ARBORETUM KÓRNICKIE
ROCZNIK XXIX — 1984

KRYSTYNA PRZYBYŁ

# Disease of poplar caused by the fungus *Ceratocystis fimbriata* Ell. et Halst. II. Morphology of the pathogen*

## INTRODUCTION

The fungus *Ceratocystis fimbriata* Ell. et Halst. was first isolated by Halsted in 1890 from diseased sweet potato (after Hunt 1956). Presently the fungus is known as a pathogen of such plant species as *Coffea arabica* L., *Hevea rubber* L., *Platanus orientalis* L., *Populus tremuloides* Mixch., *Prunus domestica* L., *Prunus americana* L., *Prunus amygdalis* Batsch., *Prunus persica* L., *Theobroma cacao* L., (Martin 1949, Olson and Martin 1949, Feazeli and Martin 1950, Schieber and Sosa 1960, Parkinson 1964, Zalasky 1965, Webster and Butler 1967, De Vay et al. 1968, Smith 1970, Panconesi 1976, 1977, Panconesi and Nambi 1978).

The first study on the occurrence of *C. fimbriata* in Poland on stems of three poplar clones (P. 'NE-44', P. 'NE-49', P. 'Kórnik 5') was published in 1977 (Gremmen and De Kam 1977). Then Przybył (1980 and 1983) made a review of poplar plantations in 26 Forest Districts in various parts of Poland. The fungus *C. fimbriata* was isolated from 38 trees of seven poplar clones.

It is the purpose of the present investigation to present the morphology of the fungus *C. fimbriata* isolated from poplars. The studies were performed using an electron and a light microscope.

## MATERIAL AND METHODS

Studies on the morphology of the fungus have been conducted on 38 isolations from trees of seven poplar clones made in the years 1978 and 1979 (Przybył 1980, 1983).

---

* This paper is a part of Ph. D. thesis carried out in the Institute of Dendrology Polish Academy of Sciences in Kórnik, under supervision of Dr F. Młodzianowski from the Department of Botany, A. Mickiewicz University in Poznań. This study was supported by research program fund MR. II/16, coordinated by the Institute of Dendrology, Kórnik.

Sterile hyphae of the fungus *C. fimbriata* have been picked up from pure cultures and stained with Methyl Blue in lactophenol. Measurements of the length and width of 50 conidia of each type (cylindrical, barrel shaped, oval thick-walled and ascospores) were made from all the isolations of the fungus using an ocular micrometer.

In order to demonstrate the presence of lysosomes in spores and hyphae of the fungus use was made of a technique depending on the identification of acid phosphatase (Armentrout et al. 1968, Wilson et al. 1970). The fungus was cultured at a temperature of 29°C on a potato culture. After 36 hours and 2 weeks of culturing hyphae of the fungus were placed for a period of 24 hours in a solution with the following composition 0.6 g plumbic nitrate in 500 ml 0.05 M acetate buffer (pH 4.5) and 50 ml 0.1 M sodium glycerophosphate. The material after washing in distilled water was transferred to a 0.5% aqueous solution of ammonium sulphate for a period of 3 min. The hyphae of the fungus were observed under a light microscope in a few drops of distilled water.

Acid phosphatase in hyphae and conidia of the fungus *C. fimbriata* has been also identified by the method of Pearse (1957). For the purpose hyphae of the fungus growing on a potato medium have been filtered after prior violent shaking with a mixture made as follows: to 20 mg of $\alpha$-naphtylo sodium phosphate dissolved in 20 ml of 0.1 M acetate buffer (pH 5.0) 20 mg of dinitrite salt of Fast Blue RR (4-benzoiloamino-2,5-dimethoxyaniline) was added. Then the material was incubated at 37°C for 3 hours. After washing the material with distilled water observations were made under a light microscope.

A control in both the methods was composed of a mycelium onto which the incubation medium devoid of the substrate was filtered on.

Observations of the lysosomes were also conducted in a fluorescence microscope using UGl (1,5) and UGl (3,5)+BG12 (2,5) filters and 0.01% Nile Blue (Holcomb et al. 1967).

In order to observe in a transmission electron microscope, the hyphae of *C. fimbriata* together with pieces of the potato medium were fixed in 5% glutaric aldehyde in 0.01 M phosphate buffer (pH 6.8) at a temperature of 4°C for a period of 12 hours. After that time the material was washed in the phosphate buffer and then fixed again in 2% osmium tetroxide ($OsO_4$) dissolved in a 0.1 M phosphate buffer for two hours at a temperature of 4°C.

A part of the material was also fixed in a 1% solution of potassium permanganate ($KMnO_4$) for a period of 20 min. After that time the material was washed trice with distilled water.

Dehydration of the samples fixed in glutaric aldehyde and in potassium permanganate has been accomplished through a series of transfers to acetone. Then the material was transferred to propylene oxide

for 10 min and then serially into increasingly concentrated epone mixtures 25%, 50%, 70% and 100% dissolved in propylene oxide. The composition of the epone mixture was as follows: Epon 812 MNA (Methyl nadic anhydride), DDSA (Dodecymyl succinic anhydride) and the accelerator DMP-30 (2, 4, 6-tri dimethylar inomethyl phenol) in proportions 3 : 2 : 1.

The material was sectioned on a LBK microtome type Ultratom III. A part of the sections was contrasted with a solution of uranyl acetate and plumbic citrate. Preparations made in this manner were observed in an JEOLCO electron microscope type 7A using an 80KV potential.

The negatives were made on an ORWO film type RU-2 at various magnifications.

Material intended for observation in a scanning electron microscope was fixed in the manner described above. After dehydration and drying, the sections were fixed to the basal slides and dusted with carbon and gold.

The observations were made with a scanning electron microscope type JSM-VS.

## RESULTS

### DESCRIPTION OF THE FUNGAL COLONIES

In the first four days of culturing, both on the surface as well as deep inside the medium white hyphae developed. Free ends of growing hyphae in the medium were strongly branched. After seven days of culturing, both the fungal colony and the medium took on an olive-brown colour, which with the passage of time turned dark-brown. After two weeks it was possible to observe on the medium surface single perithecia. With time their number increased. After two months numerous perithecia formed concentric rings on the surface of the medium.

In the period from one to four weeks of culturing the fungus produced a characteristic fruity smell, mostly resembling the smell of bananas.

### CHARACTERIZATION OF THE HYPHAE AND CONIDIA

Hyphae of the fungus, both growing on the surface and inside the medium were divided by transverse septa. Two types of hyphae were recognized: those growing in the substrate, morphologically unspecialized hyphae from 2 - 3 μm wide and endoconidiophore-hyphae 4 - 6 μm wide. Within the endoconidiophore-hyphae conidia developed, cylindrical and barrel shaped. Within the medium only cylindrical conidia were

observed. After 10 days of culturing a third type of conidial spores was observed, namely oval (thickwalled) ones, which formed on ends of hyphae.

