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Scanning ultrastructural ontogeny of cleistothecia in the powdery mildew Microsphaera alphitoides

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Morphogenesis of cleistothecia in *Microsphaera alphitoides* Griff. et Maubl. (*Erysiphales, Ascomycetes*) on naturally infected leaf of oak was investigated with the scanning electron microscope. All phases of the life cycle of this species have been described. Some comparisons between methods of preparation of the material have been made.

INTRODUCTION

Microsphaera alphitoides Griff. et Maubl. is a very frequent species which causes the plant disease commonly referred to as powdery mildew. Only a few kinds of powdery mildew have been studied with SEM. Genus *Microsphaera* appeared only in papers by Y u k a w a, K a t a m o t o, T o k a h a s h i (1971) and by H a r r i s, R o t h (1974). The first deals with *M. euonymi-japonicus* and the second – with *Microsphaera* sp. collected from *Quercus stellata* leaves on dried unifixed material. The present study therefore was undertaken to obtain data on the phases of cleistothecial morphogenesis and were provided on fresh fixed material.

MATERIALS AND METHODS

Naturally infected by *Microsphaera alphitoides* leaves of *Quercus robur* were collected from the Ojców National Park (South of Poland, Cracow-Wieluń Upland) in 1984. They were fixed for 30 minutes in 3% glutaraldehyde in 0,2 M phosphate buffer, pH 7.2. The fixed material was rinsed several times in the same buffer and dehydrated in a 30 to 100% ethanol series (10 at each step). The samples were at the critical point dried with CO_2, coated with carbon and gold, and observed with a JSM-35 scanning electron microscope (SEM) at 25 kV. A few naturally dried pieces of infected leaves were not fixed in the above described way, but only coated with carbon and gold and also examined with SEM.

RESULTS

All stages of *Microsphaera alphitoides* life cycle have been found on naturally infected oak leaves from Ojców. Powdery mildew colonies were macroscopic and appeared as powdery white mass on the leaf surface. Both the upper and lower epidermis has been infected by hyphae ca. 2-3 μm thick. Hyphae, growing on the surface of leaves, develop attachments to the cuticle, called appressoria (Pl. I. *A*.) and penetrate host tissue by haustoria – the feeding organ. The asexual reproductive cycle was completed by the production of ellipsoidal conidiospores. This state, called *Oidium quercinum* Thüm, has been described by several authors (C e j p, S k a l i c k ý 1954; B l u m e r 1967 and others). Cleistothecia were initiated by plasmogamy of a single mycelial bulbous like cell ca. 3-4 μm in diameter (Pl. I. *B*) and a spherical cell ca. 1.2-2 μm in diameter functioning as antheridia (Pl. I. *C*, *D*). As ascogonium develops, it undergoes further division, forming ascogonial hyphae (Pl. II. *A*, *B*). In further development hyphae near the ascogonium produce branches (Pl. II. *C*, III. *A*) which begin to form the cover of ascogonial hyphae. They become more septate and swollen than those from which they have arisen. The fruit-bodies at this phase are ca. 30-40 μm in diameter. On the lower surfaces of the ascocarp they form attachments to the surface of leaf from vegetative hyphae. In Pl. III. A we can find a hyphe growing and branching on the surface of ascocarp. Such hyphae probably make a net of ridges visible at the end of ontogeny. But as they appear the ascocarp becames covered by interstitial mucilage visible on the surface of the fruit-body which appears in SEM as a membranous material. As the growing appendages arised there are remnants of this mucilage at the top of them (Pl. IV. *A*). Pl. IV-VI show completely developed fruit-bodies. Appendages are directed at the begining down to the surface of the leaf and their ends are entangled into a net or vegetative hyphae (Pl. IV. *B*). Later the appendages are changing their position upwards (Pl. IV. *C*). Pl. IV. *D* shows different positions of appendages. As cleistothecium matures it becames marked by ridges (Pl. V. *A*). In fixed material (Pl. V. *B*) appendages ornamentation is hardly visible but if unfixed specimens are considered it becomes more distinct (Pl. V. *C*, *D*). In dried material endings of appendages in particular collapse (Pl. V. *D*).

DISCUSSION

In this investigation ontogeny of cleistothecia in *Microsphaera alphitoides* was followed in the SEM. It may help in considering similarities and diferences

Plate I

A – appresoria (×3000); *B* – ascogonium attached to the hyphae (×3000); *C* – fusion of ascogonium and antherydium (× 5000); *D* – male cells produced by hyphae (× 2500)

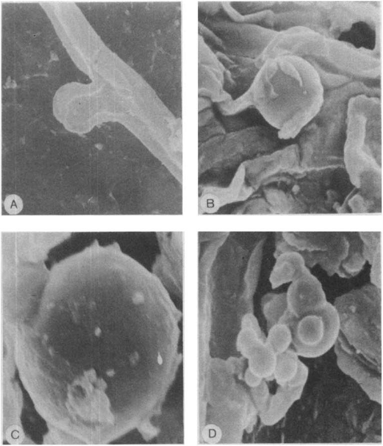


Plate II

A, B – ascogonial hyphae begining to arise from ascogonium (*A* – × 5000, *B* – × 3900); *C* – hyphae from nearly vegetative cells growing toward and around ascogonium × 1200)

