UNIVERSIDADE FEDERAL DE GOIÁS
ESCOLA DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

EFICÁCIA DE *Cordyceps javanica* SOZINHO OU EM COMBINAÇÃO COM INSETICIDAS QUÍMICOS NO CONTROLE DE *Bemisia tabaci* MEAM1 E PERSISTÊNCIA EM FOLHAS DE SOJA

HELOIZA ALVES BOAVENTURA

Orientadora:
Drª. Eliane Dias Quintela

Agosto – 2019

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HELOIZA ALVES BOAVENTURA

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Dissertação apresentada à Coordenação do Programa de Pós-Graduação em Agronomia, da Universidade Federal de Goiás, como exigência para obtenção do título de Mestra em Agronomia. Área de concentração: Fitossanidade

Orientadora:

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Goiânia, GO – Brasil
2019

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Boaventura , Heloiza Alves
EFICÁCIA DE Cordyceps javanica SOZINHO OU EM COMBINAÇÃO COM INSETICIDAS QUÍMICOS NO CONTROLE DE Bemisia tabaci MEAM1 E PERSISTÊNCIA EM FOLHAS DE SOJA [manuscrito] / Heloiza Alves Boaventura . - 2019.
XCIV, 94 f.

Orientador: Prof. Eliane Dias Quintela.
Dissertação (Mestrado) - Universidade Federal de Goiás, Escola de Agronomia (EA), Programa de Pós-Graduação em Agronomia, Goiânia, 2019.

1. virulência. 2. epizootia. 3. bioproduto. 4. formulação . 5. fatores ambientais. I. Quintela, Eliane Dias , orient. II. Título.

CDU 631/635

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ATA DE DEFESA DE DISSERTAÇÃO

Aos trinta dias do mês de agosto do ano de dois mil e dezenove (30.08.2019), às 13h30min, na Embrapa Arroz e Feijão, em Santo Antônio de Goiás, reuniu-se a Banca Examinadora composta pelos membros: Drª. Eliane Dias Quintela - Orientadora e Presidente da Banca, Drª. Janayne Maria Rezende e Prof. Dr. Éverton Kort Kamp Fernandes, para a realização da sessão pública da defesa de Dissertação intitulada: "Eficácia de *Cordyceps javanica* sozinho ou em combinação com inseticidas químicos no controle de *Bemisia tabaci* MEAM1 e persistência em folhas de soja", de autoria de **Heloiza Alves Boaventura**, discente do curso de **Mestrado**, na área de concentração em **Fitossanidade**, do Programa de Pós-Graduação em Agronomia da UFG. A sessão foi aberta pela presidente, que fez a apresentação formal dos membros da Banca e deu início às atividades relativas a defesa da Dissertação. Passou a palavra a mestranda que em quarenta minutos apresentou o seu trabalho. Após a exposição, a candidata foi arguida pelos membros da banca. Terminada a fase de arguição, procedeu-se à avaliação da defesa. De acordo com Resolução CEPEC 1403/2016, de 10 de junho de 2016 que regulamenta os Programas de Pós-Graduação *Stricto Sensu* na UFG, a Banca Examinadora considerou a Dissertação "**APROVADA**", com as correções recomendadas, estando integralmente cumprido este requisito para fins de obtenção do título de **MESTRA** em Agronomia, na área de concentração em **FITOSSANIDADE**, pela Universidade Federal de Goiás. A mestranda poderá efetuar as modificações sugeridas pela Banca Examinadora e encaminhar nova versão eletrônica da Dissertação à Secretaria do PPGA, no prazo máximo de trinta dias após a data da defesa. A Banca Examinadora recomendou a publicação de artigo(s) científico(s), oriundo(s) dessa Dissertação, em periódicos de circulação nacional e, ou, internacional, depois de acatadas as modificações sugeridas. Para finalizar, a Presidente agradeceu os membros examinadores, congratulou-se com a mestranda e encerrou a sessão às 16:30 min, para constar, eu Welinton Barbosa Mota, secretário do PPGA, lavrei a presente Ata que depois de lida e aprovada, será assinada pelos membros da Banca Examinadora, em quatro vias de igual teor.

Drª. Eliane Dias Quintela
Presidente da Banca – Embrapa Arroz e Feijão

Drª. Janayne Maria Rezende
Membro – AgBitech

Prof. Dr. Éverton Kort Kamp Fernandes
Membro – ICB/UFG

Aos meus pais Hélio e Ilza,

DEDICO

AGRADECIMENTOS

A Deus, em primeiro lugar, por me abençoar na busca pelos meus sonhos sendo meu guia, minha força e minha luz em todos os momentos.

Ao meu pai Hélio, pelo amor e apoio incondicional, por nunca medir esforços para a realização deste sonho.

A minha mãe Ilza, pelo amor incondicional, por ser o meu porto seguro e estímulo durante esta caminhada, enxugar as minhas lágrimas e me incentivar a seguir sempre em frente.

Aos meus irmãos Marcos e Carlos, por todo amor, companheirismo, amizade, incentivo e apoio, por sempre demonstrarem o orgulho que sentem por mim.

Aos meus sobrinhos Lucas e Lavínia, por me receberem sempre com o sorriso mais lindo e me encherem com tanto amor.

Ao meu namorado Luiz Fernando, por estar sempre ao meu lado, acreditando em mim e me incentivando a lutar sempre pelos meus sonhos.

A minha orientadora Eliane Quintela, por acreditar em mim e me incentivar a ser sempre melhor, por todo ensinamento, incentivo e orientação ao decorrer destes anos e por ser um exemplo profissional pra mim.

Aos amigos da Embrapa Arroz e Feijão, Edmar Cardoso (*in memorian*), José Francisco Arruda, Gerusa Souza, Edson Djalma, Genoquinha Vaz e Leandro Ribeiro pelo apoio, incentivo, ensinamentos, paciência, amizade e enorme ajuda. Em especial ao José Francisco por toda ajuda e trabalho com as análises estatísticas.

Aos amigos que conquistei durante o mestrado e que me apoiaram e sempre incentivaram: Klênia Pacheco, Muriel Rizental, André Cirilo, Enio Santos, Gabriella Godoi, Letícia Rafaelly, Sara Guimarães, Amanda Ferreira, Renata Coutinho, Larissa Sousa, Camila Regina, Mariana Aguiar, Rafael Silverio e Cristiane Ferreira.

A Embrapa Arroz e Feijão e ao Laboratório de Patologia de Insetos, pela infra-estrutura que permitiu o desenvolvimento deste trabalho.

A Universidade Federal de Goiás, pela oportunidade de realizar o curso de Mestrado.

Ao CNPq pela concessão de bolsa de estudos para o Mestrado.

A todos, que de forma direta ou indireta contribuíram para a realização deste trabalho. Muito obrigada!

“Foi o tempo que dedicastes à tua rosa que a fez tão importante.”

(Antoine de Saint-Exupéry)

SUMÁRIO

RESUMO GERAL

BOAVENTURA, H. A. **Eficácia de *Cordyceps javanica* sozinho ou em combinação com inseticidas químicos no controle de *Bemisia tabaci* MEAM1 e persistência em folhas de soja.** 2019. 94 f. Dissertação (Mestrado em Agronomia: Fitossanidade) - Escola de Agronomia, Universidade Federal de Goiás, Goiânia, 2019[1].

A mosca-branca, *Bemisia tabaci* causa danos diretos pela sucção de seiva e injeção de toxinas, e indiretos pela fumagina e transmissão de mais de 300 espécies de vírus as plantas. *Cordyceps javanica* foi encontrado causando epizootias naturais e é uma alternativa para o manejo de populações resistentes de mosca-branca. Os objetivos deste trabalho foram: (1) determinar a suscetibilidade de todos os estágios ninfais de *B. tabaci* a três isolados de *C. javanica* observando o efeito das diferentes condições climáticas na virulência em casa telada; (2) avaliar a eficiência e persistência de *C. javanica* e a associação do fungo com inseticidas químicos no controle de mosca-branca. Não foram observadas diferenças na suscetibilidade entre os isolados de *C. javanica* para as ninfas de 1º, 2º e 3º instar; as mortalidades variaram de 63,7-87,8% a 5×10^7 conídios mL^{-1}. O 4º ínstar foi menos suscetível aos isolados (≤ 15,5% de mortalidade). No entanto, os adultos que emergiram de ninfas tratadas no 4º ínstar foram altamente suscetíveis (mortalidade e micose de adultos variaram de 75,6 a 93,2%). Em nosso estudo, a temperatura foi mais prejudicial para a virulência de *Cordyceps* em ninfas de *Bemisia* do que a umidade relativa. O isolado BRM 27666 foi selecionado para testes de eficiência em condições de campo devido a habilidade de crescer extensivamente sobre a superfície da folha e produzir uma quantidade elevada de conídios sob condições úmidas. Em todos os experimentos, utilizou-se um pulverizador pressurizado de CO_2 com barras dropleg (pulverizam as folhas debaixo para cima). A porcentagem de ninfas esporuladas variou entre 47 – 59,1% e 25,4 – 41,3% para o fungo não formulado (3 aplicações de 1×10^{12} conídios ha^{-1}) e formulado (1 aplicação de 2×10^{11} conídios ha^{-1}), respectivamente, após 28 dias. A mortalidade de ninfas aumentou após ação conjunta com parasitoides (67,9 - 81,6%) em tratamentos com fungo. A persistência dos conídios de *C. javanica* foi significativamente reduzida em 48 h. Após combinação do fungo com os inseticidas químicos a porcentagem de micose variou entre 20,2 a 35% após 26 dias, exceto o fungo + piriproxifeno (≤ 9,4%). De forma geral, após ação conjunta dos inimigos naturais a mortalidade de ninfas nos tratamentos com fungo variou entre 90,3 a 98,1%. O alto potencial de *C. javanica* no controle de mosca-branca foi demonstrado em nossos experimentos de campo e casa telada. Com base nisto, *C. javanica* BRM 27666 foi selecionado como isolado promissor no controle de mosca-branca. Um bioproduto a base de conídios de *C. javanica* foi desenvolvido e está em fase de registro no Brasil para o controle de mosca-branca em diversas culturas.

Palavras-chave: virulência, epizootia, fatores ambientais, formulação, bioproduto

[1]Orientadora: Eliane Dias Quintela

GENERAL ABSTRACT

BOAVENTURA, H. A. **Efficacy of *Cordyceps javanica* alone or in combination with chemical insecticides in the control of *Bemisia tabaci* MEAM1 and persistence in soybean leaves.** 2019. 94 f. Dissertation (Masters in Agronomy: Plant Health) - Escola de Agronomia, Universidade Federal de Goiás, Goiânia, 2019[2].

The whitefly *Bemisia tabaci* causes direct damage by sap suction and toxin injection, and indirect damage by fumagine and transmission of more than 300 species of viruses to plants. *Cordyceps javanica* has been found to cause natural epizootic diseases and is an alternative for the management of resistant whitefly populations. The objectives of this study were: (1) to determine the susceptibility of all *B. tabaci* nymphal stages to three *C. javanica* isolates by observing the effect of different climatic conditions on screened virulence; (2) evaluate the efficiency and persistence of *C. javanica* and the association of the fungus with chemical insecticides in the control of whitefly. No differences in susceptibility were observed between C. javanica isolates for 1st, 2nd and 3rd instar nymphs; mortalities ranged from 63.7-87.8% to 5×10^7 conidia mL^{-1}. The 4th instar was less susceptible to isolates (≤15.5% mortality). However, adults who emerged from 4th instar nymphs were highly susceptible (adult mortality and ringworm ranged from 75.6 to 93.2%). In our study, temperature was more detrimental to *Cordyceps* virulence in *Bemisia* nymphs than relative humidity. The BRM 27666 isolate was selected for field efficiency tests because of its ability to grow extensively on the leaf surface and produce a high amount of conidia under wet conditions. In all experiments, a pressurized CO_2 spray with dropleg bars was used (they spray the leaves upwards). The percentage of sporulated nymphs ranged from 47 - 59.1% to 25.4 - 41.3% for unformulated (3 applications of 1×10^{12} conidia ha^{-1}) and formulated (1 application of 2×10^{11} conidia ha^{-1}), respectively, after 28 days. Nymph mortality increased after joint action with parasitoids (67.9 - 81.6%) in fungal treatments. The persistence of *C. javanica* conidia was significantly reduced at 48 h. After combining the fungus with the chemical insecticides, the percentage of mycosis ranged from 20.2 to 35% after 26 days, except the fungus + pyriproxyfen (≤9.4%). In general, after the joint action of natural enemies, the mortality of nymphs in fungal treatments ranged from 90.3 to 98.1%. The high potential of *C. javanica* in whitefly control has been demonstrated in our field and screenhouse experiments. Based on this, *C. javanica* BRM 27666 was selected as a promising isolate in whitefly control. A *C. javanica* conidia-based mycoinsecticide has been developed and is under registration in Brazil for the control of whitefly in several crops.

Key words: virulence, epizootics, environmental factors, formulation, mycoinsecticide

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[2]Orientadora: Eliane Dias Quintela

1 INTRODUÇÃO GERAL

A mosca branca *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) é uma das pragas mais invasivas e prejudiciais de uma grande variedade de culturas hortícolas, ornamentais e de campo em todo o mundo, causando grandes perdas econômicas (De Barro et al., 2011). Os danos causados à planta hospedeira podem ocorrer diretamente pela alimentação da seiva do floema e injeção de toxinas, e indiretamente, pela excreção de "honeydew" que favorece o crescimento do fungo *Capnodium* sp. que escurece a folha e causa o sintoma conhecido como "fumagina" e, principalmente, pela transmissão de vírus às plantas (Oliveira et al., 2001; Barbosa et al., 2002; Stansly e Natwick, 2010; Navas-Castillo et al., 2011). *B. tabaci* é vetor de mais de 300 espécies de vírus (Hanssen et al., 2010; Gilbertson et al., 2015), incluindo Begomovirus (família Geminiviridae), Crinivirus (família Closteroviridae), e Carlavirus (família Betaflexiviridae) no Brasil. Begomoviroses tem impedido significativamente o cultivo de feijão e tomate, particularmente em climas quentes e secos (De Faria et al., 2016; Souza et al., 2018).

B. tabaci é um complexo de espécies crípticas englobando grupos morfologicamente indistinguíveis, mas ecologicamente e geneticamente distintos (Xu et al., 2010; De Barro et al., 2011; Kanakala e Ghanim, 2015), composto por pelo menos 43 espécies biológicas crípticas (De Barro et al., 2011; Tay et al., 2017). Estas espécies diferem-se em características como variedade de plantas hospedeiras, capacidade de causar distúrbios de plantas, atração por inimigos naturais, expressão de resistência e a capacidade de transmissão do vírus da planta (Bedford et al., 1994; Brown et al., 1995; Perring, 2001; Horowitz et al., 2005). A *B. tabaci* Middle East-Asia Minor I - MEAM1 e a Mediterrâneo são as espécies mais invasivas e destrutivas do mundo (De Barro et al., 2011; Pan et al., 2012; Polston et al., 2014).

No Brasil, foram identificadas as espécies MEAM1 (biótipo B), Novo Mundo, Novo Mundo 2 (biótipo A) e mais recentemente a Mediterrâneo (biótipo Q) (Barbosa et al., 2015). No entanto, *B. tabaci* MEAM1 é uma praga economicamente significativa no Brasil,

e infesta culturas incluindo soja, algodão, feijão, melão, tomate e mais recentemente milho (Inoue-Nagata et al., 2016; Quintela et al., 2016). Após a introdução desta espécie, no início da década de 1990, severos surtos populacionais ocorreram em diversos cultivos (Lourenção e Nagai, 1994). A propagação e o aumento da população de *B. tabaci* MEAM1 no Brasil têm sido favorecidos pelo sistema agrícola (com três safras anuais), o grande número de plantas hospedeiras e o clima tropical (Quintela et al., 2016).

O manejo de altas infestações da mosca-branca é muito difícil, e o controle resume-se principalmente a aplicação de inseticidas químicos (Palumbo et al., 2001; Liang et al., 2012; Zheng et al., 2017). Porém, o excesso de aplicações tem resultado na seleção de indivíduos resistentes a diversos grupos químicos, incluindo os organofosforados, carbamatos, piretroides, ciclodienos, reguladores de crescimento e neonicotinoides (Elbert e Nauem, 2000; Ahmad et al., 2002; Silva et al., 2009; Yuan et al., 2012; Basit et al., 2013; Horowitz e Ishaaya, 2014; Cardoso, 2014). Além disso, o número decrescente de novos inseticidas registrados, seus efeitos colaterais prejudiciais a organismos não-alvo, as restrições legais quanto ao seu uso seguro e seus riscos ambientais encorajaram a adoção de técnicas de controle adicionais, incluindo o controle biológico (Mascarin et al., 2013).

Os fungos entomopatogênicos são importantes componentes no controle biológico de insetos-praga, e estão entre os mais importantes inimigos naturais das moscas brancas (Lacey et al., 1996; Faria and Wraight, 2007). Diferentemente da maioria dos patógenos que precisam ser ingeridos, os fungos entomopatogênicos penetram diretamente no hospedeiro através da cutícula, o que é vantagem para o manejo da mosca-branca e outros insetos sugadores (Faria e Wraight, 2001; Mascarin et al. 2013, 2015). Os principais fungos entomopatogênicos de *B. tabaci* incluem *Cordyceps* spp., *Lecanicillium* spp., *Beauveria bassiana* (Balsamo) Vuillemin and *Aschersonia* spp. (Ascomycota: Hypocreales) (Faria e Wraight, 2001; Wraight et al., 2007; Lacey et al., 2008; Mascarin et al., 2013). No entanto, apesar da alta suscetibilidade das ninfas de mosca-branca, *B. bassiana* nunca foi isolado de *Bemisia* spp. em condições naturais (Wraight et al. 1998).

Cordyceps javanica tem sido considerado um candidato promissor para o controle biológico de moscas-brancas devido sua importância como regulador natural de populações de *B. tabaci*, alta virulência, facilidade de produção em meios sólidos ou líquidos e capacidade de causar epizootias naturais (Jackson et al., 1997; Lozano-Contreras et al., 2007; Cabanillas e Jones, 2009; Zhu e Kim, 2011; Mascarin et al., 2013, 2018). Epizootias de *C. javanica* foram observadas sobre ninfas e adultos de mosca-branca no feijoeiro, soja,

tomate e goiabeira no Distrito Federal e Goiás na safra 2012/2013 (Quintela et al., 2016). Dez destes isolados coletados foram testados sobre ninfas de *B. tabaci* em condições de laboratório e casa telada na Embrapa Arroz e Feijão. Com base nos critérios de virulência, produção de conídios e tolerância à radiação UV-B, três isolados de *C. javanica,* BRM 27666, BRM 27714 e BRM 27715, foram selecionados para estudos posteriores (Mascarin et al., 2018; Quintela, E. D. dados não publicados).

No primeiro capítulo, determinamos a suscetibilidade de todos os estágios ninfais de *B. tabaci* aos três isolados de *C. javanica*, selecionados anteriormente, observando o efeito das diferentes condições climáticas na virulência de *C. javanica* em casa telada. O potencial dos entomopatógenos no controle da mosca-branca tem sido focado em todos os estágios da vida (Cabanillas e Jones, 2009a), pois o estágio de desenvolvimento dos insetos é um dos fatores mais importantes, pois influenciam a patogenicidade e virulência dos isolados (Qiu et al., 2013). Moscas-brancas de todos os ínstares são infectadas por *C. fumosorosea* (Tian et al., 2015). No entanto, as ninfas mais jovens são consideradas mais suscetíveis que ninfas mais velhas, ovos e adultos (Wraight et al., 2000; Cabanillas e Jones 2009; Zhang et al., 2018). Baixa mortalidade foi observada em os ovos tratados com *C. fumosorosea* (Lacey et al., 1999; Gindin et al., 2000; Mascarin et al., 2013).

Temperatura e umidade relativa têm sido reconhecidos como importantes fatores que afetam a capacidade de fungos entomopatogênicos em infectar seus hospedeiros (Pu e Li, 1996; Arthurs e Thomas, 2001; Bouamama et al., 2010; Tian et al., 2014). Uma vez na cutícula do inseto, os conídios geralmente invadem o corpo do hospedeiro dentro de 24 h. Durante o processo inicial de infecção os fungos são suscetíveis a muitos fatores ambientais, mas dentro do corpo do hospedeiro, continuam sendo afetados pela temperatura e, indiretamente, pela umidade (Jaronski, 2010). Embora exista uma exigência de alta umidade para a germinação de conídios, os insetos podem ser infectados com umidade relativamente baixa (Lazzarini et al., 2006; Samish et al., 2014). Por exemplo, Wraight et al. (2000) observaram que *C. fumosorosea* infectou ninfas de mosca-branca de terceiro instar em folhas de hibisco a 25-30% de umidade. A temperatura ótima para infecção, crescimento do micélio e esporulação dos entomopatógenos geralmente variam entre 23-28°C, mas a maioria deles tem uma ampla gama de tolerância à temperatura (Goettel et al., 2000; Lacey et al., 2001; Jaronski et al., 2010). *Cordyceps* sp., por exemplo, é capaz de crescer em temperaturas relativamente altas, o que sugere que ele é naturalmente tolerante (Cabanillas e Jones, 2009b).

No segundo capítulo, avaliamos a eficiência e persistência de *C. javanica* formulado e não formulado e a associação com diferentes inseticidas químicos no controle de mosca-branca em condições de campo. Além disso, a persistência dos conídios em folhas de soja também foi avaliada. Embora haja muitos estudos sobre o controle de *B. tabaci* com fungos entomopatogênicos em laboratório e casa telada (Wraight et al., 1998; Cabanillas e Jones, 2009a; Zhu e Kim, 2011; Mascarin et al., 2013, 2018; Tian et al., 2015; Zhang et al., 2017), poucos estudos são desenvolvidos a nível de campo (Akey and Hennerberry, 1996; Ruiz-Vega and Aquino-Bolaños, 1999; Wraight et al., 1996, 2000; Ruiz and Medina, 2001; Azevedo et al., 2005). Além disso, não há estudos recentes que avaliam o potencial de *Cordyceps* no controle de ninfas de mosca-branca em condições de campo. Os entomopatógenos, ao serem aplicados no campo, estão sujeitos a uma série de fatores bióticos e abióticos que podem ter influência na sua sobrevivência, propagação e infecção no hospedeiro. Fatores como umidade, temperatura, precipitação e radiação solar são limitantes para a eficiência dos entomopatógenos no campo (McCoy et al. 2002; Franco, 2005; Fernandes et al., 2015). No entanto, a radiação solar, particularmente os componentes UV-A e UV-B, é um dos principais fatores de mortalidade dos conídios na superfície foliar e é largamente responsável pela baixa persistência do fungo (Zimmermann, 2007; Jaronski, 2010). A persistência dos conídios é importante porque, no mínimo, um patógeno precisa persistir no ambiente por tempo suficiente para infectar o hospedeiro alvo (Shapiro-Ilan et al., 2012).

A formulação na qual os conídios são aplicados é de fundamental importância para o sucesso do biocontrole, pois mantém a viabilidade, virulência e efetividade dos patógenos em condições de campo (Camargo et al., 2016). Produtos formulados, contendo materiais como óleos, umectantes, protetores UV e nutrientes para estimular a germinação e o crescimento, têm grande potencial para fornecer resultados melhores e mais consistentes (Burges, 1998; Wraight et al., 2001). Além disso, a associação de fungos entomopatogênicos com inseticidas químicos pode aumentar a mortalidade de pragas, pois atuam como "estressores" e aumentam a eficiência dos entomopatógenos, diminuindo o impacto ambiental de inseticidas sintéticos e reduzindo a pressão de seleção para populações resistentes (Quintela et. al. 2013, Santos et. al. 2017). Efeito sinérgico foi observado na mortalidade de ninfas de mosca-branca tratadas com *C. fumosorosea* combinado com os inseticidas spirotetramat, imidacloprid e thiamethoxam (Zou et al., 2014).

Um inseto pode adquirir conídios diretamente através da pulverização ou indiretamente pelo contato com uma superfície contaminada por fungos (Jaronski, 2010). No entanto, ninfas de mosca-branca são sésseis em superfícies abaxiais das folhas, o que dificulta a aplicação dos conídios com pulverizadores convencionais (Faria e Wraight, 2001; Mascarin et al., 2016). Para superar esse obstáculo, Wraight et al. (2000) utilizou um pulverizador eletrostático que forneceu conídios a ninfas em culturas de cucurbitáceas nos EUA através de aplicações em intervalos de 4 a 5 dias. Em nosso estudo, uma barra de pulverização do tipo "Dropleg" que pulveriza as folhas debaixo para cima foi utilizada para maximizar a eficiência devido a aplicação direta sobre o alvo.

Diante disso, o objetivo geral do trabalho foi avaliar a eficiência de *Cordyceps javanica* no controle de mosca-branca em condições de casa telada e campo. Nossos objetivos específicos foram: (1) Determinar a suscetibilidade dos estádios ninfais de *B. tabaci* a três isolados de *C. javanica*, previamente selecionados, em casa telada; (2) Determinar o efeito das variações climáticas (temperatura e umidade) na virulência dos isolados em casa telada; (3) Avaliar a eficiência de *C. javanica* não formulado e nas formulações WP e WG no controle de *B. tabaci* em condições de campo; (4) Avaliar a persistência dos conídios de *C. javanica* formulado e não formulado em folhas de soja; (5) Avaliar a eficiência da associação de *C. javanica* não formulado a inseticidas químicos no controle de *B. tabaci* em condições de campo.

CAPÍTULO 1

SUSCETIBILIDADE DE TODOS OS ESTÁGIOS NINFAIS DE *Bemisia tabaci* MEAM1 (HEMIPTERA: ALEYRODIDAE) A TRÊS ISOLADOS BRASILEIROS DE *Cordyceps javanica* (Hypocreales: Cordycipitaceae) EM CASA TELADA SOB TEMPERATURA E UMIDADE VARIÁVEIS[1]

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2 Susceptibility of all nymphal stages of *Bemisia tabaci* Biotype B (Hemiptera: Aleyrodidae) to three Brazilian isolates of *Cordyceps* sp. (Hypocreales: Cordycipitaceae) in a screenhouse under variable temperature and moisture conditions

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Abstract

The susceptibility of 1st to 4th instars of *Bemisia tabaci* (Hemiptera: Aleyrodidae) to three isolates of *Cordyceps* sp. (Hypocreales: Cordycipitaceae) was evaluated in screenhouse experiments under variable temperatures and moisture conditions. No differences in susceptibility to the *Cordyceps* sp. isolates were observed among 1st, 2nd and 3rd instar nymphs with respect to median lethal time (LT_{50}) values. Confirmed mortalities ranged from 63.7-87.8% when the isolates were tested at 5×10^7 conidia mL^{-1}. The 4th instar was the least susceptible to the fungal isolates (≤36.6% mortality). However, 60.0 to 99.5% of the

adults that emerged from 4th instar nymphs previously treated with the fungus succumbed to the infection. Temperature was more detrimental to *Cordyceps. sp.* virulence towards *B. tabaci* nymphs than relative humidity (RH). At similar RH, median LT_{50} for 1st instar (9.4 days) was higher than for 3rd instar (5.3 days) when the fungus was tested at 5×10^7 conidia mL^{-1}; minimal temperatures of ≥12.6 °C compared to ≥17.0 °C were registered for experiments with 1st and 3rd instars, respectively. However, temperatures ≥35 °C for 4 to 6 hours daily did not affect the efficacy of the fungus against nymphs. *Cordyceps* sp. showed high virulence to all life stages of *B. tabaci* at relatively low RH, and an ability to grow extensively over the leaf surface and to produce high amounts of conidia on infected hosts. These attributes certainly boost its potential as an important pest control component of *B. tabaci* Biotype B, especially for management of populations resistant to synthetic insecticides.

