Cryphonectria parasitica in Sessile Oak in Hungary

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Abstract – Since 1999 occurrence of chestnut blight fungus (*Cryphonectria parasitica*) has been observed in sessile oak in Western and South-Western regions of Hungary in young and middle aged *Quercus petraea* stands mixed with *Castanea sativa*. Incidence and impact of the disease, vegetative compatibility type diversity of the pathogen, occurrence of the natural hypovirulence in oak, conversion capacity of the local hypovirulent strains and development of the symptoms in inoculated oak trees were studied in order to investigate the conditions of the biological control based on the hypovirulence in oak. In 2003 survey plots were designated in 12 infected oak stands. The rate of infection varied up to 23.02% and the mortality rate up to 5.76%. Isolations were performed in 2004 from survey plots and in 2006 from larger areas of two forest management units. In 2004 all isolates from oak were of virulent character, while 15.38% of the isolates showed hypovirulent features in 2006. Totally 15 VC types were delimited among the 174 isolates. The VC type diversity varied between 1 to 5 types/plot or forest subcompartment. Five local hypovirulent isolates showed full conversion capacity to the selected virulent strains of different VC types *in vitro*. Early appearing and progressive increasing of the symptoms were observed in the oak trees inoculated with virulent strains, however more than half of the trees healed during the second year after the inoculation. Some hypovirulent inoculations caused superficial altering of the suberisation, showing the establishment of the hypovirulent fungus in the bark. Our results show favourable conditions for successful application of the preventive control by disseminating the hypovirulent strains in young oak stands.

***Cryphonectria parasitica* / *Quercus petraea* / VC types / hypovirulence / conversion / symptom development / biological control**

Kivonat – ***Cryphonectria parasitica* kocsánytalan tölgyön Magyarországon.** A szelídgesztenye kéregrákját okozó *Cryphonectria parasitica* kocsánytalan tölgyön való tömeges előfordulását 1999 óta tapasztaljuk Nyugat- és Dél-Dunántúlon, fiatal és középkorú, gesztenyével elegyes kocsánytalan tölgy állományokban. A kórokozó elleni biológiai védekezés feltételeinek megismerése céljából vizsgáltuk a betegség előfordulását és jelentőségét, a kórokozó vegetativ kompatibilitási típusait, a hipovirulencia természetes előfordulását, a helyi hipovirulens törzsek konvertáló kapacitását és a tünetek kifejlődését mesterségesen fertőzött fákban. 2003-ban mintaterületeket jelöltünk ki 12 állományban, amelyeken a fertőzöttségi arány 23,02%-ig, a mortalitás pedig 5,76%-ig változott. 2004-ben a mintaterületek fertőzött fáiról, 2006-ban pedig két erdészet nagyobb területéről gyűjtöttünk izolátumokat. Hipovirulens törzseket a tölgyön 2004-ben nem találtunk, ezzel szemben 2006-ban a tölgyről származó izolátumok 15,38%-a hipovirulens jellegűnek bizonyult. A 174 izolátumból álló populációban összesen 15 VC típust különítettünk el. A VC típusok száma 1 és 5 között változott az egyes mintaterületekben, illetve erdőrészletekben. Öt helyi hipovirulens izolátum teljes konverziós kapacitást mutatott különböző VC típusú virulens törzsekkel szemben. Mesterséges fertőzési kísérlet első évében a tünetek gyors kifejlődését és fokozódását tapasztaltuk, de a fertőzés utáni második

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évben a fák több mint felénél a nekrózisok begyógyultak. A hiopvirulens oltások egyrészénél a parásodás felületi megváltozását észleltük, ami a hipovirulens gomba sikeres megtelepedésére utal. Eredményeink azt bizonyítják, hogy a hipovirulens törzsek terjesztése általi megelőző védekezés sikeres alkalmazásának feltételei kedvezőek a fiatal tölgy állományokban.

