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Ultrastructure and Development of *Anthracoidea elynae* Ustilospores

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Abstract

The aim of the study was to examine the ultrastructure of *Anthracoidea elynae* ustilospores isolated from *Kobresia myosuroides* (Vill.) Fiori plant ovaries, harvested in the Bucegi Mountains, Romania. Samples examination was performed using scanning (SEM) and transmission (TEM) electron microscopy. The results showed that *A. elynae* ustilospores had a dynamic ultrastructure, because their three-layered cell wall, nucleus shape, lipid and glycogen accumulations in the cytoplasm changed at each developmental stage. In conclusion, according to the ultrastructural changes, *A. elynae* ustilospores development may be divided into three stages.

Keywords: *Anthracoidea elynae*, SEM, smut fungus, TEM, ustilospores

Introduction

Species of *Anthracoidea* produce plant smuts that parasitize members of the Cyperaceae family, including *Carex, Carpha, Fuirena, Kobresia, Schoenus, Scirpus,* and *Uncinia* (VANKY 2002). *Anthracoidea elynae* (H. Sydow) I. Kukkonen forms sori in plant ovaries and produces medium-sized spores that are roughly disc-shaped, medium-to-dark reddish brown, and have a faint hyaline sheath on the flat sides. *A. elynae* is parasitic on the circumpolar Cyperaceae *Kobresia myosuroides* (Vill.) Fiori, which is found in the arctic mountains of Europe, Asia, and North America (VANKY 1994). The aim of this study was to examine the ultrastructure of *A. elynae* ustilospores in different developmental stages by scanning (SEM) and transmission electron microscopy (TEM).

Materials and methods

Chemicals

Glutaraldehyde, Resin (Epon 812), Lead citrate, Uranyl acetate, Bismuth subnitrate (Electron Microscopy Sciences, Fort Washington, USA); Sticky carbon tabs, Colloidal carbon coated grids (Agar Scientific, Cambridge, England).

Micro-organisms

The fungus *A. elynae* was isolated from Kobresia myosuroides (Vill.) Fiori plant ovaries and identified by Dr. M. Parvu. A voucher specimen (CL 659754) was deposited at the Herbarium of Babes-Bolyai University of Cluj-Napoca, Romania. Plants were harvested in the Bucegi Mountains, Romania, during the summer of 2004, by Dr. M. Puscas.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

The ustilospores of *A. elynae* were fixed in 2.7% glutaraldehyde (in phosphate-buffered saline for 90 min). For SEM, the samples were critical-point dried in liquid CO_2, mounted on sticky carbon tabs, and sputter-coated with gold (10 nm). For TEM, the fixed and dried samples were infiltrated with resin, then deposited onto colloidal-carbon-coated copper grids, and negatively stained with lead citrate and uranyl acetate. The grids were examined by SEM with a JEOL JSM 5510 LV electron microscope (Vanky, 1994) and by TEM with a JEOL JEM 1010 electron microscope (Japan Electron Optics Laboratory Co., Tokyo, Japan) (Hayat, 2000). Glycogen granules were stained with bismuth subnitrate and examined by TEM (Hayat, 2000).

Results and discussion

Although the development of *A. elynae* ustilospores is a continuous process, ultrastructural examination allowed separation into three stages: young, mature and senescent, similar to the development of sclerotia in *Botrytis* cinerea (Nair and Martin, 1987). In SEM micrographs, the young ustilospores had a disc shape with a slightly convex median area (Fig. 1); the mature ustilospores were also disc-shaped, but with a flat or slightly concave median area (Fig. 2). In all developmental stages of *A. elynae* ustilospores, SEM examination showed smooth or nearly smooth margins that were minutely verruculose.

