

# Diversity of *Phytophthora* species broadleaved nurseries and plantations in Serbia

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# 1. Introduction

- *Phytophthora* species are fungi like organisms within the kingdom Chromista/Stramenopiles and “**SAR**” supergroup (Beakes et al. 2015);
- They can infect various plant tissues, and they are responsible for **several devastating epidemics**;
- One of the main reasons for their ubiquitous distribution in the world is **introduction from nurseries into natural and planted forests** via living plants (e.g. on the roots, in the soil and plant tissues (Brasier and Jung 2006; Scott et al. 2013; Jung et al. 2016)).

## 2. Aims of the research

- Since the nurseries are one of the main sources of *Phytophthora* infections, the aim of this study was to **determine the presence** of these pathogens in different nurseries in Serbia;
- The second aim was to determine the **possible link** between the **nursery** and selected **planted forests**, where the plants originated from studied nurseries.

### 3. Material and methods

#### 3.1. Studied objects and recorded symptoms

- Ten different nurseries that produce forestry material were sampled across Serbia, over 100 samples from 11 different hosts were taken;
- In addition, over 20 ornamental nurseries with woody and ornamental plants were tested as well;
- Various symptoms in nurseries were recorded.



- Also, ten different planted forests with known nursery that delivered the plants were selected and sampled;
- Four planted forests did not show the symptoms, while six showed the symptoms indicative for *Phytophthora* infections.