***Cryphonectria parasitica* / *Quercus petraea* / VC típusok / hypovirulencia / konverzió / tünetek kifejlődése / biológiai védekezés**

1 INTRODUCTION

Chestnut blight caused by *Cryphonectria parasitica* (Murrill) M.E. Barr was found first time in Hungary in 1969 in locality Nemeshetés county Zala (Körtvély 1970). The distribution and impact of the disease was investigated by the MÉM Laboratory for Chestnut Protection founded in order to find measures to reduce the spread of the epidemic (Eke - Gál 1975). Later investigations started since 1995 assessed the recent impact of chestnut blight in Hungary, occurrence of vegetative compatibility types and of the natural hypovirulence (Radócz et al. 1997). Successful application of the biological control based on the hypovirulence was performed in chestnut orchards and gardens in Sopron and surroundings (Vidóczi et al. 2005). Mass occurrence of the *C. parasitica* infection in oak stands (*Quercus petraea* (Mattuschka) Liebl.) was first observed in 1999 in Southwest Hungary (Surd 11D), where the assessment stated 14.9% infection and 5.7% mortality rate in 2001 (Gáncs 2002). Preliminary observations and data of the State Forest Service indicated that the disease is wide-spread in oak in South-western and Western regions of Hungary. Systematic investigations were started in 2003 in order to know the real impact of the disease and to find managing solution of this new forest protection problem.

Decreased virulence of *C. parasitica* strains containing cytoplasmatic dsRNS (*Cryphonectria hypovirus* 1, CHV1) constitutes the basis of the biological control of the pathogen. The isolates containing CHV1 are white or less pigmented in culture and do not form pycnidia or very sparsely only. These strains cause only superficial, non lethal symptoms in the bark of the hosts. The CHV1 can be transmitted from hypovirulent strains to the virulent ones by hyphal anastomosis, therefore the virulent strains become converted to hypovirulent. The transmission of the CHV1 happens only between the vegetative compatible partners belonging to the same VC type. The vegetative incompatibility system in *C. parasitica* is regulated by five to seven so-called *vic* gene. Compatible fungal strains belonging to the same VC type have identical alleles at all *vic* loci (Anagnostakis 1983, Bissegger et al 1997). The transmission of hypoviruses is negatively correlated to the number of *vic* genes that are different between VC types (Liu - Milgroom 1996). Increasing of the VC type diversity in the subpopulations of the fungus is possible by the recombination of *vic* genes during the sexual reproduction. The great diversity of the VC types influences negatively the success of the biological control. Natural hypovirulence occurs in many European countries and biological control programs were carried out in chestnut (Turchetti – Maresi 1991, Juhasova – Bernadovicova 2001, Heiniger – Rigling 1994, Vidóczi et al. 2005). Occurrence of *C. parasitica* in oak species also has been reported in some countries of Europe and USA (Torsello et al. 1994, Bissegger – Heiniger 1991, Luisi et al. 2002, Juhasova – Kulcsarova 2002, Radócz – Tarcali 2005), but the biological control in field has not been tried out in the field in connection with oaks.

2 MATERIALS AND METHODS

2.1 Incidence and impact of the disease in oak

Survey plots with numbered trees were established in 12 infected forest subcompartments distributed in 4 forestry districts. The rate of infection was assessed in the years 2003 and 2004 using an estimation scale: 1-trees free from symptoms, 2-trees with one perennial canker, 3-trees with more perennial cankers or diffuse canker, 4-dead trees. General healthy state and social position of the trees were also assessed as well as the position of the cankers: in trunk and/or in branches.

2.2 Isolation, native hypovirulence

Bark samples were taken from infected oak trees and some chestnut trees during the field evaluations. In 2004 samples were taken from all infected oak trees inside the survey plots. In 2006 regular field investigations and bark samplings were carried out in the area of two forest management units: Iharos (SEFAG Forestry and Timber Industry Co.) situated in South-Western Hungary and Hegyvidék (TAEG Co. Sopron) in the North-Western area of Hungary. The field investigations aiming to discover the infection of oak trees covered all oak stands presumed as threatened by the disease (oak stands of young and medium age mixed with chestnut). Bark samples were taken mostly from infected oak trees but also from some chestnut trees in order to investigate the VC type diversity of the subpopulations of the pathogen.

