The development of population structure of *Cryphonectria parasitica* on European chestnut (*Castanea sativa* Mill.) in the Experimental Castanetarium Horné Lefantovce, observed over a 12-year study period

K. Adamčíková, M. Kobza, G. Juhásová

Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plants Biology, Slovakia

ABSTRACT: We studied occurrence of chestnut blight disease in the Experimental Castanetarium Horné Lefantovce, SW Slovakia. The study ran in years 2006–2007 on a set consisting of 889 chestnut trees growing in the clonal orchard on Biological Plot 105. From this number, 857 trees were found healthy without disease symptoms. The chestnut blight disease was identified on 32 of them. The infected trees were examined for presence of pycnidia and perithecia of the causal agent. In all positive cases, the observed morphological characters indicated virulency of the obtained isolates. No hypovirulent isolate was detected on the evaluated experimental plot. In total, six vegetative compatibility (vc) types were specified in the sample consisting of 31 isolates. Our vc types corresponded to the European vc types EU 2, EU 12, EU 13, EU 14, EU 17, EU 19. Two vc types – EU 2 and EU 19 – were dominant. Vc type EU 19 accounted 35.5% and EU 2 32.2% of isolates. The vc type EU 19, which was the most frequent one in the evaluated site, was detected in Slovakia for the first time.

Keywords: chestnut blight; *Castanea sativa*; vegetative compatibility types

In Slovakia, European chestnut (*Castanea sativa* Mill.) is grown mainly as a decorative woody plant (Benčať 1960). In forestry, it is used as a production species contributing at the same time to improvement of stand conditions, primarily in circumstances when coppice forests are turning in high forests (Tokár 1998). The success in growing this precious introduced woody plant in our climatic conditions depends on thorough understanding of its biological and ecological properties. For this purpose, the Experimental Castanetarium Horné Lefantovce was established in 1965, focussing on comprehensive research of this woody plant under uniform ecological conditions at the site (Benčať, Tokár 1971).

The Castanetarium with an area of 14.38 ha is situated at 220–250 m a.s.l., in the Tríbeč Mts., at locality Ferdinandka, 20 km north of the Nitra town.

Since 1976, the European chestnut in Slovakia has been threatened by *Cryphonectria parasitica* (Murr.) Barr. This pathogen destroys whole coppices and orchards (Juhásová 1999). The first occurrence of the fungus *C. parasitica* in the Castanetarium Horné Lefantovce was recorded in 1995, on two trees in the clonal orchard on Biological Plot 105. By November 2003, the fungus was also found on plots 28, 29 (Č1, Č2), 53, 54 (K1, K5), 42 (DP5) (Tokár et al. 2004).

Up to the year 2003, damage to the trees in the Castanetarium caused by the chestnut blight fungus was rather low. No biological protection was necessary to apply (Tokár et al. 2004).

The aim of this study was to evaluate the health state of the chestnut trees in Castanetarium Horné Lefantovce with regard to the incidence of chestnut blight, and to acquire more detailed data on population structure of this fungus.

MATERIAL AND METHODS

Specification of damage degree

Disease incidence and severity was assessed on each tree growing in the studied clonal orchard on Biological Plot 105. The disease severity was clas-

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sified with a 6-point scale designed based on estimation of the proportion of attacked (necrotized) leaves and dead branches and the number and size of cankers:

Degree 0 – healthy trees, showing no disease symptoms.

Degree 1 – concave cavities on branches or stump sprouts, smaller and yellow leaves.

Degree 2 – dry brown-coloured leaves on branches or stump sprouts. Considerable colour changes (reddish discoloration) in affected spots on smooth stem bark and smooth branches.

Degree 3 – progressive foliar necrosis on branches and/or stump sprouts. Longitudinal cracks in infected stem and branch bark. Fan-shaped mycelium of the fungus is easy-to-detect under the bark.

Degree 4 – more than 2/3 of tree crown with dry branches. Longitudinal strips of affected bark peel off from tree stem and branches. The fungus produces pycnidia and also perithecia.

Degree 5 – the whole tree crown is dry and manifests many large cankers on stem and branches.

The assembled data were classified to the above-specified categories, and the final index of health condition (I_{HC}) was calculated as follows:

$$I_{HC} = (1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5)/n$$

where:
n – total number of evaluated trees,
$n_1,...n_5$ – numbers 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 5).