The cell wall of fungal spores may have one or more layers. *Botrytis cinerea* conidia (Parvu *et al*., 2008) and *Sporisorium sorghi* teliospores (Alexopoulos *et al*., 1996) have a double-layered cell wall, with two layers of different thickness and electrodensity, but *Gymnosporangium sabinae* teliospores have a single-layered cell wall (Parvu, unpubl.). The cell wall of *A. elynae* is a dynamic structure that is subject to change, depending on the life stage of the fungus (Peberdy, 1990). TEM micrographs showed that *A. elynae* ustilospores had a three-layered cell wall. In young and mature ustilospores, the external gelatinous layer was weakly electrondense, thinner at the edge and thicker at the median area (Fig. 3). The middle layer was more electrondense and had a uniform thickness (Fig. 4), and the inner layer had the weakest electrondensity and was attached to the plasmalemma. In senescent *A. elynae* ustilospores, the electron density of the outer cell wall layer was weaker and the inner layer was thicker than in the young and mature ustilospores (Fig. 5). Young spores of *Anthracoidea* are surrounded by a gelatinous coat which disappears at maturity (Vanky, 1994), but our results showed that the external cell wall layer of *A. elynae* ustilospores was present in all stages of development (Fig. 3-6).

A. elynae ustilospores have a single nucleus, because karyogamy occurs very early in their formation, so that even the youngest ustilospores are uninucleate and diploid, as in some species of *Ustilaginales*. In other *Ustilaginales* species, ustilospores remain binucleate until late in their development (Alexopoulos *et al*., 1996). TEM micrographs of *A. elynae* ustilospores showed the nucleus changing shape with developmental stage. Young and mature *A. elynae* ustilospores had a single sferic nucleus with a nucleolus (Fig. 3 and 4), while the senescent ustilospores had a lobate nucleus, with extensions between lipid granules (Fig. 5). Numerous small lipid granules of uniform size were also found in the cytoplasm of young ustilospores (Fig. 3). Mature ustilospores had oval-to-round shaped mitochondria under the plasmalemma or near the nucleus, and more lipid granules than young ustilospores (Fig. 4). Senescent ustilospores had large lipid granules, generated by fusion, with variable shapes (Fig. 5).

Glycogen granules have been identified in the conidia of *Magnaporthe grisea* (Thines *et al*., 2000) and *Colletotrichum lagenarium* (Tsuji *et al*., 2003), the aeciospores of the rust *Puccinia distincta* (Weber and Davoli, 2002) and in *Ustilago nuda* smut teliospores (Van Laere and Fransen, 1989). TEM micrographs showed that *A. elynae* ustilospores also contain glycogen deposits (Fig. 6 and 7).