Keywords: Entomopathogenic fungus, Silverleaf whitefly, Relative humidity, *Phaseolus vulgaris*, Biological control

2.1 Introduction

The whitefly *Bemisia tabaci* Biotype B (Gennadius, 1989) (Hemiptera: Aleyrodidae) is one of the most devastating tropical and sub-tropical agricultural pests, and it affects many agricultural crops, including vegetables and ornamentals (Stansly & Naranjo 2010). Damage to the host plant may be caused directly by feeding on phloem sap, and indirectly by the large amounts of sticky honeydew that promote the growth of saprophytic fungi that, in turn, causes cosmetic injury and impairs photosynthesis (Stansly & Natwick 2010). In addition, *B. tabaci* is a vector of more than 300 species of virus (Navas-Castillo *et al* 2011, Gilbertson

et al 2015). In some crops the resulting viral diseases are growth-limiting factors that may cause total crop loss (Lapidot *et al* 2014).

In Brazil, the propagation in the population of the *B. tabaci* biotype B has been favored by the agricultural system (up to three growing seasons per year), by the presence of a large diversity of differing host plants and the tropical climate (Quintela *et al* 2016). The control of this insect is primarily achieved with synthetic insecticides that have already resulted in the selection of resistant populations in many regions of the world (Horowitz & Ishaaya 2014, Basit 2019).

Entomopathogenic fungi provide alternatives for the sustainable management of whiteflies, mainly because they are less harmful to the environment and can be included in strategies for the management of insecticide-resistant populations (Lacey *et al* 1996, 2008, Cuthbertson *et al* 2012). Among several groups of biocontrol agents for whiteflies and other sap-sucking insects, entomopathogenic fungi possess the unique ability to infect their host directly through the integument and have proven to be fundamental in Integrated Pest Management (IPM) strategies because they can cause frequent epizootics (Faria & Wraight 2001, Lacey *et al* 1996, 2008, Quintela *et al* 2016).

The complex of *Cordyceps* species formerly classified in *Isaria*, (Kepler *et al* 2017), including *C. fumorosea* and *Cordyceps. sp.* (Hypocreales: Cordycipitaceae), are the most prevalent fungi attacking whiteflies in the field worldwide (Faria & Wraight 2001, Lacey *et al* 2008). Massive epizootics were observed as the predominant factor controlling populations of *Bemisia* sp. (Lacey *et al* 1996, Quintela *et al* 2016). Several isolates of *Cordyceps* spp. have been tested against *B. tabaci*, and most of the studies for fungal screening were conducted in laboratory conditions (Vidal *et al* 1997, Wraight *et al* 1998, James *et al* 2003, Scorsetti *et al* 2008, Cabanillas & Jones 2009, Huang *et al* 2010, Mascarin *et al* 2013, 2014, 2018, Tian *et al* 2016). These studies have invariably been conducted under

constant temperature and high humidity conditions in containers with minimal or no ventilation (Wraight *et al* 2000). In these favorable conditions, similar or small differences in rankings for median lethal concentrations or median lethal times were observed, thereby making it difficult to select the best isolates for field use (Wraight *et al* 1998).

For this study, three isolates of *Cordyceps* sp., BRM 27666, BRM 27714 and BRM 27715, collected from nymphs and adults during epizootic conditions (Quintela *et al* 2016), were selected based on the criteria of virulence to 2nd instar nymphs, conidial production and tolerance to UV-B radiation in laboratory (Mascarin *et al* 2018) and screenhouse conditions (Santos *et al* 2017, Quintela *et al* unpublished data).

A comprehensive review by Jaronski (2010) showed that several abiotic factors, including temperature and humidity, affect the efficacy of entomopathogenic fungi in foliar applications. Thus, the research with the three selected *Cordyceps* isolates reported here was conducted in screenhouse conditions under variable temperature and moisture conditions with the principal objectives: (1) to identify the most promising isolate for further development; (2) to determine the susceptibility of all nymphal stages of *B. tabaci* to these fungal isolates; (3) to determine the effect of fluctuating temperatures and relative humidities in a screenhouse during rainy and dry seasons on the virulence of these isolates to nymphs.

2.2 Materials and methods

Our screenhouse studies were conducted at the Brazilian Agricultural Research Corporation (Embrapa Rice and Beans) located at Santo Antônio de Goiás, Goiás state (Central Brazil) (16°30’24,57”S, 49°17’06,53”W) and were the result of a collaboration between Embrapa and Lallemand (Patos de Minas, Minas Gerais, Brazil), under a Collaborative Research and Development Agreement.

2.2.1 Insect colony

The whitefly *B. tabaci* used in all experiments was identified as Biotype B by molecular gene sequence markers from mtDNA cytochrome oxidase I (mtCOI) (Quintela *et al* 2016). The whiteflies used were originated from a colony reared on bean plants (*Phaseolus vulgaris* L., cv. Pérola) maintained under screenhouse conditions at Embrapa Rice and Beans Research Station in Santo Antônio de Goiás, GO, Brazil (16°28′00″S, 49°17′00″W; 823 m a.s.l).

2.2.2 Fungal strains and preparations

The *Cordyceps* sp. isolates BRM 27666 (=CG 1282), BRM 27714 (=CG 1283) and BRM 27715 (=CG 1284) were obtained from infected *B. tabaci* nymphs and adults collected from soybean in Porangatu-GO, and from common bean plants in Planaltina-GO and Santo Antônio de Goiás-GO (Central Brazil), respectively. All isolates were preserved in liquid nitrogen and deposited at the Invertebrate Fungal Collection at Embrapa Genetic Resources and Biotechnology, Brasília-DF, Brazil. Although these isolates were previously identified as *Cordyceps javanica* by Mascarin *et al* (2018) based on sequencing of the β-tubulin gene, identification of species within this genus is currently based on multigenic phylogeny (Mongkolsamrit *et al* 2018), and therefore they are treated as *Cordyceps* sp. in this study. Conidia were grown on potato-dextrose-agar (PDA) for 7-10 days and immediately suspended in 10 mL of sterile aqueous solution of 0.01% (v/v) Tween 80 into 50-mL plastic centrifuge tubes. The suspension was vigorously agitated on a vortex mixer for 1 min and filtered through two layers of 30-μm pore-sized nylon cheesecloth. The filtered suspension (10 ml) was vortexed again for 1 min before application, and conidial concentrations were enumerated by haemocytometer (Brightline Improved Neubauer, New Optik®, Brazil) at 400× magnification. Conidial germination for all isolates exceeded 98% on PDA after 18 h

120 at 26 °C. Only conidia with germ tubes greater than conidial diameter were considered
121 germinated.

122 ### 2.2.3 Virulence of *Cordyceps* sp. isolates to 1st, 2nd and 3rd instar nymphs

123 Five experiments were conducted to compare the virulence of the isolates BRM 27666, BRM
124 27714 and BRM 27715 to 1st, 2nd and 3rd instar nymphs of *Bemisia tabaci*. Information about
125 tested concentrations, estimated mean number of conidia per mm^2 and per nymph,
126 experimental periods, assessment days, mean temperatures and relative humidities are
127 described in Table 1. The experiments were conducted during the fall/winter (dry) and
128 spring/summer (rainy) seasons to determine the effect of different climatic conditions
129 (temperatures and air humidities) on the virulence of these isolates to nymphs.

130 For all experiments, experimental units were represented by two 10-day-old bean plants
131 (*Phaseolus vulgaris*, cv. Pérola) grown in plastic pots filled with Oxisol soil (2 L) and kept
132 in a screenhouse (9 × 8 m) covered with a fine screen fabric (50 mesh). Adult-infested plants
133 were shaken and placed near of pest-free bean plants (10 days old with two primary leaves)
134 for six to eight hours to allow oviposition. This procedure provided more than 100 eggs per
135 leaf. The adult whiteflies were removed, and newly infested plants moved to another
136 screenhouse until nymphs reached the 1st instar (0.24–0.32 mm in length and 0.12–0.24 mm
137 in width), 2nd instar (0.30–0.44 mm in length and 0.18–0.36 mm in width), 3rd instar (0.40–
138 0.60 mm in length and 0.24–0.40 mm in width) and 4th instar (0.60–0.94 mm in length and
139 0.34–0.74 mm in width), respectively (Quintela 2004). In all experiments, treatments were
140 applied to the abaxial side of primary leaves containing nymphs with a microsprayer (0.3
141 mm needle, Paasche® airbrush type H-set) connected to a vacuum pump and calibrated to
142 250 µl per leaf in an even coverage. Controls consisted of nymphs sprayed with a surfactant
143 solution (0.01% Tween 80, Vetec Química Fina Ltda., Rio de Janeiro, RJ, Brazil).

For each date, a different leaf of each pot was collected for nymphal mortality assessment under a dissecting stereomicroscope (Leica) at 40× magnification. For the fungal infection confirmation, a small red dot was marked with permanent marker pen on the leaf next to the dead nymphs. Nymphs that became desiccated or developed yellowish symptoms with mycelial or conidial growth on the insect cadaver were considered dead by *Cordyceps* sp.. The leaves were incubated inside Petri dishes (90 mm) with a wet cotton added to the leaf petiole for four days in a growth chamber of the BOD (Biochemical Oxygen Demand) at 26 °C, 80-90% RH with 12-hr photoperiod. The dead marked nymphs presenting sporulation (i.e., mycosed insects) were also considered infected by the fungus.

In the experiment carried out with 1st instar, established at the fall season, we observed low nymphal mortality five days after spraying. Temperatures in the screenhouse at the beginning of this experiment were lower than 15 °C (12.6 °C at day 1). To determine if the low nymphal mortality was due to the lower temperatures, leaves were collected from treated and untreated plants and held at 26 °C, 80-90% RH with 12-hr photoperiod. Nymphal mortality was then determined at days 9, 10, 11, 12, 16 and 28 after spraying.

All experiments were conducted in a *completely randomized design* with four replicates, each consisting of two seedlings per pot (i.e., four primary leaves). The temperature and relative humidity in the screenhouse were monitored at one hour interval by two dataloggers (Hobo® U12-012, Onset Computer Corp. Ltd., Massachusetts). One datalogger was placed in the center of the screenhouse and the other near the screenhouse entrance. Small variations were observed for the datalogger measurements.

2.2.4 Virulence to 4th instar nymphs and to emerged adults

Bioassays were conducted from June 21 to August 3, 2018, to compare the virulence of *Cordyceps* isolates to 4th instar nymphs and to the adults that emerged from treated nymphs. The fungus at 5×10^8 conidia mL^{-1} was applied to the abaxial side of primary leaves of bean

169 plants containing 4th instar nymphs with a microsprayer. Controls consisted of nymphs
170 sprayed with a surfactant solution (0.01% Tween 80). The bean plants were held in a
171 screenhouse for 24 h in a completely randomized design with four repetitions. Each
172 repetition consisted of two seedlings per pot (i.e., four primary leaves). To determine if the
173 fungal infections of adults were due the infection of 4th instar or if the adults got
174 contaminated by the conidia present on the leaves, 24 h after spraying, three leaves from
175 each repetition (four repetitions/treatment; 12 leaves per treatment) were collected from
176 treated and untreated bean plants. Then, three procedures were used: 1) The leaf was held
177 alone in Gerbox-type box (11 x 11 x 3.5 cm) allowing the contact of emerging adults with
178 the treated leaf. In this procedure the objective was to determine if the emerged adults could
179 be infected by contact with conidia on the leaf; 2) addition of an untreated leaf inside the
180 gerbox box containing the treated leaf. In this case, the objective was to verify adult
181 mortalities when the emerged adults contacted both treated and untreated leaves; 3) transfer
182 of fungus-treated 4th instars to untreated leaves before adult emergence. The objective was
183 to evaluate only the adult mortalities, infected during the nymphal stage. In order to do so,
184 twenty five nymphs with "red-eye" (known as the pupal stage) from each leaf were removed
185 and transferred to an untreated leaf. To avoid damaging the nymphs, they were carefully
186 removed with a very fine entomological pin when the 4th instar stopped feeding (near adult
187 emergence). The leaves were held inside a plastic Gerbox-type box (11 x 11 x 3.5 cm) with
188 a wet cotton ball at 26 °C, 90% RH and 12-hr photoperiod. After seven days, living and dead
189 nymphs and adults were evaluated under a dissecting stereomicroscope (Leica) at 40×
190 magnification. Nymphs that became desiccated or developed yellowish symptoms with
191 mycelial or conidial growth on the insect cadaver were considered dead by *Cordyceps* sp.
192 Adults were considered infected by the fungus when mycelial or conidial growth was
193 observed on the insect cadaver.

2.2.5 Statistic analyses

The virulence of all three fungal isolates was expressed and compared in terms of percent mortality, confirmed mortality (% insect cadavers with fungal sporulation), and mean lethal time (LT_{50}) for the different nymphal lifestages.

For the experiments with 1st, 2nd and 3rd instar nymphs, overall and confirmed mortality curves were adjusted according to non-linear models and compared using the Wilcoxon-Mann-Whitney test ($P<0.05$). This non-parametric statistical method was used for comparison of two unpaired groups to verify whether or not they belong to the same population and when the requirements for application of Student's t test were not met. To estimate the median lethal time (LT_{50}) for the isolates for 1st, 2nd and 3rd instars non-linear models (Log-logistic, Logistic or Weibull) were fitted and values compared by the overlap of their 95% confidence intervals (95% CI) using the Package 'drc' (Ritz *et al* 2015). LT_{50} values were not estimated for treatments where mortality did not reach 50%.

For the experiment with 4th instar nymphs a factor analysis was performed through an analysis of variance by the F test to determine the main effects of the procedures regarding adult infection, treatments and their interactions on overall and confirmed mortalities of nymphs and adults that emerged from the treated nymphs. The model was considered additive because the factorial analysis for the interaction treatment/procedure was not significant (slopes were parallel) (e.g., mortality = effect of treatment + effect of procedure). The means were compared by the Tukey test ($P<0.05$). Statistical software R version 3.1.2 (R Core Team 2016) was used for all analyses (R Core Team 2016).

2.3 Results

For all five experiments, the percentage of cadavers with fungal sporulation (i.e., confirmed mortality) was very similar to nymphal mortalities. Thus, all figures for nymphal mortality

are reported as supplementary files. P-values of the comparisons of mortality and confirmed mortality curves and estimates of parameters of non-linear models and median lethal time (LT_{50}) for *B. tabaci* nymphs after treatment with *Cordyceps* sp. at different concentrations are also included as supplementary files on Tables 1, 2, 3, 4 and 5.

2.3.1 Virulence of *Cordyceps* sp. isolates to1st to 3rd instar nymphs

2.3.1.1 Rainy season

During the rainy season, the temperatures and relative humidities registered at one hour intervals at screenhouse were very similar for the experiments conducted with 1st, 2nd and 3rd instars (Fig. 1, Table 1). No infected 1st instar nymphs were observed on the controls (Fig. 2 A to C). There was no difference for 1st instar confirmed mortalities among the three isolates at 5×10^5, 5×10^6 and 5×10^7 conidia mL^{-1} (Fig. 2 A to C; Table 2 supplementary file). LT_{50} values were similar for all isolates (Table 3 supplementary file). Estimated LT_{50} values showed that at 5×10^6 and 5×10^7 conidia mL^{-1} mortality of nymphs took place between 7.1–8.2 days and between 5.1–5.6 days, respectively (Table 3 supplementary file). For 2nd instar nymphs, confirmed mortalities for BRM 27666, BRM 27715 and BRM 27714 at 5×10^5 conidia mL^{-1} were very low (0.3 to 9.0%), and they were statistically similar to the control, with no recorded mortality (Fig 3 A; Table 2 supplementary file). However, at 5×10^6 and 5×10^7 conidia mL^{-1} the three isolates were significantly different from the untreated control (Fig. 3 B, C; Table 2 supplementary file). Confirmed mortality for BRM 27666 at 5×10^6 conidia mL^{-1} was significantly higher than for BRM 27714 (Fig. 3 B). At the other concentrations, there were no differences among the tested isolates (Fig. 3 A, C; Table 2 supplementary file). Since nymphal mortality by the fungus at 5×10^5 and at 5×10^6 conidia mL^{-1} were below 50%, the median time to death was not estimated. At 5×10^7 conidia mL^{-}

[1], the LT_{50} ranged from 4.1 to 4.7 days, and no differences were observed among the isolates (Table 3 supplementary file).

Because 2nd instar nymphal mortalities by *Cordyceps* at 5×10^5 and at 5×10^6 conidia mL^{-1} were inferior to 50%, concentrations $\geq 1 \times 10^7$ conidia mL^{-1} were tested against 3rd instar nymphs. The three isolates at all concentrations caused confirmed mortalities of nymphs that were statistically different from the controls (Fig. 4 A to D; Table 2 supplementary file). No differences were observed among the three isolates (Fig. 4 A to D; Table 2 supplementary file). At 7 days after treatment, confirmed mortalities ranged from 59.4-71.6%, 63.7-80.8%, 74.4-85.4% and 69.2-84.3% for concentrations of 1×10^7, 5×10^7, 1×10^8 and 5×10^8 conidia mL^{-1}, respectively (Fig. 4 A, B, C, D). The median lethal times (LT_{50}) ranged from 3.5 to 4.7 days for all isolates, and no differences were observed among them (Table 3 supplementary file).

2.3.1.2 Dry season

For the experiments conducted during the dry season, the mean temperature was 24.9 °C (ranged from 12.6 to 41.8 °C), and the mean relative humidity was 56.3% (ranging from 17.4 to 85%) (Fig. 1, Table 1). The experiment with first instar nymphs in the fall season (lower temperatures and drier conditions) resulted in lower rates of confirmed mortalities than during the rainy season (Fig. 2 A to C). Although the mean temperature (25.1 °C) for 1st instar in the dry season was similar to that for the rainy season (25.7 °C), higher variation in temperature (from 12.6 to 41.8 °C) was observed in the dry season (Table 1). In fact, the minimum temperatures were below 17 °C most of the time, and within the 72 hours post-inoculation the minimum temperature was <13 °C (Fig. 1). The mean relative humidity was also lower (56.5%; range 17.4-85.0%) in the dry season than in the rainy season (77.8%; range 43.4-94.7%) (Fig. 1, Table 1). Due to the lower mortality at the beginning of the experiment, the evaluations of overall and confirmed mortalities were conducted until adult

emergence (28 days after fungal application). In fact, confirmed mortalities began at seven days with the highest fungal concentration (5×10^7 conidia mL^{-1}) (Fig. 2 F). For all fungal isolates and concentrations, mortalities were significantly different from the controls. There were no differences among the isolates regarding percentage of infected nymphs (Fig. 2 D to F; Table 2 supplementary file).

Estimated LT_{50} values showed that a dose of 5×10^6 conidia mL^{-1} killed 1st instar nymphs between 15.7-18.4 days while at 5×10^7 conidia mL^{-1} death occurred between 8.9-9.9 days. There were no significant differences for LT_{50} among the isolates (Table 3 supplementary file). Because of the lower temperature in the screenhouse during the experiment with 1st instar nymphs in the dry season, the LT_{50} values were significantly higher (15.7-18.4 days) than those in the rainy season (7.1-8.2 days) at the concentration 5×10^6 conidia mL^{-1}.

Due to the slow rate of mortality for the experiment in the dry season, leaves were collected from treated and untreated plants at day five after treatment and incubated in the BOD at 26 °C, 80-90% RH with 12-hr photoperiod. In these constant temperature and moisture conditions, that are suitable for the fungus, mortalities were higher when compared with the experiment conducted in the screenhouse (Fig. 2 D to F). In the BOD, confirmed mortalities ranged from 25.4-65.8%, 74.8-87.0% and 94.7-100% at the concentrations 5×10^5, 5×10^6 and 5×10^7 conidia mL^{-1}, respectively, ten days after treatments (Fig. 2 G to I). Mortalities were significantly different for all fungal isolates when compared to the untreated control (<2.6%) (Fig. 2 G to I, Table 2 supplementary file). At 5×10^5 conidia mL^{-1}, no differences for mortality percentages were observed among the isolates (Fig. 2 G, Table 2 supplementary file). Higher mortalities were observed for BRM 27666 than for BRM 27715 and BRM 27714 at 5×10^6 conidia mL^{-1} (Fig. 2 H; Table 2 supplementary file). At this concentration, BRM 27715 was significantly less virulent than BRM 27666 and BRM 27714. At 5×10^7

conidia mL^{-1}, the mortality percentage reached 100% for all isolates at 10 days after treatment (Fig. 2 I).

When the experiment with 3rd instar nymphs was repeated in the dry season, a mild winter season was observed with a mean temperature of 24.6 °C (range 14.6–42.3 °C), similar to that in experiment conducted during the summer season (26.2 °C; range 19.5–42.3 °C). However, mean relative humidity for this experiment was lower (56.8%; range 22.6–79.6) than the 72.0% (range 28.9-90.9%) seen during the experiment run in the rainy season (Fig. 1, Table 1). In the first 72 hours, favorable conditions were observed for the infection of nymphs by the fungus, since temperatures were in the range of 16.5-38.2 °C, and relative humidities were 28.2-77.8%.

Mortality percentages were significantly different for the three isolates at all concentrations when compared with the controls (Fig. 4 E to H, Table 2 supplementary file). No differences for confirmed mortality were observed among the isolates at the four concentrations tested. Confirmed mortality for the isolates ranged from 30.9-54.4%, 71.9-83.5%, 83.6-88.6% and 79.5-87.9% seven days after fungal sprays at 1×10^7, 5×10^7, 1×10^8 and 5×10^8 conidia mL^{-1}, respectively (Fig. 4 E to H).

LT_{50} values were similar for the isolates at all concentrations tested and ranged from 4.7 to 8.3 days (Table 3 supplementary file). Although intervals for the LT_{50} values (3.5 to 4.7 days) were shorter for the experiment conducted during the summer season than for the experiments conducted during the winter season (4.7 to 8.3 days), no significant differences were observed among them (Table 3 supplementary file).

2.3.2 Virulence to 4th instar nymphs and to emerged adults

The fourth instar was the least susceptible to the fungal isolates (≤36.6% mortality), even when tested at 5×10^8 conidia mL^{-1} (≈16.2×10^3 conidia per mm^2) (Table 2). All dead nymphs

were infected by the fungus (Table 2). Although 4th instar mortality by the fungus was low, the adults that emerged from fungus-treated nymphs were highly susceptible to all three isolates of *Cordyceps* sp. (Table 4 supplementary file). Overall and confirmed mortalities of adults ranged from 66.6 to 100.0% and 60.0 to 99.5.0%, respectively, and the values were significantly different from the control groups (Table 2).When the three procedures were tested to determine if the fungal infection of adults was due the infection of 4th instar nymphs (removal of nymphs from the treated leaves before adult emergence) or if adults became infected by conidia present on the leaves (adults contacted treated and untreated leaves after emergence), the factorial analysis showed that the interaction of fungal isolates x procedures was not significant (Table 4 supplementary file). In other words, the tested procedures had no effect on nymphal and adult mortality by the isolates. However, nymphal and adult mortalities and confirmed mortalities were significantly affected by the treatments. These results showed that the infection of the adults by the fungus probably occurred before moulting (during the nymphal stage).

2.3.3 Symptoms and signs of fungal infection on whitefly nymphs

Signs of initial infection on 1st to 3rd instar nymphs by *Cordyceps* sp. occurred about three to four days after inoculation. *Cordyceps* sp. isolates were able to promote elevated levels of mortality (Fig. 5 D to H). The initial external growth of *Cordyceps* sp. on some nymphs arose primarily from the pleural region (Fig. 5 A, B). Most dead nymphs became dessicated and developed a yellowish appearance due to mycelial growth on the insect cadaver (Fig. 5 C). A characteristic white circle of dense spoulation was also observed around colonized cadavers. Colonization of the substrate surrounding colonized nymphs, either on the leaf substrate or on Petri dish, was generally very extensive (Fig. 5 D). If maintained under continuous high humidity conditions, the sporulating growth would cover the cadaver,

forming structures resembling tiny cotton balls (Fig. 5 E, F). The ability to produce a large quantity of conidia and rapidly to colonize several millimeters of the surrounding substrate were particularly distinct for isolate BRM 27666. Extensive external hyphal development by this isolate covered the leaf surface and produced conidia that could infect either nymphs or adults that emerged from infected nymphs (Fig. 5 G, H).

2.4 Discussion

Several isolates of *Cordyceps* spp. have been tested against *B. tabaci*, and most of the studies for isolate screenings were conducted in laboratory conditions (Vidal *et al* 1997, Wraight *et al* 1998, James *et al* 2003, Scorsetti *et al* 2008, Cabanillas & Jones 2009, Huang *et al* 2010, Mascarin *et al* 2013, 2014, 2018, Tian *et al* 2016). These studies have invariably been conducted under constant temperature and high humidity conditions in containers with minimal or no ventilation (Wraight *et al* 2000). In these favorable conditions, similar or small differences in rankings for median lethal concentrations or median lethal times were observed, thus making it difficult to select the best isolates for field use (Wraight *et al* 1998). In addition, the selected isolate would not be necessarily the most adapted under variable climatic conditions. For example, Mascarin *et al* (2013, 2018) selected the isolate BRM 14526 (= CG 1228), based on multi-stress resistance, mass production and virulence attributes in the laboratory. However, when three experimental assays were conducted in a screenhouse, results showed that this isolate was the least virulent in a group of ten isolates that included those used in our experiment (BRM 27666, BRM 27714 and BRM 27715) (Santos *et al* 2017, Quintela *et al* unpublished data).

The three fungal isolates of *Cordyceps* sp. used in the current study were all highly infectious to 1st to 3rd instars of *B. tabaci* and caused high levels of mortality in screenhouse conditions. The similar virulence of these fungal isolates was expected since they were selected as the

most virulent from among ten isolates in previous screenhouse studies (Santos *et al* 2017, Quintela *et al* unpublished data). However, postmortem hyphal growth and sporulation of BRM 27666 were more visible than with the other two isolates. In fact, BRM 27666 hyphae rapidly covered the dead hosts, extended to several millimeters surrounding infected nymphs, and produced large quantities of conidia (Fig. 5 G).

No differences in susceptibility to the *Cordyceps* sp. isolates were observed among 1st, 2nd and 3rd instar nymphs in terms of LT_{50} values, and mortalities ranged from 63.7-87.8% when the fungus was tested at 5×10^7 conidia mL^{-1} ($\approx 16.2 \times 10^2$ conidia per mm^2) during the rainy season. In addition, the three first instars were more susceptible to the fungus than were 4th instar nymphs (≤15.5% mortality), even when the fungus was tested at 5×10^8 conidia mL^{-1}. Several other studies also showed that 4th instar nymphs of *B. tabaci* were less susceptible to fungal infections than younger instars (Osborne *et al* 1990, James *et al* 2003, Cabanillas & Jones 2009, Zhang *et al* 2018). Although 4th instar mortality by the fungus was low, the adults that emerged from these treated nymphs were highly susceptible to the three isolates of *Cordyceps* sp. (overall and confirmed mortalities of adults ranged from 66.6 to 100.0% and 60.0 to 99.5% for BRM 27666, BRM 27715 and BRM 27714, respectively). The results also showed that the infection of the adults by the fungus likely occurred before moulting (during the nymphal stage). Adults start to emerge from treated nymphs 3-4 days after fungal application, time sufficient for fungal germination and penetration on 4th instar. Accordingly, scanning electron microscopy studies by Tian *et al* (2015) revealed that conidia of *C. fumosorosea* germinated and produced appressoria within 24 h, and hyphae penetrated the cuticle and entered into the host within 48 h. Besides, Cabanillas & Jones (2009) demonstrated that mortality of the nymphs did not occur in the developmental stage to which the fungus was applied, instead development proceed, and the insects die after one or more moults. Emergence of infected adults from treated 4th instar its an important factor related

to *Cordyceps* sp. epizootics, showing its capacity to disperse on its own in the environment and infect subsequent hosts (Shapiro-Ilan *et al*. 2012).

