

Germination Behavior of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) on *Bemisia tabaci* (Hemiptera: Aleyrodidae) Nymphs¹

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Abstract *Beauveria bassiana* (Balsamo) Vuillemin is an important entomopathogenic fungus of *Bemisia tabaci* (Gennadius) biotype B. The pathogenesis of an isolate (C#1) of *B. bassiana* in *B. tabaci* was observed on the body using scanning electron microscopy, whereas the development of the fungus inside the whitefly body was studied using light microscopy with histological sections of infected insect bodies. The infection process was closely associated with the specific regions of the whitefly nymph surface. Conidia accumulate in high densities on the intersegmental membranes. In the vasiform orifice area, conidia seldom adhere to the cuticle except in areas adjacent to the dorsum, but seldom ($6.3\% \pm 1.2$) germinate. Germinating conidia and some germ tubes with mucoid materials were seen in the lateral areas 10 h ($85.0\% \pm 4.5$) after conidia application. Appressoria began to form on the cuticle in 12 h, and more appressoria were formed on the dorsum and the intersegmental membrane areas of the dorsal surface. Germinated conidia could be observed in the same area 24 h later. Penetration of the cuticle occurred 36 h ($79.2\% \pm 6.9$) after conidia application. After germination, the germ tubes oriented to the cuticle and penetrated quickly. Fungal blastospores were observed under the cuticle 36 and 48 h after inoculation and eventually filled the body of the nymph. The nymphs died by the time a red pigment appeared in their bodies, ≈ 96 h after inoculation. By 120 h after treatment, large quantities of conidia were found on the surfaces of the whitefly nymphs.

Key Words *Bemisia tabaci*, *Beauveria bassiana*, surface topography, SEM, infection

The tobacco whitefly, *Bemisia tabaci* (Gennadius), is one of the most important pests of various field crops, vegetables and ornamental crops around the world. Apart from inflicting direct damage by extracting juices from plants, it transmits various plant viral diseases, and secretes honeydew that supports sooty mold growth that reduces plant photosynthesis (Osborne and Landa 1992, Faria and Wraight 2001, Varma and Malathi 2003). The development of pesticide resistance by *B. tabaci* and the increasing regulation of chemical pesticide use for their control have sparked interests, research and investments in biological control with parasitoids, predators, and mycopathogens, such as *Beauveria bassiana* (Balsamo) Vuillemin.

Beauveria bassiana is a common entomopathogen of many different insects (McCoy et al. 1988) and has proved to be a promising entomopathogenic fungus for

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microbial control of the tobacco whitefly under laboratory, greenhouse and field conditions (Osborne and Landa 1992, Landa et al. 1994, Lacey et al. 1996, Wraight et al. 2000, 1998, Quesada-Moraga et al. 2006, Liu and Stansly 2009). Studies have been conducted to better understand the toxicology of the toxins and insecticidal proteins that *B. bassiana* produces in vivo (Fugent et al. 2004, Vey et al. 2001) to use this entomopathogen more effectively in pest management programs. Attempts to improve isolate virulence and survival time in the field has not been successfully achieved (Quesada-Moraga et al. 2006).

Our objective of this study was to elucidate the germination and infection processes of the conidia on the third instar *B. tabaci* nymphs. This is crucial to understand the action mode on the target insect pests and to evaluate the biological control potential of the *B. bassiana* to whitefly in fields.

Materials and Methods

The tobacco whitefly, *Bemisia tabaci* (Gennadius) biotype B, was used as the host insect for these studies. Insect colonies were cultured on cabbage, *Brassica oleracea* var.

