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BIOLOGICAL CONTROL OF WHITEFLIES WITH ENTOMOPATHOGENIC FUNGI

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ABSTRACT

This paper is a review of 3 fungi (*Aschersonia aleyrodis* Webber, *Verticillium lecanii* (Zimmerman) Viégas and *Paecilomyces fumosoroseus* (Wize) Brown & Smith that are being evaluated for the management of injurious polyphagous whiteflies and was presented as part of a conference on the potential for biological control in the Caribbean. The prospect for the utilization of biopesticides based on entomopathogenic fungi is promising. The need to develop alternatives to conventional pesticides has become apparent in recent years because two of the major whitefly pest species, *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood), have developed resistance to many of the insecticides used for their control.

RESUMEN

Se revisan en este manuscrito 3 especies de hongos: *Aschersonia aleyrodis* Webber, *Verticillium lecanii* (Zimmerman) Viegas y *Paecelomyces fumosoroseous* (Wize) Brown

& Smith los cuales estan siendo evaluados para el manejo de moscas blancas polifagas. La futura ultilizacion de hongos como biopesticidas se considera muy prometedora. La necesidad de desarrollar alternativas a los pesticidas convencionales se hace cada vez mas aparente dado que las dos especies mas importantes de mosca blanca, *Bemisia tabaci* (Gennadius) y *Trialeurodes vaporariorum* (Westwood) han desarrollado resistencia a muchos de los insecticidas que se utilizan para su control.

Chemical control of whiteflies (Homoptera: Aleyrodidae) is generally very difficult because of their morphological and autecological characteristics (waxy substances as a component of the cuticle, colonization of the underside of leaves, rapid development of very dense populations, etc.). Furthermore, whiteflies represent a group of insects with the ability to develop populations that are highly resistant to pesticides. Predators, parasitoids and/or entomopathogenic microorganisms have been therefore intensively studied for biological control of whiteflies and have been successfully utilized in biological control against several whitefly species, especially the greenhouse whitefly. The prospect for the utilization of biopesticides based on entomopathogenic fungi is promising. This paper is a review of 3 fungi that are being evaluated for the management of injurious polyphagous whiteflies and was presented as part of a conference on the potential for biological control in the Caribbean.

Sweetpotato Whitefly and Greenhouse Whitefly

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius), and greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), are considered major pests of economically important crops worldwide. *B. tabaci* feed on cotton, cucurbits, lettuce, soybean, tomatoes and over 500 ornamentals, garden plants and weeds. In recent years, its importance as a pest of field crops has increased and it has also become one of the most economically damaging pests of greenhouse crops (Osborne 1988, Osborne et al. 1990a, 1990b, 1990c, Hoelmer et al. 1991). *T. vaporariorum* is a serious pest of vegetables and numerous ornamental plants grown in greenhouses and has been reported to feed on about 250 species of plants throughout the world. Both species are also major pests of urban vegetable gardens in many states (Greathead 1986, Vet et al. 1980).

The life cycles of both species consist of the egg, four nymphal instars (scales), and the adult. The egg is spindle-shaped, mostly deposited on the underside of new foliage. The first instar nymph hatches from the egg and crawls over the leaf surface until it finds a suitable place to feed. This nymphal stage is oval with well-developed legs and antennae and is pale green in color. The second and third instar nymphs are flattened, scale-like in form, and generally transparent with legs and antennae non-functional. The second through fourth nymphal stages are sedentary throughout their developmental period. The fourth instar nymph is divided into three substages: the early fourth is flattened and translucent to opaque-whitish (substage one); the transitional fourth is thickened, opaque and ensheathed with wax (substage two); the pharate adult is similar to substage two except that the red eyes of the developing adult are clearly visible and the body becomes increasingly yellow as the adult develops inside (substage three). The adult whitefly emerges from a slit in the exoskeleton of the fourth instar nymph (Lopez-Avila 1986, Gill 1990).

Both adults and nymphs of whiteflies normally occur and feed on the underside of leaves. Dense populations can adversely affect plant growth as well as deposit copious amounts of honeydew. Often, a black mold grows on the honeydew which reduces photosynthesis and especially the aesthetic quality of ornamental plants. Furthermore, viral plant diseases are also commonly vectored by *B. tabaci* adults (Cohen 1990). In

Florida, *B. tabaci* is also responsible for new vegetable and foliage disorders of unknown etiology (Osborne et al. 1990a, 1990b, Yokomi et al. 1990, Hoelmer et al. 1991).

Control of both species of whitefly by conventional chemical means has become more difficult in recent years. Many crops grown in greenhouses receive as many as two applications of different insecticides per week to suppress populations. As a result, highly resistant populations of both species are common worldwide with residual and ground water contamination becoming major problems and concerns to the public. These factors are forcing researchers to look for alternative means of control.

Greenhouse crops with controlled temperature, high relative humidity and reduced solar radiation offer an excellent opportunity for pest control with entomogenous fungi (van Lenteren & Woets 1988), especially with several species of deuteromycetes. Both *Aschersonia aleyrodis* Webber and *Verticillium lecanii* (Zimmerman) Viégas represent common fungal pathogens of whiteflies. Recent data indicate that *Paecilomyces fumosoroseus* (Wize) Brown & Smith might play an important role among fungal biopesticides used against whiteflies and other major pests of vegetable and ornamental plants grown under greenhouse condition or in the field, especially with the humid conditions of the Caribbean region (Osborne et al. 1990c).

Host Range and Natural Occurrence of Major Entomopathogenic Fungi of Whiteflies

The entomopathogenic fungus, *A. aleyrodis*, is the most frequently studied species from about 50 taxa which represent the genus *Aschersonia* (Petch 1921, Procenko 1967, Fransen 1987). *Aschersonia aleyrodis* along with *A. flava*, *A. flavocitrina*, *A. goldiana*, *A. placenta* and *A. viridans* belong to the group Aleyrodiicolae, which includes specific pathogens of whiteflies.

Aschersonia aleyrodis naturally occurs as a pathogen of several whitefly species in the subtropical region of the western hemisphere. Natural epizootics were noted in populations of *Aleurocanthus woglumi* Ashby, *Aleurothrixus floccosus* (Maskell), *B. tabaci*, *B. giffardi* (Kotinsky), *Dialeurodes citri* (Ashmead), *D. citrifolii* (Morgan), *Tetraleurodes acaciae* (Quaintance), *Trialeurodes abutiloneus* (Haldeman) and *T. vaporariorum* (Petch 1921, Berger 1921, Mains 1959, Fransen 1987).

Verticillium lecanii is a cosmopolitan species first reported as a pathogen of the scale insect *Coccus viridis* (Green) (Hall 1976, 1981) and was described under several names (*Cephalosporium lecanii*, *C. aphidicola*) (Samson & Rombach 1985). Gams (1971) had revised the taxonomy of the species and placed it into the genus *Verticillium*, mainly because of the arrangement of its conidiogenous cells in regular whorls.

Verticillium lecanii is well known as an entomopathogenic fungus, but it is not restricted to insect hosts. It has been predominately recorded as a pathogen of the homopterans, particularly of aphids, whiteflies and coccids (Gams 1971, Hall 1976, 1980). Less frequent are reports of hosts in other insect orders (e.g., Orthoptera, Hemiptera, Thysanoptera, Coleoptera, Lepidoptera and Hymenoptera) (Gams 1971, Hall 1980, McCoy et al. 1988). It is also known as a pathogen of non-insect hosts (e.g., arachnids, either of tetranychid or eryiophid mites) (Gams 1971, Kanagaratnam et al. 1981) as well as a hyperparasite of several phytopathogenic fungi, mostly rusts (e.g., *Uromyces dianthi*, *U. appendiculatus*, *Puccinia graminis*) and powdery mildew (Hall 1981, Deacon 1983). However, as a means of biological control it has been most often tested and/or used against aphids, whiteflies and thrips in greenhouses.

Paecilomyces fumosoroseus is a cosmopolitan species reported as a pathogen of many different insect hosts. The fungus was described under several names (*Isaria fumosoroseus* Wize, *Spicaria aphodii* Vuill., *S. cossus* Portier & Sartory, *P. hibernicus* Kennelly & Grimes, and *P. isarioides* Inagaki) (Samson & Rombach 1985). The genus

Paecilomyces was described by Bainier and is closely related to *Penicillium*. The characteristics used to separate these two genera are that species of *Paecilomyces* lack green colored colonies and they have short cylindrical phialides which taper into long necks. The latest revision of this genus was by Samson (1974) in which he placed 31 species into two sections; *Paecilomyces* and *Isarioidea*. *P. fumosoroseus* belongs to the section *Isarioidea* as do several of the entomopathogenic species.