In two week old colonies of the fungus *C. fimbriata* the majority of hyphae have had a dark brown pigmentation. In this type of hyphae a thickening of the wall towards the cell lumen was observed (Fig. 1). Hyphae which were dark brown in colour were referred to as pigmented. On the other hand hyphae devoid of the dark stain were called "nonpigmented".

Along the walls and in apical parts of the hyphae, both nonpigmented and pigmented ones, bodies were found which have had a gold-yellow fluorescence and acid phosphatase activity.

### CYLINDRICAL CONIDIA

Cylindrical conidia form within transparent thinwalled, bottle-shaped hyphae the length of which fluctuated within the limits 25 - 100 μm and width between 3 and 7 μm. After detachment of the string of conidia at the end of these hyphae two projections were observed which were elongations of the wall (Fig. 2).

The length of cylindrical conidia from all the isolated strains varied between 8.5 and 32.8 μm and width between 2.7 and 7.5 μm. After formation the conidia were little differentiated in shape. They maintained the characteristic for this type of conidia cylindrical shape, narrowing at both ends (Fig. 2).

In older cultures of the fungus the cylindrical conidia have had a greater variability in shape, some darkenings and vacuolisations (Fig. 3). Also in this type of spores blisterlike thickenings of the cell wall towards the inside of the cell were observed.

After 36 hours of culturing the fungus in some conidia bodies were found which have a positive reaction for acid phosphatase and a golden-yellow fluorescence. After two weeks of culturing, the majority of cylindrical conidia have had numerous, agglomerating bodies giving a positive reaction for acid phosphatase and a specific fluorescence for lysosomes. Younger and older cylindrical conidia germinated easily.

### BARREL SHAPED CONIDIA

Barrel shaped conidia, similarily as cylindrical conidia are formed by endoconidiophore hyphae (Fig. 4). In the case of barrel shaped conidia, these hyphae were shorter (20 - 85 μm) and wider (6 - 8 μm) than those yielding cylindrical conidia.

The length of barrel shaped conidia in all the isolations of the fungus was within the range 6.4 - 13 μm, while the width varied from 4 to 9.3 μm. In dark brown colonies the barrel shaped conidia have had

thickenings of the cell walls towards the cell lumen and numerous vacuoles (Fig. 5).

In barrel shaped conidia, similarily as in cylindrical conidia acid phosphatase activity was observed as well as the presence of bodies with a golden-yellow fluorescence.

### OVAL (THICKWALLED) CONIDIA

Oval conidia were characterized by having a thicker cell wall compared to that in cylindrical and barrel shaped conidia. They formed on the tips of hyphae (Fig. 6). Wall of these spores was a continuation of the wall of the hyphae forming them, the length of which varied from 11.2 to 74 μm and the width from 3.5 to 7.8 μm. The length of spores in all the isolated strains varied between 7.0 and 24.8 μm and the width between 5.1 and 15.2 μm. These conidia became strongly vacuolized with time, as a result of which they became more transparent and lost their characteristic light brown pigmentation.

### ULTRASTRUCTURE — NONPIGMENTED HYPHAE

Nonpigmented hyphae of the fungus *C. fimbriata* were characterized by an electronally white cytoplasm surrounded by a plasmalemma having an undulating outline in cross section.

The cell wall of the hyphae was composed of three layers. The middle layer differed in having an electronally white structure differing from the external and inner layers. The nucleus was encased in a double membrane with an electronally very dark nucleolus. It differed in size from all other cell organelles (Fig. 7). In the nonpigmented hyphae membraneous bodies were most common (referred to in English as: membranous structures — Jeng and Hubbes (1980) membranous body — Harris and Taber (1973), vesicular membrane complex — Howard and Maxwell (1975), in Italian as: corpo lamellare — Mutto et al. (1978)) of variable size and shape. More common were bodies containg a complex of short membranes in the form of tubuli and small vesicles (Fig. 7). Besides this type of membranous bodies there were also such, the internal structure of which was composed of appropriately smaller size 1 - 2 oval or irregular vesicles (Fig. 8). On this photo it is possible to see within the cytoplasm an electronally lighter complex of this type of membranous bodies. Similar formations to those of the internal vesicles of the membraneous bodies were observed in the spaces between the plasmalemma and the cell wall and not infrequently in the space of the strongly thickened part of the plasmalemma (see pigmented hyphae, Fig. 16). In hyphae with such an appearance it was possible to observe near the transverse septa

some uniform, electronally light bodies encased in membranes, which resembled vacuoles in appearance.

It was possible to observe also Woronin bodies near the primary septa. They were frequently found opposite pores in the transverse septum (Fig. 9). These organelles were oval in shape or globular and contained a homogenous matrix.

Among other cell organelles typical for young hyphae it was possible to observe elongate, oval or frond-like mitochondria (Fig. 10), a plasmatic reticulum (Fig. 11) dictyosomes (Fig. 11) and single, globular osmiophilic bodies (Fig. 8) with a variable electron density. These bodies occurred most commonly in the apical parts of the hyphae. In cytoplasm of nonpigmented hyphae also single osmiophilic inclusions were found (Fig. 7).

### PIGMENTED HYPHAE

Whole hyphae characterized by a brown pigmentation as well older parts of the same hypha, have had an electronally dark cytoplasm surrounded in cross-section by a slightly undulating plasmalemma (Fig. 12). The external-most layer of the cell wall of pigmented hyphae was surrounded by numerous electronally dark granula (Fig. 13).

On the other hand on the outside of the cell walls of nonpigmented hyphae there were only single electronally dark granulations. In pigmented hyphae, similarily as in nonpigmented ones a nucleus with a nucleous and numerous membranous bodies were found in which various sized osmiophilic inclusions occurred (Fig. 13). Besides the two types of membranous bodies discussed when describing the ultrastructure of non-pigmented hyphae, here also the presence of composite bodies was observed which in cross section had concentrically arranged structures. They resemble mielin like bodies (Fig. 14).

Agglomerations of membranous bodies were also observed in the terminal parts of the hyphae (Fig. 15). They were composed of shorter and longer membranes in the form of flattened, small vesicles. Possibly the longer double bands represent the endoplasmic reticulum.

In pigmented hyphae as well as in nonpigmented ones it was possible to observe in the spaces between the plasmalemma and the cell wall and in the spaces of the swallen parts of the plasmalemma, some formations similar to the internal vesicles of membranous bodies (Fig. 16). On this photo it is possible to see bodies resembling vacuoles lying near the transverse septa. In the electronally dark cytoplasm characterising this type of hyphae, besides the organelles mentioned it was possible also to observe Woronin bodies which were located near the forming transverse septum, separating the younger part of the hyphae from the maternal hyphae, bands of the endoplasmic reticulum and single oval osmiophilic bodies (Fig. 17).

