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„THESIS OF DOCTORAL (PHD) DISSERTATION"

EXAMINATION OF *CRYPHONECTRIA PARASITICA* (MURRILL) M.E. BARR IN THE CARPATHIAN-BASIN

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1. INTRODUCTION

„Chestnut blight" disease caused by the fungus *Cryphonectria parasitica* (Murr.) Barr [syn: *Endothia parasitica* (Murr.) And.] (anamorf: *Endothiella* sp.) results in great damages of the chestnut stands throughout the World. I have been studying the Central European subpopulations of this fungus, since 1999.

First at the beginning of the XXth century it destroyed almost the whole American chestnut [*Castanea dentata* (Marsh.) Borkh.] populations on 4 million hectares in the USA. In the middle of the last century the pathogen was transferred into Europe (BIRAGHI, 1946), infected and destroyed the European chestnut (*Castanea sativa* Mill.) populations in the West-European countries. Then the disease spread towards Central- and Eastern-European chestnut territories and arrived at the Carpathian-basin too. First symptoms of chestnut blight were reported on European chestnut in Hungary in 1969 (KÖRTVÉLY,1970). Chestnut stands in Hungary have already been seriously damaged by this disease over the following 30 years. Up to now this disease becoming the most common disease for European chestnut. However, the importance of the disease is still increasing because it is able to infect other tree species of *Fagaceae* plant family (oaks, beech). Therefore *C. parasitica* could be a potentially more serious parasite for our forests. Until 1998, the fungus was only detected on chestnut in Hungary. Then the blight symptoms were also detected on some young sessile oak (*Quercus petraea*) trees in mixed chestnut forest near Zengővárkony (RADÓCZ and HOLB, 2002).

By 1974, the fungus was detected in all of the Hungarian chestnut populations, and it was also reported in some nurseries (EKE and GÁL, 1975). Chestnut trees of the examined Hungarian subpopulations were infected at the ratio 60-70 % in general (RADÓCZ, 1977), but in some places infection level was higher than 90 % (Radócz et al., 1997). Later on, the fungus was identified in Slovakia (JUHÁSOVÁ, 1976), in Romania (FLOREA and POPA, 1988) and in the Sub-Carpathian region of Ukraine (RADÓCZ, 2001). Nowadays, the Middle- and East-European regions are reported as the "frontline" of the epidemic of *C. parasitica*.

Successful protection against *Cryphonectria parasitica* is a very difficult question. Conventional control methods against the fungus are not applicable with a great success because of the extreme pathogenici of the fungus, and the other charasteristics of sites and host-plants. Professional mechanical treatments could delay the spread of the infection, but it is not sufficient. Some tests were carried out to adapt a resistant species [*Castanea mollissima*(Bl)] in Europe, but it was not adequate. There was the problem that this Chinese chestnut reacted to the European climate badly, on the other hand its nut quality were worse than European chestnut has.

Discovery of Italian and French experts in the 1950's was a break-through in the development of the effective protection against the pathogen. In 1950, Biraghi noticed superficial (recovered) necrosis on some chestnut trunks. Abnormal strains of the pathogen were isolated from naturally recovered cankers by Grente in 1964. It showed a reduced sporulation and pigmentation „white cultural strain". Its infecting capacity was considerably lower than the other strains, and was justified by laboratory examinations (GRENTE, 1965). This strain was called: hipovirulent.

Hipovirulence of *C. parasitica* is caused by an unencapsidated dsRNA virus of the genus Hypoviridae. It is located in the cytoplasm of the fungus (HILLMAN at al., 1995). Infection by the *Cryphonectria hypovirus* (CHV) causes a reduction of fungal virulence ranging from avirulence to almost normal virulence. Hypovirulent fungal strains produce nonlethal, superficial cankers that are restricted to the outer parts of the bark and do not destroy the vascular cambium of the host tree. These characteristics of hipovirulent strains are transmissible into virulent („wild") strains via hyphal anastomosis, resulting in conversion of the recipient fungal isolate to the hypovirulent morphology. Vegetative compatibility is necessary between the virulent and hypovirulent strains to transmit CHV (ANAGNOSTAKIS and WAGGONER, 1981). The genom of the fungus which is responsible for the vegetative incompability was identified by Anagnostakis, and this discovery gave a basis for vegetative compatibility test (ANAGNOSTAKIS, 1977). Isolates of the fungus can be divided into different vegetative compatibility groups (VCG-s) by using this method. Hipovirulent forms of *Cryphonectria parasitica* occur in nature, but it usually happens many years after the appearence of virulent fungul strains. For example *C. parasitica* was detected in Switzerland in 1948, but hipovirulent strains was detected only after 27 years, in 1975 (BAZZIGHER et al., 1981).

Practical application of hipovirulent strains is an efficient biological method to protect chestnut trees against *Cryphonectria parasitica.* The method using hipovirulent strains was also adapted in Hungary and applied in chestnut plantations with good results. But it is not adapted yet in the case of oak infections by *C. parasitica*.