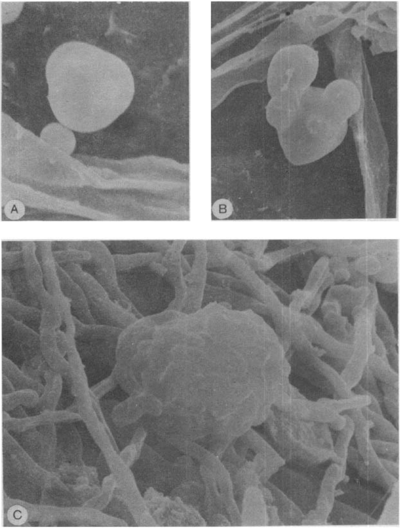
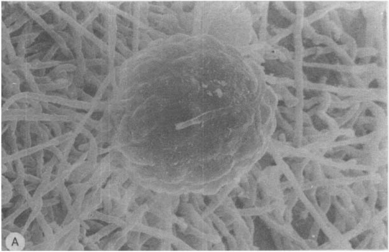


Plate III

A – the beginning of production of external seath by hyphae branching on the surface of the ascocarp. Note a few hyphae attaching ascocarp to the surface of leaf (× 500); *B* – upper surface of oak leaf with a various stages of *M. alphitoides* (× 200)



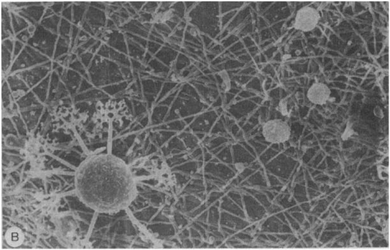


Plate IV

A – the begining of formation of appendages. Note membranous material on ascocarp and the top of appendages (× 600); *B* – mature fruit-body with appendages entangled into a net of vegetative hyphae (× 500); *C* – mature ascocarp with appendages upwards (× 330); *D* – a few mature ascocarps in a various stage of position of appendages (× 170)

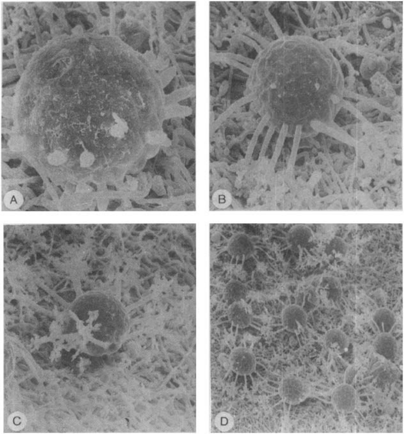
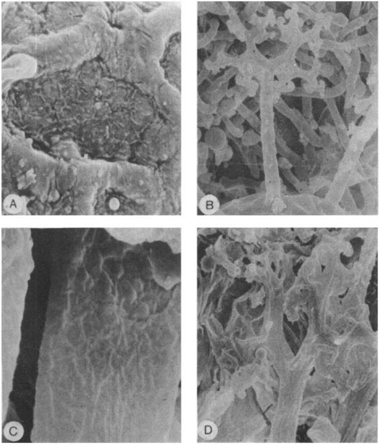


Plate V

A – the mature ascocarp surface with ridges (× 3000); *B* – dichotomously branched ends of appendages in fixed material (× 900); *C* – the basal part of appendage after drying of material (× 6000); *D* – the end of appendage after drying of material. Note the collaption of hyphae (× 1600)



between this and related species. For example, the identity of *M. alphitoides* and *M. hypophylla* Nevod. has not been decided up till now (S a ł a t a 1985; S p e e r 1973). As we know from other papers and from our investigation *M. alphitoides* posessed less and shorter appendages then in *M. hypophylla*. Their hook like ends are also very characteristic.

As we know from previous papers (cited by S a ł a t a 1985) these species like *Erysiphe sordida*, *Phyllactinia guttata*, *Sphaerotheca mors-uvae* and others are belived to be apomictic because of degeneration of the antheridial nucleus. In our study we only observed the external process of fusion of ascogonium and minute antheridium which is not easily visible using the light microscope.

This investigation carried out on fixed fresh as well as on naturally dried material has revealed the volume of the scanning electron microscope in the following developmental events in fungi. Working on dried material we found possibilities of deformations of hyphae, appendages and others.

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Ontogeneza kleistotecjów Microsphaera alphitoides Griff. et Maubl. obserwowana w mikroskopie skanningowym

S t r e s z c z e n i e

W pracy opisano kolejne etapy cyklu życiowego *Microsphaera alphitoides* (mąkulka dębowa), pospolitego pasożyta dębów. Infekcja rozpoczyna się od porośnięcia liści grzybnią przyczepiającą się do powierzchni skórki za pomocą przylg oraz wytwarzającą haustoria wrastające do wnętrza tkanek.

Rozmnażanie rozpoczyna się fuzją anterydium i askogonium, różniących się od siebie rozmiarami. Z askogonium wyrastają strzępki askogeniczne, podczas gdy sąsiadujące z nim strzępki wegetatywne tworzą okrywę (ścianę owocnika). Dojrzały owocnik osiąga średnicę około 100 μm i opatrzony jest charakterystycznymi, wielokrotnie dychotomicznie rozgałęzionymi przyczepkami o hakowatych zakończeniach. Praca ilustrowana jest kilkunastoma zdjęciami z mikroskopu skaningowego wykonanymi z preparatów uzyskanych z materiału utrwalonego jak i wysuszonego.