The presence of reproductive structures on the bark was evaluated too.

Isolation of the fungus

The study material was collected from all infected trees. Surface disinfection of the sampled bark containing mycelium consisted of a 20 min treatment with a 0.15% NaClO water solution followed by washing with distilled water. The cultivation medium was a 3% maltose agar. The plates were incubated at 25°C in the dark. Morphological characters of the culture were scored 14 days later, according to Grente (1971, 1981).

Determination of vegetative compatibility types

Vegetative compatibility (vc) testing was carried out on isolates younger than 10 days. The vc test was made according to Cortesi et al. (1996) on a potato dextrose agar green (PDAg) medium according to Powell (1995). The vc tests were also carried out on malt agar according to Anagnostakis (1977). The European tester strains for *C. parasitica* were used for assessing vc type – based on the mycelial-barrage response.

The diversity of vc types was expressed by the ratio of the number of vc types to the total sample size (S/N), and the Shannon diversity index:

$$H' = \Sigma p_i \times \ln p_i$$

where:
p_i – the frequency of the i^{th} vc type.

The same indices were also used by Anagnostakis et al. (1986).

RESULTS AND DISCUSSION

We studied a set of 889 trees growing in the clonal orchard on Biological Plot 105 in the Experimental Castanetarium Horné Lefantovce. The research ran in 2006–2007. 857 trees without symptoms of chestnut blight disease were found; the disease thus affected 32 trees (Table 1). From this number, 14 trees manifested only the first symptoms of the disease; the infection has not developed yet. Six trees (3 with damage degree 4 and 3 with damage degree 5) were seriously infected (Table 1). The overall index of health condition (I_{HC}) in the clonal orchard on Biological Plot 105 was 0.0776.

In 2004, 1433 chestnut trees were evaluated on biological plots J7 (69 trees), B1 (123), K3 (75), D8 (204), SPL5 (17), SPL7 (58), HL12 (96), MK6 (79), R6 (90), HL (42), HL17 (79), MK7 (37), RD (88), RO3 (71), HL19 (103), HL18 (44), MK8 (45), R2 (50), RO4 (33), HL14 (30). The chestnut blight disease was diagnosed only in 3 of them: one tree on Biological Plot RD was classified as damaged to degree 5 (40-year-old tree grown from seed transported from the Radošina locality), two trees on Biological Plot R2 were assessed as damaged to degree 1 and 5, respectively (40-year-old trees cultivated from seed from the Radošina locality).

The first occurrence of the fungus *C. parasitica* in the Castanetarium Horné Lefantovce was recorded in 1995, on two trees in the clonal orchard on Biological Plot 105. By November 2003 the fungus was found also on plots 28, 29 (Č1, Č2), 53, 54 (K1, K5), 42 (DP5) (Tokár et al. 2004).

Table 1. List of infected chestnuts, their damage degree, list of isolates, presence of reproductive structures and vc type diversity of the fungus *Cryphonectria parasitica* studied in the clonal orchard on Biological Plot 105 in the Castanetarium Horné Lefantovce

EU vc type (%)	Number of isolate	Number of tree	Damage degree	Reproductive structures
EU 2 (32.2%)	7	176	1	
	8	185	3	
	9	267	1	
	11	403	4	pyc
	20	630	2	
	21	638	3	pyc
	22	692	1	
	23	693	4	pyc
	26	752	5	pt
	27	753	1	
EU 13 (9.7%)	5	102	2	
	10	273	1	
	12	415	2	
EU 12 (12.9%)	2	27	1	
	6	174	1	pyc
	30	803	5	pt
	31	822	1	
EU 14 (3.2%)	25	737	3	
EU 17 (6.5%)	28	780	1	
	32	839	1	
EU 19 (35.5%)	1	21	2	
	3	71	1	
	4	79	2	pyc
	13	492	1	pyc
	14	551	2	
	15	557	2	
	16	597	4	pyc
	17	609	1	pyc
	18	623	2	
	19	627	3	pyc
	24	723	1	pyc
–	29	787	5	pt

Damage degree – the six categories specified according to the damage degree to crown and number of cankers, pyc – pycnidium, pt – perithecium, EU vc type according to Cortesi et al. (1998)

During more than 10 years, the disease spred from two infected trees to 32 trees on Biological Plot 105 as well as on other biological plots.

