Prospection and Fungal Virulence Associated with Mahanarva spectabilis (Hemiptera: Cercopidae) in an Amazon Silvopastoral System

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Prospection and fungal virulence associated with *Mahanarva spectabilis* (Hemiptera: Cercopidae) in an Amazon silvopastoral system

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Abstract

In Brazil, pastures of *Brachiaria* grasses are often attacked by the spittlebug *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae). Biological control of this pest insect is rarely used, in part because of a lack of diversity in commercialized pathogens effective against such pests. However, fungal infection of *M. spectabilis* has been noted in some tropical silvopastoral systems, which rarely have problems with pest insects. This study surveyed the fungi found in association with *M. spectabilis* in a silvopastoral system in Brazil and made a preliminary assessment of their virulence. Infected spittlebugs were collected in a silvopastoral system in Brazil, from which 5 types of fungi were isolated and identified by morphological analysis. Conidia of each wild fungus and a commercial strain of *Metarhizum anisopliae* (Metschn.) Sorokin (Clavicipitaceae) were diluted in a 1% NaCl solution at a concentration of 1×10^4 conidia per mL. In the laboratory, eggs and nymphs of *M. spectabilis* were placed in acrylic boxes within a climate-controlled chamber at 25 °C. In a non-acclimatized greenhouse, eggs and nymphs were placed on potted *Brachiaria decumbens* Stapf (Poaceae). Solutions of the 6 fungi and a control (pure saline) were applied to *M. spectabilis* in both conditions (acrylic box in laboratory and potted *Brachiaria* in a greenhouse). The most virulent fungi (UFMG 11443 and 11444) caused more than 90% of unviable eggs and mortality of nymphs. Other fungi tested (UFMG 11440, 11441, and 11442) were equally or more effective than the commercial *M. anisopliae*, causing over 50% unviable eggs or nymph mortality. All isolated fungi showed potential for use as biological control agents against *M. spectabilis*.

Key Words: entomopathogenic fungus; pasture spittlebug; mortality; biological control

Resumo

Pastagens com *Brachiaria* spp. geralmente sofrem ataques severos pela cigarrinha *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae). O controle biológico desse inseto tem sido pouco utilizado, principalmente porque há poucos entomopatógenos comercialmente disponíveis. Entretanto, *M. spectabilis* infectada por fungos tem sido encontrada em alguns sistemas silvipastoris (SSP) tropicais, os quais raramente apresentam problemas com insetos-praga. Este estudo é uma prospecção de fungos encontrados em associação com *M. spectabilis* e uma análise preliminar de sua virulência. Cigarrinhas infectadas foram coletadas em um SSP no Brasil, das quais cinco tipos de fungos foram isolados e identificados por análises morfológica e molecular. Conídios de cada um dos fungos selvagens e de uma linhagem comercial de *Metarhizum anisopliae* (Metschn.) Sorokin (Clavicipitaceae) foram diluídos em solução de NaCl 1% a uma concentração de 10^4 conídios/mL. No laboratório, ovos e ninfas de *M. spectabilis* foram colocados em caixas de acrílico, no interior de uma câmara climática a 25°C. Em uma casa-de-vegetação não-climatizada, ovos e ninfas foram depositados na base de plantas de *Brachiaria decumbens* Stapf (Poaceae) cultivadas em vasos. As seis soluções com fungos e um controle com solução salina pura foram aplicados sobre *M. spectabilis* em ambas condições. Os fungos mais virulentos foram codificados como UFMG 11443 e 11444, ambos causando mais de 90% de inviabilidade dos ovos e mortalidade de ovos edas ninfas. Os demais fungos UFMG 11440, 11441 e 11442 foram igualmente ou mais eficientes que a cepa comercial de *M. anisopliae*, com mais de 50% de ovos inviáveis ou mortalidade das ninfas. Todos os fungos selvagens isolados mostraram potencial como agentes de controle biológico para uso contra *M. spectabilis.*

Palavras Chave: fungos entomopatogênicos; cigarrinha-das-pastagens; mortalidade; controle biológico

The Brazilian cattle industry has been widely based on extensive pasture systems. Native vegetation is usually removed and replaced by monocultures of exotic grasses (Murgueitio et al. 2003; Fajardo 2009), principally African species of *Brachiaria* (Cezar et al. 2005). Given that low complexity and food web biodiversity in monocultures favor pest outbreaks of herbivorous insects (Andow 1991), pasturelands of a single exotic grass are highly susceptible to pest damage (Fajardo 2009; Zuluaga et al. 2011). An alternative to traditional pasture monocul-

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tures has been silvopastoral systems (SPSs), which are characterized by a combination of trees, shrubs, forage, and animals in the same area (Sousa et al. 2011; Araújo et al. 2013; Aguirre et al. 2016). Multiple microhabitats and plant resources generated by the greater vegetational diversity and associated with improvements in soil structure and moisture in such silvopastoral systems may increase biodiversity in their food webs (Chara et al. 2011; Aguirre et al. 2016), including the diversity of entomopathogenic fungal species and their genetic variants (Meyling et al. 2009; Ormond et al. 2010). Increased local biodiversity in SPSs (Zuluaga et al. 2011) also promotes species coexistence and population stability (Murgueitio et al. 2014), as well potentially reducing pest outbreaks. The integrated management of natural resources in an SPS enables high animal production combined with environmental protection and conservation (Silva 2008).

Spittlebugs are 1 of the major insect pests of forage grasses across tropical America (Thompson 2004; Domínguez et al. 2016), especially species of *Deois* and *Mahanarva*. The spittlebug *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) feeds on grasses of the genera *Brachiaria* and *Pennisetum* (Auad et al. 2010; Fonseca et al. 2016). Both nymphs and adults damage the host plant by sucking sap and injecting toxins (Resende et al. 2013; Aguiar et al. 2014; Fonseca et al. 2016), causing physiological disorders (Byers & Wells 1966; Souza et al. 2008; Ferreira et al. 2013), and reducing forage production (Auad et al. 2007; Resende et al. 2013). This damage makes *M. spectabilis* 1 of the factors limiting the productivity of extensive livestock pasturing in Brazil (Grisoto 2014).

