Detection and Survival of *Phytophthora ramorum* in Rhododendron Root Balls and Survival in Rootless Substrates

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Problem

- In Europe Rhododendron is an important host for *P. ramorum*
- Symptom development on Rhododendron weeks/months after import

Consequences

► spread of *P. ramorum* via plant trade

► high financial losses for the nursery managers

example:

1 Rhodo. (30-40 cm) = ca. 20 € / 25 US$
10,000 plants = 200,000 € / 251,600 US$
Questions

1 - Can *P. ramorum* survive
   ♣ in rootless substrate?
   ♣ in root balls of latently infected Rhododendron?

2 - How can latent presence of *P. ramorum* be detected in the field?
Experiment 1 – **Survival in soil / substrate without plants**

**Methods**

Rhododendron leaves infected with *P. ramorum*

- Incubation outdoor for **2 - 33 months**
  (quarantine conditions)

- Sampling of the forest soil

- Incubation box

- Nylon mesh bags with 15 leaf discs each
Experiment 1 – *Survival in soil / substrate without plants*

**Methods**

Rhododendron leaves infected with *P. ramorum*

- **Methods**
  - undisturbed forest soils
  - Rhododendron substrate
  - incubation outdoor for 2 - 33 months (quarantine conditions)

- nylon mesh bags with 15 leaf discs each

- recoverability was tested on PARP medium
Experiment 1 – **Survival in soil / substrate without plants**

**Results**

- Recovery possible until the end of the study (33 months after burial)
- Seasonal effect: lowest reisolation rate in late autumn and in summer
- Low temperatures increase the recovery rate
Experiment 2 – **Survival in artific. inocul. Rhod. root balls**

**Methods**

- lift the plant from the pot
- place a (wounded) bait leaf on the bottom of the container
- place the plant back
- raise the water in the saucer to ca. 2cm
- collect the bait leaves after 3-5 days and check for *Phytophthora* infection
- continue incubation between moist towels if needed

Modification of the Rhododendron leaf test developed by the team of Kurt Heungens, Belgium
Experiment 2 – **Survival in artific. inocul. Rhod. root balls**

**Methods**

Examples for detection methods used with the baits

- **microbiological techniques**
- **serological techniques**
- **molecular techniques**

**On-site kits**

- Direct isolation
- PCR
Experiment 2 – Survival in artific. inocul. Rhod. root balls

Results

- No disease symptoms neither on the roots nor on the upper plant parts

- Recovery possible in root balls inoculated with sporangia, infected leaf pieces or zoospores (only second expt.)

- Recovery was possible from x% of the root balls:
  - ♣ after 4 months: 53 %
  - ♣ after 7 months: 33 %

- Positive correlation between inoculum rate and recovery rate
Chlamydomspores were present in 63.9 % of the root samples
Experiment 3 – **Survival in root balls of commercial Rhodo.**

**Methods**

- five Rhododendron cultivars from commercial nurseries
- marketable plants in 1.5L containers, bought in Oct 2008
- no disease symptoms during the last two official inspections

- **R. ‘Catawbiense Grandiflorum’**
- **R. ‘Catawbiense Boursault’**
- **R. ‘Cunningham’s White’** (mother plants from the greenhouse) = negative control
- **R. ‘insigne hybride ‘Brigitte’**
- **R. ‘yakushimanum hybride ‘Sneezy’**
Experiment 3 – **Survival in root balls of commercial Rhodo.**

**Methods**

- for the two years of the study the Rhododendron plants were cultivated in the glasshouse (JKI) at outdoor temperatures
- symptom development and sampling of the root balls monthly during 2 years (start: Oct 2008)

**Monthly sampling:**
- plants of each cultivar were divided into two clusters
- per cluster the root ball samples of each plant were mixed
- when a cluster was tested positive, it was divided into subclusters

**Final sampling:**
- with the new single pot sampling method
Experiment 3 – **Survival in root balls of commercial Rhodo.**

**Results** (two years after purchase)

- only a single *R. ‘Cunningham‘s White* showed **disease symptoms** in Jan 2009, isolation and PCR **positive for P.ramorum**
- all other plants of all cultivars remained **asymptomatic** during the two years of the study

![Root balls of commercial Rhodo.](image)
**Experiment 3 – Survival in root balls of commercial Rhodo.**

## Results

<table>
<thead>
<tr>
<th>Species</th>
<th>n =</th>
<th>&lt;2 yr</th>
<th>2 yr</th>
<th>Positive Single Plants</th>
<th>Positive Pools</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. 'Catawb. Boursault'</em></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1 P + 1 Pr</td>
<td></td>
</tr>
<tr>
<td><em>R. 'Catawb. Grandiflorum'</em></td>
<td>48</td>
<td>1</td>
<td>0</td>
<td>6 Pr</td>
<td></td>
</tr>
<tr>
<td><em>R. 'Cunningham's White'</em></td>
<td>53</td>
<td>6</td>
<td>1</td>
<td>1 Pr</td>
<td></td>
</tr>
<tr>
<td><em>R. insigne hybride 'Brigitte'</em></td>
<td>51</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>R. yakushimanum hybride 'Sneezy'</em></td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

P = *Phytophthora* spec., Pr = *P. ramorum*
Summary

*P. ramorum* can survive not only in forest soils but also in potting soil

- Root infection seems to cause no root symptoms in Rhododendron
- *P. ramorum* can survive in asymptomatic Rhododendron roots (*latent presence in root balls of marketable plants*)
- It is confirmed that *P. ramorum* produces chlamydospores in infected Rhodo. roots
Summary

Visual inspection is not effective to detect *P. ramorum* in the field in case there is latent root infection.

The modification of the Rhododendron bait test is a non-destructive and simple to handle method for detection of *P. ramorum* in potted plants on container stands.
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**Thank you very much!**
Thank you for your attention