# The Role of Phytophthora Species in the Decline of Black Walnut Stands

Judit KOVÁCS[a*] – Ferenc LAKATOS[a] – Ilona SZABÓ[a]

[a]Institute of Silviculture and Forest Protection, University of West-Hungary, Sopron, Hungary

**Abstract** – The paper reports on the occurrence and impact of *Phytophthora* species in two declining black walnut stands in West-Hungary. The health condition of both stands was investigated and soil samples were taken from the rhizosphere of the trees in order to isolate *Phytophthora* species. The species identity of the isolates was determined by morphological and molecular methods. *Phytophthora cactorum*, *Phytophthora plurivora* and *Phytophthora polonica* were found as responsible for the decline of the stands.

**Keywords**: black walnut / decline / *Phytophthora plurivora* / *Phytophthora cactorum* / *Phytophthora polonica*

## 1. INTRODUCTION

Some *Phytophthora* species are known as pathogens of walnut (*Juglans* species), affecting seedlings and mature trees, too: *Phytophthora cactorum* (LEBERT & COHN) SCHRÖT., *Phytophthora cinnamomi* (KRÖBER & MARWITZ), *Phytophthora citrophthora* (R. E. SMITH & E. H. SMITH) LEONIAN, *Phytophthora cryptogea* (PETHYBRIDGE & LAFFERTY), *Phytophthora megasperma* (DRECHSLER), *Phytophthora parasitica* (DASTUR) and *Phytophthora plurivora* JUNG & BURGESS) (ERWIN & RIBEIRO 1996).

In the early 2000s, decline of the black walnut trees (*Juglans nigra*) was observed in South-Danubian floodplain forests in Gemenc. The canopy of over 80 years old trees showed drying symptoms in spite of adequate soil humidity and lack of any other visible reason of the decline. *Phytophthora* species were isolated from the soil. *Phytophthora cactorum* and *Phytophthora plurivora* were the most frequent occurring species. The pathogenicity of these species was confirmed by inoculation of black walnut seedlings. It was concluded that the decline of the stands was caused by these *Phytophthora* species (SZABÓ & LAKATOS 2008). Since that time similar symptoms were observed in old black walnut stands in some other regions of Hungary, too.

## 2. MATERIALS AND METHODS

In June 2011 the health condition of two West-Hungarian black walnut stands was investigated. The 1st stand is situated near Kapuvár, the 2nd one near Sárvár (Table 1.).
The trees showed declining symptoms: sparse crown, drying branches, and small, yellowish discoloured leaves. No bleeding lesions occurred on the trunks as specific symptom of *Phytophthora* infection.

*Table 1. Data of the investigated black walnut stands*

| | **I. Stand** | **II. Stand** |
|---|---|---|
| **Location** | Kapuvár 10 A | Sárvár 5 L |
| **Age** | 73 years | 58 and 78 years |
| **Number of investigated trees** | 20 | 10 |
| **Date** | 19. June 2011. | 3. June 2011. |

The health condition of the trees was evaluated based on the crown symptoms using the following 4-pointed scale:

1. Healthy crown
2. Less than 20% of the crown is dying. Some leaves are yellowish.
3. 20-50% of the crown is dying. Leaves with yellowish discolouration in larger groups.
4. More than 50% of the crown is dead. Yellow leaves in large groups, or the remaining foliage are yellow.

Soil samples were taken from the rhizosphere of each investigated tree with a final volume of 1 l.

The isolation of the Phytophthoras was performed by using healthy, young *Rhododendron* and *Prunus laurocerasus* leaves as baits. The leaves were disinfected with 10% NaOCl solution. After a few days spots appeared on the leaves. *Phytophthora* species were isolated from these spots on selective agar media (Figure 1).

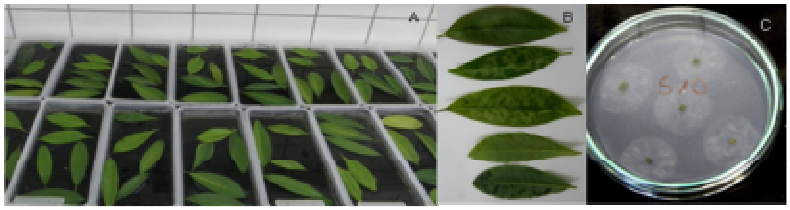


*Figure 1. The isolation: A. Leaf baiting method. B. Spots on the baits. C. Phytophthora colonies on selective agar media*

The morphological features were studied on cultures grown on carrot agar, at 20°C, in the dark. The formation of sporangia was induced by non-sterile soil solution. Daily growth rate, colony patterns and morphology of microscopic structures were investigated.

The molecular identification of the isolates was made by sequencing the ITS1-5.8S-ITS2 region of the rDNA. The extraction of DNA and the PCR was made with the REDExtract N-Amp Plant Kit (Sigma-Aldrich), according to the user's guide of the manufacturer. The PCR was made with ITS6 and ITS4 primer pair, in an Eppendorf Mastercycler Personal PCR machine. The alignment of the sequences was made with the ClustalX program. Homologous were searched in the NCBI database using BLASTN program.

## 3. RESULTS

### 3.1. Health condition of the trees

The health condition of the trees is shown in Figure 2.

