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Reproductive structures of *Cryphonectria parasitica*

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ABSTRACT: Reproductive bodies of *Cryphonectria parasitica* – pycnidia and perithecia were studied. Pycnidia and perithecia were imbedded into Spurr's medium and semithin sections were prepared on ultramicrotome. Sections were observed under light microscopy and studied structures were documented by photographs.

KEYWORDS: *Cryphonectria parasitica*, pycnidia, perithecia.

Introduction

Cryphonectria parasitica (MURRILL) M. E. BARR (=*Endothia parasitica*, Valsaceae) that causes the chestnut blight disease is one of the most dangerous parasitic fungi. It attacks also another trees except of chestnut. The fungus was recorded for first time in Slovakia in the region of Prašice – Duchonka, county of Topoľčany (JUHÁSOVÁ 1983) in 1976.

Leaves of attacked branches on blighted chestnut trees become yellow and remain on the tree even after they got dry making visible contrast with leaves on healthy branches. First symptoms of the blight on the smooth stems and on the branches are distinct colour changes and depressions (JUHÁSOVÁ 1999).

On the attacked trees a marked boundary can be seen between the healthy and the attacked part of the host. Later bark cracks, swells and peels longitudinally. The cankers are most visible on young trees and smooth branches. It is rather difficult to distinguish cracking of bark of old trees caused by the blight, because it cracks naturally. A reliable symptom in this case is attacked stem coppices, which are numerous in the place of infection. The stem coppices present the same symptoms like attacked trees. Mycelium of the

fungus has yellow colour, fan shape and is located under bark of the host (JUHÁSOVÁ 1999).

The fungus produces both red-orange pycnidia (anamorph) and concolours perithecia (teleomorph). Because of same colour it is hard to distinguish pycnidia and perithecia macroscopically.

Material and Methods

Origin of isolates

Samples of the bark tissues with perithecia were taken from a chestnut tree from Pezinok - Medvedie jazerá site (Slovakia, Malé Karpaty mountains). Pycnidia were taken from Petri plates with 2% malt agar.

Fixation

Samples were fixed in 5% solution of glutaraldehyde for 5 hours at the laboratory temperature and then they were washed six-times for 10 minutes in 0.1 M phosphate buffer solution (pH =7). Samples were postfixed in 2% osmium acid for 2 hours at laboratory temperature. Then, the samples were washed six-times for 10 minutes in 0.1 M phosphate buffer solution again and they were stored in 0.2 M phosphate solution at lowered temperature over night (at 4°C in refrigerator). The samples were washed two-times for 10 minutes in 0.1 M phosphate solution and were derived in upward acetone range next day: 15 minutes in 30% acetone, 30 minutes in 50% acetone, 70% acetone, 90% acetone and in 100% acetone. The samples do not have to dry during acetone change in tubes.

Suffusion

The samples were transferred into labelled caps and were suffused with Spurr's suffused medium (SPURR 1969). They were kept to polymerise at temperature 60°C for 48 hours.

Cutting and colouring of semithin sections

The hardened blocks were slashed with blade so, that the object in block was in the top of the pyramid. Semithin sections were cut by a glass knife for a light microscope. The thickness of semithin sections was 0,5-1 µm. They were done on ultramicrotone Tesla BS-490. Semithin sections were coloured using the quick method of double colouring by toluidin blue and basic magenta (LUX 1981).

The size of spores was detected according to 300 measurements of pycnospores and 30 measurements of ascospores from 3 collections during the season in 1998.

Results and Discussion

Cryphonectria parasitica is a blight pathogen, which grows superficially on the bark of attacked trees in its first stages. Later it penetrates into lower layers of the host (cambium). The fungus does not decay the wood.

Pycnidia of irregular shape are produced on the surface of bark and are submerged in red-orange stromata. Each is filled by conidiophores and several thousands of vital pycnospores (Fig. 2a). Pycnospores are discharged from a mature pycnidium together with slimy and sticky substance as red-orange thread-like formations, when the conditions are favourable. They are hyaline, allantoid, 2-3 × 1-1.5 µm.

Perithecia are formed either in stroma with old pycnidia or in new stroma on the bark of infected host tree (Fig. 1). Each stroma contains only few perithecia (Fig. 2b). They are pyriform with slender neck and ostiolum in the apical part (Fig. 2c). Periphyses located below the ostiolum help release of ascospores from the mature perithecium (Fig. 2d). Basal part of a perithecium contains asci (30-60 × 6-8 µm) with eight two-celled ascospores (7-9 × 4-4.5 µm) and paraphyses (Fig. 2e).

Delimitation of *Cryphonectria* and *Endothia* was confirmed by MICALES & STIPES (1987). They introduced also photographs illustrating reproductive bodies of *Cryhonectria parasitica:* two-celled, ellipsoid to ovoid ascospores and perithecia with oblique necks converged within the ectostromatic disk before growing to the stromatal surface. Configuration of the stroma was valsoid with an immersed entostroma and an erumpent ectostroma.

