POPULATION STRUCTURE OF *CRYPHONECTRIA PARASITICA* IN THE MODRÝ KAMEŇ REGION

Gabriela Juhásová, Katarína Adamčíková*, Marek Kobza*, Emília Ondrušková**

Institute of Forest Ecology SAS Zvolen, Branch of Woody Plants Biology Nitra
Akademická 2, 949 01 Nitra, Slovak Republic

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Abstract: The European chestnut was evaluated for Chestnut blight disease in the Modrý Kameň region, in 2010. A total of 1321 trees and sprouts were selected. There were 140 healthy trees, without any symptoms of *Cryphonectria parasitica* infection. Nearly 90% of the evaluated trees and sprouts were diseased. The health condition index (I_{HC}) on 11 experimental plots varied from 2.31 to 4.03. Every collected sample was identified as having *C. parasitica.* All isolates had the orange culture morphology; all isolates were virulent. Among the isolates, 8 vc types were detected. Vc type EU-2 was dominant, it comprised 37.14% of the tested isolates. Two other vc types: EU 1 and EU 12 were frequently detected.

Key words: Chestnut blight disease, health condition, virulence, vc types, Slovakia

INTRODUCTION

The biggest area with *Castanea sativa* (Mill.) in Slovakia is in Modrý Kameň. The trees are grown in orchards and also in forests. *C. sativa* yield plenty of fruits (Benčať 1960). The trees are predominantly spread on all east and west sides of the surrounding hills between 250–500 m above mean sea level (AMSL). The estimated number of trees growing on these hills is 1,500–2,000 (Benčať 1960).

The chestnut blight fungus *Cryphonectria parasitica* (Murr.) M. E. Barr. has caused a decline in all the chestnut stands in cultivated areas in the world.

The fungus *C. parasitica* was declared a quarantine pest A2 by the EPPO (European Plant Protection Organization) and it also has a quarantine value in the NAPPO (North American Plant Protection Organization) and IAPSC (Inter African Phytosanitary Council) classification. This fungus has occurred in Modrý Kameň since 1991 (Juhásová *et al.* 2006).

The aim of this study was (1) to evaluate the health state of European chestnut, (2) to gain more detailed data about the population structure of *C. parasitica* in the Modrý Kameň region, and (3) to follow the development of vc type diversity in time.

MATERIALS AND METHODS

The health state of *C. sativa*

In the region of Modrý Kameň 11 experimental sites were selected in 2010, to evaluate the health state of European chestnut. A minimum of 100 trees were evaluated on each site. The total number of 1,321 trees was evaluated. According to Juhásová and Bernadovičová (2001) the health state of trees was assessed (Table 1).

The degree of injury was expressed by the index of the health condition I_{HC}. The index is defined as follows:

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

where:
n – total number of evaluated trees,
$n_1 \ldots n_5$ – number of trees in the corresponding category of injury (Table 1).
I_{HC} is given for all the 11 experimental sites with chestnut blight disease.

Table 1. Grading of injury category of *C. sativa* with description of *C. parasitica* symptoms for each degree of injury (Juhásová and Bernadovičová 2001)

Category of injury	Symptoms of *C. parasitica* infection
0	tree healthy, no symptoms
1	foliage thin, yellow
2	dry, brown leaves; initial cankers with reddish discoloration
3	dry, brown leaves; cankers with cracks, cankers on main and on root shoots
4	>2/3 of the crown with dry branches; bark peels in longitudinal strips
5	the whole crown is dry; big cankers on the stem and branches

*Corresponding address:
gabriela.juhasova@savzv.sk, katarina.adamcikova@savzv.sk, marek.kobza@savzv.sk, ondruskova@savzv.sk

