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PATHOGENICITY OF *METARHIZIUM ANISOPLIAE* VAR. *ACRIDUM* TO THE FALSE SPIDER MITE *BREVIPALPUS PHOENICIS* (ACARI: TENUIPALPIDAE)

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The flat mite, *Brevipalpus phoenicis* (Geijkes), is a polyphagous pest found in many subtropical and tropical regions of the world (Childers et al. 2003). *Brevipalpus phoenicis* is recognized as the most economically harmful virus vector species in citrus areas where *Citrus leprosis* Virus (CiLV) has been reported. So far, chemical sprays have been the main approach adopted to control the mite in citrus (Rodrigues & Machado 2003). Otherwise, fungal pathogens are frequently found causing diseases and epizootics in mite populations (Alves 1998; Van Der Geest et al. 2000). Recently, the potential to control *Brevipalpus* populations by spraying with the fungus *Hirsutella thompsonii* Fisher was suggested (Rosas-Acevedo & Sampedro 2000; Rossi 2002). However, the development of *H. thompsonii* as a bio-acaricide has been hampered by difficulties in mass producing aerial conidia. In contrast, the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* Driver & Milner is easily produced in large scale and has been developed as a mycoinsecticide in several countries (Magalhães et al. 2000; Lomer et al. 2001). This fungus is tolerant of high temperatures, an important characteristic for pathogens developed for tropical agroecosystems. We studied the pathogenicity of *M. anisopliae* var. *acridum* against *B. phoenicis* to evaluate its potential use as an acaricide.

Mites used in this study were 5-15 days old adult females derived from clonal lineage established from a single female isolated from a mite colony collected from citrus in Plant City, Florida (Rodrigues et al. 2004). The fungus assayed was the isolate CG423 of *M. anisopliae* var. *acridum*, produced on SDAY medium, harvested, and dried according to Magalhães & Boucias (2004). The viability of the conidia used in the bioassays was determined by plate assay to be >95%. The bioassay unit was a 90-mm diameter Petri dish containing a 60-mm diameter plate glued to the bottom. A 1.5-cm diameter circular leaf arena clipped from *Ligustrum lucidum* Aiton was placed over a layer of cotton wool which was saturated with water. Twenty mites were transferred to the leaf arena. Two h later the pathogen was applied at a concentration of 10^8 conidia/ml in a 200-µl suspension applied by air flow with a pressure of 150 psi with the aid of a spray tower (Pereira 1991). Mite survival was monitored daily and infection confirmed by examining the specimens with an epifluorescence microscope. Dead mites were stained with Calcofluor White (Sigma) (1 mg/ml) for 30 min and washed three times. To prevent rapid fading, the stained material was immersed in a mounting medium containing DABCO (Sigma) (100 mg), glycerine (30 ml) and Hepes (10 ml) for microscopic examination.

In preliminary experiments, the mites were sprayed with fungus + 0.5% Tween 20 (v/v) (polyoxyethylenesorbitan monolaurate, P-1379 (Sigma)) in deionized water, water + 0.5% Tween 20, and control (no treatment). Each treatment was repeated five times. Observations showed very low survival rates (<23%) 24 h after treating the mites with the fungus + Tween 20 but also with the Tween 20 alone. Otherwise, the mortality rates in the controls (water treatment) were insignificant, indicating acaricidal activity by the wetting agent. Therefore, we tested the direct mortality to *B. phoenicis* with 0.5, 0.05, 0.005, 0.0005, and 0.00005% of Tween 20 to determine a safe concentration for subsequent studies. The results from a linear regression confirmed that mite survival was dependent on the Tween 20 concentration ($P < 0.001$) (Zar 1998). When Tween 20 was applied at a concentration 0.5%, survival at 24 h after inoculation was lower than 35%. In a separate experiment, similar mortality was observed when the leaf substrates were treated with Tween 20 prior to transferring the test mites (data not shown).

Survival of adult female mites treated with 0.00005% Tween 20 was not significantly different from the control and was therefore used in the fungal assays. The fungus was able to germinate and penetrate the treated adult female mites (Fig. 1). The SEM observations confirmed these findings. At 8 days post-treatment, the fungus caused 90% mortality (Fig. 2), where infection levels were confirmed by fluorescence microscopy.

