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Susceptibility of *Brevipalpus phoenicis* to entomopathogenic fungi

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Abstract The pathogenicity of 52 isolates from several fungus species was studied for the false spider mite *Brevipalpus phoenicis*. In addition, the main stages during the course of infection by *Hirsutella thompsonii*, by far the most virulent pathogen, were studied by means of light and electron microscopy. Adult mites were confined to arenas prepared with citrus leaves in acrylic dishes containing agar–water. Conidial suspensions containing 10^8 conidia/ml were applied, except for *H. thompsonii*, where a concentration of 10^7 conidia/ml was used. The *H. thompsonii* isolates caused higher mortality, with indices higher than 90%. Observations under the scanning electron microscope (SEM) were performed at 0, 6, 12, 24, 48, 72, and 120 h after application of a *H. thompsonii* suspension containing 10^7 conidia/ml. Twenty-four hours after inoculation, *H. thompsonii* conidia were observed attached to the mite's integument. The conidia germinated and penetrated through the base of the setae on the hysterosoma. Colonization occurred after 48 h, as evidenced by mortality. Conidiogenesis occurred after 120 h, with the development of mycelium and conidiophores emerging from the posterior and anterior parts of the mite.

Keywords False spider mite · Tenuipalpidae · *Hirsutella thompsonii* · Microbial control

Introduction

Citrus is one of the most important crops in Brazil, because of fruit and juice concentrate exports. The industry generates all sorts of services and other

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production-related activities (Neves 2000). One of the main obstacles limiting yield increase in citrus are pests, in particular *Brevipalpus phoenicis* (Geijskes, 1939) (Acari: Tenuipalpidae), known as the false spider mite and "flat mite." Other crops that are susceptible to *B. phoenicis* injuries in Brazil are coffee (*Coffea arabica*) (Chagas et al. 2003) and passion fruit (*Passiflora edulis*) (Kitajima et al. 1997).

This species is a polyphagous mite and a total of 486 plants were identified as hosts (Childers et al. 2003a). The importance of *B. phoenicis* is directly associated with its capacity to transmit leprosis virus, a localized-action rhabdovirus (Gonzáles 1975; Oomen 1982; Childers et al. 2003b). The virus causes chlorotic spots and necrosis on leaves, branches, and fruits, pronounced fruit shedding, and decreases in fruit weight. Depending on the intensity of infestation by the pest, the plant may become unproductive or die (Guinado and Silvério 1992; Rodrigues et al. 1994).

The most important control strategy against the disease consists of reducing the mite population through application of chemical acaricides. In 1999, the estimated costs of the control of this pest were US$ 75 million, in the State of São Paulo (Brazil) only (FNP, 2000). Intensive pesticide applications aimed at reducing mite populations in citrus have caused the development of resistance to some products and increased residues in fruits (Omoto 1998; Alves et al. 2000). These problems, together with the resurgence of pests, have transformed brazilian citriculture into a low-sustainability activity.

Microbial control agents appear desirable alternatives within the IPM context. In addition to contributing to pest control, they limit the development of resistance and minimize impact on the environment. Fungi are the most important entomopathogens that occur in phytophagous mite populations; they can be introduced, increased, or protected at locations where they are already present (Alves 1998).

Among mite-attacking fungi the most relevant examples are the Entomophthorales species and the Deuteromycetes species *Hirsutella thompsonii*, an important pathogen of eriophyoid mites (Van der Geest et al. 2000). The Entomophthorales are the most important, since they cause epizootics under field conditions. However, their mass production is still incipient, making their practical use difficult to achieve. On the other hand, even though mitosporic fungi are not frequently observed causing epizootics in the field, they can be easily produced and could be used under the inundative strategy.

Therefore, as an initial stage in a microbial control program, virulent lines with suitable characteristics to be used as microbial products can be selected by laboratory bioassays (Alves 1998). The objectives of this research were to evaluate the susceptibility of *B. phoenicis* to isolates of entomopathogenic fungi and to observe the biological cycle of one isolate on the mite.

