Chestnut Blight and its Biological Control in the Sopron Hills, Hungary

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Abstract – The distribution of chestnut blight caused by *Cryphonectria parasitica* was surveyed in the forests of Sopron Hills. The infection rate was different in the forest subcompartments ranging up to 35%. Six vegetative compatibility types were detected, two of them were wide spread and present all of the hills. Converted hypovirulent strains were tested for practical control of the disease. More than 90% of the treated trees healed in eight years after the inoculation in the experimental plot.

***Cryphonectria parasitica* / vc type / biological control**

Kivonat – A szelídgesztenye kéregrákja és az ellene való biológiai védekezés a Soproni-hegységben. Vizsgálatunk tárgya a *Cryphonectria parasitica* által előidézett szelídgesztenye-kéregrák a Soproni-hegység területén. Megállapítottuk, hogy a fertőzöttség mértéke változó, maximálisan 35%. A gyűjtött kéregmintákból a kórokozó hat különböző vegetatív kompatibilitási típusát különítettünk el. Közülük kettő széleskörűen elterjedt, jelen van a hegység egész területén. A betegség leküzdésére konvertált hipovirulens törzseket alkalmaztunk. A kísérleti területen a kezelt fák több mint 90%-a nyolc évvel az első kezeléseket követően meggyógyult.

***Cryphonectria parasitica* / vc típusok / biológiai védekezés**

1 INTRODUCTION

The causal agent of chestnut blight the ascomycete *Cryphonectria parasitica* was introduced into Europe from Asia, mediated in America. A few decades ago the disease reached Sopron Hills and caused epidemic infections and destruction of chestnut trees in plantations and semi-natural forest associations (Körtvély 1970). The control of the disease and the stop of the epidemic are possible only by one effective biological method based on dissemination of the hypovirulent strains of the pathogen. The cytoplasm of the hypovirulent strains contains double-stranded RNA (later named *Cryphonectria hypovirus 1*, CHV-1) that is related to the reduction of the virulence of these strains. The dsRNA can be transmitted to a virulent strain in laboratory and natural conditions, too. After this transmission the recipient virulent strain will become hypovirulent. The tree can overcome the attack of hypovirulent strains, the

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cambium and phloem remain living under the hypovirulent cankers. The transmission of CHV-1 is possible only between the vegetative compatible strains. By this reason the VC type diversity of the fungus populations is a cardinal condition of the success of the biocontrol. The biological control based on the hypovirulence was tried out successfully in practice in many European countries (Grente and Berthelay-Sauret 1978, Turchetti and Maresi 1991, Juhasova – Bernadovicova 2001, Robin – Heiniger 2001, Heiniger – Rigling 1994).

2 MATERIALS AND METHODS

2.1 Distribution of the disease

In autumn 1996 investigations were carried out on 4070 hectares of Sopron Hills (Sopron, Ágfalva and Harka localities). The determination of infection was performed by linear sampling in each forest subcompartment. Bark samples for further laboratory investigations were collected from each subcompartment where the disease occurred.

2.2 VC type diversity of the pathogen

The pathogen was isolated from the bark samples and a strain collection was established for later vegetative compatibility tests. A number of 51 isolates were analyzed by vegetative compatibility test using the pairing method developed by Anagnostakis (1977) (*Figure 1*). The delimited VC types were than identified by pairing with the EU tester strains 1-31 (Cortesi et al. 1998).