Most of the host records for *P. fumosoroseus* are from Lepidoptera, Coleoptera, and Diptera (Bajan 1973, Fargues & Robert 1985, Maniania & Fargues 1984, Poprawski et al. 1985, Rodriguez-Rueda & Fargues 1980, Zimmerman 1986). The first report of *P. fumosoroseus* being a pathogen of whiteflies was from China, where the fungus was isolated from *T. vaporariorum* after natural epizootics occurred on this insect in greenhouses in Beijing (Fang et al. 1983). These new isolates of *P. fumosoroseus* were given the trinomial var. *beijingensis*. Recently, an isolate of *P. fumosoroseus* highly virulent to sweetpotato whitefly and a broad range of other pests has been isolated in Florida. This isolate has caused dramatic natural epizootic in populations of *B. tabaci* in both greenhouses and open shade-cloth protected structures. This strain (PFR 97 - collection of entomopathogenic fungi, University of Florida, CFREC Apopka, Dr. L. S. Osborne) is currently being evaluated in the laboratory and under greenhouse conditions (Osborne et al. 1990b, Osborne et al. 1990c).

Mode of Infection of Major Fungi Pathogenic to Whiteflies

The most susceptible stages of whiteflies to infection by *A. aleyrodis* are the immatures (scales), especially the first, second and third nymphal instars. All substages of the 4th nymphal instar are less susceptible. First instar nymphs may be infected if hatched from eggs treated by the conidial suspension of *A. aleyrodis* (Fransen et al. 1987). No infection caused by *A. aleyrodis* was noted on whitefly eggs and infection of adults is rare (Landa 1982, Samson & McCoy 1983, Fransen 1987). Landa (1982) noted an infection of greenhouse whitefly adults when *A. aleyrodis* was applied as a part of an IPM program on greenhouse cucumbers. Infection of adults expresses itself as mycelial growth between the head and prothorax, with typical sporulation occurring later. Recently, an infection of *B. tabaci* adults caused by *A. aleyrodis* (strain China 3, collection of entomopathogenic fungi, Dr. D. Boucias, University of Florida, Gainesville) was obtained in the laboratory (Landa & Osborne, unpublished data).

The mode of infection of the various entomopathogenic deuteromycetes is basically quite similar. A typical infection cycle is as follows: conidial attachment, germination, penetration, vegetative growth and conidiogenesis. The infection process for *A. aleyrodis* starts when the one-celled, fusiform conidia (Fig. 1) attaches to the surface of the host's body. After a swelling and germination phase, the germ tube penetrates the cuticle. The very early stage of infection is followed by visible color changes of the infected host. Compared with a healthy host (usually whitish to yellowish green), an infected nymph turns to a light yellow color and the body appears much less translucent than uninfected ones. The first visible sign of infection is followed by external growth of mycelia, which usually takes place all around the body of an infected host (Fig. 2). Later, the fungus produces vigorous hyphal growth and mat-like pustules which cover the entire surface of the host body (Fig. 3). The fungus covered host is white, but soon turns to an orange-reddish color as conidiogenesis begins. Conidia of *A. aleyrodis* are produced in slime by phialids which are arranged in cavities or pycnidia (Fig. 4). A conidial mass is visible as orange-red slimy droplets on a surface of pustules (Berger 1921, Landa 1982, Samson & McCoy 1983, Ramakers & Samson 1984, Samson & Rombach 1985, Fransen 1987). The intensive orange-reddish color of the conidial mass is caused by presence of carotenoids (mostly beta-carotene) the synthesis of which is

Fig. 1. Conidia of *A. aleyrodis* attached to the body surface of a greenhouse whitefly (*Trialeurodes vaporariorum*) the nymph - (SEM, 5000 ×).

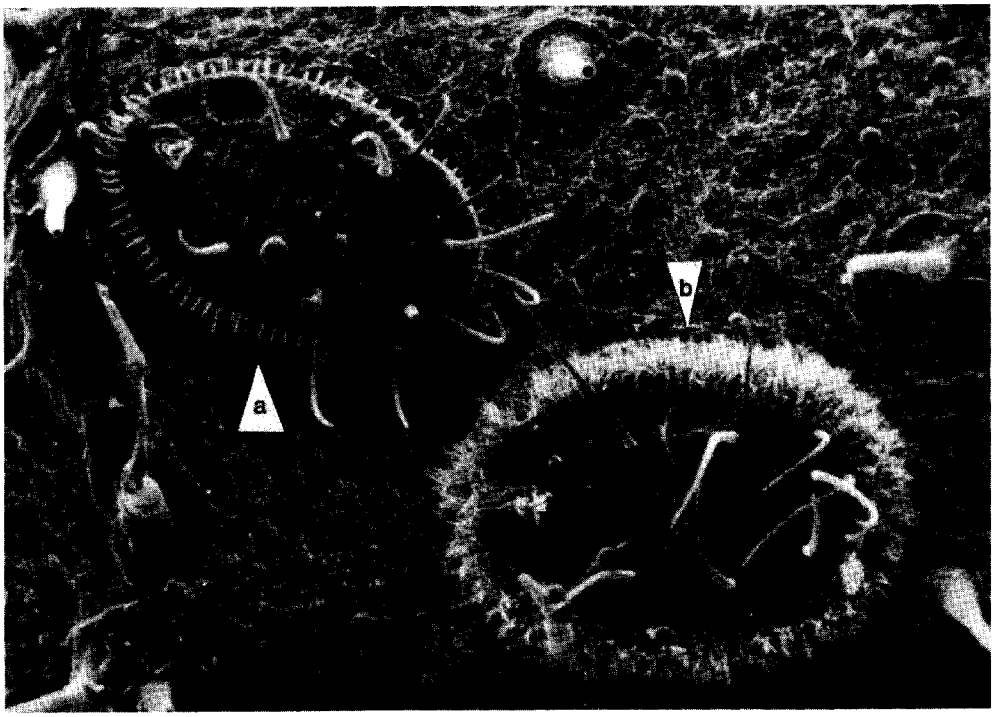

Fig. 2. Late 4th instar nymphs of greenhouse whitefly a) healthy b) infected with *A. aleyrodis*, the fungal growth is visible alongside the body of the mummified dead host (5 days after initiation of infection) (SEM, 95 ×).

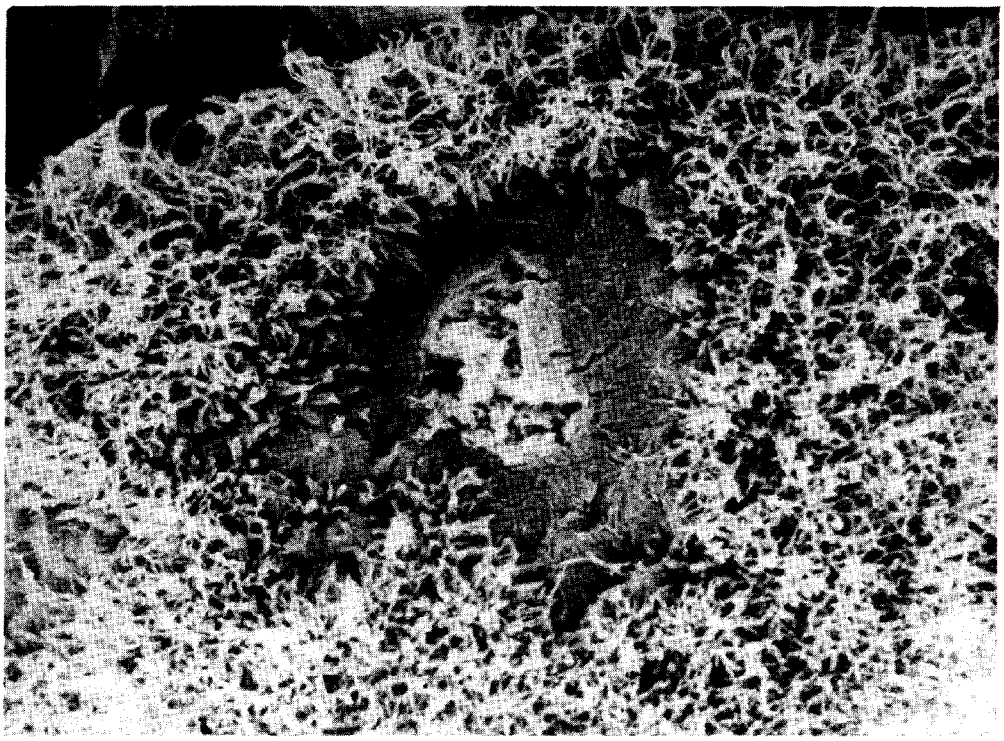

Fig. 3. The pycnidium of *A. aleyrodis* formed in the mycelium mat which covers the entire surface of the mummified nymph of greenhouse whitefly, the initial conidia are produced in central slimy mass (7 days after initiation of infection) (SEM, 400 ×).