The isolation was performed on PDA after surface sterilization of the bark pieces using NaOCl solution (2 g/l active chlorine). The virulent or hypovirulent character of the isolates was estimated on the presence or lack of pigmentation and pycnidia in two week old cultures.

2.3 Vegetative compatibility

The vegetative compatibility was determined based on the formation or lack of merging barrage between the isolates in pairing test performed on PDA amended with pH indicator (Anagnostakis 1977, Powell 1995). The investigation of VC type diversity of the subpopulations was performed in several steps. First isolates from each forest subcompartiment were compared together, than one isolate was selected from each distinguished VC type and compared with the selected isolates from the other subcompartiments. Finally, the representative isolates of different VC types were paired with the tester strains EU 1-31 (Cortesi et al. 1998).

2.5 Conversion capacity of the hypovirulent strains

Native hypovirulent strains were selected for testing their conversion capacity against the local virulent isolates belonging to the most frequent and wide-spread VC types present in the forest subcompartments designated for the future field trials of the biological control. Six hypovirulent isolates were paired with 11 selected virulent strains in three repetitions. Conversion capacity of the hypovirulent strains was evaluated on the scale below:

- \- no conversion
- \+ partial conversion: the growth reduction of the virulent strain is less than 50%
- ++ good conversion: the growth reduction of the virulent strain is more than 50%
- +++ full conversion: the virulent strain is converted to hypovirulent in 100%

2.4 Inoculation tests

The inoculation experiment was performed in field in a *Q. petraea* stand of 11 years old near to Sopron, under permanent control. In May 2005 a number of 80 trees were inoculated with virulent isolates of *C. parasitica*, 10 trees with hypovirulent isolates and 10 trees were assigned as control. Four inoculation points were made in each tree. Holes of 5 mm diameter were bored in the bark and mycelial plugs from *C. parasitica* culture were placed in, than the wounds were covered with wax. Pieces of sterile medium were placed into the wounds of the control trees. The evaluation was effected at two weeks, 4 months, one year and two years after the inoculation. The symptoms cracking, deformation, sap flux, appearance of the stromata etc. were recorded and the length of phloem necrosis measured. The trees with developing stromata were regularly removed.

3 RESULTS AND DISCUSSION

3.1 Incidence and impact of the disease

The symptoms of *Cryphonectria parasitica* appeared mostly in form of perennial cankers in oak (*Figures 1 and 2*), however diffuse cankers also were observed in some weakened trees and dead trees killed by the fungus also occurred. Sexual stromata of the fungus were observed in most of the trees. Results of the field investigation are shown in the *Table 1*. In the year 2003 the rate of infection varied between 2.44 and 23.08% in the survey plots, mean 12.05%. The mortality was meanly 2.14% and varied up to 5.75%. In 2004 both the infection and mortality rates increased a bit in each plot, the mean values were 13.84 and 2.76% respectively. Trees with one perennial canker and with more perennial or diffuse cankers occurred approximately in the same rate, both a bit higher in 2004 than in the precedent year. Although the rate of mortality was not too high, the damages caused by the fungus are considerable, because of losses in quality timber due by deformed logs with large perennial cankers (*Figures 3 and 4*).