### MORPHOLOGY OF THE PERITHECIUM

Perithecium is composed of a base and a neck ending in ostiolar hyphae (Fig. 18 and 19). The globular or conical base having a smooth, dark brown or black surface, was covered by mottled hyphae, usually conidiophores, producing numerous conidia. Dimentions of the bases of mature perithecia in all the isolated strains varied between 150 - 240 × × 150 - 360 μm. Necks were 750 - 800 μm long and were wider at the base (25 - 38 μm), narrowing towards the top (15 - 30 μm) (Fig. 19). The number of ostiolar hyphae was variable within the limit 8 to 22. Width of the ostiolar hypha was 2.3 μm. The neck was formed by parallel hyphae outgrowing from the wall of the perithecium. These hyphae constituted the external layer of the neck and surrounded the internal space filled with ascospores (Fig. 20), which oozed out from the perithecium through this neck in drops of a jelly-like mass.

### ASCOSPORES

Mature ascospores were transparent, oval and formed projections at both their ends. The ascospores with the projections resembled a small "hat" in appearance, which is the characteristic form for this type of spores (Fig. 21). The length of ascospores was between 4 and 7.2 μm and the width between 2.5 and 6.5 μm. Sexual spores as well as cylindrical conidia germinated readily. Ascospores germinated on a potato medium at a temperature of 29°C already after 24 hours of incubation (Fig. 22).

## DISCUSSION

### CHARACTERISATION OF THE COLONIES

Webster and Butler (1967) have compared the morphology of *C. fimbriata* strains isolated from the following host plants: *Ipomea batatas* Lam., *Theobroma cacao* L., *Coffea arabica* L., *Platanus* L., *Populus tremuloides* Michx., *Quercus ellipsoidalis* Hill., *Prunus domestica* L., *Prunus persica* Batchs. and *Prunus amygdalis* Batchs. These authors have divided the colonies of the isolated of the fungus into three groups. The criterium for the division were such traits as pigmentation of the colony and type of growth of the perithecium. Strains isolated from the poplar clones in Poland have to be included on the basis of the dark olive colour of the colonies and the growth of the perithecium in concentric rings in the second group described by Webster and Butler (1967) in which were included strains of the fungus isolated from the trembling aspen and the fruit trees. The traits of the strains isolated from poplars are in agreement with the description give by Hunt (1956).

SIZE AND OCCURRENCE OF THE CONIDIA

In the cultures of strains of the fungus *C. fimbriata* isolated from poplars it was found that the imperfect stage of the fungus is characterized by three types of conidia: cylindrical, barrel shaped and oval (thick-walled).

In the literature there is agreement as concerns the endoconidial origin of cylindrical conidia. Also no discrepancies were found with these opinions in the spores originating from strains of the fungus isolated from poplars.

The length of the cylindrical conidia in all the strains of *C. fimbriata* isolated from poplars (range 8.5 - 32.8 μm) lies within the length given jointly for isolates from fruit trees, coffee, aspen and plane trees (range 6.8 - 36.9 μm as given by Webster and Butler 1967). The data given above differ from those reported for cylindrical conidia of *C. fimbriata* by such investigators as Pontis (1951), Campbell (1960), Zalasky (1965) and Panconesi (1973).

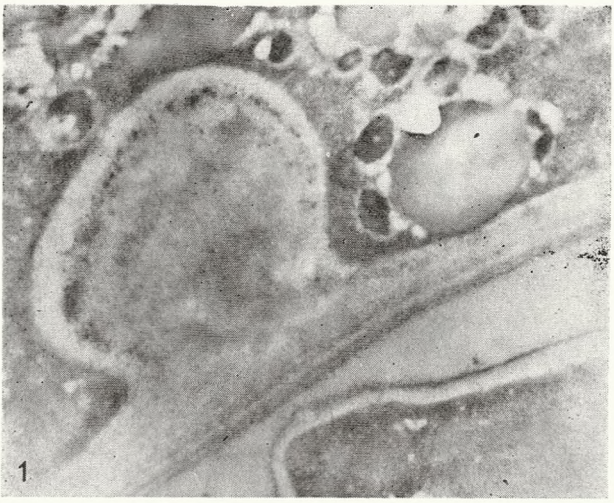
Barrel shaped conidia of *C. fimbriata* ("doliform endoconidia" of Webster and Butler (1967), barrel shaped endoconidia of Zalasky (1965)) similarily as the cylindrical conidia originate inside tubular hyphae.

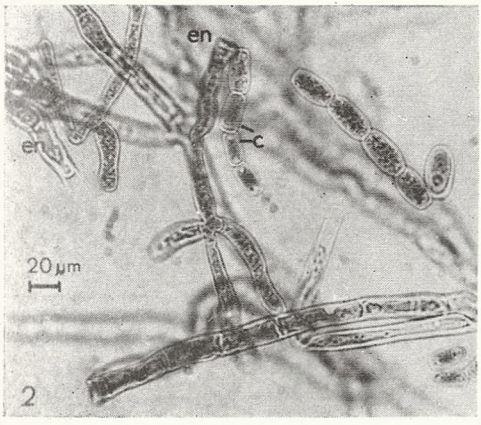
Barrel shaped conidia were observed in isolates of *C. fimbriata* obtained from fruit trees, oaks, plane trees and aspen. Their occurrence was not observed in isolates from coffee, cacao and sweet potatoes (Webster and Butler 1967).

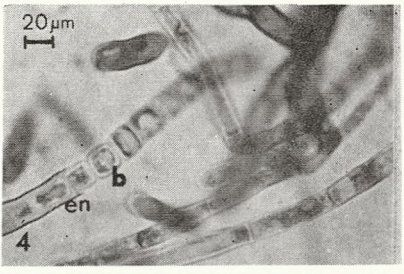
Length and width of this type of conidia in all the isolated strains from poplar clones vary within the following limits: length 6.4 - 13 μm and width 4.0 - 9.3 μm. These magnitudes differ only slightly from the dimentions of barrel shaped conidia of *C. fimbriata* isolated from other hosts (Campbell 1960, Zalasky 1965, Webster and Butler 1967). On the other hand considerable discrepancies were found between the dimentions of these conidia in strains of the fungus isolated from poplars as given by Hunt (1956) who reported 9 - 16×6.0 - 13 μm. It is suggested that the cause of the discrepancy lies in the placement by that author of both the barrel shaped conidia and the oval (thick-walled) ones in one group.