According to our experiences the susceptibility of oaks to the pathogen is more moderate than the chestnut. Until now, chestnut blight infection occured principally on young oak trees mostly in mixed populations with chestnut. But this situation can change in the future and the destruction of the pathogen on oaks could be more serious. In that case, *Cryphonectria parasitica* can be potentially a more serious pathogen in our forests. This is also well known that there are 21.6 % fine oaks and 10.75 % austrian oak (*Quercus cerris*) within the Hungarian forest territory. One of the most important varietes is the sessile oak (*Quercus petraea*) which covers near 10 % of our forest-land and this tree species is important both economically and ecologically.

2. OBJECTIVES OF THE RESEARCH

Main goals of our studies were the following:

- measuring damages on European chestnut (*Castanea sativa*) and on oaks (*Quercus spp.*) caused by the fungus *Cryphonectria parasitica* in the Carpathian-Basin (in North-Transylvania - Romania, in Sub-Carpathia - Ukraine, in Slovakia and in Hungary), population dynamic studies based on datas from Bakonya test site,
- laboratory investigations of the collected samples and isolates,
- pathogenical examination on branches of different oak trees,
- examinations of hypovirulent strains to study their capacity for the practical biological protection, evaluation and analysis of the results achieved,
- comparative analysis of data realized from different chestnut growing areas of the Carpathian-Basin.

3. METHODS

Field investigations

Field examinations were done continuously between 2001 and 2006 on 7 test sites near the town of Baia-Mare (North-Transylvania), on 9 test sites in Carpathian Ukraine, on 3 sites in Slovakia and in 10 subpopulations of 3 regions in Hungary. Usually 100-100 randomly selected trees were examined on a test site. In those populations, where the number of the trees were less than 100, every tree was examined. In one oak population (Bakonya 16 I) a test site had been marked out containing150 oak trees.

Infection rates (I%) were checked during the field examinations according to the applied international method (RADÓCZ, 1997) on chestnuts, as well as according to a modified method (West-Hungarian University, Institute of Forest. and Forest Protection) on oaks. Moreover, bark samples were collected from the infected or „suspicious looking" trees by a disinfected sharp scalpel for laboratory identifications and further analysis.

Methods of laboratory examinations

Surface sterilized bark samples were cultivated on the surface of a potato-dextroz-agar (PDA) media. Samples were incubated for 7 days in a climated chamber, then the mycelia were cultivated again on the PDAmb media. 10 days later, virulent and hypovirulent isolates were seperable visually. Then, vegetative compatibility tests were done on the PDA-Powel media. The isolates were paired first to each other, then to EU-tester strains. Vegetatively compatible isolates were classified as the same Vegetative Compatibility Group (VCG). Those isolates which formed a visible barrage zone at the edge of the mycela were classified into different VCG-s. As the last step of the laboratory examination, conversion tests were done involving the virulent isolates and Hungarian hypovirulent strains. Conversion capacity of the hypovirulent strains was determined according to the valuation scale elaborated by Radócz (1995).

Pathogenical examinations on chestnut and oak branches

Virulence of different isolates were examined on the dormant sticks of different oak species (*Quercus* spp.) and chestnut (*Castanea sativa*). Branches were cultivated with virulent isolates. The experiments were done in 4 repetitions. The treated branches were inoculated for 36 days, then were measured the caused necroses. The necroses were mostly ellipse shaped. Shorter and longer diagonals of the ellipses were measured, and the size of the damaged bark surface was determined using the ellipse calculation formula $[(a \times b) \times \pi]$.

Field treatments with hipovirulent strains

Infected trees were applicated with hipovirulent isolates which had the capacity to convert virulent isolates during the laboratory examinations. Cankers were grafted round with hipovirulent strains, and the treated trees were examined one year later, and then yearly.

Hipovirulent treatments were done at Pécsbányatelep, at Nagymaros and at Nagykanizsa. Test sites were selected containing 25-25 trees, and field efficiency of the hipovirulent treatments were checked.

A special treatment was done at the Pécsvárad-Zengővárkony chestnut area, where 1000 chestnut trees were treated with hipovirulent strains.

Statistical tests

Infection rate development were estimated by linear trend analysis using the data from Baia Mare and from Ukraine. Comparison of the results of the pathogenical tests were done by Mann-Whittney U-test and Kruskall-Wallis test. Analyses of the size of the cankers were done by the scattering calculating method.

4. MAIN RESULTS OF THE DISSERTATION

Results of the field examinations

Cryphonectria parasitica was reported in Romania on chestnut in 1984, but there were not any other examinations. Our investigation was the first near Baia Mare. 7 chestnut sites were studied betweeen 2002-2006.

