Susceptibility of *Tetranychus ogmophallos* (Acari: Tetranychidae) to *Beauveria bassiana* and *Metarhizium anisopliae*

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Abstract

Tetranychus ogmophallos Ferreira & Flechtmann (Acari: Tetranychidae) is an emergent pest in peanut (*Arachis hypogaea* L. [Fabaceae]) and an important pest of forage peanut (*A. pintoi* Krapov. & W.C. Greg. [Fabaceae]). The susceptibility of *T. ogmophallos* to *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae) and *Metarhizium anisopliae* (Metschnikof) Sorokin (Clavicipitaceae) was evaluated under laboratory and greenhouse conditions. In the laboratory, adult *T. ogmophallos* females were treated with *B. bassiana* and *M. anisopliae* using a Potter's tower, and in the greenhouse, bio-insecticides were applied on infested peanut plants. Both fungal suspensions containing 10^8 conidia per mL caused greater mortality in adult *T. ogmophallos* females. At 7 d after treatment under laboratory conditions, *B. bassiana* resulted in 100% mortality and *M. anisopliae* almost 100% mortality. Bio-insecticides substantially reduced the mite population density under greenhouse conditions when applied to infested peanut plants, with mite populations reduced 84% and 92% by *B. bassiana* and *M. anisopliae*, respectively. Under greenhouse conditions, the increase in the number of mites per plant observed at 15 d after treatment suggests the need to repeat the entomopathogen application. Our results demonstrate that *B. bassiana* and *M. anisopliae* are promising biological agents to control *T. ogmophallos*. The practical application of this work for managing *T. ogmophallos* population on peanut crop is discussed.

Key Words: peanut red mite; *Arachis hypogaea*; *Arachis pintoi*; microbial control; forage peanut

Resumo

Tetranychus ogmophallos Ferreira & Flechtmann (Acari: Tetranychidae) é uma praga emergente na cultura do amendoim (*Arachis hypogaea* L. [Fabaceae]) e uma importante praga de amendoim forrageiro (*A. Pintoi* Krapov. & W.C. Greg. [Fabaceae]). A suscetibilidade de *T. ogmophallos* aos bioinseticidas *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae) e *Metarhizium anisopliae* (Metschnikof) Sorokin (Clavicipitaceae) foi avaliada em laboratório e em casa de vegetação. No laboratório, fêmeas adultas de *T. ogmophallos* foram tratadas com *B. bassiana* e *M. anisopliae* sob torre de Potter e em casa de vegetação, os bioinseticidas foram aplicados sobre plantas de amendoim infestadas e avaliações foram realizadas durante 15 dias. Suspensões de ambos os fungos contendo concentração de 10^9 conídios para mL causaram alta mortalidade de ácaros. No experimento em laboratório 7 d após as aplicações, *B. bassiana* causou 100% de mortalidade e *M. anisopliae* causou mortalidade próximo de 100%. Os fungos reduziram substancialmente a densidade populacional do ácaro quando aplicados sobre plantas infestadas, com reduções que atingiram cerca de 84% para *B. bassiana* e 92% para *M. anisopliae*. Em condições de casa-de-vegetação, o aumento no número de ácaros por planta observado aos 15 d após as aplicações reflete a necessidade de realizar uma nova aplicação dos entomopatógenos. Concluiu-se que *B. bassiana* e *M. anisopliae* são promissores agentes no controle biológico do ácaro *T. ogmophallos*. A aplicação prática deste trabalho para o manejo de *T. ogmophallos* na cultura do amendoim será discutida.

Palavras Chave: ácaro-vermelho-do-amendoim; *Arachis hypogaea*; *Arachis pintoi*; controle microbiano; amendoim forrageiro

Peanut red mite *Tetranychus ogmophallos* Ferreira & Flechtmann (Acari: Tetranychidae) is considered an important emergent pest in Brazil because it causes economic and environmental losses (Lourenção et al. 2001; Melville 2015). This mite is found only on *Arachis* spp. plants in Brazil; therefore, it has quarantine importance for many countries (Bonato et al. 2000; Migeon & Dokeld 2007).

