Genetic Diversity of *Cryphonectria parasitica* Population in the Štiavnicko-Krupinská Subpopulation in Slovakia

Katarína ADAMČÍKOVÁ, Gabriela JUHÁSOVÁ and Marek KOBZA

Institute of Forest Ecology of the Slovak Academy of Sciences, Branch of Woody Plant Biology Nitra, Nitra, Slovak Republic

Abstract

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

The structure of *Cryphonectria parasitica* populations was evaluated in the Štiavnicko-krupinská subpopulation in Slovakia. Among 215 isolates, four vegetative compatibility groups (vcg) were detected that correspond to the European vc groups EU-12, EU-2, EU-13 and EU-8. The number of vcg at single sites varied between one and four. One vc group (EU-12) predominated in the Štiavnicko-krupinská subpopulation; it comprised 93% of all isolates and was found at each site. Two vcg were represented by only one or two isolates. The observed vcg frequencies were compared with expected vcg frequencies. The χ^2 test showed that the observed vcg frequencies differed significantly from the frequencies expected under random mating.

Keywords: chestnut blight; *Cryphonectria parasitica;* vc group; *Castanea sativa*

An important factor influencing the success in biological protection of chestnut trees (*Castanea sativa* Mill.) against chestnut blight caused by the fungus *Cryphonectria parasitica* (Murr.) Barr is the vegetative compatibility system of this pathogen (Anagnostakis 1988).

Vegetative incompatibility is a self-nonself recognition system in filamentous fungi regulating the formation of heterokaryonts and the transmission of cytoplasmic elements between strains (Bégueret *et al*. 1994; Biraghi 1946). In most filamentous ascomycetes, incompatibility is controlled by allelic interactions: two strains are incompatible when they have different alleles at one or more vegetative incompatibility loci (Bégueret *et al*. 1994).

Vegetative incompatibility of *C. parasitica* is controlled by at least six unlinked vegetative incompatibility (vic) loci, each with two alleles (Cortesi & Milgroom 1998). From these six vic loci, it is possible to obtain 64 genotypes ($2^6 = 64$) corresponding to 64 vegetative compatibility (vc) groups (Cortesi *et al*. 1996; Cortesi & Milgroom 1998). Compatible fungal strains share identical alleles at all vic loci and are assigned to the same vc group (vcg). The hypovirus is readily transmitted between strains that belong to the same vcg (Anagnostakis & Waggoner 1981; Cortesi *et al*. 2001). Transfer of dsRNA can also occur between strains that belong to different vc groups, but more slowly and less frequently (Cortesi *et al*. 2001). The success of transmis-

Supported by the Grant Agency for Science VEGA, Grant No. 2/4020/04, and by Science and Technology Assistance Agency under the contract No. APVT-51-015602.

sion is negatively correlated to the number of different vc genes between the recipient and the hypovirulent strain (Liu & Milgroom 1996). The success of biological control of chestnut blight is practically assured, when the hypovirus has been transmitted into a virulent strain. The main source of vcg diversity is sexual recombination between polymorphic vic loci.

The aim of this study was to gain more detailed data about the population structure of *C. parasitica* in the Štiavnicko-krupinská subpopulation in Slovakia.

MATERIAL AND METHODS

Material sampling and isolation. The isolates were obtained from the Štiavnicko-krupinská subpopulation of the fungus in Slovakia. *C. parasitica* occurs there at four sites: Modrý Kameň, Stredné Plachtince, Horné Plachtince and Príbelce. Altogether 215 samples were collected during 1997–2002 (Modrý Kameň 108, Stredné Plachtince 71, Horné Plachtince 34, and Príbelce 2).

Bark samples (4–5 cm) were cut from chestnut blight cankers. The samples were immersed for 20 min in 0.1% NaClO for surface disinfecting, followed by washing in distilled water. Small pieces of stroma (0.5 × 0.5 cm) were placed on 2% malt agar. The isolates were incubated at 25–27°C. Isolates less than 10 days old were used for the vc test.

Vc test and estimation of vcg diversity. For the vc test we used the PDAg (Potato dextrose agar green) medium described by Powell (Powell 1995). Vc tests for *C. parasitica* were done according to Cortesi *et al.* (1996).