Nursery 1. Symptomatic *Robinia* plants







Nursery 1. Stand with *Quercus petraea*





Nuresry 2. Poplar plants in nursery in central Serbia





Sampling of symptomatic poplar plants in nursery 3 in central Serbia





Sampling of beech and Hungarian oak in Nursery 4 in central Serbia





Nursery 5. Sampling of *Quercus rubra* (2+0) in central Serbia





Nursery 5. *Fraxinus excelsior* healthy nursery stand





Nursery 5. *Acer pseudoplatanus* declining nursery stand





Nursery 7. Persian walnut (*Juglans regia*) plants in forestry nursery



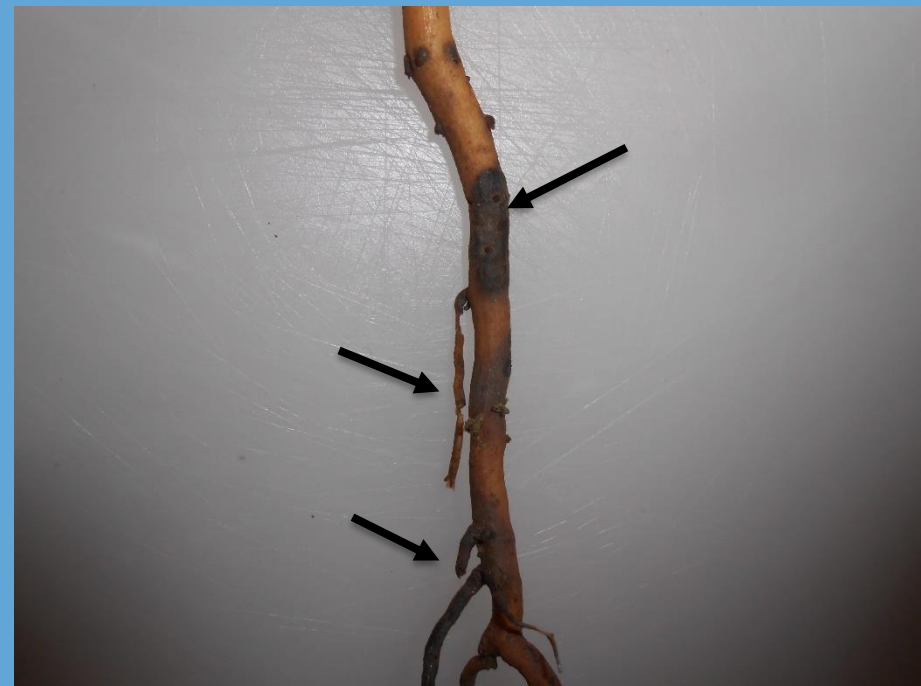


Nursery 21. Declining *Tilia* plants 4-years old





*Magnolia* sp. In ornamental nursery No. 23







Nursery 24. Declining, three-years-old cherry laurel plants





Three-years-old planted beech forest in central Serbia

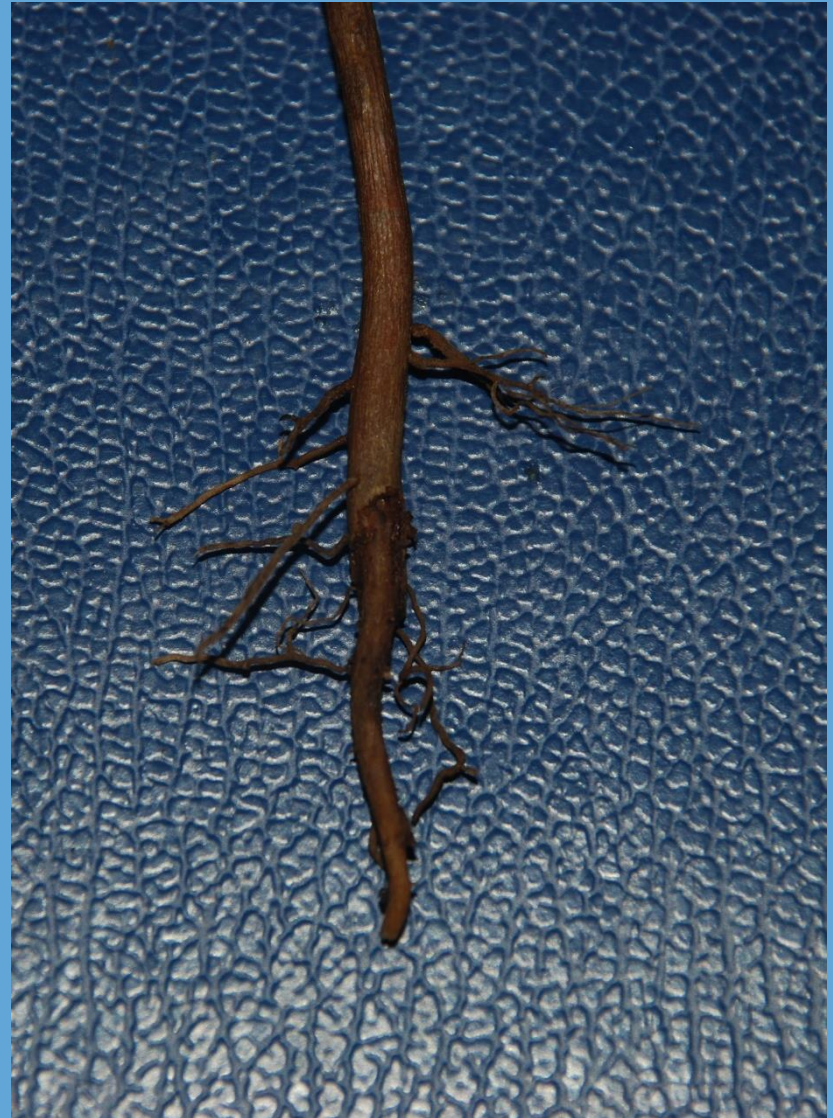




Declining, 4-years-old plantation of black locust (*Robinia pseudoacacia*) in sout. Serbia







Symptomatic plants from two-years-old *Fraxinus excelsior* planted forest  
- cankers and root rot





Declining plants of *Juglans regia* in four-years-old planted forest  
- Dieback, cankers, root rot and root loss

## 3.2. Sampling and isolation

- Sampling and isolation were performed using the **baiting method** (Jung et al. 1996; Jung 2009);
- Necrotic spots on the baits were plated onto selective V8A-PARPNH selective media;
- Collected dead plants were baited as well;
- Necrotic tissue samples from the edges of necrotic zones were plated directly onto PARPNH agar media.





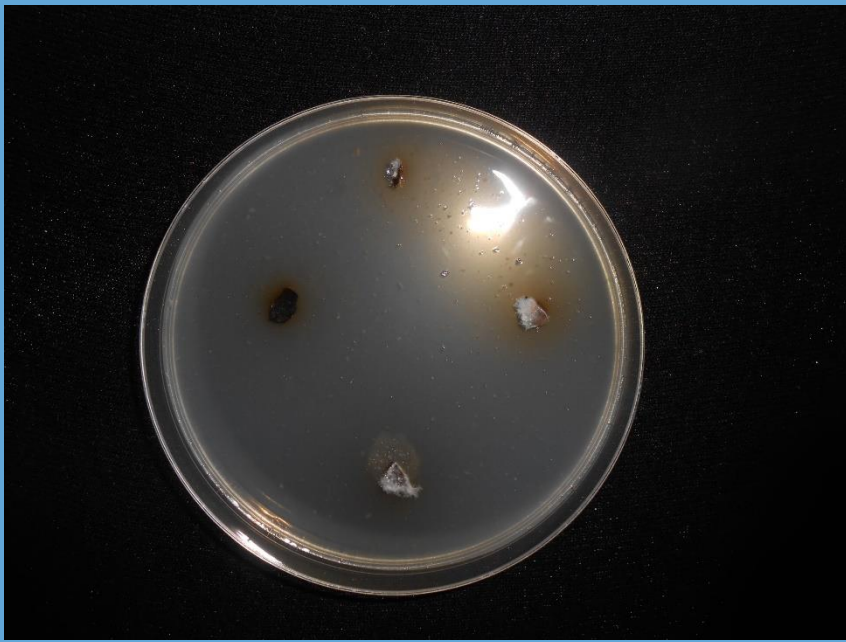




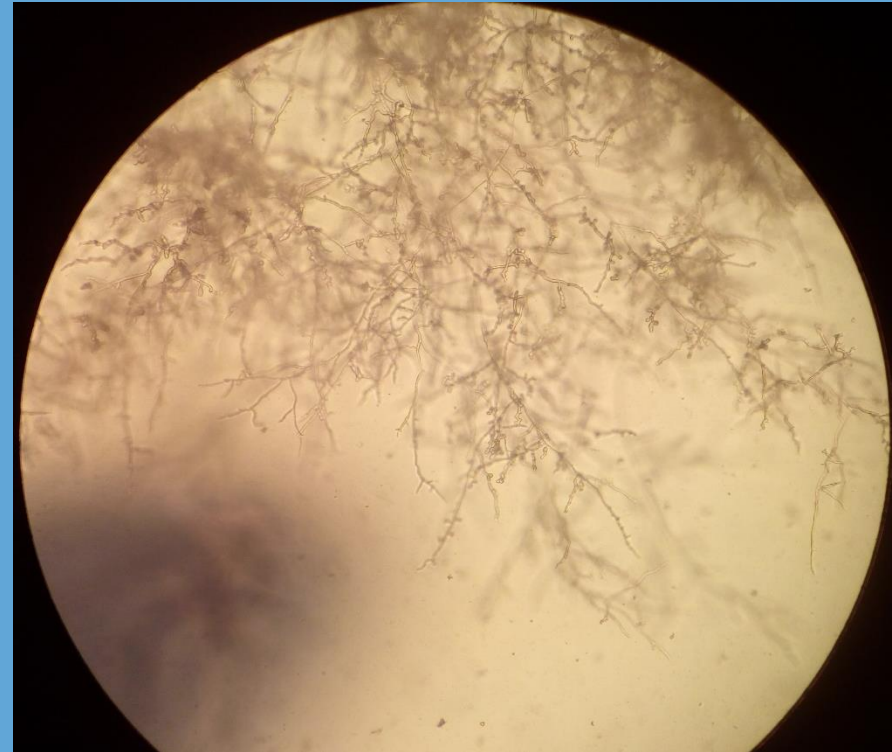
Plating of necrotic pieces onto  
selective agar media-PARPNH



