Virulence of the insect-pathogenic fungi *Metarhizium* spp. to Mormon crickets, *Anabrus simplex* (Orthoptera: Tettigoniidae)

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Abstract

The Mormon cricket (MC), *Anabrus simplex* Haldeman, 1852 (Orthoptera: Tettigoniidae), has a long and negative history with agriculture in Utah and other western states of the USA. Most *A. simplex* populations migrate in large groups, and their feeding can cause significant damage to forage plants and cultivated crops. Chemical pesticides are often applied, but some settings (e.g. habitats of threatened and endangered species) call for non-chemical control measures. Studies in Africa, South America, and Australia have assessed certain isolates of *Metarhizium acridum* as very promising pathogens for Orthoptera: Acrididae (locust) biocontrol. In the current study, two isolates of *Metarhizium robertsii*, one isolate of *Metarhizium brunneum*, one isolate of *Metarhizium guizhouense*, and three isolates of *M. acridum* were tested for infectivity to MC nymphs and adults of either sex. Based on the speed of mortality, *M. robertsii* (ARSEF 23 and ARSEF 2575) and *M. brunneum* (ARSEF 7711) were the most virulent to instars 2 to 5 MC nymphs. *M. guizhouense* (ARSEF 7847) from Arizona was intermediate and the *M. acridum* isolates (ARSEF 324, 3341, and 3609) were the slowest killers. ARSEF 2575 was also the most virulent to instar 6 and 7 nymphs and adults of MC. All of the isolates at the conidial concentration of 1×10^7 conidia ml^{-1} induced approximately 100% mortality by 6 days post application of fungal conidia. In conclusion, isolates ARSEF 23, ARSEF 2575, and ARSEF 7711 acted most rapidly to kill MC under laboratory conditions. The *M. acridum* isolates, however, have much higher tolerance to heat and UV-B radiation, which may be critical to their successful use in field application.

Introduction

The Mormon cricket (MC) (*Anabrus simplex* Haldeman, 1852), a katydid of the family Tettigoniidae (Orthoptera), has one generation per year; and it is geographically restricted to the western USA (primarily Utah, Nevada, Wyoming, Idaho, Colorado, Montana, and Arizona) (Pfadt, 1994). MC is a large flightless insect. The adults weigh up to 3 g and reach up to 50 mm in length (fig. 1a). These insects have the ability to migrate up to 80 km during their lifetime of approximately 6 months (MacVean, 1991; Pfadt, 1994; Gwynne, 2001; Simpson *et al.*, 2006). Noisy populations (listen to Supplementary material 1) of this insect found on the western slope of the Rocky Mountains and westward tend to aggregate into migratory bands (fig. 1b, c, Supplementary material 2) that can extend up to 16 km in length in sagebrush-dominated rangeland (Lorch and Gwynne, 2000; Gwynne, 2001; Bailey *et al.*, 2005; Lorch *et al.*, 2005; Sword *et al.*, 2005), and their feeding can cause significant damage to forage plants and cultivated crops (MacVean, 1991).

MC bands were recorded as early as 1825 by trappers in Utah. The first record of serious damage occurred in 1848, the initial farming season in Utah by newly arrived Mormon settlers. The *A. simplex* insects were present in very large numbers and caused devastation to crops such as alfalfa, cucumbers, corn, beans, small grains, seeds, grasses, and vegetables (Hartley, 1970). Accordingly, the common name of this insect became 'Mormon crickets'. The intervention of local seagull populations in June 1848, known to the Mormon pioneers as the 'Miracle of the Seagulls', greatly reduced MC populations that year (Hartley, 1970; Quinn, 1997; Schwarz, 1998). More than 150 years later, this insect periodically still causes serious agricultural losses in Utah and nearby states. A monument in Salt Lake City's Temple Square commemorates the seagulls that protected desperately needed food and forage from the hoards of MCs in the late 1840s (Schwarz, 1998). Because of their long, negative history with this insect, Western USA agriculturists react strongly to large MC populations and frequently seek advice and help from state and federal agencies for its control.
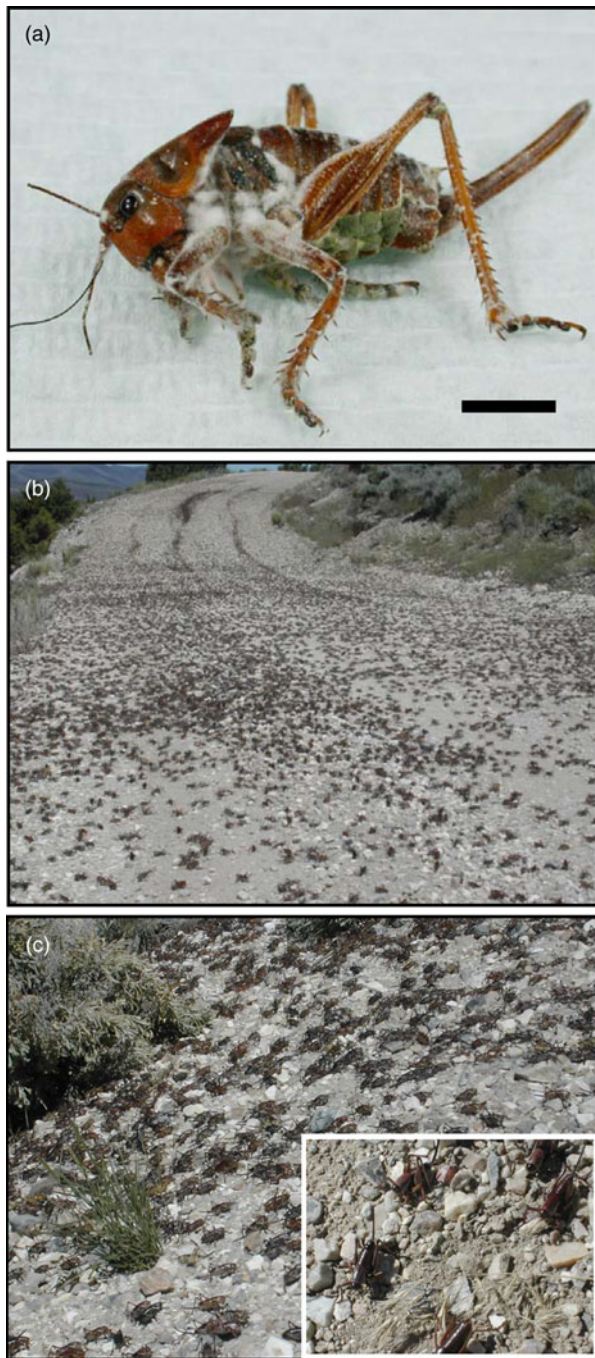
Figure 1. (a) Conidial production of *M. acridum* (ARSEF 324) on a dead *A. simplex* adult. Bar = 1 cm. (b) Migratory band of MCs in NE Utah in 2006. (c) Migratory band of MCs in NE Utah in 2006. Inset: Close-up of individuals in lower band.

