’	31-I-2011	U.F. Library West
33	29°39.049’	82°20.547’	31-I-2011	U.F. Library West
34	29°39.049’	82°20.547’	31-I-2011	U.F. Library West

SUPP. TABLE S2:

Replicate	Latitude	Longitude	Date	Nearest Building/Structure
1	29°38.945'	82°20.874'	10-IV-2011	U.F. Ben Hill Griffin Stadium
	29°38.945'	82°20.874'	10-IV-2011	U.F. Ben Hill Griffin Stadium
	29°38.945'	82°20.874'	10-IV-2011	U.F. Ben Hill Griffin Stadium
	29°38.945'	82°20.874'	10-IV-2011	U.F. Ben Hill Griffin Stadium
	29°38.945'	82°20.874'	10-IV-2011	U.F. Ben Hill Griffin Stadium
	29°38.677'	82°21.676'	10-IV-2011	U.F. Student Agricultural Gardens
	29°38.677'	82°21.676'	10-IV-2011	U.F. Student Agricultural Gardens
	29°38.677'	82°21.676'	10-IV-2011	U.F. Student Agricultural Gardens
	29°38.677'	82°21.676'	10-IV-2011	U.F. Student Agricultural Gardens
	29°38.677'	82°21.676'	10-IV-2011	U.F. Student Agricultural Gardens
	29°33.430'	82°26.108'	10-IV-2011	Residential Housing
	29°33.430'	82°26.108'	10-IV-2011	Residential Housing
	29°33.430'	82°26.108'	10-IV-2011	Residential Housing
	29°33.430'	82°26.108'	10-IV-2011	Residential Housing
	29°33.430'	82°26.108'	10-IV-2011	Residential Housing
2	29°39.054'	82°20.557'	1-V-2011	U.F. Library West
	29°39.054'	82°20.557'	1-V-2011	U.F. Library West
	29°39.054'	82°20.557'	1-V-2011	U.F. Library West
	29°39.054'	82°20.557'	1-V-2011	U.F. Library West
	29°39.054'	82°20.557'	1-V-2011	U.F. Library West
	29°38.673'	82°21.670'	1-V-2011	U.F. Student Agricultural Gardens
	29°38.673'	82°21.670'	1-V-2011	U.F. Student Agricultural Gardens
	29°38.673'	82°21.670'	1-V-2011	U.F. Student Agricultural Gardens
	29°38.673'	82°21.670'	1-V-2011	U.F. Student Agricultural Gardens
	29°38.673'	82°21.670'	1-V-2011	U.F. Student Agricultural Gardens
	29°33.430'	82°26.108'	1-V-2011	Residential Housing
	29°33.430'	82°26.108'	1-V-2011	Residential Housing
	29°37.570'	82°19.390'	1-V-2011	Treehouse Village Condominiums
	29°37.570'	82°19.390'	1-V-2011	Treehouse Village Condominiums
	29°37.570'	82°19.390'	1-V-2011	Treehouse Village Condominiums
3	29°39.050'	82°20.250'	29-V-2011	U.F. Matherly Hall
3	29°39.050'	82°20.250'	29-V-2011	U.F. Matherly Hall
	29°39.050'	82°20.250'	29-V-2011	U.F. Matherly Hall
	29°39.050'	82°20.250'	29-V-2011	U.F. Matherly Hall
	29°39.050'	82°20.250'	29-V-2011	U.F. Matherly Hall
	29°39.050'	82°20.250'	29-V-2011	U.F. Matherly Hall
	29°38.673'	82°21.670'	29-V-2011	U.F. Student Agricultural Gardens
	29°38.673'	82°21.670'	29-V-2011	U.F. Student Agricultural Gardens
	29°38.673'	82°21.670'	29-V-2011	U.F. Student Agricultural Gardens
	29°38.673'	82°21.670'	29-V-2011	U.F. Student Agricultural Gardens
	29°38.673'	82°21.670'	29-V-2011	U.F. Student Agricultural Gardens
	29°37.570'	82°19.390'	29-V-2011	Treehouse Village Condominiums
	29°37.570'	82°19.390'	29-V-2011	Treehouse Village Condominiums
	29°37.570'	82°19.390'	29-V-2011	Treehouse Village Condominiums
	29°37.570'	82°19.390'	29-V-2011	Treehouse Village Condominiums
	29°37.570'	82°19.390'	29-V-2011	Treehouse Village Condominiums
4	29°38.945'	82°20.874'	1-VI-2011	U.F. Ben Hill Griffin Stadium
	29°38.945'	82°20.874'	1-VI-2011	U.F. Ben Hill Griffin Stadium
	29°38.945'	82°20.874'	1-VI-2011	U.F. Ben Hill Griffin Stadium
	29°38.945'	82°20.874'	1-VI-2011	U.F. Ben Hill Griffin Stadium
	29°39.054'	82°20.557'	1-VI-2011	U.F. Library West
	29°39.054'	82°20.557'	1-VI-2011	U.F. Library West

SUPP. TABLE S2: (CONTINUED)

Replicate	Latitude	Longitude	Date	Nearest Building/Structure
	29°39.054'	82°20.557'	1-VI-2011	U.F. Library West
	29°38.370'	82°21.460'	1-VI-2011	U.F. Bat Houses
	29°38.370'	82°21.460'	1-VI-2011	U.F. Bat Houses
	29°38.370'	82°21.460'	1-VI-2011	U.F. Bat Houses
	29°38.370'	82°21.460'	1-VI-2011	U.F. Bat Houses
	29°38.673'	82°21.670'	1-VI-2011	U.F. Student Agricultural Gardens
	29°38.673'	82°21.670'	1-VI-2011	U.F. Student Agricultural Gardens
	29°38.673'	82°21.670'	1-VI-2011	U.F. Student Agricultural Gardens
	29°37.570'	82°19.390'	1-VI-2011	Treehouse Village Condominiums
	29°37.570'	82°19.390'	1-VI-2011	Treehouse Village Condominiums
	29°37.570'	82°19.390'	1-VI-2011	Treehouse Village Condominiums
	29°33.430'	82°26.108'	1-VI-2011	Residential Housing
	29°33.430'	82°26.108'	1-VI-2011	Residential Housing