Perithecia occur in autumn and during favourable humidity conditions also in beginning of winter. Fructification of perithecia depends on rainfall. Large number of perithecia growing during humid weather is not affected on air temperature either on age of mycelium (CEJP 1957). Perithecia are formed in the stromata in cankers on hosts, and the black perithecial necks usually become visible in autumn (ANDERSON 1914). ANDERSON (1914) reported that stromata with perithecial initials could be found in August in cankers started in June, but that perithecia were not visible on the surface of stromata until autumn. Ascospores are probably the primary inoculum responsible for establishing the new cankers each season (ANAGNOSTAKIS & KRANZ 1987, MILGROOM, MACDONALD & DOUBLE 1991). They are discharged during the period of rain when the temperature rises above 11.1°C for at least 3 days. HEALD, GARDNER & STUDHALTER (1915) reported discharge of ascospores to continue for as long as 14 hours and stop after the end of rain in the field and to continue for as many as 25 days in the laboratory. Perithecia persist during the entire year.

The fungus can overwinter as mycelium in the host tissue, or as pycnospores and ascospores. Stroma grows with the same intensity on the living trees as on the dead wood. The fungus is extremely virulent and pycnospores are more virulent and dangerous that ascospores. Dispersal of chestnut blight depends on climatic conditions while during more suitable conditions the fungus produces more generations of pycnospores within a year. Ascospores germinate usually in the spring and may cause also new infections.

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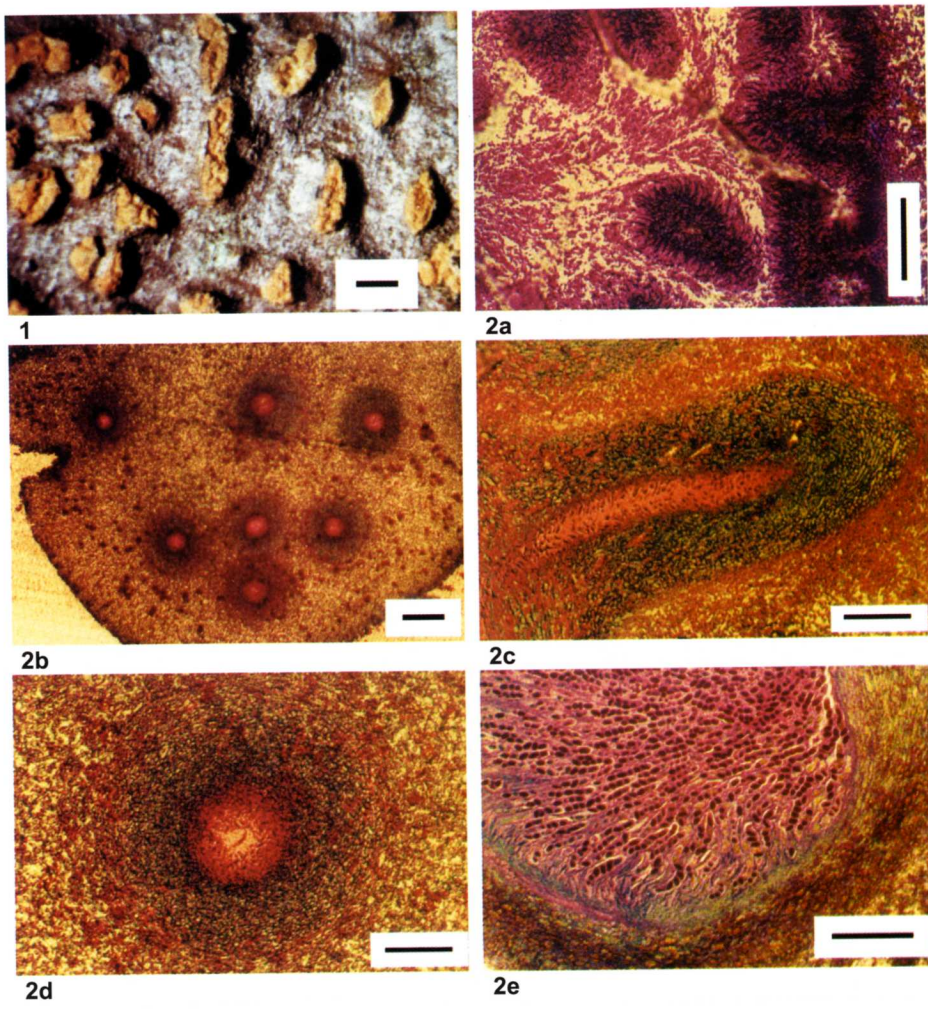

Fig. 1. Red-orange stroma with perithecia located on the bark of infected host (bar = 1 mm).
Fig. 2a. Transverse section of pycnidium fill by pycnospores, which are 2-3 × 1-1.5 μm large (bar = 50 μm).
Fig. 2b. A few perithecia are situated in each stroma (bar = 100 μm).
Fig. 2c. Longitudinal section of ostiolum of a perithecium of *Cryphonectria parasitica* (bar = 50 μm).
Fig. 2d. Transverse section of ostiolum. The ostiolum is fill by periphyses that relieve releasing of ascospores from the mature perithecium (bar = 50 μm).
Fig. 2e. Transverse section of perithecium. Basal part of perithecium contains ascae with 8 two celled ascospores (bar = 50 μm).