Tetranychus ogmophallos causes reduction in the productivity of peanut, *Arachis hypogaea* L. (Fabaceae), and when infestation occurs in the early crop stages, it may cause plant death (Melville 2015). This mite also causes damage in forage peanut, *Arachis pintoi* Krapov. & W.C. Gregory (Fabaceae), used as an ornamental plant and for animal feed (Ferreira & Flechtmann 1997). Bonato et al. (2000) verified that *T. ogmophallos* survived in the laboratory on a diet of bean leaves and soybeans plants. In addition, *T. ogmophallos* fertility was higher on a diet of bean leaves compared to peanut leaves.

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In recent years, several *T. ogmophallos* outbreaks have been observed in peanut producing areas in the state of São Paulo, Brazil, which produces approximately 90% of the national peanut production (IBGE 2016). Several factors are identified as responsible for outbreaks, such as the banning of some broad spectrum insecticides and acaricides, frequent dry spells, use of new varieties with characteristics that may favor the development of mite populations, and the hormesis effect caused by new pesticides (Guedes & Cutler 2014; Guedes et al. 2016).

Applications of acaricides to reduce peanut red mite populations in peanuts crops have been the only mite control measure used. However, this strategy has caused great negative impact on environment and human health (Guedes et al. 2016). As an alternative to synthetic pesticides, fungal entomopathogens are considered to be potential agents to control mite populations. For example, Chandler et al. (2000) collected records of 58 fungal species infecting at least 73 mite species. Among fungal entomopathogens, *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae) and *Metarhizium anisopliae* (Metschnikof) Sorokin (Clavicipitaceae) species stand out (Oliveira et al. 2004; Lacey 2017; Moro et al. 2011). To date, few pathogens have been developed as commercial acaricides due to mass production issues or poor performance under field conditions (Arthurs & Bruck 2017).

Numerous mite species, including tetranychids, are infected by entomopathogenic fungi, predominantly Entomophthorales (e.g., *Neozygites floridana*) and Hypocreales (e.g., *B. bassiana*, *Isaria* spp. (formerly *Paecilomyces*), *M. anisopliae*, *Hirsutella thompsonii* (Fischer), *Cladosporium cladosporioides* (Fresen), *Cephalosporium diversiphialidum* Balazy, and *Lecanicillium lecanii* species (Zimmermann) [formerly *Verticillium lecanii* (Zimmermann)]) (Chandler et al. 2000; Barreto et al. 2004; Oliveira et al. 2004; Maketon et al. 2008; Bugeme et al. 2009; Moro et al. 2011; Sanjaya et al. 2013; Geroh et al. 2015; Lacey 2017; Nakai & Lacey 2017).

Beauveria bassiana and *M. anisopliae* caused between 22 and 82% mortality among adult *Tetranychus evansi* Baker and Pritchard (Prostigmata: Tetranychidae) females under laboratory conditions (Bugeme et al. 2008; Wekesa et al. 2005). Both entomopathogens cause > 65% mortality among *Tetranychus urticae* (Koch) (Acari: Tetranychidae), which is a very important agricultural pest in Brazil (Alves et al. 2002; Tamai et al. 2002; Bugeme et al. 2009; Shi & Feng 2009; Moro et al. 2011; Cerqueira et al. 2017). Six *B. bassiana* isolates and 7 *M. anisopliae* isolates were pathogenic to *Tetranychus kanzawai* (Kishida) (Acari: Tetranychidae), causing > 85% mortality under laboratory conditions, evidencing high potential as a biological control agent (Sanjaya et al. 2013).

The use of fungal entomopathogens for the control of phytophagous mites is restricted to a few species (Lacey 2017), and there are no known reports of fungal entomopathogens for control of *T. ogmophallos*. Thus, the purpose of this study was to evaluate the susceptibility of *T. ogmophallos* to *B. bassiana* and *M. anisopliae* under laboratory and greenhouse conditions.

Material and Methods

COLLECTION AND DEVELOPMENT OF *TETRANYCHUS OGMOPHALLOS*

Tetranychus ogmophallos colonies used in experiments were sourced originally from a peanut field (*A. hypogea* cv. 'Granoleico'), located at the Faculty of Agriculture and Veterinary Sciences (UNESP/FCAV), Campus of Jaboticabal, São Paulo, Brazil (21.25611°S, 48.31611°W). Mites were reared on peanut plants in 8 L pots (with fertilized soil) and kept at 25.3 °C, 79.3% RH, and 12:12 h (L:D) pho-

toperiod. Plants were replaced monthly by healthy plants simply by touching 1 plant to the other for mite migration. Irrigation was performed with manual irrigator every 2 d in order to guarantee sufficient moisture for the development of plants.