Materials and methods

Rearing and maintaining *B. phoenicis* in the laboratory

The mite was reared on ripe fruits of "Pêra Rio" and/or "Valence" varieties (*Citrus sinensis*) maintained in the laboratory. The mites were collected from an unsprayed citrus grove located in Piracicaba, State of São Paulo (Brazil), and have been mantained in the laboratory since 2000.

The fruits were collected in a pesticide-free citrus grove, washed in running water, and dried with paper towels in order to clean and disinfest the oranges. Each fruit was submitted to a melted paraffin bath covering 2/3 of its total area, leaving an arena of approximately 4 cm diameter on the surface. This arena was delimited with a sticky barrier (Tanglefoot®, Grand Rapids, MI, USA) to prevent mites from escaping. Most fruits selected showed citrus scab symptoms on the skin, since this condition favors mite maintenance because of their cryptical habit (Childers et al. 2003b).

The fruits were then infested with 50 adult mites and placed in plastic boxes ($28 \times 43 \times 12$ cm) with a perforated styrofoam bottom. The boxes were maintained in an air-conditioned room (25 ± 5°C; 12-h photophase, and 75 ± 10% RH) for a 20-day period. After this period, old fruits were replaced with new ones.

Selection of entomopathogenic fungal isolates

The isolates used in the selection bioassays came from the laboratory's ''Pathogen Bank'' and were stored in a freezer (– 12°C) in the form of pure conidia. Fifty-two isolates were tested: *Beauveria bassiana* (16), *Beauveria brongniartii* (1), *Metarhizium anisopliae* (18), *Paecilomyces lillacinus* (5), *Paecilomyces fumosoroseus* (2), *Paecilomyces farinosus* (1), *Hirsutella thompsonii* (4), *Lecanicillium lecanii* (1), *Lecanicillium muscarum* (1), and *Sporothrix* sp. (3). These isolates came from different localities in the Southeastern, Northeastern, Central-Western, and Southern regions of Brazil, and were isolated from several host species and from the soil (Table 1).

A sample from each isolate tested was initially transferred to Petri dishes containing complete culture medium (0.36 g KH_2PO_4, 1.05 g $NaHPO_4{\cdot}7H_2O$, 0.60 g $MgSO_4{\cdot}7H_2O$, 1.0 g KCl, 10.0 g glucose, 5 g yeast extract, 20 g agar, 1,000 ml sterile water), previously autoclaved at 120°C for 20 min. After inoculation, the dishes were maintained for 10 days under controlled condition (26 ± 0.5°C; 12 h photophase and 70 ± 10% RH) for pathogen growth and sporulation.

The bioassays were performed using leaves of variety ''Pêra-Rio'' collected from a pesticide-free citrus grove. After cleaning and disinfesting, 2.6 cm diameter arenas were cut out with a metal hole punch. Each arena was placed on an acrylic plate (3.5 cm diameter) containing 5 ml of a solidified agar–water mixture (2%). In order to fix the leaf onto the agar and allow it to last longer, a border was made on the leaf using 2 ml of the agar–water mixture (2%), thus delimiting a confinement arena. Twenty *B. phoenicis* teneral females showing high mobility were transferred to each arena from the stock colony.

After preparing the arenas and transferring the mites, the plates were sprayed with a suspension containing 10^8 conidia/ml for all isolates tested, with the exception of *H. thompsonii*, whose isolates were tested at a concentration of 10^7 conidia/ml. Five plates containing 20 mites were sprayed for each isolate, totaling 100 mites per treatment. The spray was performed in a Potter Spray Tower (Burkard Manufacturing, Rickmansworth, Hertfordshire, United Kingdom), calibrated at 1.034 bar and 2 ml of the conidial suspensions were used (0.2 μl suspension/cm^2).