Fungal entomopathogens are viable agents for biological control of insects (Alston *et al.*, 2005; Li *et al.*, 2010; Garrido-Jurado *et al.*, 2020; Rangel *et al.*, 2020). *Metarhizium* spp. fungi have been extensively studied and used in several countries for locust and grasshopper control (Bateman *et al.*, 1996; Bidochka *et al.*, 1997; Blanford and Thomas, 2001; Hunter *et al.*, 2001; Lomer *et al.*, 2001; Faria *et al.*, 2002; Enserink, 2004; Li *et al.*, 2010; Keyser *et al.*, 2017). Entomopathogenic fungi have been field tested for MC control (Streett and Woods, 2001; Foster *et al.*, 2011; Keyser *et al.*, 2017). Microsporidian and nematode

entomopathogens also have been tested for control of MC in laboratory and field studies (MacVean and Capinera, 1991, 1992). In the current study, several species of *Metarhizium* were tested in the laboratory for virulence to MCs. The goal of this study was to compare the virulence of selected isolates to different developmental stages of the MC. The selected *Metarhizium* isolates demonstrated high virulence against *A. simplex*.

Materials and methods

The fungal isolates

The isolates were obtained from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF); Robert W. Holley Center for Agriculture & Health, Cornell University Campus, Ithaca, NY, USA, and from Earth BioSciences, Lansing, CT, USA. In addition, DWR 200 (= ARSEF 7847) was originally isolated from western USA soil in our laboratory by baiting with *Galleria mellonella* larvae and/or dodine selective medium (Zimmermann, 1986; Goettel and Inglis, 1997; Rangel *et al.*, 2010a). The insect hosts and geographical origins of the isolates are listed in table 1.

The isolate ARSEF 3341 of *Metarhizium acridum* (= IMI 330189) is the active constituent of one of the biopesticides used in Africa for locust control (Lomer *et al.*, 2001).

Isolate ARSEF 7711 (*Metarhizium brunneum* from Austria) was provided by Earth BioSciences, FairWeld, CT (= Earth BioSciences F52; IMI 385045). It has been sold for insect control by Novozymes Biologicals Inc., Salem, VA (Bruck *et al.*, 2005; Bruck and Donahue, 2007).

The insect

MCs (*A. simplex*), at different stages of development, were collected in rangeland and surrounding areas in Tooele and Box Elder counties (UT) as well as the Curlew National Grasslands Reserve, Oneida county (ID). The field-collected insects were first held for 3–7 days in the laboratory and fed Flucker's® high calcium cricket feed (Flucker Laboratories, Baton Rouge, LA, USA), sagebrush leaves, sunflower seeds, and a variety of organically produced green leaves such as mixed baby greens, baby romaine salad, and baby spinach salad (Earthbound Farm®, CA, USA). In addition, high-protein dry fish food (TetraMin® Tetra Holding Inc., Blacksburg, VA, USA) and a gelled water source with calcium (Fluker's® Cricket Quencher, Baton Rouge, LA, USA) were provided. This gelled water prevents the insect from drowning. Cannibalization is a minor problem for MC with ready access to protein (Simpson *et al.*, 2006), and most of the insects reached adulthood. The stage of insect development was determined by measurements of body and femur length (Pfadt, 1994). Use of field collected insects was required because, despite numerous attempts by our and other research groups, no MC laboratory colonies have been successfully established.

Conidia production and inoculum preparation

The fungal isolates were grown on 23 ml potato dextrose agar (PDA) medium (Difco Laboratories, Detroit, MI, USA) supplemented with 1% yeast extract ($1 \, g \, l^{-1}$) (Technical, Difco) in Petri dishes (polystyrene, $95 \times 15 \, mm^2$, Fisherbrand®, Pittsburg, PA, USA) and incubated in the dark at $28 \pm 1°C$ for 14 days. Conidia were harvested and suspended in 10 ml of 0.1% Tween

Table 1. Fungal isolates

Isolates	Host/substrate	Geographic origin
M. acridum		
ARSEF 324	*Austracris guttulosa* [Orthoptera: Acrididae]	Queensland, Australia
ARSEF 3341	= IMI 330189. *Ornithacris cavroisi* [Orthoptera: Acrididae]	Niger
ARSEF 3609	*Patanga succincta* [Orthoptera: Acrididae]	Thailand
M. guizhouense		
ARSEF 7847	= DWR 200 from soil	Arizona, USA
M. brunneum		
ARSEF 7711	= F52. Earth BioSciences, Lansing, CT [Lepidoptera: Tortricidae]	Austria
M. robertsii		
ARSEF 23	*Conoderus* sp. [Coleoptera: Elateridae]	North Carolina, USA
ARSEF 2575	*Curculio caryae* [Coleoptera: Curculionidae]	South Carolina, USA

80 solution in 15-ml tubes (modified polystyrene, Corning®, USA). The suspensions were vortexed, and conidial concentrations estimated by hemocytometer counts. A 100 ml conidial suspension with concentration adjusted to 1×10^7 conidia ml^{-1} was prepared for each isolate. Suspensions of all isolates were used immediately for bioassay tests. The conidial viabilities of all suspensions used were high (usually ca. 95%).

Virulence assays (bioassays)

The immersion method was used to expose older MCs (instars 6 and 7 and adults of either sex) to conidial suspensions. The insects, in groups of six, were cold immobilized (ca. 5°C) (Gillespie *et al.*, 2000) for 1 h and dipped into the conidial suspension (1×10^7 conidia ml^{-1} in 0.1% Tween 80) for 15 s at ambient temperature. The control-insect group was dipped in Tween 80 (0.1%) solution. However, for small MCs (instar 2 to 5), due to high control mortality from the dipping method, the insects were sprayed to run-off with conidial suspensions or with Tween 80 (control). The dipping method is known to reduce the medium lethal time for *Beauveria bassiana* in *Chilo partellus* (Lepidoptera: Pyralidae) to 1 day vs. 3 days for the direct spray method (Tefera and Pringle, 2003).

Immediately after treatment, the insects were transferred individually to cloth-net-covered glass jars with a layer of sterile white sand and provided with food as described above. The jars were covered with cloth netting and held in moisture glass chambers (1-cm of deionized H$_2$O in each chamber) at 28 ± 1°C. The relative humidity of the moisture chambers was above 80%, as indicated by humidity indicator strips glued inside the jars (Humidicator paper HJH-650 Hydrion, Micro Essential Laboratory, New York, USA, that changes color in response to humidity). Mortality was assessed daily; dead insects were collected, surface sterilized in 5% Clorox (= 0.25% sodium hypochlorite) for 10 min, and maintained in humid chambers. Development of

Metarhizium on a cadaver was assessed to confirm mycosis. Thirty insects were used for each fungal treatment. The experiments were repeated at least two times, but most were repeated three times on three different dates, as shown in the figure captions. The varied number of repetitions in the different experiments was due to the variability in availability of field-collected insects.