Biological control with predators, parasitoids, or pathogens (Hajek 2004) is an important means of insect pest management in agricultural ecosystems (Pedigo & Rice 2009). Pathogens used to control insects include bacteria, viruses, and fungi, which are usually host-specific (Vega & Kaya 2012). Thousands of strains of entomopathogenic fungi have been isolated, from species in more than 50 genera (Khan et al. 2012; Hussain et al. 2014); however, the use of fungi-based bioinsecticides for management remains uncommon (Chaurasia et al. 2016). The success of a mycoinsecticide depends on several criteria, but the 1st is its virulence level (Hussain et al. 2014).

Entomopathogenic fungi can be used to suppress insect populations through both inundative and inoculative releases (Mahdavi et al. 2013), and strains of *Beauveria bassiana* (Bals.–Criv.) Vuill. (Cordycipitaceae) and *Metarhizium anisopliae* (Metschn.) Sorokin (Clavicipitaceae) have been successfully used to control many spittlebug species (Hemiptera: Cercopidae) in pasture grasses and sugarcane (Destefano et al. 2004, Domínguez et al. 2016). Although nematodes also can be used to control spittlebugs, their inability to penetrate spittlebug eggs and their need to encounter the host (Batista et al. 2014) makes the use of mycoinsecticides a more efficient approach to control such insects. *Beauveria bassiana* and *M. anisopliae* remain the most common fungi used as mycoinsecticides (Zimmermann 2007; Lubeck et al. 2008; Hussain et al. 2014; Domínguez et al. 2016). However, highly diverse tropical ecosystems are assumed to contain many potentially useful species, including new entomopathogenic fungi (Ghazoul & Sheil 2010), and prospecting for such unknown fungi may increase the role of biological control in sustainable agriculture.

Although supporting studies are lacking, the occurrence of spittlebug population outbreaks appears to be less common in SPSs than in exotic grass monocultures. In addition, fungal infections in *M. spectabilis* have frequently been noted in some tropical SPSs, such as those located in ecotones between the Amazon and Cerrado biomes in Brazil. Therefore, it is of interest to know if spittlebugs are under better natural control in such SPSs because their increased plant diversity benefits natural enemies and enhances the biological control of pest insects (Barbosa 1998; Pickett & Bugg 1998). In this study, we hypothesized that *M. spectabilis* populations are being regulated by entomopathogenic fungi in SPS areas. With this in mind, we set out to survey the fungi found in association with *M. spectabilis* and to make an assessment of the virulence of the collected fungi.

Materials and Methods

COLLECTING INFECTED SPITTLEBUGS

Pasture spittlebugs infected by fungi were collected at the Monaliza Farm, in western Maranhão State (Brazil), at 05.5255556°, 047.4430556°. The farm is comprised of 800 ha, of which 500 ha are grass pasture (*Brachiaria brizantha* (A. Rich.) Stapf (Poaceae), cultivar 'Marundu') in a silvopastoral systems that was developed from natural regeneration of native shrubs and trees since 1998. The farm is located in a transitional area of vegetation (ecotone) between the Amazon and Cerrado biomes. The local climate is tropical sub-humid, with the rainier season occurring from Nov to Apr, but there is no very dry period. The annual average rainfall is 1,530 mm, with Mar being the most humid month (315 mm) and Jul the driest (7 mm). The annual average temperature ranges from 26 to 27 °C. In the coolest months (Jun and Jul), temperatures sometimes drop to 16 °C in the early morning, but they can reach 40°C throughout the day (INMET 2016).

Spittlebugs were collected from Feb to May 2015. Individuals visibly infected by fungi were taken directly from plants with a fine paintbrush and placed in previously autoclaved 1.5 mL microcentrifuge tubes. Specimens were transported at ambient temperature from the field to the laboratories, where they were stored at 0 °C.

IDENTIFYING SPITTLEBUGS AND FUNGI

Taxonomic identification of the infected spittlebugs that we collected was completed at the National Dairy Cattle Research Center, of the Brazilian Agricultural Research Corporation (CNPGL/EMBRAPA), Minas Gerais, Brazil. *Mahanarva spectabilis* identification was confirmed with the aid of a taxonomic key and a taxonomic reference collection of common pasture spittlebugs. The fungi from field-collected spittlebugs were isolated and identified at the Fungal Biomolecule Systematics Laboratory of the Federal University of Minas Gerais (UFMG), Minas Gerais, Brazil.

Ten infected *M. spectabilis* were used to isolate each fungi. A fungal fragment was removed from each infected host with the aid of a platinum needle. The fragment was placed into a Petri dish prepared with Sabouraud dextrose agar culture medium (4% dextrose, 5% casein, and 15% agar) (Alves 1998). After inoculum growth, different colony morphotypes were isolated through successive inoculations in Petri dishes. The colonies were photographed from the top and bottom, and the fungi were then characterized and grouped according to colony morphology, including color, texture, edge type, and radial growth rate on Sabouraud dextrose agar (Fröhlich et al. 2000). The collected fungi also were mounted on glass slides for structural microscopic analysis. After genus-level identification based on morphological traits with the aid of taxonomic keys, duplicate samples of the fungi were preserved. Using this procedure, we collected 5 morphospecies in 3 genera, which were placed in glass tubes with 10 mL distilled water (Castellani 1967). Then, the glass tubes were placed in 20% sterile glycerol filled cryotubes and stored at −80 °C.

PREPARATION OF INOCULATION SOLUTIONS

Samples of the 5 isolated fungi were used to produce conidia to make inoculation solutions. Fungi were cultured in Petri dishes on Sabouraud dextrose agar (Alves 1998) and cultures were incubated in a

climate-controlled chamber (28 ± 2 °C) for 7 to 10 d for growth and conidiogenesis. Conidia were collected from these cultures with a platinum loop and placed in saline solution (1% NaCl) (Almeida et al. 2005). For each fungal biotype, serial dilutions were made to obtain a concentration of 1×10^4 conidia per mL. As a reference control, a commercial formulation of *M. anisopliae* (Metarhyd) was prepared at the same concentration of 1×10^4 conidia per mL.

VIRULENCE UNDER LABORATORY CONDITIONS

The relative virulence of the field-collected fungi and the commercial control species was determined for eggs and nymphs of *M. spectabilis*, which were obtained from a spittlebug colony maintained by the Laboratory of Entomology at the CNPGL/EMBRAPA. For virulence tests, we used eggs at the last stages before hatching and 4th and 5th instar nymphs (last stages before adults). The virulence experiments were carried out in the Laboratory of Insect–Plant Interaction, Department of Biosystems Engineering, Federal University of São João del Rei (UFSJ), Minas Gerais, Brazil.