The diversity of vcg at each site was expressed by the Shannon diversity index: $H' = -\Sigma p_i \times \ln p_i$, where p_i is the frequency of the i-th vc group (Shannon & Weaver 1949).

Comparison of observed and expected vc group frequencies. The vcg frequencies observed at the sites Modrý Kameň and Stredné Plachtince were compared with vcg frequencies that would be expected from random mating. The comparison between the observed and expected vcg frequencies was done according to Hoegger *et al.* (2000). Since vcg EU-2 was found only once it was omitted from this test.

RESULTS

Vc test and vcg diversity. Four vc groups were detected among the 215 isolates sampled, corresponding to the European vc groups EU-12, EU-2, EU-13 and EU-8 (Table 1). Each isolate was assigned unambiguously to a unique vc group;

Table 1. Results of vegetative compatibility testing of *Cryphonectria parasitica* isolates of the Štiavnicko-krupinská subpopulation

Site	Year of evaluation	EU-2	EU-8	EU-12	EU-13
Modrý Kameň	1997	0	2	19	0
	1998	1	0	34	1
	2001	0	0	22	7
	2002	0	0	20	2
Stredné Plachtince	1997	0	0	7	0
	1998	0	0	18	0
	2001	0	0	26	2
	2002	0	0	18	0
Horné Plachtince	1997	0	0	6	0
	2001	0	0	7	0
	2002	0	0	21	0
Príbelce	2002	0	0	2	0
Štiavnicko-krupinská subpopulation		1	2	200	12

Table 2. Vc group diversity in four *C. parasitica* populations of the Štiavnicko-krupinská subpopulation

	M. Kameň	S. Plachtince	H. Plachtince	Príbelce
N = number of isolates	108	71	34	2
Vc type diversity				
S = number of vc groups	4	2	1	1
H_{vc} [a]	0.47	0.13	0	0
Number of polymorphic vc loci[b]	5	2	0	0

[a]H_{vc} – Shannon diversity index; $H_{vc} = -\Sigma p_i \times \ln p_i$, where p_i is the frequency of the *i*-th vc group (Shannon & Weaver 1949)

[b]according to Cortesi and Milgroom (1998)

no isolate was compatible with more than one vc group. The numbers of vc groups at individual sites varied between one and four. One vc group (EU-12) was dominant in the Štiavnicko-krupinská subpopulation; it represented 93% of all examined isolates and was found at each site. Two vc groups were represented by only one or two isolates.

At the site Modrý Kameň, all four vc groups were present (Table 1). One vc group (EU-12) was dominant at this site and comprised 87.9% of all isolates. Vc group EU-13 was the second abundant vc group, with 9.3% of the isolates. The two other vc groups detected at this site were represented by only one (EU-2) or two isolates (EU-8). At the Stredné Plachtince site (Table 1), two vc groups occurred with frequencies of 97% (EU-12) and 3% (EU-13). At the sites Horné Plachtince and Príbelce only one vc group (EU-12) was detected (Table 1).

The diversity of vc groups was expressed by the Shannon diversity index (H_{vc}, Table 2). At Horné Plachtince and Príbelce, where only one vc group was present, there was the lowest possible value of diversity ($H_{vc} = 0$). The Shannon diversity index was also very low at site Stredné Plachtince ($H_{vc} = 0.13$). The highest value of vc group diversity was determined for Modrý Kameň ($H_{vc} = 0.47$).

Comparison of observed and expected vc group frequencies. To test whether vc groups occurred at frequencies that would be expected from random mating, the observed and the expected vc group frequency distributions at Modrý Kameň and Stredné Plachtince were compared (Table 3). The expected vc group frequencies were calculated on the basis of the allele frequencies determined for six vic loci (Table 4). According to these results, two additional vc groups could be expected at Stredné Plachtince. These are vc groups EU-11 and EU-42 with the same frequency of distribution (2.87%). Based on the number of polymorphic vic loci, eight vc groups could be expected at Modrý Kameň. The expected frequency of vc groups EU-11 and EU-42 was relatively high, but no isolate with these vc groups was actually found. The expected number of isolates in vc groups which did not achieve one isolate in re-calculation to the total number of isolates from a single site were pooled in the χ^2 test. This showed that the observed vc group frequencies at Modrý Kameň differed significantly