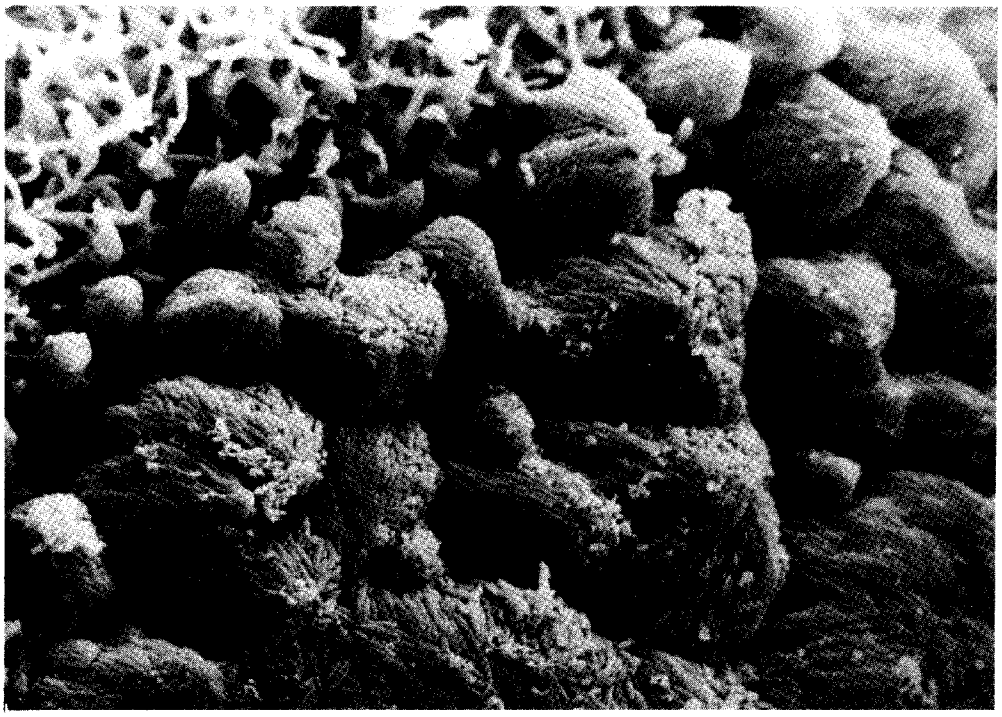

Fig. 4. Large quantities of conidia present in pycnidia of *A. aleyrodis* at the end of the infection cycle (SEM, 1000 ×).

induced by light (Landa et al. 1989). Under optimal conditions, the first symptoms of infection occur within 24 to 48 h, a vigorous hyphal growth may take place within 4 to 6 days and production of the conidial mass occurs usually from 7 to 9 days after initiation of infection (Solovej & Kolcov 1976, Ramakers & Samson 1984, Landa 1982, 1984, Fransen 1987, Rombach & Gillespie 1988).

Aschersonia aleyrodis is a common representative of the entomopathogenic mycoflora of plantation tree crops (e.g., citrus) in tropical and subtropical regions (Berger 1921, Petch 1921, Mains 1959), but the nature of the epizootics it produces is not yet satisfactorily understood. Samson & McCoy (1983) suggest that production of conidia in a slimy-mass indicates that the conidia are dispersed by free water (dew, rain) or by animals. Landa (in press) noted the presence of mycophagous mites (*Acalvolia* sp.) on leaves with infected nymphs of the common citrus whitefly *D. citri*. The number of *Acalvolia* mites on the leaves correlated with the number of infected host nymphs. These mites fed on the sporodochia of this fungus and were able to develop and reproduce having *A. aleyrodis* as its only food source. Also, *Acalvolia* mites lay eggs beside whitefly eggs and may transfer conidia of *A. aleyrodis* to whitefly eggs when ovipositing. All these data indicate that this mite may play an important role in *A. aleyrodis* dissemination.

The range of the whitefly stages infected by *V. lecanii* and *P. fumosoroseus* is broader than that infected by *A. aleyrodis*. Both of these fungi cause infections of all immature stages and adults of both the greenhouse and sweetpotato whitefly. Furthermore, *P. fumosoroseus* infects whitefly eggs.

The infection process of either *V. lecanii* or *P. fumosoroseus* is not known in detail. In general, these fungi are dispersed as conidia, either by air or water movement or by other insects and/or mites. The growth of mycelium around an infected host and subsequent sporulation of the fungus may result in further dispersal and secondary infections caused by these fungi. This spread is probably caused by the casual contact between infected and healthy individuals. This process plays an important role particularly in the dense colonies of some pests (whiteflies, aphids, mites etc.). Natural epizootics are common for both species in the field and greenhouse.

Once the conidia attaches to the host cuticle, it germinates and initial growth is apparently saprophytic on the outside of the host (Samson & Rombach 1985). Infection of the host is initiated by an invasion hypha growing through natural orifices and/or between body segments. *Verticillium lecanii* forms a cotton-like whitish aerial mycelium on the infected host. Conidiophores produced on the mycelium of *V. lecanii* bear awl-shaped phialids arranged in a characteristic verticillate manner (Fig. 5). Conidia are of various shapes (cylindrical to ellipsoidal) and are aggregated in mucus (Fig. 6) (Gams 1971, Hall 1981, Samson & Rombach 1985).

Paecilomyces fumosoroseus produces colonies which are white at first but change to shades of pink and become light gray when conidia are present. Conidiophores arise from the basal growth or from aerial hyphae, and have verticillate branches which bear whorls of 3 to 6 phialids which in turn bear cylindrical to fusiform conidia with rounded ends. Conidia are born in long chains and fully sporulated colonies (or cultures on an artificial media) have a powdery gray appearance (Fig. 7) (Samson 1974).

The infection cycles of *V. lecanii* and especially *P. fumosoroseus* are somewhat faster than that of *A. aleyrodis*. The infection cycle of *P. fumosoroseus* is particularly rapid. First, symptoms of infection caused by this fungus are apparent within 24 to 48 h after the conidia contact the insect. Recently, TEM and SEM studies revealed that *P. fumosoroseus* conidia attached to the dorsum of the insect and hyphae are present in the host hemocoel within 24 h. The mycelium is present on the dorsum of the whitefly body within 48 hours and sporulation occurs within 72 hours (Storey, McCoy & Osborne, unpublished data). Under optimal conditions, the first visual sign of infection may be

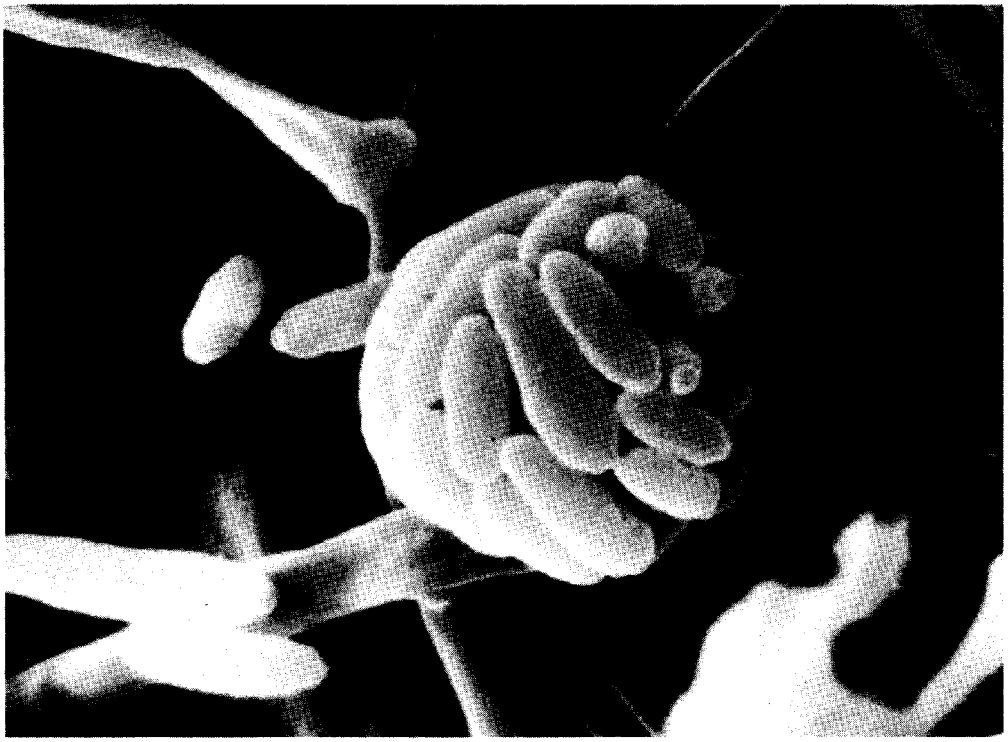

Fig. 5. *Verticillium lecanii* - a detail of the ellipsoidal conidia arranged in the terminal spherical heads of phialids with no mucilaginous substances on the surface (SEM, 2 400 ×).