The infected trees were examined to detect the presence of pycnidia and perithecia. The pycnidia were observed on all infected trees. The perithecia

were present on trees number 752, 787, 803, all of damage degree 5. Sexual reproduction is supposed to have played an important role in creating the current structure of the fungus population.

The samples for laboratory examination were taken from each infected tree (32). The isolation of *C. parasitica* was successful in 31 cases. The cankers on tree number 787 (damage degree 5) on Biological Plot 105 were old, and the pathogen was not living any longer. For these reasons, the isolation of the fungus was not successful.

Morphological characters of the studied isolates (yellow mycelium, sporulation after 96–140 hours of cultivation), allow us to conclude that all isolates had the orange culture morphology (Grente 1971, 1981), which means that all isolates were virulent. No hypovirulent isolate was detected.

Each isolate was assigned unambiguously to one particular vc type; no isolate was compatible with more than one vc types. In the examined set consisting of 31 isolates we detected the following six vc types (European vc types classification): EU 2, EU 12, EU 13, EU 14, EU 17, EU 19 (Table 1). Two vc types EU 2, EU 19 were dominant. The vc types EU 19 and EU 2 comprised 35.5% and 32.2% of isolates, respectively. After 13 years, vc type diversity in the Castanetarium Horné Lefantovce increased from former 1 (Juhásová et al. 2005) to current 6 vc types. The ratio of numbers of vc types to sample size (S/N) was 0.19. The value of Shannon diversity index (H´) was 1.51.

We obtained the highest number of vc types per locality in Slovakia. In Slovakia, the average number of vc types per locality is 2.3. Only one other Slovak locality (Arboretum Mlyňany) had the same vc type diversity in 2005 (Juhásová et al. 2005).

In summary, in 2004, ten vc types (EU 2, EU 5, EU 8, EU 12, EU 13, EU 14, EU 15, EU 16, EU 17, and EU 25) were confirmed at 23 localities in Slovakia showing the presence of *C. parasitica*. The most frequent types were EU 12 (46.60%) and EU 13 (33.93%). EU 12 was dominant in 11 localities, EU 13 in 5 localities (Juhásová et al. 2005). In the Castanetarium Horné Lefantovce, these two vc types (EU 12, EU 13) were represented only by a few examples, 13% and 9.7% of the tested isolates, respectively. The isolates of the fungus obtained during the former investigation carried out in the Castanetarium were all compatible among themselves and were also compatible with the European vc type EU 12 (Juhásová et al. 2005).

The vc type EU 12 is the dominant vc type in southern and eastern Europe, with exception of Turkey (Robin, Heiniger 2001). The vc type EU 12 accounted for 95% of Macedonian isolates, 85% of Greek isolates and 86% of the isolates in Sicily (Heiniger et al. 1998). All the Romanian and Ukrainian isolates were assigned to the vc type EU 12 (Radócz 2001). Also in Bosnia-Herzegovina EU 12 is the dominant vc type (Robin, Heiniger 2001).

In our study locality, the occurrence of EU 13 was low, in contrast to the fact that it is one of four most frequent vc types in the Carpathian basin (Radócz 2001) and Slovakia on 5 from 23 localities with chestnut blight occurrence. In north Italy (Corniglio) 20% of a sample comprised vc type EU 13 (Cortesi et al. 1998). This vc type was found in several cases in Bregalia in Switzerland (Bazzigher 1981). EU 13 was also identified as one of the dominant vc types in Austria (Robin, Heiniger 2001). This vc type is rare in other European countries.

The second most frequent vc type in the research locality was EU 2 (10 isolates, 32.2%). In Slovakia, this vc type can be found in 8 localities, its frequency values, however, low (Juhásová et al. 2005). The type EU 2 is dominant in western and north-western Europe and in Austria, the south-west neighbour of Slovakia (Robin, Heiniger 2001). This vc type was found in two Hungarian sites, as well: Velem and Nemeshetés (Radócz 2001). EU 2 was the dominant vc type also in northern Italy, Switzerland (Cortesi et al. 1998) and south-eastern France (Heiniger et al. 1998). In Germany, the vc type EU 2 comprised more than 50% of the isolates.

