

Aschersonia aleyrodidis, a fungal pathogen of whitefly

I. Scanning electron microscopy of the development on the citrus whitefly

By R. A. SAMSON and C. W. MCCOY

Abstract

Scanning electron microscope studies were conducted on the larvae of the citrus whitefly, *Dialeurodes citri* (Ashmead), naturally infected with the fungus *Aschersonia aleyrodidis* Webber. Conidiogenous cells of *A. aleyrodidis* are phialidic, producing conidia in a basipetal sequence. Conidia are one-celled, fusiform, smooth-walled propagules produced in slime. Conidiogenesis appears early in the infection process soon after the hyphae rupture the dorsal cuticle and produce mat-like pustules of white mycelia on the host surface.

1 Introduction

Of the species of *Aschersonia* that are parasitic on scale insects (Coccidae) and whiteflies (Aleyrodidae), *A. aleyrodidis* Webber is the most common. Epizootics for *A. aleyrodidis* have been reported in several humid subtropical and tropical regions (MCCOY 1978; QUEZADA 1974). PETCH (1921) and MAINS (1959) included the species in their taxonomical studies, but provided only poor descriptions.

A. aleyrodidis has attracted much interest as a bio-insecticide. In Florida, a major campaign to control the citrus whitefly, *Dialeurodes citri* (Ashmead), was successfully conducted early in this century. A number of methods of disseminating the fungus, which included artificial propagation and spore-spraying were utilized to establish and/or maintain the fungus in citrus groves (BERGER 1907; ROLFS and FAWCETT 1908; BERGER 1921; FAWCETT 1944). Today, both citrus whiteflies, *D. citri* and *D. citrifolii* Morgan, are considered under biological control in Florida (MCCOY 1978).

Experiments on the culture and the utilization of *A. aleyrodidis* to control citrus whitefly were also conducted in Japan (OHO and SATOH 1966; OHO 1967). Best results were obtained when the fungus was sprayed on the eggs or larval instars of the whitefly (SADAMATSU and SEKI 1966).

Recent invasion of *D. citri* in citrus regions surrounding the Black Sea has resulted in the introduction of different species and strains of *Aschersonia*. *A. placenta* Berk. & Br., a species closely-related to *A. aleyrodidis*, has given excellent microbial control in these areas under favourable weather conditions (PONOMARENKO et al. 1975). It has also been tested for use against the immature stages of *Trialeurodes vaporariorum* (Westw.) in the U.S.S.R. (PRIMAK and CHIZHIK 1975; OSOKINA and IZHEVSKII 1976; KOGAN and SERYPIN 1978).

When considering the potential of *Aschersonia* spp. as a microbial control agent, it is important to gain knowledge about the biology of this fungus in relation to its host. This paper presents a morphological study on the mode of

infection of the citrus whitefly by *A. aleyrodalis* and on conidiogenesis. Scanning electron microscopy was used to illustrate the complex structures of this fungus.

2 Material and methods

Citrus leaves with infected whitefly larvae exhibiting various stages of fungal development were collected in a commercial citrus grove near Lake Alfred, Florida in July 1979. Initially, individual whiteflies were examined with a dissecting microscope to determine the exact stage of development. Small sections of citrus leaf with the infected larvae were prepared for scanning electron microscopy according to the technique described by SAMSON et al. (1979).

3 Results and discussion

All developmental instars of the citrus whitefly are found on the under surface of newly-formed leaves where one or more stages usually can be found throughout the year. Eggs and adult citrus whitefly were never observed to be infected with *A. aleyrodalis*, while infection was detected in motile crawlers and young pupae, but mainly in second and third stage larvae. SADAMATSU and SEKI (1966) reported a high incidence of infection in larvae when the fungus was sprayed on eggs and larvae but no infection of pupae. Since it is difficult to distinguish between third stage larvae and pupae of *D. citri*, our observations on pupal infection are considered inconclusive.

The healthy larvae of *D. citri* are thin, translucent, elliptical, scale-like insects with a whitish to yellowish green colour. Only the first instar is mobile. They generally lack leaf-inhabiting microflora. The micrograph in fig. 1, however, does show some hyphae, but these probably belong to saprophytic fungi of the phyllosphere of the citrus leaves. It is interesting, that in this study, conidial attachment of *A. aleyrodalis* to healthy insects was not observed with the SEM.