Germination-speed assay

In an effort to explain the lower-than-expected virulence (speed to kill) of ARSEF 7847, the germination speed of this isolate was compared with that of ARSEF 2575 using the methods described elsewhere (Rangel *et al.*, 2004). Conidia of both isolates were collected after 14 days of growth and immediately suspended at concentration of 1×10^5 conidia ml^{-1}. The suspensions were vortexed, and 40 µl were inoculated (dropped, but not spread) on 4 ml of PDA medium in polystyrene Petri dishes (35×10 mm^2). The plates were kept at 28°C in the dark. Plates were observed and scored for germination every 2 h from 2 to 28 h after conidial inoculation. This experiment was repeated three times with different cultures and on different dates.

Statistical analyses

The effect of treatment on mortality was assessed using a one-way analysis of variance (ANOVA) with a randomized complete block design. Mortality data were arcsine-square-root transformed prior to analysis to better meet assumptions of normality and homogeneity of variance. The mean of each treatment was compared to the mean of the control using Dunnett's test. Separate analyses were made for mortality on days 3, 4, and 5 for each of the four age classes (instars 2–3, instars 4–5, instars 6–7, and adults). Data analyses were generated using the MIXED procedure in SAS/STAT software, Version 9.1.3 of SAS System for Windows.

Results and discussion

MC mortality after exposure to *Metarhizium robertsii* was remarkably fast in comparison with that reported for the desert locust, *Schistocerca gregaria* with *M. acridum* (ARSEF 3341) (Bateman *et al.*, 1993, 1996; Gillespie *et al.*, 2000; Hunt and Charnley, 2011). For *M. robertsii* isolate ARSEF 2575 at concentration of 1×10^7 conidia ml^{-1}, death of MCs started 1 day after conidial exposure, and by day 4 the mortality reached 100% (fig. 2). With the locust (*S. gregaria*), deaths caused by *M. acridum* (ARSEF 3341) started on day 3 after inoculation at conidial concentration of 3.75×10^7 conidia ml^{-1} and 100% mortality occurred on day 12 (Bateman *et al.*, 1996). In tests of additional isolates at 3.75×10^7 conidia ml^{-1}, Bateman *et al.* (1996) found that the medium lethal time (LT$_{50}$) was 4.8 days for the isolate ARSEF 2575 (referred to as isolate number I91 613 = ME1); however, for the isolates ARSEF 324 and ARSEF 3341, the LT$_{50}$ were 3.7 and 4.4 days, respectively. Rapid MC mortality was also observed with *B. bassiana* in laboratory assays and 100% mortality occurred on day 6 at conidial concentration of 1×10^6 conidia ml^{-1} (Streett and Woods, 2001). Accordingly, the MC is very susceptible to *M. robertsii* ARSEF 2575. By comparison, we found that 100% of plum curculio larvae (*Conotrachelus nenuphar*) dipped in the ARSEF 2575 conidial suspension of 1×10^7 conidia ml^{-1} for 15 s were killed by 8 days post exposure (Alston *et al.*, 2005).
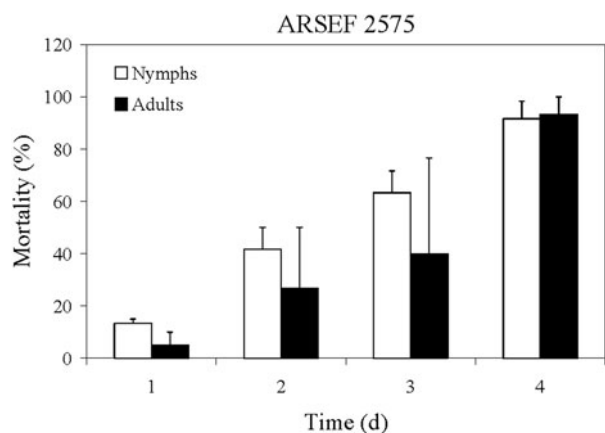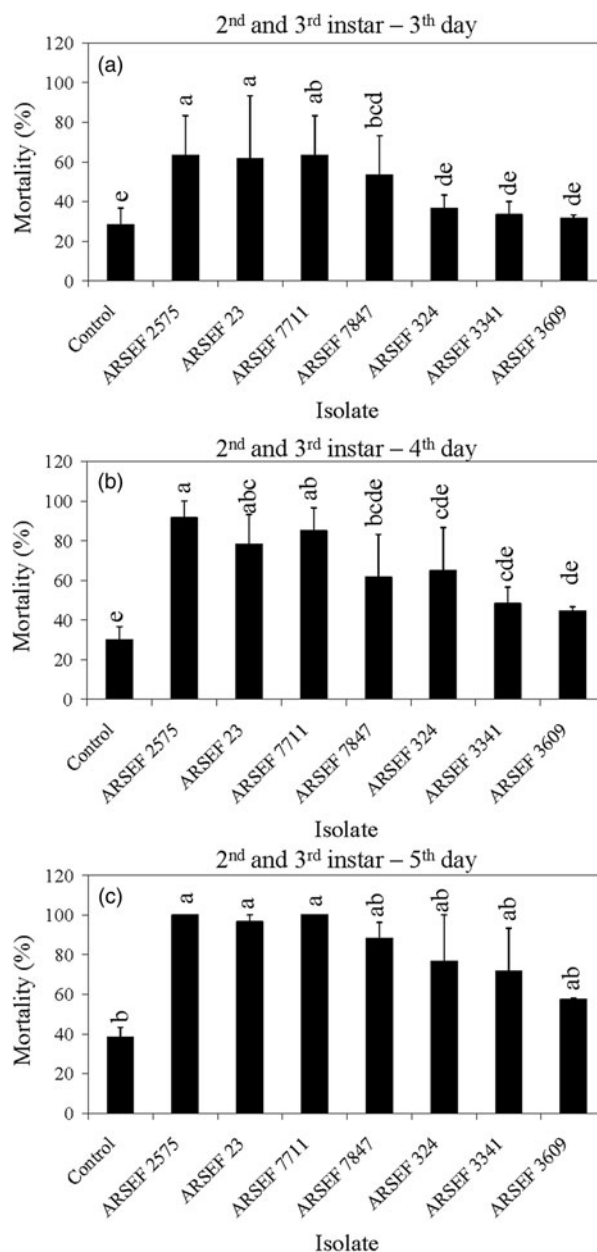
Figure 2. Mean percentage mortality of *A. simplex* nymphs (instars 2 and 3) and adults exposed to *M. robertsii* (ARSEF 2575) conidial suspensions (10^7 conidia ml^{-1}). Error bars are standard error of two or three independent experiments.