Fungal efficacy is intrinsically mediated by abiotic factors, most noticeably humidity, temperature, rainfall and solar radiation (McCoy *et al* 2002, Jaronski 2010, Fernandes *et al* 2015). On humidity, an increasing number of studies indicated that sufficient moisture exists within the microhabitat of many insect hosts or within the microenvironment of the host's body surface to support infection independent of ambient moisture conditions (Ferron 1977, Riba & Marcandier 1984, Ramoska 1984, Marcandier & Khachatourians 1987, Fargues *et al* 1997, Boulard 2002). A study by Wraight *et al* (2000) also showed that a moisture-saturated environment was not required for *C. fumosorosea* infection of *B. tabaci* nymphs on excised hibiscus leaves, and that it could infect third-instar nymphs incubated at 25%–30% RH. Our data also indicated an ability of *Cordyceps* sp. to infect whitefly nymphs in relatively low moisture conditions. In our screenhouse studies, RH >70% was observed for 4-5 h daily in the experiments conducted in the dry season and, in the rainy season, RH >80% was registered for 10-12 h daily (Fig.1). In addition, the mean minimum daily RH for dry season experiments was 28.3% (range 22.6–35.2%) and 42.2% (range 34.4–54.7%) for rainy season experiments.

Low temperature seemed to be more detrimental for *Cordyceps* virulence to *Bemisia* nymphs than RH. The mean time to kill the nymphs by *Cordyceps* at 5×10^7 conidia mL^{-1} was higher for 1st instar (9.4 days) than 3rd instar (5.3 days) although both experiments were conducted at similar RH [means of 56.5% (range 17.4–85.4%) and 56.8% (range 22.6–79.7%), respectively]. The main difference between the two experiments was related to temperature. In the beginning of the experiments (48 h), minimal temperatures were ≥12.6 °C compared with temperatures ≥17.0 °C for experiment with 1st and 3rd instar, respectively. In addition, when leaves were transferred to constant temperature (26 °C), 1st instar mortalities by

Cordyceps sp. resumed quickly at all concentrations. The results of our study corroborated previous findings that temperatures below 16 °C slow germination and growth rates for most of the fungal entomopathogens (Vidal *et al* 1997, Ihara *et al* 2008) and thus affect their virulence. For winter crops, when lower temperatures are expected in the field, this fungus will still kill whitefly nymphs although in a slower rate.

The *Cordyceps* spp. are known to be mesophilic since they grow over a range of 8 to 30–32 °C, with thermal optima ranging from 20 to 30 °C, and limits at 35 °C and their thermal tolerance were related to their history, including the geo-climatic origin (Fargues *et al* 1992, Mietkiewski *et al* 1994, Vidal *et al* 1997, Fargues & Bon 2004). In the present study, we observed that temperatures ≥35 °C for 4 to 6 hours daily [mean maximum daily temperature for dry season experiments was 37.3% (range 31.5–40.8%)] did not affect the efficacy of the three isolates of *Cordyceps* sp. against nymphs. Then, the tolerance of these isolates to high temperature, low virulence at lower temperatures, and also the ability to infected nymphs in relatively low moisture conditions are probably due to their geographical origin. These isolates were collected from Goiás state of Brazil, a tropical climate with two distinct seasons, five months of drought (May to September) and seven months of rain (October to April), and average annual temperature of 23ºC, that can reach up to 39°C in September and October (INMET, 2020).

In Brazil, "rainy season" soybeans are sown from October through December over an area of 35 million hectares (IBGE 2020), and can support large whitefly populations. Then, adults of *B. tabaci* that emerge from several wild plants that can host the whitefly in the off-season (May to September) will colonize soybean and other cultivated plants grown at the beginning of rainy season (Quintela *et al* 2016). Sucessive plantings of soybeans and other suitable crops (cotton, common bean, tomatoes etc) provide continuous food resources for multiple whitefly generations. In addition, with soybean senescence, adults migrate to late-planted

soybean cultivars and other crops cultivated after the soybean harvest. Along with the drought period in some years (January-February), huge whitefly 'clouds' are usually seen during these months. According to the results of our screenhouse experiments, it can be assumed that *Cordyceps* sp. will be an important component to reduce populations of 1st to 4th instar of *B. tabaci* biotype B in the field: at low densities (for the first whitefly infected crops, November through December) and also at high densities and overlapping generations (January through March) in field conditions in hot, humid and relatively dry climate areas. Further, this fungus can cause significant adult mortality (60.0 to 99.5%) when sprayed on 4th instar, and epizootics could be favored by dissemination of the fungus by the infected adults.

The ability of *Cordyceps* sp., particularly BRM27666, to grow extensively over the leaf surface and to produce a large number of conidia under humid conditions are attributes that certainly boost its capacity to spread rapidly through whitefly populations. The sporulation of entomopathogenic fungi on a host is an important characteristic because conidia serve as a source of pathogen inoculum for horizontal transmission, ecosystem dissemination and infection cycle development (Lacey *et al* 2008, Cabanillas & Jones 2009, Hesketh *et al* 2010, Jaronski 2010, Borisade & Magan 2014, Shapiro-Ilan *et al* 2012).

The use of a mycoinsectide is an important pest control component, especially for management of populations resistant to synthetic insecticides. Besides its high virulence to all life stages reported in here, *Cordyceps* sp. causes massive epizootics and is easy to mass-produce (Lacey *et al* 1996, Mascarin *et al* 2013, 2015, Quintela *et al* 2016).

Acknowledgments

The authors thank to Edmar Cardoso de Moura (in memoriam) and Edson Djalma for technical support. We also thank Ana Lúcia Delilabera Faria for assistance with the cited

references. This work was possible through financial support from Lallemand grant nº. 0978009 and Embrapa grant nº. 021107005. To the National Council for Scientific and Technological Development (CNPq) for support with the scholarship for the first author nº. 132678/2017-9.

Author contributions

HB and EQ designed the studies. HB and ES performed the bioassays. HB and JFS analyzed the data. HB, EQ and RH wrote the manuscript. All authors reviewed and approved the manuscript before submission.

Conflict of Interest

The authors declare that they have no conflict of interest. Although the project was partially financed by Lallemand grant nº. 0978009 and a product containing aerial conidia of *Cordyceps sp.* BRM27666 is under development by this company through a partnership with Embrapa, all research and subsequent steps until publication were conducted independently by the latter.

2.5 References

Basit M (2019) Status of insecticide resistance in *Bemisia tabaci*: resistance, cross resistance, stability of resistance, genetics and fitness costs. Phytoparasitica 47:207-225. https://doi.org/10.1007/s12600-019-00722-5

Borisade OA, Magan N (2014) Growth and sporulation of entomopathogenic *Beauveria bassiana, Metarhizium anisopliae, Isaria farinosa* and *Isaria fumosorosea* strains in relation to water activity and temperature interactions. Biocontrol Sci Technol 24:999–1011. https://doi.org/10.1080/09583157.2014.909007

Boulard T, Mermier M, Fargues J, Smits N, Rougier M, Roy JC (2002) Tomato leaf boundary layer climate: implications for microbiological whitefly control in greenhouses. Agr Forest Meterol 110:159–176. https://doi.org/10.1016/S0168-1923(01)00292-1

Cabanillas E, Jones WA (2009) Pathogenicity of *Isaria* sp. (Hypocreales: Clavicipitaceae) against the sweet potato whitefly B biotype, *Bemisia tabaci* (Hemiptera: Aleyrodidae). Crop Prot 28:333–337. https://doi.org/10.1016/j.cropro.2008.11.015

Cuthbertson AGS, Buxton JH, Blackburn LF, Mathers JJ, Robinson KA, Powell ME, Fleming DA, Bell HA (2012) Eradicating *Bemisia tabaci* Q biotype on poinsettia plants in the UK. Crop Prot 42:42–48. https://doi.org/10.1016/j.cropro.2012.08.009

Faria M, Wraight SP (2001) Biological control of *Bemisia tabaci* with fungi. Crop Prot 20: 767–778. https://doi.org/10.1016/S0261-2194(01)00110-7

Fargues J, Maniania NK, Delmas JC, Smits N (1992) Influence de la température sur la croissance in vitro d'hyphomycétes entomopathogénes. Agronomie 12:557-564. https://doi.org/10.1051/agro:19920708

Fargues J, Goettel MS, Smits N, Ouedraogo A, Rougier M (1997) Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. Mycologia 89:383–392. https://doi.org/10.1080/00275514.1997.12026797

Fargues J, Bon MC (2004) Influence of temperature preferences of two *Paecilomyces fumosoroseus* lineages on their co-infection pattern. J Invertebr Pathol 87:94–104. https://doi.org/10.1016/j.jip.2004.07.001

Ferron P (1977) Influence of relative humidity on the development of fungal infection caused by *Beauveria bassiana* (Fungi Imperfecti, Moniliales) in imagines of *Acanthoscelides obtectus* (Col.: Bruchidae). Entomophaga 22, 393–396. https://doi.org/10.1007/BF02373264

Gilbertson RL, Batuman O, Webster CG, Adkins S (2015) Role of the insect Supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. Annu Rev Virol 2:67–93. https://doi.org/10.1146/annurev-virology-031413-085410

Hesketh H, Roy HE, Eilenberg J, Pell JK, Hails RS (2010) Challenges in modelling complexity of fungal entomopathogens in semi-natural populations of insects. BioControl 55:55–73. https://doi.org/10.1007/s10526-009-9249-2

Horowitz AR, Ishaaya I (2014) Dynamics of biotypes B and Q of the whitefly *Bemisia tabaci* and its impact on insecticide resistance. Pest Manag Sci 70:1568–1572. https://doi.org/10.1002/ps.3752

Huang Z, Sahar F, Ren S, Ali S (2010) Effect of *Isaria fumosorosea* on *Eretmocerus* sp. nr. *furuhaskii* (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pakistan J Zool 42:121–127

Ihara F, Toyama M, Mishiro K, Yaginuma K (2008) Laboratory studies on the infection of stink bugs with *Metarhizium anisopliae* strain FRM515. Appl Entomol Zool 43:503–509. https://doi.org/10.1303/aez.2008.503

Instituto Brasileiro de Geografia e Estatística (IBGE). Available in https://sidra.ibge.gov.br/home/lspa/brasil>. Accessed in june 29 2020.

Instituto Nacional de Meteorologia. Estação Meteorológica de observação de Superfície Automática (INMET). Available in

http://www.inmet.gov.br/portal/index.php?r=estacoes/estacoesAutomaticas. Accessed in August 1, 2020.

James RR, Buckner JS, Freeman TP (2003) Cuticular lipids and silverleaf whitefly stage affect conidial germination of *Beauveria bassiana* and *Paecilomyces fumosoroseus*. J Invertebr Pathol 84:67–74. https://doi.org/10.1016/j.jip.2003.08.006

Jaronski ST (2010) Ecological factors in the inundative use of fungal entomopathogens. BioControl 55:159–185. https://doi.org/10.1007/s10526-009-9248-3

Kepler RM, Luangsa-ard JJ, Hywel-Jones NL, Quandt CA, Sung GH, Rehner SA, Aime MC, Henkel TW, Sanjuan T, Zare R, Chen M, Li Z, Rossman AY, Spatafora JW, Shrestha B (2017) A phylogenetically-based nomenclature for *Cordycipitaceae* (Hypocreales). IMA Fungus 8:335–353. https://doi.org/10.5598/imafungus.2017.08.02.08

Lacey LA, Fransen JJ, Carruthers RI (1996) Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agentes. In: Gerling D, Mayer RT (ed) *Bemisia* 1995: Taxonomy, Biology, Damage, Control and Management. Intercept, Andover, UK, pp 356–456

Lacey LA, Wraight SP, Kirk AA (2008) Entomopathogenic fungi for control of *Bemisia tabaci* Biotype B: Foreign exploration, research and implementation. In: Gould J, Hoelmer K, Goolsby J (ed) Classical Biological Control of *Bemisia tabaci* in the United States - A review of interagency research and implementation. Springer, Netherlands, pp 33–69

Lapidot M, Legg JP, Wintermantel WM, Polston JE (2014) Management of whitefly-transmitted viruses in open-field production systems. In: Advances in Virus Research. Elsevier, pp 147–206

Marcandier S, Khachatourians GG (1987) Susceptibility of the migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae), to *Beauveria bassiana* (Bals.) Vuillemin (Hyphomycete): Influence of relative humidity. Can Entomol 119: 901–907. https://doi.org/10.4039/Ent119901-10

Mascarin GM, Kobori NN, Quintela ED, Delalibera Jr I (2013) The virulence of entomopathogenic fungi against *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) and their conidial production using solid substrate fermentation. Biol Control 66:209–218. https://doi.org/10.1016/j.biocontrol.2013.05.001

Mascarin GM, Kobori NN, Quintela ED, Arthurs SP, Delalibera Jr I (2014) Toxicity of non-ionic surfactants and interactions with fungal entomopathogens toward *Bemisia tabaci* biotype B. BioControl 59:111–123. https://doi.org/10.1007/s10526-013-9543-x

Mascarin GM, Jackson MA, Kobori NN, Behle RW, Delalibera Jr I (2015) Liquid culture fermentation for rapid production of desiccation tolerant blastospores of *Beauveria bassiana* and *Isaria fumosorosea* strains. J Invertebr Pathol 127:11–20. https://doi.org/10.1016/j.jip.2014.12.001

569 Mascarin GM, Pereira-Junior RA, Fernandes EKK, Quintela ED, Dunlap CA, Arthurs SP
570 (2018) Phenotype responses to abiotic stresses, asexual reproduction and virulence among
571 isolates of the entomopathogenic fungus *Cordyceps javanica* (Hypocreales:
572 Cordycipitaceae). Microbiol Res 216:12–22. https://doi.org/10.1016/j.micres.2018.08.002

573 McCoy C, Quintela ED, Faria M (2002) Environmental persistence of entomopathogenic
574 fungi. In: Baur, ME, Fuxa JR (ed) Factors Affecting the Survival of Entomopathogens.
575 Louisiana State University Agricultural Center, Southern Cooperative Series Bulletin

576 Mietkiewski R, Tkaczuk C, Zurek M, Geest LPS Van der (1994) Temperature requirement
577 of four entomopathogenic fungi. Acta Mycol 29:109-120.
578 https://doi.org/10.5586/am.1994.012

579 Mongkolsamrit S, Noisripoom W, Thanakitpipattana D, Wutikhun T, Spatafora JW,
580 Luangsa-ard J (2018) Disentangling cryptic species with isaria-like morphs in
581 Cordycipitaceae. Mycologia 110:230–257. https://doi.org/10.1080/00275514.2018.1446651

582 Navas-Castillo J, Fiallo-Olive E, Sanchez-Campos S (2011) Emerging virus diseases
583 transmitted by whiteflies. Annu Rev Phytopathol 49:219–248.
584 https://doi.org/10.1146/annurev-phyto-072910-095235

585 Osborne LS (1990) Biological control of whiteflies and other pests with a fungal pathogen.
586 IOBC WPRS Bull 13:153–160. https://doi.org/10.2307/3496127

587 Quintela ED (2004) Manejo integrado de insetos e outros invertebrados pragas do feijoeiro.
588 Inf Agropec 25:113–136

589 Quintela ED, Abreu AG, Lima, JFS, Mascarin GM, Santos JB, Brown JK (2016)
590 Reproduction of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) B biotype in maize
591 fields (*Zea mays* L.) in Brazil. Pest Manag Sci 72:2181–2187. http://doi.org/10.1002/ps.4259

592 Ramoska WA (1984) The influence of relative humidity on *Beauveria bassiana* infectivity
593 and replication in the chinch bug, *Blissus leucopterus*. J Invertebr Pathol 43:389–394.
594 https://doi.org/10.1016/0022-2011(84)90085-5

595 Riba G, Marcandier S (1984) Influence de l'humidité relative sur l'agressivite et la viabilité
596 des souches de *Beauveria bassiana* (Bals.) Vuillemin et de *Metarhizium anisopliae*
597 (Metsch.) Sorokin, hyphomycétes pathogenes de la pyrale du maı¨s, *Ostrinia nubilalis* Hubn.
598 Agronomie 4:189–194

599 R Core Team (2016) R: A language and environment for statistical computing. R Foundation
600 for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

601 Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-Response Analysis Using R. PLoS ONE,
602 10(12): e0146021. https://doi.org/10.1371/journal.pone.0146021

603 Santos TTM, Quintela ED, Mascarin GM, Santana, MV (2017) Enhanced mortality of
604 *Bemisia tabaci* nymphs by *Isaria javanica* combined with sublethal doses of chemical
605 insecticides. J Appl Entomol 142:598–609. https://doi.org/10.1111/jen.12504

606 Scorsetti AC, Humber RA, De Gregório C, Lastra CL (2008) New records of
607 entomopathogenic fungi infecting *Bemisia tabaci* and *Trialeurodes vaporariorum* pests of
608 horticultural crops in Argentina. BioControl 53:787–796. https://doi.org/10.1007/s10526-
609 007-9118-9

610 Shapiro-Ilan DI, Brucky DJ, Lacey LA (2012) Principles of Epizootiology and Microbial
611 Control. In: Vega FE, Harry KK (2ed) Insect Pathology. Elsevier, pp 29–72.
612 https://doi.org/10.1016/B978-0-12-384984-7.00003-8

613 Stansly PA, Naranjo SE (2010) Bemisia: Bionomics and Management of a Global Pest.
614 Springer, Amsterdam

615 Stansly PA, Natwick ET (2010) Integrated systems for managing *Bemisia tabaci* in protected
616 on open field agriculture. In: Stansly PA, Naranjo SE (ed) *Bemisia*: Bionomics and
617 Management of a Global Pest. Springer, Amsterdam, pp 467–489

618 Tian J, Diao H, Liang L, Hao C, Arthurs S, Ma S (2015) Pathogenicity of *Isaria fumosorosea*
619 to *Bemisia tabaci*, with some observations on the fungal infection process and host immune
620 response. J Invertebr Pathol 130:147–153. https://doi.org/10.1016/j.jip.2015.08.003

621 Tian J, Diao H, Liang L, Arthurs S, Hao C, Mascarin G, Ma R (2016) Host plants influence
622 susceptibility of whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) to the entomopathogenic
623 fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae). Biocontrol Sci Technol 26:528–
624 538. https://doi.org/10.1080/09583157.2015.1129393

625 Vidal C, Fargues J, Lacey LA (1997) Intraspecific variability of *Paecilomyces*
626 *fumosoroseus*: effect of temperature on vegetative growth. J Invertebr Pathol 70:18–26.
627 https://doi.org/10.1006/jipa.1997.4658

628 Wraight SP, Carruthers RI, Bradley CA, Jaronski ST, Lacey LA, Wood P, Galaini-Wraight
629 S (1998) Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria*
630 *bassiana* against the silverleaf whitefly, Bemisia argentifolii. J Invertebr Pathol 71:217–226.
631 https://doi.org/10.1006/jipa.1997.4734

632 Wraight SP, Carruthers RI, Jaronski ST, Bradley CA, Garza CJ, Galaini-Wraight S (2000)
633 Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces*
634 *fumosoroseus* for microbial control of the silverleaf whitefly, *Bemisia argentifolii*. Biol
635 Control 17:203–217. https://doi.org/10.1006/bcon.1999.0799

636 Zhang C, Shao ZF, Han YY, Wang XM, Wang ZQ, Musa PD, Ali S (2018) Effects of
637 *Aschersonia aleyrodis* on the life table and demographic parameters of *Bemisia tabaci*. J
638 Integr Agric 17:389–396. https://doi.org/10.1016/S2095-3119(17)61773-8

Table 1 Estimated mean number of conidia per leaf and per nymph, days of nymphal mortality assessment and environmental conditions for the experiments conducted at different dates with *Cordyceps* sp. for the control of 1st to 4th nymphs of *Bemisia tabaci* at screenhouse.

Nymphal instar (nymphal size, mm^2)	Concentration tested (conidia mL^{-1})	Mean number of conidia per mm^2	Mean number of conidia per $nymph^2$	Experiment dates	Assessment days	Enviromental conditions	
						Mean temperature ºC (min-max)	Mean UR (%) (min-max)
1st (0.0405)	5×10^5	16.7	0.68	Experiment 1 January 21-28, 2016 (summer)	3, 4, 5, 6, 7	25.7 (19.9 - 36.5)	77.8 (43.4 - 94.7)
	5×10^6	1.67×10^2	6.78				
	5×10^7	1.67×10^3	67.8	Experiment 2 (screenhouse) May 21 to June 13, 2018 (fall)	4, 5, 6, 7, 11, 23	25.1 (12.6 - 41.8)	56.5 (17.4 - 85.4)
2nd (0.077)	5×10^5	16.7	1.29	Experiment 3 November 27 to December 4, 2015 (spring)	3, 4, 5, 6, 7	27.2 (20.6 to 41.7)	72.3 (28.8 - 96.7)
	5×10^6	1.67×10^2	12.9				
	5×10^7	1.67×10^3	1.29×10^2				
3rd (0.1623)	1×10^7	3.35×10^2	54.32	Experiment 4 January 30 to February 6, 2018 (summer)	3, 4, 5, 6, 7	26.2 (19.5 - 42.3)	72.0 (28.9 - 90.9)
	5×10^7	1.67×10^3	2.72×10^2				
	1×10^8	3.35×10^3	5.43×10^2	Experiment 5 May 28 to June 7, 2018 (fall)	4, 7, 8, 9, 10	24.6 (14.6 – 40.8)	56.8 (22.6 – 79,6)
	5×10^8	1.67×10^4	2.72×10^3				
4th (0.4212)	5×10^8	1.67×10^4	7.0×10^3	Experiment 6 June 21 to August 3, 2018 (Screenhouse/BOD)	7	25.2 (20.0 – 37.2)	80.1 (43.2 – 94.1)

[a] The mean number of conidia per mm^2 was estimated according to the equation: volume of fungal spray x fungal concentration/area of primary bean leaf . For each fungal concentration, 0.25 mL^{-1} was sprayed on a foliar mean area of the primary bean leaf of 7460 mm^2 (n=20).

[b] The mean number of conidia per nymph was estimated according to the equation: mean number of conidia.mm^2 (calculated for each fungal concentration, column 3) x each nymphal size, column 1.

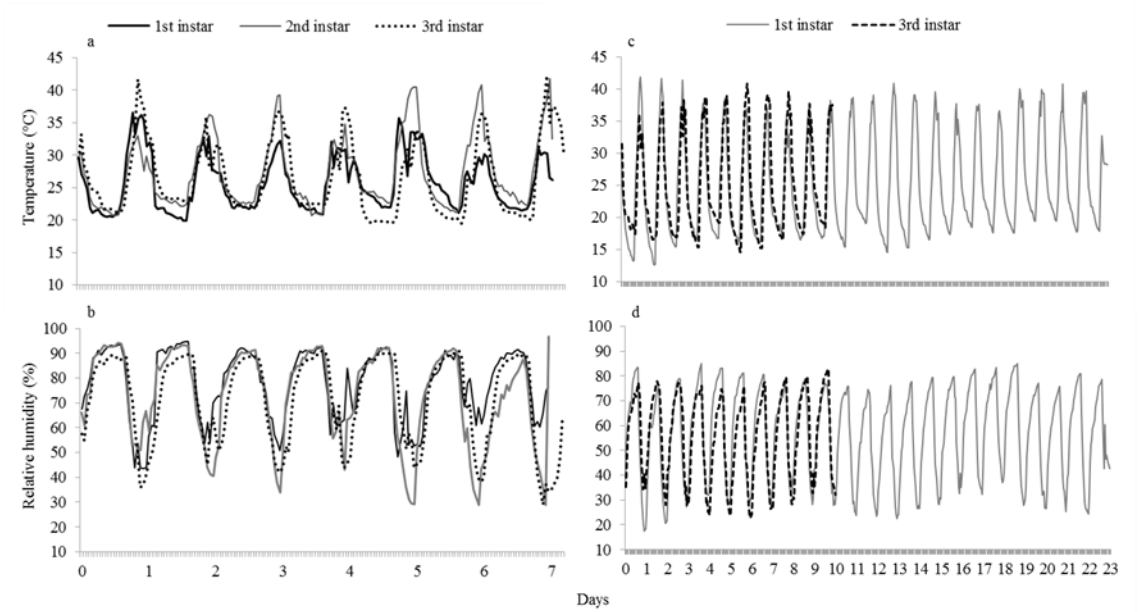
Table 2 Mean confirmed mortalities of 4th instar nymphs and overall mean mortality and confirmed mortalities of emerged adults of *Bemisia tabaci* seven days after treatment of 4th instar nymphs with three isolates of *Cordyceps* sp. at 5×10^8 conidia mL^{-1}

Procedure [a]	Control	BRM 27666	BRM 27714	BRM 27715
		Confirmed mortalities of nymphs[b, c] (%)		
1	0 ± 0 bB	8.7 ± 11.9 aAB	20.7 ± 13.5 aA	12.6 ± 15.2 abAB
2	0 ± 0 bB	11.9 ± 8.5 aA	16.1 ± 9.7 aA	3.9 ± 6.8 bB
3	6.4 ± 6.6 aC	15.6 ± 4.3 aBC	19.2 ± 9.3 aAB	36.6 ± 24.1 aA
		Mortality of adults (%)		
1	28.7 ± 18.3 aB	93.0 ± 4.7 aA	99.8 ± 4.4 aA	94.2 ± 23.3 aA
2	28.2 ± 11.9 aB	94.9 ± 3.9 aA	100.0 ± 1.1 aA	98.9 ± 16.7 aA
3	21.5 ± 6.3 aB	66.6 ± 9.4 aA	78.6 ± 15.0 aA	92.2 ± 13.9 aA
		Confirmed mortalities of adults (%)		
1	10.4 ± 15.4 aB	89.6 ± 17.7 aA	96.6 ± 4.8 abA	78.2 ± 34.7 aA
2	12.0 ± 6.9 aB	92.5 ± 14.2 aA	99.5 ± 1.1 aA	88.1 ± 18.6 aA
3	10.8 ± 14.3 aB	60.0 ± 46.4 aA	64.9 ± 27.1 bA	77.3 ± 20.8 aA

[a] Procedure 1) leaf was held alone in gerbox box allowing the contact of adults with the treated leaf after emergence; Procedure 2) addition of an untreated leaf inside the gerbox box containing the treated leaf; Procedure 3) transfer of fungal treated 4th instars to untreated leaves before adult emergence.

[b]Means followed by the same letter in the horizontal (treatments) and in vertical (procedures) are not significantly different according to the Tukey´s test at 0.05%.