In the control treatment sterile water with spreader-sticker at 0.01% (Tween 40®) was applied. In addition, a standard isolate of *B. bassiana* (ESALQ-447—ARSEF/USDA-APHIS 2722) was used in all bioassays, sprayed at a concentration of 10^8 conidia/ml.

Table 1 Entomopathogenic fungal isolates used in the selection bioassays against *Brevipalpus phoenicis*

Species	Identification (ESALQ)	Origin (Brazil)	Host
Beauveria bassiana	447	Cuiabá (MT)	*Solenopsis invicta*
Beauveria bassiana	307	Araras (SP)	*Diatraea saccharalis*
Beauveria bassiana	457	Goiás (GO)	*Euchistus heros*
Beauveria bassiana	957	Piracicaba (SP)	Unknown
Beauveria bassiana	1036	Porto Alegre (RS)	*Solenopsis* sp.
Beauveria bassiana	1083	Londrina (PR)	Soil
Beauveria bassiana	1208	Piracicaba (SP)	Auchenorryncha
Beauveria bassiana	1248	Bebedouro (SP)	Auchenorryncha
Beauveria bassiana	1249	Boa Esperança do Sul (SP)	Phasmatodea
Beauveria bassiana	1250	Piracicaba (SP)	Coleoptera
Beauveria bassiana	1258	Caçú (GO)	*Leptopharsa heveae*
Beauveria bassiana	1259	Unknown	Auchenorryncha
Beauveria bassiana	1260	Itiquira (MT)	*Leptopharsa heveae*
Beauveria bassiana	1273	Itatinga (SP)	*Stictoscarta dissimilis*
Beauveria bassiana	1284	Espírito Santo (ES)	*Rhynchophorus palmarum*
Beauveria bassiana	PL63	Piracicaba (SP)	*Atta* sp.
Beauveria brongniartii	621	Alambari (SP)	*Solenopsis saevissima*
Metarhizium anisopliae	319	Goiânia (GO)	*Mahanarva posticata*
Metarhizium anisopliae	798	Cuiabá (MT)	*Solenopsis invicta*
Metarhizium anisopliae	860	Piracicaba (SP)	*Macropsis* sp.
Metarhizium anisopliae	1037	Porto Alegre (RS)	*Solenopsis* sp.
Metarhizium anisopliae	1076	Arapongas (PR)	Soil
Metarhizium anisopliae	1104	São João do Piauí (PI)	Soil
Metarhizium anisopliae	1172	Córrego Rico (SP)	Soil
Metarhizium anisopliae	1189	Corumbá (MT)	Soil
Metarhizium anisopliae	1203	Pernambuco (PE)	Blattodea
Metarhizium anisopliae	1204	Piracicaba (SP)	*Mahanarva fimbriolata*
Metarhizium anisopliae	1220	Piracicaba (SP)	Auchenorryncha
Metarhizium anisopliae	1247	Turvínia (SP)	Soil
Metarhizium anisopliae	1256	Concórdia (SP)	Auchenorryncha
Metarhizium anisopliae	1286	Valparaíso(SP)	*Mahanarva fimbriolata*
Metarhizium anisopliae	E6	Pernambuco (PE)	*Diatraea saccharalis*
Metarhizium anisopliae	E9	Boca da Mata (AL)	*Mahanarva posticata*
Metarhizium anisopliae	PL43	Fleixeira del Estado (Bolívia)	*Mahanarva* sp.
Metarhizium anisopliae	PL47	Rio de Janeiro (RJ)	*Mahanava posticata*
Paecilomyces lillacinus	508	Piracicaba (SP)	*Solenopsis* sp.
Paecilomyces lillacinus	581	Cuiabá (MT)	*Solenopsis invicta*
Paecilomyces lillacinus	623	Cuiabá (MT)	Soil
Paecilomyces lillacinus	774	South of Brazil	*Solenospsis* sp.
Paecilomyces lillacinus	827	Piracicaba (SP)	Soil
Paecilomyces fumosoroseus	1200	Amélia Rodrigues (BA)	*Mahanarva fimbriolata*
Paecilomyces fumosoroseus	1297	Ipeúna (SP)	*Lagria vilosa*
Paecilomyces farinosus	1205	Santa Fé do Sul (SP)	*Bemisia tabaci*
Hirsutella thompsonii	1221	Itiquira (MT)	*Calacarus heveae*
Hirsutella thompsonii	1266	Pindorama (SP)	*Calacarus heveae*
Hirsutella thompsonii	1269	Caçú (GO)	*Calacarus heveae*
Hirsutella thompsonii	1282	Caçú (GO)	*Calacarus heveae*
Lecanicillium lecanii	870	Piracicaba (SP)	*Coccus viridis*
Lecanicillium muscarum	972	Piracicaba (SP)	*Coccus viridis*
Sporothrix sp.	1224	Brasília (DF)	*Leptopharsa heveae*
Sporothrix sp.	1229	Itiquira (MT)	*Leptopharsa heveae*
Sporothrix sp.	1230	Itiquira (MT)	*Leptopharsa heveae*