Table 1. Rate of C. parasitica infection in Q. petraea in the survey plots

Plot location (Forest subcompartment)	Nr. trees	2003				2004			
		Free from symptom %	One canker %	More/ diffuse cankers %	Dead trees %	Free from symptom %	One canker %	More/ diffuse cankers %	Dead trees %
Csurgónagymarton 16A	155	86.45	8.39	3.23	1.94	81.94	11.61	3.23	3.23
Iharosberény 10H	155	81.94	7.10	5.81	5.16	81.94	6.45	6.45	5.16
Iharosberény 12C	149	90.60	3.34	2.68	3.34	90.60	4.03	2.01	3.34
Kőszeg 64D	123	97.56	2.44	0	0	95.93	3.25	0	0.81
Liszó 23F	150	88.67	5.33	3.33	2.67	86.00	6.00	5.33	2.67
Nagykanizsa 56E	148	81.76	8.11	10.14	0	79.73	6.76	11.49	2.03
Pogányszentpéter 5M	139	76.98	7.19	10.07	5.75	76.98	6.47	10.79	5.76
Simonfa 11J	134	93.28	2.99	2.24	1.49	93.28	2.99	2.24	1.49
Sopron 211C	131	95.42	3.05	1.53	0	93.13	3.05	3.05	0.76
Surd 9B	118	89.83	4.24	5.93	0	84.75	5.93	7.63	1.69
Total	**1402**	**87.95**	**5.35**	**4.56**	**2.14**	**86.16**	**5.78**	**5.28**	**2.76**

Figure 1. and Figure 2. Symptoms of Cryphonectria parasitica infection in Quercus petraea

Figure 3. and Figure 4. Large perennial cankers in Q. petraea caused by C. parasitica

3.2 Isolates, occurrence of native hipovirulence

In 2004 a number of 66 pure isolates were obtained from bark samples taken from infected oak trees inside the survey plots. All of them showed virulent features, intense pigmentation and abundant production of pycnidia *in vitro*. In 2006 totally 65 isolates were obtained from oak trees in forests of Iharos and Sopron. The 15.38% of these isolates were of hypovirulent character (*Figure 5*). In 2006 isolates were taken also from chestnut in order to investigate the population structure of the pathogen in the studied areas. The rate of hypovirulence was of 51.16% in the 43 isolates from chestnut bark (*Table 2*). The occurrence of hypovirulent strains in oak is an important result of this research. No former records of the natural hypovirulence of *C. parasitica* in oak trees in Hungary. It was presumed, that only the virulent strains can infect the oak trees that are more resistant than chestnut.

The presence of hypovirulent strains in oak indicates the possibility of the biological control of the disease in oak. Since the hypovirulent fungus can stabilize in oak trees the conversion probability of virulent strains infecting them is more real. The great amount of natural hypovirulence among the isolates from chestnut indicates the reason of decreasing mortality in chestnut observed in the latest years.

Table 2. Occurrence of hypovirulence among the C. parasitica isolates from oak and chestnut

Year and location	*Quercus petraea* Total isolates No.	*Quercus petraea* Hypo-virulent No.	*Quercus petraea* Hypo-virulent %	*Castanea sativa* Total isolates No.	*Castanea sativa* Hypo-virulent No.	*Castanea sativa* Hypo-virulent %	TOTAL No.
2004 Survey plots	66	0	0	0	0	0	66
2006							
Iharos	60	9	15.0	33	19	57.57	93
Sopron	5	1	20.0	10	3	30.0	15
Total 2006	65	10	15.38	43	22	51.16	108
TOTAL 2004 and 2006	131	10	7.63	43	22	51.16	174

Figure 5. Virulent and hypovirulent isolates of C. parasitica

Figure 6. Test of vegetative compatibility

3.3 Vegetative compatibility, population structure of the pathogen

Totally 174 isolates were involved in the VC compatibility test evaluated on the presence or lack of the mycelial barriers between the cultures (*Figure 6*). Mostly 1-3 VC types were distinguished inside of each forest subcompartment and survey plot, however 5 different VC types occurred among 12 isolates from the survey plot Liszó 23F (*Table 3*) .