FUNGAL ENTOMOPATHOGENS

Two commercial products formulated with *B. bassiana* and *M. anisopliae* were used in experiments. Bio-insecticides were acquired from Koppert Brasil Company (Piracicaba, São Paulo, Brazil) and kept at −4 °C in a freezer.

LABORATORY EXPERIMENT

Under laboratory conditions, the susceptibility of *T. ogmophallos* to *B. bassiana* and *M. anisopliae* was evaluated in Dec 2015. Both treatments used a concentration of 10^8 conidia per mL, and a control treatment using deionized water. Each treatment was repeated 10 times and in all 3 treatments, Tween® 20 (LabSynth Ltda., Diadema, São Paulo, Brazil) spreader sticker (0.05%) was added.

Experimental units were prepared with Petri dishes (90 mm diam × 15 mm ht) containing a 10-mm layer with foam and hydrophilic wet cotton. *Arachis hypogea* cv. 'Granoleico' peanut leaflet without pesticide residue was placed on each Petri dish on the cotton layer. The wet cotton served as a barrier to confine the mites and preserve the leaflet's turgor. Each leaflet was transferred with the aid of a thin paintbrush, and 15 adult female mites of *T. ogmophallos* from the colony were transferred using a stereomicroscope (model Stemi 2000-C, Carl Zeiss Corporation, Jena, Germany). Treatments (fungal suspensions and deionized water) were applied directly on mites using a Potter's tower calibrated at 4 lbf.pol-2, with 0.5 mL of suspension per leaflet. After applications, the experimental units were sealed with plastic film to maintain humidity and kept in climatized chamber at 25 ± 1 °C, 70 ± 10% RH, and 12:12 h (L:D) photoperiod. Mite survival was assessed at 1, 3, 5, and 7 d after applications.

The experiment was conducted in a completely randomized design, and data were submitted to analysis of variance (ANOVA) with the averages of mite survival percentages compared by the Tukey test ($P < 0.05$) (SAS 2002).

GREENHOUSE EXPERIMENT

This experiment was conducted under greenhouse conditions (averages 25.3 °C and 79.0% RH) during the period from Nov 2015 to Feb 2016. Four treatments were evaluated: (1) deionized water + Tween® 20 spreader sticker (0.05%) (negative control treatment); (2) synthetic acaricide 'fenpyroximate' (Ortus® 50SC – Arysta Lifescience do Brasil Ind. Quim. e Agropec. Ltda., São Paulo, Brazil) at dosage of 100 mL of c.p. per 100 L (positive control treatment); (3) *B. bassiana* suspension (10^8 conidia per mL) + Tween® 20 spreader sticker (0.05%); and (4) *M. anisopliae* suspension (10^8 conidia per mL) + Tween® 20 spreader sticker (0.05%). For application of treatments, a hand sprayer with sufficient volume to provide complete plant coverage was used.

Pots with 8 L capacity containing 1 *A. hypogea* cv. 'Granoleico' peanut plant and a mixture of soil, sand, and bovine manure (2:1:1) as substrate were used. There were 10 pots for each treatment (10 replicates). At 75 d after seedling emergence, at the reproductive stage (R5), 100 adult *T. ogmophallos* females were transferred to each pre-inspected plant using a paintbrush.

Five d after mite transfer to clean plants, treatments were applied as part of a randomized block design and evaluations were performed at 5, 10, and 15 d after applications. The number of live mites in each treatment was estimated by counting mites present on 2 leaves per

plant (1 leaf in the lower stratum and 1 leaf in the upper stratum of plants). The numerical data (insect abundance) were transformed into log (x + 1) to be submitted to analysis of variance, and the average number of mites per plant was compared by the Tukey test ($P <$ 0.05) (SAS 2002). In order to calculate the efficiency of treatments, the Henderson-Tilton formula was used (Henderson & Tilton 1955). The average number of mites per plant stratum was analyzed in a 4 × 3 × 2 factorial scheme: 4 treatments (deionized water, fenpyroximate, *B. bassiana*, and *M. anisopliae*), 3 evaluation periods (5, 10, and 15 d after applications) and 2 strata.