Table 3. Comparison of observed and expected numbers of isolates among vc groups in two *C. parasitica* populations

EU vc group	Modrý Kameň		Stredné Plachtince	
	observed	expected	observed	expected
8	2	2.6	0	0
11	0	9.34	0	1.9
12	95	84.06	64	62
13	10	1.04	2	0.1
19	0	0.03	0	0
25	0	0.29	0	0
42	0	9.34	0	1.9
43	0	0.29	0	0
Total	107		66	
χ^2 test				
χ^2 value		98.033		12.885
D.F.		5		3
P		< 0.001		< 0.01

Table 4. Allele frequencies for six vegetative incompatibility (vic) loci in two *C. parasitica* populations

Site/vic loci[a]		vic 1	vic 2	vic 3	vic 4	vic 6	vic 7
Modrý Kameň	allela 1	1	0.9	1	0.1	0.97	1
	allela 2	0	0.1	0	0.9	0.03	0
Stredné Plachtince	allela 1	1	0.97	1	0.03	1	1
	allela 2	0	0.03	0	0.97	0	0

[a]according to Cortesi and Milgroom (1998)

($P < 0.001$) from the frequencies expected under random mating. The difference was significant ($P < 0.01$) also at Stredné Plachtince.

DISCUSSION AND CONCLUSION

Four vc groups were detected in a sample of 215 isolates of *C. parasitica* from four sites of the Štiavnicko-krupinská subpopulation in Slovakia. One vc group at Horné Plachtince and Príbelce, two at Stredné Plachtince and four vc groups at Modrý Kameň. One vc group (EU-12) was dominant at each site.

The fungus *C. parasitica* occurs in two other regions in Slovakia: Horná Nitra and Malé Karpaty (Juhásová 1999). Vc group diversity was also low in Horná Nitra (Adamčíková & Juhásová 2003), comparable to the low vc group diversity observed in the present study. The same vc groups were detected at the three sites Lipovník, Radošina (Horná Nitra subpopulation) and Modrý Kameň, the same vc group was dominant and with similar frequency of isolates, and vc group diversity expressed by the Shannon diversity index was also similar for the three sites. Distribution of vc groups in these two subpopulations was similar. All this indicates a homogenous infection with *C. parasitica*.

Chestnut blight had been recorded in the Malé Karpaty subpopulation as long as in the Štiavnicko-krupinská subpopulation, but the number of identified vc groups is higher. Six vc groups were identified in the Malé Karpaty subpopulation (Juhásová & Bernadovičová 2001). The reason of this higher diversity can be the geographic situation (the Malé Karpaty subpopulation is close to neighbouring Austria and Hungary, from where new vc groups could be introduced).

Vc groups represented only by one or a few isolates occurred in all subpopulations in Slovakia (Adamčíková & Juhásová 2003). The two vc groups EU-2 and EU-8, identified only at Modrý Kameň, are rare in the Štiavnicko-krupinská subpopulation. The dominant vc group in the Štiavnicko-krupinská subpopulation (EU-12) is the same at all sites. We can thus suppose the vc group EU-12 as the primary source of infection. 93% of isolates in the Štiavnicko-krupinská subpopulation belong to vc group EU-12, so do 89% of the isolates in the Horná Nitra subpopulation (Adamčíková & Juhásová 2003). This vc group is dominant in several European countries. Vc group EU-12 accounted for 95% of the Macedonian isolates, 85% of Greek isolates and for 86% of the isolates at one site in Sicily (Heiniger *et al*. 1998). All Romanian and Ukrainian isolates were assigned to this vc group (Radócz 2001). In Bosnia-Herzegovina, EU-12 is also the dominant vc group (Robin & Heiniger 2001). In Hungary, it belongs to the most widespread vc groups (Radócz 2001). According to Robin and Heiniger (2001), EU-12 is the dominant vc group in southern and eastern Europe, with the exception of Turkey.

Strains belonging to vc group EU-12 could have been the first to be introduced to the sites examined by us. The following support this hypothesis: vc group EU-12 (1) is the most frequent at the investigated sites, (2) was identified at the first infected site in Slovakia (Adamčíková & Juhásová 2003), (3) was identified also in countries of eastern Europe (Romania, Ukraine) where *C. parasitica* had only recently been introduced (Radócz 2001).

