**PHYTOPHTHORA GENUS PATHOGENS ISOLATED FROM RHODODENDRONS IN LITHUANIA**

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Abstract  
*Rhododendron* spp. plants were surveyed for *Phytophthora* infection in Lithuania during 2010 – 2016. This study aims to identify *Phytophthora* genus pathogen which infects rhododendrons in Lithuania. Samples were taken from young sick plants with visible infection symptoms. Soil sampling was performed from the rhizosphere of sick plants. DNA from soil and plant was tested for the presence of *Phytophthora* genus pathogens. Data showed positive results of *Phytophthora* genus specific probe during real-time PCR. All tested diseased leaves and soil samples have indicated *Phytophthora* sp. infection during Alert-LF® *Phytophthora* spp. analysis. The extracted DNA concentrations were not very high for *Phytophthora* species identification, but in most cases, it was high enough for further researches.

Key words: *Phytophthora* genus, *Rhododendron*, Lithuania.

Introduction  
*Rhododendrons* are popular between professional and hobbyist abreast because of the great variety (ca. 12000 cultivars) and exceptional decorativeness (Malciūtė & Naujalis, 2010). In Lithuania, rhododendrons were first introduced in the Vilnius Botanical Garden in 1814 (Skridaila, 1996). They were seldom cultivated in Lithuania until the second half of the 20th c. However, rhododendron plants have spread rapidly in Lithuania during the last thirty years (Navasaitis, 2004). Intolerance to the low and negative temperatures is one of the most important limiting factors for rhododendrons in Lithuania (Malciūtė, Naujalis, & Šiaulienė, 2011). However, there is a shortage of information on the phytosanitary state of rhododendrons in Lithuania. Several fungi were isolated from rhododendrons in Lithuania, e.g. *Erysiphe azaleae* (U. Braun) U. Braun & S. Takamatsu and *Exobasidium japonicum* Shirai causing rhododendron mildew and leaf blisters (Grigaliūnaitė & Pribušauskaitė, 2006; Lygis et al., 2010).


In Lithuania, the increasing number of rhododendrons infected by *Phytophthora* genus fungi has been reported (Table 1). *Phytophthora citricola* was first isolated in Lithuania from drying rhododendron branches and top of twigs in 2002. It was isolated in *Rhododendron*  

<table>
<thead>
<tr>
<th>Rhododendron species</th>
<th>Year, authors</th>
<th><em>Phytophthora</em> species</th>
<th>Location</th>
<th>Injuries</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. <em>catawbiense</em> ‘Grandiflorum’</td>
<td>2002; Jovaišienė, Lane</td>
<td><em>P. citricola</em></td>
<td>Marijampolė district</td>
<td>Branches and top of twigs</td>
</tr>
<tr>
<td>R. sp.</td>
<td>2004; Jovaišienė, Lane</td>
<td><em>P. cactorum</em></td>
<td>Kaunas, private collection</td>
<td>Leaf and twigs</td>
</tr>
<tr>
<td>R. sp.</td>
<td>2004; Jovaišienė, Lane</td>
<td><em>P. cactorum</em></td>
<td>Kaunas and Šiaulių Botanical Garden</td>
<td>Leaf and twigs</td>
</tr>
<tr>
<td>R. <em>catawbiense</em> ‘Grandiflorum’</td>
<td>2006; Jovaišienė, Lane</td>
<td><em>P. ramorum</em></td>
<td>Marijampolė district</td>
<td>Leaf and twigs</td>
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</tbody>
</table>
catawbiense ‘Grandiflorum’ in the Marijampolė district. *Phytophthora cactorum* was first isolated from the collection of rhododendrons in Kaunas and Šiauliai Botanical Gardens in 2004 (Jovaišienė & Lane, 2006). *P. ramorum* was first isolated on fifty shrubs of *Rhododendron catawbiense* ‘Grandiflorum’ imported from Poland in the market centre of ornamental plants in Marijampolė district (Jovaišienė & Lane, 2006). This species was described at first on oranges in Taiwan as a disease agent of brown rot in 1927 (Sawada, 1927). At present, *P. citricola* is spread in Europe and it is a disease agent of collar roots and stem canker in many economically important crops. Therefore, it is considered as an aggressive pathogen. The investigation on *Phytophthora* genus fungi started in Lithuania State Plant Protection Service in 2002. Several years later, the investigation has started in the Kaunas Botanical Garden of Vytautas Magnus University (VMU).