*P. plurivora*- pure  
culture on MEA



Isolation from tissue and checking under the light microscope (x200)







Preparation for storage of obtained isolates

## 3.4. Identification

- Observation of sexual and asexual structures;
- Comparison with previously identified species;
- Colony growth patterns;
- Comparison with morphological keys (e.g. Stamps et al. 1990; Erwin and Ribeiro 1996), as well as with recently published species description papers;
- Sequencing of ITS region of selected isolates.

## 4. Results

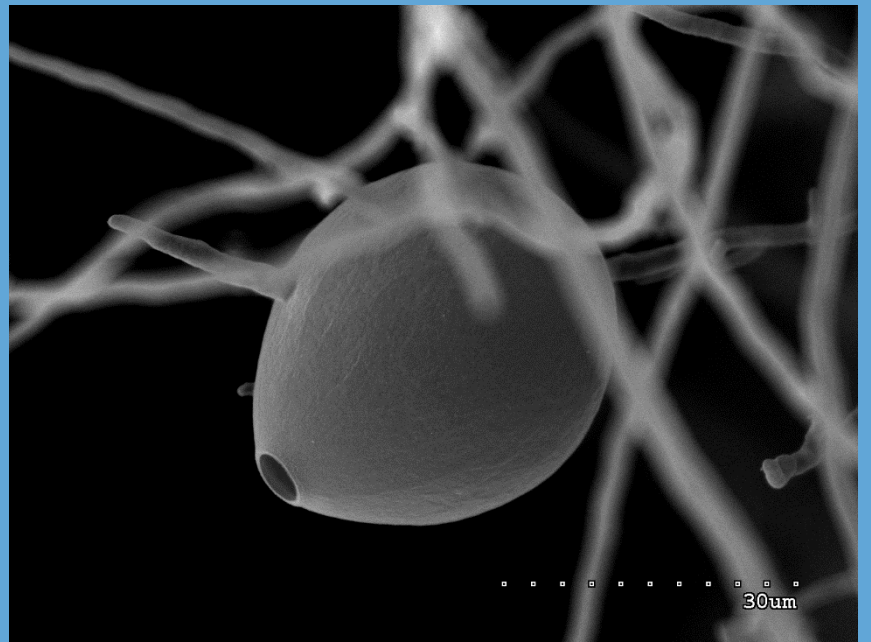
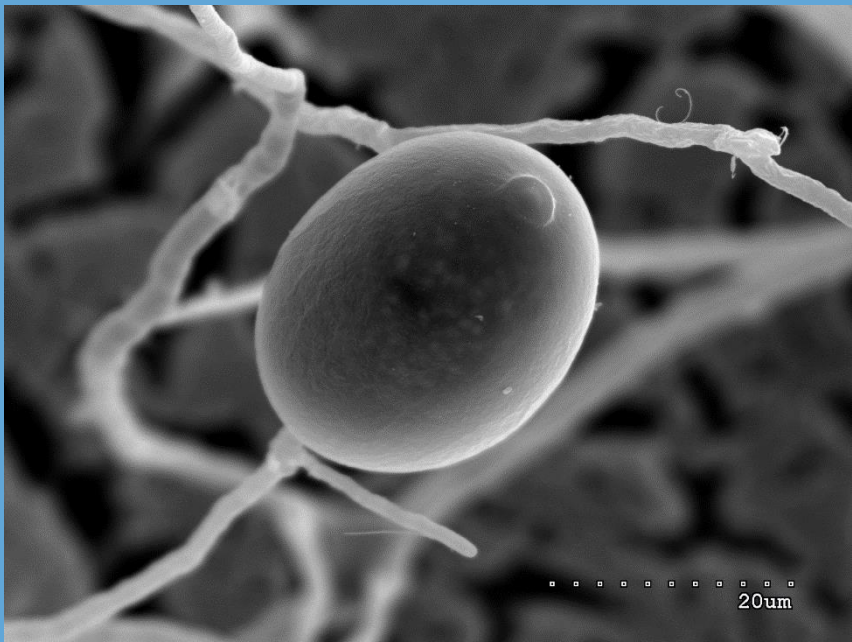
- Eight out of ten forestry nurseries were positive on the presence of *Phytophthora* species;
- While 12 out of 20 ornamental nurseries were also positive;
- After the isolation tests, 64% from tissue samples, and ca. 90% of soil samples were positive, and over 200 isolates were obtained;