It was found that *C. parasitica* was spread throughout the territory and symptoms were easily visible, but differences were observed in the destruction among the different growing sites. The ratio of the infected trees (I%) **was more then 90 %** on the most damaged site (Baia Mare-Veresvíz II. section), and the index of the infection (Ii) was also high (4,38) at that place. After the field ivestigations, laboratory examinations were carried out, which showed that only 1 VCG of the pathogen exists on the examined Romanian stands what was vegetatively compatible with the **EU-12** strain.

Between 2004-2006, our field investigations were extended over the sessile oak trees (*Quercus petraea*) on the examined chestnut areas. In 2004, **infected sessile oak trees** with bark necrosis were also found near Baia Mare. The ratio of infection on oak (I%) was less than on chestnut but it is increasing year by year (Figure 1). According to the results of the laboratory examinations oak trees were infected by the same *C. parasitica* strain (EU-12) that was detected from chestnut in Romania.

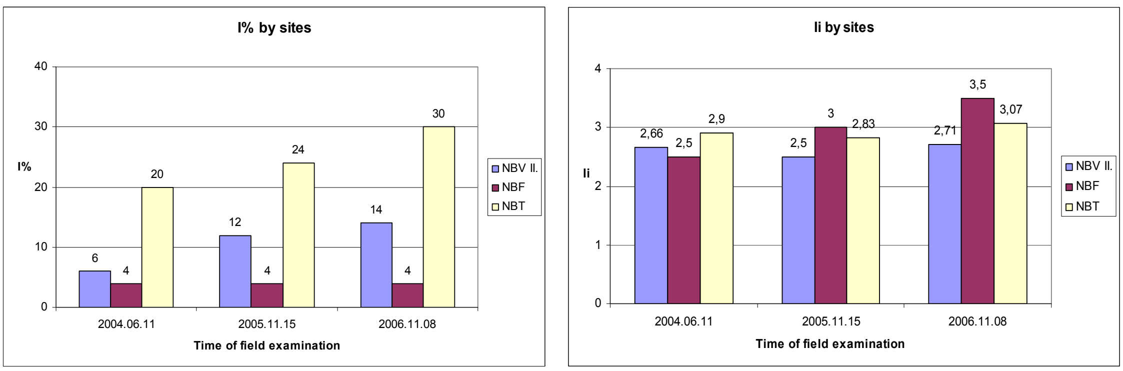


Figure 1. I% and Ii datas from sessile oaks on the Romanian test sites between 2004-2006. (NBV = Baia Mare-Veresvíz, NBF = Baia Mare-Felsőtótfalu, NBT = Baia Mare-Tauti Magherau).

In Ukraine, our examinations were done between 2001-2006, near the towns of Uzhgorod and Munkacevo. There were symptoms of *C. parasitica* found in three examined chestnut stands. This was the first report on this fungus in Ukraine. Laboratory examinations showed that every isolate from Ukraine were **virulent,** and two VCG-s were identified from the Ukrainian bark samples. Vegetative compatibility tests gave the results that **EU-12** (in Seredne) and **EU-13** (in Bobovisce and in Rostovjatitsja) fungal strains exists on the examined Ukrainian test sites. Infected oak trees by chestnut blight have not been found yet in Ukraine. Trend analysis of the Romanian and Ukrainian infection data were done at the end of the examinations. Results are presented in the Figure 3.

Romanian and Ukrainian isolates were paired with Hungarian hypovirulent strains. Only one Hungarian hypovirulent strain (PJ-2) was able to convert isolates from Romanian chestnuts. There were several Hungarian hypovirulent strains (PJ-2, L13xW31, FS8xW31, FS8x88, FS1xGA13, FS4x146, B1xBF, A3xB7) which were able to convert isolates from Romanian oaks. There was not any conversion between hypovirulent strains and Ukrainian origined isolates.

In Hungary and in Slovakia, we principally researched sessile oaks. Infected oak trees were detected in Slovakia, near Duchonka. EU-2 pathogen strain was identified from the Slovakian sites. In Hungary, three growing areas were examined. Blight symptoms were identified on two areas (Zala-county and Baranya-county). All of the isolates were virulent. EU-3 (from Iharos), EU-16 (from Pogányszentpéter), EU-9 and EU-11 (from Bakonya) fungal strains were identified from bark samples of infected oaks in Hungary. The ratio of the infection on sessile oak trees (I%) in the Hungarian test sites are visible on the diagrams of Figure 2.