The mortality of MC nymphal instars 2–5 was faster with *M. robertsii* isolates (ARSEF 23, 2575) and *M. brunneum* (ARSEF 7711) than with *M. acridum* isolates (ARSEF 324, 3341, and 3609) (figs 3, 4). For larger nymphs (instars 6–7) and adults, the *M. robertsii* isolate ARSEF 2575 also killed faster than the *M. acridum* isolates (ARSEF 324, 3341, and 3609) (figs 5, 6). In contrast, *M. robertsii* (ARSEF 2575 = IMI I91 613) and *M. acridum* (ARSEF 3609 = IMI I91614) were similarly virulent to *S. gregaria* (Bateman *et al.*, 1996). The *S. gregaria*, however, were killed much faster by *M. acridum* ARSEF 3341 (total mortality at 10 days) than *M. robertsii* (ARSEF 2575) (total mortality at 17 days) (Hunt and Charnley, 2011). However, when the locusts thermoregulated, *M. robertsii* killed faster than *M. acridum* (Hunt and Charnley, 2011). This was possibly due to the production of destruxins by *M. robertsii*, but not by *M. acridum*. *M. robertsii* (ARSEF 23), as in Bischoff *et al.* (2009) and Humber (2014), appears to possess much greater potential for the production of secondary metabolites than *M. acridum* (Gao *et al.*, 2011). Destruxin A (dtx A) produced by *M. robertsii* has been associated with the inhibition of the insect immune system (Hunt and Charnley, 2011).

Isolate ARSEF 7847 (DWR 200), based on culture characteristics and amplified fragment length polymorphism analysis, is a *M. anisopliae* s.l. (Fernandes *et al.*, 2010); however, according to sequence analysis of its elongation factor I-alpha (eEF1A), it is *Metarhizium guizhouense* (Roberts, unpublished). This isolate was relatively slow in killing MCs and was similar to *M. acridum* in this regard (figs 3, 4). The germination rate of isolate ARSEF 7847 (50% germination at 20 h) was much slower than ARSEF 2575 (50% germination at 7 h) (fig. 7), which may partially explain the slower mortality rate by ARSEF 7847. High germination rates correlate with high virulence in several entomopathogenic fungi (Altre *et al.*, 1999; Rangel *et al.*, 2008; Oliveira *et al.*, 2018; Oliveira and Rangel, 2018).

Differences in virulence of entomopathogenic fungi to insects of different ages has been reported, e.g. first-instar European corn borer (*Ostrinia nubilalis*) are more susceptible to *B. bassiana* than later larval stages (Feng *et al.*, 1985). First-instar nymphs of MC were not available for our experiments, but the susceptibility to fungi of nymphal stages 2–7 and adult were similar (fig. 2).

Isolates ARSEF 2575 and ARSEF 324, *M. robertsii* and *M. acridum*, respectively, have the potential for high conidial production
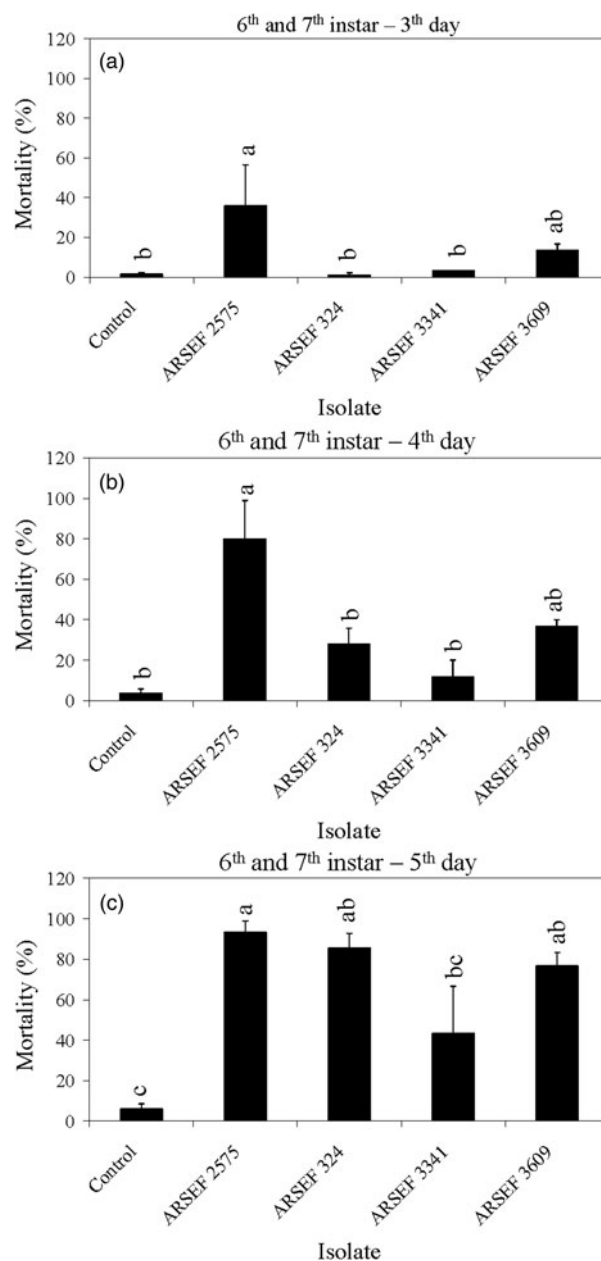

Figure 3. Mean percentage mortality of *A. simplex* nymphs (instars 2 and 3) exposed to *M. acridum* (ARSEF 324, 3341, and 3609), *M. guizhouense* (DWR 200 = ARSEF 7847), *M. brunneum* (F 52 = ARSEF 7711), and *M. robertsii* (ARSEF 23 and 2575) conidial suspensions (10^7 conidia ml^{-1}). (a) Accumulated mortality on day 3; (b) accumulated mortality on day 4; (c) accumulated mortality on day 5. Error bars are standard error of two independent experiments. Graph bars with the same letters are not significantly different ($P < 0.001$; Dunnett's test following one-way ANOVA [see section 'Materials and methods']).