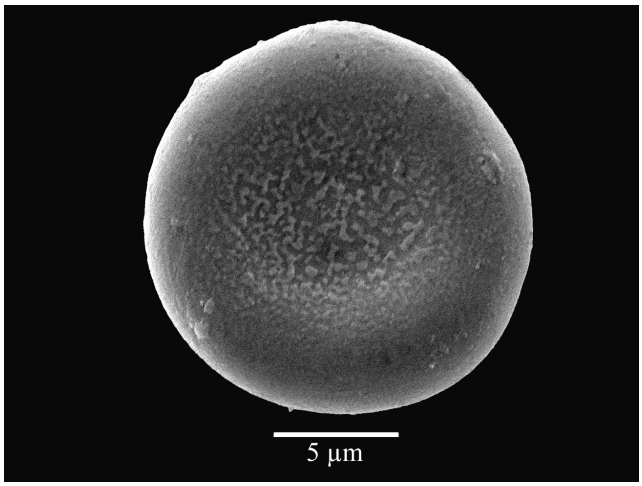

Fig. 2

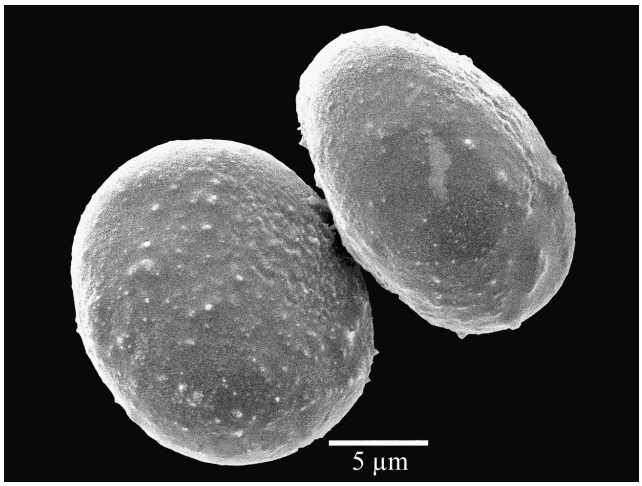

Fig. 1

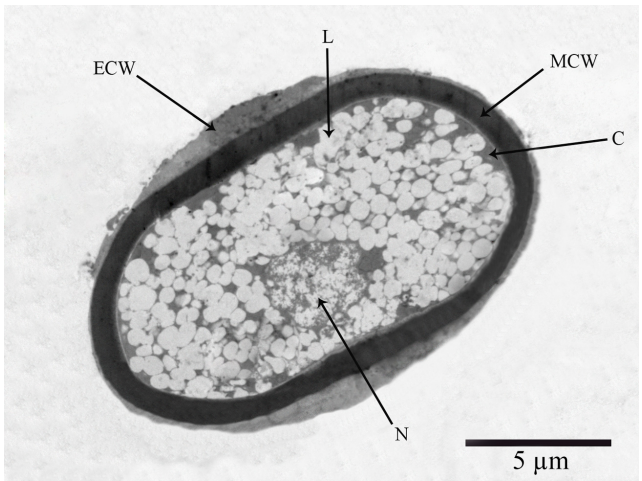

Fig. 3

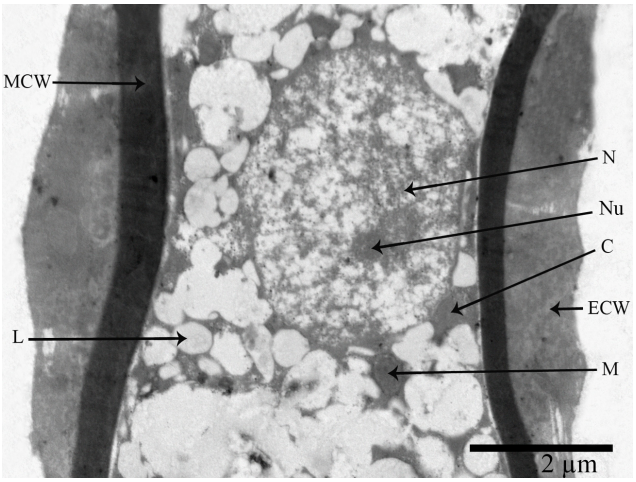

Fig. 4

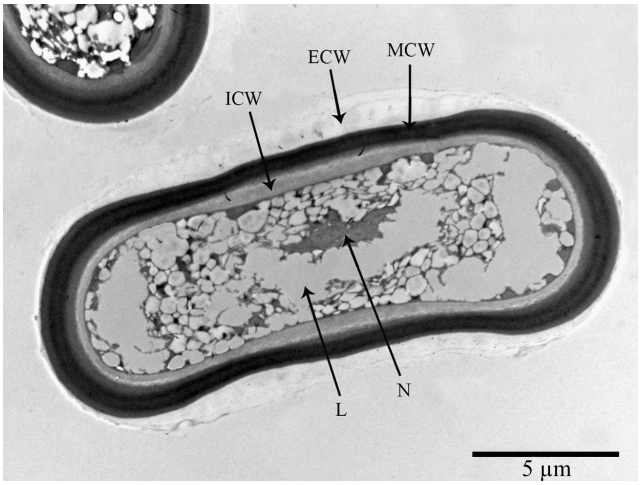

Fig. 5

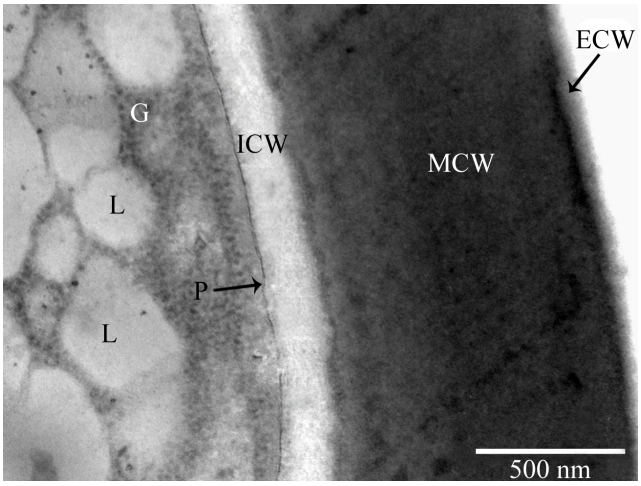

Fig. 6

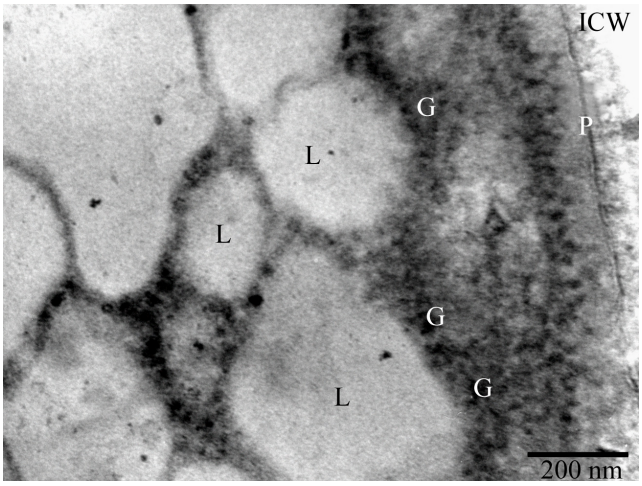

Fig. 7

Fig. 1. SEM of two young *A. elynae* ustilospores showing the disc shape, convex median area, and a minutely verruculose surface.

Fig. 2. SEM of a mature *A. elynae* ustilospore, in plane view, showing the disc shape, concave median area, and minutely verruculose surface.

Fig. 3. TEM of an *A. elynae* young ustilospore, showing a three-layered cell wall, one sferic nucleus and numerous small lipid granules. ECW, external layer of cell wall; MCW, medium layer of cell wall; ICW, inner layer of cell wall; C, cytoplasm; N, nucleus; L, lipid granules.

Fig. 4. TEM of an *A. elynae* mature ustilospore, showing ultrastructural components from the median zone. ECW, external layer of cell wall; MCW, medium layer of cell wall; ICW, inner layer of cell wall; C, cytoplasm; N, nucleus; Nu, nucleolus; L, lipid granules, M, mitochondria.

Fig. 5. TEM of an *A. elynae* senescent ustilospore, showing three-layered cell wall, lobate nucleus and fused lipid granules. ECW, external layer of cell wall; MCW, medium layer of cell wall; ICW, inner layer of cell wall; N, nucleus; L, lipid granules.

Fig. 6. TEM of *A. elynae* ustilospore coloured with bismuth subnitrate showing reserve glycogen in cytoplasm and ultrastructure of cell wall. ECW, external layer of cell wall; MCW, medium layer of cell wall; ICW, inner layer of cell wall; P, plasmalemma; L, lipid granules; G, glycogen granules.

Fig. 7. TEM of *A. elynae* ustilospore coloured with bismuth subnitrate showing reserve glycogen in cytoplasm: ICW, inner layer of cell wall; P, plasmalemma; L, lipid granules; G, glycogen granules.

Conclusions

In conclusion, *A. elynae* ustilospores have a dynamic ultrastructure, with structural components changing with developmental stage. Based on the ultrastructural changes of the three-layered cell wall, the shape of the nucleus, and lipid and glycogen accumulation in the cytoplasm, the development of *A. elynae* ustilospores can be divided into three different stages.

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