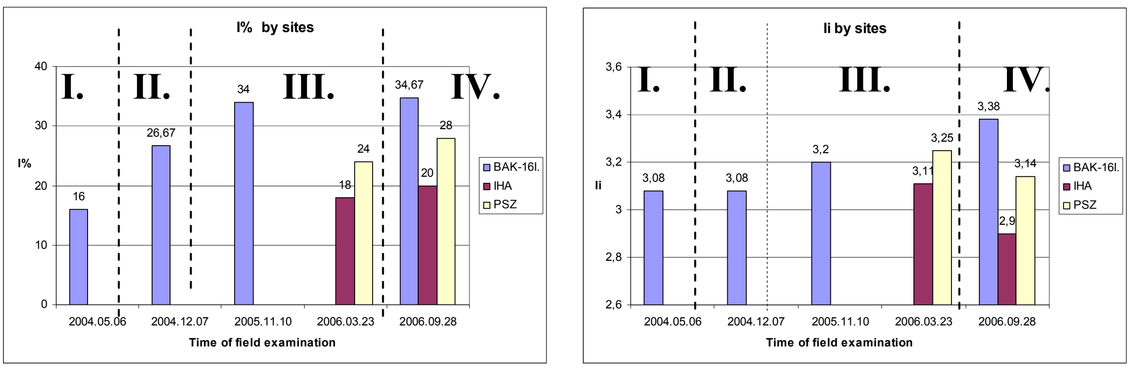
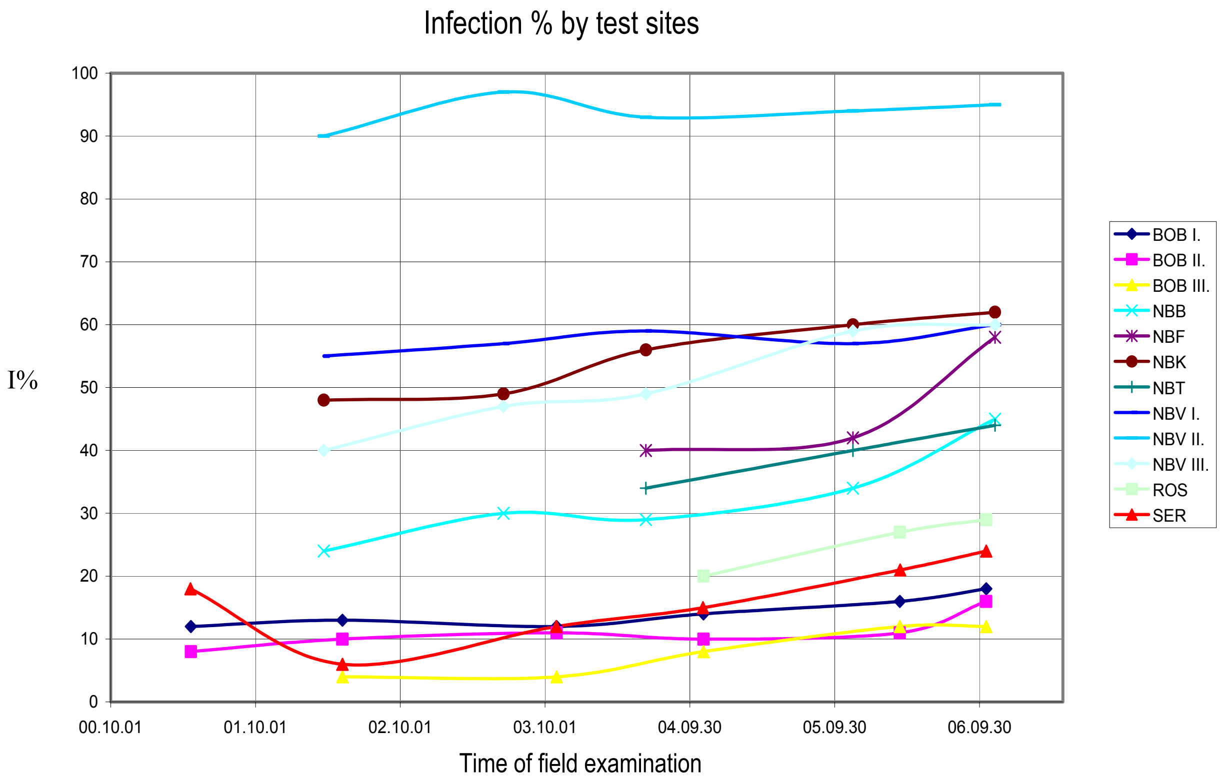


Figure 2. I% and Ii dates from sessile oaks on the South-Transdanubien growing areas between 2004-2006 (BAK = Bakonya, IHA = Iharos, PSZ = Pogányszentpéter).