The design in both the egg and the nymph experiments was to assess 7 treatments (5 wild fungi, *M. anisopliae* as a positive control, and NaCl 1% solution as a negative control), each with 10 replicates (n = 7 treatments × 10 replicates).

In the egg experiment, each replicate consisted of 10 eggs placed on a filter paper strip, which was put inside a closed acrylic box (11 × 11 × 5 cm) lined with filter paper (previously autoclaved and moistened with distilled water). With the aid of graduated pipettes, each box with eggs received 2 mL of saline solution with 1×10^4 conidia per mL (1 of the 6 fungi) or of pure saline solution as a control. Boxes with treated eggs (70 in total, 10 for each treatment) were kept in a climate-controlled chamber at 25 ± 2 °C. The boxes were inspected daily and non-viable eggs were counted under a stereomicroscope. Reduction in egg viability due to fungal infection was assessed daily by counting the number of black-colored eggs.

In the second experiment, virulence to nymphs was determined under laboratory conditions. For each replication, 10 nymphs were placed in a closed acrylic box (11 × 11 × 5 cm) lined with filter paper (previously autoclaved and moistened with distilled water). Fresh roots of *Brachiaria decumbens* Stapf (Poaceae) were placed in the box as a food source for the nymphs. The roots had been disinfected with 20% sodium hypochlorite and distilled water, according to Fröhlich et al. (2000). Each box was inoculated with 2 mL of saline solution (1%) with 1×10^4 conidia per mL (1 of the 6 fungi) or pure saline solution as control. The 70 boxes (10 for each treatment) were kept in a climate controlled chamber at 25 ± 2 °C and inspected daily under a stereomicroscope to quantify nymphal infection and mortality. Dead nymphs were removed from the box.

VIRULENCE UNDER GREENHOUSE CONDITIONS

The field virulence experiments were carried out at the Federal University of São João Del Rei (UFSJ), MG State, Brazil, in a greenhouse without climate control, covered with transparent waterproof plastic and enclosed with anti-aphid netting. The average temperature inside the greenhouse was 28 ± 4 °C and relative humidity was 65 ± 30%. Plants of *B. decumbens* were grown in 0.5 L plastic pots filled with soil fertilized with a 3-component fertilizer (nitrogen, phosphorous, and potassium). Six seeds were sown per pot and grass was left to grow for 3 mo before the experimental trial. Relative fungal virulence was assessed in both eggs and nymphs of *M. spectabilis*. The design in both tests was randomized blocks with 7 treatments (5 wild fungi, *M. anisopliae* as positive control, and 1% NaCl solution as a negative control) with 10 replicates of each treatment or control (n = 7 treatments × 10 replicates). Groups of 7 pots with grass (1 for each treatment or control) were placed in each of 10 plastic trays as plots. The trays were placed on greenhouse benches 1 m high and randomly distributed inside the greenhouse. The plants were irrigated on alternate days, and the water drained from the pots was retained inside the trays.

Ten eggs were placed on each of a series of filter papers (previously autoclaved), which were then place singly at the base of the plants in each pot. With the aid of a graduated pipette, the filter paper received 2 mL of saline solution with 1×10^4 conidia per mL or pure saline solution as a control. In order to avoid cross-contamination among treatments and escape of insects, the pots were then closed at the base of the plants with a plastic lid and gauze. After 15 days, the paper fragments were inspected under a stereomicroscope and non-viable eggs were counted. Ten days later, the filter papers were again removed to count the remaining unhatched eggs.

In the nymphal experiment, ten 4th or 5th instar nymphs were placed at the base of the plants in each pot. The base of the plants received 2 mL of saline solution with 1×10^4 conidia per mL or pure saline solution as a control. In 1 test, the solutions were added immediately after the nymph releases, before spittlebugs had formed any foam. In another test, the solutions were added 24 h after nymphs were released, after they had begun producing spittle at the bases of the grass stems. As described above, the pots were closed at the base of the plants with a plastic lid and gauze. The pots were inspected after 24, 72 and 120 h and dead nymphs were counted and removed.

STATISTICAL ANALYSES

The normality of the data was verified by Kolmogorov–Smirnov test, and homogeneity of variance was evaluated using the Bartlett test. The percentage of dead individuals at the end of the assessment period following fungal inoculation was then subjected to analysis of variance (ANOVA). Subsequently, the averages among treatments were compared by Holm–Sidak test, with a 5% significance level. Data analysis was performed using the statistical packages Graphpad Prism 5.0 (Graph Prism Inc., San Diego, CA, USA) and SigmaPlot 12 (Systat Software Inc., San Jose, CA, USA).

Results

FUNGI ISOLATED FROM SPITTLEBUGS

Five fungal biotypes were found in association with *M. spectabilis*, belonging to the genera *Penicillium, Fusarium, Mucor,* and *Metarhizium.* These fungi were deposited in the Microorganism Collection of the Federal University of Minas Gerais, Minas Gerais, Brazil (World Data Centre for Microorganisms - WDCM 1029. CM-UFMG) according to the following security codes: *Penicillium* sp. = UFMG 11440, *Penicillium* sp. = UFMG 11441, *Mucor* sp. = UFMG 11442, *Fusarium* sp. = UFMG 11443, and *Metarhizium* sp = UFMG 11444 (Fig. 1; Table 1).

VIRULENCE TO EGGS AND NYMPHS UNDER LABORATORY CONDITIONS

Nine d after inoculation, the mortality of *M. spectabilis* eggs was significantly different among the 6 fungi tested and the control solution (F = 13.7, df = 6, 69, P <0.001), and this difference could be classified into 3 virulence level groups (Fig. 2A). UFMG 11443 and UFMG 11444 were the most virulent fungi, which caused more than 90% of the eggs to be non-viable by the end of the observation period. The group of intermediate virulence,

