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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\$



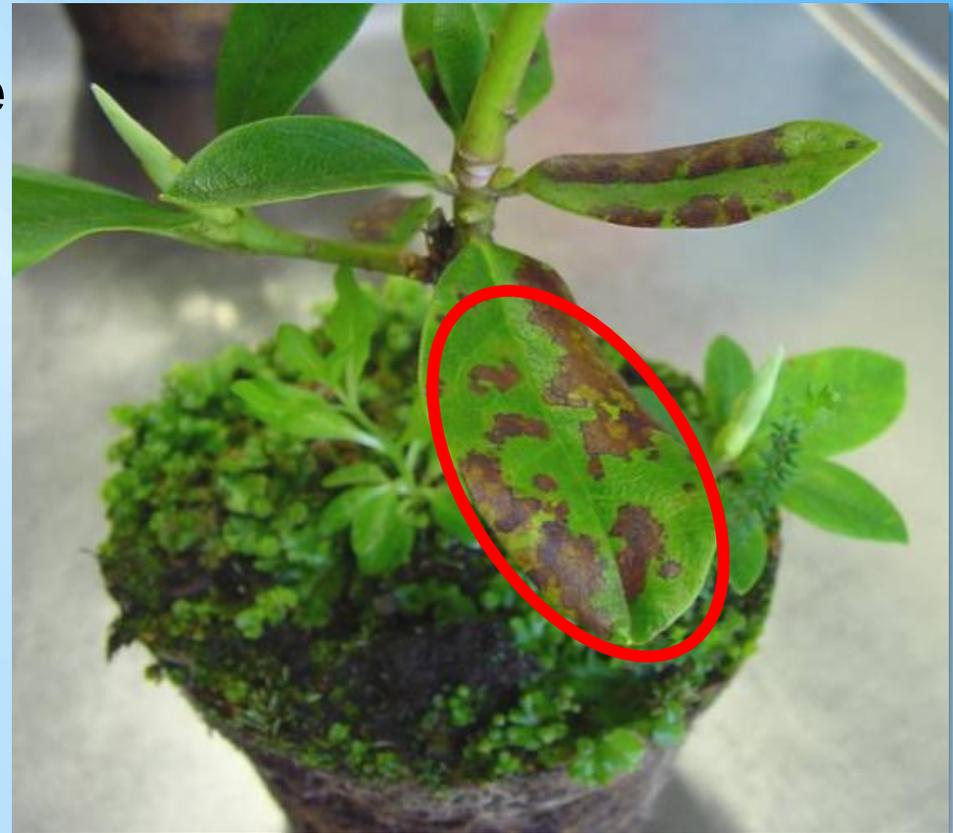
Questions

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?

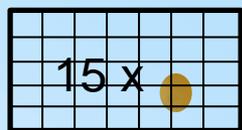


Experiment 1 – Survival in soil / substrate without plants

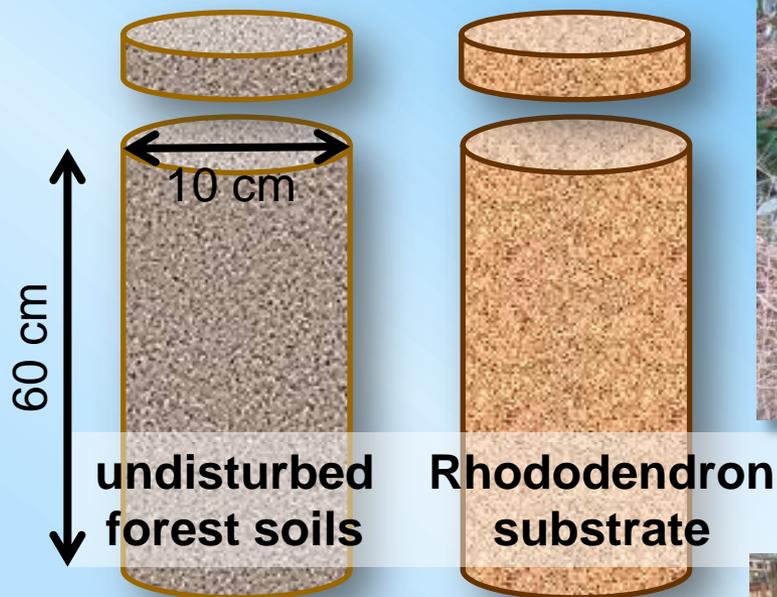
Methods



Rhododendron leaves infected with *P. ramorum*



nylon mesh bags with 15 leaf discs each



incubation outdoor for 2 - 33 months (quarantine conditions)