Results

For both fungal entomopathogens, a gradual reduction in the percentage of adult *T. ogmophallos* females was observed throughout the study period at laboratory conditions. On the first d after application, a significant reduction in the number of surviving mites in treatments with fungal entomopathogens was observed, when compared to the control ($F = 76.05$; df = 2,27; $P < 0.05$), represented by 28.3 and 15.9% of individuals surviving in treatments with *M. anisopliae* and *B. bassiana*, respectively (Fig. 1).

At 3 d after applications, the percentage of surviving adult *T. ogmophallos* females was 13.1 and 3.4% for treatments with application of *M. anisopliae* and *B. bassiana*, respectively, significantly differing from control ($F = 170.08$; df = 2,27; $P < 0.05$) (Fig. 1). The same situation was observed at 5 d after applications ($F = 562.27$; df = 2,27; $P < 0.05$), where survival of tetranychids was 5.1% (*M. anisopliae*) and 2.7% (*B. bassiana*) ($F = 404.18$, df = 2.27, $P < 0.05$), with survival of 3.9% of adult females for treatment with *M. anisopliae* and total mortality for *B. bassiana* (Fig. 1).

Regarding the efficacy of fungal entomopathogens in the greenhouse on *T. ogmophallos*, control efficacy of 68.41 and 68.68% was observed at 5 d after applications for *B. bassiana* and *M. anisopliae*, respectively (Fig. 2A). At 10 d after applications, control efficacy increased to 83.86% (*B. bassiana*) and 92.18% (*M. anisopliae*) (Fig. 2B); however, control efficacy decreased to 71.82% (*B. bassiana*) and 59.34% (*M. anisopliae*) at 15 d after applications (Fig. 2C).

The population density of *T. ogmophallos* on peanut plants, under greenhouse conditions, at 5 d after application was not significantly different when fenpyroximate (synthetic acaricide) and fungal entomopathogen treatments were compared (Fig. 2A). There was a significant differ-
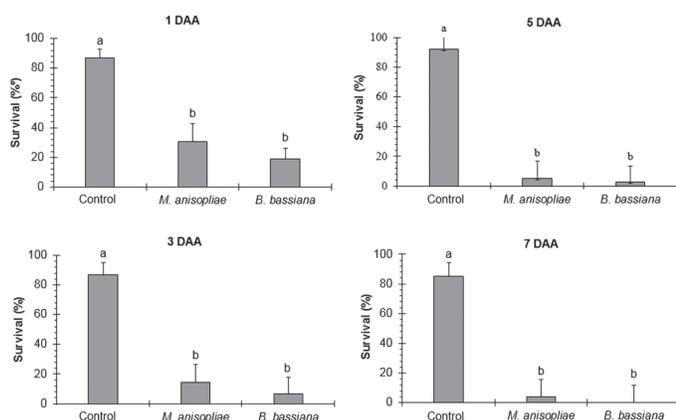
Fig. 1. Survival (%) (± SE) of adult *Tetranychus ogmophallos* females 1, 3, 5, and 7 d after application for treatments with *Beauveria bassiana* and *Metarhizium anisopliae* at laboratory conditions. Values within a time interval topped by the same letter did not differ significantly from each other by the Tukey test ($P <$ 0.05). Error bar corresponds to the standard error (± SE).
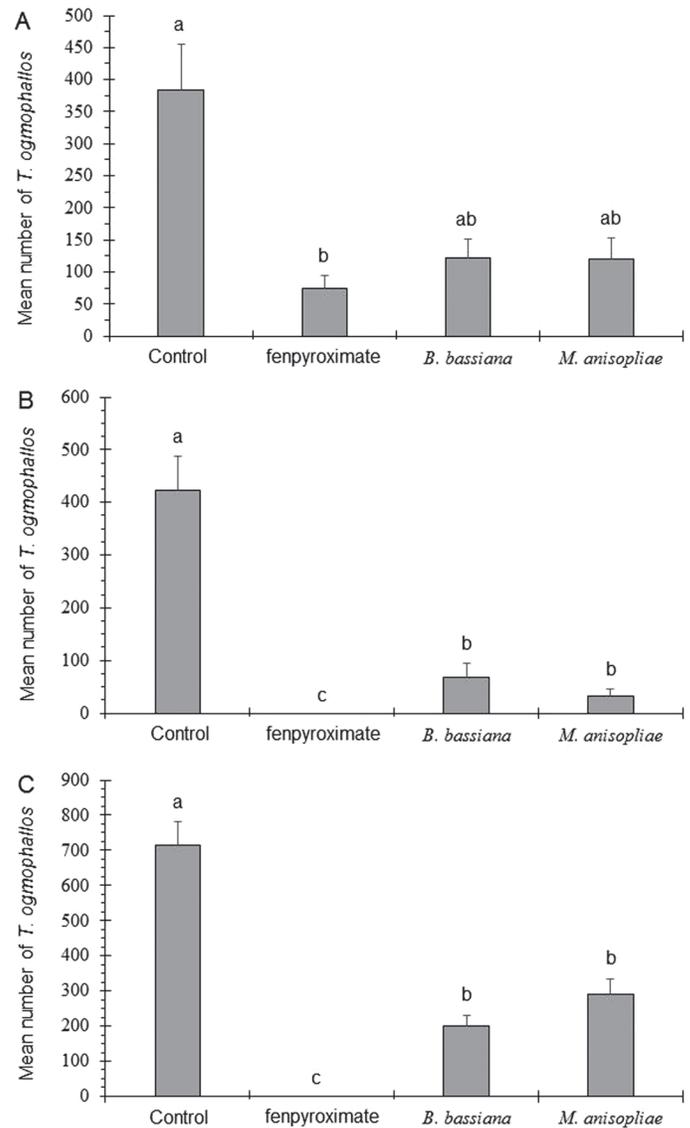
Fig. 2. Mean number (± SE) of *Tetranychus ogmophallos* on peanut leaves 5 (A), 10 (B), and 15 (C) d after application. Values within a time interval topped by the same letter did not differ significantly from each other by the Tukey test ($P < 0.05$). Error bar corresponds to the standard error (± SE).