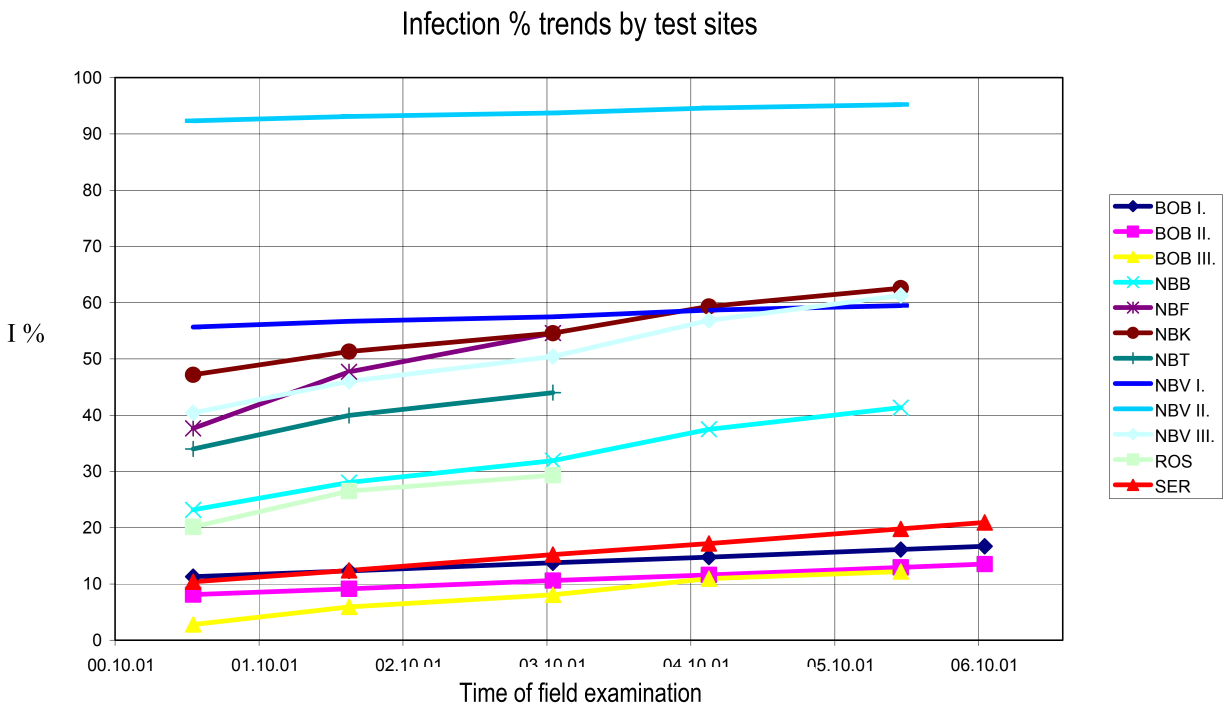


Figure 3. I % datas and infection trends from the Romanian and Ukrainian chestnut growing areas (BOB=Bobovisce, NBB=Baia Mare-Borpatak, NBF = Baia Mare-Felsőtótfalu, NBK=Baia Mare-Kőbánya, NBT= Baia Mare-Tauti Magherau, NBV=Baia Mare-Veresvíz, ROS=Rostovjatitsja, SER=Seredne).

Results of the pathogenical examinations

Pathogenical examinations were done on branch pieces of different oak species. Our main goal was to research the susceptibility of oak species to *Cryphonectria parasitica*. According to the results of this study, red oak (*Quercus rubra*) was the most susceptible to the fungus. Necroses on the branches of red oak (Figure 4) caused by *C. parasitica* were significantly bigger in size than those were on the chestnut branches. *Q. rubra* and *C. sativa* were more susceptible than other species, and *Q. petraea* and *Q. robur* also showed a great susceptibility (Figure 5, 6).

The diagrams and tables of Figure 7 present the results of my comparatory investigations analysed with the Mann-Whittney U-test and the Kruskall-Wallis test.

Figure 4. Necrosis on a branch-piece of red oak (photo:Tarcali, G.)