[c]All nymphs that died were infected by the fungus.



655 **Fig 1** Temperature (°C) and relative humidity (%) recorded at screenhouse at one hour intervals for
656 experiments with 1st, 2nd an 3rd instar at rainy season (A, B) and for experiments with 1st and 3rd instar at dry
657 season (C, D).

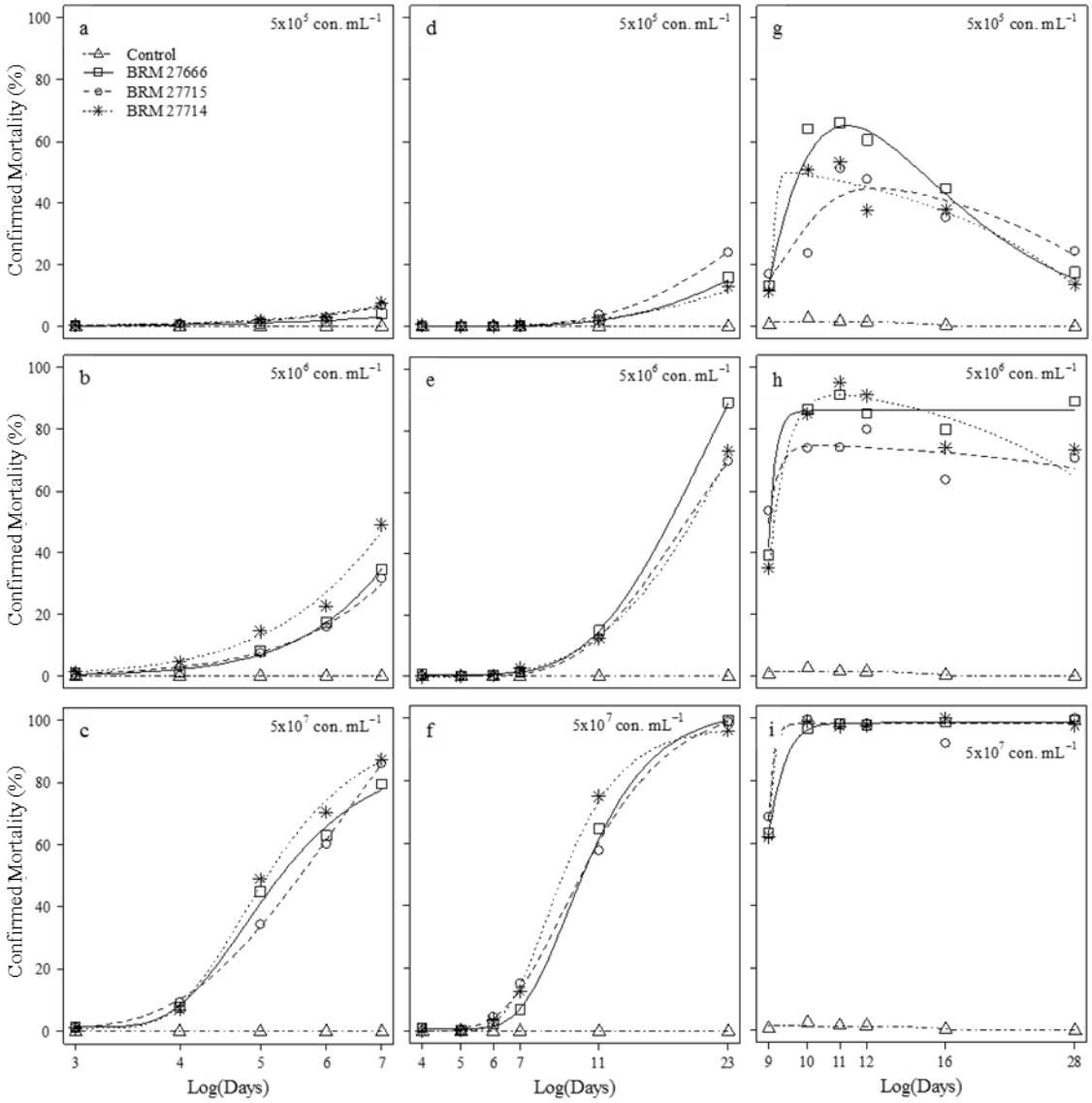


Fig 2 Confirmed mortalities at different days post-inoculation for 1st instar nymphs of *Bemisia tabaci* treated with three isolates of *Cordyceps sp.* at doses 5×10^5, 5×10^6 and 5×10^7 conidia mL^{-1} in experiments conducted in the rainy (a, b, c) and dry season (screenhouse - d, e, f and BOD - g, h, i). Curves were adjusted according to non-linear models Gompertz (a), Weibull (b, c, d, e, f), *Brain-Cousens* modified logistic (g, h) and Log-logistic. (i).

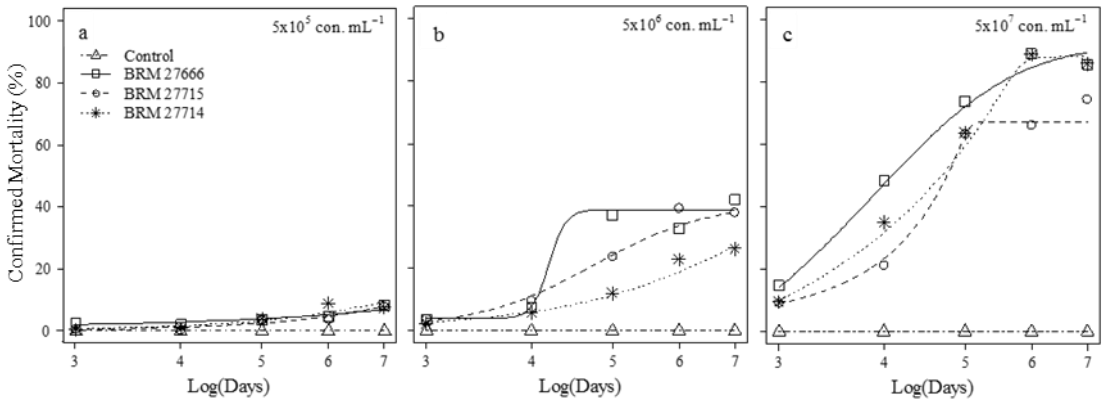


Fig 3 Confirmed mortalities at different days post-inoculation for 2nd instar of *Bemisia tabaci* treated with three isolates of *Cordyceps* sp. at doses 5×10^5, 5×10^6 and 5×10^7 conidia mL^{-1} in experiments conducted in the rainy season. Curves were adjusted according to non-linear models Gompertz (a), Log-logistic (b) and Logistic (c).

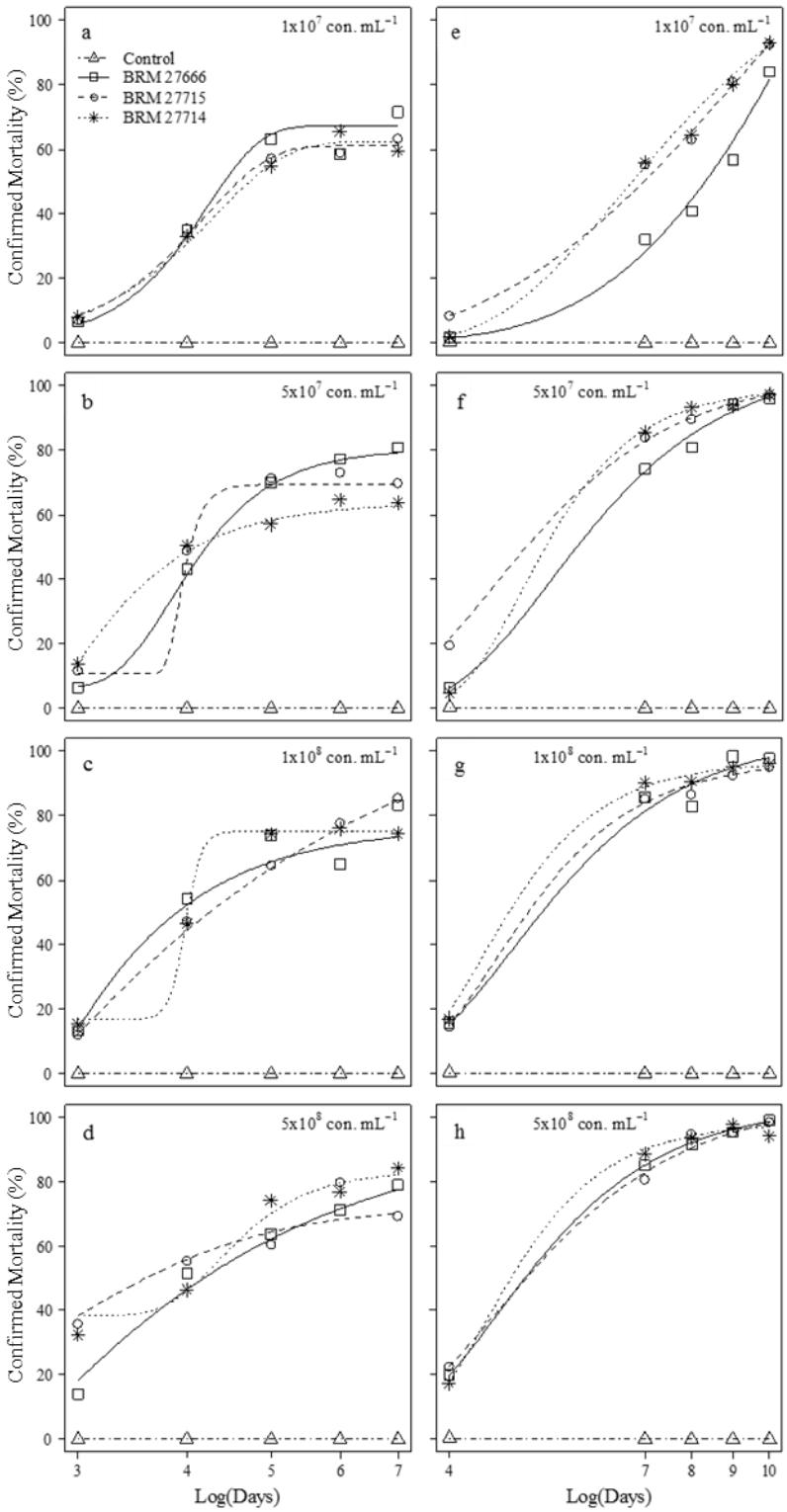


Fig 4 Confirmed mortalities at different days post-inoculation of 3rd instar of *Bemisia tabaci* by three isolates of *Cordyceps* sp. at 5×10^5, 5×10^6 and 5×10^7 conidia mL^{-1} in experiments conducted in the rainy (a, b, c, d) and dry season (e, f, g, h). Curves were adjusted according to non-linear Weibull (a, c, d, e, f, g, h) and Log-logistic models (b)

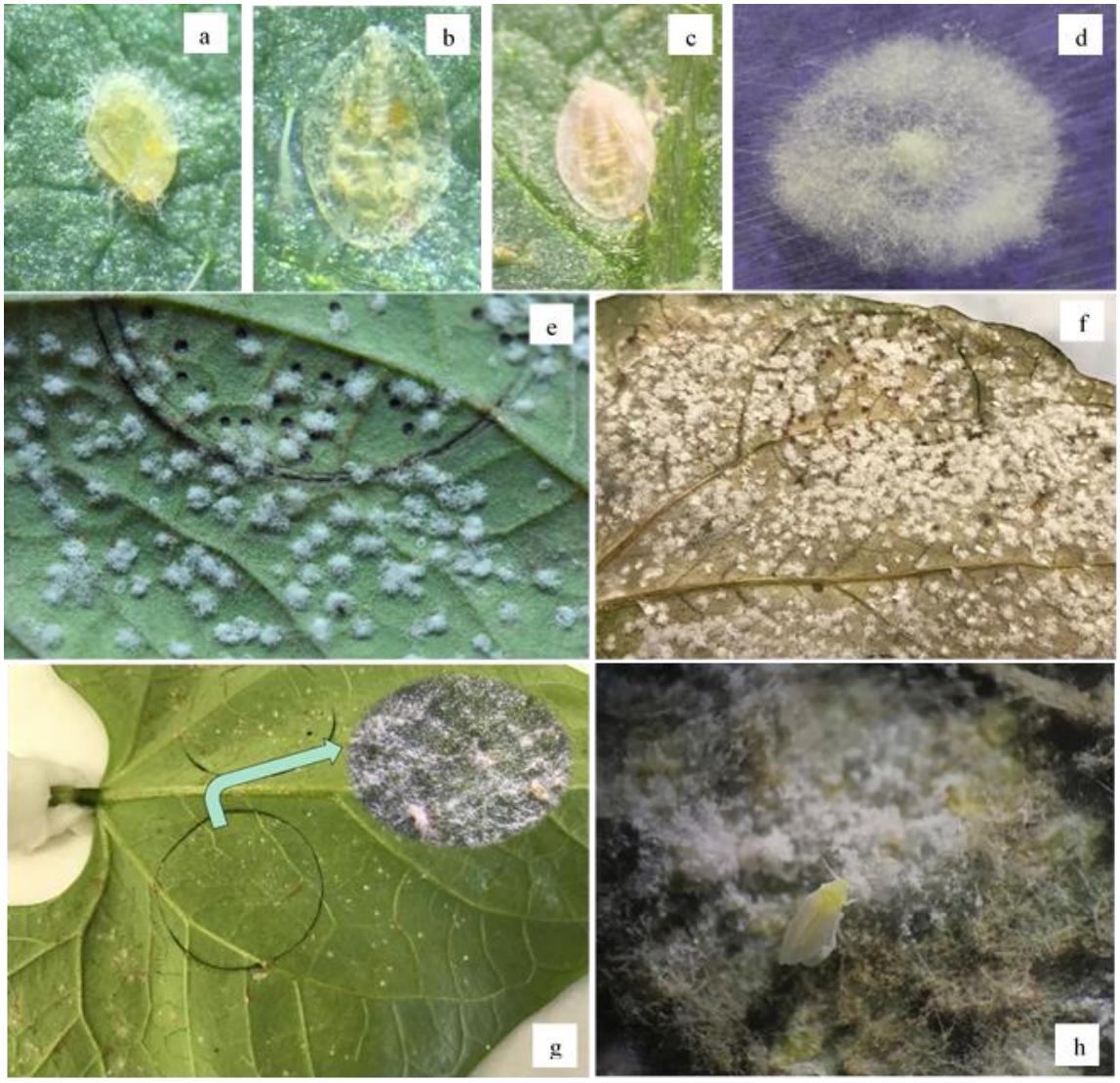


Fig 5 Pathogenicity of *Cordyceps* sp. against *Bemisia tabaci*. **(a, b)** Initial external hyphal growth of *Cordyceps* sp from the nymph pleural regions. **(c)** Dead nymph dessicated with yellowish symptoms. **(d, e)** Colonization of the substrate surrounding the nymph on Petri dish or on leaf substrate. **(e, f)** Nymphs covered with hyphae and conidia, resembling tiny cotton balls. A characteristic white circle of dense sporulation around nymphal cadavers. **(g)** Fungal growth on the leaf surface surrounding the nymph by the isolate BRM 27666. **(h)** External hyphal proliferation by BRM 27666 on the leaf surface reaching either nymphs or adults that emerged from treated nymphs.

Eletronic Supplementary Material

Table 1 P values ($P \leq$ value) of the comparisons of mortality curves for *Bemisia tabaci* nymphs after treatment with *Cordyceps* sp. at different concentrations. Wilcoxon-Mann-Whitney rank sum test was used for P values calculation. Curves were considered significant different at $P \leq 0.05$

NYMPHAL MORTALITY									
Rainy season									
1st instar									
Treatments	5×10^5 conidia mL^{-1}			5×10^6 conidia mL^{-1}			5×10^7 conidia mL^{-1}		
	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	0.2581	0.0531	0.0531	0.0141	0.0018	0.0001	0.0018	0.0001	0.0046
27666	.	0.3865	0.2581	.	0.9591	0.2786	.	0.9591	0.7984
27715	.	.	0.6665	.	.	0.3823	.	.	0.7209
2st instar									
	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	0.0625	0.7304	0.2891	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
27666	.	0.7304	0.5961	.	0.4385	0.1014	.	0.1615	0.6048
27715	.	.	1	.	.	0.1713	.	.	0.4363
3rd instar									
	1×10^7 conidia mL^{-1}			5×10^7 conidia mL^{-1}			1×10^8 conidia mL^{-1}			5×10^8 conidia mL^{-1}		
	27666	27715	27714	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
27666	.	0.4894	0.4894	.	0.5961	0.2973	.	0.9314	0.9314	.	0.8633	0.3401
27715	.	.	1	.	.	0.1859	.	.	1	.	.	0.4363
Dry season									
1st instar									
	5×10^5 conidia mL^{-1}			5×10^6 conidia mL^{-1}			5×10^7 conidia mL^{-1}		
	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	0.0326	0.0005	0.0007	<0.0001	0.0003	<0.0001	<0.0001	<0.0001	<0.0001
27666	.	0.0965	0.5291	.	0.4945	0.4291	.	0.7584	1
27715	.	.	0.2315	.	.	0.7788	.	.	0.7788
1st instar (BOD)									
	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0021	0.0021	0.0021
27666	.	0.6983	0.8347	.	<0.0001	0.0263	.	0.093	0.5887
27715	.	.	0.5053	.	.	0.0155	.	.	0.1797
3th instar									
	1×10^7 conidia mL^{-1}			5×10^7 conidia mL^{-1}			1×10^8 conidia mL^{-1}			5×10^8 conidia mL^{-1}		
	27666	27715	27714	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
27666	.	0.1534	0.1534	.	0.4184	0.3358	.	0.9197	0.6498	.	0.8403	0.801
27715	.	.	1	.	.	0.8403	.	.	0.3897	.	.	0.6139

Table 2 P values ($P\leq$ value) of the comparisons of mycosis curves for *Bemisia tabaci* nymphs after treatment with *Cordyceps* sp. at different concentrations. Wilcoxon-Mann-Whitney rank sum test was used for P values calculation. Curves were considered significant different at $P\leq0.05$

				Rainy season					
				1st instar					
Treatments	$5x10^5$ conidia mL^{-1}			$5x10^6$ conidia mL^{-1}			$5x10^7$ conidia mL^{-1}		
	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	0.4894	0.6048	0.4363	0.0281	0.0069	0.0006	0.0018	0.0001	0.0046
27666	.	0.2973	0.1903	.	0.9591	0.2786	.	0.9591	0.7984
27715	.	.	0.7304	.	.	0.3282	.	.	0.7209
				2st instar					
	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	0.0625	0.0625	0.0625	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	0.0001
27666	.	0.3401	0.6048	.	0.1164	0.0399	.	0.1615	0.7304
27715	.	.	0.7304	.	.	0.0879	.	.	0.4363
				3rd instar					
	$1x10^7$ conidia mL^{-1}			$5x10^7$ conidia mL^{-1}			$1x10^8$ conidia mL^{-1}			$5x10^8$ conidia mL^{-1}		
	27666	27715	27714	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
27666	.	0.2973	0.2973	.	0.1615	0.7304	.	0.8633	0.1359	.	1	0.3865
27715	.	.	0.8633	.	.	0.4363	.	.	1	.	.	0.4363
				Dry season					
				1st instar					
	$5x10^5$ conidia mL^{-1}			$5x10^6$ conidia mL^{-1}			$5x10^7$ conidia mL^{-1}		
	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	0.0187	0.0007	0.0029	0.0002	0.001	0.0001	<0.0001	<0.0001	<0.0001
27666	.	0.0894	0.8581	.	0.4777	0.5117	.	0.7994	0.9467
27715	.	.	0.0785	.	.	0.9467	.	.	0.841
				1st instar (BOD)					
	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
27666	.	0.6192	0.3977	.	<0.0001	0.0195	.	0.1513	0.0758
27715	.	.	0.195	.	.	0.0103	.	.	0.133
				3th instar					
	$1x10^7$ conidia mL^{-1}			$5x10^7$ conidia mL^{-1}			$1x10^8$ conidia mL^{-1}			$5x10^8$ conidia mL^{-1}		
	27666	27715	27714	27666	27715	27714	27666	27715	27714	27666	27715	27714
Control	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
27666	.	0.169	0.1389	.	0.4184	0.3897	.	0.9598	0.6836	.	0.7623	0.801
27715	.	.	1	.	.	0.8403	.	.	0.4743	.	.	0.5114

692 **Table 3** Estimates of parameters of non-linear models and median lethal time (LT50) of whitefly nymphs
693 treated with *Cordyceps* sp. isolates at different concentrations (conidia mL-1) in the rainy e dry seasons

Fungal isolate	No. insects tested	Model	Model parameters[a]					LT_{50} (d) (CI95%)
			B	C	d	e	f	
Rainy season								
			1st instar nymphs					
			5×10^6 conidia mL^{-1}					
BRM 27666	3219		4.86	-[b]	-	8.32	-	7.7 (7.0 – 8.4)
BRM 27715	2739	Weibull	4.13	-	-	8.98	-	8.2 (6.5 – 9.9)
BRM 27714	3413		4.23	-	-	7.80	-	7.1 (6.9 – 7.4)
			5×10^7 conidia mL^{-1}					
BRM 27666	3393		-5.28	0.02	0.88	4.77	-	5.3 (3.0 - 7.5)
BRM 27715	3181	Weibull	-2.61	0.01	1.67	6.01	-	5.6 (3.0 - 8.1)
BRM 27714	4399		-5.48	0.01	0.98	4.74	-	5.1 (3.0 - 7.2)
			2nd instar nymphs					
			5×10^7 conidia mL^{-1}					
BRM 27666	1434		–7.64	-0.68	0.94	1.63	0.16	4.1 (2.5 – 5.6)
BRM 27715	1313	Weibull	–108.4	0.01	0.68	1.62	0.04	4.7 (2.5 – 6.9)
BRM 27714	1568		–64.78	-0.26	0.88	1.76	0.03	4.6 (2.5 – 6.7)
			3rd instar nymphs					
			1×10^8 conidia mL^{-1}					
BRM 27666	2989		-2.15	-	0.68	4.05	-	4.5 (2.5 – 6.5)
BRM 27715	3909	Logistic	-2.17	-	0.62	3.87	-	4.5 (2.5 – 6.6)
BRM 27714	2860		-1.88	-	0.63	3.97	-	4.7 (2.2 – 7.1)
			5×10^7 conidia mL^{-1}					
BRM 27666	3085		-6.72	0.07	0.80	3.83	-	4.2 (2.5 – 5.9)
BRM 27715	3043	Weibull	-37.04	0.11	0.69	3.93	-	4.0 (2.4 – 5.6)
BRM 27714	3104		-5.00	-0.48	0.63	2.65	-	4.0 (1.9 – 6.0)
			1×10^8 conidia mL^{-1}					
BRM 27666	3300		-3.38	-1.78	0.85	0.80	-	3.9 (2.0 – 5.7)
BRM 27715	3427	Log-logistic	-1.58	-3.0	1.19	0.42	-	4.2 (2.5 – 6.0)
BRM 27714	2836		-17.76	0.16	0.75	1.37	-	4.0 (2.4 – 5.6)
			5×10^8 conidia mL^{-1}					
BRM 27666	4163		-1.44	-0.64	1.10	2.30	-	4.2 (2.5 – 5.8)
BRM 27715	2851	Weibull	-3.33	0.08	0.73	2.70	-	3.5 (2.0 – 5.0)
BRM 27714	3649		-7.15	0.39	0.84	4.34	-	4.1 (2.3 – 5.9)
Dry season								
			1st instar nymphs					
			5×10^6 conidia mL^{-1}					
BRM 27666	1503		-1.81	0.01	1.60	17.28	-	15.7 (9.9 - 21.6)
BRM 27715	1637	Weibull	-2.22	0.008	1.02	14.54	-	16.8 (9.9 -23.9)
BRM 27714	1447		-0.88	0.001	4.23	43.83	-	18.4 (9.3 - 27.6)
			5×10^7 conidia mL^{-1}					
BRM 27666	1537		-4.46	0.02	1.01	6.87	4.01	9.9 (5.4 - 14.5)
BRM 27715	1916	Log-logistic	-3.38	0.01	1.02	3.85	15.1	9.4 (5.3 - 13.5)
BRM 27714	1488		-4.48	0.003	0.97	5.38	6.75	8.9 (5.2 - 12.6)
			3rd instar nymphs					
			1×10^7 conidia mL^{-1}					
BRM 27666	1136		-1.21	-	5.80	17.38	-	8.3 (4.4 – 12.2)
BRM 27715	1251	Weibull	-1.35	-	2.48	9.87	-	7.0 (3.5 – 10.4)
BRM 27714	1377		-2.77	-	1.26	6.55	-	7.0 (3.6 – 10.3)
			5×10^7 conidia mL^{-1}					
BRM 27666	1225		-3.59	-	1.07	5.34	-	5.8 (3.3 – 8.2)
BRM 27715	1110	Weibull	-3.46	-	1.04	4.55	-	5.0 (3.2 – 6.8)
BRM 27714	1379		-5.60	-	0.99	4.92	-	5.26 (2.2 – 7.4)
			1×10^8 conidia mL^{-1}					
BRM 27666	1001		-3.58	-	1.05	4.80	-	5.2 (3.2 – 7.2)

BRM 27715	1000	Weibull	-4.56	-	0.97	4.58	-	5.0 (3.2 – 6.8)
BRM 27714	1476		-5.29	-	0.97	4.38	-	4.7 (3.2 – 6.3)
			5×10^8 conidia mL^{-1}					
BRM 27666	1480		-5.39	-	1.01	5.18	-	5.2 (3.2 – 7.1)
BRM 27715	1457	Log-logistic	-4.76	-	1.03	5.21	-	5.2 (3.2 – 7.1)
BRM 27714	1314		-7.02	-	0.98	4.96	-	5.0 (3.2 – 6.8)

[a] Model parameters: B = B is the slope factor around the "e" parameter; C = is the lowest asymrate of the curve; d = is the upper asymrate of the curve; e = is the inflection point of the curve; f = symmetry reference parameter related to hormesis.
[b]Parameter is not part of the model.

698 **Table 4** Summary of factorial analyses for nymphal mycosis, adult mortality and mycosis of *Bemisia tabaci*
699 after treatment with *Cordyceps* sp.

