

The Morphology and the Biological Control of *Cryphonectria parasitica*

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Abstract. *Cryphonectria parasitica* (Murr.) Bar [syn. *Endothia parasitica* (Murr. And.) (anamorf: *Endothiella sp.*) is the causal agent of chestnut bark disease or chestnut blight, disease which produced great damages throughout the world, for example, in Europe, the European chestnut tree (*Castanea sativa*(P.) Mill) was heavily affected. Environmental concerns have focused attention on natural forms of disease control as an effective alternative to chemical pesticides. In the chestnut blight fungus, *Cryphonectria parasitica* deals with a natural form of biological control in which the virulence of a fungal pathogen is attenuated by an endogenous viral RNA genetic element- the hypovirulent strain. In our researches we picked samples of chestnut bark from different areas in Maramures county. We've isolated the fungus on PDA medium and we've studied the morphological characteristics of the usual virulent strain and we looked for a possible hypovirulent strain in order to study its capacity for biological control. The fungus develops in the bark and in cambium where forms a yellowish or brownish stroma and produces both conidia and ascospores. The pycnidia stomata break through the lenticels producing conidia and later in the same stroma develop the perithecia which produce ascospores. Both strains of the fungus were found in the research area. The hypovirulent strain had a slower development, showed no sporulation and pigmentation "white cultural strain" and was tested *in vitro* for the capacity to convert the virulent isolates by dual culture tests.

Keywords: *Cryphonectria*, chestnut, biological control, hypovirulent, pycnidia

Introduction. *Cryphonectria parasitica* (Murr.) Bar [syn. *Endothia parasitica* (Murr. And.) (anamorf: *Endothiella sp.*) is the causal agent of chestnut bark disease or chestnut blight, disease which produced great damages throughout the world, for example, in Europe, the European chestnut tree (*Castanea sativa* (P.) Mill) was heavily affected (Gonzales-Varela and Gonzales, 2007). Environmental concerns have focused attention on natural forms of disease control as an effective alternative to chemical pesticides. In the chestnut blight fungus, *Cryphonectria parasitica* deals with a natural form of biological control in which the virulence of a fungal pathogen is attenuated by an endogenous viral RNA genetic element- the hypovirulent strain (Tarcali, 2007).

Aims and objectives. Successful protection against *Cryphonectria parasitica* is a very difficult problem because the conventional control methods against the fungus are not applicable with a great success because of the extreme fungus pathogenity and on the other hand because of the characteristics of sites and host plants. The main objective of our research were to isolate and study a local strain, a romanian one, able to convert the virulent fungus in a hypovirulent one.

Materials and methods. In our researches we picked samples of chestnut bark from different areas in Maramures county. *Cryphonectria parasitica* was isolated from natural cankers on stem and twigs infected with chestnut blight from three different locations: Dealu Crucii, Tauti and Valea Rosie. Bark tissues were removed with sterilized cork borer (5 mm diameter) and placed on PDA. After incubating for three days mycelia plugs were removed

from mycelia margin and then transferred on fresh PDA where we've studied the morphological characteristics of the usual virulent strain and we've looked for a possible hypovirulent strain in order to study its capacity for biological control. The centre of each test plate was inoculated with a 1 mm size plug and incubated on PDA at $25 \pm 2^\circ\text{C}$. Mycelial growth and changes of colors were checked four days after incubation. The ability to convert the virulent strain in a hypovirulent one was tested by dual culture test (co-culture test) on PDA medium.

Results and discussions

The fungus develops in the bark and in cambium where forms a yellowish or brownish stroma and produces both conidia and ascospores. The pycnidia stomata break through the lenticels producing conidia and later in the same stroma develop the perithecia which produce ascospores.

In our experiments we've identified and isolated both strains of the fungus which were found in the research area, in Maramures county. The hypovirulent strain had a slower development, showed no sporulation and pigmentation "white cultural strain" as we can see in figure 1.

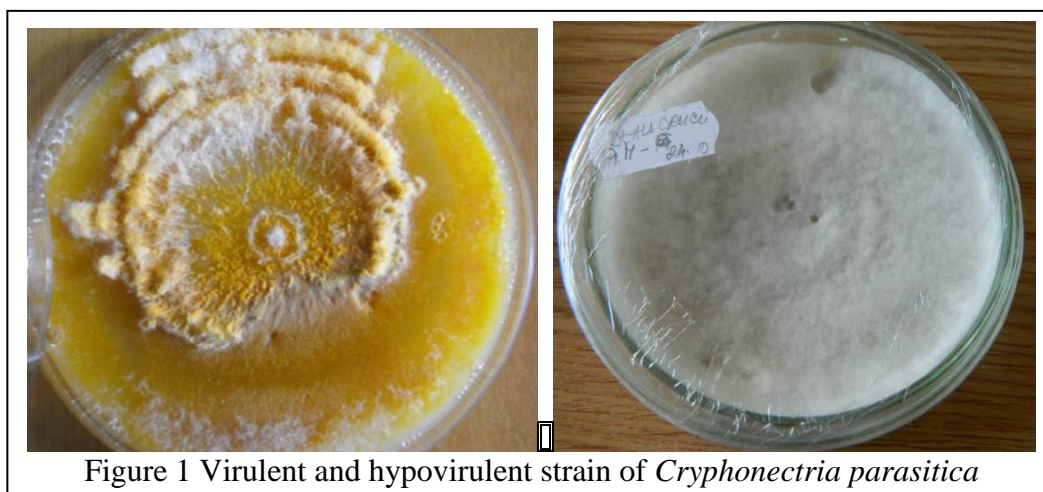


Figure 1 Virulent and hypovirulent strain of *Cryphonectria parasitica*

The hypovirulent strain was tested *in vitro* for the capacity to convert the virulent isolates by dual culture tests and we've obtained positive reaction – the two strains were compatible and the hypovirulent one converted the virulent strain as we can see in figure 2.



Figure 2 Conversion of the virulent strain

Conclusion. Our study demonstrated that in Romania, Maramures county, exists both virulent and hypovirulent strains of *Cryphonectia parasitica* and the hypovirulent one was able to convert the virulent fungus, so we can hope to have a biological control of the chestnut bark disease.

REFERENCES

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