Early infection was usually detected as a white fringe of hyphae (figs. 2-6) extending from the marginal area of the larval body and as pustules at the weak points on the dorsum (figs. 4-6). At this stage of fungal development, a few conidia were observed arising directly from what appeared to be solitary conidiogenous cells. Later the fungus produced vigorous internal hyphal growth within the hemocoel, and mat-like pustules that completely covered the larval body (fig. 7). Colonies are white at first, but take on an orange to reddish appearance as sporulation begins. A cross section of the stroma at this stage of fungal development shows no recognizable remnants of the host (fig. 9). The stroma consists of a compact accumulation of hyphae covered by thinner fertile hyphae bearing conidiogenous structures (fig. 10), which are discrete at first, later becoming confluent. At several locations, cavities were detected (figs. 10-11). In these cavities, the conidiogenous cells are formed, appearing as densely packed cylindrical to awl-shaped cells. These cells are phialidic (fig. 12-13) producing conidia in a basipetal manner. The conidia are one-celled, fusiform and smooth-walled. They are not produced in dry chains but in slime indicating a dispersal by free water, such as dew or rain or by animals. On sporulating colonies, conidial masses are readily seen as orange-red slimy droplets.

We did not observe a teleomorph of *A. aleyrodalis*, and neither did PETCH (1921) and MAINS (1959).