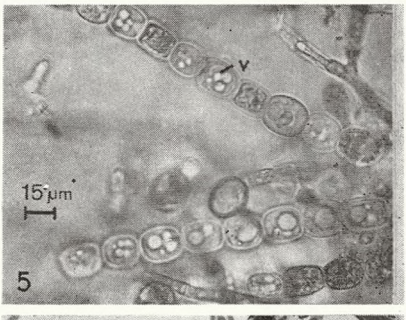
Oval conidia (thick-walled conidia — Webster and Butler 1967, parete spessa — Panconesi 1973) were observed in all strains of *C. fimbriata* isolated from seven poplar clones after 10 days of incubation at a temperature of 29°C.

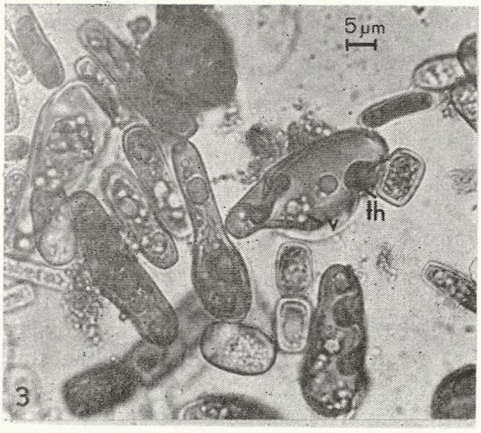
Webster and Butler (1967) report that oval conidia occurred in large numbers in strains of the fungus *C. fimbriata* isolated from such hosts as *Ipomea batatas, Coffea arabica, Platanus* sp., *Populus tremuloides, Prunus amygdalis, Prunus armeniaca, Prunus domestica, Prunus*



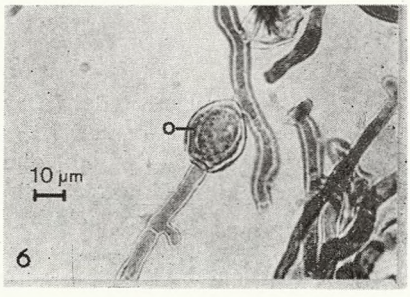
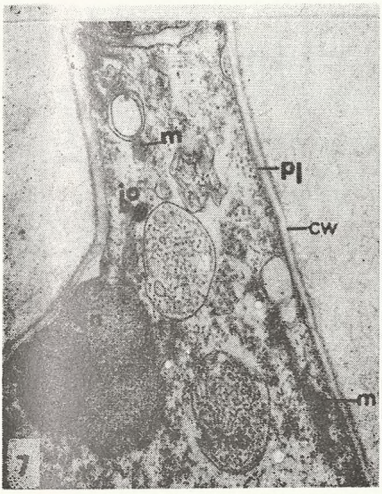
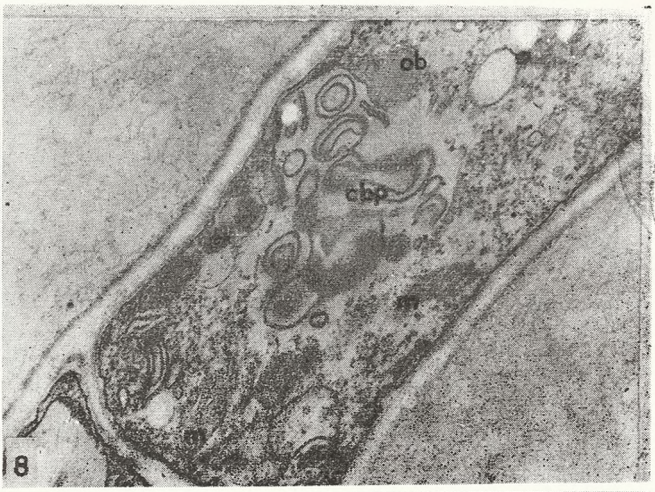




Fig. 1 - 6: 1 — Thickening of cell wall of pigmented hyphae towards the cell lumen. Transmission electrone microscope, glutaraldehyde fixation, ×8000. Micrograph by F. Młodzianowski, 2 — Endoconidiophores (*en*) producing cylindrical conidia (*c*). 3 — Cylindrical conidia in older cultures of *C. fimbriata* fungus (vacuoles — *v*, thickening of wall — *th*), 4 — Endoconidiophores (*en*) producing barrel shaped conidia (*b*), 5 — Barrel shaped conidia in older cultures of *C. fimbriata* fungus (vacuoles — *v*), 6 — Terminal origin of oval (thick walled — *o*) conidia

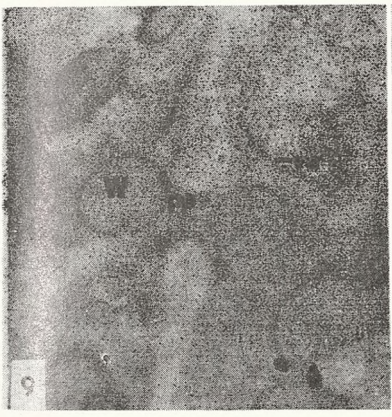
Fig. 7 - 12: 7 — Nonpigmented hyphae (plasmalemma — *pl*, cell wall — *cw*, nucleus — *n*, nucleolus — *nu*, mitochondria — *m*, membraneous body consisting of tubules and small vesicles — *mb*, osmiophilic inclusions — *io*). Transmission electron microscope, glutaraldehyde fixation, ×8000, 8 — Membraneous body consisting of 1 - 2 oval or irregular vesicles (*cbp*) in nonpigmented hyphae (mitochondria — *m*, osmiophilic bodies — *ob*). Transmission electron microscope, glutaraldehyde fixation, ×15000, 9 — Woronin body (*W*) situated opposite a pore of septum (*pp*) and endoplasmic reticulum (*re*) in a nonpigmented hyphae. Transmission electron microscope, potassium permengate fixation, ×26000, 10 — Nonpigmented hyphae (elongated and oval profiles of mitochondria — *m*, vacuoles — *v*). Transmission electron microscope, glutaraldehyde fixation, ×14000, 11 — Golgi dictyosome (*d*) and endoplasmic reticulum *re*) in a nonpigmented hyphae. Transmission electron microscope, glutaraldehyde fixation, ×14000, 12 — Nonpigmented (np) and pigmented (p) parts of hyphae. Transmission electrone microscope, glutaraldehyde fixation, ×3100. Micrographs 7 - 12 by F. Młodzianowski