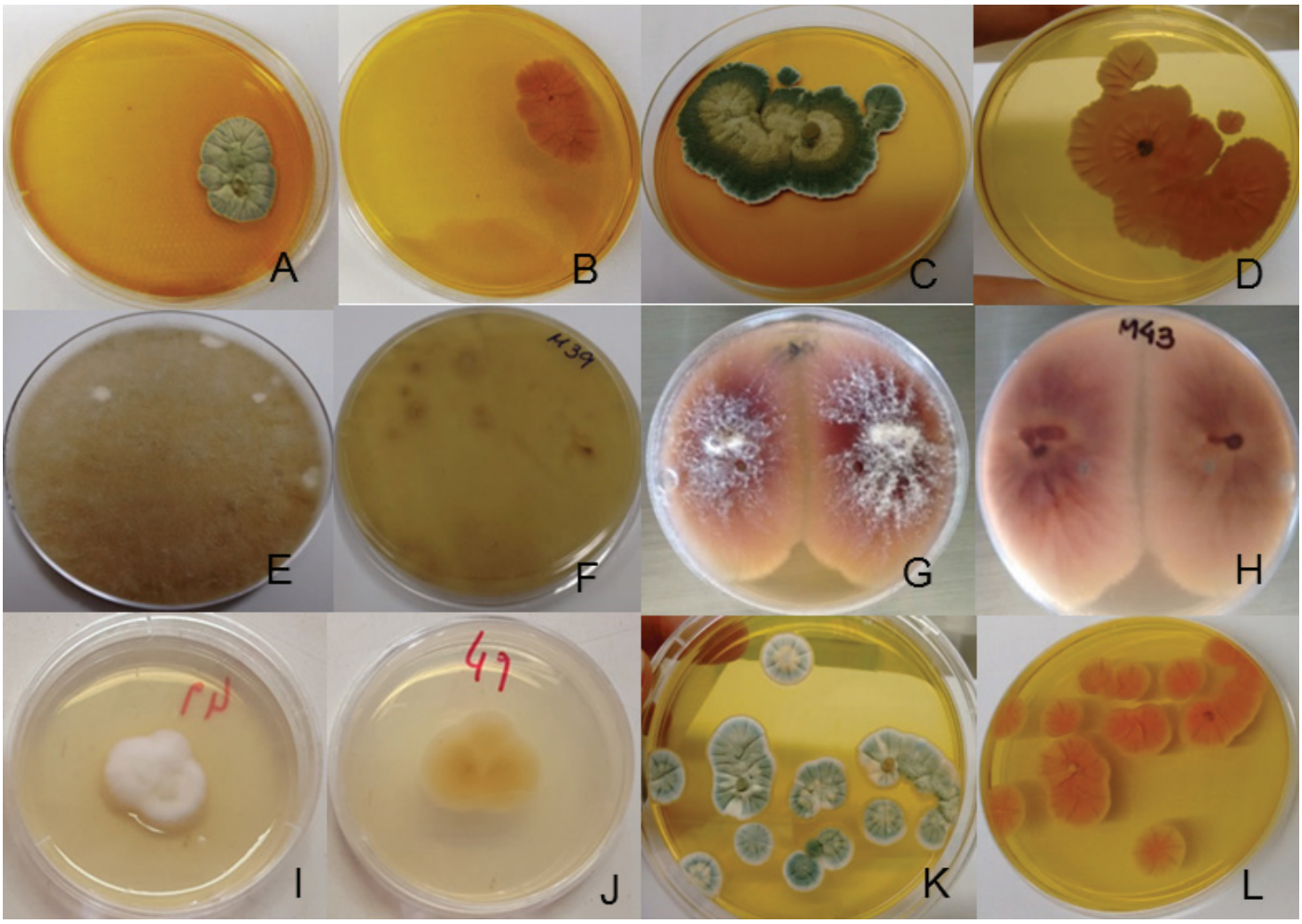

Fig. 1. Fungal colonies (photographed top and bottom), showing morphology of 5 fungi isolated from the spittlebug *Mahanarva spectabilis* in a silvopastoral system in Maranhão, Brazil (A–B = *Penicillium* sp. (UFMG 11440), C–D = *Penicillium* sp. (UFMG 11441), E–F = *Mucor* sp. (UFMG 11442), G–H = *Fusarium* sp. (UFMG 11443), and I–J = *Metarhizium* sp. (UFMG 11444) and a commercial strain of *Metarhizium anisopliae* (K–L).

was made up of the commercial strain of *M. anisopliae* and UFMG 11442, both of which caused approximately 50% of the eggs to be unviable. The 3rd group, which consisted of UFMG 11440 and UFMG 11441, was ineffective, being no different from the saline control (Fig. 2A).

Three d after inoculation, we found significant differences in nymphal mortality (F = 37.1, df = 6, 69, P <0.001), with 2 distinct groups of virulence level (Fig. 2B). Both groups caused higher nymphal mortality than the control solution, all of them killing more than 50% of nymphs by 24 h after inoculation. However, at the end of the 3-d observation period, UFMG 11440 (Group 1) killed fewer of the nymphs than the other fungi. Group 2 fungi (UFMG 11441, UFMG 11442, UFMG 11443, and UFMG 11444) were as virulent as the commercial strain of *M. anisopliae*, all killing more than 80% of the spittlebug nymphs (Fig. 2B).

VIRULENCE UNDER GREENHOUSE CONDITIONS

Under greenhouse conditions, at 25 d after inoculation, egg viability was significantly different among the treatments (F = 8.9, df = 6, 69, P <0.001). The 6 fungi showed similar levels of virulence, and all caused higher mortality than the control solution. Thus, all of them could be grouped into a single virulence category (Fig. 3A).

Similarly, by end of the test period (7 d), nymphal mortality differed significantly among treatments (F = 39.2, df = 6, 69, P <0.001). The

Table 1. Morphology of 5 fungal morphospecies that were isolated from the spittlebug *Mahanarva spectabilis* in a silvopastoral system in Maranhão, Brazil.