Characteristics of *C. parasitica*

To study the population of *C. parasitica*, samples were collected during April and August 2010, from all experimental sites. A total of 171 samples were collected. The samples were disinfected in 0.15% NaOCl (Bochemie, Czech Republic) solution for 20 minutes, and subsequently washed in distilled water. The isolates were transferred to 3% malt extract agar and incubated at 25°C in darkness for 14 days. All *C. parasitica* isolates were assessed for the presence of hypovirus using culture morphology (Grente 1981). For that assay, the isolates were incubated at 20–22°C under diffused daylight, on the laboratory bench, for 7 days. The vegetative compatibility (vc) types of isolates were determined. For vc tests, we used PDAg (Potato dextrose agar green) medium (Powell 1995) and the tests were performed according to Cortesi *et al*. (1996) with the testers from EU-1 to EU-31. The diversity of vc types was expressed by the Shannon diversity index:

$$H' = -\Sigma pi \times \ln pi$$

where:
pi – the frequency of the i-th vc type
(Shannon and Weaver 1949).

RESULTS AND DISCUSSION

The health state of *C. sativa*

In total, 1,321 trees and sprouts of European chestnut were evaluated. There were 140 healthy trees, without any symptoms of infection by *C. parasitica*. These 140 uninfected trees represents 10.59% of the evaluated trees. The majority of the healthy trees had a circumference of up to 100 cm, only 3 of them had a bigger circumference. Moreover, they were young sprouts (up to 20 cm). These sprouts were so young that the fungus did not have enough time to attack them. Nearly 90% of the evaluated trees and sprouts were diseased. A total of 397 trees with dry whole crown were assessed, and put into the 5 category of injury. The portion of trees and sprouts killed by the fungus was 28.69%. Not only young, thin sprouts and trees were killed, but also large, old trees. As much as 21% of withered trees had a circumference over 100 cm. The I_{HC} index on 11 experimental plots varied from 2.31 to 4.03 (Table 2).

Juhásová *et al.* (2005a) evaluated the health condition of *C. sativa* in the Štiavnicko-Krupinská subpopulation and they evaluated the incidence of the *C. parasitica*. The Modrý Kameň area was also included in this research. The same method for evaluation of the health state of trees was used. In the Modrý Kameň area, they evaluated 1,107 trees (in our present study 1,321). In the 0 category of injury (healthy trees) 704 trees were assessed; this represents 63.59% of the evaluated trees (Juhásová *et al*. 2005a). During the following years, the number of healthy European chestnut trees at the evaluated locality decreased 6 times. Chestnut blight development was fast at the evaluated site.

The high disease incidence at the evaluated site is not exceptional. In only 4 years, the portion of cankered sprouts rose from 37% to about 60% at two Swiss investigation sites, which is almost double (Bissegger *et al.* 1997). Within 2 years, an increase of blighted sprouts from 39 to 61% was observed in Italy (Zechini D'Aulerio and Zambonelli 1987).

Table 2. Results of the evaluation of the health state of *C. sativa* with regard to the fungus *C. parasitica*

Category of injury	Experimental site/number of trees (for each category of injury)											Total
	1	2	3	4	5	6	7	8	9	10	11	
0	0	1	2	11	19	8	16	28	29	6	20	140
1	7	0	17	16	19	10	10	9	7	28	19	142
2	4	19	5	15	15	12	16	7	19	20	12	144
3	30	60	39	13	15	17	10	12	16	10	29	215
4	29	65	25	21	13	25	22	7	7	8	43	265
5	67	31	12	25	19	28	26	37	22	28	84	379
I_{HC}	4.03	3.81	3.04	2.91	2.41	3.25	2.9	2.79	2.31	2.7	3.43	