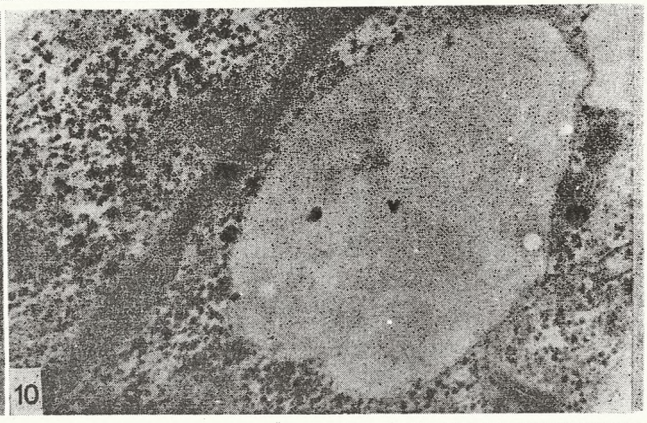
7



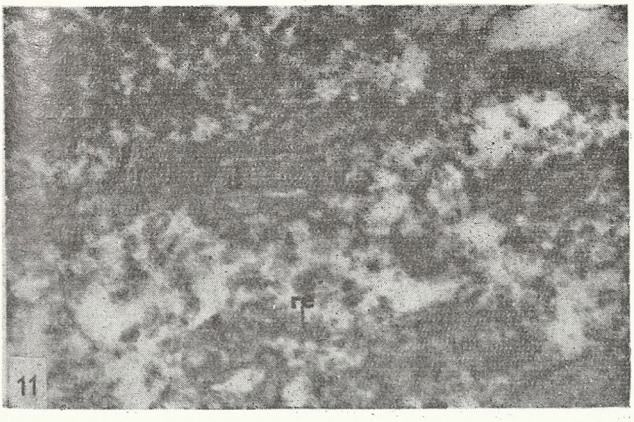
8



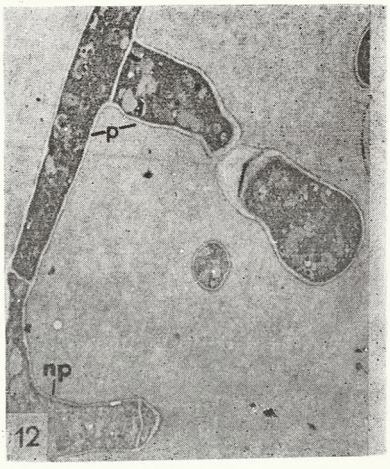
9



10

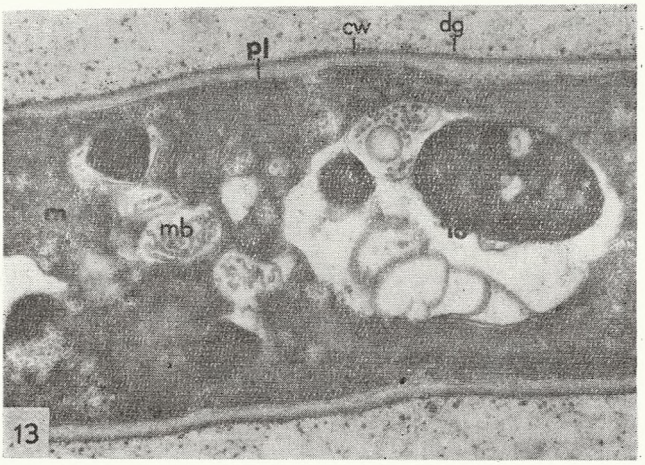


11

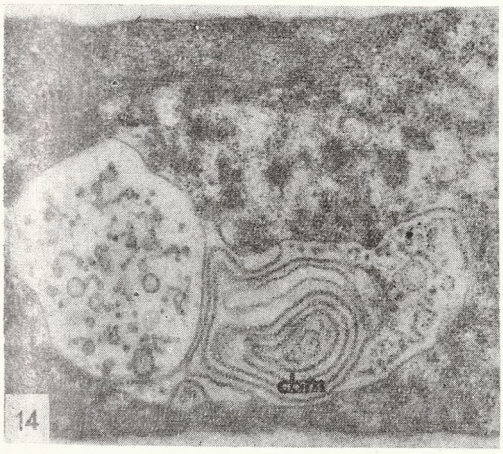


12

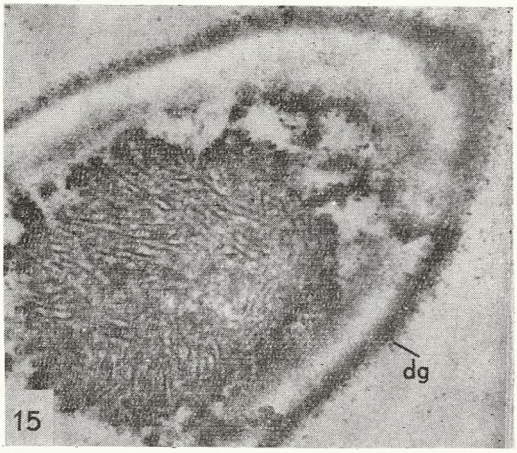
Fig. 13 - 17: 13 — (×1400), 14 (×22000) and 17 (×26000). Pigmented hyphae (plasmalemma — *pl*, cell wall — *cw*, electronally dark granules outside of wall — *dg*, oval mitochondria — *m*, mielin like body — *cbm*, membraneous body consisting of tubules — *mb*, Woronin body — *W*, osmiophilic bodies — *ob*, endoplasmic reticulum — *re*, vacuoles — *v*, osmiophilic inclusions — *io*). Transmission electron microscope, glutaraldehyde fixation, 15 — Accumulation of membraneous bodies on the cell tip of pigmented hyphae (electronally dark granules on outside of wall — dg). Transmission electron microscope, glutaraldehyde fixation, 14000, 16 — Vesicular structures (*vs*) in space between plasmalemma (*pl*) and cell wall (*cw*) and in standing apart zone plasmalemma (*pl*). Near the standing off part of plasmalemma the electronally light bodies resembling vacuoles (*v*) and Woronin bodies (*W*) are visible. Transmission electrone microscope, glutaraldehyde fixation, ×20000. Micrographs 13 - 17 by F. Młodzianowski