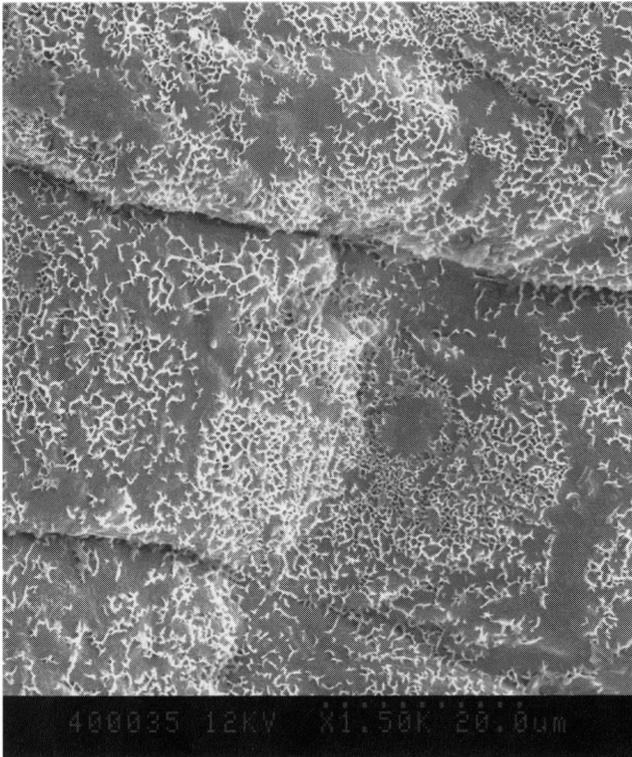


Fig. 1. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing ridges (r), protrusions (p), and sparsely scattered waxy deposits (w).

acephala L., in a greenhouse. To obtain plants with homogeneous, heavy infestations of *B. tabaci* for the experiments, uninfested cabbage plants with 4 - 5 leaves were provided to *B. tabaci* adults for oviposition. Twenty-four hours later, all adults were cleaned using a vacuum machine (Black & Decker Dust Buster, Towson, MD), and the plants with eggs and subsequent nymphs were maintained in growth chambers (GXZ-260B, Jiagnanyiqi, Ningbo, Zhejiang) at $25 \pm 1^\circ\text{C}$ with a photoperiod of 10:14h (L:D) and $60 \pm 5\%$ RH.

The *B. bassiana* isolate, C#1, used in the investigations was originally isolated from locusts, *Locusta migratoria manilensis* (Meyen), from Jiamusi, Helongjiang, China. A monoconidial culture of the isolate was selected and grown on potato dextrose agar (PDA) at 25°C in darkness. This stock culture was stored at 4°C . Conidia which had been sub-cultured for 6 times on the whitefly nymphs from the stock culture of C#1 onto PDA in glass tubes (155 long \times 15 mm diam) at 25°C in complete darkness during the first 7 days of growth and then in total light in the next 7 days of growth. Conidial suspensions for the experiments were prepared by scraping conidia from the agar surfaces of the 14-d-old cultures into an aqueous solution of 0.1% polysorbate (Tween-80™, ScienceLab.com, Houston, TX, USA). Concentration of conidia per milliliter of the suspension was determined using a counting chamber or hemocytometer under a microscope. Conidial viability

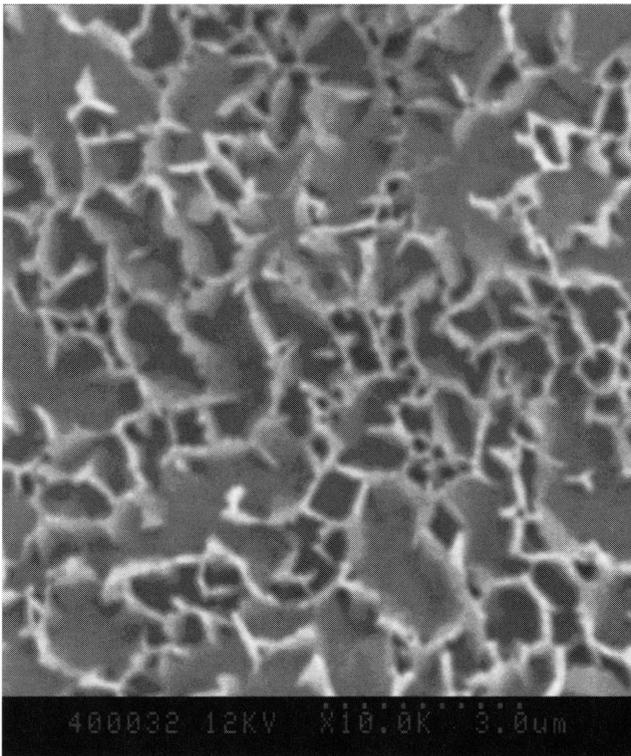


Fig. 2. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing the dorsal planar area with extensive scale and the membranes.