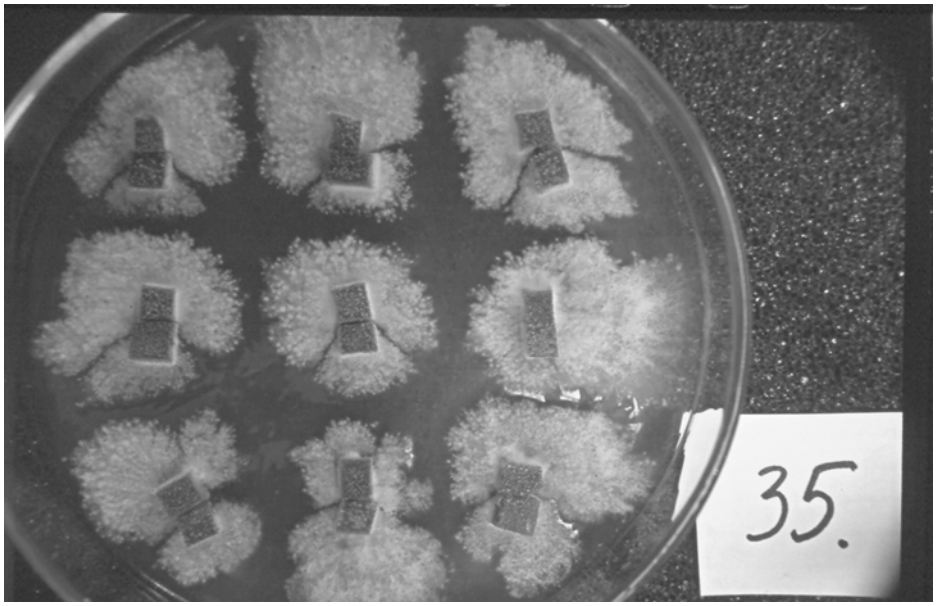

Figure 1. Compatibility test of the C. parasitica isolates

2.3 Hypovirulence, conversion of the local virulent isolates

The white color of the cultures indicates the presence of dsRNA. No native hypovirulent strains occurred among the isolates from Sopron Hills in 1996, so the identified vegetative compatibility types were converted in vitro with hypovirulent strains originated from other regions of West Hungary, where the natural hypovirulence was revealed a few years ago (Radócz et al. 1997). The character of the virulent strain turned into hypovirulent type after a short initial growth in case of positive conversion (*Figure 2*).

2.4 Prove of dsRNA content and genetic identity of the converted isolates

Molecular investigations were carried out in order to prove the presence of dsRNA in the converted strains and in some local isolates that seemed to be hypovirulent because of their intermediate morphological characteristics. The investigated strains were: S11, S18, S18xSa3, S21, S21xR5, S21xIhb2, An as hypovirulent control strain and S23 as virulent control. Isolation and analysis of dsRNA was performed by phenolic extraction method. Samples of extractions were separated by electrophoresis using 0,8% agarose gel in TBE buffer at 110 V, dsRNS molecules were stained with ethidium bromide (Wronski et al. 1997).

RAPD technique was used to determine the genotypes of the converted strains by using a version of Wronski et al. (1997). The DNA-containing supernatant was originated from the dsRNA extraction protocol. Polimerase chain reaction (PCR) was performed by six ten-base oligonucleotide primers. After PCR the DNA fragments were separated by electrophoresis using 1% agarose gel in TBE buffer at 110 V, after staining with ethidium bromide, and examined under UV light. Two virulent isolates, and three converted hypovirulent isolates were investigated. The molecular works were carried out in the laboratory of Österreichishes Forschungscentrum Seibersdorf.

2.5 Application of the biological control

Field trials with converted hypovirulent strains were carried out from autumn 1996 to 2000. In the experimental plot Ágfalva 37 diseased trees were treated. In addition, individual chestnut trees were inoculated in several gardens and orchards in Sopron. In the treated trees every canker was artificially inoculated roundly with mycelia of adequate (compatible) hypovirulent strain (*Figure 2*).

Figure 2. Inoculation holes in the bark

At the first step cork borer wounds were carried out in the living bark, in distances 4-5 cm from each other. These holes were filled with the hypovirulent inoculum (pieces of medium with mycelia), and finally the holes were closed with wax not containing fungicides. Parallel the inoculations the dried branches were removed. The inoculated trees were evaluated every year. In a few cases when the healing was uncertain the treatment was repeated using hypovirulent strains of two VC types. This was necessary because of occurrence of two VC types in the site, one dominant (SI) and one less frequent (SII). Strongly diseased, severe cankered trees were treated differently: not only cankers were treated roundly, but the stem also was inoculated vertically from the base to the branches up to the crown. Infected bark of heavy sporulated cankers was removed, in order to decrease the sources of inoculum.