Factor	Nymphal mycosis			Mortality of adults			Mycosis adults		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Treatment	5.283	3, 44	<0.0001	45.003	3, 44	<0.0001	29.880	3, 44	<0.0001
Procedure	0.087	2, 44	0.9166	6.449	2, 44	0.0035	4.069	2, 44	0.0239
Treatment x Procedure	1.965	6, 44	0.09144	1.535	6, 44	0.1893	1.488	6, 44	0.2046

700

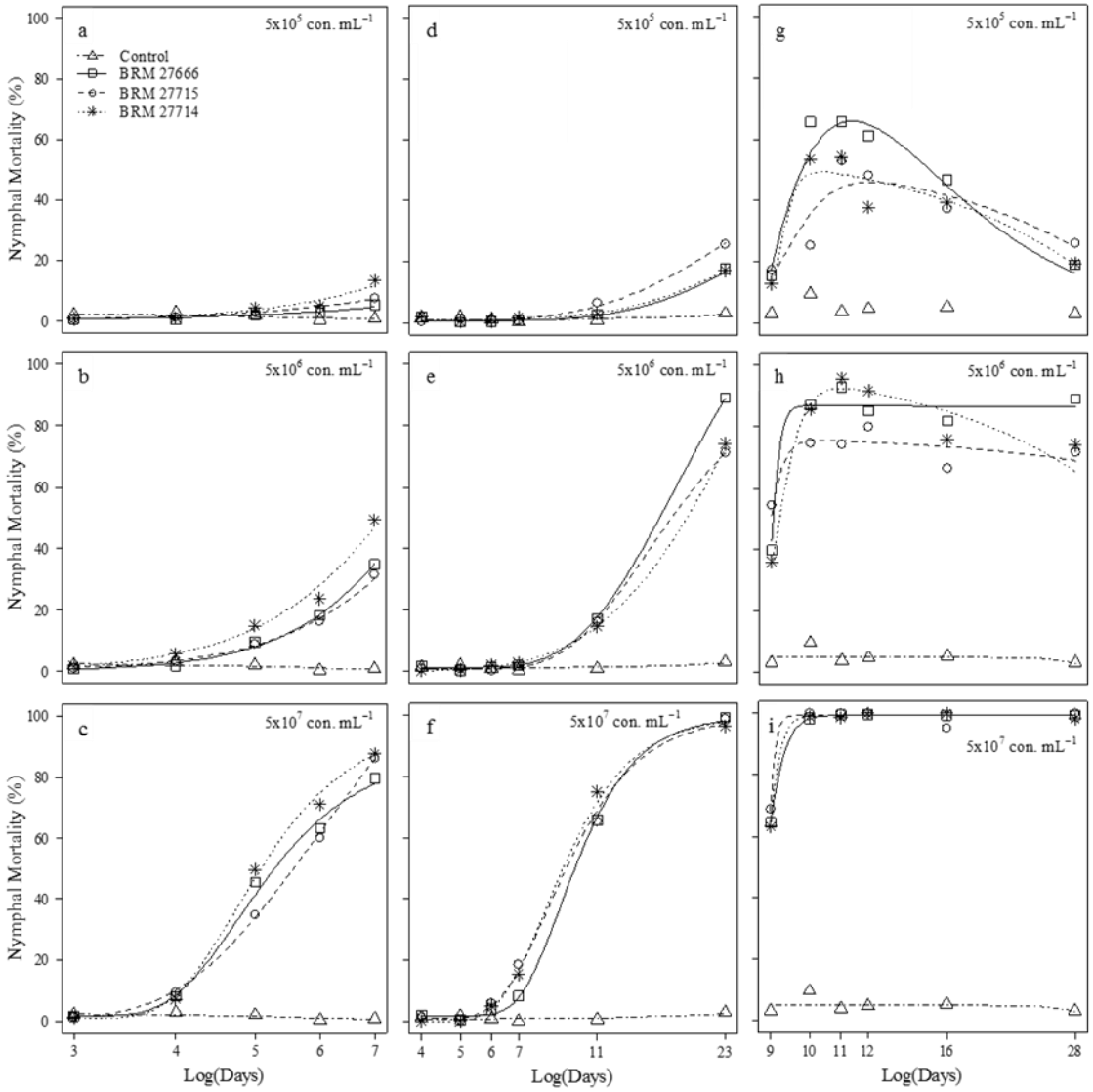
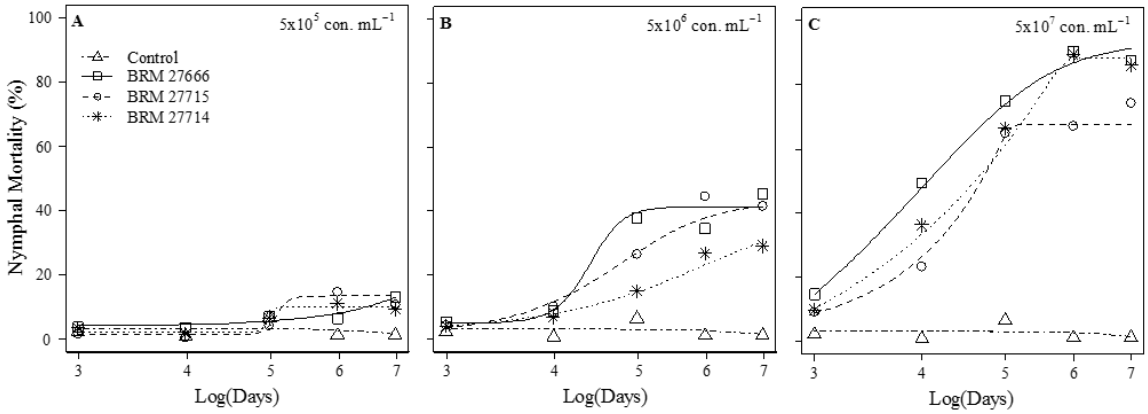
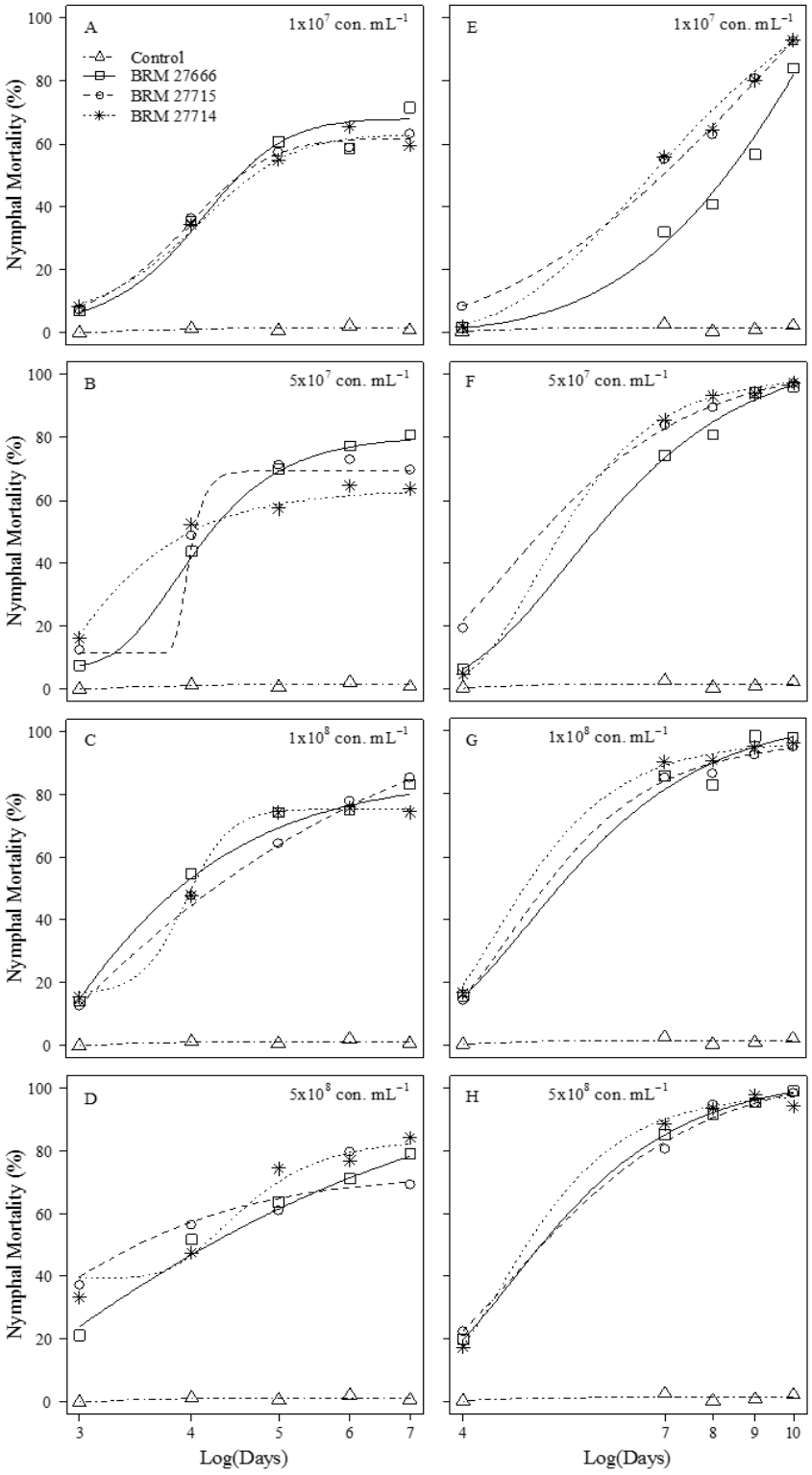


Fig 1 Cumulative mortality at different days post-inoculation for 1st instar nymphs of *B. tabaci* treated with three isolates of *Cordyceps* sp.at 5×10^5, 5×10^6 and 5×10^7 conidia mL^{-1} in experiments conducted in the rainy (a, b, c) and dry season (screenhouse - d, e, f and BOD – g, h, i). Curves were adjusted according to non-parametric models Gompertz (a), Weibull (b, c, d, e, f), Brain-Cousens modified logistic (g, h) and Log-logistic (i)



707

708 **Fig 2** Cumulative mortality at different days post-inoculation for 2nd instar of *B. tabaci* treated with three
709 isolates of *Cordyceps* sp. at 5×10^5, 5×10^6 and 5×10^7 conidia mL^{-1} in experiments conducted in the rainy
710 season. Curves were adjusted according to non-parametric models Log-logistic (a, c) and Logistic (b)



711 **Fig 3** Cumulative mortality at different days post-inoculation for 3rd instar of *B. tabaci* treated with three
712 isolates of *Cordyceps* sp. at 5×10^5, 5×10^6 and 5×10^7 conidia mL^{-1} in experiments conducted in the rainy
713 (a, b, c, d) and dry season (e, f, g, h). Curves were adjusted according to non-parametric models Logistic (a),
714 Weibull (b, d, e, f, g, h) and Log-logistic (c)

CAPÍTULO 2

EFICIÊNCIA DE CAMPO DE *Cordyceps javanica* NO CONTROLE DA MOSCA-BRANCA *Bemisia tabaci* (HEMIPTERA: ALEYRODIDAE) E PERSISTÊNCIA DE CONÍDIOS EM FOLHAS DE SOJA[1]

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[1]Capítulo elaborado conforme as normas do periódico científico Pest Management Science

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3 Field efficiency of *Cordyceps javanica* for controlling whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidade) and persistence of conidia in soybean leaves

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Abstract

Field experiments were carried out in the Embrapa Rice and Bean experimental area to evaluate the efficiency of formulated (WP and WG) and unformulated *C. javanica* BRM 27666 (pure conidia) and the combination with different insecticides to control whitefly nymphs; in addition, the conidia persistence of the formulated and unformulated fungus was evaluated in soybean leaves. In all experiments a CO_2 pressurized sprayer with dropleg bars spraying the leaves upwards reaching the nymphs was used. After three applications of the unformulated fungus at 7-day intervals at a dose of 1×10^{12} conidia ha^{-1}, the mycosis percentage ranged from 47 - 59.1% after 28 and 33 days of application. When performing a single application at the dose of 2×10^{11} conidia ha^{-1} of formulated *C. javanica* it was observed that sporulated nymphs variation between 25.4 - 41.3% on the same date. However, mortality of nymphs in fungal treatments increased significantly after joint action of *C. javanica* with parasites *Eretmocerus* sp. and *Encarsia formosa*. Nymph mortality ranged from 67.9 - 76.9% to 72.6 - 81.6% in formulated and unformulated fungus treatments, respectively. Persistence of *C. javanica* conidia was significantly reduced at 48 h. However, there was no significant difference between treatments for each experiment. When the unformulated fungus was combined with different chemical insecticides no significant difference was observed between fungal + chemical and fungus alone treatments (mycosis ranged from 20.2 - 35% at 26 days after application) except for fungus + pyriproxyfen (≤

9.4%) on all dates. After fungus action with parasitoids in the field, mortality ranged from 90.3 - 98.1% after 26 days of fungal application. Following the promising results shown in our screenhouse and field studies with *C. javanica* BRM 27666, Farroupilha Laboratory - Lallemand has developed a conidial formulation of this isolate for *B. tabaci* control in several cultures; this product is already under registration in Brazil.

3.1 INTRODUCTION

The invasive whitefly, *Bemisia tabaci* MEAM1 (Gennadius) (Hemiptera: Aleyrodidae), is worldwide known for large losses each year in several crops including bean, soybean, tomato, cotton and ornamental plants (Lapidot et al., 2014; Naranjo et al. 2010; Quintela et al., 2016). Damage are due to direct feeding on phloem sap, injection of toxins and indirectly by sugar excretion that foster the growth of saprophytic fungi that decrease the plant photosynthetic area and the commercial value of the crop (Stansly and Natwick, 2010). In addition, adults can transmit more than 300 plant viruses to commercial crops (Gilbertson et al. 2015; Navas-Castillo et al., 2011).

Since the outbreaks of *B. tabaci* MEAM1 in the early 1990s the spread and increase in population of this species have been favored by Brazil's agricultural cropping system (with three growing seasons), the large number of host plants and the tropical climate (Oliveira et al., 2001; Quintela et al., 2016). Management of whitefly infestations are primarily achieved through synthetic insecticides (Liang et al. 2012; Horowitz and Ishaaya, 2014). Repetitive insecticide sprays have increased selection pressure and accelerated development of resistance to multiple classes of insecticides in different regions of the world (Cahill et al., 1996; Horowitz et al., 2004; Naveen et al., 2017; Silva et al., 2009).

Prior to the global outbreak of *B. tabaci* MEAM1 in the early 1990s, very little attention was paid to the potential of entomopathogenic fungi for control of Bemisia (Lacey et al., 2008). In United States, unacceptable losses occurred highlighting the need for an organized, coordinated research and action effort to provide solutions to the problem and prevent the extreme losses in cotton, vegetable, ornamental and nursery production in the field and greenhouses (Oliveira et al., 2001; Lacey et al., 2008). A massive foreign exploration for natural enemies of Bemisia (1990–1996) was made by the USDA Agricultural Research Service to collect and develop fungi and other natural enemies of whiteflies (Lacey et al., 1993, 1996; Kirk and Lacey, 1996; Poprawski and Lacey, 2000;

Kirk et al. 2001). Exploration consistently revealed the complex of *Cordyceps* species formerly classified in *Isaria*, (Kepler et al., 2017), including *C. fumorosea* and *C. javanica,* are the most prevalent fungi attacking whiteflies in the field worldwide (Faria and Wraight, 2001; Lacey et al. 2008; Lacey et al. 1993, 1996; Humber 2002).

Cordyceps species complex have received the majority of attention for *B. tabaci* control due to its ability to develop epizootics in the field, virulence against *B. tabaci* life stages and notable mass production characteristics on solid substrate (Lacey et al., 2008, 2015; Mascarin et al., 2013, 2015). Extensive research has been conducted with *Cordyceps* sp. for control of whitefly, *B. tabaci,* in laboratory and screenhouse conditions in wordwide (Osborne et al., 1990a,b; Vidal et al., 1997b; Negasi et al., 1998; Wraight et al., 1998; Vidal et al., 1998; Osborne and Landa, 1994; Cabanillas et al., 2009; Mascarin et al., 2013, 2014, 2018; Boaventura et al., 2020). In field conditions, the great majority of research performed with *Cordyceps* sp. for whitefly control was conducted in curcubits, tomatoes and cotton (Akey and Hennerberry, 1996; Ruiz-Vega and Aquino-Bolaños, 1999; Wraight et al., 1996, 2000; Ruiz and Medina, 2001; Azevedo et al., 2005).

Massive epizootics of *Cordyceps javanica* were observed as the predominant factor controlling populations of *B. tabaci* in common bean, soybean, cotton, corn, guava and tomato crops, in the 2012/2013 growing season at Federal District and Goiás states in Brazil (Quintela et al., 2016). Ten isolates were collected and studies to detect the best *C. javanica* isolates were performed in laboratory (Mascarin et al., 2018) and screenhouse conditions (Quintela et al., unpublished data). Based on the criteria of virulence, conidial production and tolerance to UV-B radiation, three isolates of *C. javanica*–BRM 27666, BRM 27714 and BRM 27715–were selected.

In subsequent studies, Boaventura et al. (2020**)** assessed susceptibility of 1st to 4th instars of *Bemisia tabaci* MEAM1 to three isolates of *Cordyceps javanica*, previously selected, in a screenhouse under variable temperature and moisture conditions. The 1st, 2nd and 3rd instar nymphs were more susceptible to the three isolates of *C. javanica* than the 4th instar, but the adults that emerged from 4th instar treated nymphs were highly susceptible. There was no difference in virulence between the three isolates. However, the ability of *C. javanica* BRM27666 to grow extensively over the leaf surface and to produce a high amount of conidia under humid conditions are attributes that certainly boost its capacity to spread rapidly through whitefly populations. Based on combined attributes of high virulence and

excelent sporulation in screenhouse bioassays this isolate was selected for field-testing (Boaventura et al., 2020).

In other studies with *C. javanica* isolates, Santos et al. (2018) assessed the bioefficacy of *C. javanica* and the insect growth regulators named spiromesifen and buprofezin alone and in combination against *B. tabaci* nymphs under screenhouse conditions. The insecticides did not influence the germination and mycelial growth of the *C. javanica*. In general, the insecticide–fungus combinations increased nymphal mortalities in comparison with their single counterparts. Furthermore, Zou et al. (2014) observed synergistic effect was observed in the mortality of *B. tabaci* nymphs treated with *C. fumosorosea* combined with the insecticides spirotetramat, imidacloprid and thiamethoxam.

The research on *C. javanica* reported here was conducted under soybean field conditions with three principal objectives: (1) evaluate the efficiency of different applications of one to three sprays of unformulated *C. javanica* in *B. tabaci* control and the persistence of conidia in soybean leaves; (2) compare the efficiency of WP (wettable powder) and WG (dispersible granule) formulations on nymphs and to evaluate the conidia persistence in soybean leaves; (3) assess the efficacy of the fungus alone or in combination with chemical insecticides for *B. tabaci* control.

3.2 MATERIALS AND METHODS

The field studies reported here were conducted at the Brazilian Agricultural Research Corporation (Embrapa Rice and Beans) located at Santo Antônio de Goiás, Goiás state (Central Brazil) (16°30’24,57” S, 49°17’06,53” W) and were the result of a collaboration between Embrapa and Lallemand (Patos de Minas, Minas Gerais, Brazil) under a Collaborative Research and Development Agreement. The experiments were conducted from January to April, 2018 with mean temperature of 22.3 °C (ranged from 17.2 to 31.1 °C) (Fig. 1A), and mean relative humidity of 83.1% (ranging from 40 to 100%) (Fig. 1B).

3.2.1 Whitefly species

The whitefly *Bemisia tabaci* was identified as Middle East Asia Minor 1 (MEAM 1) (formerly biotype B) by molecular gene sequence markers from mtDNA cytochrome oxidase I (mtCOI) (Quintela et al., 2016).

3.2.2 Fungal origin and production

The *C. javanica* isolate BRM 27666 was obtained from infected nymphs *B. tabaci* collected from soybean in Porangatu, Goiás in 2013 and selected for field testing on the basis of combined attributes of high virulence and excelent sporulation in screenhouse bioassays (Boaventura et al., 2020).

The isolate was preserved in liquid nitrogen and deposited at the Invertebrate Fungal Collection at Embrapa Genetic Resources and Biotechnology, Brasília-DF, Brazil. For fungal identification, polymerase chain reaction (PCR) amplifications of the partial β-tubulin gene were performed with 1x AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, CA, USA), ∼100 ng template DNA, and 0.2 μM each of primers bt2a and bt2b (Glass and Donaldson, 1995; Mascarin et al., 2018).

Cordyceps javanica BRM27666 was used in the experiments as unformulated and formulated conidia. Unformulated conidia were produced in autoclaved parboiled rice using the solid fermentation technique mentioned by Mascarin et al. (2013) at Embrapa Rice and Beans (Santo Antônio, Go, Brazil). The conidial suspension was obtained after washing the colonized rice grains with the surfactant solution 0.01% Silwet® L-77. The formulated conidia were provided by Lallemand (Patos de Minas, Mg, Brazil). The isolate was formulated as wettable powder (WP) and water dispersible granule (WG). To determine conidial concentrations of the formulations a 0.1 g sample of each was placed in 10 mL of sterile aqueous solution of 0.01% (v/v) Tween 80 into 50-mL plastic centrifuge tubes and vigorously agitated on vortex for 15 min. Conidial concentrations were determined by haemocytometer (Brightline Improved Neubauer, New Optik®, Brazil) at 400× magnification. Conidial viability was determined after 18 h at 26 °C and exceeded 95% germination. All conidia with visible germ tubes of any length were scored as viable.

The conidial suspensions were prepared with the carrier surfactant solution 0.01% Silwet® L-77 (Momentive Performance Materials Ltd., Waterford, NY). According Mascarin et al. (2014) Silwet L-77 was compatible to *C. fumosorosea* (=*C. javanica*) resulting mostly in additive and synergistic insecticidal activity against *B. tabaci* MEAM1 nymphs.

3.2.3 Insecticides

The synthetic chemical insecticides used in the experiments were flupyradifurone (Sivanto Prime® 200 SL [Soluble Concentrate], 20% [AI], technical grade 4-[(6-chloro-3-pyridylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-one), cyantraniliprole (Benevia® OD [suspension concentrated in oil], 10% [AI], technical grade 3-bromo-1-(3-chloro-2-pyridinyl)-N-[4-cyano-2-methyl-6-[(methylamino)carbonyl]phenyl]-1 H-pyrazole-5-carboxamide), spiromesifen (Oberon® SC [Suspension concentrate], 24% [AI], technical grade 3-mesityl-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutanoate) and piryproxifen (Tiger® 100 EC [Emulsifiable Concentrate], 10% [AI], technical grade 4-fenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether. These products are currently registered for the control of *B. tabaci* in soybean in Brazil (Agrofit, 2020).

3.2.4 Field experiments

In experiment 1, different applications number of unformulated *C. javanica* at 1×10^{12} conidia ha^{-1} was sprayed once (February 28, 2018), twice (February 28 and March 7, 2018) and three times (February 28 and March 7 and 14, 2018) at 7 days intervals. In experiment 2, WP and WG formulations of *C. javanica* at 2×10^{11} conidia ha^{-1} were sprayed at February 28, 2018. The chemical insecticide flupyradifurone was used in the bioassays as a reference for comparison. In experiment 3, unformulated *C. javanica* was tested alone at 1×10^{12} conidia ha^{-1} or in combination with four chemical insecticides: flupyradifurone 100 g [AI]/ha, cyantraniliprole 50 g [AI]/ha, spiromesifen 120 g [AI]/ha and pyriproxyfen 25 g [AI]/ha. The chemical insecticides were also tested alone. All treatments were sprayed at March 12, 2018.

For all experiments, the cultivar seeded was soybean NS7550 I-PRO at 16 seeds m^{-1} with 0.50 m row spacing in January 23, 2018. Agronomic practices used were those recommended for soybean production in Brazilian savannah (‘Cerrado’) (Tuelher et al., 2016). The experimental design was a randomized block with four replicates and experimental plot with five lines of 5.0 m long and spaced 0.5 m (12.5 m^2 area).

All field applications were made using costal sprayer pressurized with CO_2 connected to a compressed-air cylinder with an operating pressure of 3 kgf/cm^2 and Dropleg^{UL} bar (Lechler) (spray from bottom to top) fitted with four sets (base + two nozzles) (model: TwinSprayCap, system Multijet, for flood nozzles). The control group was treated with surfactant solution 0.01% Silwet® L-77. Applications were made during the afternoon (after

4 p.m.), with lower incidence of solar radiation and mild temperatures. The treatments were sprayed at a rate of 200 L/ha.

To assess mortality of nymphs 20 leaflets of each parcel was collected before spraying and after 7, 14, 21 and 28 days for evaluation of live, dead and parasitized nymphs under a dissecting stereomicroscope (Leica) at 40× magnification. For the third experiment, 15 leaflets were collected up to 21 days. Nymphs were considered dead by the fungus became desiccated or developed yellowish symptoms with mycelial or conidial growth (Mascarin et al., 2013; Boaventura et al., 2020). For the fungal infection confirmation, ten leaflets were incubated inside Petri dishes with a wet cotton added to the leaf petiole for five days in a growth chamber of the BOD at 26°C, 80-90% RH with 12-hr photoperiod to evaluation of mycosis in nymphs.

The harvest of the experiments was performed at 81 DAE (harvest: May 23, 2018) at the physiological maturation point of the crop. The yield and the mass of one hundred grains were determined from plants of the useful area of each plot. After threshing the grains were weighed and the data corrected to 13% wet basis. The meteorological data were obtained at Embrapa Rice and Beans Climatological Station. The temperature in the field ranged from 17.2 to 32.1 °C (mean 22.7 °C) (Fig. 1 A) and RH from 40 to 100% (mean 58.7%) (Fig. 1B).

Conidia persistence on leaves was also performed for experiments. After spraying of treatments (Experiments 1 and 2), ten leaflets of each plot in soybean field, in the middle part of the plant totally exposed to the sun, were collected at 0, 16, 20, 24, 40 and 48 hours. In laboratory, the leaflets (10 per repetition) were cut through a 3.2 cm diameter cylinder and deposited in a 125 mL Erlenmeyer flask containing 50 mL of 0.01% Tween 80. The suspension was shaken for five minutes in a chilled incubator with Shaker orbital at 400 rpm.

Three dilutions were performed and 100 μL of each sample was added on a 9.0 cm diameter Petri dish of oat medium with antibiotic (500 mg/L) and dodine fungicide (500 ppm/L). The suspension was spread with Drigalski loop over the entire plate area. The plates were incubated for five days in a growth chamber of the BOD (Biochemical Oxygen Demand) at 26°C, 80-90% RH with 12-hr photoperiod. Then, it was evaluated the number of colony forming units (UFC) was evaluated.

3.2.5 Data analysis

For experiments 1, 2 and 3 the independent variables were tested for residual normality and homogeneity of the variance by using the Kolmogorov–Smirnov and Levene's test,

respectively. When the data were found to deviate from these assumptions, non-parametric procedures (Kruskal–Wallis) were carried out.

For conidia persistence in experiments 1 and 2 curves were adjusted according to non-parametric models and compared using the Wilcoxon-Mann-Whitney test ($P<0.05$). This non-parametric statistic method was used for comparison of two unpaired groups to verify whether or not they belong to the same population and when the requirements for application of Student's t test were not met. All statistical analyses were carried out at a 95% confidence interval using the R software (R Development Core Team, 2016).

3.3 RESULTS

3.3.1 *Cordyceps javanica* formulated and unformulated

Field trials revealed that the mortality of *B. tabaci* nymphs was affected by directly spray the fungal treatments. After 14 days the growth and sporulation of *C. javanica* hyphae was visible and all nymphs dead by the fungus were infected. During this period, rainy days and high humidity were observed, which promoted sporulation of the fungus in the field. Futhermore, a high parasitism rate caused by *Encarsia* sp. and *Eretmocerus* sp. parasitoids was observed in all treatments and increased significantly throughout the experiment.