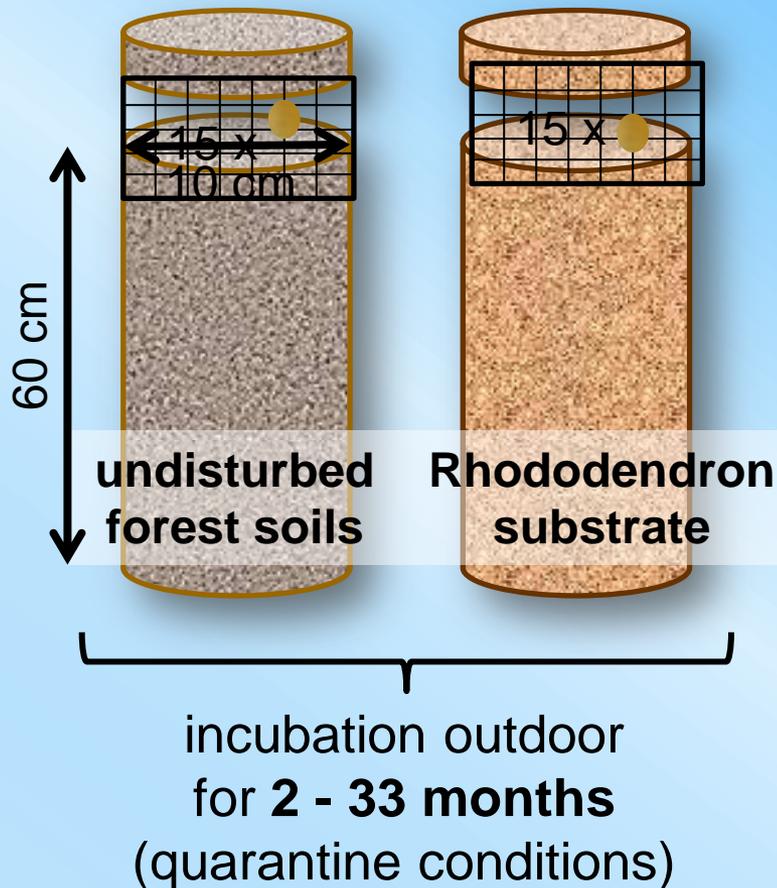


Experiment 1 – Survival in soil / substrate without plants

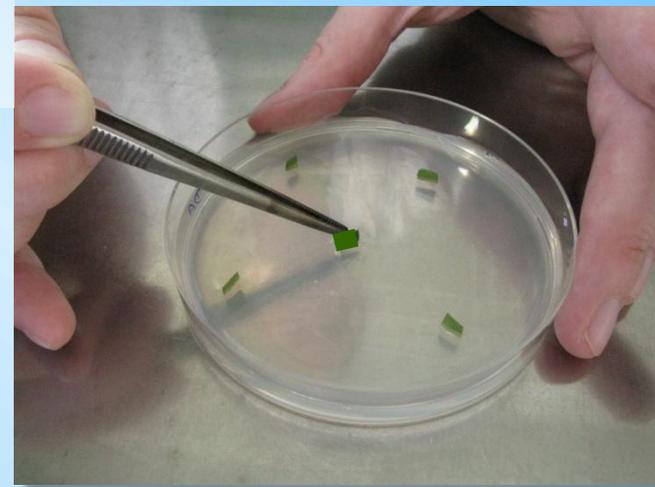
Methods



Rhododendron leaves infected with *P. ramorum*



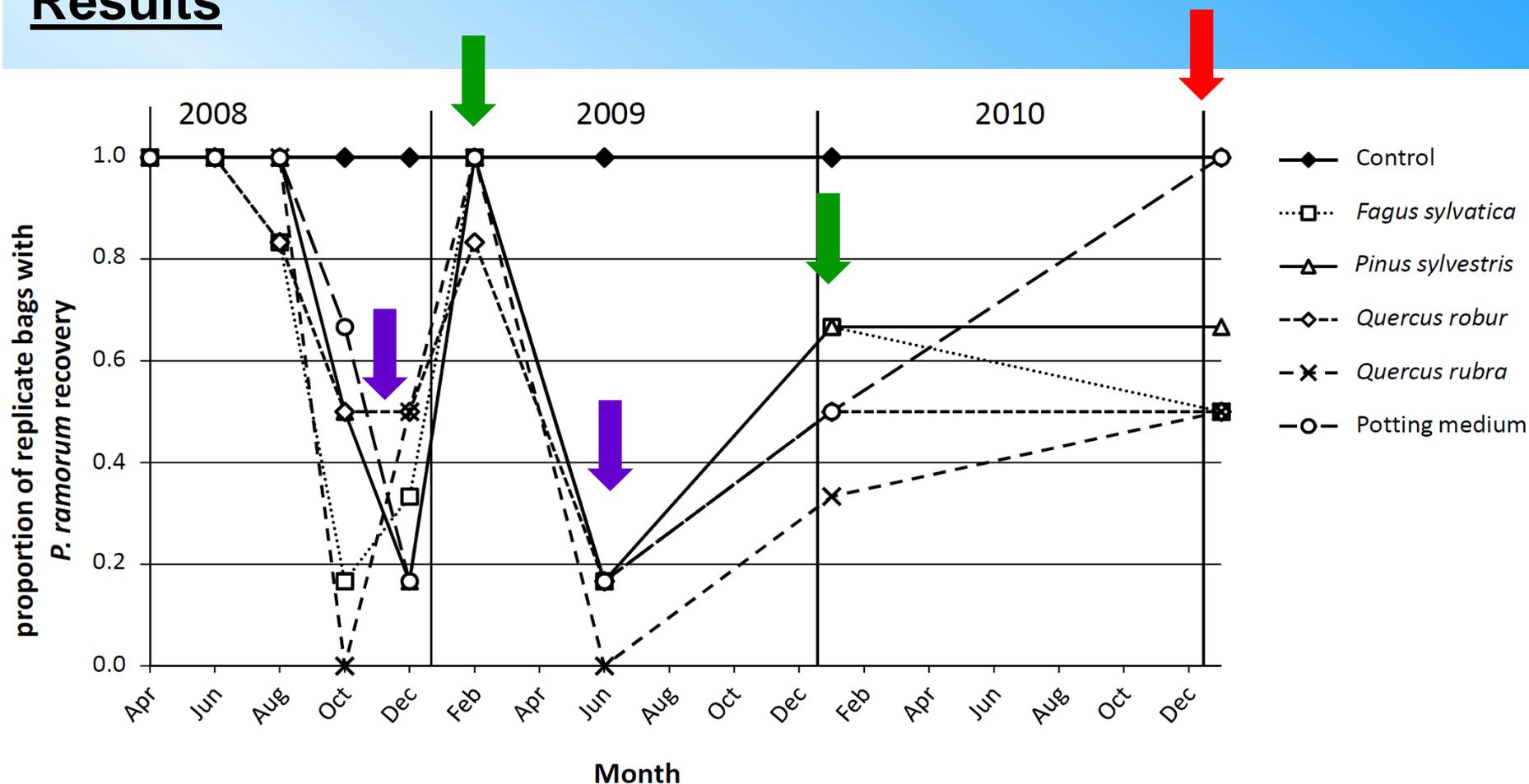
nylon mesh bags
with 15 leaf discs each



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