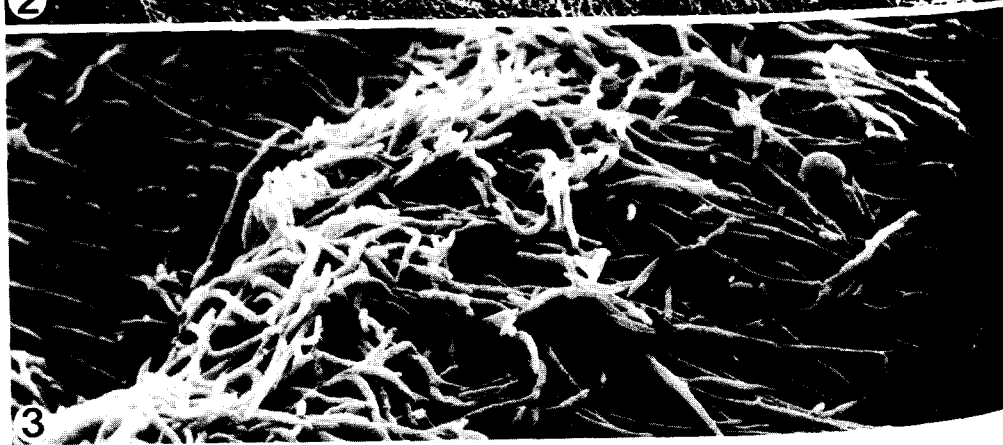
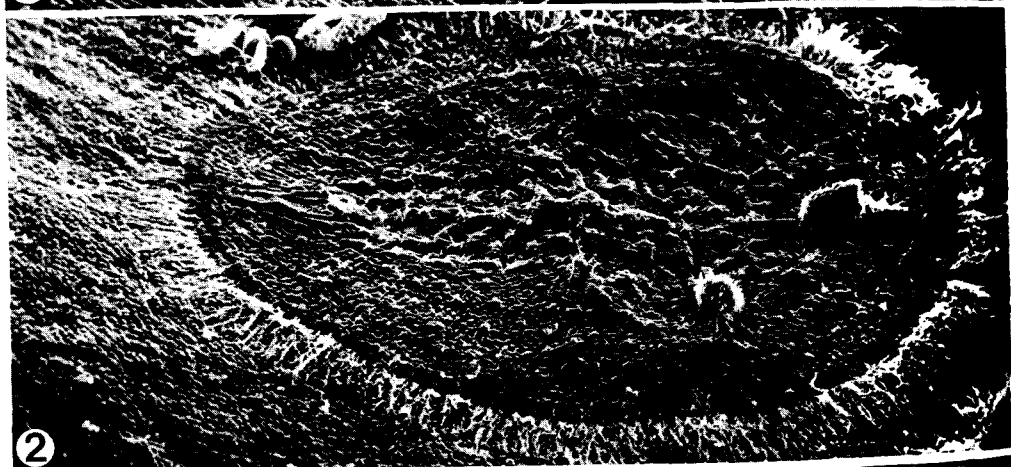