Table 3. Vegetative compatibility of the isolates from survey plot Liszó 23F

	1	2	3	4	5	6	7	8	9	10	11	12
1		-	-	-	-	-	-	-	+	-	-	-
2	-		+	+	+	-	+	+	-	+	-	-
3	-	+		+	-	-	+	+	-	+	-	-
4	-	+	+		-	-	+	+	-	+	-	-
5	-	+	-	-		-	-	-	-	-	-	-
6	-	-	-	-	-		-	-	-	-	-	-
7	-	+	+	+	-	-		+	-	+	-	-
8	-	+	+	+	-	-	+		-	+	-	-
9	+	-	-	-	-	-	-	-		-	-	-
10	-	+	+	+	-	-	+	+	-		-	-
11	-	-	-	-	-	-	-	-	-	-		-
12	-	-	-	-	-	-	-	-	-	-	-	

Isolates No. 2, 3, 4, 5, 7, 8 and 10 represent the most frequent VC type in the site (identical with EU 12 tester strain). Isolates No. 1 and 9 belong to another VC type, while isolates No. 6, 11, and 12 are different from all and each represents a separated VC type.

Totally 15 VC types were distinguished in survey plots and two larger areas of the forest management units Iharos and Sopron. 12 VC types were compatible with one of the EU 1-31 tester strains, while 3 VC types (the representative isolates from Csurgónagymarton 4B, Iharosberény 21A and Iharosberény 10B) were different from all EU testers 1-31. (*Table 4*).

Table 4. Vegetative compatibility of C. parasitica isolates compared with EU 1-31 testers

Origin of the representative isolates	EU testers											
	1	2	3	9	10	11	12	13	14	15	20	28
Sopron 211C							+	+				
Nagykanizsa 56E					+				+			
Liszó 23F	+	+					+			+	+	
Surd 9B										+		
Csurgónagymarton 4B												
Csurgónagymarton 9C				+								+
Iharosberény 21A												
Iharosberény 12D											+	
Iharosberény 17I			+						+		+	
Iharosberény 18C								+				
Iharosberény 22H	+											
Iharosberény 8F								+			+	+
Iharosberény 10B												
Iharosberény 10C											+	
Iharosberény 10G									+			
Porrog 5H											+	

3.4 Conversion capacity of the local native hypovirulent strains

Different grades of reduction of virulent growth were observed at *in vitro* conversion test (*Figure 7 and Figure 8*). Five hypovirulent isolates (Hv) proved to possess a good and total conversion capacity to the most of the virulent strains (V) involved in the test (*Table 5*). Majority of the tested virulent strains representing different VC types were converted totally by at least one hypovirulent strain. However the hypovirulent isolate 104 Hv did not convert any of the tested virulent isolates and the virulent isolate 129 V was not converted by any of the hypovirulent strains. In this case we have to try further hypovirulent strains available in our collection of isolates.

Table 5. Conversion capacity of the selected hypovirulent isolates (Hv)

	30 V	31 V	32 V	45 V	49 V	52 V	56 V	57 V	105V	107 V	129 V
74 Hv	+++	++	+	++	+++	++	+	+++	++	+++	-
89 Hv	+	++	+++	+++	++	++	++	++	+	-	-
97 Hv	+	++	++	++	++	++	+++	++	+	-	-
104 Hv	-	+	-	-	+	-	-	-	-	-	+
112 Hv	+	++	+++	+++	++	+++	+	++	+	-	+
132 Hv	-	+++	++	+++	+++	+++	+	-	+	-	-

Figure 7. Different grades of conversion (white hypovirulent strains on left)

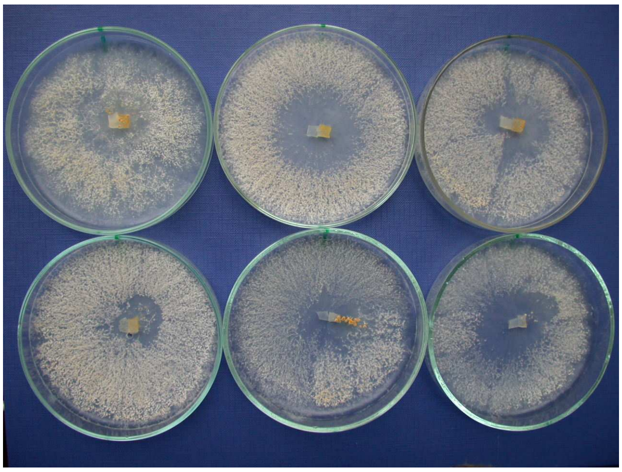

Figure 8. Examples of total conversion

3.5 Artificial inoculations, development of the symptoms

The field observations indicated that the incipient symptoms of *C. parasitica* infection are not easy to recognize in the oak trees, so a part of the infections may remain unobserved and unrecorded during the surveys. The inoculation tests aimed to observe the appearing and developing in time of the symptoms. The results are included in the *Table 6.*