In the first experiment, it was observed that parasitism was not affected by any of the treatments resembling control at all evaluation dates. Parasitism ranged from 2.8-7.3%, 19.8-32.6% and 31.8-52.5% after 14, 21 and 28 days, respectively. On all dates, fungal treatments were significantly different from control and flupyradifurone (Table 1). There was no difference in mycosis at 14 and 21 days after spraying (ranged 21.9 to 24.6% and 31.4 to 42.2%, respectively). However, it was observed that after 28 days and three applications of the fungus the number of mycosis nymphs was significantly higher compared to the fungus applied once and twice (47% and 27.7 – 35.5%, respectively) (Table 1). When nymphs were not identified as dead by the fungus or parasitoid, they were considered dead by other causes. However, there was no difference in mortality between treatments, except after 21 days (ranged 3.4 to 10.2%) (Table 1). Overall, there was no difference in the percentage of total mortality at 7 days after spraying (4.8 – 12%). However, total mortality was significantly higher in fungal treatments after 14 and 21 days after application ranging from 36-45.2 to 68-72.6%, respectively. After 28 days of application, there was no difference between treatments (64.3-84.2%) due to high parasitism rate in all treatments (Table 1).

When maintained on BOD for five days to confirm fungal infection mortality increased significantly. On all evaluation dates parasitism between treatments was similar to control except after 26 days (1.8-13.1%, 7.5-17.5 and 33.5-64.3 after 12, 19 and 33 days, respectively) (Table 2). On this date, the number of nymphs killed by parasitoids after three applications of fungus and flupyradifurone (24 and 17.7%, respectively) were significantly different from control (41%). The number of mycosed nymphs (ranged 39.5-60.5%) was significantly different from control and chemical insecticide (0 to 8%) at all evaluation dates (Table 2). There was no difference between the number of applications of unformulated fungus, except after 33 days when the number of mycosis nymphs was higher after three applications than in two. About nymphs killed by other causes, a significant difference was observed only at 19 days after application. Overall, after 12, 19 and 26 days the total mortality of nymphs was significantly higher in fungal treatments compared to control. Total mortality of nymphs after one to three applications of *C. javanica* ranged from 49.8 to 96.8% (Table 2).

In the second experiment, parasitism rate was similar between treatments for all evaluation dates (≤52.3%). There was no significant difference between WP and WG formulations (11.3-18%, 27.6-29.3% and 25.4-32.3% after 14, 21 and 28 days, respectively). However, the formulations were different from control and chemical insecticide (ranged 0.1 to 5.1%). There were no significant differences between treatments for the number of nymphs killed by other causes (≤14.7%) (Table 3). The total mortality rate of nymphs was higher for WP compared to other treatments after 7 days of application. However, after 14 days the formulations were similar (41.4-42.7%, respectively) differing from control and flupyradifurone (≤21.6%). After 21 and 28 days there was no significant difference between the treatments which, due to the high parasitism rate, varied between 39.3-68.4% and 64.3-81.6%, respectively (Table 3).

The mortality of dead and infected nymphs increased with leaf incubation in BOD for all treatments for 5 days. At all evaluation dates, the parasitism rate in the treatments was similar to the control ranging from 36.3 to 64.3% after 33 days of application (Table 4). The number of mycosis nymphs was significantly different from control and flupyradifurone on all dates. The number of mycosed nymphs was similar between formulations on all dates (ranged 42.7 to 52.7%) (Table 4). There was no difference between the number of nymphs killed by other causes (≤ 7.8%), except at 19 days. At 12 and 19 days after application, total nymph mortality was significantly higher for formulation WP (59.6%) and WP/WG (ranged

58.1 to 64.2%), respectively, compared to control (≤16.8%). After 26 days with high parasitism rate treatments differed only from chemical insecticide (62.2-80.8% and 36.1%, respectively). There was no difference in the percentage of total mortality between treatments after 33 days (ranged 64.3-90.0%) (Table 4).

3.3.1.1 Persistence of conidia

Conidia persistence was evaluated in experiments 1 and 2 after the first spraying of *Cordyceps javanica* in the field. In the first experiment (only one application in all treatments) it was observed that the initial population of leaf conidia ranged from 7.1 to 10.7 × 10^3 conidia cm^2 (Fig. 2). However, a significant reduction was observed within 48 hours after application. After 16 hours of fungus application the number of conidia ranged from 2.8 to 3.0 × 10^3 conidia cm^2. At 24 and 48 hours between 1.7 to 2.0 × 10^3 conidia cm^2 and 0.8 to 1.2 × 10^3 conidia cm^2, respectively (Fig. 2). No differences among the fungal treatments were observed for persistence of unformulated *C. javanica* conidia, but all these differed from the control (Fig. 2, Table 5).

In the second experiment the conidia population in the WP and WG formulations was initially 7.7 and 5.3 × 10^3 conidia cm^2, respectively, but reduced over time (Fig. 3). At 16 hours after application this number decreased to 3.9 × 10^3 conidia cm^2 (WP) and 2.1 × 10^3 conidia cm^2 (WG); 24 and 48 hours after application the number of conidia decreased from 2.8 to 1.4 × 10^3 conidia cm^2 for WP and from 2.6 to 0.5 × 10^3 conidia cm^2 for WG (Fig. 3). In the WP formulation, more soybean leaf conidia were recovered during the experiment. However, the persistence of conidia was similar between the two formulations tested (Fig. 3, Table 5).

3.3.2 Combination of *Cordyceps javanica* with chemical insecticides

At 7 days after application, nymphal mortality induced by *C. javanica* in combination with chemical insecticides (6.8 – 8.4%) showed no significant difference compared to fungus alone (10.6%), except for fungus with pyriproxyfen (0.8%) (Fig. 4A). There was no difference between the combination of fungus + pyriproxyfen with single applications of insecticides and control (Fig. 4A). On this date, parasitoid mortality was significantly high. The parasitism rate ranged from 18.7 to 31.7% in all treatments. There was no difference compared to the control (Fig. 4A). In combination with parasitoids, mortality in fungal treatments varied between 35.6 - 54.7 after 7 days (Fig. 4A).

At 14 days after application, mortality in treatments combining fungus with flupyradifurone, cyantraniliprole and spiromesifen were similar to fungus alone (13.8 – 24.4%) (Fig. 4B). However, *C. javanica* alone resulted in higher mycosis than the mixture of this fungus + pyriproxyfen. This treatment was statistically different, resembling only control and insecticides alone (≤ 3.5%) (Fig. 4B). The parasitism rate was similar to the control in all treatments except for the fungus alone (Fig. 4B). Overall, mortality in fungal treatments was significantly high ranging from 57.7 - 79% (Fig. 4B).

At 21 days after application, no differences in mortality percentage were observed by mixed treatments (11.1 – 19.5%) compared with the fungus applied singly (23.3%), except for the mixture with pyriproxyfen (3.7%) (Fig. 4C). Treatments with fungus alone and in combination with insecticides (except fungus + pyriproxyfen) were significantly different from control and chemical insecticides applied alone (Fig. 4C). A high parasitism rate was observed in the treatments; however, all treatments were similar to the control (Fig. 4C). In all fungal treatments the mortality of nymphs after joint action with the parasitoids ranged from 69.4 - 85.9% (Fig.4C). Under BOD conditions for 5 days the number of dead and sporulated nymphs increased significantly. It was observed that in fungal treatments the high percentage of "others" were confirmed as infected by the fungus.

When maintained in BOD for 5 days, at 12 days after application, the proportion of sporulated nymphs was not significantly affected by mixing the fungus with insecticides, except in combination with pyriproxyfen (Fig 5A). Mortality of nymphs by fungus alone or in combination with insecticides flupyradifurone, cyantraniliprole and spiromesifen was 17.8, 17.2, 12.5 and 18.5, respectively (Fig. 5A). All of these treatments were significantly different from control and insecticides applied alone (≤ 2.2%). There was no difference in parasitism rate between treatments compared to control (ranged 22.3 – 53.8%) (Fig. 5A). At 12 days after spraying, nymph mortality in fungal treatments ranged from 40.6 to 71.8% due to combination with parasitoids (Fig. 5A).

At 19 days after application, the mycosis percentage was significantly lower when *C. javanica* was combined with spiromesifen (22.2%) compared to the fungus alone (47%). However, the combinations of fungus with flupyradifurone and cyantraniliprole (30.4 and 27.1%, respectively) were similar to the fungus alone (Fig. 5B). The parasitism rate was significantly high between treatments. Only the fungus alone caused a significant reduction compared to control (Fig. 5B). Overall, the mortality caused by fungal treatments ranged between 74.7 – 95.1% (Fig. 5B).

At 26 days after application, the fungus alone caused 35% of mycosis; only the combination fungus with pyriproxyfen caused significant different mortality (9.4%). The combination of *C. javanica* with other chemical insecticides caused significantly higher mortality than control and insecticides alone (Fig. 5C). The parasitism rate was significantly higher than the other dates. Treatments with fungus alone and in combination with flupyradifurone and cyantraniliprole showed significant difference compared to control (Fig. 5C). With significantly high mortality, in the fungal treatments the control by the joint action of these natural enemies reached 98.1% (Fig. 5C).

3.4 DISCUSSION

The great majority of field tests of fungal pathogens against *Bemisia* spp. have been conducted with *C. fumosorosea* and *B. bassiana* in cucurbits, tomatoes and cotton have typically resulted in 50–70% reductions in nymphal B. tabaci populations (Lacey et al., 2008). This study is the first to report effective control of *B. tabaci* by *C. javanica* in soybean. The isolate BRM 27666 used in the current study was highly infectious to *B. tabaci* nymphs promoting high levels of mycosis in field conditions (≈ 60%). We attribute this high efficacy to several factors, including: (1) the optimal humidity and temperature conditions recorded in the field, (2) the use of a drop leg bar that sprays the fungal suspensions from bottom to top of the plants, (3) the association between fungus and parasitoid.

In addition to environmental factors, the method of application is another important factor to consider. Target insects have to acquire a sufficient number of conidia for infection to occur. High levels of whitefly nymph control were achieved by Wraight et al. (2000) in tests in which high rates of fungal conidia were applied with a portable, hand-targeted air-blast sprayer and coverage of leaf undersides was verified. Coverage on the lower leaf surface where the target insects are located is particurlaly important for *C. javanica* because their active ingredients (fungal conidia) must contact the insect cuticle for germination (Liu et al., 1999). Therefore, immature whiteflies, being sessile, need the fungus ‘‘to come to them’’ which makes it difficult to apply conidia with conventional sprayers (Jaronski, 2010; Mascarin et al., 2016). In general, the dropleg bar provide better distribution of the spray broth in the canopy and higher deposition of active ingredients on plant parts which are difficult to reach such as the lower sides of leaves and the lower parts of shoot (Rüegg and Total, 2013) which is important for the effective control of the whitefly nymphs.

Some of the best and most consistent results in the field have been obtained against nymphal whitefly populations infesting cucurbits. In tests by Wraight et al. (2000), C. *fumosorosea* and *B. bassiana* was applied against whitelfy nymphs infesting cantaloupe and honeydew melons, cucumber and zucchini squash. Control levels of 86 – 98% were achieved with both pathogens following 3 – 5 applications of low to high rates of conidia ($1.25 – 5 \times 10^{13}$ conidia ha^{-1}) at 4 – 7 days intervals using a portable air-blast sprayer. In our experiments the number of applications and the dose has been reduced. After conidial sprays of 1×10^{12} conidia ha^{-1} with conidia of unformulated fungus applied at 7 days intervals (3 applications) with a drop leg bar mycosis ranged between 47 – 59.1% after 28 and 33 days of application, respectively. The number of applications did not increase the effectiveness of the fungus, except at 28 and 33 days. After only one application of the formulations, the mycosis percentage in WP and WG treatments after application of 2×10^{11} conidia ha^{-1} ranged from 25.4 – 41.3% for the same date. Wraight et al. (2000) observed that the effective concentrations were achieved through the multiple, frequent applications with the highly efficient portable sprayers. In terms of microbial control potential, differences in leaf morphology/physiology and growth habits of the plants should also be considered; the physical and chemical characteristics of the cucurbit phylloplane and phyllosphere are highly favorable for fungal infection of these sessile, scale-like insects (Lacey et al., 2008).

Another factor observed was the high parasitism rate by the *Encarsia* sp. and *Eretmocerus* sp. parasitoids in all treatments. Many natural enemies, including parasitoids, predators and entomopathogens are responsible for the suppression of whitefly populations (Lacey et al., 2008). However, in our study the abundance of parasitoids in fungal treatments was similar to control in most cases showing that *C. javanica* did not interfere with parasitism rate. Studying the complex interaction between the parasitoid, the pathogen and the whitefly Fransen and Van Lenteren (1993, 1994) observed that both natural enemies can act complementarily. Our studies showed that in the treatments with formulated and unformulated fungus mortality ranged between 67.9 – 76.9% and 72.6 – 81.6%, respectively, what demonstrates apparent synergistic activity of *C. javanica* with the parasitoids *Eretmocerus* spp. and *Encarsia spp.*. Sterk et al. (1995a, b, 1996) also noted little or no effect of *C. fumosorosea* on *E. formosa* and several predators used for control of greenhouse whitefly. However, there may be a reduction in predation due to partial competitive exclusion. Laboratory and field studies suggest that *E. formosa* has the capacity to

discriminate between healthy and fungus-infected whiteflies and commonly avoid laying eggs in whitefly hosts infected (Lacey et al., 1996; Jazzar and Hammad, 2004).

The integration of fungi into comprehensive IPM programs will not only depend on compatibility with other biological control agents, but also with chemical methods of control, and the prevailing environmental conditions in a given cropping system and their effects on infectivity and persistence of spores (Lacey et al., 2008). Chemical insecticides might inhibit cellular and immune responses and improve fungal infection, affect the insect cuticle facilitating fungal penetration and development or might avoid removal of conidia from cuticle (Quintela and McCoy, 1998; Silva et al., 2013). In our studies, the association of *C. javanica* with pyriproxyfen reduced the fungal mycosis on *B. tabaci* nymphs. Anderson and Roberts (1983) showed that *B. bassiana* is also affected by the chemical insecticide pyriproxyfen by inhibiting the development and reproduction of this entomopathogen. However, the combination of *C. javanica* with the chemical insecticides flupyradifurone, cyantraniliprole and spiromesifen did not affect mycosis nymphs that ranged from 20.2 – 35% after 26 days of application.

In general, nymph mortality in fungus + insecticide treatments was highly significant after complementary action of parasitoids (ranged from 90.3 – 98.1% after 26 days of fungal application). The parasitism rate during the experiment was significantly high. Unlike experiments with formulated and unformulated fungus, this experiment was sprayed at different nymphal stages ($2^{nd}/3^{rd}$ instar). The egg-to-adult periods of *E. formosa* are shorter on third and fourth instar nymphs, thus indicating a better performance for development of this parasitoid on these nymphal instars of *B. tabaci*. Younger host stages are more abundant and easy to find. However, younger stages have lower amounts of nutrients, thus slowering parasitoid immature development and increasing the time of exposition to negative environmental influences (Gerling, 1990; Labbé, 2009).

The survival of conidia in the field is likely to be affected by UV light, moisture and temperature (Jaronski, 2010; McCoy et al., 1988; Tian et al., 2014). However, conidia of *C. fumosorosea* are rapidly killed by solar radiation, particularly UV-B (Fargues et al. 1996, 1997; Smits et al. 1996, Zimmermann, 2007; Mascarin et al., 2018). In our study, the persistence of *C. javanica* conidia significantly reduced at 48 hours and showed no significant difference between treatments for each experiment. Fargues et al. (1996) demonstrated that *C. fumosorosea* conidia are highly susceptible to solar radiation and even more susceptible compared to *M. anisopliae* and *B. bassiana* conidia. Furthermore, the

interaction between temperature and solar radiation also has a detrimental effect on the persistence of *C. fumosorosea* conidia (Smits et al., 1996). Therefore, the application of fungus under field conditions should be conducted at late afternoon when temperature and solar radiation are not too high and RH is high. For this reason, formulated products, containing such materials as oils, humectants, UV-protectants, and nutrients to stimulate germination and growth, have great potential to provide better, more consistent results (Burges, 1998; Wraight et al., 2001). The wettable powder (WP) and water dispersible granule (WG) formulation of *C. javanica* were tested in our experiments but its composition is unknown.

The potential of *C. javanica* BRM 27666 supports the development of a mycoinsecticide. Following the promising results shown in our screenhouse (Boaventura el al., 2020) and field studies with *C. javanica*, the isolated BRM 27666 is already under registration in Brazil by Lallemand for control of whiteflies in several cultures. It is necessary to integrate these organisms with other tools, such as predators and parasites, and cultural practices, to create sustainable, biologically based systems, where possible, and not to use the fungi by themselves (Jaronski, 2010). The use of this mycoinsecticide in integrate pest management (IPM) programs represents an eco-friendly strategy to minimize chemical insecticide resistance by *B. tabaci.*

3.5 REFERENCES

Agrofit, 2019. Sistema de Agrotóxicos Fitossanitários. Web Page: <http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons > (accessed 01.28.2019).

Akey, D.H., T.J. Henneberry. 1998. Control of silverleaf whitefly with the entomopathogenic fungi, *Paecilomyces fumosoroseus* and *Beauveria bassiana* in upland cotton in Arizona. Proceedings Beltwide Cotton Conferences, San Diego, CA, 5–9 January 1998, Vol. 2, pp. 1073–1077. National Cotton Council of America.

Anderson, T. E., Roberts, D. W. 1983. Compatibility of *Beauveria bassiana* isolate with insecticide formulations used in Colorado potato beetle (Coleoptera: Chrysomelidae) control. J. Econ. Entomol. 76, 1437-1441.

Azevedo, F. R., Guimarães, J.A., Braga Sobrinho, R., Lima, M.A.A. 2005. Eficiência de produtos naturais para o controle de *Bemisia tabaci* biótipo B (Hemiptera: Aleyrodidade) em meloeiro, Arq. Inst. Biol. 72 (1), 73-79.

Burges, H. D. 1998. Formulation of microbial biopesticides: Beneficial organisms, nematodes and seed treatments. Kluwer Academic, Dordrecht. p. 412.

Cabanillas, E., Jones, W. A. 2009. Pathogenicity of *Isaria* sp. (Hypocreales: Clavicipitaceae) against the sweet potato whitefly B biotype, *Bemisia tabaci* (Hemiptera: Aleyrodidae). Crop Prot. 28, 333–337.

Cahill, M., Gorman, K., Day, S., Denholm, I. 1996. Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). Bull. Entomol. Res. 86, 343–349.

Cuthbertson, A. G. S., Murchie, A. K. 2005. European red spider mite - an environmental consequence of persistente chemical pesticide application. International J. Environm. Sci. and Tech. 2, 287–290.

De Barro, P.J., Liu, S.S., Boykin, L.M., Dinsdale, A.B., 2011. *Bemisia tabaci*: a statement of species status. Annu. Rev. Entomol. 56, 1–19.

Fargues, J., Goettel, M.S., Smits, N., Ouedraogo, A., Vidal, C., Lacey, L.A., Lomer, C.J., and Rougier, M. 1996, 'Variability in Susceptibility to Simulated Sunlight of Conidia among Isolates of Entomopathogenic Hyphomycetes'. Mycopathol. 135, 171-181.

Fargues, J., Rougier, M., Goujet, R., Smits, N., Coustere, C., Itier, B. 1997. 'Inactivation of Conidia of *Paecilomyces fumosoroseus* by Near-ultraviolet (UVB and UVA) and Visible Radiation', J. Invertebr. Pathol. 69, 70-78.

Faria, M., Wraight, S.P., 2001. Biological control of *Bemisia tabaci* with fungi. Crop Prot. 20 (9), 767–778.

Faria, M.R., Wraight, S.P., 2007. Mycoinsecticides and mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biol. Control. 43, 237–256.

Fransen, J., van Lenteren, J.C. 1993. Host Selection and Survival of the Parasitoid *Encarsia formosa* on Greenhouse Whitefly, *Trialeurodes vaporariorum*, in the Presence of 444 R.M. Labbe et al. Downloaded By: [Canadian Research Knowledge Network] At: 20:50 21 May 2009. Hosts Infected with the Fungus *Aschersonia aleyrodis*. Entomol. Experim. Appl. 69, 239-249.

Fransen, J.J., van Lenteren, J.C. 1994. Survival of the Parasitoid *Encarsia formosa* after Treatment of Parasitized Greenhouse Whitefly Larvae with Fungal Spores of *Aschersonia aleyrodis*. Entomol. Experim. Appl. 71, 235-243.

Gerling, D., 1990. Natural enemies of whiteflies: predators and parasitoids. In: Gerling, D. (Ed.), Whiteflies, their Bionomics, Pest Status and Management. Intercept, Andover, UK, pp. 147–185.

Gilbertson, R. L., Batuman, O., Webster, C. G., Adkins, S., 2015. Role of the insect Supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. Annu. Rev. of Virol. 2, 67–93.

Glass, N.L., Donaldson, G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. 61, 1323–1330.

Horowitz, A. R., Kontsedalov, S., Ishaaya, I. 2004. Dynamics of resistance to the neonicotinoids acetamiprid and thiamethoxam in *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. 97, 2051–2056.

Horowitz, A.R., Ishaaya, I., 2014. Dynamics of biotypes B and Q of the whitefly *Bemisia tabaci* and its impact on insecticide resistance. Pest Manag. Sci. 70, 1568–1572.

Huang, Z., Ali, S., Ren, S. X., Wu, J. H. 2010. Effect of *Paecilomyces fumosoroseus* on mortality and reproduction of *Bemisia tabaci* and *Plutella xylostella*. Insect Sci. 17, 140–148.

Hu, Q.B., Ren, S.X., An, X.C., Qian, M.H., 2007. Insecticidal activity influence of destruxins on the pathogenicity of *Paecilomyces javanicus* against *Spodoptera litura*. J. Appl. Entom. 131, 262–268.

Inglis, G.D., Johnson, D.L., Goettel, M.S. 1997. Effects of temperature and sunlight on mycosis (*Beauveria bassiana*) of grasshoppers under field conditions. Environ. Entomol. 26, 400-409.

Jaronski, S.T. 2010. Ecological factors in the inundative use of fungal entomopathogens. BioControl. 55, 159-185.

Kepler, R.M., Luangsa-Ard, J.J., Hywel-Jones, N.L., et al., 2017. A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). IMA Fungus. 8 (2), 335–353.

Labbé, R.M., Gillespie, D.R., Cloutier, C., Brodeur, J. 2009. Compatibility of an entomopathogenic fungus with a predator and parasitoid in the biological control of greenhouse whitefly. Biocont. Sci. Technol. 19 (4), 429–446.

Lacey, L.A., Fransen, J.J., Carruthers, R.I., 1996. Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agentes, in: Gerling, D., Mayer, R.T. (Eds.), *Bemisia* 1995: Taxonomy, Biology, Damage, Control and Management. Andover, UK, pp. 356–456, Intercept.

Lacey, L. A., Wraight, S. P., Kirk, A. A., 2008. Entomopathogenic fungi for control of *Bemisia tabaci* Biotype B: Foreign exploration, research and implementation, in: Gould, J., Hoelmer, K., Goolsby, J. (Eds.), Classical biological control of *Bemisia tabaci* in the United States - A review of interagency research and implementation. Springer, Netherlands, pp. 33–69.

Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., Goettel, M.S. 2015. Insect pathogens as biological control agents: Back to the future. J. Invertebr. Pathol. 132, 1–41.

Liang, P., Tian, Y. A., Biondi, A., Desneux, N., Gao, X. W. 2012. Shortterm and transgenerational effects of the neonicotinoid nitenpyram on susceptibility to insecticides in two whitefly species. Ecotoxicol. 21, 1889–1898.

Liu, T,X., Stansly, P.A., Sparks Jr, A.N., Knowles, T.C., Chu, C.C. 1999. Application of Mycotrol and Naturalis-L (*Beauveria bassiana*) for management of *Bemisia argentifolii* (Homoptera: Aleyrodidade) on vegetables, corron and ornamentals in Southern United States. Subtropical Plant Sci. 51, 41-48.

Mascarin, G.M., Kobori, N.N., Quintela, E.D., Delalibera Junior, I., 2013. The virulence of entomopathogenic fungi against *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) and their conidial production using solid substrate fermentation. Biol. Control 66, 209-218.

Mascarin, G. M., Kobori, N. N., Quintela, E. D., Arthurs, S. P., Delalibera Junior, I., 2014. Toxicity of non-ionic surfactants and interactions with fungal entomopathogens toward *Bemisia tabaci* biotype B. BioControl. 59, 111–123.

Mascarin, G.M., Jackson, M.A., Kobori, N.N., et al., 2015. Liquid culture fermentation for rapid production of desiccation tolerant blastospores of *Beauveria bassiana* and *Isaria fumosorosea* strains. J. Invertebr. Pathol. 127, 11–20.

Mascarin, G.M., Jaronski, S.T. 2016. The production and uses of *Beauveria bassiana* as a microbial insecticide. World J. Microbiol. Biotechnol. 32, 177.

Mascarin, G. M., Pereira-Junior, R. A., Fernandes, E. K.K., Quintela, E. D., Dunlap, C. A., Arthurs, S. P., 2018. Phenotype responses to abiotic stresses, asexual reproduction and virulence among isolates of the entomopathogenic fungus *Cordyceps javanica* (Hypocreales: Cordycipitaceae). Microbiol. Res. 216, 12-22.

McCoy, C.W., R.A. Samson, and D.G. Boucias. 1988. Entomogenous fungi. In C.M. Ignoffo and N.B. Mandava, eds., Handbook of Natural Pesticides, Vol. V, Microbial Insecticides, Part A: Entomogenous Protozoa and Fungi, pp. 151–236. CRC Press. Boca Raton, FL.

Monzo, C., Qureshi, J.A., Stansly, P.A. 2014. Insecticide sprays, natural enemy assemblages and predation on Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). Bull. Entomol. Res. 104, 576–585.

Naranjo, S. E., Castle, S. J., De Barro, P. J., Liu, S. S. 2010. Population dynamics, demography, dispersal and spread of *Bemisia tabaci*. In: Stansly, P A, Naranjo S, eds., *Bemisia*: Bionomics and Management of a Global Pest. Springer, Dordrecht. pp. 185–226.

Naveen, N. C., Chaubey, R., Kumar, D., Rebijith, K. B., Rajagopal, R., Subrahmanyam, B., Subramanian, S. 2017. Insecticide resistance status in the whitefly, *Bemisia tabaci* genetic groups Asia-I, Asia-II-1 and Asia-II-7 on the Indian subcontinent. Scientific Reports. 7, 1–15.

Negasi, A., Parker, B.L., Brownbridge, M. 1998. Screening and bioassay of entomopathogenic fungi for the control of silverleaf whitefly, *Bemisia argentifolii*. Insect Sci. Appl. 18, 37–44.

Osborne L.S., K. Hoelmer, and D. Gerling. 1990a. Prospects for biological control of *Bemisia tabaci*. International Organization of Biological Control Western Palearctic Regional Section Bulletin. 13, 153–160.

Osborne, L.S., G.K. Storey, C.W. McCoy, and J.F. Walter. 1990b. Potential for controlling the sweetpotato whitefly, *Bemisia tabaci*, with the fungus, *Paecilomyces fumosoroseus*. Proceedings of the Vth International Colloquium on Invertebrate Pathology and Microbial Control. pp. 386–390. August 20–24, 1990, Adelaide, Australia, Society for Invertebrate Pathology, Knoxville, TN.

Osborne, L.S. and Z. Landa. 1994. Utilization of the entomogenous fungus *Paecilomyces fumosoroseus* against sweetpotato whitefly, *Bemisia tabaci*. International Organization of Biological Control Western Palearctic Regional Section Bulletin. 17, 201–206.