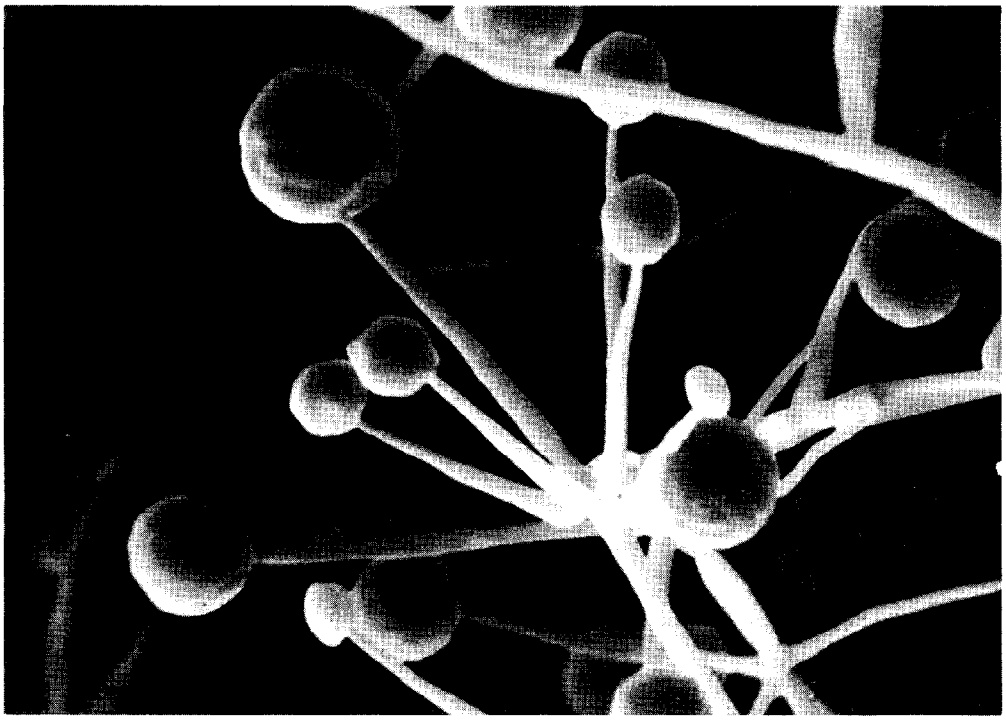

Fig. 6. *Verticillium lecanii* - conidia formed on the end of phialids of aerial mycelium, and protected with a mucilaginous substance (SEM, 1000 ×).

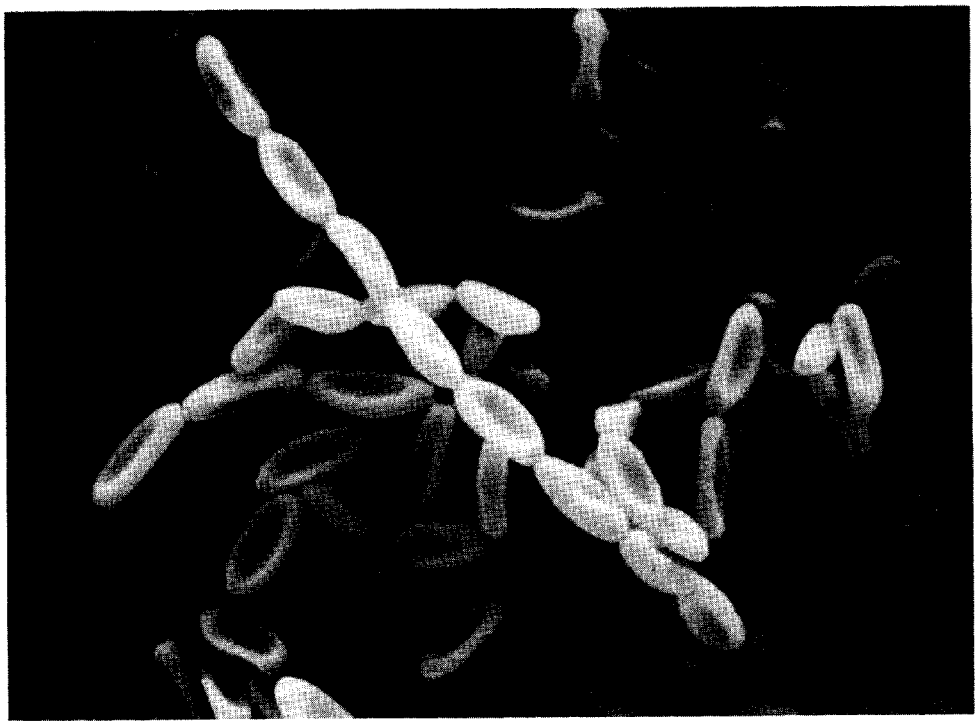

Fig. 7. Conidia of *Paecilomyces fumosoroseus* arranged in typical chains which are formed on phialids on the aerial mycelium (SEM, 2000 ×).

noted within 48 to 72 h (mycelial growth on the host surface) and maximum sporulation may occur within 5 to 7 days (Hall 1981, Landa & Jiranova 1988).

Relative humidity plays the most important role among abiotic factors affecting the infection cycle of the fungi discussed in this paper. Germination of *A. aleyrodis* conidia is retarded if relative humidity declines under 98% and is usually impaired at humidities lower than 90% (Fransen 1987). This appears to be similar for both *V. lecanii* and *P. fumosoroseus* (Gillespie 1984). Under adverse conditions, the fungus may stay dormant inside the dead host for several weeks and become active if conditions are suitable (Landa 1982). Gillespie (1984) showed that successful conidiogenesis of several deuteromycetes (*Metarhizium anisopliae* (Metschnikoff) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin, *V. lecanii*, *P. fumosoroseus*) depends on high relative humidities (95-100%), and it is not fully realized when relative humidity is below this range. As to practical utilization of entomopathogenic fungi for whitefly control, it seems critical to have at least a short period of high humidity during the initiation of the infection process (conidial attachment to the host surface, conidial swelling, germination and penetration of the host cuticle). In *A. aleyrodis*, this process is very rapid and at optimal conditions may be realized within 12 to 24 h (Rombach & Gillespie 1988). *Verticillium lecanii* requires high humidity, especially for conidial germination. During this phase, high humidity is required for a period of at least 10 to 12 h (Hall 1981, Samson & Rombach 1985). Similarly, *P. fumosoroseus* requires, for this portion of the infection cycle, a relative humidity above 95% (Osborne, unpublished data).

Compared with relative humidity, the temperature seems to be less of a limiting factor. All three fungal species grow and multiply at temperatures between 15 and 30°C, with colony growth optimal between 23 and 25°C. Germination of conidia and

growth of mycelium decline above 25°C and cease above 32°C (Gillespie 1984, Fransen 1987). Mycelial growth of *V. lecanii* ceases when it is maintained below 11°C (Hall 1981).

Besides humidity and temperature, light also affects some aspects of fungal development, particularly the last phase of the infection cycle - conidiogenesis. Gillespie (1984) noted a significant increase in the production of conidia when cultures of *P. fumosoroseus* and *V. lecanii* were exposed to fluorescent light. The same effect was noted when different strains of *A. aleyrodis* were cultured under different light conditions. In this case, besides a significant increase in the production of conidia, the amount of beta-carotene and the shape of the slime-conidial mass were significantly influenced (Landa et al. 1989).