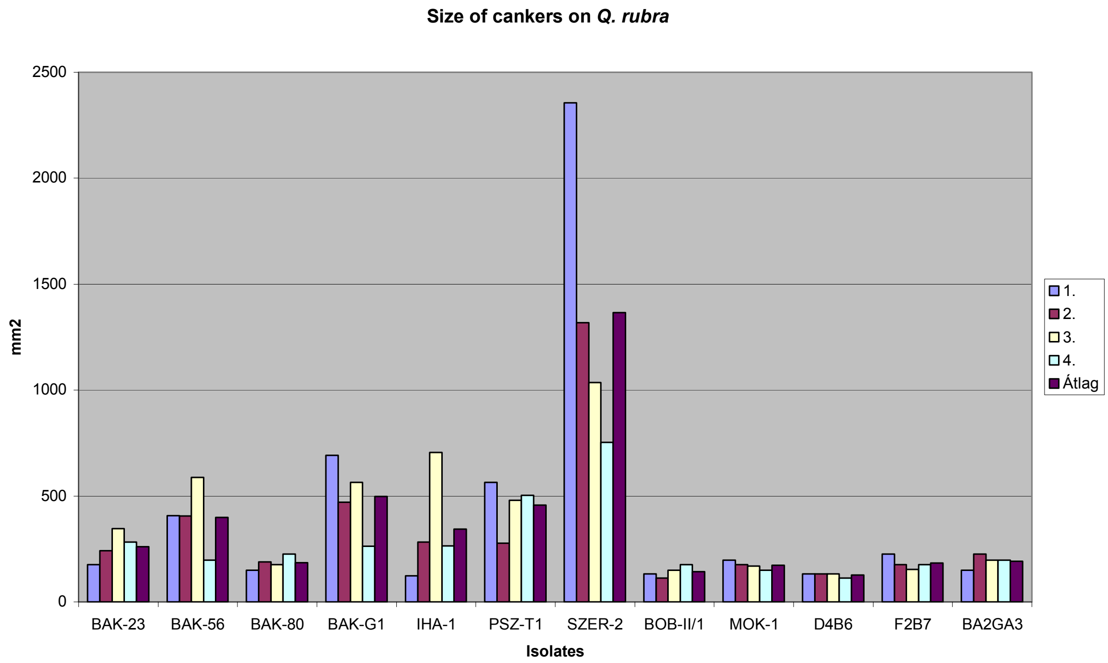


Figure 5. Measurement of necroses on *Q. rubra.*

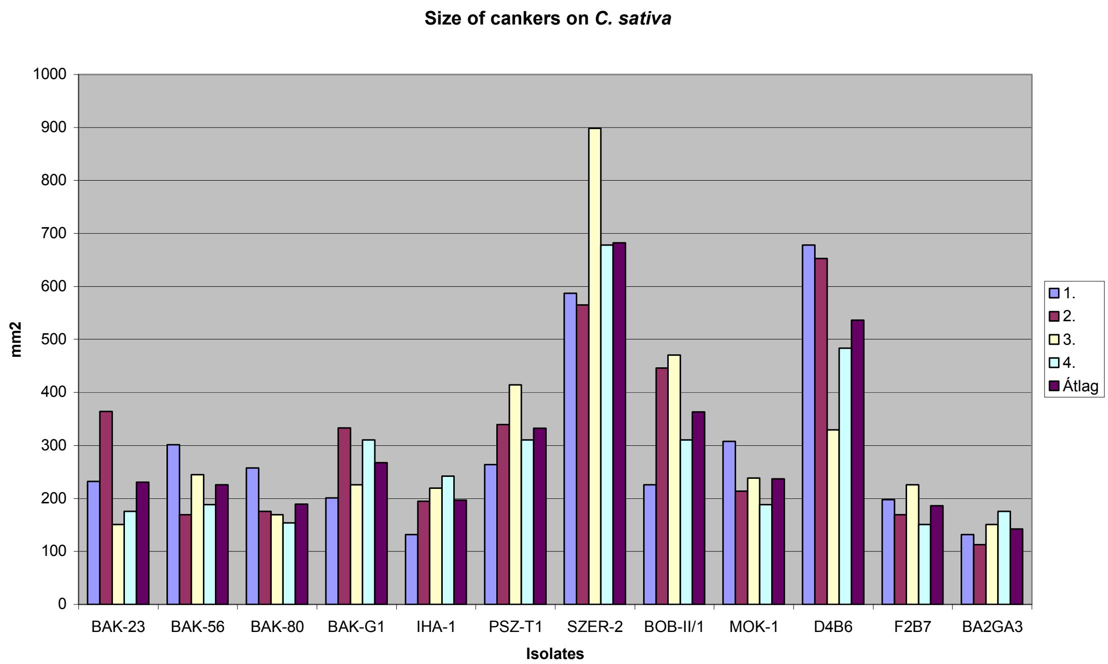
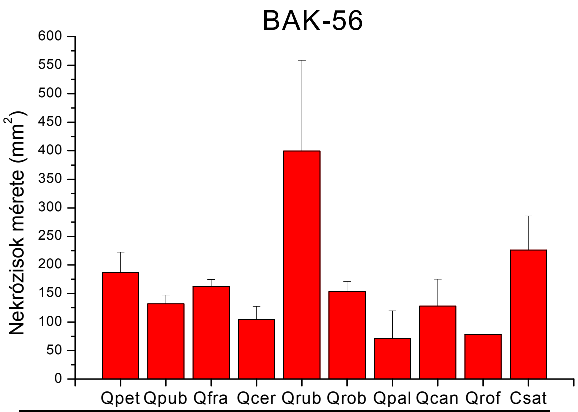
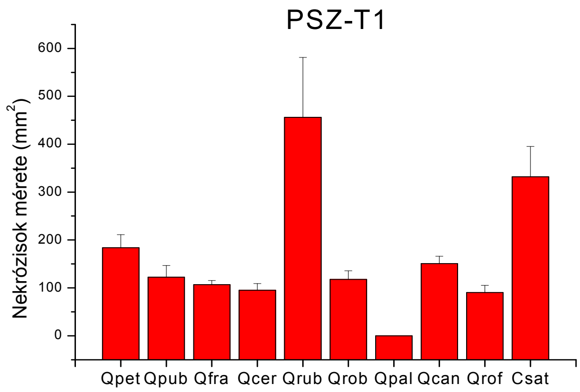