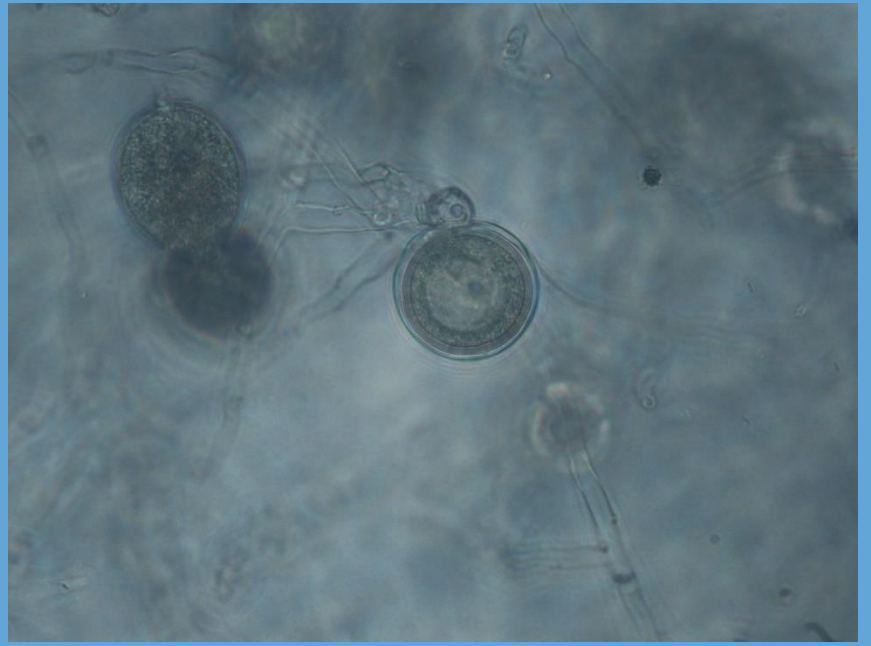
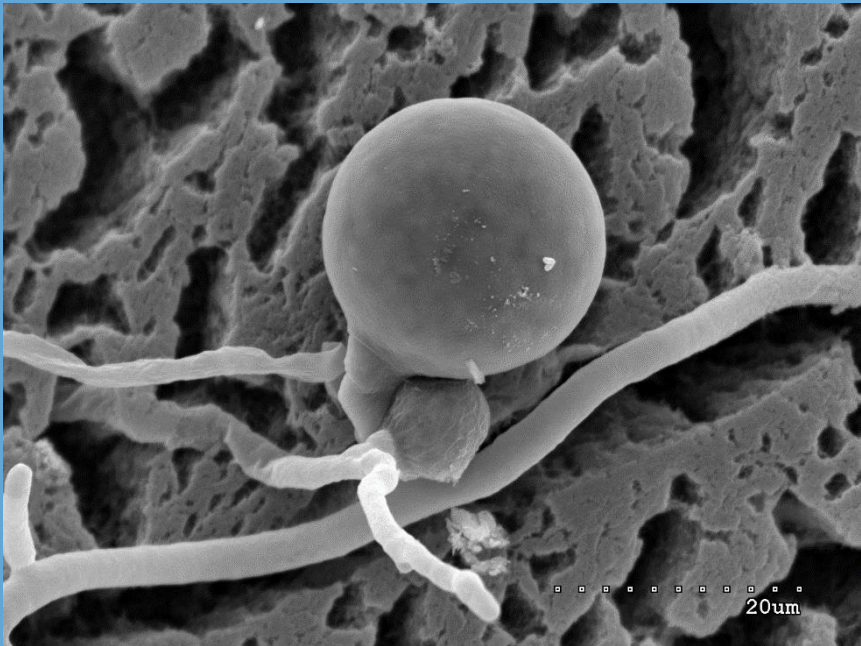
- The most positive hosts were sessile oak, beech, Persian walnut, and black locust;
- The most common species was *P. plurivora*, followed by *P. ×cambivora* and other species;
- Some rare host-pathogen combinations were recorded.

# Table 1. Isolation of *Phytophthora* species on hosts in forestry nurseries in Serbia

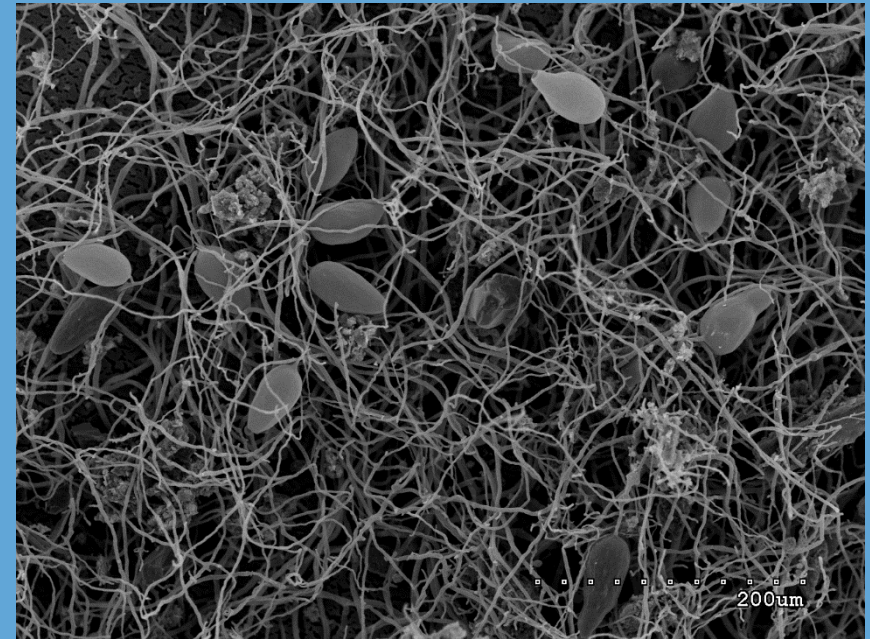
N	<i>Phytophthora</i> species	Host										
		<i>Fagus sylvatica</i>	<i>Quercus petraea</i>	<i>Q. robur</i>	<i>Q. frainetto</i>	<i>Q. rubra</i>	<i>Juglans regia</i>	<i>Robinia pseudoacacia</i>	<i>Malus sylvestris</i>	<i>Pyrus pyraister</i>	<i>Picea omorika</i>	<i>Populus</i> spp.
1	<i>P. cactorum</i>	-	-	+	+	-	+	+	-	-	+	+
2	<i>P. cambivora</i>	+	+	-	-	-	-	-	-	-	-	-
3	<i>P. cryptogea</i>	-	+	-	-	-	-	-	-	-	-	-
4	<i>P. plurivora</i>	+	+	+	-	+	+	-	-	-	-	+
5	<i>P. quercina</i>	-	+	+	+	-	-	-	-	-	-	-
6	<i>P. gonapodyides</i>	+	-	-	+	+	-	-	-	-	-	+
7	<i>P. lacustris</i>	-	-	-	-	-	+	-	-	-	-	+
8	<i>P. polonica</i>	-	-	-	-	-	-	-	-	-	-	+
9	<i>P. pini</i>	-	-	-	-	-	-	-	-	-	-	+
10	<i>Phytophthora</i> spp.	-	-	-	-	+	+	+	-	-	+	-