Utilization of *A. aleyrodis* and *V. lecanii* in Biological Control Against Whiteflies in Practice

The utilization of *A. aleyrodis* as a biological control agent has a long history. First of all, the introduction and colonization of *A. aleyrodis* into citrus plantations in Florida is a classical example of successful biological control against *D. citri* (citrus whitefly) and *D. citrifolii* (cloudy wing whitefly). The fungus was first utilized by making a conidial suspension from the rinsate of leaves with naturally infected scales. A method for production of *A. aleyrodis* conidia on semi-artificial media was developed later (Berger 1921). Presently, both *D. citri* and *D. citrifolii* are naturally controlled by this introduced fungus and regular chemical treatments are no longer needed (Samson & McCoy 1983). Similar results have been obtained with *A. aleyrodis* in the USSR after the accidental introduction of *D. citri* into the Azerbadijan region. Several strains of *A. aleyrodis* (from China, Cuba, USA, India, Japan) were successfully introduced into citrus groves by applying conidia produced on a media (Primak & Chiznik 1975, Ponomorenko et al. 1975). Other successful introductions of *A. aleyrodis* to control whiteflies on either citrus (Yen & Tsai 1969, Gao et al. 1985) or other host plants outdoors have been reported (Chen & Chen 1986, Muraleedharan 1985).

As a consequence of the success in citrus, *A. aleyrodis* was tested in greenhouses as a potential selective biological control agent against greenhouse whitefly, *T. vaporariorum*. Different strains of *A. aleyrodis* were tested as the only regulatory agent or as a component of an IPM program in combination with the parasitoid *Encarsia formosa* Gahan. Most of the experiments were conducted in the USSR (Osokina & Izevskij 1976, Solovej & Kolcov 1976), Bulgaria (Spasova et al. 1980), Sweden (Ekbom 1979), Netherlands (Ramakers & Samson 1984, Fransen 1987) and Czechoslovakia (Landa 1982, Landa & Jiranova 1988) with encouraging results. In general, the fungus was applied as a conidial suspension (1.0×10^6 to 5.0×10^7 conidia per ml) and, in some cases, infection of scales was as high as 85% (Landa & Jiranova 1988). The potential for the use of *A. aleyrodis* to control whiteflies in greenhouses is high, especially when applied in combination with the parasitoid *E. formosa*. *Aschersonia aleyrodis* is a selective pathogen which does not infect scales parasitized with *E. formosa* and the parasitoid *E. formosa* does not parasitize scales which are in the early phases of the infection cycle (Landa 1982, 1984, Fransen 1987). A combination of *E. formosa* and *A. aleyrodis* is used experimentally in Czechoslovakia as a component of IPM for greenhouse cucumber (Landa 1985, Landa & Jiranova 1989); however, there are no biopesticides based on *A. aleyrodis* currently available.

In spite of fact that *V. lecanii* is a common pathogen of whiteflies worldwide, its practical use is restricted and this fungus is used only against several pests in greenhouses. Different biopesticides based on *V. lecanii* are utilized on greenhouse crops to manage greenhouse whitefly, aphids and thrips in the Netherlands (Van der Schaaf et al. 1989), Denmark (Borregaard 1991), USSR (Solovej & Sogojan 1982), Swe-

den (Ekbom 1979), U.K. (Hall & Burges 1979, Hall 1980, 1985), Czechoslovakia (Samsinakova & Kalalova 1975, Landa & Jiranova 1989) and other countries. *Verticillium lecanii* is utilized as a component of IPM programs for cucumbers (Hall 1985, Hussey 1985, Landa & Jiranova 1989) and for ornamental plants (Wardlow 1985, Borregaard 1991). In fact, commercial biopesticides based on conidia or blastospores of different *V. lecanii* strains represent one of the few examples of entomopathogenic fungi already being utilized commercially. Several commercial formulations based on conidia of *V. lecanii* are registered in the Netherlands, Denmark and the U.K. (e.g., Mycotal - formerly a product of Microbial Resources Ltd., U.K., now of Koppert B.V., Netherlands; Vertalec - Koppert B.V.; Microgermin A and F - Chr. Hansens Bio Systems - Denmark). The blastospore formulations are used in Czechoslovakia, where *V. lecanii* is used as part of their "IPM complete-service-system" and it is not commercialized as a biopesticide for market distribution. Because of problems caused by some new pests that have been recently introduced into European greenhouses (e.g., *B. tabaci* and the western flower thrips *Frankliniella occidentalis* (Pergande)), the possibility of utilizing *V. lecanii* to manage these pests in greenhouses is being intensively studied.

Recent Experiences with *P. fumosoroseus* in Biological Control of *B. tabaci*

When compared with *A. aleyrodis* and *V. lecanii*, much less information is available about *P. fumosoroseus*. Nevertheless, results obtained during the last two years indicate that there is a high potential for the implementation of *P. fumosoroseus* into practice as a fungal biopesticide against sweetpotato whitefly under greenhouse conditions and possibly on field crops in both Florida and the Caribbean region.

As mentioned above, an extremely virulent strain of *P. fumosoroseus* was isolated from naturally infected mealybugs in Florida in 1989 (PFR 97). After isolation, preliminary experiments were conducted using conidial suspensions of PFR 97 against sweetpotato whitefly populations established on ornamentals under greenhouse conditions. The results of these experiments were positive and the University of Florida patented (Osborne 1990) and then licensed the rights to this strain to W. R. Grace & Co., Connecticut. Both parties began a cooperative research program to investigate the possibility of formulating and utilizing *P. fumosoroseus* to control various pests of horticultural plants. Regardless of the fact that this joint research project covers a broad spectrum of potential host pests, *B. tabaci* has been studied extensively.

When a modified laboratory in-vitro bioassay was used for comparison (Landa & Jiranova 1988, Osborne & Landa, unpublished data), the infection cycle of *V. lecanii* was 1 to 3 days longer than PFR 97, and the infection cycle of *A. aleyrodis* was even longer (4-7 days after PFR 97). The ability of the PFR 97 to infect whitefly eggs is unique among the entomopathogenic fungi. When exposed to the conidial suspension, eggs of sweetpotato whitefly are overgrown within 24 to 48 h and the presence of conidial chains indicates that an egg has sufficient nutrients to allow for the whole infection cycle. Furthermore, infection of adults that were treated directly or treated prior to their emergence from the last nymphal stage was frequently observed. An *in vitro* bioassay under laboratory conditions demonstrated that conidia applied within 24 h of adult eclosion could regularly infect partly or fully emerged adults. As to the immatures, younger stages are more sensitive in terms of mortality, but the 4th instar nymphs support significantly better fungal growth and sporulation. A procedure for evaluation of the *P. fumosoroseus* strain was developed which allows us to compare other strains of this entomopathogenic fungus and to evaluate basic qualitative parameters of a biopesticide containing *P. fumosoroseus* before use in experiments. This procedure includes a viability test (standard test of germination of conidia) and an in vitro

bioassay using early (substage 1) and late 4th instar nymphs (substage 3 - partly formed adult visible).

As a consequence of results obtained in laboratory studies, the first field experiments with PFR 97 were started in 1990, and other experiments were conducted in 1991. Most of experiments focused on the management of sweetpotato whitefly populations on different ornamental plants (e.g., *Hibiscus rosa-sinensis*, *Mandevilla amabilis* (*Dipladenia rosea*), *Euphorbia pulcherrima* (Poinsettia), and *Crossandra infundibuliformis*) with different strategies for the utilization of the fungus being tested.

In most of the experiments, conidial suspensions were applied to the plant surface, especially the underside of leaves, and the results have been very encouraging. *Paecilomyces fumosoroseus* significantly reduces populations of sweetpotato whitefly when applied at weekly intervals. Under normal greenhouse conditions, the establishment of infection is detectable within 7-10 days after the first treatment. After application of PFR 97 conidia, a significant increase in the number of dead scales without visible infection was noted, but when placed into control wet chambers (high relative humidity), the presence of fungus was usually observed. In contrast to laboratory observations, infection of whitefly adults is less frequent. *Paecilomyces fumosoroseus* is very compatible with some beneficials, especially with *Eretmocerus* sp. a common parasitoid of *B. tabaci* and with *Delphastus pusillus* (LeConte) a common predator of various whiteflies in the Florida region. As a part of the PFR 97 project, additional studies have been conducted. The fungus was tested using one of the common in vitro methods and found to exhibit a high tolerance to a broad spectrum of fungicides which are frequently used in protection of greenhouse foliage and ornamental plants: a high level of tolerance was observed (Osborne & Hoelmer 1990). Tolerance of PFR 97 to most of the fungicides is similar to that of *V. lecanii* (Ledieu 1985), and both of these pathogens and the fungicides may be utilized if applications are scheduled so as to minimize negative interactions. The integration of *V. lecanii* and some fungicides is already recommended for special IPM programs (Gardner et al. 1984). Also, the possibility of utilizing PFR 97 against other greenhouse pests (e.g., aphids, thrips, broad mites, two-spotted spider mites, leaf miners, mealybugs, etc.) is currently being evaluated in the laboratory and in university and grower greenhouses. All experiments with PFR are linked to the production and formulation of this fungus into a standard product to be commercialized by W. R. Grace & Co.