3 RESULTS

3.1 Distribution of the disease

During the area surveying we recognized that in forests near the city chestnut blight is common, infection rate is more than 30%, and the blight occurrence in the farer areas is only sporadic. This result indicates that the pathogen was introduced first into the gardens and orchards inside the town and suburbs from where it was spread to the neighboring forest areas.

3.2 VC type diversity

Six vegetative compatibility types of the pathogen were delimited in the Sopron Hills. Three vegetative compatibility types are dominant (SI, SII, SIII), the other types (SIV, SV, SVII) are represented with only a few isolates. Near to the hills a seventh (SVI) type was detected. Six types were compared with EU tester strains 1-31.

The SI type was compatible with EU 13, this type is dominant in most of the studied areas, and 51.2% of the isolates belong to this type. SII representing 14.3% of the isolates was compatible with EU 6, 9 and 23. It is dominant in some forest areas closed to the chestnut cultures. SIII occurs locally in the West part of the Hills but there it causes massive infection of more than 30%. It is compatible with EU 1 and 17. The locally occurring SIV is compatible with EU 12, SV and SVII represented by a few isolates are compatible with a few EU types (*Table 1*).

Table 1. VC types of C. parasitica in Sopron Hills

VC Type	Compatible EU testers	Conversion dsRNA donor
SI	13	A3 x BF
SII	6, 9, 23	IHB2, R5
SIII	1, 17	S3
SIV	12	-
SV	1, 16, 22	-
SVII	1, 25, 28	-

3.3 Conversion of the local virulent strains

The isolates from Sopron Hills showed virulent character in culture, orange pigmentation and abundantly production of pycnidia. Local virulent strains were converted in vitro by using compatible hypovirulent strains as dsRNA donors available in our strain collection, originated from other regions of Hungary namely Zala and Somogy Counties (*Table 1*). The initial growth of virulent strain is changed into hypovirulent by the successful conversion (*Figure 3*).

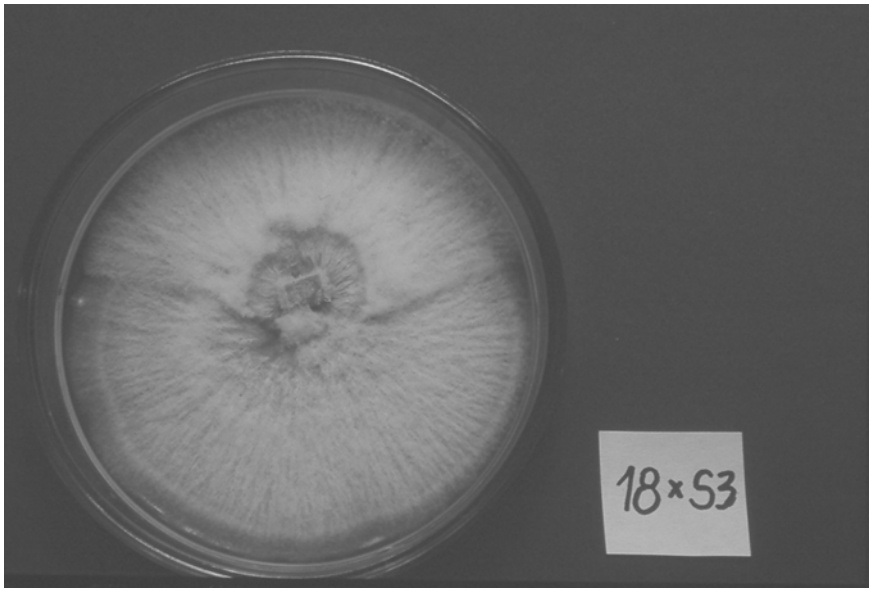

Figure 3. Successful conversion in vitro

3.4. Content of dsRNA and genetic identity of the converted strains

The conversions were verified by methods of molecular biology. The presence of dsRNA (molecular weight 12.7 kb) was detected in all of the converted strains. The dsRNA was not found in the local isolates, so the lack of natural hypovirulence was proved in the hills in 1996. The converted strains should be genetically identical with the original isolates, this fact is important for avoid the introduction of strange genotypes into the experimental plot. Genetic identity between the converted strains and original virulent strains was also demonstrated by RAPD technique with all the three converted strains.