	Colony				Microscopy	
Code for Morphospecies	Mycelium	Front color	Back color	Edge	Conidium	Wall
UFMG 11440 *Penicillium* sp.	mold	greyish green	yellowish	smooth	elliptic fusiform	smooth
UFMG 11441 *Penicillium* sp.	mold	olive green	beige	smooth	elliptic oval	smooth
UFMG 11442 *Mucor* sp.	Cottonous	brown	brown	smooth	globose	smooth
UFMG 11443 *Fusarium* sp.	cottonous	purple	purple	irregular	Elliptic fusiform	smooth
UFMG 11444 *Metarhizium* sp.	milky	green	beige	irregular	cylindrical	smooth

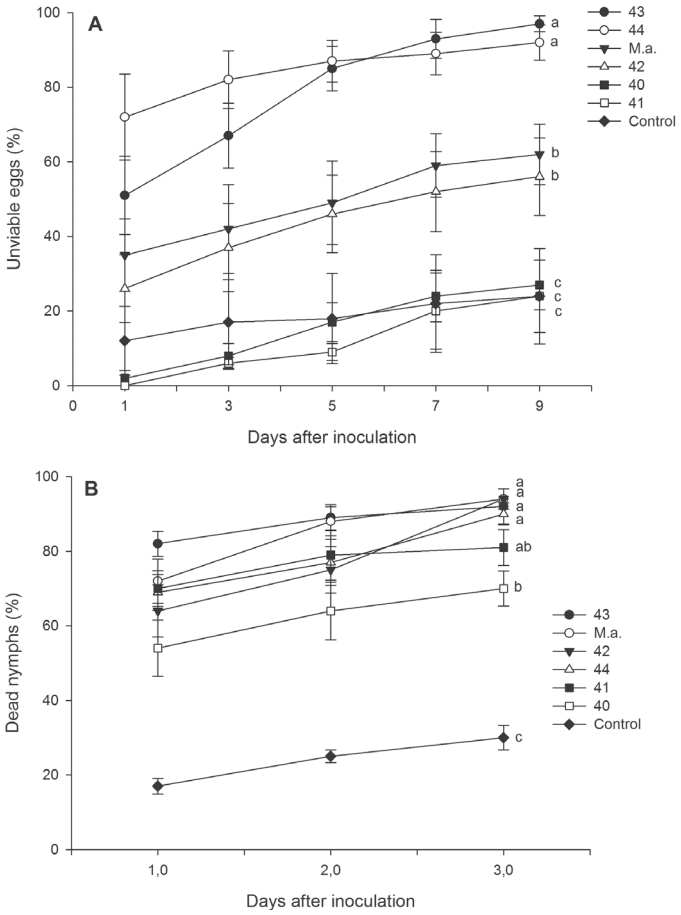

Fig. 2. Unviable eggs (A) and mortality of nymphs (B) of *Mahanarva spectabilis,* in a closed box in a climate controlled chamber, caused by wild fungi (40 to 44, being the last 2 digits of the isolate's code), a commercial strain of *Metarhizium anisopliae* (M.a) and a control saline solution. Dot and bar indicate mean ± SE of 10 replicates. Treatments with the same letter belong to the same group on the last day after inoculation, according to the Holm–Sidak post hoc test with $P < 0.05$.

same was true for nymphs treated before spittle formation ($F = 48.0$, df = 6, 69, $P < 0.001$) (Fig. 3B and C).

Under greenhouse conditions, fungi strains showed greater variation in their virulence (Fig. 3B and C) than they did in the laboratory experiment (Fig. 2B). In both inoculation conditions, before or after foaming, UFMG 11443 was the most virulent, immediately followed by UFMG 11444. The others (UFMG 11440, UFMG 11441, UFMG 11442, and the commercial *M. anisopliae*) could be grouped together (Fig. 3B and C).

Discussion

Under laboratory conditions fungal isolates UFMG 11442, UFMG 11443, and UFMG 11444 from our study all killed *M. spectabilis* eggs and nymphs as well as or better than the commercial strain of *M. anisopliae*, suggesting they have potential for use as biopesticides. Under the conditions of our greenhouse tests, all 5 of our newly collected fungi were as effective as commercial *M. anisopliae* in killing *M. spectabilis* eggs, perhaps because the observation period was longer than in the laboratory. In the greenhouse experiment, isolates UFMG 11443 and UFMG 11444 showed greater virulence against nymphs than the other fungi, including *M. anisopliae*. In addition, our study showed that encasement of nymphs in foam (spittle) did not prevent fungal infection, in contradiction to speculation in the literature (Grisoto et al. 2014).

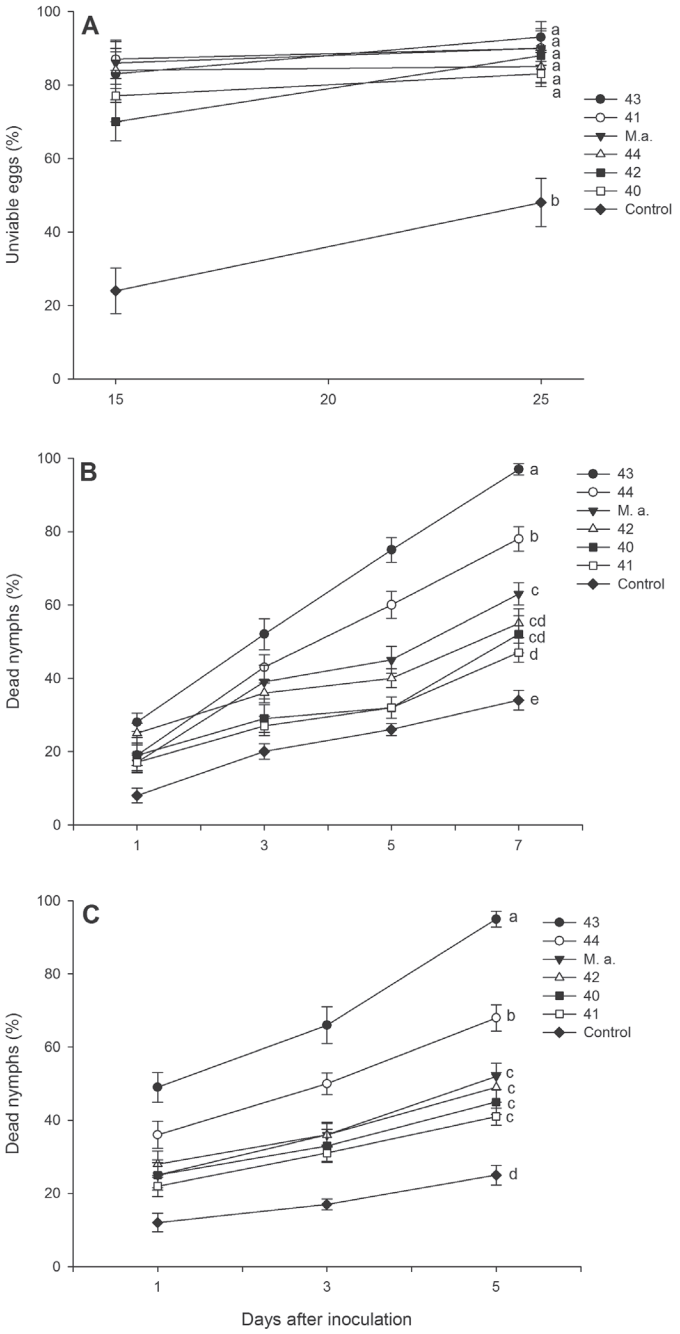

Fig. 3. Unviable eggs (A), mortality of nymphs inoculated before spittle formation (B) and nymphs inoculated after spittle formation (C) of *Mahanarva spectabilis* on *Brachiaria decumbens* grown in pots in a greenhouse, caused by wild fungi (40 to 44, being the last 2 digits of the isolate security code), a commercial strain of *Metarhizium anisopliae* (Ma) and a control saline solution. Dot and bar indicate mean ± SE of 10 replications. Treatments with the same letter belong to the same group on the last day after inoculation, according to the Holm–Sidak post hoc test with $P < 0.05$.