After the spray, the plates were maintained for a few minutes in a room to allow the suspension to dry and were then transferred to closed plastic boxes (11 × 33 × 20.5 cm) with a moistened paper towel sheet on the bottom and maintained under controlled condition (26 ± 0.5°C; 12-h photophase and 70 ± 10% RH) during the entire evaluation period. Daily evaluations were made until the sixth day after application, and the number of dead mites on each leaf disc was recorded.

The dead mites were sorted, transferred to acrylic containers holding the agar–water mixture (2%) and maintained in an incubator (26 ± 0.5°C; 12-h photophase; 70 ± 10% RH) for 5 days to confirm death by the fungus. After sporulation, each individual was transferred to a slide containing Hoyer's medium + lactophenol-cotton blue (2:1) and was observed under the optical microscope for pathogen structure visualization.

The mortality values were corrected in relation to control treatment using the formula proposed by Schneider-Orelli (1947).

Hirsutella thompsonii biological cycle stages on *B. phoenicis*

Some *H. thompsonii* (ESALQ-1269) developmental stages on *B. phoenicis* adults were studied in the laboratory by scanning electron microscopy (Zeiss Leo 435 VP). In this experiment arenas consisting of variety ''Pêra Rio'' citrus leaves were used as substrate for the mites, following procedures as described above.

Adults from the stock colony were transferred to the leaves (15 adults/leaf arena) and sprayed with a suspension containing 10^7 conidia/ml, using a Potter Spray Tower. After applying the pathogen, the plates were placed into plastic boxes with a moistened paper towel sheet on the bottom and stored under controlled condition (26 ± 0.5°C, 70 ± 10% relative humidity, and 12-h photophase).

After 0, 6, 12, 24, 48, 72, and 120 h from inoculation, each group of three plates was removed from the incubator and stored at – 40°C to cause mite death and preserve the material. A corresponding stub was prepared for each plate, containing a piece of the leaf with a variable number of mites.

After this preparation, the samples were fixated in osmium tetroxide vapor (OsO_4) for 48 h and left for 72 h in a glass dehumidifier with silica-gel to maintain relative humidity near 0%. Later, the material was sputtered with gold in a Balzers Evaporator (MED 010) for 120 s and observed under the electron microscope.

Results and discussion

Selection of entomopathogenic fungal isolates

Based on *B. phoenicis* mortality values the isolates were separated into two groups: the first combined *B. bassiana*, *B. brongniartii*, *M. anisopliae*, *Sporothrix* sp., *L. lecanii*, *L. muscarum*, and *Paecilomyces* spp. strains; the second group combined the *H. thompsonii* strains (see Fig. 1). Isolates from the second group caused 90–100% mite mortality, 6 days after inoculation. Isolates from the first group caused mite mortality of less than 30%, ranging from 0% to 29.3% after the sixth day from application. Despite the great genetic variability of these isolates, the pathogenicity and virulence of an isolate are not related to its origin. The isolate may cause high

mortality to a wide array of hosts (Moino Junior et al. 1998; Almeida et al. 1997; Tamai et al. 1999; Alves et al. 2005). This variation in mortality is frequently related to factors such as low virulence, specificity, and host tolerance, among others (Alves 1998).