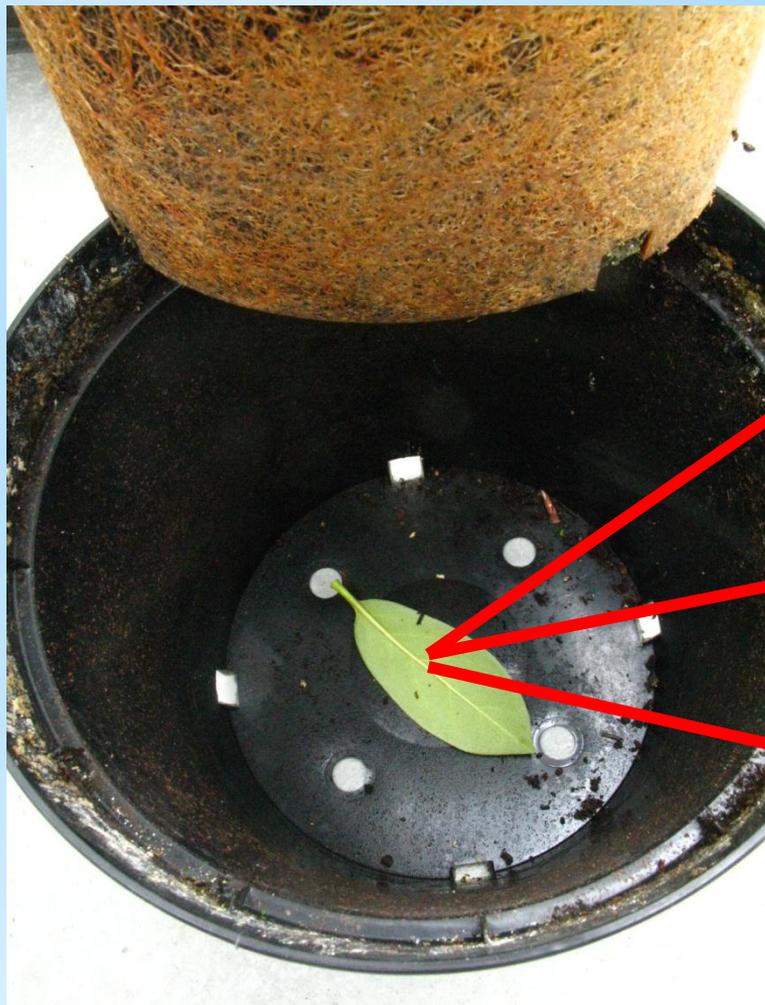


Modification of the Rhododendron leaf test developed by the team of Kurt Heungens, Belgium

- 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

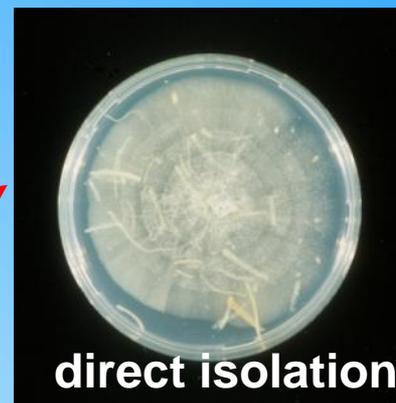
Experiment 2 – Survival in artific. inocul. Rhod. root balls