By now, the vc type EU 19, the most frequent in our evaluated site, has not been reported from any other locality in Slovakia. The vc type EU 19 was determined in Bosnia-Herzegovina, but only as rare (Trestic et al. 2001). In Italy, it was found in three northern subpopulations (Sondrio – 1 isolate, Bergamo – 1 isolate, Firenze – 2 isolates) (Cortesi et al. 1996). This vc type is also present in Swiss subpopulations of *C. parasitica* (subpopulation Lumino) (Cortesi et al. 1998). In the neighbouring countries (Hungary, Ukraine, and Czech Republic), EU 19 has not been recorded (Radócz 2001; Haltofová et al. 2005a,b; Haltofová 2006). This vc type was however detected in the *C. parasitica* population in Bartow WV in the U.S. (Milgroom, Cortesi 1999).

We detected only 1 and 2 isolates of vc types EU 14 and EU 17, respectively. EU 14 was reported only from 2 other localities in Slovakia (Modra and Bratislava-Rača) (Juhásová et al. 2005). This vc type was found in 3 northern subpopulations and in one southern subpopulation in Italy (Cortesi et al. 1996), as well as in one subpopulation in Hungary (Radócz 2001). In a low frequency, it was present in Bosnia-Herzegovina and France (Trestic et al.

2001). It was also detected in the U.S. (Milgroom, Cortesi 1999). The vc type EU 17 was identified in 5 other localities in Slovakia (Juhásová et al. 2005). From the neighbouring countries, this vc type occurred in Hungary, in a sole locality (Radócz 2001); it has been confirmed with 3 subpopulations in Italy (only four isolates in three subpopulations) (Cortesi et al. 1996) and in Switzerland (Cortesi et al. 1998). The vc type EU 17 was also detected in populations of *C. parasitica* in the U.S. (Milgroom, Cortesi 1999).

In this case, by analogy to vc genotypes (Cortesi, Milgroom 1998), 32 vc types, probably arisen by recombination, might present limits to the hypovirus spreading and to achieving success in biological control. The realistic assessment of prospects of hypovirulence-based biological control in the study site requires further monitoring of changes in vc type diversity and sexual reproduction in the studied population as well as other populations, showing polymorphism in their vc and mating types. At present, however, no preventive measures inhibiting the increase in diversity are available.

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Vývin štruktúry populácie huby *Cryphonectria parasitica* na gaštanu jedlém (*Castanea sativa* Mill.) na experimentálnej ploche Kastanetária v Horných Lefantovciach počas dvanástich rokov

ABSTRAKT: Študovali jsme výskyt rakoviny kôry gaštana jedlého v Kastanetáriu v Horných Lefantovciach (juhozápadné Slovensko). V priebehu rokov 2006–2007 sme vyhodnotili 889 stromov gaštana jedlého v klonovom sade na biologickej ploche 105. Z toho bolo 857 stromov zdravých, bez prítomnosti príznakov ochorenia. Rakovinu kôry gaštana jedlého sme detegovali na 32 stromoch. Na napadnutých stromoch sme hodnotili prítomnosť pyknídií a peritécií huby. Vo všetkých prípadoch pozitívneho nálezu bola na základe morfologických znakov určená virulencia získaných izolátov huby; pritom žiadny z izolátov nebol označený ako hypovirulentný. Z 31 izolátov huby sme určili celkom šesť vegetatívne kompatibilných (vc) skupín, ktoré zodpovedajú Európskym vc skupinám EU 2, EU 12, EU 13, EU 14, EU 17 a EU 19, z ktorých boli dominantné dva typy – EU 2 (32,2%) a EU 19 (35,5%). Na experimentálnej ploche bola najpočetnejšia vc skupina EU 19, ktorá bola na Slovensku zaznamenaná prvýkrát.

Kľúčové slová: rakoviny kôry gaštana jedlého; *Castanea sativa*; vegetatívne kompatibilné skupiny

Corresponding author:

Mgr. Katarína Adamčíková, PhD., Ústav ekológie lesa SAV Zvolen, Pobočka biológie drevín, Akademická 2, 949 01 Nitra, Slovensko
tel.: + 421 376 943 339, e-mail: katarina.adamcikova@sav.savzv.sk