Conclusions

The three pathogens discussed in this review are frequently found attacking insects in the greenhouse and in the field. Because these fungi have been found in many different environments, it is quite possible that each of these pathogens could be utilized in Florida and the Caribbean for the management of whiteflies.

Recent trends in greenhouse pest management lead us to believe that future IPM programs for arthropod pests will rely on various entomopathogenic organisms. These trends include the elevation of secondary pests to primary pest status when pesticide pressure is removed once biological control programs are implemented for other pests. Another complicating factor is the importation of new pest species without adequate biological controls available, as occurred with leafminers, thrips, and more recently for *B. tabaci*. The colonization of exotic pests has a major destabilizing influence on the IPM practices that are currently being used. Chemicals are utilized to maintain control of these new pests until methods compatible with established programs can be found. Viruses, bacteria and fungi are, for the most part, influenced much less than predators and parasitoids by many of the agricultural chemicals used in these disturbed situations, but the development of these pathogens into control tactics will take time.

The usefulness of various predators and parasitoids is reduced in many ornamental crops because of the very low aesthetic injury thresholds that currently exist. The organisms that will be most readily accepted by growers will probably be those that can be mass released on a regular basis as biological insecticides. Pathogens fit these criteria much better than do many arthropods with the possible exception of certain phytoseiid mites.

Another complicating factor with the development of any type of control program for ornamental plants is simply the diversity of plant species and varieties grown. Each plant type has its own complex of pests and sensitivities to pesticides. In general, monocultures do not exist in the ornamental industry. A few growers specialize in growing a few plant types, but the common practice is to produce a wide range of products which are often grown on the same or adjacent benches in a greenhouse. This practice of mixed cultures makes it very difficult to treat one crop with a pesticide and not affect non-target plant material.

We feel that there are excellent opportunities for the use of fungi to control whiteflies in Florida and the Caribbean. The choice of which pathogen to utilize in a specific situation can't be answered with our current knowledge; however, we think that each will find place in arthropod management programs developed in the future.

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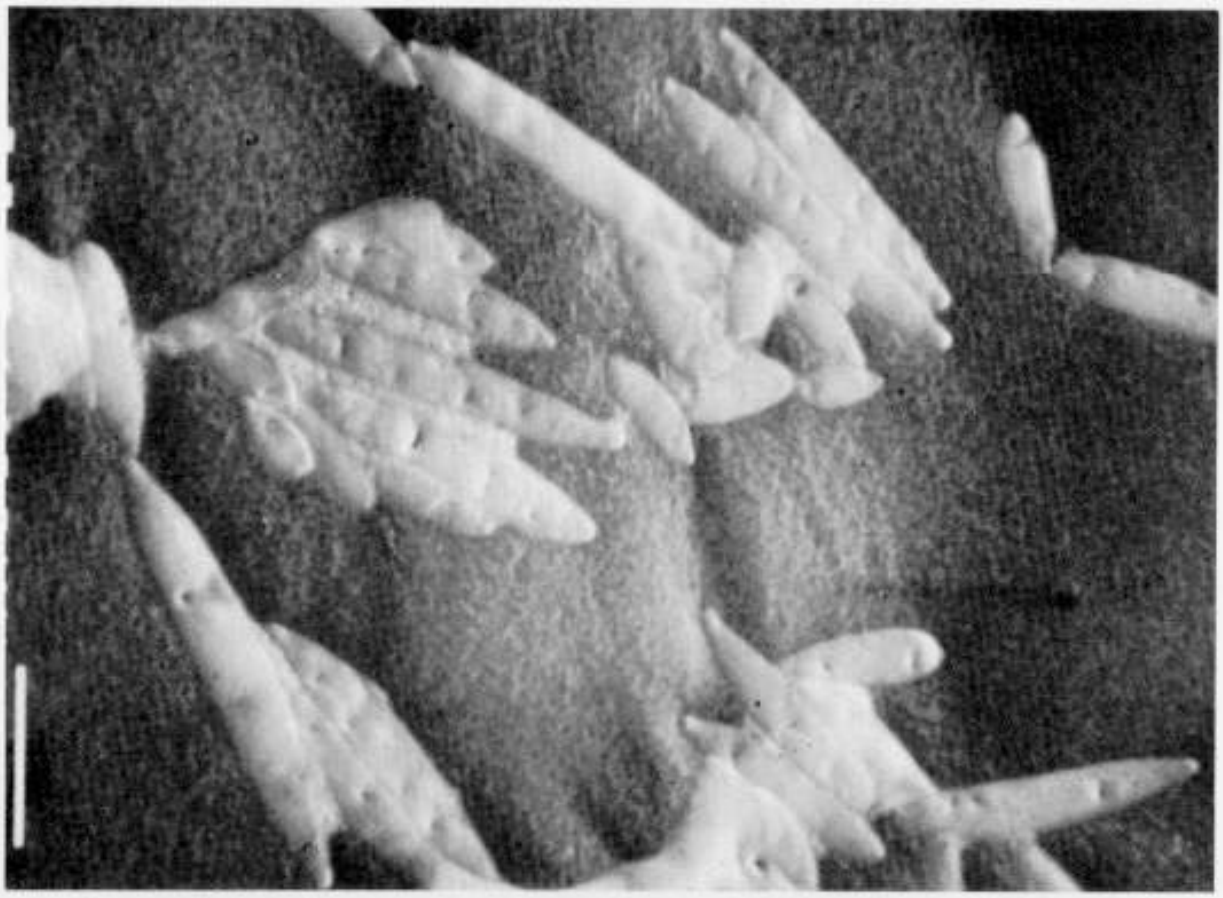

Fig. 1. Conidia of *A. aleyrodis* attached to the body surface of a greenhouse whitefly (*Trialeurodes vaporariorum*) the nymph - (SEM, 5000 ×).