*P. cactorum*- papillate sporangia



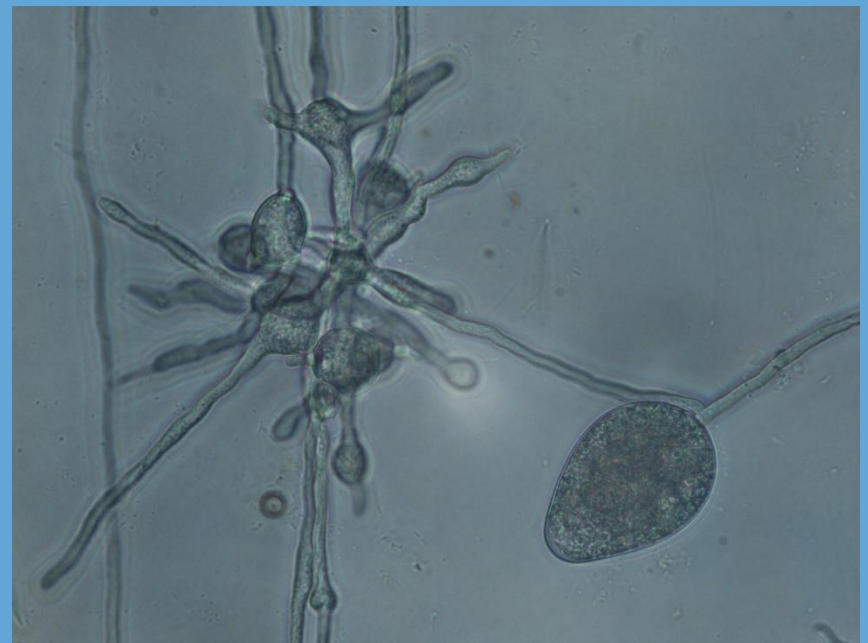




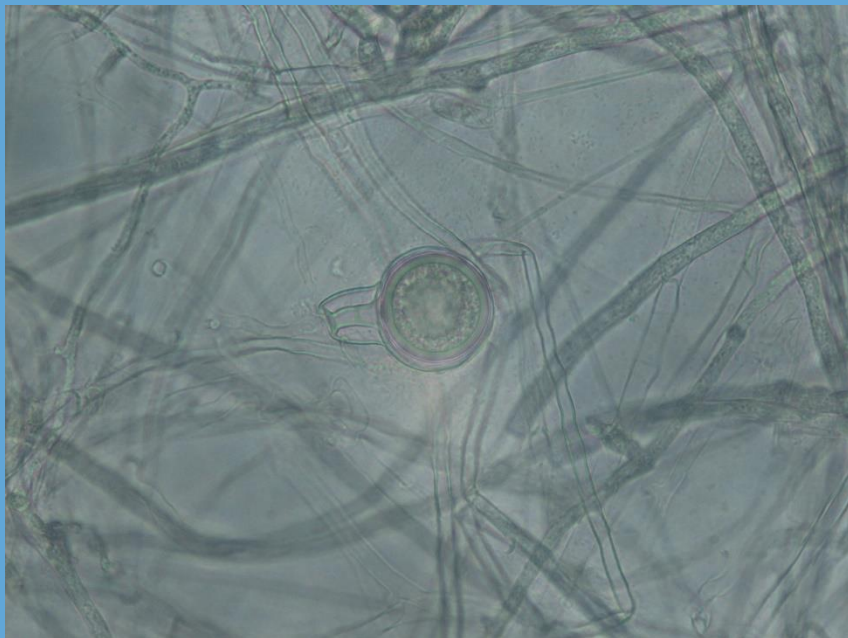
*Phytophthora citrophthora*- papillate sporangia