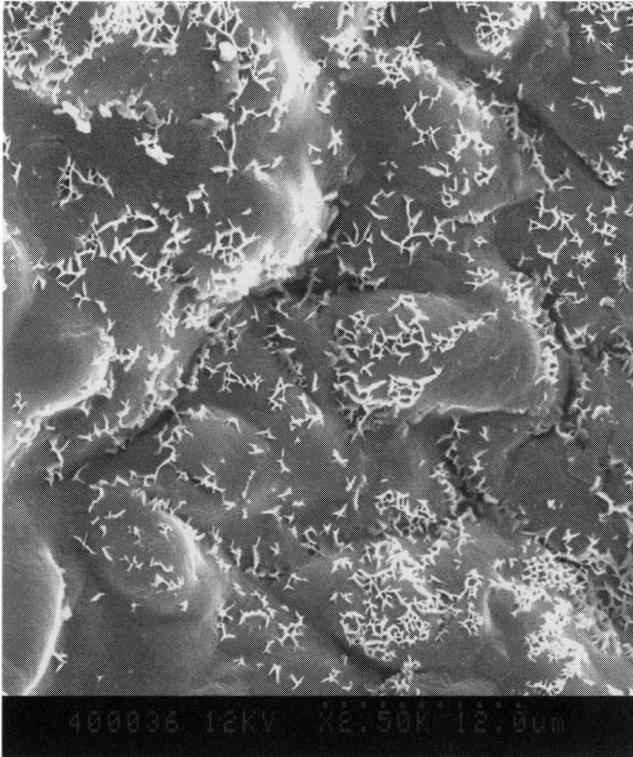


Fig. 3. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing the extensive scales on the dorsal planar area.

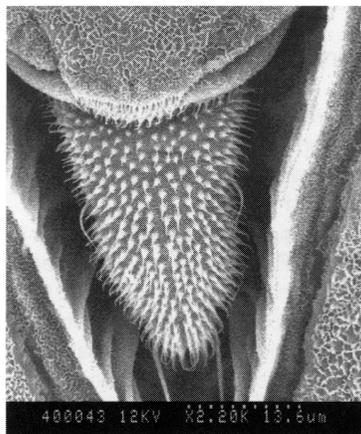


Fig. 4. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing the vasiform orifice with spinous structure.

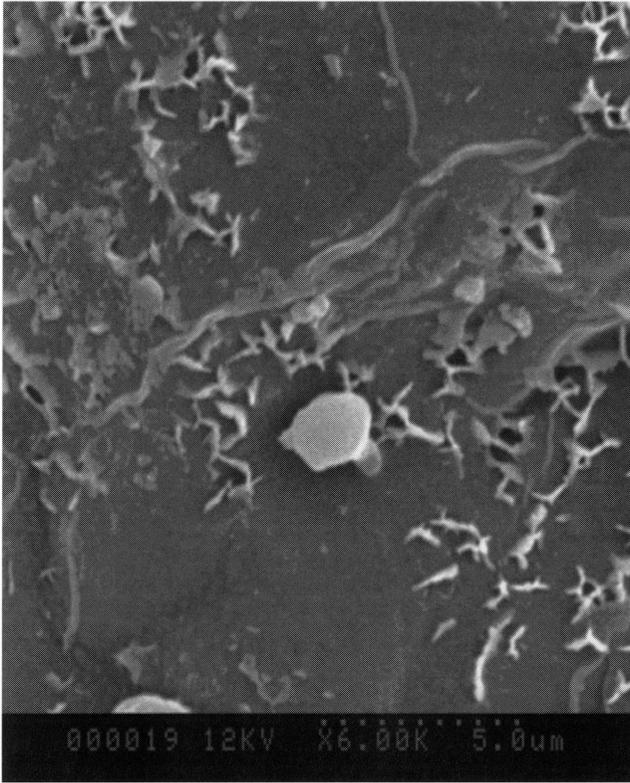


Fig. 5. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing germinating conidia with mucoid material in lateral area.

was assessed using a germination test in 0.1% glucose (w/v) solution containing 0.1% Tween-80 (v/v).