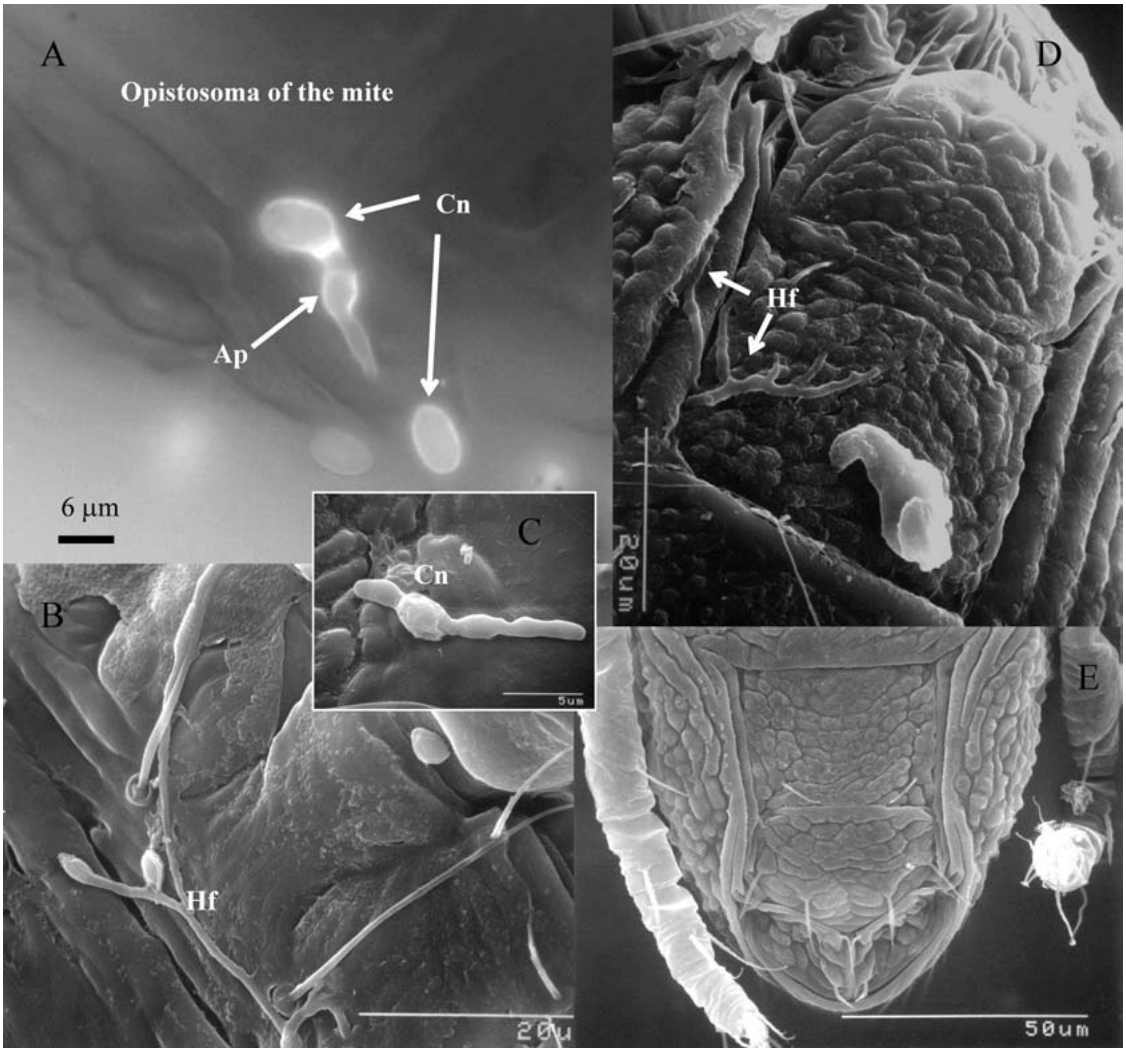

Fig. 1. (A) Microphotograph of *Metarhizium anisopliae* var. *acridum* infecting *Brevipalpus phoenicis* 3 days after inoculation by fluorescence microscopy. (B, C, and D) SEM of infection and colonization process. E) Control sprayed with water. (Cn = Conidium, Ap = Appressorium, Hf = Hypha).

Fungal infection also reduced the production of eggs by *B. phoenicis* females in comparison to treatments that received only Tween 20 or water (Table 1). These results are important and support the possibility of exploiting *M. anisopliae* var. *acridum* as a biological acaricide against *B. phoenicis*. Additional research is needed to determine the acaricidal effects of Tween 20 and similar products for false spider mite control.