on their dead insect hosts in the laboratory (e.g. fig. 1a); however, various Utah MC populations from three different years sometimes demonstrated severely reduced conidial production on their cadavers, and conidial production was mostly inside the insect. The incidence of insect mycosis was higher for instar 6 and 7 nymphs than with adults and younger nymphs for all fungal isolates. Mortality from fungal infection without external conidial production on insects has been shown by other authors (Bateman *et al.*, 1996). Apparent non-mycosis mortality (= without high outgrowth of fungus from cadavers) may indicate that
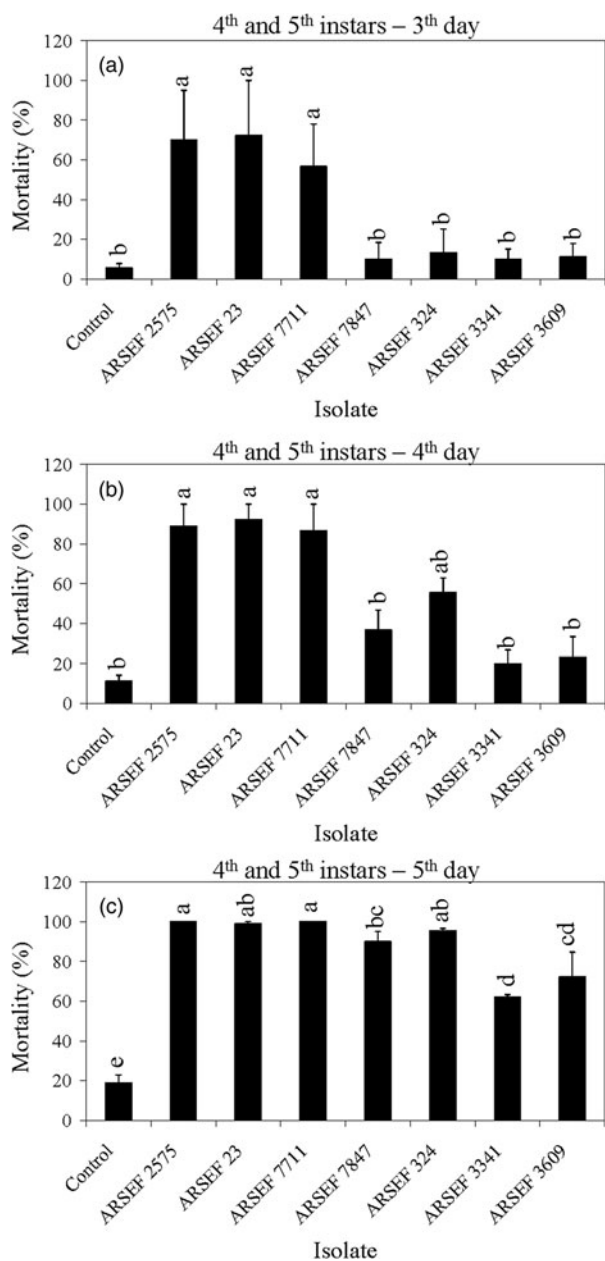
Figure 4. Mean percentage mortality of *A. simplex* nymphs (instars 4 and 5) exposed to *M. acridum* (ARSEF 324, 3341, and 3609), *M. guizhouense* (DWR 200 = ARSEF 7847), *M. brunneum* (F52 = ARSEF 7711), and *M. robertsii* (ARSEF 23 and 2575) conidial suspensions (10^7 conidia ml^{-1}). (a) Accumulated mortality on day 3; (b) accumulated mortality on day 4; (c) accumulated mortality on day 5. Error bars are standard error of three independent experiments. Graph bars with the same letters are not significantly different ($P < 0.001$; Dunnett's test following one-way ANOVA [see section 'Materials and methods']).
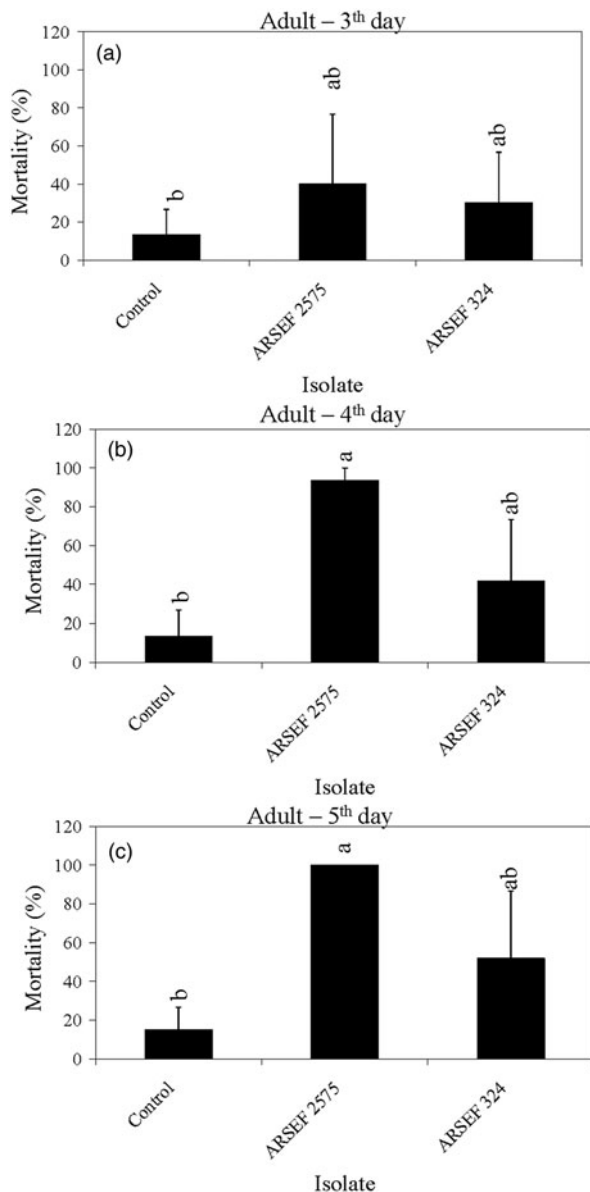
Figure 5. Mean percentage mortality of *A. simplex* nymphs (instars 6 and 7) exposed to *M. acridum* (ARSEF 324, 3341, and 3609), *M. guizhouense* (DWR 200 = ARSEF 7847), *M. brunneum* (F52 = ARSEF 7711), and *M. robertsii* (ARSEF 23 and 2575) conidial suspensions (10^7 conidia ml^{-1}). (a) Accumulated mortality on day 3; (b) accumulated mortality on day 4; (c) accumulated mortality on day 5. Error bars are standard error of four independent experiments. Graph bars with the same letters are not significantly different ($P < 0.001$; Dunnett's test following one-way ANOVA [see section 'Materials and methods']).

pathogenesis includes the action of toxins (Bateman *et al.*, 1996). This lack of fungus emergence from dead hosts was also found with one *M. anisopliae* isolate [*viz.* ESC 1 (= ARSEF 9591) from EcoScience (EPA registration no. 129056)] in a comparison of Abbott-corrected and mycosis-confirmed (conidial production on cuticle observed) mortality of *C. nenuphar* larvae (Alston *et al.*, 2005).

The mortality in controls was somewhat high in some experiments, reaching 40% mortality on the fifth day post-treatment for nymphs of instar 2 and 3 (fig. 3). High control (untreated)
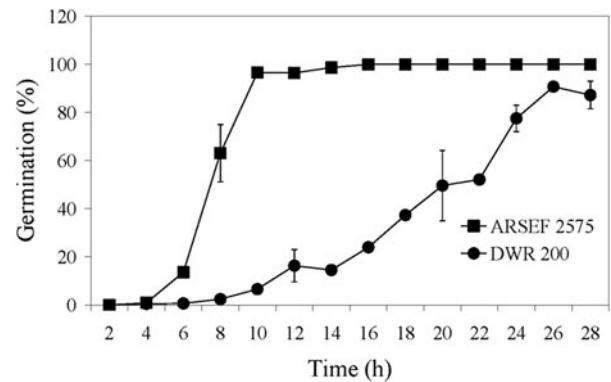
mortality in the laboratory may occur with field-collected insects, usually due to pre-existing conditions such as poor nutrition, pesticide exposure, or the presence of other pathogens. Mortality near 40% of insects in the control treatment has also been recorded in other studies (Wright *et al.*, 2005; Ansari *et al.*, 2008). For example, 44% of alates of the Formosan subterranean termite (*Coptotermes formosanus*) had died in the control treatment 4 days after treatment application (Wright *et al.*, 2005). In the current study, crickets of instar 4 to adult survived well in the control treatment with mortality below 15% (figs 4–6). For all

Figure 6. Mean percentage mortality of *A. simplex* adults exposed to *M. acridum* (ARSEF 324), and *M. robertsii* (ARSEF 2575) conidial suspensions (10⁷ conidia ml⁻¹). (a) Accumulated mortality on day 3; (b) accumulated mortality on day 4; (c) accumulated mortality on day 5. Error bars are standard error of two independent experiments. Graph bars with the same letters are not significantly different ($P < 0.001$; Dunnett's test following one-way ANOVA [see section 'Materials and methods']).