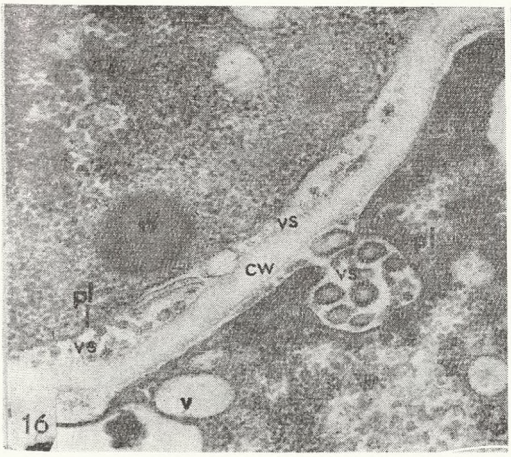
13



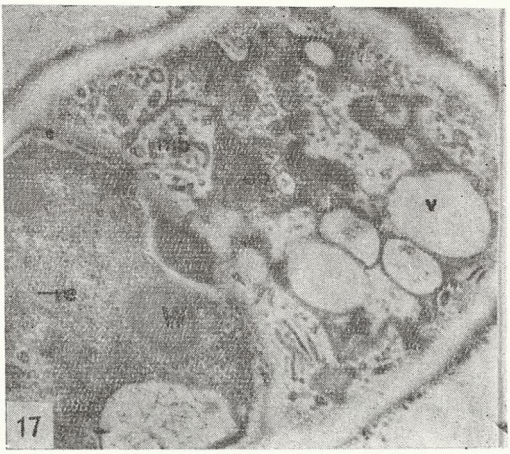
14



15



16



17

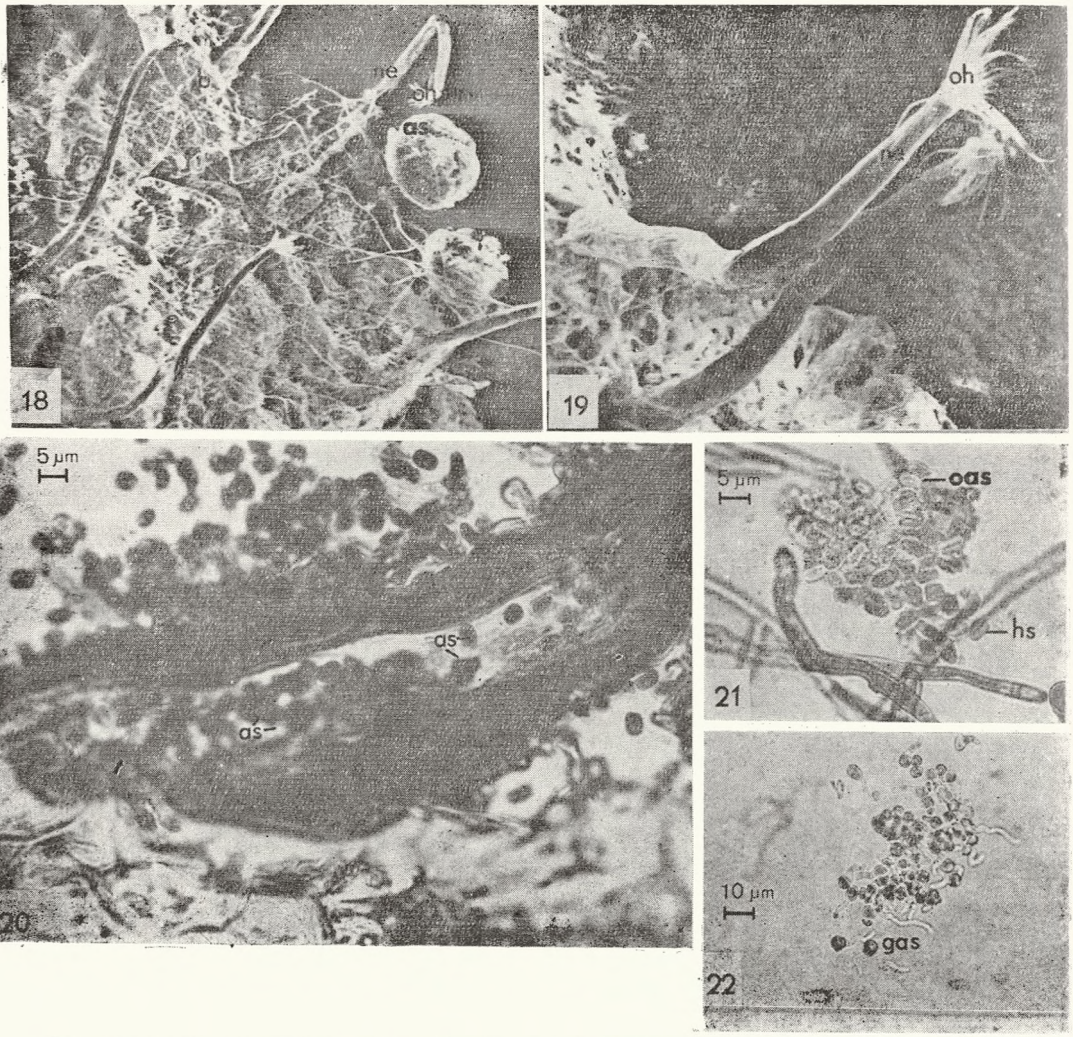



Fig. 18. Perithecium (base of perithecium — *b*, neck of perithecium — ne, ascospores — *as*, ostiolar hyphae — *ah*). Scanning electrone microscope, x100, 19 — Neck of perithecium (*ne*) and ostiolar hyphae (*oh*). Scanning electrone microscope, ×250, 20 — Neck of perithecium with ascospores (*as*). Longitudinal section, 21. and 22. Ascospores (oval — *oas*, hat-shaped — *hs*, germinating — *gas*). Micrographs 18 and 19 by F. Młodzianowski

*persica*, *Quercus ellipsoidalis* and *Theobroma cacao*. Observations of these authors are in agreement with the reports of P a n c o n e s i (1973) based on studies of *C. fimbriata* f. *platani* and P o n t i s (1951) who investigated the morphology of isolates from coffee trees. On the other hand H u n t (1956), Z a l a s k y (1965) and G r e m m e n and D e K a m (1977) when describing the morphology of the fungus *C. fimbriata* do not mention such conidia.

In the studies on the morphology of *C. fimbriata* there occur disagreements in the views on the origin of the oval (thickwalled) conidia. In the present study it has been mentioned that these conidia formed on tips of hyphae. Wall of the oval conidia, in contrast to the conidia of endoconidial origin, represented an extension of the wall of the hyphae. Similar observations were made by W e b s t e r and B u t l e r (1967). On the other hand A n d r u s and H a r t e r (1933 — after H u n t 1956), and P a n c o n e s i (1973) report endoconidial origin for these conidia. In conidial spores and in hyphae collected from the dark brown colonies of the fungus *C. fimbriata* a thickening of the cell wall towards the lumen was observed. However insufficient observations are available to explain the role or function of these thickenings in the cells.

The main criterion differentiating under electron microscopy the nonpigmented from the pigmented hyphae were the electronally dark granulations occurring on the outside of the cell wall. Similar observations were made earlier by J e n g and H u b b e s (1980) in hyphae of *C. ulmi*. These authors have suggested that the electronally dense bodies are product of protein turnover. Discussion on the nature of these bodies requires further investigations.

## CELL ORGANELLES

The criterium for distinguishing between hyphae of *C. fimbriata* under electron microscopy lies in the electronally dense granulations occurring in the cytoplasm and outside the cell.

In nonpigmented hyphae of the fungus such organelles were found as the nucleus with nucleolus, mitochondria, membraneous bodies, osmiophilic bodies (most probably lysosomes), Woronin bodies, rugged endoplasmic reticulum and dictyosomes.

In the cytoplasm of pigmented hyphae besides the granulations mentioned above there were numerous osmiophilic inclusions, membraneous bodies, Woronin bodies and the nucleus with nucleolus. Other cell organelles were difficult to observe in the electronally dense cytoplasm.

