

Situation of damage caused by *Cryphonectria parasitica* to forest stands and orchards of *Castanea sativa* by 2001 in Slovakia

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ABSTRACT: In the present paper the knowledge of evaluation of the health condition of *Castanea sativa* (Mill.) in forest stands was summarised. It was found out that the fungus *Cryphonectria parasitica* (Murr.) Barr damaged the coppices until 60 years. Degree of damage (I_{HC}) in the evaluated localities ranged from 1.11 to 3.50. Between 1992 and 2001 the biological protection in orchards by the method of hypovirulent converted strains from France was used. This method had not been used in forest stands until then. The efficiency of canker treatment ranged from 38.9% to 64.9%. On the basis of genetic variability of *C. parasitica* isolates they were classified into 4 vegetative-compatible (v-c) groups.

Keywords: *Castanea sativa*; *Cryphonectria parasitica*; hypovirulence; biological control; Slovak Republic

The fungus *C. parasitica* represents significant risks for cultivation of *Castanea sativa* in all cultivated areas (JAYNES, ELLISTON 1980; GRENTE 1981; HEINIGER, RIGLIN 1994; FRIGIMELICA, FACCOLI 1999; JUHÁSOVÁ 1999; JUHÁSOVÁ, BERNADOVIČOVÁ, 2001; RADÓCZ 2002). However, during the last three decades these chestnut stands as well as old chestnut orchards have been seriously threatened by major fungal diseases caused by *Cryphonectria parasitica* (GRENTE, BERTHELAY-SAURET 1997; JUHÁSOVÁ et al. 1997). A long-term investigation has been carried out to know the spread of diseases in greater detail and to test methods of their biological control.

Besides phytopathological studies *Castanea sativa* was evaluated with regard to the incidence of parasitic fungi also from the aspect of its cultivation (TOKÁR 2001), untraditional multiplication (IVANOVÁ 1990) and study of genetic-breeding problems (BOLVANSKÝ, MENDEL 1998). On the basis of literary data methods about the pathogen hypovirulence (GRENTE 1981; TURCHETTI, MARESSI 1991) and about the genetic relationship of fungus isolates (ANAGNOSTAKIS 1977; CORTESI et al. 1996a,b) were elaborated that were improved for plant protection (GRENTE 1981; ANAGNOSTAKIS 1997).

The aim of this paper is to give a report on the incidence and evaluation of damage caused by *Cryphonectria parasitica* at selected sites in Slovakia and on the classification of *Cryphonectria parasitica* strains distributed in Slovakia (v-c groups, virulence, hypovirulence).

MATERIAL AND METHODS

Damage caused by *Cryphonectria parasitica* has been detected in 26 localities of Slovakia and within them at 126 particular sites (Fig. 1). To achieve the objectives set up in the framework of CHESUD project 8 sites in 5 localities were selected for more detailed evaluation of the phytopathological situation caused by chestnut blight.

All sites involved in the study are situated at the altitude of 200–400 m a.s.l. The long-term average annual temperature at the sites fluctuates from 7 to 9°C (cold years 6–8°C, warm years 9–11.5°C) and long-term annual sum of precipitation is 600–800 mm (dry years 350–550 mm, wet years 750–1,100 mm). Soil types and geological bedrock at particular sites are as follows: Svätý Jur, Modra, Bratislava, Duchonka, Modrý Kameň – ochreous brown soils on gneiss, gneiss and quartzite, quartzite and andesite. Stredné Plachtince, Radošina, Horné Lefantovce – podzolic and podzolic gleyed soils on loess loam. In 2000 and 2001 a special attention was paid to the young coppice stand (age 20–25 years) at the site Prašice – Duchonka, where a new series of experimental plots (three plots each 30 × 30 m) was established. The coppice regenerated after clear cutting about 300 trees at the age from 50 to 150 years that formed the largest chestnut orchard. In 1976–1990 all trees were gradually seriously damaged by chestnut blight. The first occurrence of this disease at this site in 1976 was also the first occurrence of blight in Slovakia. Also at present a majority of the existing stump stools are damaged by chestnut blight to a different extent.

