

## The first record of *Cryphonectria parasitica* in the East Slovakia subregion

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### Abstract

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This study is reporting about the first record of a subpopulation of the chestnut blight fungus in the East Slovakia. Occurrence of the fungus *Cryphonectria parasitica* was found in the Petrovce village, district Sobrance. The fungus was successfully isolated from 11 samples. Based on the culture phenotype, all isolates were considered to be virulent. No hypovirulent strains were found. Each isolate was unambiguously assigned to a simple vc type. According to the European nomenclature, the vc type detected in Petrovce is EU 12. Hypovirulent isolates were prepared for biological control of chestnut blight in Petrovce.

### Key words

chestnut blight, *Cryphonectria parasitica*, *Castanea sativa*, vc types

### Introduction

The chestnut blight fungus *Cryphonectria parasitica* (Murr.) M. E. Barr. causes decline of whole chestnut stands in all cultivation areas in the world.

The fungus *C. parasitica* was declared as a quarantine pest A2 by the EPPO (European Plant Protection Organization) and it has also a quarantine value in the NAPPO (North American Plant Protection Organization) and IAPSC (Inter African Phytosanitary Council) classification.

European chestnut (*Castanea sativa* Mill.) is grown in 5 cultivation subregions (BENČAĎ, 1960) comprising 210 localities in Slovakia. The first record of the fungus in Slovakia is reported from the locality Prašice – Duchonka, district Topoľčany (JUHÁSOVÁ, 1983), 1976. Since this year, the fungus has been spreading in three subregions (Malokarpatsko-dolnopovažská, Inovecko-tribečská and Štiavnicko-krupinská) with 23 localities. Up to the year 2004, the fungus has not yet been found in the eastern part of the country (The East Slovakia subregion), where European chestnut is also cultivated (JUHÁSOVÁ et al., 2005).

### Materials and methods

In 2004 we evaluated the health condition of European chestnut trees in the eastern part of Slovakia, in connection with incidence of the fungus *C. parasitica*. Altogether 166 chestnut trees occur in 12 localities in the subregion East Slovakia (BENČAĎ, 1960). The degree of injury to the trees was evaluated according to JUHÁSOVÁ and BERNADOVIČOVÁ (2001). The trees classified in the category 0 were healthy, showing no symptoms of infection. The trees with dry crowns and big cankers on the stem and branches were assigned in the 5th category.

Bark samples (4–5 cm) were cut from chestnut blight cankers. The samples were immersed in 0.15% NaClO solution for 20 minutes for surface disinfection, and subsequently washed in distilled water. Small pieces of stroma (cca 0.5 x 0.5 cm) were placed on 3 % malt agar. The isolates were incubated at 25–27 °C in the dark.

According to GRENTE (1981), GRENTE and BERTHELAY-SAURET (1969a, b), virulent and hypovirulent isolates of *C. parasitica* are morphologically different in culture and distinguishable to the naked eye. Mycelium

of the virulent isolates is white, later turning to yellow or orange-yellow. About 96–140 hours after subculturing, globose red-orange pycnidia are seen in the culture. They are produced abundantly. In the hypovirulent isolates, the mycelium remains white and the production of pycnidia is low.

Isolates younger than 10 days were used for vegetative compatibility (vc) testing. The vc test was done according to CORTESI et al. (1996) on a potato dextrose agar green (PDAG) medium described by POWELL (1995). The vegetative compatibility type was assessed according to the mycelial-barrage response, using European tester strains of *C. parasitica*.

Conversion of virulent isolates of *C. parasitica* with hypovirulent strains was done on malt agar. One pair of cubes (one virulent and one hypovirulent) was placed in contact on the medium in a Petri plate, about 5 mm from the edge of the plate. The conversion is successful when the white mycelium is divided in virulent mycelium close to the interface with hypovirulent strain. The test was replicated 10 times. The aim of this test was to transfer dsRNA hypovirus from the hypovirulent strains into the virulent isolates from Petrovce and, in such a way, to obtain domestic hypovirulent isolates that can be used in biological control of chestnut blight.

## Results

The health condition of the European chestnut was evaluated for 12 localities in its cultivation subregion East Slovakia (Table 1). The fungus *Cryphonectria parasitica* was found in the locality Petrovce (N 48°42'30'', E 22°19'30''), district Sobrance. The examined chestnut trees are growing in a stand with a local name "Mladé". The first territory with chestnut occurrence is 2.61 ha large in area and belongs to private owners. The research was pursued on thirty trees chosen randomly and marked for research purposes (Table 2). The second territory is 1.67 ha large. It is a mixed forest plantation where chestnut trees represent 5% of the woody plants. All chestnuts infected by *C. parasitica* were classified in the 4<sup>th</sup> degree of injury (Table 2).

The samples were taken from different parts of infected trees (from attacked bark, from mycelium and also from pycnidia). The fungus was successfully isolated from 11 samples. All isolates had at first white coloured mycelium. Later it turned to light yellow and orange-yellow, some weeks later, to red-orange. After 5 days, pycnidia were formed in cultures. They were produced abundantly. Based on phenotype of this culture, all isolates were considered to be virulent. No hypovirulent strains were found.

Only one vc type was detected among the samples of 11 isolates. Each isolate was unambiguously assigned

to the single vc type. According to the European nomenclature, the vc type detected in Petrovce is EU 12.