Similar activity of fungi has been reported against other spittlebugs, including 54% mortality of the spittlebug *Aeneolamia postica* (Walker) from a commercial strain of *M. anisopliae* (La Torres et al. 2013). Under field conditions, various strains of *M. anisopliae* have caused mortality as high as 66 to 72% in *Aeneolamia varia* (Fabricius) nymphs (Matabanchoy et al. 2012). In another study, a commercial formulation of

M. anisopliae caused 13% less mortality in *A. postica* adults than wild fungal strains isolated in Colombia (Obando et al. 2013). Some wild fungi can be more virulent than commercial strains of *M. anisopliae,* thus unexplored potential for improved control with fungal insecticides clearly exists. In general, we found that isolated fungi UFMG 11443 and UFMG 11444 were the most promising, and future studies should examine more closely their potential for use in the biological control of insect pests.

Under the experimental conditions of our study, some locally collected wild fungi showed high virulence against *M. spectabilis* and were able to kill both eggs and nymphs quickly after application, thus would likely to control the pest and prevent severe plant damage. However, blends of isolates should be investigated, because synergism may increase host mortality (Inglis et al. 2008). Associations of fungi with other pathogens may also improve pest control (Meyling & Hajek 2010; Pell et al. 2010). For example, nematodes have been shown to have effective action against nymphs and adults of *M. spectabilis*, but they are unable to penetrate eggs (Batista et al. 2014). Fungi also can be combined with entomopathogenic bacteria, such as the *Bacillus thuringiensis* (Wraight et al. 2009; Ansari et al. 2010).

To prompt the development of wild fungi for use as biopesticides, virulence tests under laboratory and greenhouse conditions must be followed by ecological studies under field conditions to determine if any specific fungus might be limited by weather or other field conditions (Oliveira et al. 2016). For example, exposure to strong sunlight decreases fungal spore populations and hinders spore dispersal (Wraight et al. 2007). Ultraviolet radiation (UV) in particular causes direct and indirect structural and physiological damage to fungi that dramatically reduces efficiency (Nicholson et al. 2000; Braga et al. 2002, Oliveira et al. 2016). Ultimately, between the stage of prospecting for new, more effective species of fungi (as described here), and their successful use for pest control, there are many further considerations, including effects of such fungi on other beneficial insects in the crop (Santos Jr. et al. 2006), optimal timings of applications (Ugine et al. 2007), and commercial competition from chemical pesticides in terms of cost, ease of management, persistence, and required frequency of application (Lacey et al. 2015).

By prospecting for infected spittlebugs, we found 5 types of fungi infecting *M. spectabilis* in a short period of time in a relatively small pasture. Many other biotypes could still be found, considering the large territory in Brazil with this pest and habitat and the variability in the habitat among regions. The complexity of fungal ecology and the microclimatic and biotic diversification found in SPSs compared with monocultures (Ghazoul & Sheil 2010; Sousa et al. 2015) may have promoted the high diversity of fungi found infecting *M. spectabilis* in the study area. This study clearly shows that diversified agroecosystems, particularly in regions of high biodiversity regions, can provide ecosystem services to support sustainable agriculture and livestock. However, the ecological mechanisms that increase the diversity of natural enemies of insect pests and enhance natural biological control in such SPSs remain unclear.

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References Cited

Aguiar DM, Auad AM, Fonseca MG, Leite MV. 2014. *Brachiaria ruziziensis* responses to different fertilization doses and to the attack of *Mahanarva spectabilis* (Hemiptera: Cercopidae) nymphs and adults. The Scientific World Journal 2014: 1–8.

Aguirre S, Galván G, González A, González T, Bentancor A, Souza J. 2016. Sistemas de silvopastoreo en predios familiares de Colonia Gestido (Uruguay). Livestock Research for Rural Development 28: 2–21.

Almeida CV, Yara R, Almeida M. 2005. Fungos endofíticos isolados de ápices caulinares de pupunheira cultivada in vivo e in vitro. Pesquisa Agropecuária Brasileira 40: 467–470.

Alves SB. 1998. Fungos entomopatogênicos, pp. 289–381 *In* Alves SB [eds.], Controle Microbiano de Insetos. Fundação de Estudos Agrários "Luiz de Queiroz", Piracicaba, Brazil.

Andow DA. 1991. Vegetational diversity and arthropod population response. Annual Review of Entomology 36: 561–586.

Ansari MA, Shah FA, Butt TM. 2010. The entomopathogenic nematode *Steinernema kraussei* and *Metarhizium anisopliae* work synergistically in controlling overwintering larvae of the black vine weevil, *Otiorhynchus sulcatus*, in strawberry growbags. Biocontrol Science and Technology 20: 99–105.

Araújo RP, Carvalho AJCA, Araújo SAC, Ribeiro ET, Pádua FT, Carvalho CAB, Lista FN. 2013. Produção e composição química de *Brachiaria decumbens* cv. Basilisk em sistema silvipastoril sob diferentes espaçamentos com *Eucalyptus urophylla* S.T. Blake. Revista Brasileira de Agropecuária Sustentável 3: 90–98.

Auad AM, Simões AD, Pereira AV. 2007. Seleção de genótipos de capim-elefante quanto à resistência à cigarrinha-das-pastagens. Pesquisa Agropecuária Brasileira 42: 1077–1081.

Auad AM, Domingues R, Machado MA, Souza LS, Carvalho GS, Paula-Moraes SV. 2010. Genetic variability of *Mahanarva* sp. (Hemiptera: Cercopidae) collected from different sites in Brazil. Genetics and Molecular Research 9: 1005–1010.