Vc group EU-13 (12 isolates, 5.6%) was the second most frequent vc group in the Štiavnicko-krupinská subpopulation. That was similar to the Horná Nitra subpopulation (11 isolates, 6.74%) (Adamčíková & Juhásová 2003). EU-13 was one of the four most frequent vc groups in the Carpathian basin (Radócz 2001). In isolates from northern Italy (Corniglio), 20% were vc group EU-13 (Cortesi *et al*. 1998). This vc group was found several times in

Bregaglia in Switzerland (Bazzigher 1981) and at Uherský Brod in the Czech Republic (Haltofová *et al.* 2005; Haltofová 2006). This vc group is rare in other European countries.

Isolates belonging to vc groups EU-1, EU-6, EU-12 and EU-13 were the first to affect Hungarian chestnut stands (Radócz 2001). We detected the two vc groups EU-12 and EU-13 at the investigated sites of the Štiavnicko-krupinská subpopulation; it is possible that chestnut blight there had been introduced from Hungary.

Vc group EU-2, that was identified in the Štiavnicko-krupinská subpopulation, is dominant in western and north-western Europe (Robin & Heiniger 2001). EU-2 had been found only once in Modrý Kameň in 1998. It appears that this vc group did neither spread nor persist in the studied region.

The number of vc groups at sites in Slovakia is distinctly lower than in other countries in Europe. The presence of *C. parasitica* in Slovakia was detected for the first time in 1976 (Juhásová 1999), while it was introduced to southern Europe already in the 1930-s (Biraghi 1946). For example, 20 vc groups were detected in Italy according to Cortesi *et al.* (1996), 40 and some unknown vc groups were identified in France according to Robin *et al.* (2000). In these countries the fungus had enough time to increase the number of vc groups by sexual recombination between existing strains of vc groups, by introduction of new vc groups or by mutations.

Because *C. parasitica* was introduced to Slovakia nearly 40 years later, the diversity in vc groups is lower, but it can be supposed that the number of vc groups will increase with time.

In Europe there are subpopulations with low vc group diversity, similar to that of the Štiavnicko-krupinská subpopulation. Cortesi *et al.* (1998) determined the vc group diversity in 11 subpopulations in Italy and found subpopulations with low diversity. The lowest values of the Shannon diversity index were calculated for two subpopulation in southern Italy that had two (Zafferana, $H_{vc} = 0.41$) and three (Teano, $H_{vc} = 0.64$) vc groups, respectively. These values are comparable with those calculated for the site Modrý Kameň (four vc types, $H_{vc} = 0.47$). Hoegger *et al.* (2000) studied the genetic structure of newly established populations of *C. parasitica* in Switzerland, where the fungus was observed for the first time in 1986 and 1989. In the same year the fungus was observed in the Štiavnicko-krupinská subpopulation (first record at Stredné Plachtince in 1989). It is likely that these subpopulations are of the same age. The vc group diversity is comparable; one or two vc groups were identified at the Swiss sites and the Shannon diversity index ranged from 0 to 0.58 (Hoegger *et al.* 2000). The northern border of distribution of *Castanea sativa* is in Slovakia. Two populations in Michigan, outside the natural range of American chestnut, also had a low vc group diversity (Liu *et al.* 1996). Clonality seems to be a common feature of *C. parasitica* outside the major disease areas (Hoegger *et al* 2000).

Significant differences, as shown by the χ^2 test, between observed and expected frequencies of vc group distribution at the sites Modrý Kameň and Stredné Plachtince could result from a low frequency of crosses between strains. Sexual reproduction plays an important role in creating the actually observed structure in the fungus population.

References

Adamčíková K., Juhásová G. (2003): Diversity of subpopulation of *Cryphonectria parasitica* in Horná Nitra. Folia Oecologica, **30**: 149–155.

Anagnostakis S.L. (1988): Hypovirulence in chestnut blight in Connecticut improves the condition of chestnut trees. Frontiers of Plant Science, **41**: 2–3.

Anagnostakis S.L., Waggoner P.E. (1981): Hypovirulence, vegetative incompatibility, and growth of cankers of chestnut blight. Phytopathology, **71**: 1198–1202.