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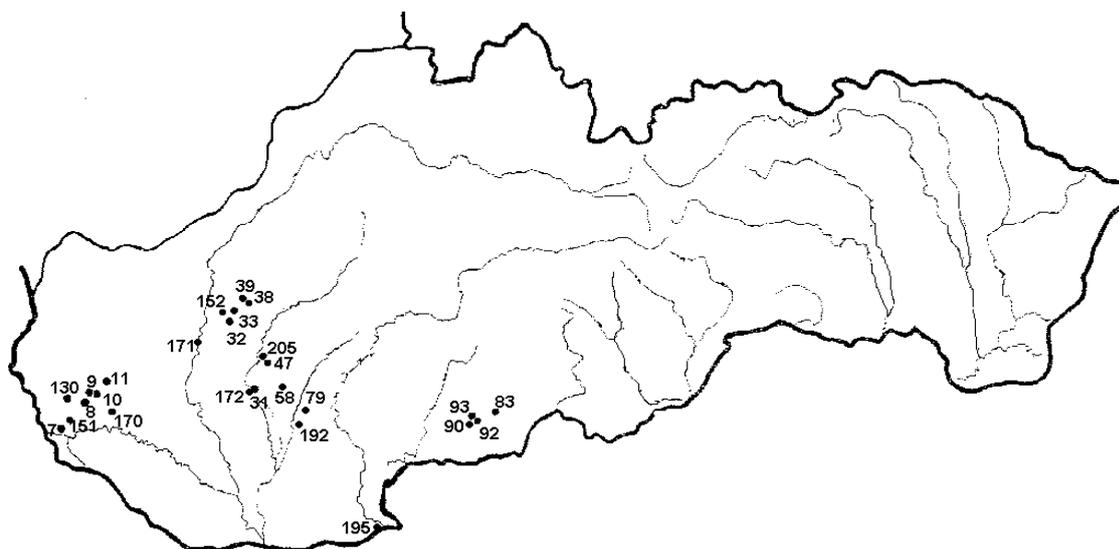


Fig. 1. Incidence of *Cryphonectria parasitica* in 26 localities of Slovakia

(7 – Bratislava, 8 – Svätý Jur, 9 – Grinava, 10 – Pezinok, 11 – Modra, 31 – Nitra, 32 – Radošina, 33 – Bojná, 38 – Podhradie, 39 – Duchonka, 47 – Horné Lafantovce, 58 – A. Mlýňany, 83 – Modrý Kameň, 90 – Dolné Pribelce, 79 – Tlstý Vrch, 92 – Stredné Plachtince, 93 – Horné Plachtince, 130 – Limbach, 151 – Rača, 152 – Lipovník, 170 – Senec, 171 – Hlohovec, 172 – Párovské háje, 192 – Podhájska, 195 – Štúrovo, 205 – Nitr. Streda)

Evaluation of damage degree

The investigated trees were classified into 6 categories according to the degree of crown damage and the number of canker wounds:

Degree 0 – trees are healthy, no symptoms of disease.

Degree I – concaves on branches and/or stump sprouts, leaves becoming yellow (Fig. 2).

Degree II – dry brown leaves on branches and/or stump sprouts. Marked coloured changes on the smooth bark of the stem and smooth branches at the place of infection.

Degree III – dry brown leaves on branches and/or stump sprouts. At the place of infection, the bark on the stem and on branches cracks longitudinally downward. The fan shaped mycelium of fungus is well visible under the bark (Fig. 3). On older trees the rough bark cracks and canker wounds appear mainly on the stem and stump sprouts.

Degree IV – more than 2/3 of dry branches in the crown of the tree. The bark peels in longitudinal strips on the stem and also on branches. The fungus produces pycnidia and also perithecia (Figs. 4 and 5).

Degree V – the whole crown of the tree is dry, marked canker wounds on the stem and on branches.

The data collected according to the above categories were used to calculate the index of health condition (I_{HC}):

$$I_{HC} = \frac{1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5}{n}$$

where: n – total number of evaluated trees,

n_1, \dots, n_5 – number of trees in particular injury categories.