3.5 Biological control

Efficiency of hypovirulent control against blight in field trials was investigated from 1996 to 2000. In 8 years 90% of treated trees recovered. The margins of the virulent lesions were healed and growth of the canker was stopped in the bark. (*Figure 4*). More than 90% of the treated trees healed in eight years after the inoculation in the experimental plot (*Figure 5*). The combined inoculations with two hypovirulent strains also were efficient. The strongly diseased, severe cankered trees treated by vertically inoculation in the stem also healed partially because of a protection zone created in the bark, which is not available for the virulent strains, so the water and nutrition transport is ensured and the trees survive the attack.

Figure 4. Healed cankers

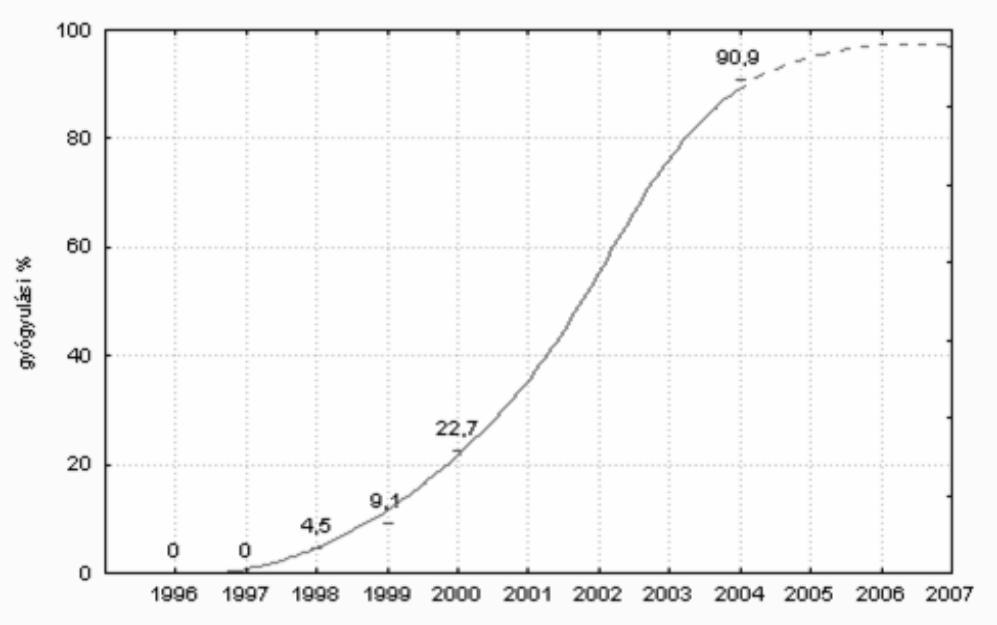

Figure 5. Healing rate of the treated trees in time

4. DISCUSSION

The aim of this study was to characterize the population of *C. parasitica* and to adapt the biological control onto the Sopron Hills. According to our results and the data regarding the distribution of VC types in Europe and Hungary (Robin – Heiniger 2001, Radócz 2001) we deducted to the spread of the epidemic. The first wave of the blight was entered from the Kőszeg Hills to the Sopron Hills. Later the second wave of epidemic has reached the hills from farer South-Western Transdanubia possible by anthropogenic effect. Then a vegetative compatibility type dominant in Austria occurred in the forests close to the border, this type has local area, but great infection capacity. The sexual propagation of the pathogen has begun.

During the treatments it was found that year for year new vegetative compatibility types appear, so the biological control needs continuous survey on population structure of the pathogen. The natural spread of hypovirulence started after the treatments by a meanly rate of approx. 2 m a year. In the knowledge of population structure of the fungus the first experiments in Ágfalva were extended and a practical biocontrol programme started in the chestnut stands in the surroundings of Sopron. Since *C. parasitica* causes necrotic cankers and destructions also on sessile oak, and in Sopron Hills the blight is common in oak forests mixed with chestnut, more investigations are needed on spreading of the pathogen and the hypovirulence on oak species.

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