Methods



Examples for detection methods used with the baits

microbiological techniques



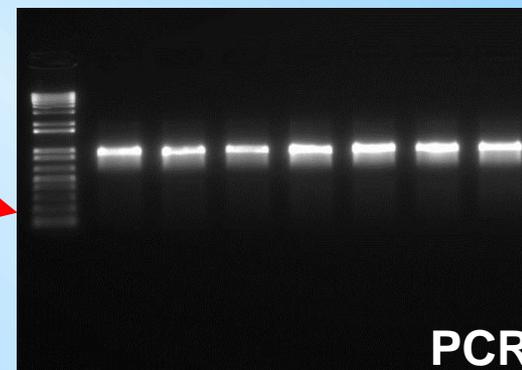
direct isolation

serological techniques



On-site kits

molecular techniques



PCR

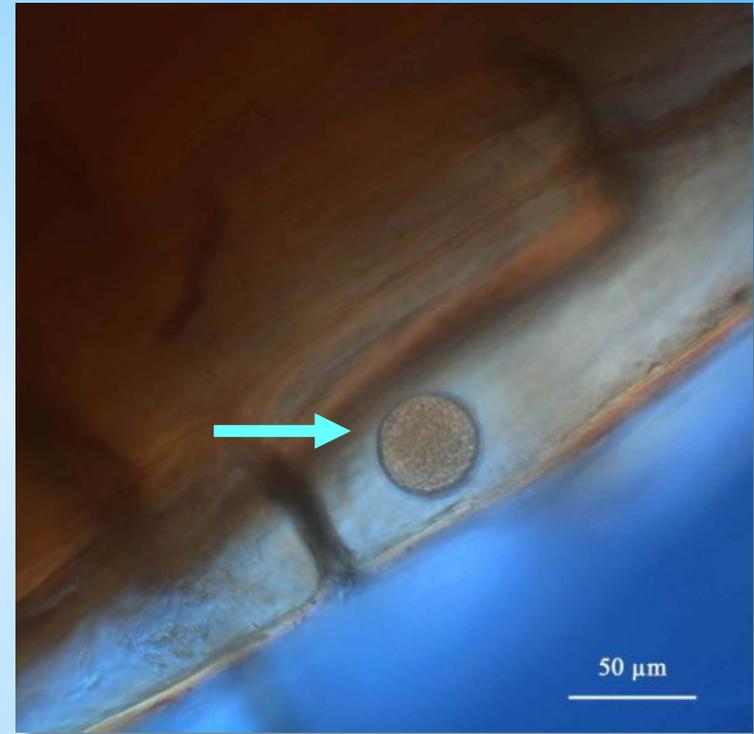
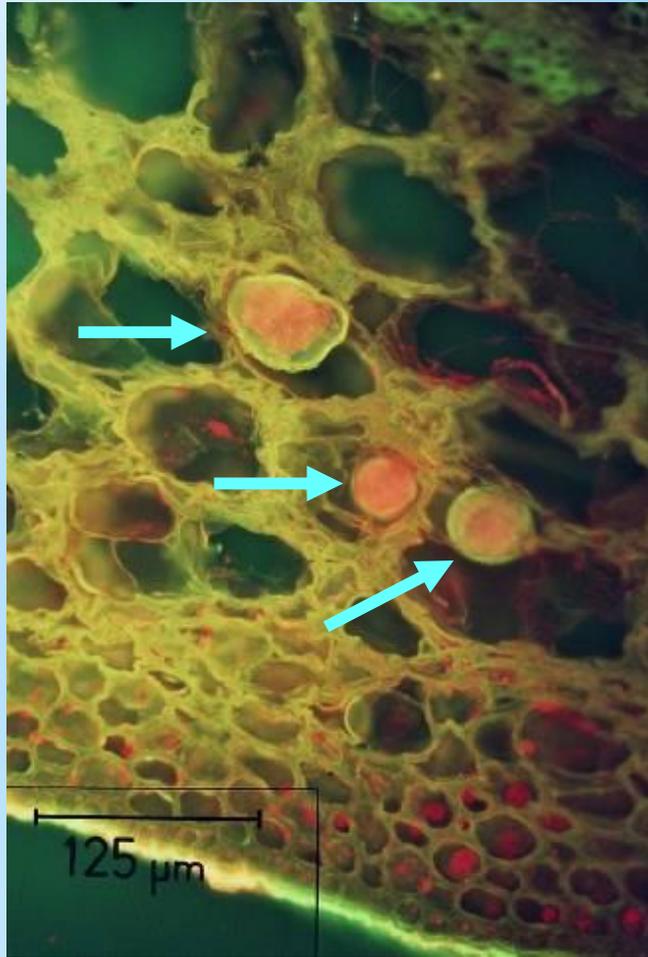
Experiment 2 – Survival in artific. inocul. Rhod. root balls

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**

Experiment 2 – Survival in artific. inocul. Rhod. root balls

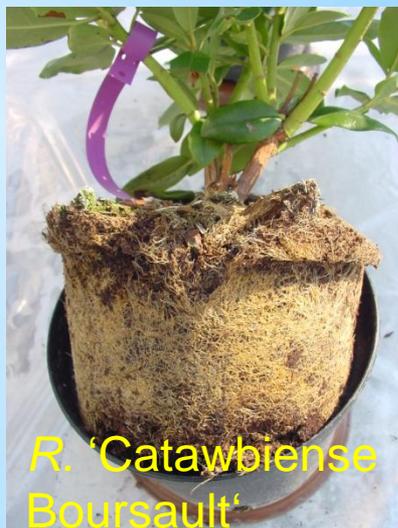
Results



Chlamydospores were present in 63.9 % of the root samples

Experiment 3 – Survival in root balls of commercial Rhodo.

Methods



= negative control



- 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

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



R. 'Catawb.
Boursault'



R. 'Catawb.
Grandiflorum'



R. 'Cunningham's
White'



R. insigne
hybride
'Brigitte'



R. yakushimanum
hybride 'Sneezy'

Experiment 3 – Survival in root balls of commercial Rhodo.

Results



R. 'Catawb.
Boursault'



R. 'Catawb.
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n =	5	48	53	51	50
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<2 yr	0	1 P + 1 Pr	6 Pr	1 Pr	0
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2 yr	0	3 P	1 P + 1 Pr	0	0
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positive single plants

positive pools

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

Acknowledgement

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- All the nice colleagues who helped with advices, ideas etc.

Thank you very much!



Thank you for your attention