Intraspecific variation was observed among the *B. bassiana* and *M. anisopliae* isolates, because the mean mortality values observed on days 3 and 6 after application were 2% and 10.4%, and 3.9% and 15.1%, respectively. There are few reports in the literature on the natural occurrence of these fungi in phytophagous mites, but they are found in ticks (Chandler et al. 2000). Still, some mite species are very susceptible to these fungi.

The ESALQ-447 *B. bassiana* isolate, used as a standard in the experiments, yielded a mean corrected mortality of 9.7% on day 6 after application. For *T. urticae*, this isolate caused 22.5% mortality on the sixth day after application, thus being considered low virulent against this species (Tamai et al. 1999). In *P. oleivora*, however, this fungus caused 91.4% mortality when applied at a concentration of 1×10^8 conidia/ml (Alves et al. 2005). In some insect species, the same isolate presents good effectiveness and has been selected as promising in microbial control programs (Stimac et al. 1989; Alves 1998).

The *M. anisopliae* isolates ESALQ-1104, ESALQ-1203, and ESALQ-1204, and *L. muscarum* isolate ESALQ-972 caused a considerable increase in mite mortality after the third day from inoculation, but on day 6 live mites with infection symptoms could still be observed.

The main advantage of using *M. anisopliae* or *L. muscarum* to control *B. phoenicis* in the field is the ease of large-scale production, allowing the use of these agents in an inundative manner (Alves and Pereira 1998). Pathogenic but low-virulence

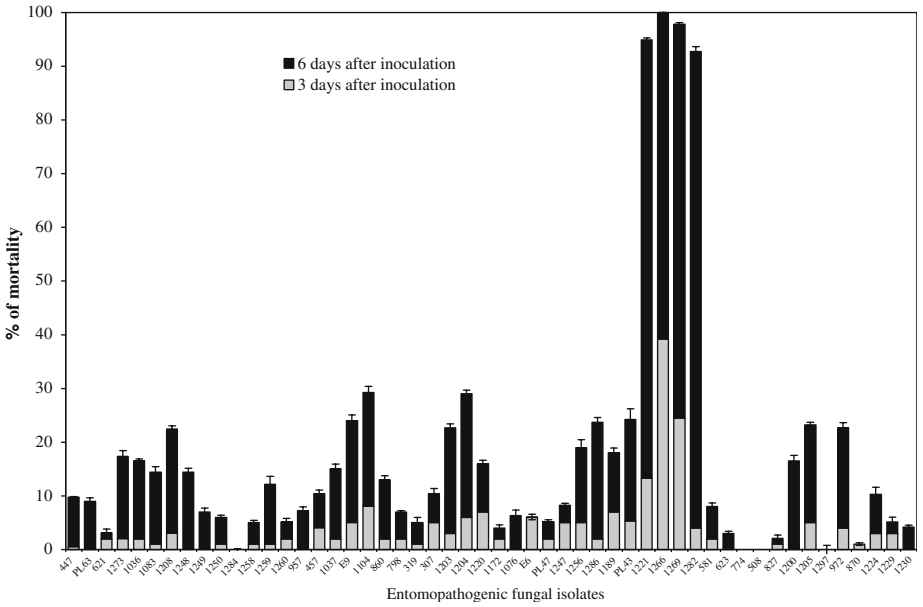

Fig. 1 Corrected mortality (± SEM) of *Brevipalpus phoenicis* inoculated with different fungal isolates after 3 and 6 days from inoculation

lines can be used against mites that cause direct damage to the crop, as long as the interval between application and effective control does not compromise crop yield. However, the adoption of this strategy against *B. phoenicis* in citrus could be risky due to the mite's capacity to transmit a rhabdovirus (Childers et al. 2003b). Because this is a persistent-circulative mode of transmission, the time between virus acquisition by the mite and virus transmission is shorter than the time required for mite infection and subsequent death, and this could compromise disease control effectiveness. On the other hand, the change in behavior of hosts infected by fungi, particularly in relation to reduced feeding, is an aspect that may reduce the rate of virus transmission by the mite (Hajek and Leger 1994). Therefore, the association of these fungi with compatible acaricides, as long as accompanied by a synergistic effect, could be evaluated for resistance management (Alves et al. 2000).