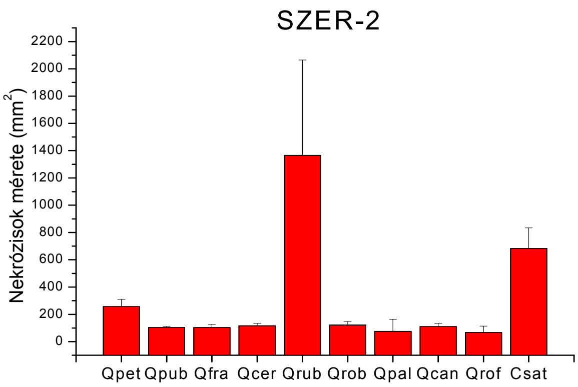
Figure 6. Measurement of necroses on *C. sativa*.



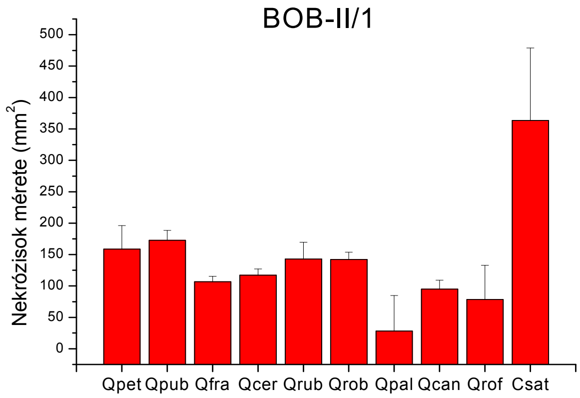
BAK 56	Qpet	Qpub	Qfra	Qcer	Qrub	Qrob	Qpal	Qcan	Qrof	Csat
Qpet										
Qpub	*									
Qfra	*	*								
Qcer	*		*							
Qrub		*	*	*						
Qrob	*			*	*					
Qpal	*	*	*		*	*				
Qcan	*				*					
Qrof	*	*	*	*	*	*		*		
Csat		*		*	*	*	*		*	



PSZ T1	Qpet	Qpub	Qfra	Qcer	Qrub	Qrob	Qpal	Qcan	Qrof	Csat
Qpet										
Qpub	*									
Qfra	*									
Qcer	*									
Qrub	*	*	*	*						
Qrob	*				*					
Qpal	*	*	*	*	*	*				
Qcan	*		*	*	*	*	*			
Qrof	*				*		*	*		
Csat	*	*	*	*	*	*	*	*	*	



SZER 2	Qpet	Qpub	Qfra	Qcer	Qrub	Qrob	Qpal	Qcan	Qrof	Csat
Qpet										
Qpub	*									
Qfra	*									
Qcer	*									
Qrub	*	*	*	*						
Qrob	*				*					
Qpal	*				*					
Qcan	*				*					
Qrof	*			*	*					
Csat	*	*	*	*	*	*	*	*	*	



BOB II/1	Qpet	Qpub	Qfra	Qcer	Qrub	Qrob	Qpal	Qcan	Qrof	Csat
Qpet										
Qpub										
Qfra	*	*								
Qcer		*								
Qrub			*							
Qrob			*	*						
Qpal	*	*			*	*				
Qcan	*	*			*	*				
Qrof	*	*			*	*				
Csat	*	*	*	*	*	*	*	*	*	

Figure 7 The infectedness of different species (based on the size of necroses) and the results of paired comparison with various applicated isolates. Mann-Whittney U-test. A * means the significant differences (p<0,05), n=4.

Examinations by using hypovirulent strains

Hipovirulent treatments were done at Pécsbányatelep, at Nagymaros and at Nagykanizsa. Test sites were selected on 2002 in all places containing 25-25 trees for the treatments and containing other 25-25 trees as controls (without treatments). The trees of the test sites were checked between 2003-2005. Average development of the necroses caused by *C. parasitica* stopped almost totally as the result of hypovirulent strains application. Growing of the cankers continued intensively on the control trees.

A special treatment was done between 2001-2003 at Pécsvárad-Zengővárkony, where 1000 chestnut trees were treated with hipovirulent strains, as the curative treatment, and measure the natural spreadig capacity of hypovirulent strains. Treatments were checked one year later, when the efficiency was 87 %.

According to the results of the examinations the rate of hypovirulent/virulent strains has increased. It was 1,38 in the first year of the application, but it became 1,68 by the second year.

Comparative analysis among data of different chestnut growing areas of the Carpathian-basin.