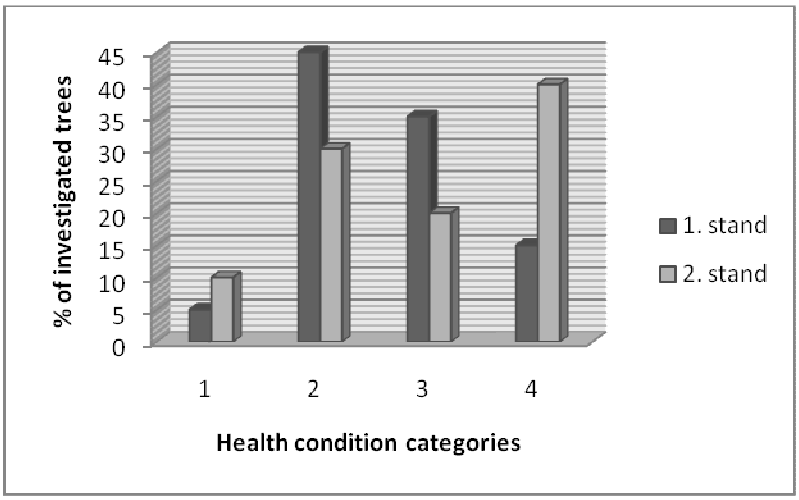


*Figure 2. The health condition of the stands. Categories are described in the text.*

In the 1st stand, 45% of the investigated trees belonged to the category '2' and 35% to the category '3'. Healthy and completely dead trees were rare. In the 2nd stand 40% of the investigated trees belonged to the category '4' (severe symptoms). The health condition of the 2nd stand was worse than that of the 1st stand.

### 3.2. The isolation

We collected 20 soil samples in the 1st stand. Isolation was successful from 15 samples. We got 28 *Phytophthora* isolates from this site. We got 18 isolates from 10 soil samples in the 2nd stand.

### 3.3. Species identification and species composition in the stands

The colony patterns of the isolates were variable. Rosette, petalloid, stellate, smooth and apressed colony patterns could be seen (Figure 3.).

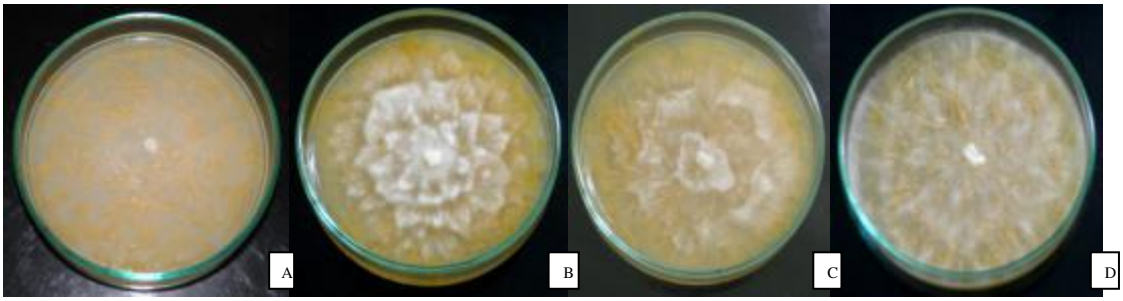


*Figure 3. Colony patterns: A: Apressed. B: Rosette. C: Petalloid. D: Stellate*

These colony patterns and the various microscopic features (Figure 4.) suggested that we have 2 or 3 different morphological types. However, the daily growth rates of the isolates were similar.

For molecular identification 13 isolates were chosen from the 1st stand and 10 from the second stand, originating from different trees and representing various morphological types. Three species were identified via molecular methods: *Phytophthora plurivora*, *Phytophthora cactorum* and *Phytophthora polonica*.

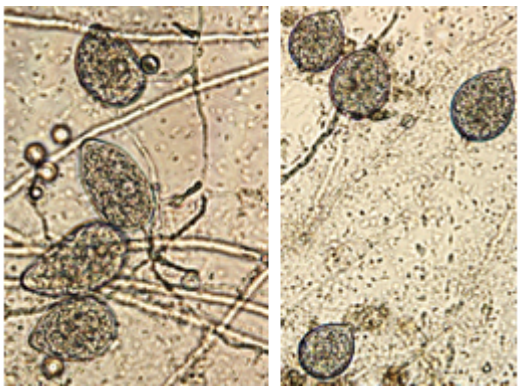

*Figure 4. Different sporangia types*

The species composition of the stands was different. *Phytophthora cactorum* was the dominant species in the 1st stand (9 isolates from 13 sequenced ones), while *Phytophthora plurivora* occurred preponderantly in the 2nd stand (8 isolate from 10). The only *Phytophthora polonica* isolate was found in the 2nd stand (Figure 5).

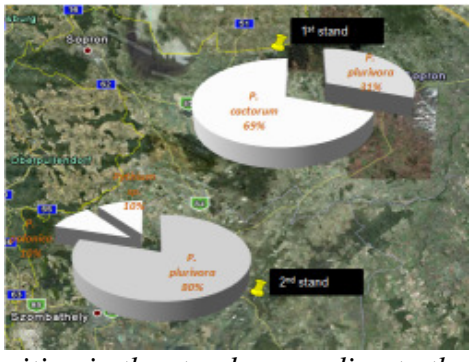


*Figure 5. Species composition in the stands according to the molecular identification*

## 4. SUMMARY

The presence of Phytophthora species was approved in the rhizosphere of the trees in both investigated stands. The most frequent species were *Phytophthora plurivora* and *Phytophthora cactorum.* Differences were revealed in the species composition of the sites. Our aims are to keep tabs on the changes in the health condition of the stands, and to confirm the pathogenicity of the isolates representing the identified *Phytophthora* species.

**Acknowledgements**: We thank TÁMOP 4.2.1.B-09/1/KONV-2010-0006 for supporting our research.

### References

ERWIN, Donald C. – RIBEIRO, Olaf. K. (1996): Phytophthora Diseases Worldwide. APS Press, St. Paul, Minnesota.

SZABÓ, Ilona – LAKATOS, Ferenc (2008): Phytophthora species isolated from declining forests in Hungary, Növényvédelem 44 (12): 607-613.