

Phytophthora species in the decline of Black Walnut stands

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The healthy state of two West-Hungarian Black Walnut stands was investigated in June 2011. Both stands showed wilting symptoms. *Phytophthora* species were isolated from soil samples. Three *Phytophthora* species were identified using morphological and molecular features: *Phytophthora plurivora*, *Phytophthora cactorum* and *Phytophthora polonica*. These species are supposed to be responsible for the symptoms and the decline of the trees.

Introduction

Among the pathogens of black walnut (*Juglans nigra* L.) there are some *Phytophthora* species, which can cause the decline of older black walnut stands even by adequate site and growth conditions. According to the previous studies of our Institute, the most common *Phytophthora* species in the soil of dying black walnut trees are *Phytophthora cactorum* (Lebert & Cohn) Schröt. 1886 and *Phytophthora plurivora* (Jung & Burgess 2009) in Hungary. The pathogenicity of these species was previously confirmed to young black walnut trees.

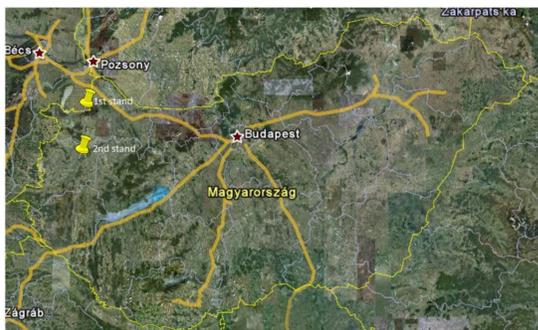


Figure 1.: The black walnut stands.

Materials and Methods

The health condition of two black walnut (*Juglans nigra* L.) forests was investigated in June 2011 (Figure 1.). Both stands are located in West-Hungary. The 1st stand is situated near Kapuvár, the 2nd stand near Sárvár, both North-West-Hungary.

Decline symptoms were observed as follows: the crown of dying trees was sparse with dead branches; the leaves were smaller as usual, often with yellowish discolouration (Figure 2).



Figure 2.: Symptoms in the 1st and in the 2nd stand.

The crown of trees with different health condition was investigated and evaluated with a four-point scale. In the 1st stand 20, in the 2nd 10 trees were assessed. Soil samples were taken from the rhizosphere of the trees, with a final volume of 1 litres. *Phytophthora* cultures were isolated on selective agar media by using the leaf baiting method with *Rhododendron* and *Prunus laurocerasus* leaves (Figure 3).

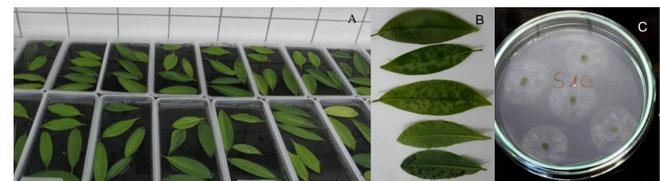


Figure 3.: The isolation method: A-B.: *Prunus laurocerasus* leaves as baits, C.: *Phytophthora* cultures on selective agar media.

Isolates were grown on carrot agar in dark, at 20 °C for studying the morphological features. The formation of sporangia was induced by flooding the cultures with non-sterile soil extract. The molecular identification of species was performed by sequencing a 810 bp fragment of the ITS1-5.8S-ITS2 region of the rDNA and searching homologues in the GenBank Database.

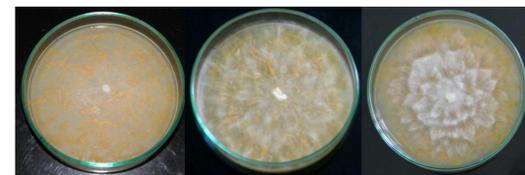


Figure 4.: Growth patterns: A; apressed.; B; stellate; C; rosette on CA.

Results

Differences between species:	<i>Phytophthora plurivora</i>	<i>Phytophthora cactorum</i>	<i>Phytophthora polonica</i>
<i>Phytophthora plurivora</i>	-	85 loci	97 loci
<i>Phytophthora cactorum</i>	85 loci	-	87 loci
<i>Phytophthora polonica</i>	97 loci	87 loci	-

Figure 5: Differences between isolates and species according to their sequences

Three different colony growth patterns were observed: stellate, rosette and apressed (Figure 4). The growth rate of the isolates was also slightly different. The microscopic features of the isolates showed clearly three different species, that was also proved by the molecular results. The cultures from the 2nd stand belong to 2 species: from 9 identified isolates, 8 isolates are *Phytophthora plurivora*, 1 isolate is *Phytophthora polonica* (Belbahri et al. 2006). From the 1st stand 13 isolates were identified: 9 isolates are *Phytophthora cactorum*, and 4 isolates are *Phytophthora plurivora* (Figure 6.). The identification of further isolates is in progress.

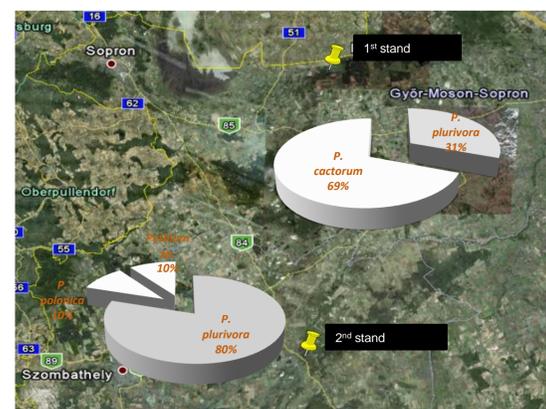


Figure 6.: The frequency of *Phytophthora* species in the stands.

Acknowledgement

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