ence between the treatments with chemical acaricide and the negative control with water + adjuvant (Fig. 2A) ($F = 10.48$; df = 3,36; $P < 0.05$).

At 10 d after applications, a reduction in the number of *T. ogmophallos* was observed in treatments with entomopathogens, significantly differing from the other treatments ($F = 30.61$; df = 3,36; $P < 0.05$). In this evaluation, the number of mites per plant in treatments with *B. bassiana* and *M. anisopliae* was 68.3 ± 26.1 mites per plant and 33.1 ± 12.3 mites per plant, respectively. There were increases in mite densities at 15 d after applications in treatments with microorganisms; however, the mite densities were significantly lower compared to the negative control ($F = 50.6$; df = 3,36; $P < 0.05$) (Fig. 2C). In this evaluation, there were 201.2 ± 29.6 mites per plant in the treatment with *B. bassiana* and 290.3 ± 42.2 mites per plant in the treatment with *M. anisopliae*. In the treatment with fenpyroximate, no mites were found on plants at evaluations performed at 10 and 15 d after applications (Figs. 2B, C).

At 5 d after application, the miticide treatments were affected by plant stratum, with fewer mites present in the upper region of

Table 1. Mean number (± SE) of *Tetranychus ogmophallos* in the lower and upper regions of peanut plants 5, 10, and 15 d after application (DAA).

Treatments	Lower region of the plant		
	5 DAA	10 DAA	15 DAA
Negative control	272.5 ± 57.1 Aa	256.6 ± 52.8 Aa	228.9 ± 45.3 Aa
fenpyroximate	75.2 ± 18.7 Ab	0.0 Ab	0.0 Ab
Beauveria bassiana	121.1 ± 29.4 Ab	37.1 ± 20.0 Ab	64.2 ± 18.3 Ab
Metarhizium anisopliae	120.1 ± 33.1 ABb	31.0 ± 12.6 Bb	219.5 ± 41.7 Aa
	Upper region of the plant		
Negative control	110.9 ± 54.8 Ba	166.5 ± 53.9 Ba	485.1 ± 52.46 Aa
fenpyroximate	0.0 Ab	0.0 Ab	0.0 Ac
Beauveria bassiana	0.0 Bb	31.2 ± 17.7 Bb	137 ± 20.6 Ab
Metarhizium anisopliae	0.0 Ab	2.1 ± 0.2 Ab	70.8 ± 40.1 Abc

Means followed by the same lowercase letter in the column and upper case in the row did not differ significantly from each other by the Tukey test ($P < 0.05$). The comparison of the statistical analysis was performed for each region of the plant.

the plant. However, at this time there were no significant differences among the miticide treatments. At 10 d after application, possibly due to the drastic population reduction in all treatments, no difference in the number of mites per plant stratum was observed (Table 1). At 15 d after applications, *B. bassiana* displayed better control in the lower stratum, but *M. anisopliae* did not differ from the negative control in the lower stratum. In the upper region of the plant, the result was opposite to that observed in the lower stratum, as evidenced by the low population density of *T. ogmophallos* in the *M. anisopliae* treatment (Table 1).