This study was aimed to identify *Phytophthora* genus pathogen infecting rhododendrons in Lithuania.

**Materials and Methods**

During 2010 – 2016 at least 6 samples were taken from each place which had sick *Rhododendron*. Samples were taken from young sick plants with visible infection symptoms, e.g., top and leaf wilting, leaf blotch and longitudinal twisting, leaf browning along the main vessel, and twig necrosis. Soil sampling was performed from the rhizosphere of sick plants. The sampling place are Kaunas Botanical Garden nursery and Alytus Park.

The taken samples are stored in the zip bags. They could be stored under +4 °C for a longer period. Leaves are washed with a tap water one time, branches and pieces of the stem are washed two times. Dry parts of plants are soaked into the tap water for 24 hours, washed two times. Samples, cut from a necrosis border are cut into 5x5 mm pieces and put into at least four Petri dishes with growing medium under sterile environment (Jung, Blaschke, & Neumann, 1996).

The soil baiting is performed in a bath, which is poured with distilled water for 2/3 of its volume. On the water surface fresh oak or rhododendron leaves are placed. The bath is left for 3 – 5 days maintaining light/dark schedule and 18 °C temperature. The leaves are removed from the bath, washed out with tap water, divided into two parts and placed into the growing media. The isolation and identification of *Phytophthora* genus fungi is much more difficult in comparison to the other microscopic fungi (Werres et al., 2001). Therefore, various laboratory tests should be performed for the identification at the species level.

Malt extract agar (MEA) was used for the identification of *Phytophthora* species. MEA medium is produced with chloramphenicol. The prepared medium is autoclaved under 120 °C for 20 min. (Erwin & Ribeiro, 2005).

The incubation time was 1 – 3 days in darkness maintaining 24 °C. On the third day, *Phytophthora* hives are usually visible from the bottom side of the plate. Hives with medium pieces are transferred to water agar (WA) medium with capsicum or hemp seeds, which stimulate the formation of sporangia, which are formed within a few days (Jung, Blaschke, & Neumann, 1996).

The *Phytophthora* genus fungi identification at the species level is performed according to descriptors (Erwin & Ribeiro, 2005; Gallegly & Hong, 2008).

Soil and plants samples before DNA extraction were tested for the presence of *Phytophthora* sp. using Alert-LF® *Phytophthora* spp. ELISA devices (Neogen Corporation). The soil probes were taken around roots from sick *Rhododendron* plants.

The DNA from leaves was extracted using NucleoSpin® Plant II kit (Mec...
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The extracted DNA concentrations were not very high for *Phytophthora* species identification, but in most cases, it was high enough for further researches. *Phytophthora* genus pathogens spread is favoured by environmental conditions: soil flooding and excess moisture, droughts, and temperature extremes (Erwin & Ribeiro, 2005). Also, an intensive international trade of living plants accelerates the spreading of alien species over long distances (Jung et al., 2005).

### Conclusions

1. The common symptoms of *Phytophthora* infected rhododendrons – top and leaf wilting, leaf spots and twig necrosis.
2. All tested diseased leaves and soil samples have indicated *Phytophthora* sp. infection during Alert-LF® *Phytophthora* spp. analysis.
3. All tested samples can be considered as containing *Phytophthora* DNA. The extracted DNA concentrations were not very high for *Phytophthora* species identification, but in most cases, it was high enough for further researches.

### References


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<table>
<thead>
<tr>
<th>Rhododendron species</th>
<th>City, place</th>
<th>Part of the plant or soil</th>
<th>DNA [ng ml⁻¹]</th>
<th>Ratio of absorbance 260/280</th>
<th>Ratio of absorbance 260/230</th>
<th>Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. catawbiense</em></td>
<td>Kaunas Botanical Garden</td>
<td>leaves</td>
<td>2.75</td>
<td>2.42</td>
<td>0.92</td>
<td>17.70</td>
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<td>5.10</td>
<td>1.16</td>
<td>0.52</td>
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<tr>
<td><em>R. catawbiense</em></td>
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<td>5.78</td>
<td>2.90</td>
<td>1.41</td>
<td>25.62</td>
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</table>