Even applied at a concentration 10 times lower than the other fungi (10^7 conidia/ml), the *H. thompsonii* isolates were effective to control *B. phoenicis*. This result indicates greater virulence of this species, per infective unit, compared to the others. The application of suspensions containing 10^6 conidia/ml of different *Hirsutella* spp. isolates against *Varroa destructor*, a parasitic mite on bees, provided mortality rates of 97% (7 days after application), similar to those obtained with the application of 10^8 conidia/ml of *M. anisopliae*, *Lecanicillium* spp., *B. bassiana*, and *Paecilomyces* spp. (Shaw et al. 2002). This result is interesting because it demonstrates the high specificity of the genus *Hirsutella* to Acarina.

With the exception of isolate ESALQ-1282 expressive mortality rate increases were observed on day 3 after application of the pathogen. On the last evaluation day, the total, cumulative, and corrected mortality indices were higher than 90% for isolates ESALQ-1221, ESALQ-1282, and ESALQ-1269, and 100% for ESALQ-1266 (Table 2).

The high virulence and specificity of *H. thompsonii* against mites could be related to a series of events resulting from the pathogen–host relations cycle. The action of secondary metabolites produced during the colonization process of *Hirsutella* spp. could be one of the factors responsible for the promptness with which this fungus is capable of killing *B. phoenicis* adults, as already observed for other organisms (Vey et al. 1993; Alves 1998; Omoto and McCoy 1998).

The mites infected by *M. anisopliae*, *B. bassiana*, *B. brongniartti*, *Paecilomyces* spp., and *Sporothrix* sp. showed alterations in integument color and movement, with conspicuous ventral stiffening of the legs. When disturbed by the touch of a brush or by direct incidence of light, the diseased individuals exhibited a behavior of ''throwing'' their bodies back, maintaining only the hind legs adhered to the substrate. For *L. muscarum*, on the fifth day from inoculation, the mites showed

Table 2 Cumulative and corrected mortality (± SEM) of *Brevipalpus phoenicis* inoculated with a *Hirsutella thompsonii* suspension containing 10^7 conidia/ml

Isolates	Days after inoculation				
	2	3	4	5	6
1221	4.0 ± 0.63	13.3 ± 0.55	45.1 ± 1.81	68.4 ± 1.66	95.9 ± 0.45
1266	1.0 ± 0.40	39.2 ± 2.52	78.5 ± 1.64	98.9 ± 0.20	100 ± 0.0
1269	2.0 ± 0.40	24.5 ± 2.42	74.5 ± 1.71	94.7 ± 0.45	97.9 ± 0.24
1282	0.0 ± 0.0	4.0 ± 0.20	52.0 ± 1.60	81.8 ± 1.03	92.8 ± 0.87

mycelial growth across the whole idiosoma region and sluggishness of movement, besides the previously described symptoms. All dead individuals showed ventrally stiffened legs and alterations in the color of the integument.

The symptoms and signs of the disease caused by *H. thompsonii* were also observed from the third day of inoculation in dying or dead mites, which were very similar to what was verified with the other fungus species. Under the optical microscope, the presence of hyphae could be observed inside and outside the body of the host, with the presence of conidiophores emerging through the mouth and hind openings. According to the literature, when infected by *H. thompsonii*, the eriophyid mites *P. oleivora*, *Aceria vaccinii*, and *C. heveae* show similar symptoms to those observed in *B. phoenicis* (Lipa 1971; Baker and Neunzig 1968; Tanzini et al. 2000).