Barbosa P [ed.].1998. Conservation Biological Control. Academic Press, San Diego, California.

Batista ESP, Auad AM, Andaló V, Monteiro CMO. 2014. Virulence of entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) to spittlebug *Mahanarva spectabilis* (Hemiptera). Arquivos do Instituto Biológico 81: 145–149.

Braga GU, Rangel DE, Flint SD, Miller CD, Anderson AJ, Roberts DW. 2002. Damage and recovery from UV-B exposure in conidia of the entomopathogens *Verticillium lecani* and *Aphanocladium album*. Mycologia 94: 912–920.

Byers RA, Wells HD. 1966. Phytotoxemia of coastal bermuda grass caused by the two-lined spittlebug *Prosapia bicincta* (Homoptera: Cercopidae). Annals of the Entomological Society of America 59: 1067–1071.

Castellani A. 1967. Maintenance and cultivation of common pathogenic fungi in distilled water. The Journal of Tropical Medicine and Hygiene 42: 181–184.

Cezar I M, Queiroz HP, Thiago LRLS, Cassales FLG, Costa FP. 2005. Sistemas de produção de gado de corte no Brasil: uma descrição com ênfase no regime alimentar e no abate. Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Gado de Corte, Campo Grande, Brazil, https://www.embrapa.br/gado-de-corte/busca-de-publicacoes/-/publicacao/326307/sistemas-de-producao-de-gado-de-corte-no-brasil-uma-descricao-com-enfase-no-regime-alimentar-e-no-abate (last accessed 5 Mar 2017).

Chara JD, Giraldo C, Caro M. 2011. Servicios Ambientales de la Biodiversidad en Paisajes Agropecuarios. Centro para la Investigación en Sistemas Sostenibles de Producción Agropecuaria, Cali, Colombia.

Chaurasia A, Yaqoob L, Owais W, Gupta US. 2016. Effect of certain entomopathogenic fungi on oxidative stress and mortality of *Periplaneta americana.* Pesticide Biochemistry and Physiology 127: 28–37.

Destefano RHR, Destefano SAL, Messias CL. 2004. Detection of *Metarhizium anisopliae* var. anisopliae within infected sugarcane borer *Diatraea saccharalis* (Lepidoptera, Pyralidae) using specific primers. Genetics and Molecular Biology 27: 245–252.

Domínguez CH, Guzmán-Franco AW, Carrillo-Benítez MG, Alatorre-Rosas R, Rodríguez-Leyva E, Villanueva-Jiménez JA. 2016. Specific diversity of *Metarhizium* isolates infecting *Aeneolamia spp*. (Hemiptera: Cercopidae) in sugarcane plantations. Neotropical Entomology 45: 80–87.

Fajardo D. 2009. Influencia de sistemas silvopastoriles en la diversidad de aves en la cuenca del río La Vieja, Colombia. Recursos Naturales y Ambiente 58: 9–16.

Ferreira RB, Moraes JC, Auad AM, Fonseca MG. 2013. Interaction of spittlebug and forage grass under different carbon dioxide concentrations. Journal of Pest Science 86: 161–166.

Fonseca MG, Auad AM, Resende TT, Hott, MC, Borges, CAV. 2016. How will *Mahanarva spectabilis* (Hemiptera: Cercopidae) respond to global warm-