Figure 7. Germination speed of *M. robertsii* (ARSEF 2575) and *M. guizhouense* (DWR 200 = ARSEF 7847) conidia on PDAY medium at 28°C. Error bars are standard deviation of three independent experiments.

and other isolates evaluated by hazard ratios (Cox proportional hazards regression model) (Keyser *et al.*, 2017).

High mortality in laboratory bioassays, however, often has not translated to high levels of pest control in the field (Sosa-Gomez and Moscardi, 1998; Streett and Woods, 2001). Despite slower MC mortality with the *M. acridum* isolates in the laboratory, high levels of MC kill eventually were obtained with these isolates; and, importantly, the *M. acridum* isolates all had considerably higher conidial tolerance to heat (45°C) and UV-B radiation (Moore *et al.*, 1993; McClatchie *et al.*, 1994; Rangel *et al.*, 2005; Rangel *et al.*, 2006; Souza *et al.*, 2014; Dias *et al.*, 2018; Rangel and Roberts, 2018) as well as mycelial thermotolerance (Rangel *et al.*, 2010b) than any of the *M. robertsii* isolates.

Conclusions

Both *M. acridum* and *M. robertsii* have good potential for MC control, with *M. acridum* isolates having the distinct advantage due to their host specificity limited to Orthoptera (e.g. locusts and MCs and excluding non-target beneficial insects).

Dedication. The lead author Drauzio E. N. Rangel and his wife Alene Alder-Rangel dedicate this study to his dear PhD advisor, adoptive father, and friend Dr. Donald W. Roberts, who is also an author of this article.

Insect pathology lost an extremely important scientist on 2 May 2021 when Dr. Donald W. Roberts (1933–2021) passed away at 88 due to natural causes. Originally from Phoenix, Arizona, USA, Don was inspired to study insect diseases as a teenager when he first saw nucleopolyhedrosis virus infecting caterpillars in cotton fields.

age groups, cricket mortality in the control treatment was always less than mortality caused by *M. robertsii* or *M. acridum*. The results indicate that most of the *M. robertsii* isolates were more virulent (in terms of speed of kill) than the *M. acridum* isolates against MCs, but all isolates tested eventually killed 100% of the MCs exposed to them. *M. brunneum* (ARSEF 7711), registered by US EPA as a myco-insecticide, was similar to two *M. robertsii* isolates (ARSEF 23 and ARSEF 2575) in virulence to instar 2–3 and 4–5 MC nymphs (figs 3, 4). Isolate ARSEF 7711 also is highly virulent to other pest-insect species [e.g. the cabbage maggot, *Delia radicum* (Diptera: Anthomyiidae)] (Bruck *et al.*, 2005).

Comparative ranking of the ten isolates examined here by time-to-kill bioassays remained consistent in a study of these

When studying for his B.S. at Brigham Young University (1957), a mycology teacher, Dr Kent McKnight, sparked his fascination in combining the disciplines of entomology and mycology. He then obtained his M.S. at Iowa State University (1959), working on the chemical and biological control of *Evora hemidesma*. For his PhD from the University of California at Berkeley (1964), he studied under the father of insect pathology, Edward Steinhaus. Don's PhD dissertation focused on destruxins of *Metarhizium anisopliae*.

Don spent most of his career (1965–1997) at the Boyce Thompson Institute (BTI) in New York where he helped build the world-renowned Insect Pathology Resource Center. Don's enthusiasm for insect pathology helped convert other scientists at BTI to take up the study of various insect pathogens. Early in his career, Don conducted pioneering research on the entomopoxvirus *Amsacta moorei* and fungal pathogens *Coelomomyces* and *Metarhizium*.

Although Don and his group worked with virtually every type of insect pathogen, his favorite was always fungal insect-pathogens, especially *Metarhizium anisopliae*. Don's group conducted basic, applied, and molecular studies on this fungus, making it a premier model. Fittingly, when *M. anisopliae* was divided into several new species in 2008, the type that he had studied most was named *Metarhizium robertsii* in honor of Don.

Don was a passionate promoter of insect pathology and biological control world-wide. He journeyed to 64 countries promoting biological control, making presentations, conducting research, and collecting pathogens, especially in the developing world. In these travels, Don collected fungi and contributed significantly to the ARSEF fungal collection in Ithaca, NY. He also supervised research projects in India, Nigeria, the Philippines, and Brazil. Don's research was pivotal for the early development of Brazil as a leader in biological control of insects using fungal pathogens.

Don retired from BTI, and at the age of 65, became a Research Professor for the Department of Biology at Utah State University, where he remained for 22 years (1997–2019). He focused on the effects of solar UV radiation and heat on entomopathogenic fungi. He had a large grant to investigate using insect-pathogenic fungi to control Mormon crickets. Don delved into a completely new subject initiating a research program on the parasite that causes Whirling Disease in fish. Part of the reason for this Utah location was to be closer to his children and grandchildren.

He is a founding member of the Society for Invertebrate Pathology (SIP), and this Society has always been very close to his heart. He attended and presented at almost every SIP meeting from the first in 1968 until 2017. He took a leading role in organizing the annual SIP meetings in 1983, 1984, and 2009. He served in a multitude of ways including chairing various committees as well as serving as Vice President (1986–88), President (1988–90), and Treasurer (1990–92). He was honored to present the SIP Founders lecture in 1982 and was recognized as the Founders Lecture Honoree in 2009. His life stories are detailed in a new book (Alder-Rangel, 2021).

Don's greatest legacy will be the people that he trained. With the help of his wife Mae, he took excellent care of his staff on a personal and professional level; earning the nickname 'Uncle Don.' A talented storyteller, he enjoyed using stories about his own adventures as parables to teach others about both science and life. All of his personal and professional activities have been the direct outgrowth of his deeply held belief that it is his duty to help improve the lives of other people. He was a very supportive person that encouraged his family, those who had the privilege to work with him, and many others to explore the world and make it a better place. In this spirit of service to and love for all, and in his professional dedication, Don was a role model worthy of emulation.

Don was also the star of the dance floor and a talented photographer. Donald Roberts leaves behind his wife Mae Roberts, two children, and three grandchildren, as well as innumerable scientific descendants that will pass on his passion for insect pathology.

References

Alder-Rangel A (2021) Dr Donald W. Roberts: International Insect Pathologist. In press.