Cabbage leaves infested with third-instar *B. tabaci* nymphs were selected and immersed in a conidial suspension of 1×10^8 conidia/ml for 10 sec. Twenty nymphs each were removed from the leaves with a soft brush at 8 and 10 h, and then at 12-h intervals after the fungus was applied for 6 d.

The histopathology of the fungus on the surface of the insects was observed using scanning electron microscopy (SEM). Insects ($n = 30$) used for these observations were removed from the treated cabbage leaves and prepared for SEM observation using the following procedure: nymphs were fixed in 3% glutaraldehyde for 8 h, then rinsed in H_3PO_4 buffer, postfixed in 1% osmium tetroxide for 1 h, dehydrated in a series of graded ethanol, and transferred to isoamyl acetate for CO_2 critical point drying. Prepared nymphs were observed using a Hitachi H-570 SEM.

The internal infection process was studied using histological cross sections of infested nymphs. Nymphs ($n = 30$) were removed from the infested cabbage leaves and immersed and fixed in Bouin's solution for 24 h, rinsed in 50% methanol 3X followed by immersion in 70% methanol, at which point the yellow coloration of the specimens

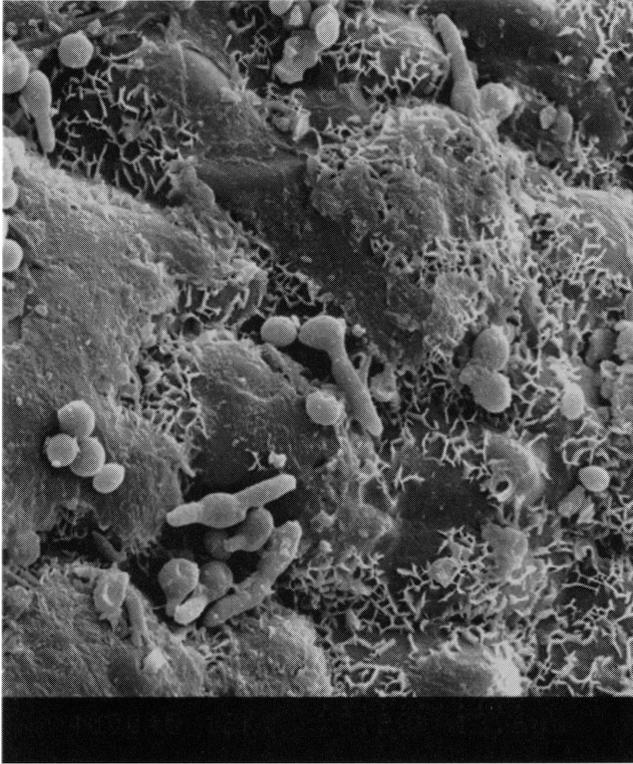


Fig. 6. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing unidirectional germinating conidia in the lateral area.

completely disappeared. Nymphs were then dehydrate by immersion in serial dilutions of 75, 80 and 95% ethanol (3X each) followed by immersion in 100% ethanol for 10 min. Specimens were embedded in paraffin and serially sectioned into 8- μ m sections using a rotary microtome. Sections were mounted on glass slides, stained with Kings Hematoxylin-Eosine solution, and examined at 600X using a transmission light microscope. Selected fungal and insect structures observed in the microscopic studies were photographed (DSC-F707, Sony, Tokyo, Japan).

Results

SEM micrographs showed that the cuticular surface of third-instar *B. tabaci* nymphs varies with respect to body regions. The lateral areas and the peripheral margins of the dorsal cuticle are covered with numerous knurs (knots) and sparsely scattered waxy deposits (Figs. 1, 2). The ventral area of the body, including the compound eyes, mouthparts, legs, and abdomen, is characterized by dense waxy deposits and a relatively flat surface (Fig. 3). The vasiform orifice, an ovate triangular opening on the dorsum of the last abdominal segment, has a pronounced caudal furrow and extensive spinous structures (Fig. 4).