Discussion

Beauveria bassiana and *M. anisopliae* were pathogenic to *T. ogmophallos*, which is the first report on the pathogenicity of these fungi to this mite species. Other studies also have reported the pathogenicity of *B. bassiana* and *M. anisopliae* to tetranychid species, such as *T. urticae* (Alves et al. 2002; Tamai et al. 2002; Shi & Feng 2009; Moro et al. 2011), *T. evansi* (Wekesa et al. 2005), *T. kanzawai* (Sanjaya et al. 2013), *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) (Barreto et al. 2004), and *Oligonychus yothersi* (McGregor) (Acari: Tetranychidae) (Oliveira et al. 2002, 2004).

The high mortality of *T. ogmophallos* females observed for all treatments (Fig. 1) may be related to the concentration of the conidial suspensions used (Alves et al. 2002). At concentrations of 10^5, 10^6, and 10^7 conidia per mL of *B. bassiana*, aiming to control adult *T. urticae* females under laboratory conditions, Alves et al. (2002) observed mortality below 45.0%, while at concentration of 10^8 conidia per mL, the mortality rate of these tetranychids was 74.4%. Geroh et al. (2015) also observed a lower number of individuals surviving the use of more concentrated *B. bassiana* conidia suspensions, with mortality of *T. urticae* adults ranging from 45.6 to 91.0% using concentrations between 10^5 and 10^{12} conidia per mL.

The methodology used also should be considered an important point in relation to values found for the survival rate of *T. ogmophallos*. According to Geroh et al. (2015), the application of an entomopathogen directly on mites was more reliable in assessing potential mortality of *T. urticae* when compared to treatment of food.

Relative to plant strata, there were not many differences in the effectiveness of the entomopathognes for the first 10 d after treatment. After 15 d, *B. bassiana* was more effective in the lower stratum (as compared to *M. anisopliae*) (Table 1). In tomato cultivation under greenhouse conditions, Wekesa et al. (2005) observed that *B.*

bassiana was efficient in controlling *T. evansi* in the upper and lower strata of plants, when compared to treatments with *M. anisopliae* at 7 and 14 d after applications. Stratum-specific responses may be due to the variation in moisture and temperature throughout the canopy of plants. For example, Shi & Feng (2009) evaluated 2 *B. bassiana* and 2 *M. anisopliae* isolates for spider mites in cotton in China and obtained desirable control over a period of 30 to 35 d with efficacy > 80%. High humidity in the canopy and moderate daily mean temperatures were advantageous to control.

This mite has caused many problems for forage peanut, which is used for animal feed (Ferreira & Flechtmann 1997; Santos 2016). Biological control with fungal entomopathogens may prove to be a useful tool to control *T. ogmophallos* in forages because the use of fungal entomopathogens presents minimal risks to human and animal health when compared to the use of synthetic pesticides (Zimmerman 2007; Hu et al. 2016).

The results of our research indicate that both entomopathogenic fungi have potential for managing *T. ogmophallos* in peanut crop. However, bioassays in the field are required to prove the real potential of these entomopathogens. In the field, the entomopathogenic fungi may be influenced by abiotic factors, principally temperature, relative humidity, and solar radiation, which may affect the development and reproductive parameters of these microorganisms (Bugeme et al. 2008; Oliveira et al. 2016).

The time of spraying entomopathogenic fungi also is a limiting factor for use of these microorganisms in the field to control the genus *Tetranychus*. High temperature and low relative humidity may be responsible for the failure of microbial control with entomopathogenic fungi. In the state of São Paulo, Brazil, the peanut crop is cultivated in the summer season, with medium temperature and high relative humidity, which makes it possible to use the formulated products based on *M. anisopliae* and *B. bassiana*, with the recommendation to spray in the evening, at temperatures between 25 to 35 °C and relative humidity of 60% (Koppert 2018a, b).

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