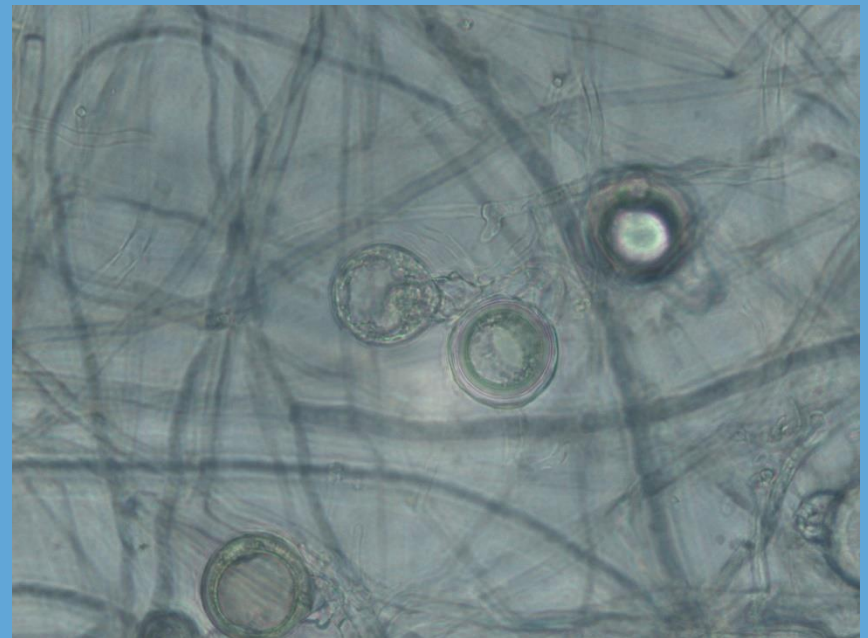
*P. cryptogea*- nonpapillate sporangium



*P. cryptogea*- swellings and sporangium



*P. cryptogea*- oogonium and antheridium



*P. cryptogea*- mature and aborted oogonia





*P. gonapodyides*- sporangia



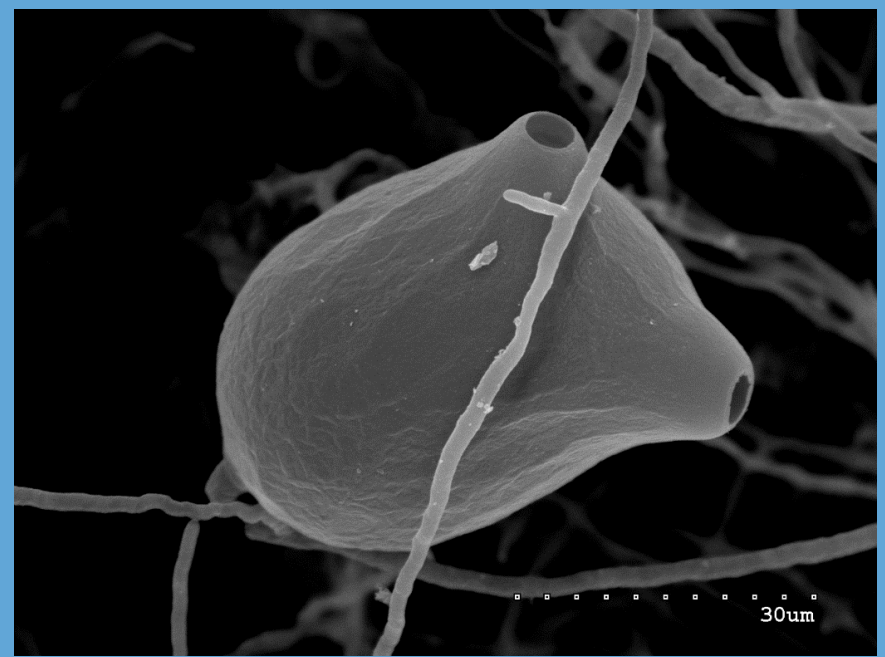
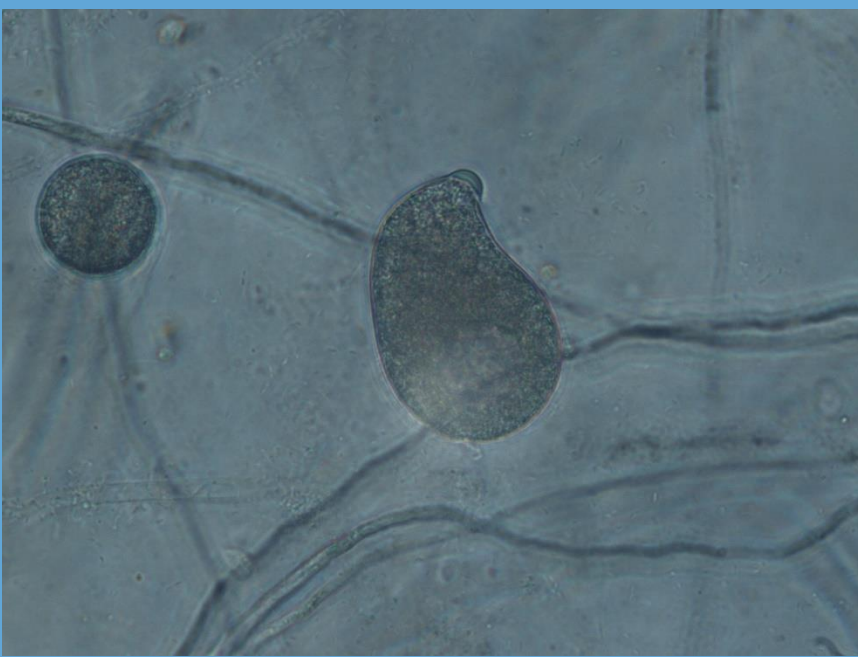
*P. gonapodyides*- mature and empty sporangia