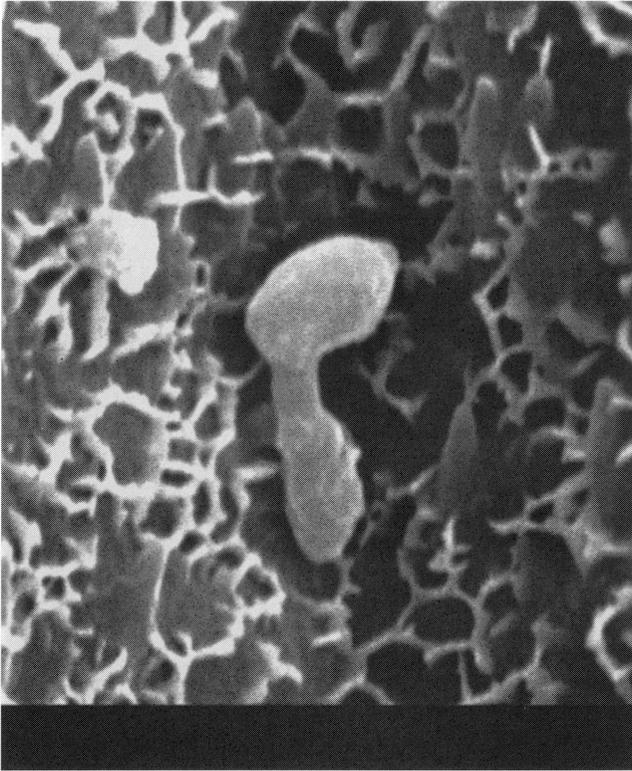


Fig. 7. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing appressorium structures in the dorsal planar area.

The process of conidial germination differs with respect to body region or area. Conidia accumulate in high densities on the intersegmental membranes; most of these conidia germinate unidirectionally (Fig. 5). However, at the irregularly crenulated margin, conidia geminate both bidirectionally and unidirectionally (Fig. 6). In the vasiform orifice area, conidia seldom adhere to the cuticle except in areas adjacent to the dorsum, where we observed conidia, but seldom ($6.3\% \pm 1.2$) found germinating conidia.

Germinating conidia, as well as some germ tubes with mucoid materials, were seen in the lateral areas 10 h ($85.0\% \pm 4.5$) after the conidial suspensions were applied on the whitefly nymphs. Twelve hours after inoculation, appressoria began to form on the cuticle, and more appressoria were formed on the dorsum and the intersegmental membrane areas of the dorsal surface (Figs. 7, 8, 9). Bidirectionally-germinated conidia could be observed in the same area 24 h later (Fig. 6). Penetration of the cuticle was observed 36 h ($79.2\% \pm 6.9$) after the conidia were applied to the whitefly nymphs (Fig. 10).

After germination, the germ tubes oriented to the cuticle and penetrated quickly (Figs. 9, 10). Fungal blastospores were observed under the cuticle 36 h and 48 h after inoculation (Fig. 11) and eventually filled the body of the nymph (Fig. 12). The nymphs died by the time a red pigment appeared in their bodies, approximately 96 h after of



Fig. 8. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing germ tube orientate to the cuticle.

inoculation. After that, fungal hyphae grew into muscle tissue and the internal organs. Mycelia also were observed growing back through the cuticle to the exter of the insect and eventually forming conidiophores and producing a few conidia. By 120 h after treatment, large quantities of conidia were found on the surfaces of the whitefly nymphs.

Discussion

The physicochemical characteristics of insect cuticle reportedly affect germination of entomopathogenic fungi, including *B. bassiana*. Ferron (1985) argued that chitin and certain fatty acids in the cuticle were required to meet the carbon needs of germinating conidia on the insect cuticle. Some cuticular components, such as medium-chain and short-chain fatty acids, are reported to be inhibitory to conidial germination (Pedersen 1970, Rolinson 1954, Teh 1974). According to James et al. (2003), the long-chain wax esters produced by whitefly nymphs could affect the germination of *B. bassiana* conidia on the tobacco whitefly by reducing moisture availability to the conidia on the cuticle surface. We surmise that the *B. bassiana* conidia germinated on the whitefly nymphal cuticle 10 h after inoculation, releasing specific enzymes that digested and degraded the insect cuticle as the germ tube penetrated the cuticle.