ing? Journal of Insect Science 16: 32, doi: https://doi.org/10.1093/jisesa/iew005.
Fröhlich J, Hyde KD, Petrini 2000. Endophytic fungi associated with palms. Mycological Research 104: 1202–1212.
Ghazoul J, Sheil D. 2010. Tropical Rain Forest Ecology, Diversity, and Conservation. Oxford University Press, Oxford, UK.
Grisoto E, Vendramim JD, Lourenção AL, Usberti Filho JA, Dias CTDS. 2014. Biology of *Mahanarva fimbriolata* on forage grasses. Ciência Rural 44: 1043–1049.
Hajek A. 2004. Natural Enemies: an Introduction to Biological Control. Cambridge University Press, Cambridge, UK.
Hussain A, Rizwan-ul-Haq, Al-Auedh, Al-Jabr. 2014. Mycoinsecticides: potential and future perspective. Recent Patents on Food, Nutrition and Agriculture 6: 45–53.
Inglis GD, Duke GM, Goettel MS, Kabaluk JT. 2008. Genetic diversity of *Metarhizium anisopliae* var. *anisopliae* in southwestern British Columbia. Journal of Invertebrate Pathology 98: 101–113.
INMET (Instituto Nacional de Meteorologia). 2016. Instituto Nacional de Meteorologia, Brasilía, Brazil, http://www.inmet.gov.br (last accessed 5 Mar 2017).
Khan S, Guo L, Maimaiti Y, Mijit M, Qiu O. 2012. Entomopathogenic fungi as microbial biocontrol agent. Molecular Plant Breeding 3: 63–79.
La Torres CM, Cortez MH, Ortiz GCF, Capello GS, De la Cruz PA. 2013. Caracterización de aislamientos nativos de *Metarhizium anisopliae* y su patogenicidad hacia *Aeneolamia postica*, en Tabasco, México. Revista Colombiana de Entomología 39: 40–46.
Lacey LA, Grzywacz D, Shapiro-Ilan DI, Frutos R, Brownbridge M, Goettel MS. 2015. Insect pathogens as biological control agents: Back to the future. Journal of Invertebrate Pathology 132: 1–41.
Lubeck I, Arruda W, Souza BK, Stanisçuaski F, Carlini CR, Schrank A, Vainstein MH. 2008. Evaluation of *Metarhizium anisopliae* strains as potential biocontrol agents of the tick *Rhipicephalus* (*Boophilus*) *microplus* and the cotton stainer *Dysdercus peruvianus*. Fungal Ecology 1: 78–88.
Mahdavi V, Saber M, Rafiee-Dastjerdi H, Mehrvar A. 2013. Susceptibility of the Hymenopteran parasitoid, *Habrobracon hebetor* (Say) (Braconidae) to the entomopathogenic fungi *Beauveria bassiana* Vuillemin and *Metarhizium anisopliae* Sorokin. Jordan Journal of Biological Sciences 6: 17–20.
Matabanchoy JAS, Bustillo AEP, Castro UV, Mesa NCC, Moreno CAG. 2012. Eficácia de *Metarhizium anisopliae* para controlar *Aeneolamia varia* (Hemiptera: Cercopidae), en caña de azúcar. Revista Colombiana de Entomología 38: 177–181.
Meyling NV, Hajek AE. 2010. Principles from community and metapopulation ecology: application to fungal entomopathogens. Biocontrol 55: 39–54.
Meyling NV, Lübeck M, Buckley EP, Eilenberg J, Rehner SA. 2009. Community composition, host range and genetic structure of the fungal entomopathogen *Beauveria* in adjoining agricultural and seminatural habitats. Molecular Ecology 18: 1282–1293.
Murgueitio E, Chará JDO, Barahona RR, Cuartas CAC, Naranjo JFR. 2014. Intensive silvopastoral systems (ISPS), mitigation and adaptation tool to climate change. Tropical and Subtropical Agroecosystems 17: 501–507.
Murgueitio E, Ibrahim M, Ramírez E, Zapata A, Mejía CE, Casasola YF. 2003. Usos del suelo en fincas ganaderas: guía para el pago de servicios ambientales en el proyecto. Enfoques Silvopastoriles Integrados para el Manejo de Ecosistemas, Centro para la Investigación en Sistemas Sostenibles de Producción Agropecuaria (CIPAV; Cali, Colombia), Centro Agronómico Tropical de Investigación y Enseñanza (CATIE; Turrialba, Costa Rica), and Nitlapan-UCA (Managua, Nicaragua).
Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P. 2000. Resistance of *Bacillus* endospores to extreme terrestrial end extraterrestrial environments. Microbiology and Molecular Biology Reviews 64: 548–572.
Obando B, Johanna A, Bustillo P, Alex E, Castro V, Ulises MC, Nora C. 2013. Selección de cepas de *Metarhizium anisopliae* para el control de *Aeneolamia varia* (Hemiptera: Cercopidae). Revista Colombiana de Entomología 39: 26–33.
Oliveira MT, Monteiro AC, Scala Jr NL, Barbosa JC, Mochi DA. 2016. Sensibilidade de isolados de fungos entomopatogênicos às radiações solar, ultravioleta e à temperatura. Arquivos do Instituto Biológico 83: 1–7.
Ormond EL, Thomas APM, Pugh PJA, Pell JK, Roy HE. 2010. A fungal pathogen in time and space: the population dynamics of *Beauveria bassiana* in a conifer forest. FEMS Microbial Ecology 74: 146–154.
Pedigo LP, Rice ME. 2009. Entomology and Pest Management, 6th edition. Pearson Prentice-Hall, Upper Saddle River, New Jersey
Pell JK, Hannam JJ, Steinkraus DC. 2010. Conservation biological control using fungal entomopathogens. Biocontrol 55:187–198.
Pickett CH, Bugg RL [eds.]. 1998. Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests. University of California Press, Berkeley, California.
Resende T, Auad AM, Fonseca MG, Souza SF, Ribeiro DS, Silva SEB. 2013. The damage capacity of *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) adults on *Brachiaria ruziziensis* pasture. Scientific World Journal 1–6.
Santos Jr HJG, Marques EJ, Barros R, Gondim Jr MGC. 2006. Interação de *Metarhizium anisopliae* (Metsch.) Sorok., *Beauveria bassiana* (Bals.) Vuill. e o parasitoide *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) sobre larvas da traca-das-cruciferas, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Neotropical Entomology 35: 241–245.
Silva HD. 2008. Benefícios sócio-econômicos e ambientais dos sistemas agrossilvipastoris. Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Florestas, http://sigam.ambiente.sp.gov.br/sigam3/Repositorio/222/Documentos/EPBio/EPBio_27_Helton.pdf (last accessed 5 Mar 2017).
Sousa LF, Maurício RM, Gonçalves LC, Borges I, Moreira GR. 2011. Cinética de fermentação ruminal in vitro da forrageira *Brachiaria brizantha* cv. Marandu em sistema silvipastoril. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 6: 382–391.
Sousa LF, Maurício RM, Paciullo DSC, Silveira SR, Ribeiro RS, Calsavara LH, Moreira GR. 2015. Forage intake, feeding behavior and bio-climatological indices of pasture grass, under the influence of trees, in a silvopastoral system. Tropical Grasslands – Forrajes Tropicales 3: 129–141.
Souza JC, Silva RA, Reis PR, Queiroz DS, Silva DB. 2008. Cigarrinhas-das-pastagens: histórico, bioecologia, prejuízos, monitoramento e medidas de controle. Empressa de Pesquisa Agropecuária de Minas Gerais Circular Téchnia 42: 1–8.
Thompson V. 2004. Associative nitrogen fixation, C4 photosynthesis, and the evolution of spittlebugs (Hemiptera: Cercopidae) as major pests of neotropical sugarcane and forage grasses. Bulletin of Entomological Research 94: 189–200.
Ugine TA, Wraight SP, Sanderson JP. 2007. Effects of manipulating spray application parameters on efficacy of the entomopathogenic fungus *Beauveria bassiana* against western flower thrips, *Frankliniella occidentalis*, infesting greenhouse impatiens crops. Biocontrol Science and Technology 17: 193–219.
Vega FE, Kaya HK. 2012. Fungal entomopathogens, pp. 171–220 *In* Vega FE, Kaya HK [eds.], Inset Pathology, 2nd edition. Academic Press, San Diego, California.
Wraight SP, Inglis GD, Goettel MS. 2007. Fungi, pp. 223–248 *In* Lacey LA, Kaya HK [eds.], Field Manual of Techniques in Invertebrate Pathology, 2nd edition. Springer, Dordrecht, The Netherlands.
Wraight SP, Lacey LA, Kabaluk JT, Goettel MS. 2009. Potential for microbial biological control of coleopteran and hemipteran pests of potato. Fruit, Vegetable and Cereal Science and Biotechnology 3: 25–38.
Zimmermann G. 2007. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Science and Technology 17: 879–920.
Zuluaga AF, Glraldo C, Chará J. 2011. Servicios ambientales que proveen los sistemas silvopastoriles y los beneficios para la biodiversidad. Manual 4, Proyecto Ganadería Colombiana Sostenible, GEF, Banco Mundial, FEDEGAN, CIPAV, Fondo Accion, TNC. Bogotá, Colombia, http://www.cipav.org.co/pdf/4.Servicios.Ambientales.pdf (last accessed 5 Mar 2017).