I_{HC} – index of health condition

Characteristics of *C. parasitica*

Samples were collected from well accessible cankers, mostly from trees assessed in the 1–3 injury category. All collected samples were identified as *C. parasitica* (based on symptoms and microscopic investigation of fruiting bodies), but not all yielded the isolate of this fungus. Several cankers were old and the pathogen could not be isolated from them. Isolation from 171 samples yielded 150 positive *C. parasitica* cultures (87.7%). All isolates had the orange culture morphology described by Grente (1981) and sporulated after 96–140 h of cultivation. This means that all isolates were virulent. In 1998, the first hypovirulent isolates determined by their morphology were obtained from other locations as well as from Modrý Kameň, but no dsRNA was detected in these isolates (Juhásová *et al*. 2005b). The time between the introduction of *C. parasitica* and the natural appearance of hypovirus in Europe lasts about 20 to 30 years (Robin and Heiniger 2001). The disease was first determined in Modrý Kameň in 1991 (Juhásová *et al.* 2006), however, so far no natural appearance of hypovirus has been observed. The natural introduction of the hypovirus at the studied locality in the near future may be expected to be discovered if there are not any barriers for its spreading.

Eight vc types were detected among 109 isolates, corresponding to the European vc types EU-1, EU-2, EU-3, EU-7, EU-11, EU-12, EU-28 (Table 3). Four isolates (each compatible with other 8th vc types) were not compatible with available 31 EU testers from our vc type database. Vc type EU-2 was dominant, it comprised 37.14% of tested isolates. There were two other frequent vc types detected (EU-1 and EU-12) which comprised 30.48% and 25.71% of tested isolates, respectively. The next four vc types (EU-3, EU-7, EU-11, EU-28) were represented variously from 1 to 3 isolates.

Table 3. Vc type diversity of *C. parasitica* populations at the Modrý Kameň subpopulation and comparison of present and previous results

	Modrý Kameň [b]	Modrý Kameň [c]
N	109	108
S	8	4
H_{vc} [a]	1.45	0.47

[a]H_{vc} – Shannon diversity index; H_{vc} – –Σpi × ln pi, where: pi is the frequency of the i-th vc type (Shannon and Weaver 1949)
[b]results from present study; [c] results according to Adamčíková *et al*. (2006); N – number of isolates; S – number of vc types

Four vc types were determined at Modrý Kameň during the years 1997-2002 with the dominant being vc type EU-12 (87.9%). Three other vc types (EU-13, EU-2, EU-8) were detected in lower numbers (Adamčíková *et al.* 2006).

Over the years, the genetic diversity of the studied *C. parasitica* population has changed. The vc type EU-2 is dominant at present, whereas in the past it was very rare. Vc type EU-2 is dominant in western and north-western Europe (Robin and Heiniger 2001). EU-2 was found only once in Modrý Kameň in 1998. It appeared that this vc type neither spread nor persisted in the studied region (Adamčíková *et al*. 2006). This hypothesis was not confirmed, because the number of obtained EU-2 isolates expanded within the time, this vc type spread in this locality. It appears that this vc type was newly established or introduced in that time, a into the studied locality.

The EU-12 vc type is dominant in several European countries (Greece, Macedonia, Bosnia Herzegovina, Hungary) and also EU-1 belongs to the major vc types in Turkey, Bosnia Herzegovina, Hungary, Italy, Switzerland and France (Robin and Heiniger 2001).

Two vc types (EU-8 and EU-13) determined from 1991 to 2002, were not detected in the present study. The supposition of vc type persistence or carrying over at a locality could be confirmed for these two vc types. They disappeared as the years passed, but new vc types formed.

The expected vc type frequencies were calculated at Modrý Kameň on the basis of the allele frequencies determined for the six vic loci of *C. parasitica*. Besides the four determined vc types, five other vc types (EU-11, EU-19, EU-25, EU-42 and EU-43) were expected (Adamčíková *et al.* 2006). From these vc types, only one EU-11 was detected in the present study. The vc type testers for EU-42 and EU-45 are not included in our database. It may be supposed, that one of these two vc types (EU-42, EU-45) could be compatible with the 8th undetermined vc type in Modrý Kameň.