The value of the index may vary from 0 (all trees healthy) to 5 (all trees with the heaviest damage of degree V).

Cryphonectria parasitica was isolated according to GRENTE and BERTHELAY-SAURET (1997). Virulent isolates (Fig. 6) of *C. parasitica* were obtained from a transition zone between the healthy and affected parts of the host, from mycelium, pycnospores and ascospores. We use several kinds of culture media, the best results were obtained with 3% malt agar (JUHÁSOVÁ, BERTHELAY-SAURET 1993).

Determination of v-c groups

In 1990, eight Slovak virulent isolates of this fungus were tested with ten hypovirulent strains from France at the Institute of Plant Pathology at Clermont Ferrand, INRA. They detected 2 v-c groups in Slovakia. In 1991–1996 the number of v-c groups did not increase. During 1997–2001 using CORTESI et al. (1996a) method the number of v-c groups increased to 8 (Table 4). It was found out that more v-c groups occurred in one locality.

Determination of canker type of chestnut blight

In this evaluation the following categories were assumed: a) old damage (old branches killed), b) new damage (dry leaves on killed twigs, limbs and branches, in percentage low (0–10%), medium (10–30%), high (up to 30%).

Biological control using hypovirulent strains of *Cryphonectria parasitica*

Between 1992 and 1998 in selected localities in Slovakia cankers were treated with converted hypovirulent strains of the fungus *Cryphonectria parasitica*. The hypovirulent strains A (10 + 2,103) and D (9 + 2,106) from Plant Pathology at Clermont Ferrand (JUHÁSOVÁ,



Fig. 2. *Castanea sativa* Mill. with dry branches. A tree damaged by the fungus *Cryphonectria parasitica* (Murr.) Barr. ranked to degree I of damage

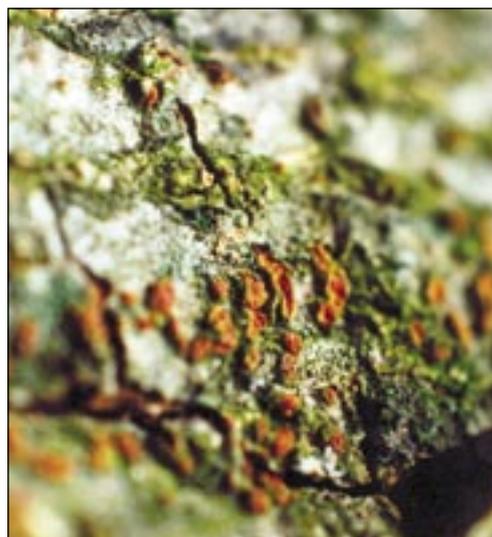


Fig. 4. The stromata of the fungus *Cryphonectria parasitica* (Murr.) Barr are formed on the damaged branches and stem. The dimensions of stromata with perithecia were 3–5 mm in length and 1–3 mm in width. They are formed in the winter and early spring months



Fig. 3. Characteristic yellow fan-shaped mycelium developed between bark and cambium



Fig. 5. Pycnospores are discharged from a mature pycnidium as red-orange thread-like formations when the conditions are favourable (appropriate temperature and humidity)

BERTHELAY-SAURET 1993) were used (Fig. 7). Biological control of *Castanea sativa* according to Grente's method (1981) was modified for our conditions (Fig. 8). Prior to the treatment, the attacked trees were classified in dependence on the degree of damage into 5 degrees as described above. Cankers on trees damaged to degree 1–3 of damage were treated. Those with damage of degree 4–5 were not suitable for any treatment any longer.