Eleven hypovirulent isolates were used in conversion testing. The conversion was successful with 9 hypovirulent isolates (marked A3 x BF, C-2, R-6, R-5, R5 x 2, FS4 x 146, V-4, IHB2, R-2). From virulent isolates from Petrovce we prepared hypovirulent isolates that can be used in biological control of chestnut blight.

Table 1. Occurrence of European chestnut according to BENČAĽ (1960) and occurrence of chestnut blight in the East Slovakia subregion

No. of locality	Name of locality	Number of trees	Occurrence of chestnut blight
1	Lucia Baňa	3	–
2	Šemša	1	–
3	Skároš	2	–
4	Košice	4	–
5	Ličartovce	3	–
6	Uzovský Šalgov	2	–
7	Kalnište	27	–
8	Slanec	9	–
9	Malé	40	–
10	Ozorovce Michalovce	20	–
11	Vinné	3	–
12	Petrovce	50	+

–: absence of the fungus *Cryphonectria parasitica*,  
 +: presence of the fungus *Cryphonectria parasitica*

Table 2. Occurrence of chestnut blight in Petrovce

No. of tree	Circumference of trunk (in mm)	Occurrence of <i>Cryphonectria parasitica</i>	Degree of injury <sup>a</sup>
1	252	–	0
2	158	–	0
3	83	–	0
4	147	–	0
5	149	–	0
6	166	–	0
7	231	–	0
8	310	–	0
9	245	–	0
10	223	–	0
11	222	–	0
12	164	–	0
13	193	–	0
14	188	–	0

Table 2. Continued

No. of tree	Circumference of trunk (in mm)	Occurrence of <i>Cryphonectria parasitica</i>	Degree of injury <sup>a</sup>
15	161	–	0
16	213	–	0
17	181	+	4
18	153	–	0
19	185	+	4
20	180	–	0
21	133	+	4
22	140	–	0
23	194	–	0
24	200	+	4
25	135	+	4
26	95	–	0
29	235	–	0
30	300	–	0

– absence of chestnut blight cankers, + presence of chestnut blight cankers, <sup>a</sup> according to JUHÁSOVÁ and BERNADOVIČOVÁ (2001)

## Discussion

*Cryphonectria parasitica* was found for the first time in Slovakia in 1976 in Prašice. Since then, it has been spreading and causing serious damage to 21 sites. Until 2004, no occurrence of chestnut blight was reported from the subregion East Slovakia (JUHÁSOVÁ et al., 2005). This study deals with the first examination of the East Slovakia subpopulation of the chestnut blight fungus.

*C. parasitica* was first observed in Europe in Italy in 1938, and from there the disease was spreading rapidly over Italy and to the neighbouring chestnut growing areas. It attacked first the largest compact areas, later also scattered chestnut stands (HEINIGER and STADLER, 1990). The same situation is in Slovakia. Chestnut blight fungus was observed first in one of the largest orchards in Slovakia (Prašice, 1976). Then it spread to small neighbouring chestnut stands (Podhradie in 1979, Radošina in 1990, Lipovník in 1992, Bojná in 1995, Nitra 1995, JUHÁSOVÁ et al., 2005). We supposed it would be similar in the East Slovakia subregion. Petrovce is the site with the highest number of chestnut trees in the East Slovakia subregion (BENČAĽ, 1960). We assume that the disease could spread to other sites in this subregion (in dependence of wind, biotic vectors).

Today only the scattered stands of chestnuts in the Netherlands, the coppice stands in southern UK and isolated, solitaire trees in orchards and botanic gardens

are free of the blight in Europe (ROBIN and HEINIGER, 2001). It is possible chestnuts in the sites in the East Slovakia subregion will also be free of blight.

All isolates of the chestnut blight fungus were uniform and compatible with the vc type EU 12. According to JUHÁSOVÁ et al. (2005), the vc type EU 12 was dominant in 18 sites in Slovakia and only this one vc type was present in 6 sites. This vc type was also determined in the site Duchonka where the chestnut blight was observed for the first time in Slovakia.

The vc type EU 12 is the dominant vc type in southern and eastern Europe, apart from Turkey (ROBIN and HEINIGER, 2001). Vc type EU 12 accounted for 95% of the Macedonian isolates, 85% of Greek isolates and for 86% of the isolates at a site in Sicily (HEINIGER et al., 1998). RADÓCZ (2001) reported about chestnut blight in 6 sites in Ukraine, which are near to Petrovce. All the Ukrainian isolates were virulent and were assigned to the vc type EU 12. The Romanian isolates of *C. parasitica* were also assigned to the vc type EU 12. In Hungary, the vc type EU 12 belongs to the most widespread vc types (RADÓCZ, 2001). Also in Bosnia-Herzegovina, the EU 12 is the dominant vc type (ROBIN and HEINIGER, 2001).

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## Prvý výskyt huby *Cryphonectria parasitica* vo Východoslovenskej podoblasti

### Súhrn

Práca predstavuje prvý záznam rakoviny kôry gašтана jedlého vo Východoslovenskej podoblasti. Huba *Cryphonectria parasitica* bola zistená na lokalite Petrovce, okres Sobrance. Úspešne bola izolovaná z 11-tich vzoriek. Všetky kultúry boli označené ako virulentné na základe ich fenotypu v in vitro podmienkach. Hypovirulentné kmene huby sa nenašli. Všetky izoláty z Petroviec boli jednoznačne zadelené do jednej vc skupiny, ktorá podľa Európskej nomenklatúry zodpovedá vc skupine EU 12. Na ošetrovanie rakovinových rán formou biologickej ochrany boli pripravené hypovirulentné izoláty.