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Summary

Metarhizium anisopliae var. *acridum* Driver & Milner isolate CG423 was demonstrated to be pathogenic to the false spider mite *Brevipalpus phoenicis* Geijskes (Acari: Tenuipalpidae). Effects on mite survival and egg production were assessed. The fungus was able to infect treated adult mites at least 4 days after inoculation and reached 90% mortality by the 8th day. We also demonstrated that Tween 20 shows acaricidal activity at low concentrations to *B. phoenicis*.

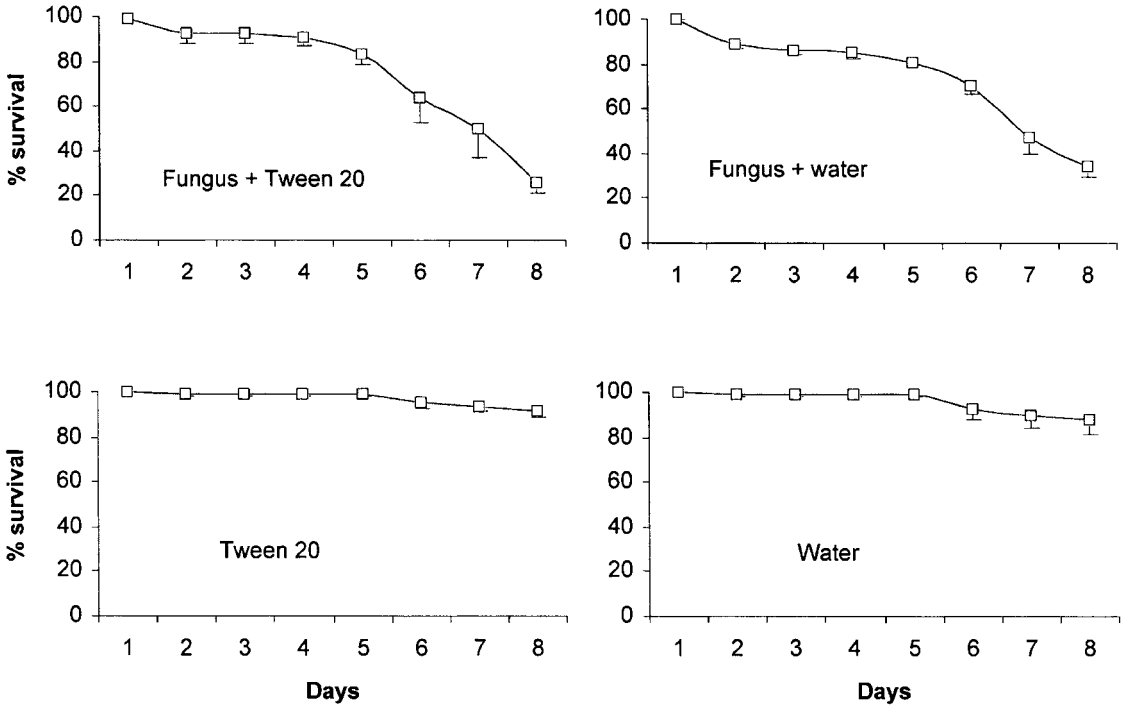

Fig. 2. Survival curves of *Brevipalpus phoenicis* females treated with *Metarhizium anisopliae* var. *acridum* suspended in Tween 20 and water. The fungus suspension was sprayed at concentration of 10^8 conidia/ml and 0.00005% Tween 20. Vertical bars = Standard Error.

TABLE 1. EGG PRODUCTION OF *Brevipalpus phoenicis* FEMALES BY THE 4TH DAY AFTER INOCULATION WITH *Metarhizium anisopliae* VAR. *acridum*. AVERAGE OF FIVE REPLICATES.

Treatment	Females alive	Eggs produced	Eggs/Female (S.E.)
Water (W)	19.8 (±0.2) a*	17.2 (±4.1) a*	0.87 (±0.21) a
Tween 20 (T)	18.6 (±0.8) a	9.64 (±3.7) ab	0.51 (±0.20) ab
Fungus + W	17.0 (±0.4) a	4.2 (±0.2) b	0.25 (±0.01) b
Fungus + T	18.2 (±0.9) a	3.8 (±1.5) b	0.20 (±0.08) b

*Means followed by the same letter do not differ at $P < 0.05$; Tukey ($\alpha = 0.05$); S.E. = Standard Error.

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