Alston DG, Rangel DEN, Lacey LA, Golez HG, Kim JJ and Roberts DW (2005) Evaluation of novel fungal and nematode isolates for control of *Conotrachelus nenuphar* (Coleoptera: Curculionidae) larvae. *Biological Control* **35**, 163–171.

Altre JA, Vandenberg JD and Cantone FA (1999) Pathogenicity of *Paecilomyces fumosoroseus* isolates to diamondback moth, *Plutella xylostella*: correlation with spore size, germination speed, and attachment to cuticle. *Journal of Invertebrate Pathology* **73**, 332–338.

Ansari MA, Brownbridge M, Shah FA and Butt TM (2008) Efficacy of entomopathogenic fungi against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis*, in plant-growing media. *Entomologia Experimentalis et Applicata* **127**, 80–87.

Bailey NW, Gwynne DT and Ritchie MG (2005) Are solitary and gregarious Mormon crickets (*Anabrus simplex*, Orthoptera, Tettigoniidae) genetically distinct? *Heredity* **95**, 166–173.

Bateman RP, Carey M, Moore D and Prior C (1993) The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. *Annals of Applied Biology* **122**, 145–152.

Bateman R, Carey M, Batt D, Prior C, Abraham Y, Moore D, Jenkins N and Fenlon J (1996) Screening for virulent isolates of entomopathogenic fungi against the desert locust, *Schistocerca gregaria* (Forskal). *Biocontrol Science and Technology* **6**, 549–560.

Bidochka MJ, St. Leger RJ and Roberts DW (1997) Mechanisms of deuteromycete fungal infections in grasshoppers and locusts: an overview. In Goettel MS and Johnson DL (eds), *Microbial Control of Grasshoppers and Locusts*. Memoirs of the Entomological Society of Canada, pp. 213–224.

Bischoff JF, Rehner SA and Humber RA (2009) A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia* **101**, 512–530.

Blanford S and Thomas MB (2001) Adult survival, maturation, and reproduction of the desert locust *Schistocerca gregaria* infected with the fungus *Metarhizium anisopliae* var *acridum*. *Journal of Invertebrate Pathology* **78**, 1–8.

Bruck DJ and Donahue KM (2007) Persistence of *Metarhizium anisopliae* incorporated into soilless potting media for control of the black vine weevil, *Otiorhynchus sulcatus* in container-grown ornamentals. *Journal of Invertebrate Pathology* **95**, 146–150.

Bruck DJ, Snelling JE, Dreves AJ and Jaronski ST (2005) Laboratory bioassays of entomopathogenic fungi for control of *Delia radicum* (L.) larvae. *Journal of Invertebrate Pathology* **89**, 179–183.

Dias LP, Araújo CAS, Pupin B, Ferreira PC, Braga GÚL and Rangel DEN (2018) The Xenon Test Chamber Q-SUN® for testing realistic tolerances of fungi exposed to simulated full spectrum solar radiation. *Fungal Biology* **122**, 592–601.

Enserink M (2004) Can the war on locusts be won? *Science (New York, N.Y.)* **306**, 1880–1882.

Faria MR, Magalhães BP, Alves RT, Schimidt FGV, Tavares da Silva JB and Frazão H (2002) Effect of two dosages of *Metarhizium anisopliae* var. *acridum* against *Rhammatocerus schistocercoides* Rehn. *Pesquisa Agr Brasil Pesqui Agropecu* **37**, 1531–1539.

Feng Z, Carruthers RI, Roberts DW and Robson DS (1985) Age-specific dose mortality effects of *Beauveria bassiana* (Deuteromycotina, Hyphomycetes) on the European corn borer, *Ostrinia nubilalis* (Lepidoptera, Pyralidae). *Journal of Invertebrate Pathology* **46**, 259–264.

Fernandes EKK, Keyser CA, Chong JP, Rangel DEN, Miller MP and Roberts DW (2010) Characterization of *Metarhizium* species and varieties based on molecular analysis, heat tolerance and cold activity. *Journal of Applied Microbiology* **108**, 115–128.

Foster RN, Jaronski S, Reuter KC, Black LR, Schlothauer R, Harper J and Jech LE (2011) Simulated aerial sprays for field cage evaluation of *Beauveria bassiana* and *Metarhizium brunneum* (Ascomycetes: Hypocreales) against *Anabrus simplex* (Orthoptera: Tettigoniidae) in Montana. *Biocontrol Science and Technology* **21**, 1331–1350.

Gao Q, Jin K, Ying SH, Zhang Y, Xiao G, Shang Y, Duan Z, Hu X, Xie XQ, Zhou G, Peng G, Luo Z, Huang W, Wang B, Fang W, Wang S, Zhong Y, Ma LJ, St Leger RJ, Zhao GP, Pei Y, Feng MG, Xia Y and Wang C (2011) Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. *PLoS Genetics* **7**, e1001264.

Garrido-Jurado I, Resquín-Romero G, Yousef-Naef M, Ríos-Moreno A and Quesada-Moraga E (2020) Soil drenching with entomopathogenic fungi for control of the soil-dwelling life stages and adults of the same generation of

Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* **110**, 242–248.

Gillespie JP, Burnett C and Charnley AK (2000) The immune response of the desert locust *Schistocerca gregaria* during mycosis of the entomopathogenic fungus, *Metarhizium anisopliae* var *acridum*. *Journal of Insect Physiology* **46**, 429–437.

Goettel MS and Inglis GD (1997) Fungi: hyphomycetes. In Lacey LA (ed.), *Manual of Techniques in Insect Pathology*. San Diego: Academic Press, pp. 213–249.

Gwynne DT (2001) *Katydids and Bush-Crickets: Reproductive Behaviour and Evolution of the Tettigoniidae*. Ithaca, NY: Cornell Univ. Press.

Hartley WG (1970) Mormons, crickets, and seagulls: a new look at an old story. *Utah Historical Quarterly* **38**, 224–239.

Humber RA (2014) USDA-ARS Collection of Entomopathogenic Fungal Cultures – Catalog of Strains Ithaca, USDA-ARS Biological Integrated Pest Management Research, Robert W. Holley Center for Agriculture and Health.

Hunt VL and Charnley AK (2011) The inhibitory effect of the fungal toxin, destruxin A, on behavioural fever in the desert locust. *Journal of Insect Physiology* **57**, 1341–1346.

Hunter DM, Milner RJ and Spurgin PA (2001) Aerial treatment of the Australian plague locust, *Chortoicetes terminifera* (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). *Bulletin of Entomological Research* **91**, 93–99.

Keyser CA, Fernandes ÉKK, Rangel DEN, Foster RN, Jech LE, Reuter KC, Black LR, Jaronski S, Flake DD, Evans EW and Roberts DW (2017) Laboratory bioassays and field-cage trials of *Metarhizium* spp. isolates with field-collected Mormon crickets (*Anabrus simplex*). *Biocontrol* **62**, 257–268.