According to previous investigations performed in Hungary, 18 VCG-s were identified up to this time (RADÓCZ, 2004). The most common strains are in Hungary: EU-12, EU-13, EU-16. In Slovakia were detected 8 VCG-s (JUHÁSOVÁ et al., 1999). The more typical strains in Slovakia EU-12 and EU 13, like in Hungary. In Romania exist only one VCG (EU-12). The parasite got into Ukraine in the recent years, but there are identified at least two VCG-s (EU-12 and EU-13). Result of investigations showed that in Central European relations EU-12 and EU-13 are the most frequent VCG-s.

It is evident now, that *Cryphonectria parasitica* also seriously threatens oaks. On the basis of our results 4 pathogen strains (EU-3, -9, -11, -16) infected oaks in Hungary. In Romania one strain (EU-12) was detected on oaks, than it was on chestnut at that places. In Slovakia the fungus infected oaks in two growing sites according to the Slovakian experts, but we managed to detect only one place the infection (at Duchonka - EU-12 VCG). Threatened oak trees by *C. parasitica* have not been found yet in the Ukrainian sites. VCG-s identified in our examinations from different growing areas of the Carpathian-Basin are shown in Table 1.

Table 1 Vegetative compatibility groups identified on growing areas of the Carpathian-Basin (tested with EU-1–31. strains)

Test sites		EU – TESTER STRAINS / EU-1 — 31 /																														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
CHESTNUT	NBB	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NBV-I	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NBV-II	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NBV-III	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NBK	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NBF	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NBT	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	SER	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BOB-I	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BOB-II	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BOB-III	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ROS	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MK	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BAK-16I/ G-1	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PSZ/G-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PM	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OAK	NBV-II	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NBF	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NBT	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BAK-16I/ T-23	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BAK-16I/ T-80	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	IHA	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PSZ/T-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D4B	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

5. NEW RESULTS AND THEIR PRACTICAL USE

1. Field examinations were done on European chestnut (*Castanea sativa*) and on oaks (*Quercus* spp.) in North-Transylvania, in Sub-Carpathia, in Slovakia and in some Transdanubien growing sites. *Cryphonectria parasitica* infects chestnut populations in these regions. But in Ukraine we reported first (in 2001) this fungus on chestnut. We also detected the pathogen on oaks from Romania (in 2004) and it was also the first report. Our investigations were the first in chestnut populations at Baia Mare and on Sub-Carpathia to measure the infection rates of *C. parasitica*.

C. parasitica infection were detected in several Hungarian and Slovakian sites on sessile oaks. The fungus spred on all growing areas of the Carpathian-basin on chestnut up to this time, and it was detected from more and more sites on oaks too, except the Ukrainian territories.

2. Bark samples were collected from the infected chestnut and sessile oak trees for laboratory examinations. These investigations of *Cryphonectria parasitica* infection were the first in the Romanian and in the Ukrainian chestnut growing sites. According to the results of the laboratory examinations one VCG of the pathogen exists only on chestnut on the examined Romanian areas. It was vegetatively compatible with the EU-12 strain, and this strain was identified from Romanian oak bark samples too. Two VCG-s (EU-12, EU-13) of the fungus were detected from the Ukrainian chestnut bark samples. On the basis of the results it is possible to say that the Romanian and Ukrainian chestnut growing areas became infected by *C. parasitica* from Slovakia terriories because of the detected two VCG-s belong to the most common Slovakian VCG-s. It seams that the limited number VCG-s in Ukraine and in Romania are the key factors for succesful field applications of hypovirulent strains.

Most of detected fungal strains in the examined Hungarian sessile oak populatios were the same which were also identified on chestnuts of that sites. In the Slovakian examinations the results and conlusions were similar.

3. Pathogenical examination was done on 9 oak species, using chestnut as a control. The experiment made it clear that most oak species are susceptible to the pathogen (to various extend). On the basis of the results it can be noted that red oak

(*Quercus rubra*) is extremelly susceptible, but the sessile oak (*Quercus petraea*) and the pedunculate oak (*Quercus robur*) are also showed a certain susceptibility.

4. Virulent strains of the pathogen were detected only on the examined Ukrainian and Romanian sites up to this time. It is possible that hypovirulent strains naturally develop, but on the basis of the experiences from Western-Europe this process may require even decades. Quick adapting of hypovirulent strains which were detected in the other regions of the Carpathian-basin can be a solution to control the fungus on that territories. According to the results of our laboratory examinations there is a Hungarian hypovirulent strain (PJ-2) which was able to convert fungal isolates from Romanian chestnuts. Moreover there were several Hungarian hypovirulent strains (PJ-2, L13xW31, FS8xW31, FS8x88, FS1xGA13, FS4x146, B1xBF, A3xB7) which were able to convert isolates from Romanian sessile oaks.

5. We set up an experiment site in order to examine the measure the natural spread of hypovirulent strains. We came to the conclusion that by the end of the first year after the treatment, the hypovirulent/virulent rate was set to 1.38. This indicator has changed to 1.68 by the 2 year examination. This result obviously indicates the measure of the natural spred of hypovirulent strains.

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