In the hyphae of the fungus *C. fimbriata* three types of membraneous bodies were observed. In nonpigmented hyphae they were composed of short membranes in the form of tubuli and various size ve-

sicles. More numerous were tubular bodies. In pigmented hyphae besides the sporadic appearance of the bodies mentioned above there were also mielin like bodies, which in cross-section form concentric rings. The various size tubules and vesicles representing the content of these bodies were found in the swollen part of the plasmalemma and in the space between the plasmalemma and the wall of the transverse septum. For bodies lying between the cell wall and the plasmalemma Moore and McAlear (1961 after Woźny and Młodzianowski 1972) proposed the name lomasomes.

Structure of the membranous bodies in the apical part of the pigmented hyphae differs from the structure of bodies known under the name Spitzenkörper or apical body reported in fungi of class *Ascomycetes* such as *Neurospora crassa* and *Monilia fructigena* by Backett et al. (1974) and Turian (1978).

Membraneous bodies were observed also in hyphae of other fungal species from the genus *Ceratocystis* such as *C. fagacearum, C. fimbriata* f. *platani* and *C. ulmi* (Armentrout et al. 1968 and Jeng and Hubbes 1980). Last of the authors mentioned above claims that the system of membraneous bodies is much more developed in nonpigmented hyphae than in pigmented ones.

Lysosomes in hyphae of the majority of fungi studied, such as in *C. fagacearum* are agglomerated only in tips. On the other hand single fluorescing bodies occurred near a forming septum and in the centre of the cell (Armentrout et al. 1968). In the latter case these bodies according to the authors mentioned above were similar in appearance to Woronin bodies. In hyphae of the fungus *C. fimbriata* isolated from poplars it was also possible to observe bodies that have a golden-yellow fluorescence and acid phosphatase activity. These bodies were localized in the terminal part of the hyphae and along the lateral walls. Under electron microscopy it was possible to see in hyphae of *C. fimbriata* isolated from poplars numerous oval bodies differing in electron density.

Comparing the observations made under fluorescent microscope with those under light microscope, in which the reaction for acid phosphatase was observed it is possible suspect that the single membraned, oval bodies seen under a transmission electron microscope were lysosomes. The question of the identification of the lysosomes can only be solved through cytochemical reactions observed in an electron microscope. The occurrence of lysosomes in fungi is considered by some authors to be of special significance in the host-pathogen interaction (Pitt and Combes 1968).

### PERITHECIA AND ASCOSPORES

Fruiting bodies referred to as perithecia have been produced by all the isolated strains of *C. fimbriata* from poplar clones. The dimentions of individual elements of this fungus (base: 150 - 240×150 - 360 μm; neck: 750 - 800 μm×basal width 25 - 38 μm, apical width 15 - 30 μm) were most akin to the dimentions reported earlier by Hunt (1956) and Panconesi (1973). On the other hand the number of ostiolar hyphae mentioned in the literature varies: 8 - 15 (Hunt 1956), 8 - 16 (Zalasky 1965), 7 - 16 (Webster and Butler 1967), 9 - 17 (Panconesi 1973). The number of ostiolar hyphae observed in strains of the fungus *C. fimbriata* isolated from poplars varied from 8 to 23. It is possible that in this case the observations using a scanning electron microscope have permitted a more accurate counting of the number of ostiolar hyphae.

Informations concerning the shape of the ascospores reported in the literature by the authors mentioned above are in agreement with the observations which were made in the present investigation. Also the number of ascospores in the strains of *C. fimbriata* isolated from poplar clones fit within the ranges reported by all authors dealing with the morphology of this fungus.

### ACKNOWLEDGEMENT

I wish to thank the late Doc. dr hab. Fortunat Młodzianowski for the valuable suggestions and discussions during the conduct of these experiments and for help with electron microscopy.

### SUMMARY

Studies on the morphology of the fungus *C. fimbriata* isolated from poplars have been conducted using light and electron microscopy. The studies covered 38 strains isolated in the years 1978 and 1979 from a dosen or so poplar clones.

The imperfect stage of the fungus is characterized by conidia of the following dimentions: cylindrical conidia 8.5 - 32.8 μm×2.7 - 7.5 μm, barrel shaped conidia 6.4 - 13 μm×4.0 - 9.6 μm and oval conidia (thick-walled) 7.0 - 24.8 μm×5.1 - 15.2 μm. The perfect stage consisted of perithecia with ascospores 4.0 - 7.2 μm×2.5 - 6.5 μm in size.

The main criterion differentiating nonpigmented hyphae from pigmented ones of the fungus *C. fimbriata* on the level of the electron microscope were the electronally dense granulations lying in the cytoplasm and outside the cells. In nonpigmented hyphae the following

organelles were observed: nucleus with nucleolus, mitochondria, membraneous bodies, osmiophilic bodies (probably lysosomes), Woronin bodies, rugged endoplasmic reticulum and dictyosomes. In the electronally dense cytoplasm of pigmented hyphae besides numerous osmiophilic inclusions there were a nucleus with nucleolus, membraneous bodies and Woronin bodies.

Institute of Dendrology
62-035 Kórnik

## LITERATURE

1. Armentrout Y. N., Smith G. G., Wilson C. L., 1968. Spherosomes and mitochondria in the living fungal cell Ann. J. Bot: 55: 1062 - 1067.
2. Backett A., Heath J. B., McLoughlin D. J., 1974. An Atlas of Fungal Ultrastructure. London.
3. Campbell R. N., 1960. Some sap-stain fungi in Minnesota. Plant Dis. Reptr. 44: 625 - 628.
4. De Vay J. E., Lukezic F. L., English H., Trujillo E. E., Moller W. J., 1968: *Ceratocystis* canker of deciduous fruit trees. Phytopathology 58: 949 - 954.
5. Feazeli G. D., Martin W. J., 1950. Studies on *Ceratostomella fimbriata* from sweet potato and sycamore. Phytopathology 40: 787 (Abstr.).
6. Gremmen J., Kam M. De. 1977. *Ceratocystis fimbriata* a fungus associated with poplar canker in Poland. Eur. J. For. Path. 7: 44 - 47.
7. Harris J. L., Taber W. A., 1973. Ultrastructure and morphogenesis of the synnema of *Ceratocystis ulmi.* Can. J. Bot. 51: 1565 - 1571.
8. Holcomb G. E., Hildebrandt A. C., Evert F., 1967. Staining and acid phosphatase reactions of spherosomes in plant tissue culture cells. Amer. J. Bot. 54: 1204 - 1209.
9. Howard R. J., Maxwell D. P., 1975. Cytology of *Ceratocystis ulmi.* Phytopathology 65, 816 - 819.
10. Hunt J., 1956. Taxonomy of the Genus *Ceratocystis.* Lloydia 1: 1 - 58.
11. Jeng S., Hubbes M., 1980. Ultrastructure of *Ceratocystis ulmi* I. Hyphae and ascocarp peridium. Eur. J. For. Path. 10: 16 - 27.
12. Martin W. J., 1949. Mouldy rot of tapping panels *Hevea rubber trees.* U.S.D.A. Circular 798.
13. Mutto S., Ferrata M., D'Ambra V., 1978. Ricerche al Microscopic Elettronico sul Legno di Platano Invaso da *Ceratocystis fimbriata* f. *platani.* Phytopath. Z. 91: 39 - 51.
14. Olson E. O., Martin W. J., 1949. Relationship of *Ceratostomella fimbriata* from the *Hevea rubber* and sweet potato. Phytopathology 39: 17 (Abstr.).
15. Panconesi A., 1973: Il rinvenimento della *Ceratocystis fimbriata* (Ell. and Halst.) Davidson f. *platani* Walter su Platano. Riv. di Patol. Veget. IV: 167 - - 188.
16. Panconesi A., 1976. La pericolosita della *Ceratocystis fimbriata* (Ell. et Halst.) Davidson f. *platani* Walter in rapporto alle operazioni di potatufo. Riv. di Pat. Veg. IV: 20 - 33.
17. Panconesi A., 1977. La Tracheomicosi del Platano in Versilia. Inform. Fitopat. 8: 43 - 46.