Li ZZ, Alves SB, Roberts DW, Fan MZ, Delalibera I, Tang J, Lopes RB, Faria M and Rangel DEN (2010) Biological control of insects in Brazil and China: history, current programs and reasons for their successes using entomopathogenic fungi. *Biocontrol Science and Technology* **20**, 117–136.

Lomer CJ, Bateman RP, Johnson DL, Langewald J and Thomas MB (2001) Biological control of locusts and grasshoppers. *Annual Review of Entomology* **46**, 667–702.

Lorch PD and Gwynne DT (2000) Radio-telemetric evidence of migration in the gregarious but not the solitary morph of the Mormon cricket (*Anabrus simplex*: Orthoptera: Tettigoniidae). *Naturwissenschaften* **87**, 370–372.

Lorch PD, Sword GA, Gwynne DT and Anderson GL (2005) Radiotelemetry reveals differences in individual movement patterns between outbreak and non-outbreak Mormon cricket populations. *Ecological Entomology* **30**, 548–555.

MacVean C (1991) Mormon crickets: a brighter side. *Rangelands* **12**, 234–235.

MacVean CM and Capinera JL (1991) Pathogenicity and transmission potential of *Nosema locustae* and *Vairimorpha* n. sp. (Protozoa: Microsporida) in Mormon crickets (*Anabrus simplex*; Orthoptera: Tettigoniidae): a laboratory evaluation. *Journal of Invertebrate Pathology* **57**, 23–36.

MacVean CM and Capinera JL (1992) Field evaluation of two microsporidian pathogens, an entomopathogenic nematode, and carbaryl for suppression of the Mormon cricket, *Anabrus simplex* Hald. (Orthoptera: Tettigoniidae). *Biological Control* **2**, 59–65.

McClatchie GV, Moore D, Bateman RP and Prior C (1994) Effects of temperature on the viability of the conidia of *Metarhizium flavoviride* in oil formulations. *Mycological Research* **98**, 749–756.

Moore D, Bridge PD, Higgins PM, Bateman RP and Prior C (1993) Ultra-violet radiation damage to *Metarhizium flavoviride* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. *Annals of Applied Biology* **122**, 605–616.

Oliveira AS and Rangel DEN (2018) Transient anoxia during *Metarhizium robertsii* growth increases conidial virulence to *Tenebrio molitor*. *Journal of Invertebrate Pathology* **153**, 130–133.

Oliveira AS, Braga GUL and Rangel DEN (2018) *Metarhizium robertsii* illuminated during mycelial growth produces conidia with increased germination speed and virulence. *Fungal Biology* **122**, 555–562.

Pfadt RE (1994) Mormon cricket. In USDA-ARS (ed.) Field Guide to Common Western Grasshoppers. Laramie, WY: Animal and Plant Health Inspection Service and Wyoming Agricultural Experiment, US Department of Agriculture Station, pp. 1–4.

Quinn DM (1997) *The Mormon Hierarchy: Extensions of Power*. Salt Lake City: Signature Books.

Rangel DEN and Roberts DW (2018) Possible source of the high UV-B and heat tolerance of *Metarhizium acridum* (isolate ARSEF 324). *Journal of Invertebrate Pathology* **157**, 32–35.

Rangel DEN, Braga GUL, Flint SD, Anderson AJ and Roberts DW (2004) Variations in UV-B tolerance and germination speed of *Metarhizium anisopliae* conidia produced on artificial and natural substrates. *Journal of Invertebrate Pathology* **87**, 77–83.

Rangel DEN, Braga GUL, Anderson AJ and Roberts DW (2005) Variability in conidial thermotolerance of *Metarhizium anisopliae* isolates from different geographic origins. *Journal of Invertebrate Pathology* **88**, 116–125.

Rangel DEN, Butler MJ, Torabinejad J, Anderson AJ, Braga GUL, Day AW and Roberts DW (2006) Mutants and isolates of *Metarhizium anisopliae* are diverse in their relationships between conidial pigmentation and stress tolerance. *Journal of Invertebrate Pathology* **93**, 170–182.

Rangel DEN, Alston DG and Roberts DW (2008) Effects of physical and nutritional stress conditions during mycelial growth on conidial germination speed, adhesion to host cuticle, and virulence of *Metarhizium anisopliae*, an entomopathogenic fungus. *Mycological Research* **112**, 1355–1361.

Rangel DEN, Dettenmaier SJ, Fernandes EKK and Roberts DW (2010a) Susceptibility of *Metarhizium* spp. and other entomopathogenic fungi to dodine-based selective media. *Biocontrol Science and Technology* **20**, 375–389.

Rangel DEN, Fernandes EKK, Dettenmaier SJ and Roberts DW (2010b) Thermotolerance of germlings and mycelium of the insect-pathogenic fungus *Metarhizium* spp. and mycelial recovery after heat stress. *Journal of Basic Microbiology* **50**, 344–350.

Rangel DEN, Piedrabuena AE, Roitman I and Messias CL (2020) Laboratory and field studies for the control of Chagas disease vectors using the fungus *Metarhizium anisopliae*. *Archives of Insect Biochemistry and Physiology* **105**, e21745.

Schwarz FD (1998) 1848 – Miracle of the birds (Sea gulls save crops of Utah's Mormon pioneers by devouring plague of crickets). *American Heritage* **49**, 104–106.

Simpson SJ, Sword GA, Lorch PD and Couzin ID (2006) Cannibal crickets on a forced march for protein and salt. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 4152–4156.

Sosa-Gomez DR and Moscardi F (1998) Laboratory and field studies on the infection of stink bugs, *Nezara viridula*, *Piezodorus guildinii*, and *Euschistus heros* (Hemiptera: pentatomidae) with *Metarhizium anisopliae* and *Beauveria bassiana* in Brazil. *Journal of Invertebrate Pathology* **71**, 115–120.

Souza RKF, Azevedo RFF, Lobo AO and Rangel DEN (2014) Conidial water affinity is an important characteristic for thermotolerance in entomopathogenic fungi. *Biocontrol Science and Technology* **24**, 448–461.

Streett DA and Woods SA (2001) *Beauveria bassiana* for Mormon crickets. In Branson D and Redlin B (eds), *Grasshoppers: Their Biology, Identification and Management*. USDA-ARS-APHIS. Laramie: University of Wyoming, pp. VII 6-1-5.

Sword GA, Lorch PD and Gwynne DT (2005) Insect behaviour: migratory bands give crickets protection. *Nature* **433**, 703.

Tefera T and Pringle KL (2003) Effect of exposure method to *Beauveria bassiana* and conidia concentration on mortality, mycosis, and sporulation in cadavers of *Chilo partellus* (Lepidoptera: Pyralidae). *Journal of Invertebrate Pathology* **84**, 90–95.

Wright MS, Raina AK and Lax AR (2005) A strain of the fungus *Metarhizium anisopliae* for controlling subterranean termites. *Journal of Economic Entomology* **98**, 1451–1458.

Zimmermann G (1986) The 'Galleria bait method' for detection of entomopathogenic fungi in soil. *Journal of Applied Entomology* **102**, 213–215.