Hirsutella thompsonii biological cycle stages on *B. phoenicis*

After applying a conidial suspension of *H. thompsonii* (ESALQ-1269), we observed some conidia attached to the citrus leaf and also to the dorsal part of the mite's integument, especially on lateral depressions of the propodosoma and hysterosoma (Fig. 2a, b). The conidia were sphere-shaped, approximately 2.3 μm in diameter, marked with protuberances across the entire surface, similar to those observed by McCoy et al. (1984). Although conidia with these characteristics are considered "typical" of *H. thompsonii*, some variation may occur within the same species (Baker and Neuzing 1968). The conidia are covered with a mucilaginous substance that protects against desiccation, facilitates their adherence to the host surface, and helps the infection process (Van der Geest et al. 2000; Alves 1998).

Germination started between 0 h and 6 h from inoculation. At this stage, we observed some conidia attached near the marginal setae of the mite's hysterosoma, and also some germinated conidia with the apparent formation of an appressorium (Fig. 2c). These structures are considered adaptations of the microorganism, which concentrates physical energy and high metabolic and enzymatic activity over a small area of the host to make the penetration process more effective (Hajek and Leger 1994). Because the *B. phoenicis* integument is thick and quite coarse throughout, the formation of these structures during conidium germination may have been stimulated. This process has been demonstrated for *M. anisopliae* infecting *Manduca sexta* larvae (Lepidoptera: Sphingidae) (Leger et al. 1991).

The main sites of penetration of *H. thompsonii* on *B. phoenicis* were the base of the setae on the hysterosoma, due to the large number of germinated conidia present at those locations. Between 6 h and 12 h from inoculation, most conidia attached to the mite's integument showed the characteristic formation of germ tubes (Fig. 2d). Germination time and velocity are variable traits among different species of entomopathogenic fungi, and may occur in at least 12 h at temperatures between 23°C and 30°C for most Deuteromycetes (Alves 1998).

Mycelial and hyphal growth were observed between 24 h and 48 h from inoculation at some regions of the dorsum of *B. phoenicis*, especially on the intersegmental setae, foreleg, and gnathosoma regions. During this period, the mean cumulative mortality indices were 24.5% on the third day (72 h) and 74.5% on the fourth day (96 h) after application of the pathogen, indicating that intense invasion by the fungus in the mite's hemocoel and events that culminated in mite death