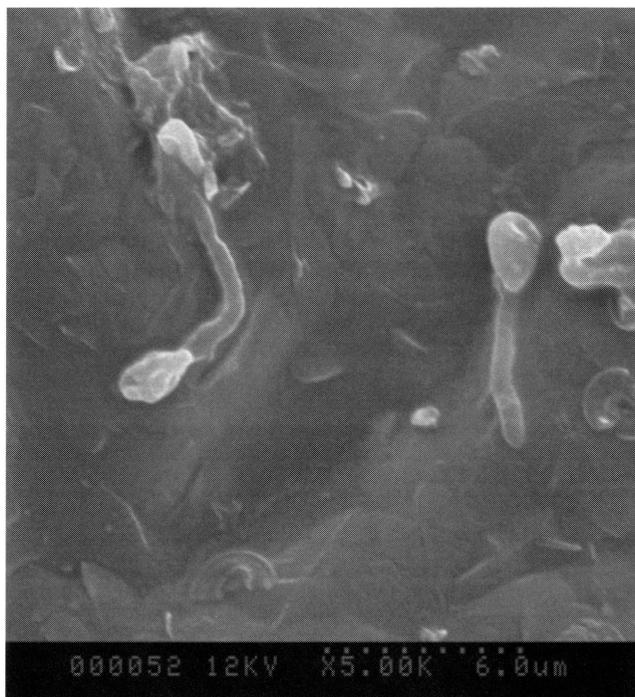


Fig. 9. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing penetrated hypha on the cuticle (36 h).

He et al. (2005) reported that the pathogenicity of *B. bassiana* to the leaf beetle, *Phaedon brassicae* Baly (Coleoptera: Chrysomelidae), was enhanced with increased relative humidity. Liu and Stansly (2009) also found that high relative humidity is critical for pathogenicity of *B. bassiana* on *B. tabaci* nymphs. James et al. (2003) noted that the infection rate of germinated *B. bassiana* conidia placed on whitefly nymph cuticle was higher than that germination rate of ungerminated conidia placed on the cuticle. They attributed this result to reduced conidial germination on the cuticle of *B. tabaci* nymphs. We also observed some ungerminated conidia on host cuticle. This lack of germination may be due to lack of viability of those conidia, conidia-host cuticle interactions, relative humidity near the cuticular surface, or any combination.

It has been reported that some compounds secreted by fungi are the components of signal transduction pathways that are required for fungi sensing the environment (Pedrini et al. 2007). In our study, we observed different conidial germination behaviors in different areas of the cuticle surface of *B. tabaci* nymphs, so perhaps different cuticular components or physical characteristics interact with the conidia causing the various germination responses observed. However, we do not know the exact cuticular chemicals inducing these differences, and this was beyond the scope of our study. Having observed the germination behavior of different fungal isolates, Talaie-Hassanloui et al. (2006) reported that conidia with only one germ tube can provide a greater physical force and accumulation of enzymes for penetration and, thus, possessed

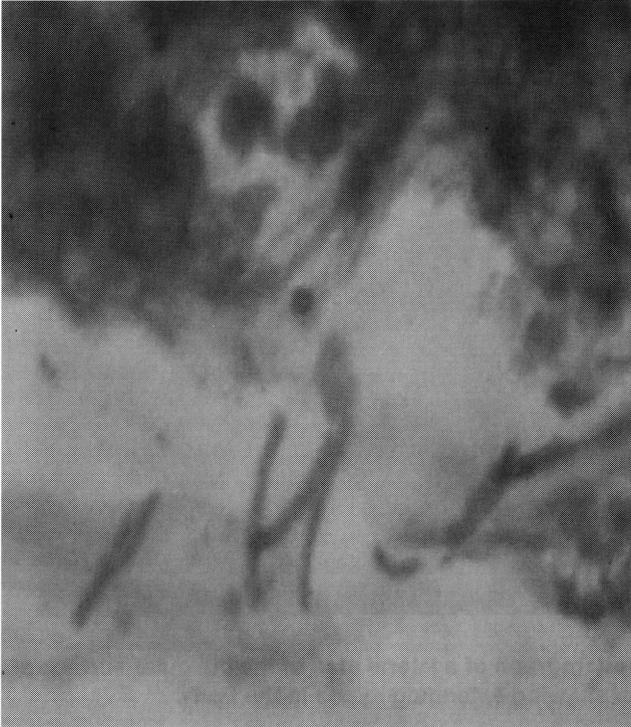


Fig. 10. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing extending blastopores under the cuticle.

greater virulence toward the tobacco whitefly than other isolates. Therefore, additional investigations should be conducted to elucidate the physicochemical effects of the cuticle on conidial germination to enhance the pathogenicity of *B. bassiana* on *B. tabaci* nymphs.