RESULTS

Evaluation of the spread and incidence of *Cryphonectria parasitica*

The incidence of *Cryphonectria parasitica* was proved in 26 localities of Slovakia (Fig. 1). The evaluation of damage caused by this fungus at selected sites in 1999 showed a medium-high extent of damage at all sites

including those where cankers were treated with hypovirulent strains but at these sites the damage was slightly higher (Table 1). In 2000 the extent of damage slightly increased at all sites except one but the damage increase was higher at sites that were not treated (Limbach orchard and Modrý Kameň-Prše). The distribution of new

Table 1. Health condition of the European chestnut in selected stands and orchards damaged by chestnut blight

Locality – site	Index of health condition (I_{HC})	
	1999	2000
Modrý Kameň-Medvecká I.	1.25	1.32
Modrý Kameň-Medvecká II.	1.11	1.11
Modrý Kameň-Prše	1.79	2.20
Lipovník	1.74	2.30
Limbach – orchard	2.36	3.50
Limbach – forest stand	1.77	1.86

Cankers were treated with converted hypovirulent strains from France



Fig. 6. The fungus *Cryphonectria parasitica* produces in nature two forms of mycelium – virulent and hypovirulent. Virulent mycelium is orange-yellow in colour



Fig. 7. White hypovirulent mycelium of *Cryphonectria parasitica* (Murr.) Barr.

damage at the studied sites according to the categories pointed to the marked prevalence of low damage at all sites (Table 2). However, the largest differences between sites were in the proportion of trees with great damage (from 1 to 16%), which indicates the fast development of the disease at some sites. In the same locality (Modrý Kameň) particular sites differ in the severity of damage.

Determination of canker type

Normal cankers prevailed at all sites under study, except three. Presence of intermediate cankers was the same at all sites (Table 3). As only natural virulent strains are formed in Slovakia, all hypovirulent and intermediate strains were isolated from cankers treated with hypovirulent strains of French origin.

Determination of v-c groups

In 1990, eight Slovak virulent isolates of *Cryphonectria parasitica* were tested by 10 hypovirulent strains



Fig. 8. Biological control of chestnut blight with pellets of hypovirulent mycelium



Fig. 9. Healing canker. Callus was formed on the periphery of canker, virulent mycelium was reduced

from France, Institute of Plant Pathology at Clermont Ferrand. Two v-c groups were detected in Slovakia. In 1991 and 1996 the number of v-c groups did not increase. In 1997–2001 using CORTESI et al. (1996a) method, the number of detected v-c groups increased to eight (Table 4). It was found out that more v-c groups occurred in one locality. In eight localities (9, 10, 31, 39, 92, 93, 150, 151) the isolates were classified into one

Table 2. Distribution of new damage among trees at the selected sites by the categories: low (0–10%), medium (11–30%), high (above 30%)

Locality – site	0–10%	11–30%	Above 30%	Number of trees
Modrý Kameň-Medvecká I.	95	0	5	100
Modrý Kameň-Medvecká II.	97	2	1	100
Modrý Kameň-Prše	80	6	14	100
Pezinok – Schwartz garden	95	5	0	100
Modra	77	7	16	100
Lipovník	72	22	6	100
Limbach – orchard	34	8	8	50
Limbach – forest stand	83	6	11	100

v-c group. At site No. 130 two v-c groups were detected, at six sites (7, 8, 11, 32, 152, 170) three v-c groups were detected. The highest number (4) of v-c groups was in locality 83. The number of v-c groups was different at the particular sites (Modrý Kameň – 4, Pezinok – 1, Modra – 3, Lipovník – 4 and Limbach – 2 v-c groups).

Results of biological control by hypovirulent strains of *Cryphonectria parasitica*

During the years 1991 and 2001, 4,680 cankers on 3,122 trees were treated with converted hypovirulent strains. The treatment was carried out with balls of hypovirulent strains manufactured by GRENTE (1981) under the licence provided only for the experimental use in the Slovak Republic (Fig. 8). The results of canker treatment of stump sprouts in Duchonka locality were as follows. In 1992–1998 we treated 976 cankers. The efficiency of treatment ranged from 38.9 to 64.9% (Fig. 9).

We observed that in 1999–2001 canker types (ii) and (iii) (by TURCHETTI, MARESSI 1991) prevailed in this

locality. The occurrence of *Cryphonectria parasitica* in relation to sprout thickness was compared on three plots (Fig. 2). On all plots, the relatively highest proportion of damaged trees was among thick stems (with the girth above 30 cm). Light damage (degree I) prevailed and occurred mostly on medium thick stems (with the girth from 10.5 to 30 cm).