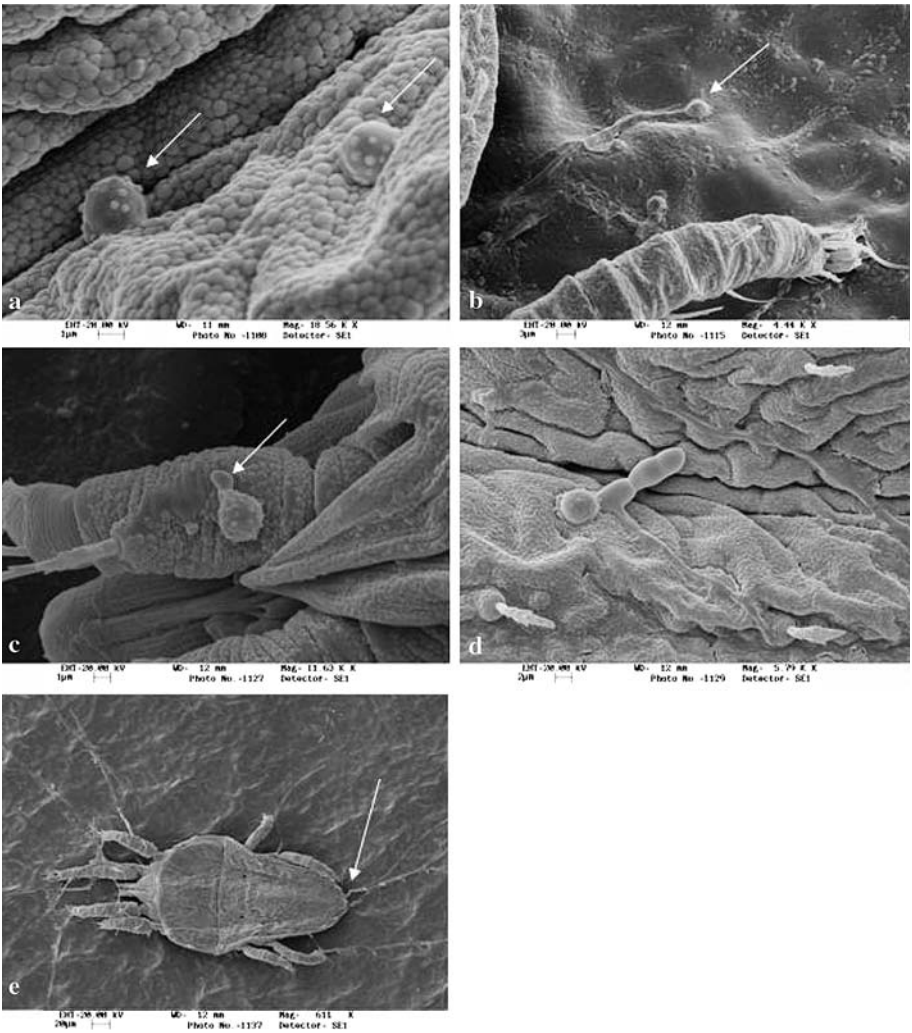

Fig. 2 (**a**) *Hirsutella thompsonii* conidia attached to the integument of *Brevipalpus phoenicis*. (**b**) Germinated conidium attached to a citrus leaf. (**c**) Conidium attached to the gnathosoma region of *B. phoenicis* with apparent formation of appressorium. (**d**) Conidium between 6 h and 12 h from inoculation, with the presence of germ tube. (**e**) Conidiogenesis and horizontal fungus dissemination 120 h after inoculation

occurred during that period (24 h). These events are described as a combination of mechanical damages that occur due to fungus growth, host nutrient depletion, and action of toxic metabolites (Hajek and Leger 1994). Colonization time also varies between 72 h to 120 h depending on host, pathogen, and environmental conditions (Alves 1998).

The extrusion and conidiogenesis of *H. thompsonii* occurred 120 h after application (Fig. 2e). Many hyphae were observed emerging from the gnathosoma and opisthosoma of *B. phoenicis*, probably from the oral, anal, and/or genital openings. The dead mites showed all legs clearly stiffened ventrally, with a ''wilted'' appear-

ance. The *H. thompsonii* conidiophores formed during the conidiogenesis process consisted of a single phialide of approximately 270 μm to which the conidium was attached. Non-typical phialides may also occur in this fungus (McCoy et al. 1984). Once out of the body of the host, the pathogen showed intense growth throughout the substrate, with the formation of mycelium that extended up to 0.5 cm from the mite practically in all directions. In the eriophyid mite *C. heveae*, the hyphae and conidiophores formed from dead mites were found at distances up to 15 times the body length (Tanzini et al. 2000). These characteristics of the pathogen can favor the dissemination of its reproductive structures in the environment, facilitating the horizontal transmission of the disease in a population.

Greater data precision about the biological cycle of the fungus can only be obtained with the combined use of electron/fluorescence microscopy techniques.

Concluding remarks

In laboratory bioassays, the false spider mite, *B. phoenicis*, presented low susceptibility to several species of mitosporic fungi frequently used as microbial control agents, such as *B. bassiana* and *M. anisopliae*. However, isolates of *H. thompsonii* were highly virulent to *B. phoenicis* adults. These observations indicate the potential of *H. thompsonii* as a sustainable environment friendly alternative to chemical acaricides for the control of *B. phoenicis* in citrus orchards.

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