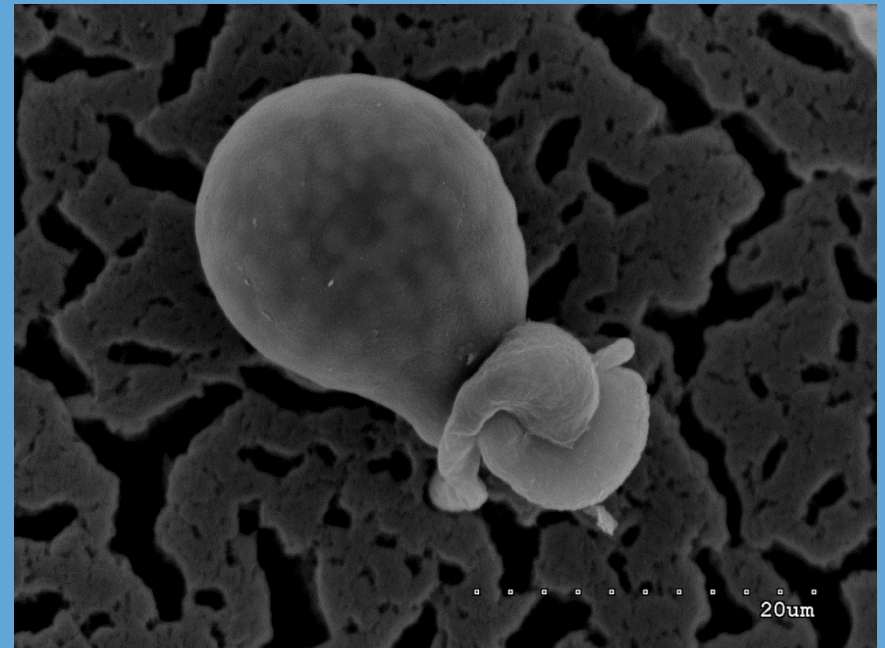
*P. lacustris*- sporangia



*P. lacustris*- mature and empty sporangia



*Phytophthora quercina*- papillate sporangia

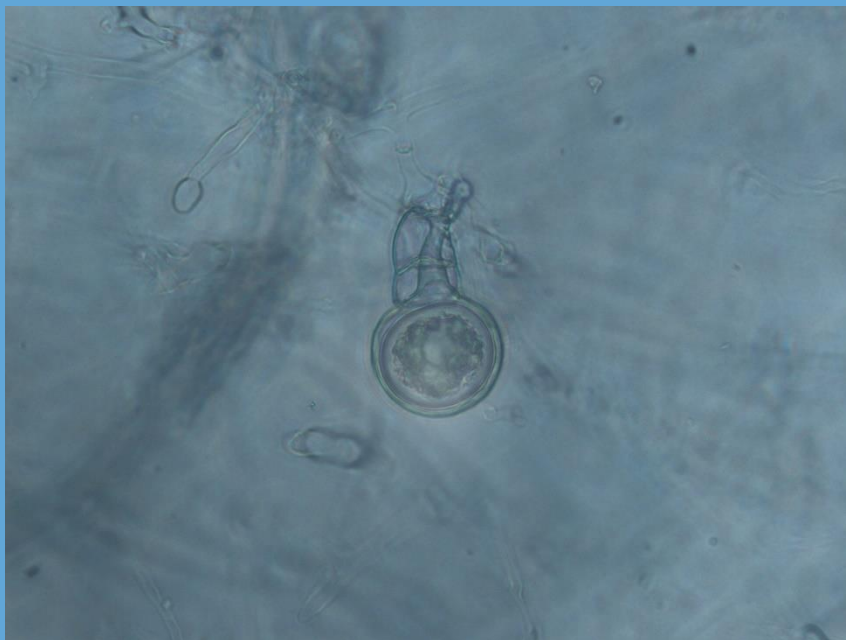


*Phytophthora quercina*- oogonium and antheridium





*Phytophthora cambivora*- nonpapillate sporangia

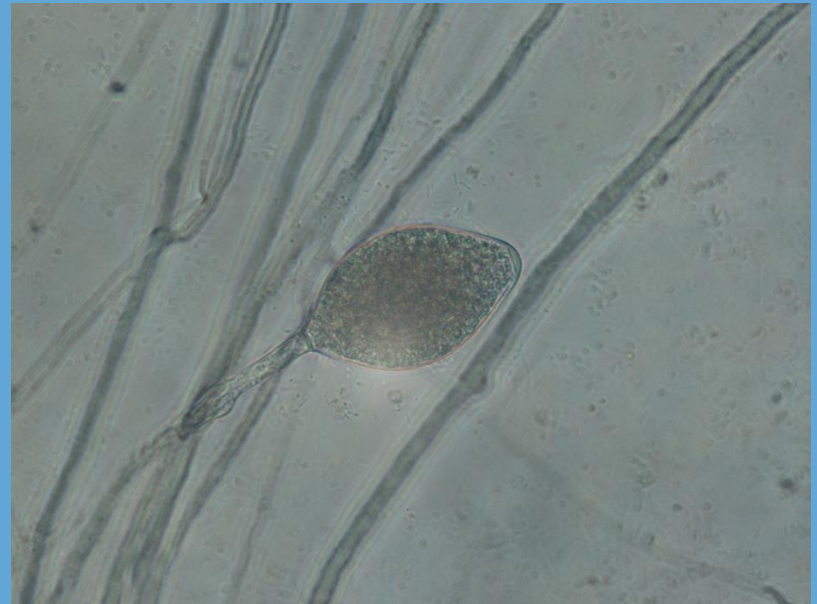


*Phytophthora cambivora*- oogonia and two-celled antheridia





*P. plurivora*- semipapillate sporangia



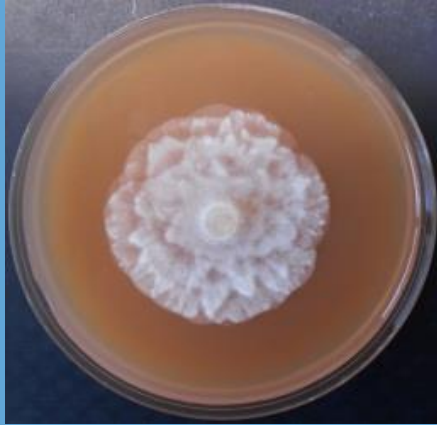
*P. plurivora*- semipapillate sporangium