DISCUSSION

The fungus *Cryphonectria parasitica* is equally dangerous in Slovakia as in the whole area of chestnut cultivation in the world. During 26 years, when it occurred in our country, it caused great damage similar to that reported by JAYNES and ELLISTON (1980), GRENTE (1981), HEINIGER and RIGLING (1994), FRIGIMELICA and FACCOLI (1999), RADÓCZ (2002). Considering that during 1990–2001 the originator of the infection was isolated, its genetic relationship was determined according to vegetative compatible groups, harmful spread was noticed. Biological protection with the converted hypovirulent strain of

Table 3. Occurrence of canker types and level of their presence at selected sites

Locality – site	Level of the presence of canker types		
	Normal	Abnormal	Intermediate
Modrý Kameň-Medvecká I.	+	++	+
*Modrý Kameň-Medvecká II.	+	+++	+
*Modrý Kameň-Prše	+++	+	+
Pezinok – Schwartz garden	+++	+	+
*Modra	+++	+	+
*Lipovník	+++	+	+
*Limbach – orchard	+++	+	+
*Limbach – forest stand	+++	+++	+

*Cankers were treated with converted hypovirulent strains from France low (+), medium (++) and high (+++) level of presence

Table 4. Occurrence of different v-c types of *Cryphonectria parasitica* at various sites over Slovakia in 1976–2000

1990–1997 A		1997–2000 B								2000 C				
I	II	I	II	III	IV	V	VI	VII	VIII	I	II	III	IV	V
Designation of the site														
7	8	7	170	170	11	170	7	83	152	9	11	77	31	8
32	9	39	7	152			8			10		32		
33	10	152	150	151			11			151		39		
93	11	92	83	83								47		
151	38	83	8	32								83		
152	39	8	32	11								92		
47	86	32		9								130		
	92	93		10								152		
	130	31		130										
	31	130												

A: ANAGNOSTAKIS (1987), GRENTE (1981)

B: CORTESI et al. (1996a,b)

C: JUHÁSOVÁ, ADAMČÍKOVÁ (KULCSÁROVÁ), RADÓCZ (1999)

French origin was realised. The method of isolation was proved according to GRENTE and BERTHELAY-SAURET (1997). Originally specified two v-c groups according to ANAGNOSTAKIS (1977) were enlarged to eight groups by the modified method from CORTESI et al. (1996b).

The biological protection was realised according to GRENTE (1981) transferring French hypoviruses into our chestnut orchards. No similar results were found in available literature. In most countries canker wounds are treated with hypovirulent strains of domestic origin. Some results from the solution of the project in the framework of COST G-4 were also published in HEINIGER and RIGLING (1994). They reported about general situation in chestnut damage in Europe.

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Situácia v poškodení lesných porastov a sádov gaštanu jedlého (*Castanea sativa* Mill.) spôsobenom hubou *Cryphonectria parasitica* (Murr.) Barr do roku 2001 na Slovensku

ABSTRAKT: Poškodenie lesných porastov a sádov gaštanu jedlého (*Castanea sativa* Mill.) spôsobené hubou *Cryphonectria parasitica* sme zaznamenali na 26 lokalitách Slovenska (v rámci nich na 126 stanovištiach). Podľa poškodenia koruny a podľa počtu zaznamenaných rakovinových rán sme napadnuté stromy rozdelili do šiestich kategórií. Získané výsledky z jednotlivých kategórií sme použili na výpočet zdravotného stavu (I_{HC}). Stupeň poškodenia dreveniny na sledovaných lokalitách sa pohyboval od 1,11 do 3,50. V rámci biologickej ochrany sa v sadoch počas rokov 1992–2001 používala metóda, v ktorej sa využívali konvertované hypovirulentné kmene huby *Cryphonectria parasitica* získané z Francúzska. Účinnosť ošetrenia rakovinových rán sa pohybovala od 38,9 % do 64,9 %. Na základe genetickej variability izolátov huby sme ich zatriedili do štyroch vegetatívne-kompatibilných skupín.

Kľúčové slová: *Castanea sativa*; *Cryphonectria parasitica*; hypovirulencia; biologická ochrana; Slovensko

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