The diversity of vc types was expressed by the Shannon diversity index (H_{vc}, Table 3). Its value is H_{vc}= 1.45. The H_{vc} calculated for this locality in 2002 was markedly lower (H_{vc} = 0.47, Adamčíková *et al.* 2006). But it is still lower than in similar small area populations in Europe (Bissegger *et al.* 1997) or in the states of Virginia and Connecticut in the US (Anagnostakis and Kranz 1987). However an exact comparison is difficult because of the different sampling methods and sampling size in each study.

After about 10 years, vc type diversity in Modrý Kameň increased to the current 8 vc types and also the Shannon diversity index increased nearly three times. Our study indicates that vc type diversity has increased in this region. The rise of vc type diversity might be caused by sexual recombination between existing vc types, mutations that generated new vc types, or the introduction of new vc types, probably from the other nearby *C. parasitica* population in the region, or from Hungary.

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REFERENCES

Adamčíková K., Juhásová G., Kobza M. 2006. Genetic diversity of *Cryphonectria parasitica* population in the Štiavnicko-krupinská subpopulation in Slovakia. Plant Protect. Sci. 42 (4): 119–124.

Anagnostakis S.L., Kranz J. 1987. Population dynamics of *Cryphonectria parasitica* in mixed-hardwood forest in Connecticut. Phytopathology 77 (5): 751–754.

Benčať F. 1960. Rozšírenie gaštana jedlého (*Castanea sativa* Mill.) a jeho stanovištné podmienky na Slovensku. [Distribution of European chesnut (*Castanea staiva* Mill.) and its stand conditions in Slovakia]. Biologické práce, Bratislava: Vydavateľstvo SAV, 172 pp.

Bissegger M., Rigling D., Heiniger U. 1997. Population structure and disease development of *Cryphonectria parasitica* in European chestnut forests in the presence of natural hypovirulence. Phytopathology 87 (1): 50–59.

Cortesi P., Milgroom M.G., Bisiach M. 1996. Distribution and diversity of vegetative compatibility types in subpopulations of *Cryphonectria parasitica* in Italy. Mycol. Res. 100 (9): 1087–1093.

Grente J. 1981. Les Variants Hypovirulents de l*Endothia parasitica*, et la Lutte Biologique Contre le Chancre du Chataignier. Clermont Ferrand: INRA, 194 pp.

Juhásová G., Bernadovičová S. 2001. *Cryphonectria parasitica* (Murr.) Barr and *Phytophthora* spp. in chestnut (*Castanea sativa* Mill.) in Slovakia. For. Snow Landsc. Res. 76 (3): 373–377.

Juhásová G., Adamčíková K., Robin C. 2005a. Results of biological control of chestnut blight in Slovakia. Phytoprotection 86 (1): 19–23.

Juhásová G., Adamčíková K., Kobza M. 2006. The chestnut blight fungus *Cryphonectria parasitica* in Slovak Republic. Mikologia i Fitopatologija 40 (4): 346–355.

Juhásová G., Kobza M., Adamčíková K. 2005b. Diversity of *Cryphonectria parasitica* (Murr.) Barr vegetative compatibility (vc) types in Slovakia. Acta Hortic. 693: 635–640.

Powell W.A. 1995. Vegetative incompatibility and mycelial death of *Cryphonectria parasitica* detected with a pH indicator. Mycologia 87 (5): 738–741.

Robin C., Heiniger U. 2001. Chestnut blight in Europe: Diversity of *Cryphonectria parasitica*, hypovirulence and biocontrol. For. Snow Landsc. Res. 76 (3): 361–367.

Shannon C.E., Weaver W. 1949. The Mathematical Theory of Communication. Urbana, University of Illinois Press, Champaign, 117 pp.

Zechini D'Aulerio A., Zambonelli A. 1987. Virulenza ed ipovirulenza di *Endothia parasitica* in un castagneto dafrutto. Monti Boschi 38 (2): 20–24.