Bazzigher G. (1981): Selection of blight-resistant chestnut trees in Switzerland. European Journal of Forest Pathology, **11**: 199–207.

Bégueret J., Turcq B., Clave C. (1994): Vegetative incompatibility in filamentous fungi: *het* genes begin to talk. Trends in Genetics, **10**: 441–446.

Biraghi A. (1946): Il cancro del castagno causato da *Endothia parasitica*. Italian Agriculture, **8**: 406–411.

Caten C.E. (1972): Vegetative incompatibility and cytoplasmic infection in fungi. Journal of General Microbiology, **72**: 221–229.

Cortesi P., Milgroom M.G. (1998): Genetics of vegetative incompatibility in *Cryphonectria parasitica*. Applied and Environmental Microbiology, **64**: 2988–2994.

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

Cortesi P., Rigling D., Heiniger U. (1998): Comparison of vegetative compatibility types in Italian and Swiss

populations of *Cryphonectria parasitica*. European Journal of Forest Pathology, **28**: 167–176.

Cortesi P., McCulloch CH.E., Song H., Lin H., Milgroom M.G. (2001): Genetic control of horizontal virus transmission in the chestnut blight fungus, *Cryphonectria parasitica*. Genetics, **159**: 107–118.

Haltofová P. (2006): Vegetative compatibility groups of *Cryphonectria parasitica* (Murrill) M.E. Barr in the Czech Republic. Advances of Horticultural Science, **20**: 55–58.

Haltofová P., Palovčíková D., Jankovský L. (2005): Distribution and health condition of sweet chestnut (*Castanea sativa* Mill.) in the Czech Republic. Acta Horticulturae, **693:** 159–164.

Heiniger U., Cortesi P., Colinas C., Perlerou C., Rigling D., Robin C., Sotirovski M., Uscuplic M. (1998): Diversity of vegetative compatibility groups of *Cryphonectria parasitica* in Europe. Poster presented at 7th International Congress of Plant Pathology, August 6–16, 1998, Edinburg, Scotland; http://www.wsl.ch/forest/wus/phytopath/abstracts/abedin1.html

Hoegger P.J., Rigling D., Holdenrieder O., Heiniger U. (2000): Genetic structure of newly established populations of *Cryphonectria parasitica*. Mycological Research, **104**: 1108–1116.

Juhásová G. (1999): Hubové choroby európskeho gaštana jedlého. Veda, Bratislava.

Juhásová G., Bernadovičová S. (2001): *Cryphonectria parastica* (Murr.) Barr and *Phytophthora* spp. in chestnut (*Castanea sativa* Mill.) in Slovakia. Forest Snow and Landscape Research, **76**: 373–377.

Liu Y.C., Cortesi P., Double M.L., MacDonald W.L., Milgroom M.G. (1996): Diversity and multilocus genetic structure in populations of *Cryphonectria parasitica*. Phytopathology, **86**: 1344–1451.

Liu Y.C., Milgroom M.G. (1996): Correlation between hypovirus transmission and the number of vegetative incompatibility (vic) genes different among isolates from a natural population of *Cryphonectria parasitica*. Phytopathology, **86**: 79–86.

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

Radócz L. (2001): Study of subpopulations of the chestnut blight (*Cryphonectria parasitica*) fungus in the Carpathian basin. Forest Snow and Landscape Research, **76**: 368–372.

Robin C., Anziani C., Cortesi P. (2000): Relationships between biological control, incidence of hypovirulence, chestnut blight severity and the population structure of *Cryphonectria parasitica* in France. Phytopathology, **90**: 730–737.

Robin C., Heiniger U. (2001): Chestnut blight in Europe: Diversity of *Cryphonectria parasitica*, hypovirulence and biocontrol. Forest Snow and Landscape Research, **76**: 361–367.

Shannon C.E., Weaver W. (1949): The Mathematical Theory of Communication. Urbana, University of Illinois Press, Champaign.

Received for publication September 22, 2006
Accepted after corrections November 30, 2006

Corresponding author:

Mgr. Katarína Adamčíková, Ph.D., Ústav ekológie lesa SAV, Pobočka biológie drevín, Akademická 2, 949 01 Nitra, Slovenská republika
tel.: + 421 377 335 738, e-mail: nrueadam@savba.sk, katarina.adamcikova@sav.savzv.sk