Quintela, E. D., McCoy, C. W. 1998. Synergistic effect of imidacloprid and two entomopathogenic fungi on the behavior and survival of larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in soil. J. Econ. Entomol. 91, 110-122.

Quintela, E. D., Abreu, A. G., Lima, J. F. dos. S., Mascarin, G. M., Santos, J. B. dos, Brown, J. K., 2016. Reproduction of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) B biotype in maize fields (*Zea mays* L.) in Brazil. Pest Manag. Sci. 72 (11), 2181–2187.

R Development Core Team. 2017. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Ruiz-Vega, J. and T. Aquino-Bolaños. 1999. Manejo de *Bemisia tabaci* mediante barreras vivas y *Paecilomyces* en Oaxaca, Mexico. Man. Integr. Plagas. 52, 68–73.

Ruiz V.J. and Z.J. Medina. 2001. Avances en el manejo integrado de *Bemisia tabaci* en tomate y chile en Oaxaca, Mexico. Manejo Integrado de Plagas 59: 34–40.

Silva, L. D., Omoto, C., Bleicher, E., Dourado, P. M. 2009. Monitoring the susceptibility to insecticides in *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) Populations from Brazil. Neotrop. Entomol. 38 (1), 116–125.

Silva, R. A., Quintela, E. D., Mascarin, G. M., Barrigossi, J. A. F., Lião, L. M. 2013. Compatibility of conventional agrochemicals used in rice crops with the entomopathogenic fungus *Metarhizium anisopliae*. Sci. Agric. 70 (3), 152–160.

Smits, N., Fargues, J., Rougier, M., Goujet, R., Itier, B. 1996. Effects of temperature and solar radiation interactions on the survival of quiescent conidia of the entomopathogenic Hyphomycete *Paecilomyces fumosoroseus* (Wize) Brown and Smith, Mycopathol. 135, 163-170.

Sterk, G., Bolckmans, K., Jonghe, V., Wael, L., Vermeulen, L. 1995a. Side-effects of the microbial insecticide PreFeRal WG (*Paecilomyces fumosoroseus*, strain Apopka 97) on *Bombus terrestris*. Mededelingen - Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent. 60, 713–717.

Sterk, G., Bolckmans, K., van de Veire, M., Sels, B., Stepman, W. 1995b. Side-effects of the microbial insecticide PreFeRal (*Paecilomyces fumosoroseus*, strain Apopka 97) on different species of beneficial arthropods. Mededelingen – Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent. 60, 719–724.

Sterk, G., Bolckmans, K., Eyal, J. 1996. A new microbial insecticide, *Paecilomyces fumosoroseus* strain Apopka 97, for the control of the greenhouse whitefly. Brighton Crop Protection Conference: Pests & Diseases, v. 2. Proceedings of an International Conference, Brighton, UK, November 18–21, 1996, pp. 461–466. British Crop Protection Council, Thornton Heath, UK.

Torres, L.C., Souza, B.D., Lourenção, A.L., Costa, M.B., Amaral, B.B., Carbonell, S.A.M., Chiorato, A.F., Tanque, R.L. 2012. Resistência de genótipos de feijoeiro a *Bemisia tabaci* biótipo B. Bragantia. 71, 346–354.

Tuelher, E.S., Silva, E.H., Hirose, E., Guedes, R.N.C., Oliveira, E.E. 2016. Competition between the phytophagous stink bugs *Euschistus heros* and *Piezodorus guildinii* in soybeans. Pest Manag. Sci. 72, 1837–1843.

Vidal, C., J. Fargues, and L.A. Lacey. 1997a. Intraspecific variability of *Paecilomyces fumosoroseus*: effect of temperature on vegetative growth. J. Invertebr. Pathol. 70, 18-26.

Vidal, C., L.A. Lacey, and J. Fargues. 1997b. Pathogenicity of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with a description of a bioassay method. J. Econ. Entomol. 90, 765–772.

Vidal, C., Osborne, L.S., Lacey, L.A., Fargues, J. 1998. Effect of host plant on the potential of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) for controlling the silverleaf whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae) in greenhouses. Biol. Control. 12, 191–199.

Wraight, S.P., R.I. Carruthers, and C.A. Bradley. 1996. Development of entomopathogenic fungi for microbial control of whiteflies of the *Bemisia tabaci* complex. Proceedings V Siconbiol Simpósio de Controle Biológico, Foz. de Iguacu, Brazil, June 9–14,1996, pp. 28–34.

Wraight, S.P., Carruthers, R.I., Bradley, C.A., Jaronski, S.T., Lacey, L.A., Wood, P., Galaini-Wraight, S., 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. J. Invertebr. Pathol. 71, 217–226.

Wraight, S.P., Carruthers, R.I., Jaronski, S.T., Bradley, C.A., Garza, C.J., Galaini-Wraight, S. 2000. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces*

fumosoroseus for microbial control of the silverleaf whitefly, *Bemisia argentifolii*. Biol. Control. 17, 203–217.

Wraight, S.P., Jackson, M.A.; Kock, S.L. 2001. Production, stabilization and formulation of fungal biocontrol agents. In: Butt, T., Jackson, C., Magan, N. (Eds). Fungi as Biocontrol Agents: Progress, Problems and Potential Bristol, UK: CAB International, 253-88.

Zimmermann, G. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Tech. 17, 553-596.

Table 1. Number of live and dead nymphs (parasitoids, *C. javanica* - unformulated and other causes) before spraying and 7, 14, 21 and 28 days after spraying. Same letters indicate that mean are not statistically different by the Friedman test (P≤0.05).

Treatments	Number of nymphs (20 leaves)					
	Total nymphs (Alive + Dead)	Alive	Parasitized	Mycosed	Dead other causes	Total dead (%)
Before spraying						
One spray	340 ± 217 a	327.0 ± 208.0 a	0	0	13.7 ± 9.9 a	-
Two sprays	306 ± 120 a	301.0 ± 120.0 a	0	0	5.2 ± 1.3 a	-
Three sprays	255 ± 303 a	247.0 ± 306.0 a	0	0	7.7 ± 5.8 a	-
Flupyradifurone	306 ± 104 a	299.0 ± 101.0 a	0	0	7.0 ± 3.0 a	-
Control	297 ± 230 a	289.0 ± 227.0 a	0	0	8.0 ± 9.8 a	-
7 days after spray						
One spray	191 ± 116.4 a	160.0 ± 98.6 a	0	-[1]	30.5 ± 18.0 a	16.1 ± 1.4 a
Two sprays	173 ± 73.5 a	151.0 ± 60.1 a	0	-	22.2 ± 16.6 a	11.6 ± 5.6 a
Three sprays	174 ± 135.8 a	148.0 ± 112.2 a	0	-	26.0 ± 23.8 a	13.0 ± 4.2 a
Flupyradifurone	132 ± 82.2 a	119.0 ± 74.4 a	0	-	12.2 ± 14.1 a	7.5 ± 9.1 a
Control	172 ± 100.2 a	161.0 ± 102.9 a	0	-	11.5 ± 11.7 a	7.6 ± 7.0 a
14 days after spray						
One spray	197 ± 93.3 a	106.0 ± 39.3 a	10.0 ± 9.1 a	57.0 ± 46.2 a	24.0 ± 20.9 a	40.2 ± 21.6 a
Two sprays	224 ± 85.6 a	142.0 ± 54.2 a	19.5 ± 18.3 a	54.0 ± 22.0 a	7.8 ± 10.3 a	36.0 ± 3.5 ab
Three sprays	184 ± 105.9 a	100.0 ± 55.3 a	19.0 ± 13.1 a	51.2 ± 36.4 a	13.5 ± 19.5 a	45.2 ± 7.1 a
Flupyradifurone	106 ± 59.6 a	88.0 ± 46.2 a	5.2 ± 6.2 a	0.2 ± 0.5 b	13.0 ± 19.4 a	17.4 ± 9.9 c
Control	125 ± 61.0 a	100.0 ± 53.2 a	11.2 ± 8.3 a	2.2 ± 3.9 b	11.8 ± 9.1 a	21.6 ± 7.9 bc
21 days after spray						
One spray	305 ± 120.2 a	93.5 ± 46.7 a	88.0 ± 30.2 a	100.5 ± 73.3 a	22.7 ± 16.6 a	68.0 ± 11.5 a
Two sprays	202 ± 65.9 ab	53.5 ± 13.9 ab	51.5 ± 26.7 ab	93.2 ± 25.3 a	3.5 ± 5.7 b	72.6 ± 4.4 a
Three sprays	192 ± 84.9 b	63.0 ± 36.8 ab	57.5 ± 29.8 ab	65.5 ± 29.1 a	6.5 ± 7.7 ab	68.9 ± 8.1 a
Flupyradifurone	92 ± 65.4 c	50.0 ± 35.4 b	34.2 ± 31.3 b	4.7 ± 5.5 b	3.0 ± 3.6 ab	39.3 ± 18.3 b
Control	182 ± 82.0 bc	86.0 ± 38.0 a	85.5 ± 51.8 ab	9.5 ± 9.1 b	0.7 ± 1.5 b	52.5 ± 4.7 b
28 days after spray						
One spray	176 ± 56.2	28.2 ± 12.5 a	94.8 ± 25.5 a	52.2 ± 24.6 b	0.7 ± 1.5 a	84.2±4.2 a
Two sprays	180 ± 110.4	49.2 ± 58.5 a	68.0 ± 24.3 a	52.5 ± 24.6 b	10.5 ± 19.7 a	77.3±12.6 a
Three sprays	204 ± 92.3	30.2 ± 10.9 a	76.8 ± 49.8 a	91.2 ± 45.1 a	6.2 ± 11.2 a	82.1±9.5 a
Flupyradifurone	90 ± 45.8	23.0 ± 15.9 a	59.2 ± 30.5 a	3.2 ± 3.9 c	4.5 ± 7.7 a	72.9±16.3 a
Control	153 ± 77.5	48.5 ± 27.5 a	99.8 ± 64.4 a	4.2 ± 4.4 c	0.2 ± 0.5 a	64.3±21.3 a

Table 2. Number of live and dead nymphs (parasitoids, *C. javanica* - unformulated and other causes) at 12, 19, 26 and 33 days after spraying. Same letters indicate that mean are not statistically different by the Friedman test (P≤0.05).

Treatments	Number of nymphs (10 leaves)					
	Total nymphs (Alive + Dead)	Alive	Parasitized	Mycosed	Dead other causes	Total dead (%)
			12 days after spray			
One spray	56.0 ± 12.8 a	29.0 ± 17.7 a	1.25 ± 1.9 a	25.2 ± 13.8 a	0.50 ± 1.0 a	49.8 ± 29.2 ab
Two sprays	52.5 ± 22.6 ab	26.0 ± 11.6 a	2.25 ± 1.9 a	23.8 ± 10.7 a	0.50 ± 0.6 a	50.8 ± 8.54 ab
Three sprays	45.5 ± 36.9 ab	19.2 ± 16.1 ab	3.50 ± 4.4 a	21.8 ± 15.7 a	1.0 ± 1.4 a	58.9 ± 9.8 a
Flupyradifurone	13.0 ± 8.0 c	10.5 ± 7.6 b	2.25 ± 2.9 a	0.0 b	0.25 ± 0.5 a	20.7 ± 31.5 bc
Control	32.2 ± 12.6 bc	28.0 ± 11.6 ab	2.25 ± 2.1 a	1.0 ± 1.4 b	1.0 ± 1.4 a	14.2 ± 10.8 c
			19 days after spray			
One spray	93.2 ± 37.3 a	32.5 ± 11.6 a	10.7 ± 11.1 a	50.0 ± 28.0 a	0.0 b	61.1 ± 20.0 ab
Two sprays	141.8 ± 45.6 a	57.2 ± 28.6 a	17.7 ± 5.1 a	66.0 ± 17.6 a	0.7 ± 0.5 a	62.0 ± 10.6 abc
Three sprays	94.2 ± 40.7 a	27.5 ± 15.0 a	12.2 ± 8.5 a	54.5 ± 24.1 a	0.0 b	71.5 ± 6.6 a
Flupyradifurone	43.5 ± 15.8 b	26.2 ± 18.4 a	13.7 ± 20.0 a	0.7 ± 1.5 b	2.7 ± 4.3 ab	33.2 ± 41.9 bc
Control	57.8 ± 26.0 b	47.0 ± 19.4 a	9.7 ± 8.7 a	1.0 ± 0.8 b	0.0 b	16.8 ± 10.0 c
			26 days after spray			
One spray	134.2 ± 55.7 a	18.5 ± 18.2 a	48.0 ± 13.8 a	67.0 ± 52.8 a	0.2 ± 0.5 a	84.6 ± 15.6 a
Two sprays	108.5 ± 33.7 a	13.5 ± 8.0 a	28.0 ± 14.1 ab	67.0 ± 14.9 a	0.0 a	87.8 ± 4.4 a
Three sprays	75.8 ± 53.8 ab	15.2 ± 8.1 a	20.0 ± 14.5 b	40.0 ± 36.1 a	0.5 ± 0.6 a	77.9 ± 4.2 ab
Flupyradifurone	35.2 ± 18.1 b	21.8 ± 10.5 a	11.0 ± 9.1 b	1.0± 1.1 b	1.5 ± 2.4 a	36.1 ± 11.1 c
Control	87.2 ± 45.1 ab	31.2 ± 13.0 a	53.2 ± 36.2 a	2.5 ± 5.0 b	0.2 ± 0.5 a	62.2 ± 7.9 bc
			33 days after spray			
One spray	87.5 ± 30.0 a	3.0 ± 5.3 a	46.2 ± 14.5 a	37.7 ± 26.4 ab	0.5 ± 0.6 a	96.8 ± 5.8 a
Two sprays	78.8 ± 34.6 a	11.8 ± 15.0 a	35.2 ± 10.6 a	31.2 ± 14.9 b	0.5 ± 1.0 a	88.3 ± 10.4 a
Three sprays	89.2 ± 28.5 a	5.5 ± 4.2 a	31.5 ± 14.0 ab	52.2 ± 18.5 a	0.0 a	93.0 ± 6.1 a
Flupyradifurone	27.5 ± 13.5 b	10.5 ± 8.7 a	16.2 ± 6.8 b	0.7 ± 0.9 c	0.0 a	64.3 ± 16.6 b
Control	75.5 ± 60.5 ab	10.0 ± 12.3 a	55.5 ± 57.1 ab	9.2 ± 10.2 c	0.7 ± 0.9 a	80.0 ± 23.2 a

Table 3. Number of live and dead nymphs (parasitoids, *C. javanica* - formulated and other causes) before spraying and 7, 14, 21 and 28 days after spraying. Same letters indicate that mean are not statistically different by the Friedman test (P≤0.05).

Treatments	Number of nymphs (20 leaves)					
	Total nymphs (Alive + Dead)	Alive	Parasitized	Mycosed	Dead other causes	Total dead (%)
Before spraying						
WP	176 ± 118 a	164.0 ± 112.0 a	0	0	11.0 ± 13.7 a	-
WG	308 ± 347 a	298.0 ± 340.0 a	0	0	10.0 ± 7.4 a	-
flupyradifurone	306 ± 104 a	299.0 ± 101.0 a	0	0	7.0 ± 3.0 a	-
Control	297 ± 230 a	289.0 ±227.0 a	0	0	8.0 ± 9.8 a	-
7 days after spray						
WP	193 ± 112.3 a	151.0 ± 84.3 a	0	-[1]	42.0 ± 31.2 a	20.3 ± 7.3 a
WG	222 ± 117.0 a	192.0 ± 96.3 a	0	-	30.5 ± 25.2 a	12.8 ± 5.7 b
flupyradifurone	132 ± 82.2 a	119.0 ± 74.4 a	0	-	12.2 ± 14.1 a	7.4 ± 9.2 b
Control	172 ± 100.2 a	161.0 ± 102.9 a	0	-	11.5 ± 11.7 a	7.6 ± 7.0 b
14 days after spray						
WP	133 ± 104.2 a	77.2 ± 60.0 a	15.5 ± 6.2 a	15.7 ± 16.0 a	24 ± 32.7 a	41.4 ± 4.7 a
WG	193 ± 185.8 a	87.8 ± 52.8 a	12.5 ± 14.5 a	62.2 ± 86.7 a	31 ± 42.2 a	42.7 ± 19.5 a
flupyradifurone	106 ± 59.6 a	88.0 ± 46.2 a	5.2 ± 20.9 a	0.2 ± 0.5 b	13 ± 19.4 a	17.4 ± 9.9 c
Control	125 ± 61.0 a	100.0 ± 53.2 a	11.2 ± 8.3 a	2.2 ± 3.9 b	12 ± 9.1 a	21.6 ± 7.9 b
21 days after spray						
WP	176 ± 80.2 a	51.0 ± 25.4 b	59.2 ± 31.5 a	57.7 ± 56.4 ab	7.7 ± 12.9 a	68.4 ± 12.4 a
WG	183 ± 114.8 a	60.5 ± 18.0 ab	62.8 ± 37.3 a	60.0 ± 62.6 a	0 a	62.0 ± 12.8 a
flupyradifurone	92 ± 65.4 a	50.0 ± 35.4 b	34.2 ± 31.3 a	4.7 ± 5.5 b	3.0 ± 3.6 a	39.3 ± 18.3 a
Control	182 ± 82.0 a	86.0 ± 38.0 a	85.5 ± 51.8 a	9.5 ± 9.1 b	0.7 ± 1.5 a	52.5 ± 4.7 a
28 days after spray						
WP	154 ± 87.1 ab	39.8 ± 38.5 a	65.5 ± 41.2 a	45.7 ± 46.7 a	3.5 ± 6.3 a	71.5 ± 18.1 a
WG	190 ± 98.6 a	31.2 ± 16.3 a	89.5 ± 35.2 a	69.0 ± 64.2 a	0 a	81.6 ± 9.9 a
flupyradifurone	90 ± 45.8 b	23.0 ± 15.9 a	59.2 ± 30.5 a	3.2 ± 3.9 b	4.5 ± 7.7 a	72.9 ± 16.3 a
Control	153 ± 77.5 ab	48.5 ± 27.5 a	99.8 ± 64.4 a	4.2 ± 4.4 b	0.2 ± 0.5 a	64.3 ± 21.3 a

Table 4. Number of live and dead nymphs (parasitoids, *C. javanica* - formulated and other causes) at 12, 19, 26 and 33 days after spraying. Same letters indicate that mean are not statistically different by the Friedman test (P≤0.05).

Treatments	Number of nymphs (10 leaves)					
	Total nymphs (Alive + Dead)	Alive	Parasitized	Mycosed	Dead other causes	Total dead (%)
12 days after spray						
WP	54.0 ± 27.8 a	23.8 ± 22.6 a	6.5 ± 8.5 a	21.8 ± 17.9 a	2.0 ± 1.4 a	59.6 ± 25.1 a
WG	33.2 ± 9.8 a	15.0 ± 15.0 a	3.0 ± 4.1 a	14.5 ± 9.1 a	0.7 ± 1.5 a	57.4 ± 34.5 ab
Flupyradifurone	13.0 ± 8.0 b	10.5 ± 7.6 a	2.2 ± 2.9 a	0 b	0.2 ± 0.5 a	20.7 ± 31.6 ab
Control	32.2 ± 12.6 a	28.0 ± 11.6 a	2.2 ± 2.1 a	1.0 ± 1.4 b	1.0 ± 1.4 a	14.2 ± 10.8 b
19 days after spray						
WP	72.0 ± 25.8 a	31.5 ± 17.3 a	13.2 ± 18.8 a	28.0 ± 16.4 a	0.0 b	58.1 ± 7.9 a
WG	109.5 ± 111.9 ab	35.0 ± 27.3 a	12.2 ± 14.4 a	62.0 ± 72.3 a	0.2 ± 0.5 ab	64.2 ± 10.9 a
Flupyradifurone	43.5 ± 15.8 b	26.2 ± 18.4 a	13.7 ± 20.0 a	0.7 ± 1.5 b	2.7 ± 4.3 a	33.2 ± 41.9 ab
Control	57.8 ± 26.0 ab	47.0 ± 19.4 a	9.7 ± 8.7 a	1.0 ± 0.8 b	0 b	16.8 ± 10.0 b
26 days after spray						
WP	62.5 ± 16.5 a	19.8 ± 4.3 a	19.8 ± 4.3 a	23.0 ± 13.9 a	0 a	66.0 ± 13.4 a
WG	109.0 ± 47.8 a	18.8 ± 18.7 a	18.8 ± 18.7 a	45.0 ± 24.3 a	0 a	80.8 ± 21.2 a
Flupyradifurone	35.2 ± 18.1 b	21.8 ± 10.5 a	21.8 ± 10.5 a	1.0 ± 1.1 b	1.5 ± 2.4 a	36. 1 ± 11.1 b
Control	87.2 ± 45.1 a	31.2 ± 13.0 a	31.2 ± 13.0 a	2.5 ± 5.0 b	0.2 ± 0.5 a	62.2 ± 7.9 a
33 days after spray						
WP	86.5 ± 35.4 a	12.0 ± 13.1 a	41.5 ± 21.0 a	31.0 ± 28.3 a	2.0 ± 2.7 a	87.2 ± 12.8 a
WG	75.8 ± 45.0 a	5.0 ± 2.9 a	37.5 ± 25.3 a	31.7 ± 25.6 a	1.5 ± 2.4 a	90.0 ± 8.9 a
Flupyradifurone	27.5 ± 13.5 a	10.5 ± 8.7 a	16.2 ± 6.8 a	0.7 ± 0.9 b	0 a	64.3 ± 16.6 a
Control	75.5 ± 60.5 a	10.0 ± 12.3 a	55.5 ± 57.1 a	9.2 ± 10.2 ab	0.7 ± 0.9 a	80.0 ± 23.2 a

Table 5. P values (*P*≤ value) of comparisons of conidia persistence curves after spraying of unformulated *Cordyceps javanica* and in formulations WP and WG (1, 2 and 3 applications) under field conditions. The Wilcoxon-Mann-Whitney test was used to calculate the P values. The curves were considered significant at P≤0.05.

Treatments	Unformulated			
	UF 1x	UF 2x	UF 3x	flupyradifurone
Control	0.0021	0.0021	0.0021	0.3939
UF 1x	-	0.9372	0.8182	0.0021
UF 2x	-	-	1	0.0021
UF 3x	-	-	-	0.0021
	Formulations			
	WP	WG	flupyradifurone	
Control	0.0021	0.0021	0.3939	
WP	-	0.2403	0.0021	
WG	-	-	0.0021	

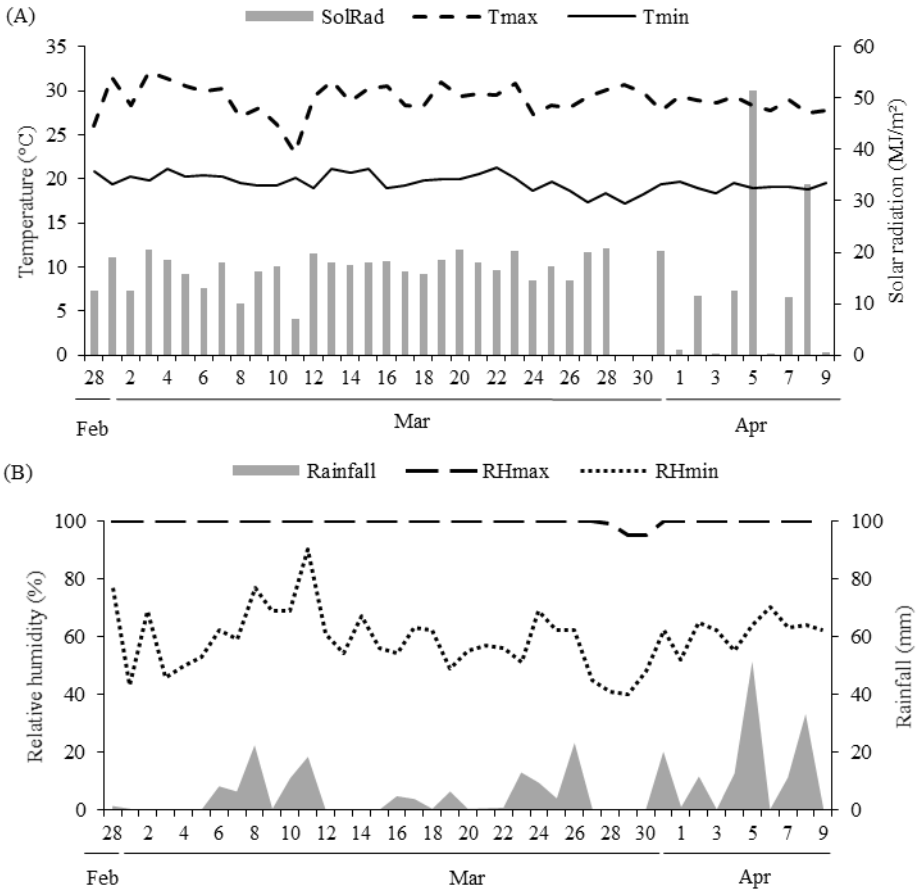

Figure 1. Temperature, solar radiation (A), relative humidity and rainfall (B) at daily intervals in the experimental area during the study of efficacy of *C. javanica*, from late February to April of 2018.

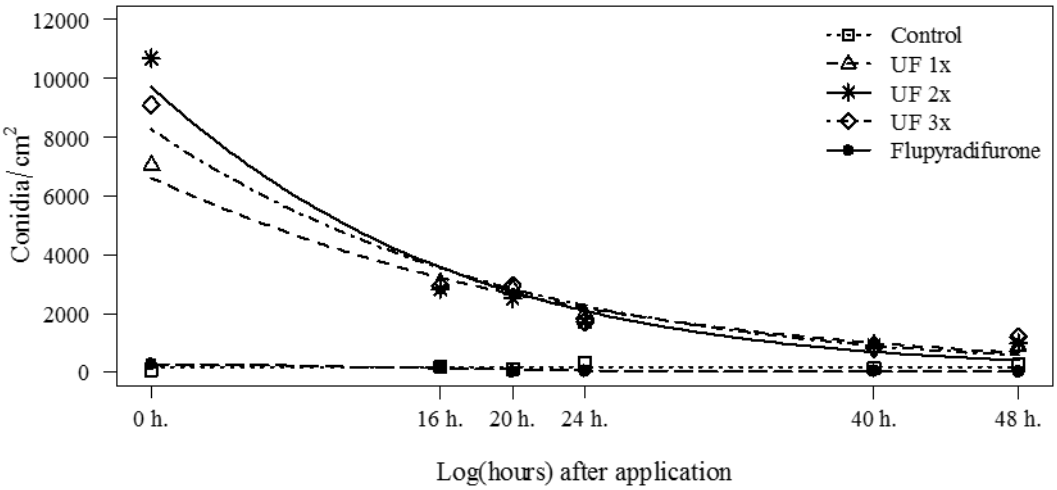

Figure 2. Persistence of *C. javanica* conidia in soybean leaves after application of unformulated fungus in the field. Curves were adjusted according to non-parametric models and compared using the Wilcoxon-Mann-Whitney test ($P<0.05$).