18. Panconesi A., Nambi V., 1978. La *Ceratocystis fimbriata* del Platano: aspetti biologici e possibilitá di lotta. Inform. Fitopat. 11/12: 17 - 27.
19. Parkinson B. W., 1964. Studies in the etiology of *Ceratocystis* canker of stone fruits. M. S. Thesis, Univ. California.
20. Pearse A. G. E., 1957. Histochemia teoretyczna i stosowana. Warszawa.
21. Pitt D., Combes C., 1968. The disruption of lysosomelike particles of *Solanum tuberosum* cells during infections by *Phytophtora erytroseptica* Pethybr. J. Ge. Microbiol. 53: 197 - 204.
22. Pontis R. E., 1951. A canker disease of the coffea tree in Columbia and Venezuela. Phytopathology 41: 178 - 184.
23. Przybył K., 1980. *Ceratocystis fimbriata* Ell. et Halst. — sprawca nowej choroby topoli. Sylwan 8: 47 - 54.
24. Przybył K., 1984. Disease of poplar caused by *Ceratocystis fimbriata* Ell. et Halst. I. Isolation of *Ceratocystis fimbriata*, symptoms of disease and evaluation of resistance of poplar clones resulting from artificial infection. Arboretum Kórnickie XXIX: 89 - 103.
25. Schieber E., Sosa O. N., 1960. Cacao canker in Guatemala incited by *Ceratocystis fimbriata*. Plant Dis. Reptr. 44: 672.
26. Smith W. H., 1970. Tree pathology. A short introduction. Academic Press. New York, London.
27. Turian G., 1978. The 'Spitzenkörper', centre of the reducing power in the growing hyphal apices of two septomycetous fungi. Experienta 34: 1277 - 1279.
28. Webster R., K., Butler E. E., 1967. A morphological and biological concept of the species *Ceratocystis fimbriata*. Can. J. Bot. 45: 1457 - 1468.
29. Wilson C. L., Stiers D. L., Smith G. G., 1970. Fungal lizosomes or spherosomes. Phytopathology 60: 216 - 227.
30. Woźny A., Młodzianowski F., 1972. Struktury pograniczne (lomasomy i plasmalemmasomy). Wiad. Bot. XVI: 91 - 98.
31. Zalasky H., 1965. Morphology of *Ceratocystis fimbriata* in aspen. Can. J. Bot. 43: 623 - 627.

## *Choroba topoli wywołana przez grzyb Ceratocystis fimbriata Ell et Halst.* II. *Morfologia patogenu*

### Streszczenie

Badania nad morfologią grzyba *C. fimbriata* wyizolowanego z topoli prowadzono z zastosowaniem mikroskopów świetlnego i elektronowego. Badaniami objęto 38 szczepów wyizolowanych w latach 1978 i 1979 z pni drzew kilkunastu klonów topoli.

Stadium niedoskonałe grzyba charakteryzują następujące zarodniki: cylindryczne (8,5 - 32,8 μm×2,7 - 7,5 μm), beczułkowate (6,4 - 13 μm×4,0 - 9,6 μm) oraz owalne (grubościenne — 7,0 - 24,8 μm×5.1 - 15,2 μm).

Na stadium doskonałe składają się otocznie z askosporami (4,0 - 7,2 μm×2,5 - - 6,5 μm).

Głównym kryterium odróżniającym niepigmentowane i pigmentowane strzępki grzyba *C. fimbriata* na poziomie mikroskopu elektronowego były leżące w cytoplazmie i na zewnątrz komórki elektronowo gęste ziarnistości. W strzępkach niepigmentowanych stwierdzono takie organelle, jak: jądro z jąderkiem, mitochondria, ciała błoniaste, ciała osmofilne (prawdopodobnie lizosomy), ciała Woronina,

szorstkie retikulum endoplazmatyczne i diktiosomy. W elektronowo gęstej cytoplazmie strzępek pigmentowanych oprócz licznych osmofilnych inkluzji obserwowano jądro z jąderkiem, ciała błoniaste i ciała Woronina.

## *Заболевание тополей вызванное грибом Ceratocystis fimbriata* Ell. et Halst.
## *II. Морфология патогена*

Резюме

Исследования морфологии гриба *C. fimbriata* изолированного с тополей проводили с помощью светового и электронного микроскопов. Изучалось 38 штаммов грибов изолированных в 1978 и 1979 гг. со стволов более десяти клонов тополей.

Несовершенная стадия гриба характеризуется следующими конидиями: цилиндрическими (8.5 - 32.8×2.7 - 7.5 мкм), похожими на боченки (6.4 - 13×4.0 - 9.6 мкм) и овальными с толстыми стенками (7.0 - 24.8×5.1 - 15.2 мкм).

Совершенной стадией является перитеций с аскоспорами (4.0 - 7.2×2.5 - 6.5 мкм).

Главным критерием отличия пигментированных и непигментированных гифов гриба *C. fimbriata* на уровне электронного микроскопа были находящиеся в цитоплазме и вне клеток плотные для электронов зерна. В лишенных пигментов гифах обнаружены следующие органеллы: ядро с ядрышком, митохондрии, мембранные тельца, осмиофильные тельца (вероятно лизосомы), тельца Воронина, шереховатый эндоплазматический ретикулум и диктиосомы. В плотной для электронов цитоплазме пигментированных гифов кроме многочисленных осмиофильных включений наблюдали также ядро с ядрышком, мембранные тельца и тельца Воронина.