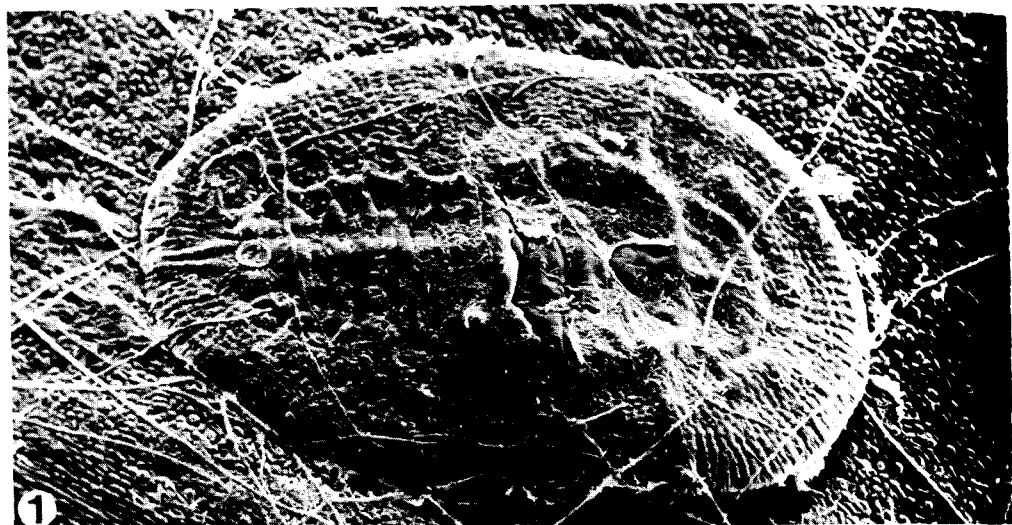
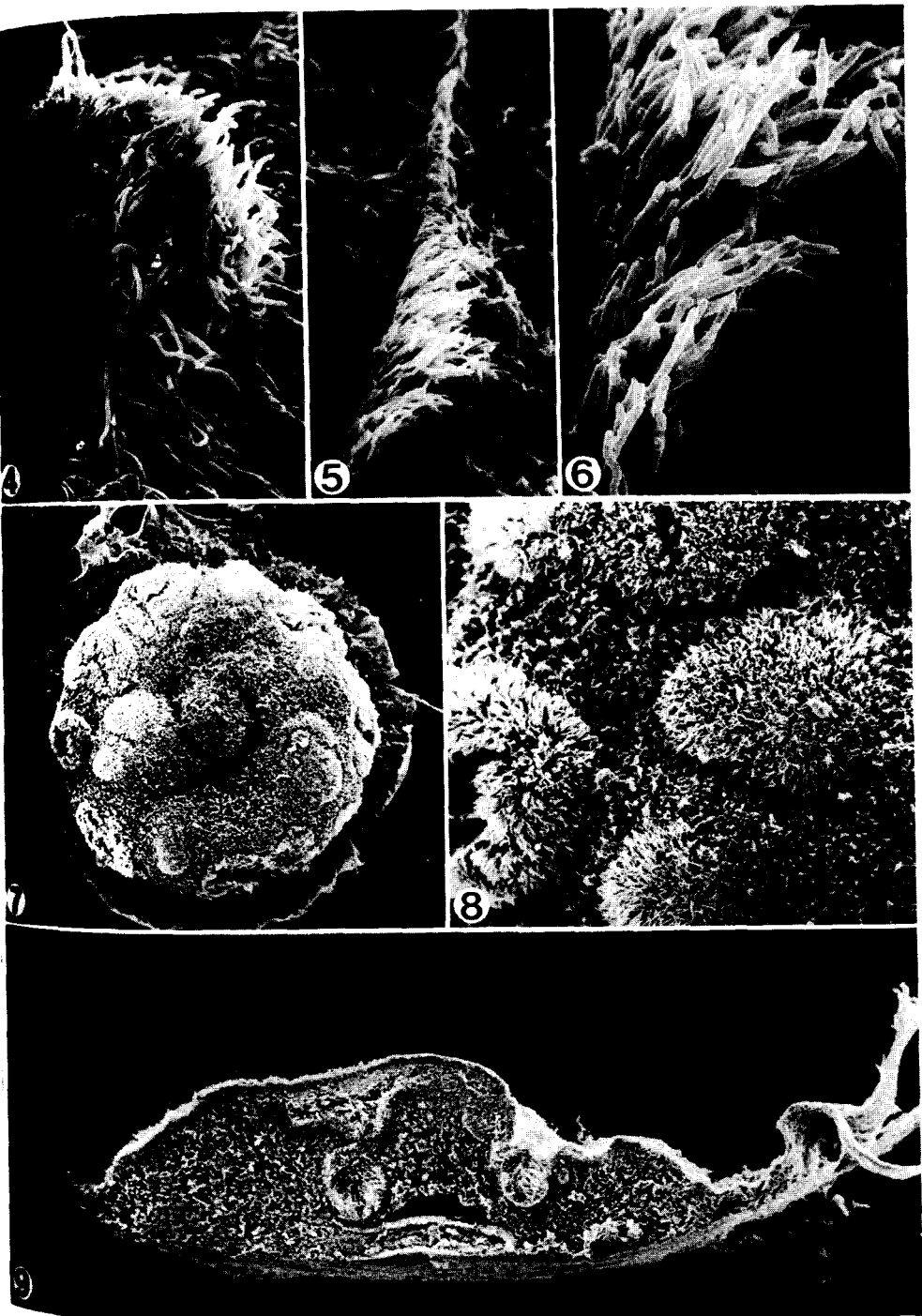