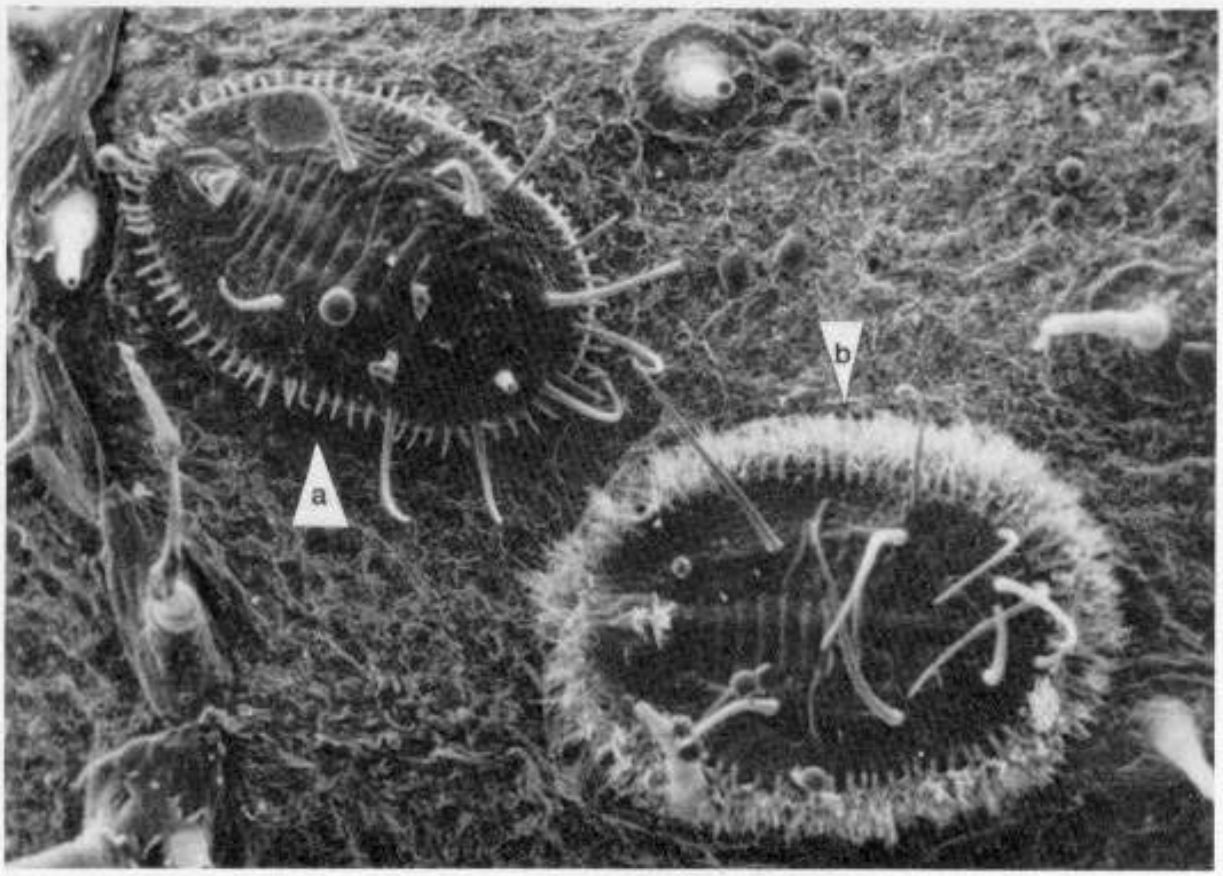

Fig. 2. Late 4th instar nymphs of greenhouse whitefly a) healthy b) infected with *A. aleyrodis*, the fungal growth is visible alongside the body of the mummified dead host (5 days after initiation of infection) (SEM, 95 ×).

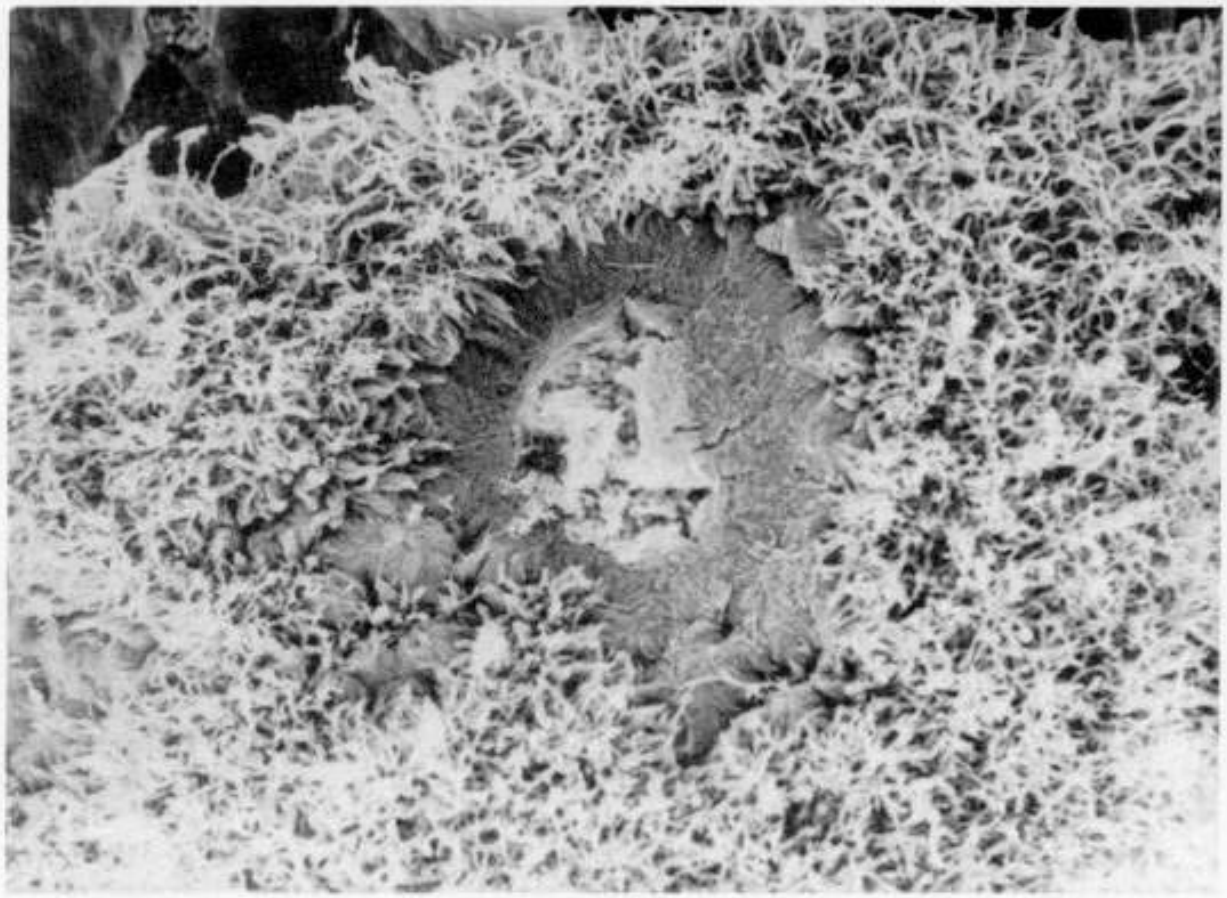

Fig. 3. The pycnidium of *A. aleyrodis* formed in the mycelium mat which covers the entire surface of the mummified nymph of greenhouse whitefly, the initial conidia are produced in central slimy mass (7 days after initiation of infection) (SEM, 400 ×).

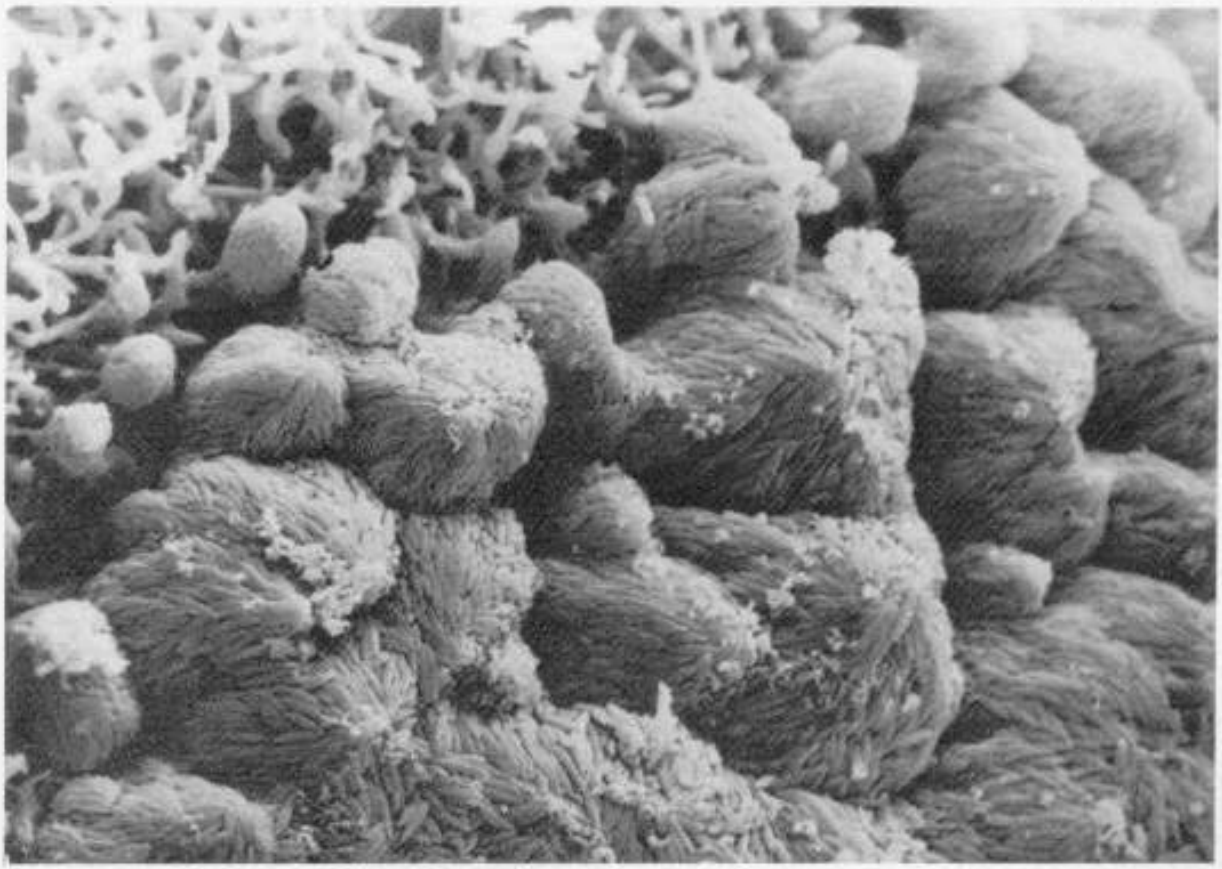

Fig. 4. Large quantities of conidia present in pycnidia of *A. aleyrodis* at the end of the infection cycle (SEM, 1000 ×).