Fargues (1984) proposed that conidial adhesion occurs in 3 successive stages. In our study, germinating conidia and various appressorial structures were observed in 10 and 12 h after inoculation, indicating that the conidia have successfully adhered to the host cuticle. Blastospores were observed under the body wall at 36 h after inoculation, demonstrating that the host cuticle had been successfully penetrated by the germinating conidia. We, however, did not directly observe germ tubes actually penetrating the cuticle. The results of Charnley (1984) are similar to ours. In this condition, we assume that the adhesion should have taken place 10 h after inoculation. Whitefly nymphs infected by *B. bassiana* turn an intensive red color 96 h after inoculation, a phenomenon also observed by Wraight et al. (1998) who attributed it to the red pigment of oosporein produced by the fungus and possesses antibiotic properties and perhaps aids in the progression of the disease condition.

Sporulation of the C#1 strain on *B. tabaci* nymphs occurs 96 h after the host was inoculated with fungal conidia. This demonstrates that the C#1 strain of *B. bassiana* has the capability of completing its life cycle within 96 h, and has a considerable epizootic potential under natural environmental conditions.

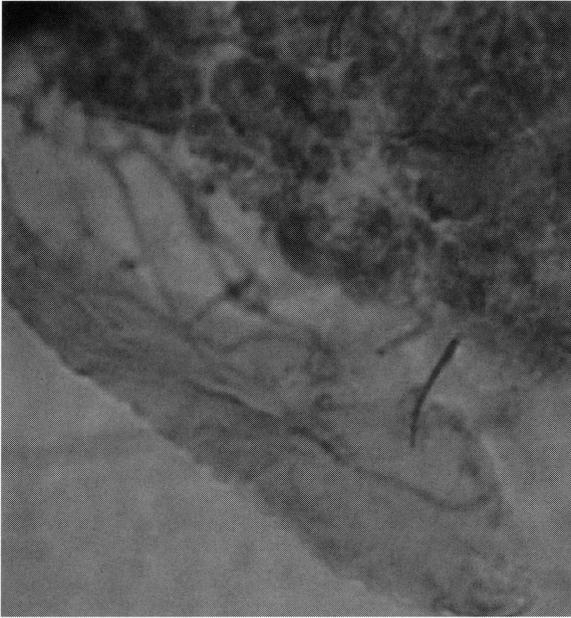


Fig. 11. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing extending hypha in the body.

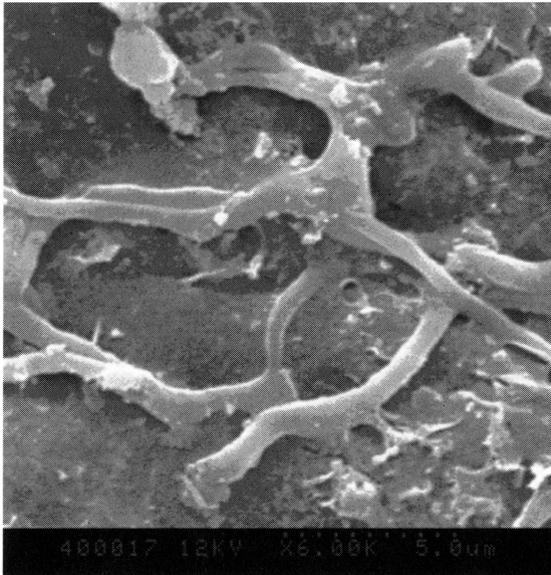


Fig. 12. SEM micrograph of a lateral area of the cuticular surface of *Bemisia tabaci* showing germinating conidia oriented to the membrane.

Acknowledgments

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