Fig. 1. Healthy larva of *Dialeurodes citri*. The hyphae seen on the micrograph probably do not belong to *Aschersonia*, but to saprophytic fungi on the phylloplane (x110). – Fig. 2. Early stages of infection, characterized by the fringe of hyphae, originating from the underside of a larva (x80). – Fig. 3. Detail of the fringe of hyphae, showing the adherence to the leaf surface. Some conidia can be detected (x930)



Figs. 4-6. Appearance of pustules and ridges of *Aschersonia* hyphae, following rupture of the larval body. Some solitary phialides with conidia are present (4: x580, 5: x570, 6: x1500). - Fig. 7. Completely parasitized larva (x35). - Fig. 8. Detail of fig. 7 showing pustules of conidiogenous structures (x140). - Fig. 9. Section through the larval body completely parasitized and killed by *A. aleyrodis*. No remnants of the larval body can be observed (x60)

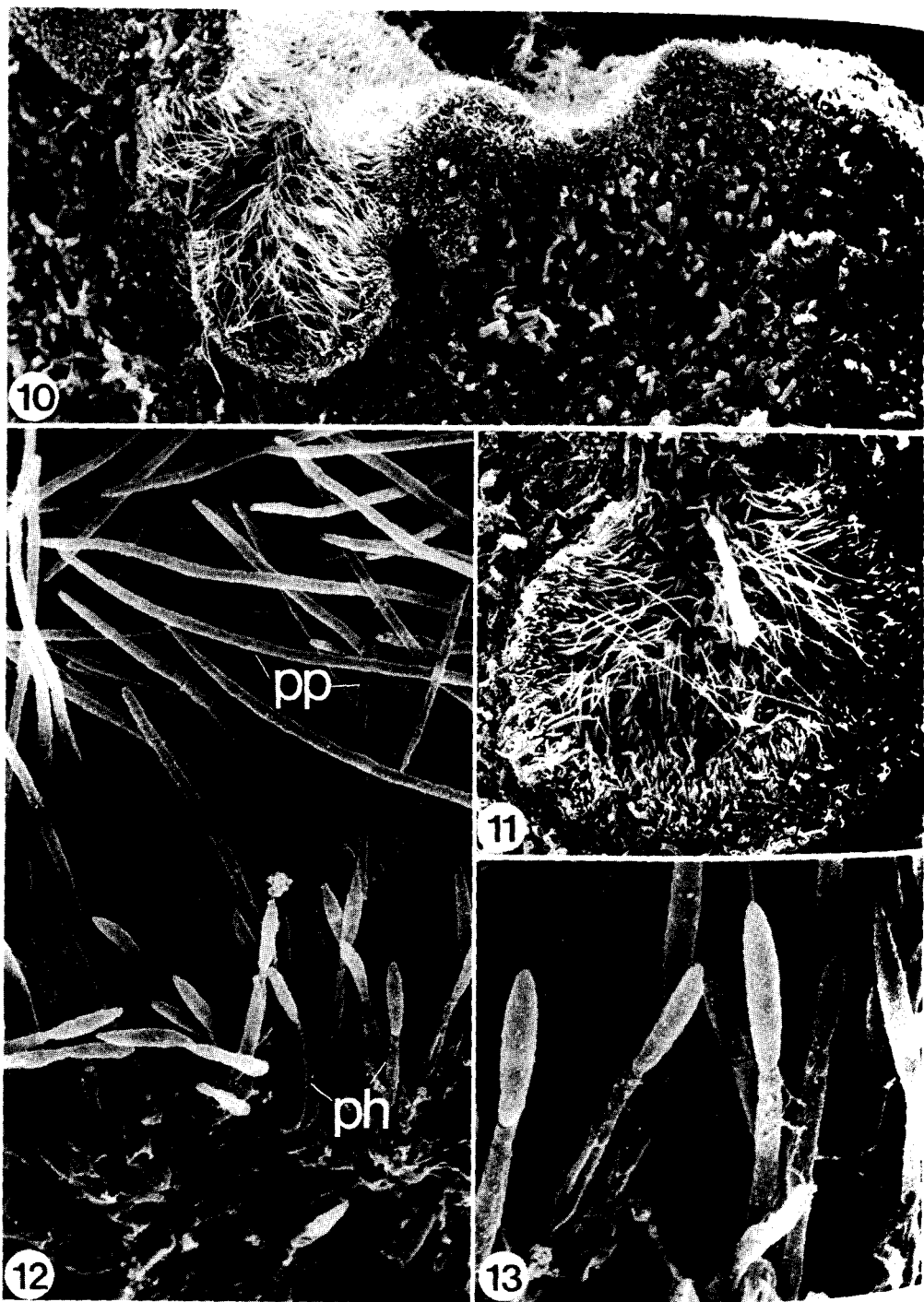


Fig. 10. Detail of the killed larva showing vegetative thick hyphae and thinner hyphae of upper layer and fertile areas (x250). - Fig 11. Pycnidium of *A. aleyrodis*, showing paraphyse and conidiogenous structures (x340). - Figs. 12-13. Detail of pycnidium, showing paraphyse (pp), phialides (ph) and conidia (12: x2500, 13: x5000)

Besides the fact, that *A. aleyrodis* can be easily introduced as a bio-insecticide against whitefly species in subtropical and tropical climates, a potential also exists for developing the fungus as a microbial control agent in greenhouse environments in moderate climates. Promising results of experiments with *A. aleyrodis* in an integrated control project with *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) have been reported (RAMAKERS and SAMSON 1983).

The isolation and cultivation of *A. aleyrodis* is relatively simple on artificial media and a mass production of inoculum is feasible (SAMSON et al., in prep.).

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Zusammenfassung

Aschersonia aleyrodis, eine Pilzkrankheit der Mottenschildläuse. I. Rasterelektronen-Mikroskopie der Entwicklung der Citrus-Mottenschildlaus

Larven der Weißen Fliege auf Citrus wurden nach natürlicher Infektion mit *Aschersonia aleyrodis* mit dem Rasterelektronenmikroskop untersucht. Die konidiogenen Zellen von *A. aleyrodis* sind Phialiden und produzieren Konidien in basipetaler Folge. Die Konidien sind einzellig, spindelig, glattwandig und werden durch Schleim zusammengehalten. Die Konidienbildung tritt früh im Infektionsprozeß auf, bald nachdem die Hyphen die dorsale Cuticula durchbrochen und Matten weißen Myzels gebildet haben.

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Age-dependent resistance to malathion in adult almond moths, *Ephestia cautella* (Walker)

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Abstract

Malathion resistance of adult *E. cautella* is inversely related to adult age. The reason seems to be abrasion and exhaustion caused by flight and mating activities. The process is slowed down and resistance remains high for 4 days or more when the emerging moths are held at 10 °C before treatment. The effects of these phenomena on *Ephestia* control and on estimates of resistance in field populations are discussed.

1 Introduction

The almond moth, *Ephestia cautella*, is one of the most serious pests of stored food commodities. Damage is caused not only by larval feeding, but also by spinning webs which hold grains together and cause severe technical problems in granaries and flour mills. The primary method of *Ephestia* control has been application of malathion before and during grain storage. In the United States and Australia very high levels of malathion resistance were reported - in some cases, LD₅₀ could not be reached even with 100 % malathion (ZETTLER et al. 1973; ZETTLER 1982; ATTIA 1976; ATTIA et al. 1979). All moth resistance data known to us, with one exception (COGAN 1982), were obtained by topical application of the pesticide to large (5-instar) larvae, although warehouse treatments are often aimed at adults.

Our interest in moth resistance is related to the idea that insecticide resistance may be prevented (or may be reduced if the population is already resistant) by immigration of susceptible males at chosen intervals between pesticide treatments (WOOL 1971). Encouraging results were obtained from laboratory experiments with flour beetles (WOOL and MANHEIM 1980; WOOL and NOIMAN 1983). Since adult moths do not feed on the stored products we are currently testing the idea with *Ephestia* in the hope that if found effective, the method may be introduced without fear of damage by the migrant males.