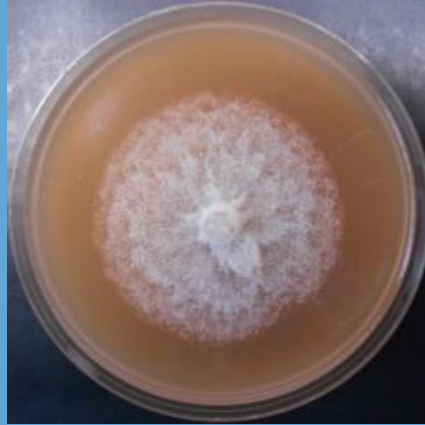
*P. plurivora*- sporangium with three peaks



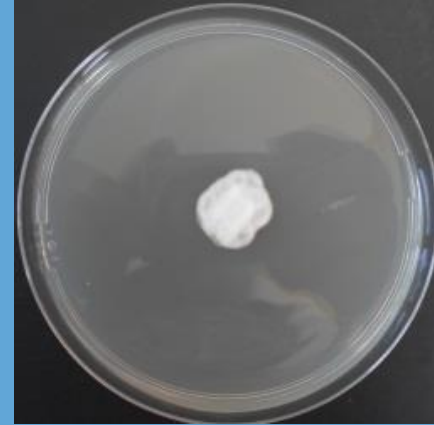
*P. plurivora*- oogonium and antheridium



*P. plurivora*- V8A, after 7 days in the dark at 20°C



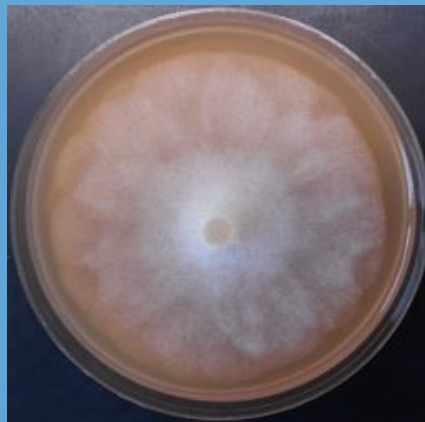
*P. quercina*- V8A, after 20 days in the dark at 20°C



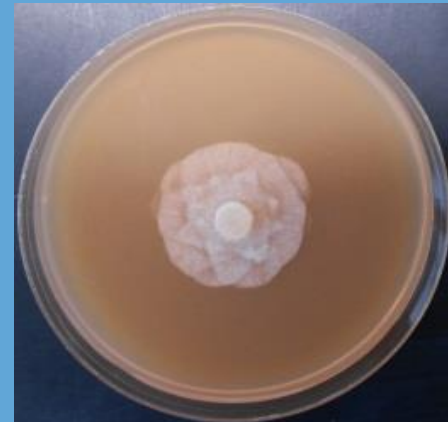
*P. quercina*- PDA, after 7 days in the dark at 20°C



*P. citrophthora*- V8A, after 7 days in the dark at 20°C



*P. cryptogea*- V8A, after 7 days in the dark at 20°C



*P. gonapodyides*- V8A, after 7 days in the dark at 20°C





ELECTRONIC  
DIGITAL CALIPER



- The most typical introduction path was recorded in the case of *Q. robur* and *Q. petraea*, where clear **nursery-forest** link was recorded:

**Table 2.** Presence of *Phytophthora* species in planted forests- Case study of *Quercus petraea* and *Q. robur* in northern Serbia

Number	Species	Host		Total
		<i>Q. petraea</i>	<i>Q. robur</i>	
1	<i>P. citrophthora</i>	1	0	1
2	<i>P. cryptogea</i>	1	0	1
3	<i>P. plurivora</i>	7	8	15
4	<i>P. gonapodyides</i>	0	1	1
5	<i>P. quercina</i>	1	0	1
6	<i>P. syringae</i>	3*	0	3
Total		13	9	22

\*lost isolates

**Table 3.** Comparison of presence of *Phytophthora* species in planted forests and nurseries

N	Species	<i>Q. petraea</i>		<i>Q. robur</i>	
		Number of obtained isolates			
		Planted forests	Nursery	Planted forests	Nursery
1	<i>P. cactorum</i>	0	0	0	1
2	<i>P. citrophthora</i>	1	0	0	0
3	<i>P. cryptogea</i>	1	2	0	0
4	<i>P. cambivora</i>	0	1	0	0
5	<i>P. plurivora</i>	7	5	8	6
6	<i>P. gonapodyides</i>	0	0	1	0
7	<i>P. quercina</i>	5	4	0	2
8	<i>P. syringae</i>	3	0	0	0





*Q. robur* - 6-years-old planted forest



*Q. petraea* - 12-years-old planted forest



*Q. petraea*- 4-years –old planted forests



*Q. robur*- destroyed root of 6-years-old plant



- Changing of nursery production policy could be discussed, due to numerous failures in production of healthy living nursery stocks across the Europe and North America (Jung et al. 2016);
- Wider scientific and practice consensus is essential, as well as information of importance of these and similar studies.

# Acknowledgements:



## Coauthors of this work:

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