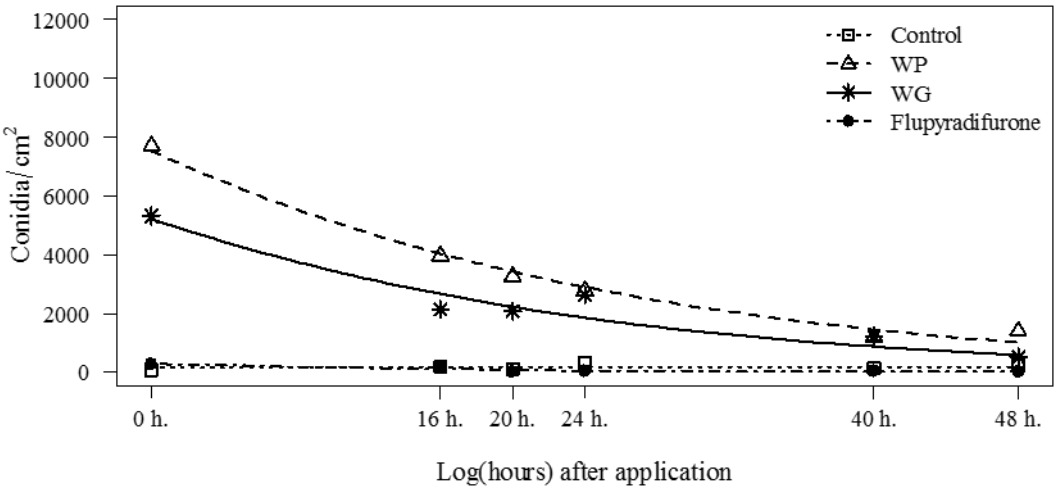

Figure 3. Persistence of *C. javanica* conidia in soybean leaves after application of formulations WP and WG in the field. Curves were adjusted according to non-parametric models and compared using the Wilcoxon-Mann-Whitney test ($P<0.05$).

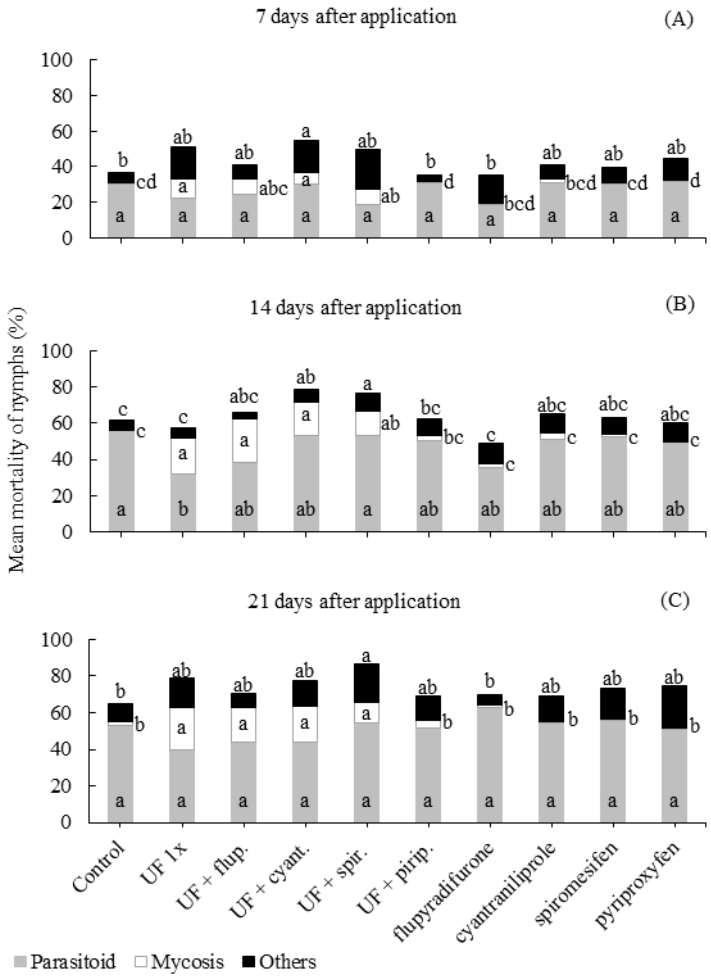

Figure 4. Percentage of mortality of nymphs by parasitoid, *C. javanica* in association with chemical insecticides and others causes at 7 days (A), 14 days (B) and 21 days (C) after spraying. Same letters indicate that mortality (%, mean) are not statistically different by the Kruskall-wallis test ($P \leq 0.05$).

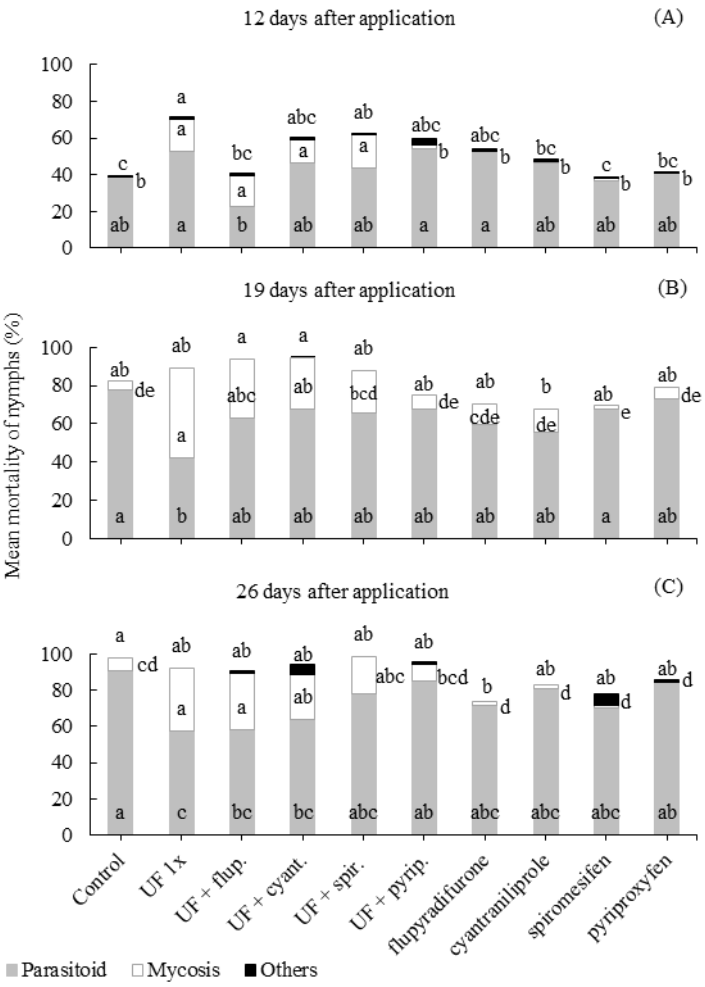

Figure 5. Percentage of mortality of nymphs by parasitoid, *C. javanica* in association with chemical insecticides and other causes at 12 days (A), 19 days (B) and 26 days (C) after spraying in field and five days of incubation in BOD. Same letters indicate that mortality (%, mean) are not statistically different by the Kruskall-wallis test ($P \leq 0.05$).

4 CONSIDERAÇÕES FINAIS

C. javanica demonstrou alto potencial de controle de ninfas de mosca-branca em condições de casa telada e campo. A habilidade de *C. javanica*, particularmente BRM27666, crescer extensivamente sobre a superfície da folha e produzir uma grande quantidade de conídios sob condições úmidas são atributos que certamente aumentam sua capacidade de se espalhar rapidamente através de populações de mosca-branca. Com base nisto, *C. javanica* BRM 27666 foi selecionado e um bioproduto a base de conídios deste fungo foi desenvolvido e está sendo registrado para o controle de mosca-branca em diversas culturas. O uso deste bioproduto é um importante componente no controle de *B. tabaci*, especialmente para o manejo de populações resistentes a inseticidas sintéticos. No entanto, a utilização de *C. javanica* deve ser vista como uma ferramenta do manejo integrado de pragas (MIP). Nenhum agente (químico ou biológico) sozinho é capaz de controlar altos surtos de populações de mosca-branca. Por isso, é importante a ação conjunta de métodos de controle biológico como fungos e parasitoides, por exemplo, que visem diminuir a incidência da mosca-branca e, consequentemente, evitar o excesso de aplicações de inseticidas químicos.

5 REFERÊNCIAS

AHMAD, M. et. al. Cotton whitefly (*Bemisia tabaci*) resistance to organophosphate and pyrethroid insecticides in Pakistan. **Pest Management Science**, Oxford, v. 58, p. 203-208, 2002.

AKEY, D.H.; HENNEBERRY, T.J. **Control of silverleaf whitefly with the entomopathogenic fungi,** *Paecilomyces fumosoroseus* **and** *Beauveria bassiana* **in upland cotton in Arizona**. Proceedings Beltwide Cotton Conferences, San Diego, CA, 5–9 January 1998, v. 2, pp. 1073–1077. National Cotton Council of America, 1998.

AZEVEDO, F. R.; GUIMARÃES, J.A.; BRAGA SOBRINHO, R.; LIMA, M.A.A. Eficiência de produtos naturais para o controle de *Bemisia tabaci* biótipo B (Hemiptera: Aleyrodidade) em meloeiro, Arquivos do Instituto Biológico, v. 72 (1), p. 73-79, 2005.

BARBOSA, F. R.; SIQUEIRA, K. M. M.; SOUZA, E. A.; MOREIRA, W. A.; HAJI, F. N. P; ALENCAR, J. A. Efeito do controle químico da mosca-branca na incidência do vírus do-mosaico-dourado e na produtividade do feijoeiro. **Pesquisa Agropecuária Brasileira**, v. 37, n. 6, p. 879-883, 2002.

BARBOSA, L. F.; YUKI, V. A.; MARUBAYASHI, J. M.; DE MARCHI, B. R.; PERINI, F. L.; PAVAN, M. A.; DE BARROS, D. R.; GHANIM, M.; MORIONES, E.; NAVAS-CASTILLO, J.; KRAUSE-SAKATE, R. First report of *Bemisia tabaci* mediterranean (Q biotype) species in Brazil. **Pest Management Science**, v. 71, n. 4, p. 501-504, 2015.

BEDFORD, I.D.; BRIDDON, R.W.; BROWN, J.K.; ROSELL, R.C.; MARKHAM, P.G. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. **Annals of Applied Biology**, v. 125, p. 311-325, 1994.

BROWN, J.K.; FROHLICH, D.R.; ROSELL, R.C.; The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? **Annual Review of Entomology**, v. 40, p. 511-534, 1995.

BURGES, H.D. Formulation of microbial biopesticides: beneficial organisms, nematodes and seed treatments. **Kluwer Academic**, Dordrecht, p. 412, 1998.

CABANILLAS, E., JONES, W.A. Pathogenicity of *Isaria* sp. (Hypocreales: Clavicipitaceae) against the sweet potato whitefly B biotype, *Bemisia tabaci* (Hemiptera: Aleyrodidae). **Crop Protection**, v. 28, p. 333–337, 2009a.

CABANILLAS, H.E.; JONES, W.A. Effects of temperature and culture media on vegetative growth of an entomopathogenic fungus *Isaria* sp. (Hypocreales:

Clavicipitaceae) naturally affecting the whitefly, *Bemisia tabaci* in Texas. **Mycopathologia**, v. 167, p. 263-271, 2009b.

CAMARGO, M.G.; NOGUEIRA, M.R.S.; MARCIANO, A.F.; PERINOTTO, W.M.S.; COUTINHO-RODRIGUES, C.J.B.; SCOTT, F.B.; ANGELO, I.C.; PRATA, M.C.A.; BITTENCOURT, V.R.E.P. *Metarhizium anisopliae* for controlling *Rhipicephalus microplus* ticks under field conditions. **Veterinary Parasitology**, v. 223, p. 38-42, 2016.

CARDOSO, M. DOS S. Efeito de plantas hospedeiras na suscetibilidade de *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biótipo B a inseticidas químicos. Universidade Federal de Goiás, 2014.

DE BARRO, P. J.; LIU, S. S.; BOYKIN, L. M.; DINSDALE, A. B. *Bemisia tabaci*: A statement of species status. **Annual Review of Entomology**, v. 56, p. 1-19, 2011.

DE FARIA, J.; ARAGAO, F.; SOUZA, T.; QUINTELA, E.; KITAJIMA, E.; RIBEIRO, S.D.G. Golden mosaic of common beans in Brazil: Management with a transgenic approach. **APS Features**, p. 1-14, 2016.

ELBERT, A.; NAUEN, R. Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. **Pest Management Science**, v. 56, p. 60-64, 2000.

FARIA, M. R.; WRAIGHT, S.P. Biological control of *Bemisia tabaci* with fungi. **Crop Protection**, v. 20, p. 767-778, 2001.

FARIA, M.R.; WRAIGHT, S.P. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. **Biological Control**, v. 43, p. 237-256, 2007.

FERNANDES, E.K.K.; RANGEL, D.E.N.; BRAGA, G.U.L.; ROBERTS, D.W. Tolerance of entomopathogenic fungi to ultraviolet radiation: a review on screening of strains and their formulation. **Current Genetics**, v. 61 (3), p. 427-440, 2015.

FRANCO, M. P. J. Tolerância de conídios às radiações UV-A e UV-B em função do tempo de cultivo de fungos entomopatogênicos. 2005. 42 f. Dissertação (Mestrado em Microbiologia Agropecuária) - Faculdade de Ciências Agrárias e Veterinárias, Unesp, Jaboticabal, 2005.

GILBERTSON, R. L.; BATUMAN, O.; WEBSTER, C. G.; ADKINS, S. Role of the insect Supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. **Annual Review of Virology**, v. 2, p. 67-93, 2015.

GINDIN, G.; GESCHTOVT, N.U.; RACCAH, B.; BARASH, I. Pathogenicity of *Verticillium lecanii* to different developmental stages of the silverleaf whitefly, *Bemisia argentifolii*. **Phytoparasitica**, v. 28, n.3, p. 229-239, 2000.

GOETTEL, M.S.; INGLIS, G.D.; WRAIGHT, S.P. Fungi. In: LACEY, L.A.; KAYA, H.K. (Eds). Field manual of techniques in invertebrate pathology. Norwell, MA: **Kluwer Academic Publishers**. p. 255-82, 2000.

HANSSEN, I.M.; LAPIDOT, M.; THOMMA, B.P.H.J. Emerging viral diseases of tomato crops. **Molecular Plant-Microbe Interactions**, v. 23, p. 539–548, 2010.

HOROWITZ, A.R.; KONTSEDALOV, S.; KHASDAN, V.; ISHAAYA, I. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. **Archives of Insect Biochemistry Physiology**, v. 58, p. 216-225, 2005.

INOUE-NAGATA, A.K.; LIMA, M.F.; GILBERTSON, R.L. A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. **Horticultura Brasileira**, v. 34, p. 8-18, 2016.

JACKSON, M. A; et al. Liquid culture production of desiccation tolerant blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus*. **Mycological Research**, Great Britain, v. 101, n. 1, p. 35-41, 1997.

JARONSKI, S.T. Ecological factors in the inundative use of fungal entomopathogens. **BioControl**, v. 55, p. 159-185, 2010.

KANAKALA, S.; GHANIM, M. Advances in the genomics of the whitefly *Bemisia tabaci*: An insect Pest and a virus vector. In Short Views on Insect Genomics and Proteomics. **Springer international publishing**, pp. 19-40, 2015.

LACEY, L.A.; FRANSEN, J.J.; CARRUTHERS, R. Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents. In: GERLING, D.; MAYER, R.T. (Eds.). *Bemisia*, 1995. Taxonomy, Biology, Damage, Control and Management. **Intercept**, Andover, UK, pp. 401-433, 1996.

LACEY, L. A.; KIRK, A. A.; MILLAR, L.; MERCADIER, G.; VIDAL, C. Ovicidal and larvicidal activity of conidia and blastospores of *Paecilomyces fumosoroseus* Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with a description of a bioassay system allowing prolonged survival of control insects. **Biocontrol Science and Technology**, Canada, v. 9, n. 2, p. 9-18, 1999.

LACEY, L.A.; FRUTOS, R.; KAYA, H.K.; VAIL, P. Insect pathogens as biological control agents: do they have a future? **Biological Control**, v. 21, p. 230-248, 2001.

LACEY, L.A.; WRAIGHT, S.P.; KIRK, A.A. Entomopathogenic fungi for control of *Bemisia* spp.: foreign exploration, research and implementation. In: GOULD, J.K.; HOELMER, K.; GOOLSBY, J. (Eds.), Classical Biological Control of *Bemisia tabaci* in the USA: A Review of Interagency Research and Implementation**. Springer**, Dordrecht, pp. 33–69, 2008.

LAZZARINI, G. M. J.; ROCHA, L. F. N.; LUZ, C. Impact of moisture on in vitro germination of *Metarhizium anisopliae* and *Beauveria bassiana* and their activity on *Triatoma infestans*. **Mycological Research**, v. 110 (4), p. 485–492, 2006.

LOURENÇÃO, A. L.; NAGAI, H. Surtos populacionais de *Bemisia tabaci* no Estado de São Paulo. **Bragantia**, Campinas, v. 53, n. 1, p. 53-59, 1994.

MASCARIN, G. M.; KOBORI, N. N.; QUINTELA, E. D.; DELALIBERA JÚNIOR, Í. The virulence of entomopathogenic fungi against *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) and their conidial production using solid substrate fermentation. **Biological Control**, v. 66 (3), p. 209-218, 2013.

MASCARIN, G.M.; JACKSON, M.A.; KOBORI, N.N.; et al. Liquid culture fermentation for rapid production of desiccation tolerant blastospores of *Beauveria bassiana* and *Isaria fumosorosea* strains. **Journal Invertebrate Pathology**, v. 127, p. 11-20, 2015.

MASCARIN, G.M., JARONSKI, S.T. The production and uses of *Beauveria bassiana* as a microbial Insecticide. **World Journal Microbiology and Biotechnology**, v. 32, p. 177, 2016.

MASCARIN, G. M.; PEREIRA-JUNIOR, R. A.; FERNANDES, E. K.K.; QUINTELA, E. D.; DUNLAP, C. A.; ARTHURS, S. P. Phenotype responses to abiotic stresses, asexual reproduction and virulence among isolates of the entomopathogenic fungus *Cordyceps javanica* (Hypocreales: Cordycipitaceae). **Microbiology Research**, v. 216, p. 12-22, 2018.

MCCOY, C.; QUINTELA, E.D.; FARIA, M. Environmental persistence of entomopathogenic fungi. In: BAUR, M.E.; FUXA, J.R. (Eds). **Factors affecting the survival of entomopathogens**. Louisiana State University Agricultural Center, Southern Cooperative Series, Bulletin, 2002.

NAVAS-CASTILLO, J.; FIALLO-OLIVÉ, E.; SÁNCHEZ-CAMPOS, S. Emerging virus diseases transmitted by whiteflies. **Annual Review of Phytopathology**, v. 49, p. 219–248, 2011.

OLIVEIRA, M.R.V.; HENNEBERRY, T.J.; ANDERSON, P. History, current status and collaborative research projects for *Bemisia tabaci*. **Crop Protection**, v. 20, p. 709-723, 2001.

PALUMBO, J. C.; HOROWITZ, A. R.; PRABHAKER, N. Insecticidal control and resistance management for *Bemisia tabaci*. **Crop Protection**, v. 20, p. 739-765, 2001.

PAN, H.P.; CHU, D.; YAN, W.Q.; SU, Q.; LIU, B.M.; WANG, S.L.; WU, Q.J.; XIE, W.; JIAO, X.G.; LI, R.M.; YANG, N.; YANG, X.; XU, B.Y.; ZHANG, Y.J. Rapid spread of Tomato yellow leaf curl virus in China is aided differentially by two invasive whiteflies. **PLoS One**, v. 7, e34817, 2012.

PERRING, T.M. The *Bemisia tabaci* species complex. **Crop Protection**, v. 20, p. 725-737, 2001.

POLSTON, J. E.; DE BARRO, P.; BOYKIN, L. M. Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabacis* species complex. **Pest Management Science,** v. 70, p. 1547-1552, 2014.

QIU, J.; SONG, F.; MAO, L.; TU, J.; GUAN, X. Time-dose-mortality data and modeling for the entomopathogenic fungus *Aschersonia placenta* against the whitefly *Bemisia tabaci*. **Canadian Journal of Microbiology**, v. 59 (2), p. 97-101, 2013.

QUINTELA, E. D.; MASCARIN, G. M.; SILVA, R.A.; BARRIGOSSI, J.A.F.; MARTINS, J. F. S. Enhanced susceptibility of *Tibraca limbativentris* (Heteroptera: Pentatomidae) to *Metarhizium anisopliae* with sublethal doses of chemical insecticides. **Biological Control**, v. 66, p. 56-64, 2013.

QUINTELA, E.D.; ABREU, A.G.; LIMA, J.F.S.; et al. Reproduction of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) B biotype in maize fields (*Zea mays* L.) in Brazil. **Pest Management Science**, v. 72 (11), p. 2181–2187, 2016.

RUIZ-VEGA, J. AND T. AQUINO-BOLAÑOS. Manejo de *Bemisia tabaci* mediante barreras vivas y *Paecilomyces* en Oaxaca, Mexico. **Manejo Integrado de Plagas**, v. 52, p. 68-73, 1999.

RUIZ V.J.; Z.J. MEDINA. Avances en el manejo integrado de *Bemisia tabaci* en tomate y chile en Oaxaca, Mexico. **Manejo Integrado de Plagas**, v. 59, p. 34–40, 2001.

SAMISH, M.; ROT, A.; MENT, D.; BAREL, S.; GLAZER, I.; GINDIN, G. Efficacy of theentomopathogenic fungus *Metarhizium brunneum* in controlling the tick *Rhipicephalus annulatus* under field conditions. **Veterinary Parasitology**, v. 206, p. 258-266, 2014.

SANTOS, T. T. M. DOS; QUINTELA, E. D.; MASCARIN, G. M.; SANTANA, M.V. Enhanced mortality of *Bemisia tabaci* nymphs by *Isaria javanica* combined with sublethal doses of chemical insecticides. **Journal of Applied Entomology**, v. 142, p. 598-609, 2017.

SHAPIRO-ILAN, D. I.; W. A. GARDNER, L. WELLS, AND B. W. WOOD. Cumulative impact of a clover cover crop on the persistence and efficacy of *Beauveria bassiana* in suppressing the pecan weevil (Coleoptera: Curculionidae). **Environmental Entomology**, v. 41, p. 298-307, 2012.

SILVA, L. D.; OMOTO, C.; BLEICHER, B.; DOURADO, P. M. Monitoramento da suscetibilidade a inseticidas em populações de *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) no Brasil. **Neotropical Entomology**, Londrina, v. 38, n. 1, p. 116-125, 2009.

SOUZA, T.L.P.O.; FARIA, J.C.; ARAGÃO, F.J.L.; DEL PELOSO, M.J.; FARIA, L.C.; WENDLAND, A.; AGUIAR, M.S.; QUINTELA, E.D.; MELO, C.L.P.; HUNGRIA, M.; et al. Agronomic performance and yield stability of the RNA interference-based-resistant common bean. **Crop Science**, v. 58, p. 579-591, 2018.

STANSLY, P. A.; NATWICK, E. T. Integrated systems for managing *Bemisia tabaci* in

protected on open field agriculture. In: STANSLY, P.A.; NARANJO, S.E. (Eds.). *Bemisia*: **Bionomics and Management of a Global Pest**. 1. ed. Springer: Amsterdam. 2010, v. 1, cap. 17, p. 467-489.

TAY, W. T.; ELFEKIH, S.; POLASZEK, A.; COURT, L. N.; EVANS, G. A.; GORDON, K. H. J.; DE BARRO, P. J. Novel molecular approach to define pest species status and tritrophic interactions from historical *Bemisia* specimens. **Scientific Reports**, v. 7, p. 429, 2017.

TIAN, J.; HAO, C.; LIANG, L.; MA, R. Effects of temperature and relative humidity on conidial germination of *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) IF-1106 and pathogenicity of the fungus against *Bemisia tabaci* (Homoptera: Aleyrodidae), v. 33 (3), p. 668-679, 2014.

TIAN, J.; DIAO, H.; LIANG, LI,; ARTHURS, S.; MA, R. Pathogenicity of *Isaria fumosorosea* to *Bemisia tabaci*, with some observations on the fungal infection process and host immune response. **Journal of Invertebrate Pathology**, v. 130, p. 147-153, 2015.

XU, J.; DE BARRO, P. J.; LIU, S. S. Reproductive incompatibility among genetic groups of *Bemisia tabaci* supports the proposition that the whitefly is a cryptic species complex. **Bulletin of Entomological Research**, v. 100, p. 359-366, 2010.

WRAIGHT, S.P.; CARRUTHERS, R.I.; BRADLEY, C. A. 1996. **Development of entomopathogenic fungi for microbial control of whiteflies of the *Bemisia tabaci* complex**. Proceedings V Siconbiol Simpósio de Controle Biológico, Foz. de Iguacu, Brazil, June 9–14,1996, pp. 28–34.

WRAIGHT, S.P.; CARRUTHERS, R.I.; BRADLEY, C.A.; JARONSKI, S.T.; LACEY, L.A.; WOOD, P.; GALAINI-WRAIGHT, S. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. Journal Invertebrate Pathology, v. 71, p. 217-226, 1998.

WRAIGHT, S.P.; CARRUTHERS, R.I.; JARONSKI, S.T.; BRADLEY, C.A.; GARZA, C.J.; GALAINI-WRAIGHT, S. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silverleaf whitefly, *Bemisia argentifolii*. **Biological Control**, v. 17, p. 203–217, 2000.

WRAIGHT, S.P., JACKSON, M.A.; KOCK, S.L. Production, stabilization and formulation of fungal biocontrol agents. In: BUTT, T; JACKSON, C; MAGAN, N. (Eds). **Fungi as Biocontrol Agents: Progress, Problems and Potential Bristol**, UK: CAB International, p. 253-88, 2001.

WRAIGHT, S.P.; INGLIS, G.D.; GOETTEL, M.S. Fungi. In: LACEY, L.A., KAYA, H.K. (Eds.). Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and Other Invertebrate Pests.
Springer, Dordrecht, The Netherlands, pp. 223-248, 2007.

ZHANG, C.; ALI, S.; MUSA, P.D.; WANG, X.M.; QIU, B.L. Evaluation of the pathogenicity of *Aschersonia aleyrodis* on *Bemisia tabaci* in the laboratory and greenhouse. **Biocontrol Science and Technology**, v. 27 (2), p. 210–221, 2017.

ZHENG, H.X.; XIE, W.; WANG, S.L.; WU, Q.J.; ZHOU, X.M.; ZHANG, Y.J. Dynamic monitoring (B versus Q) and further resistance status of Q type *Bemisia tabaci* in China. Crop Protection, v. 94, p. 115-121, 2017.

ZIMMERMANN, G. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. **Biocontrol Science and Technology**, v. 17, p. 553-596, 2007.

ZHANG, C.; SHAO, Z. F.; HAN, Y. Y.; WANG, X. M.; WANG, Z. Q.; MUSA, P. D., ALI, S. Effects of *Aschersonia aleyrodis* on the life table and demographic parameters of *Bemisia tabaci*. **Journal of Integrative Agriculture**, v. 17 (2), p. 389–396, 2018.

ZHU, H.; KIM, J. J. Susceptibility of the tobacco whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype Q to entomopathogenic fungi. Biocontrol Science Technology, v. 21, p. 1471-1483, 2011.

ZOU, C., LI, L.; DONG, T.; ZHANG, B.; HU, Q. Joint action of the entomopathogenic fungus *Isaria fumosorosea* and four chemical insecticides against the whitefly *Bemisia tabaci*. **Biocontrol Science and Technology**, Oxford, v. 24, n. 3, p. 315–324, 2014.