At two weeks after the inoculation symptoms of cracking and deformation appeared in 28.75% of the trees inoculated with virulent strains and even the stromata of the fungus developed in 6.25% of the trees. The number of trees with symptoms and stromata increased progressively in the first year. Healing of the initial lesions was observed in more than half of trees during the second year after inoculation (*Figure 9*). A rate of 56.25% of the inoculated trees apparently recovered in two years after the inoculation. The amount of 2.50 – 2.50% for the perennial cankers and dead tress is not relevant because of the regularly removing of the trees with stomata. The mean length of the phloem necrosis increased in the first year (21.48 mm to 33.94 mm), while in the second year decreasing of the average length was observed (15.24 mm) due by the callus building and healing of the lesions.

Table 6. Developing of the symptoms in Q. petraea inoculated with virulent C. parasitica

Date of inoculation: 2005.05.05	Date of evaluation Frequency of the symptoms (%)			
Symptoms	2005.05.20.	2005.09.08.	2006.06.20	2007.05.05
Cracking, deformation	28.75	51.25	20.00	-
Sap flux	8.75	1.25	-	-
Stromata (cumulative)	6.25	22.50	38.75	38.75
Healing (callus building)	-	52.50	5.00	-
Recovered (closed lesions)	-	-	35.00	56.25
Perennial canker	-	-	-	2.5
Dead	-	-	2.5	2.5
Mean necrosis length mm	21.48	33.94	15.24	-

The wounds recovered during the first year in the trees inoculated with hyporulent strains and control trees, no or minimum phloem necrosis occurred. Slight alteration of suberization was observed in some trees inoculated with hypovirulent strain indicating the presence of the hypovirulent fungus in the outer bark (*Figure 10*).

Figure 9. Healed virulent inoculations

Figure 10. Changing suberisation in the hypovirulent inoculations

4 DISCUSSION

Q. petraea is one of the most valorous forest tree species in Hungary covering an important amount of about 12% of the forested areas. At present infection of *C. parasitica* occurs in sessile oak in young and middle aged stands mixed with *Castanea sativa*. Regarding the excellent epidemic capacity of the pathogen, the threat of spreading of the disease is real to larger areas of oak forests towards the distribution of chestnut.

The natural hypovirulence occurs in 7.63% in oak and 51.16% in chestnut in the investigated areas. Decreasing of the disease severity in chestnut trees is a consequence of the natural spreading of the hypovirulent strains containing CHV. Since these strains are rare in oak trees, their dissemination may be a good way to decrease the incidence of the infection. The native hypovirulent strains possess a very good conversion capacity against the local virulent strains. The VC type diversity of the subpopulations of the pathogen is not too high in the most of the sites at this time. These circumstances offer favourable conditions for biological control of the disease in oak. The number of VC types is increasing in consequence of sexual reproduction, so continuous survey on population structure of the pathogen is needed for the success of the biological control.

Curative and preventive biological control technologies are known in the case of chestnut: inoculation with compatible hypovirulent material around the virulent bark lesions and dissemination of hypovirulent strains by inoculating sprouts, seedlings and healthy trees. Both procedures help the natural spread of the hypovirulent strains of the pathogen. Changes in population structure of the fungus favouring the rate of the hypovirulent strains occurs in natural way, too, but this process is slow and generally starts late after the establishment of the virulent strains. Introduction and dissemination of vegetative compatible hypovirulent strains capable to natural spread in oak trees seems to be the most practicable plan for the preventive control of the disease in oak stands.

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