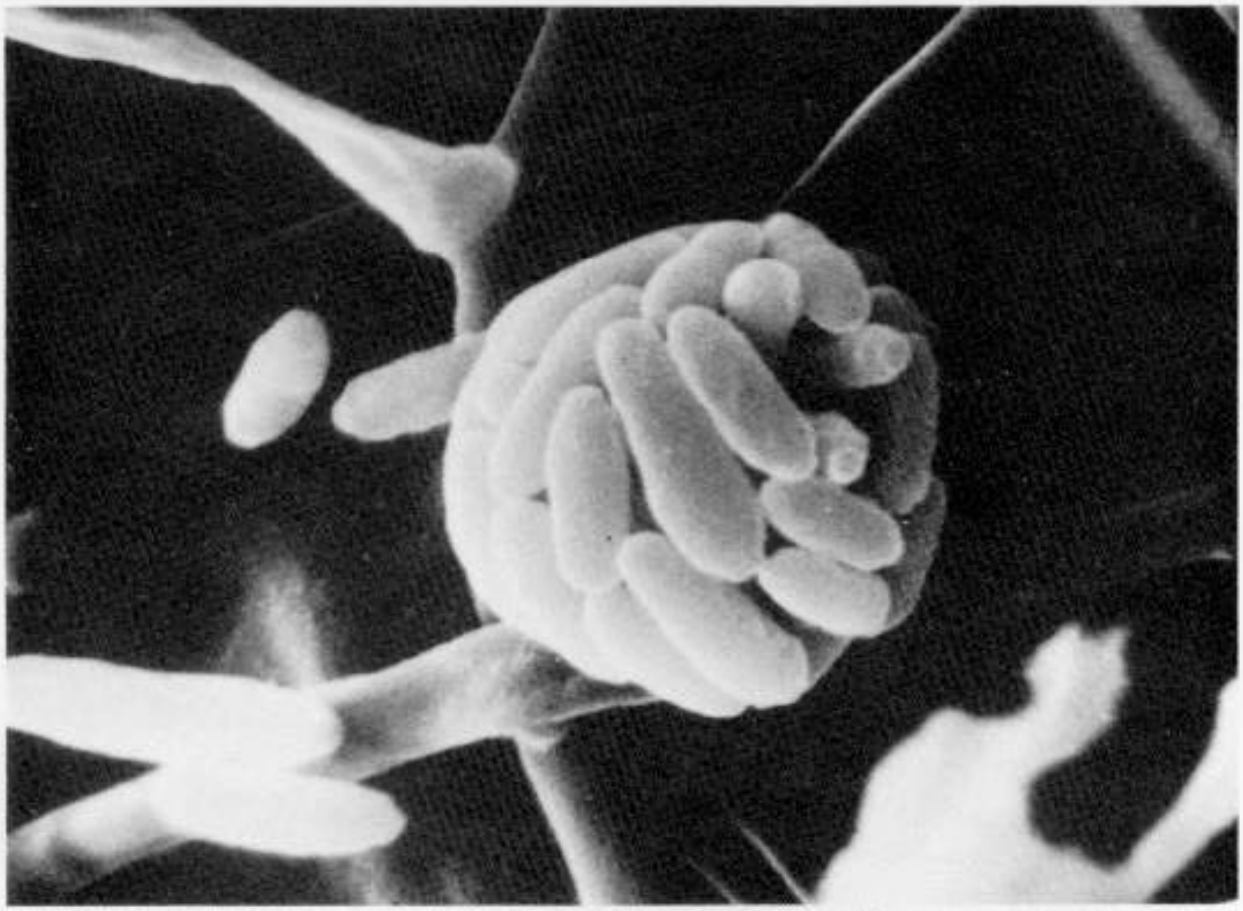

Fig. 5. *Verticillium lecanii* - a detail of the ellipsoidal conidia arranged in the terminal spherical heads of phialids with no mucilaginous substances on the surface (SEM, 2 400 ×).

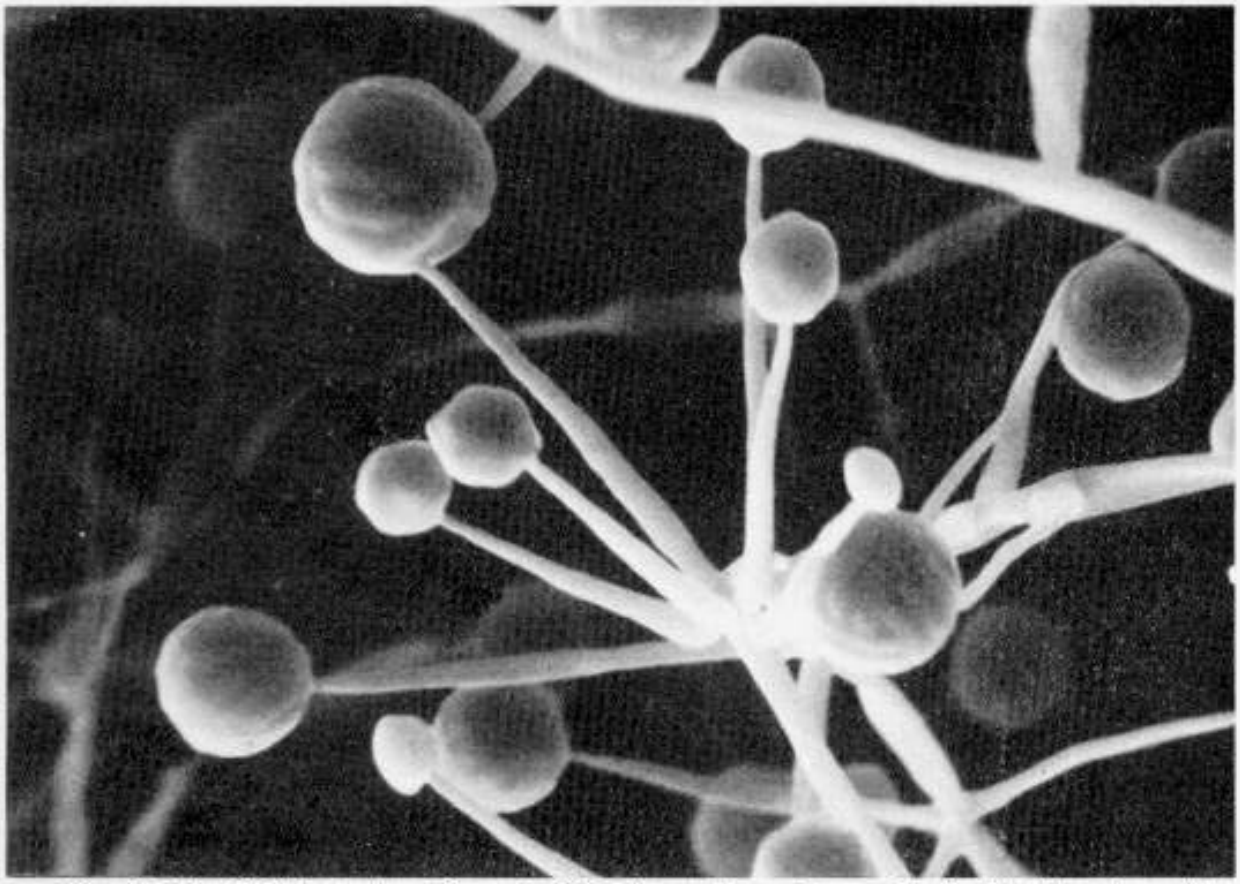

Fig. 6. *Verticillium lecanii* - conidia formed on the end of phialids of aerial myceelium, and protected with a mucilaginous substance (SEM, 1000 ×).