

National Reference Laboratory
for Plant Health



Instituto Nacional de
Investigação Agrária e
Veterinária, L.P.



Laboratories

Acarology
Bacteriology
Entomology
GMO & Molecular Biol
Herbology
Mycology
Nematology
Seeds
Virology



MINISTÉRIO DA AGRICULTURA
E DO MAR

INIAV Nematology Lab (Oeiras, Lisboa)

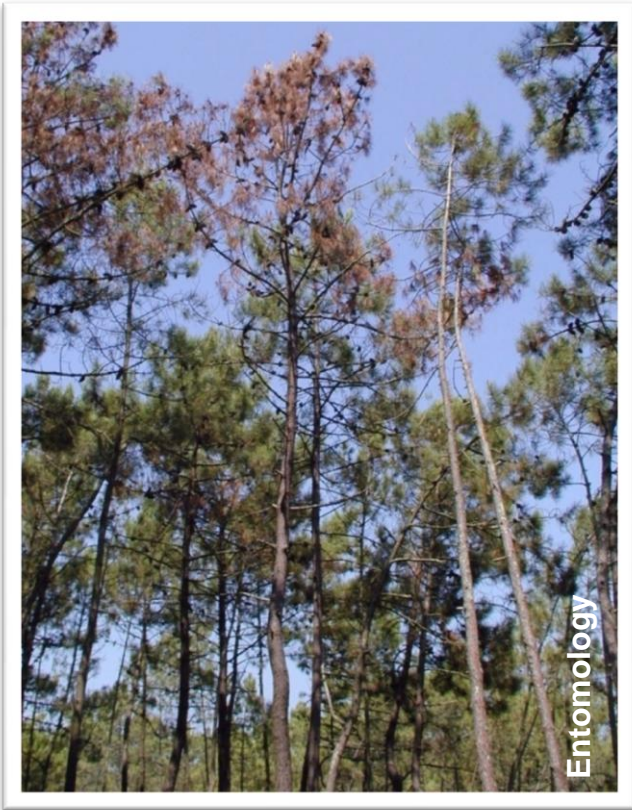
Maria L. Inácio



**EPPPO Workshop on
Maintenance of Nematode Collections
Wageningen (NL),
05-06 Sept 2017**

- PWN maintenance & inoculum production

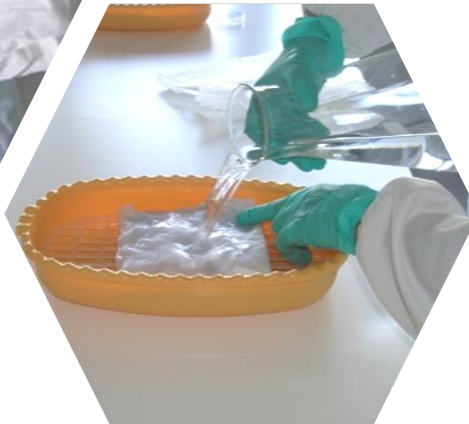
Bursaphelenchus xylophilus



Establishing a new isolate of *Bursaphelenchus xylophilus*



Baermann funnel
(modified)

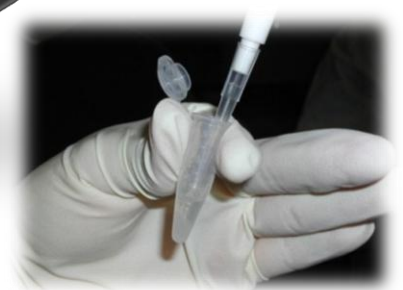
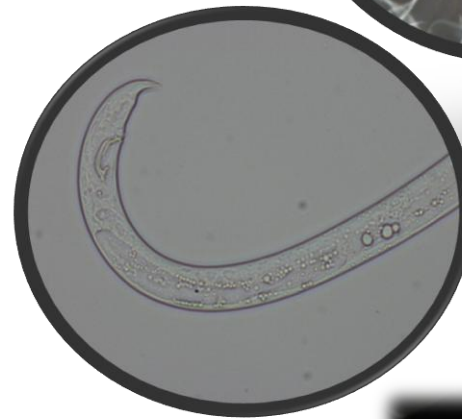
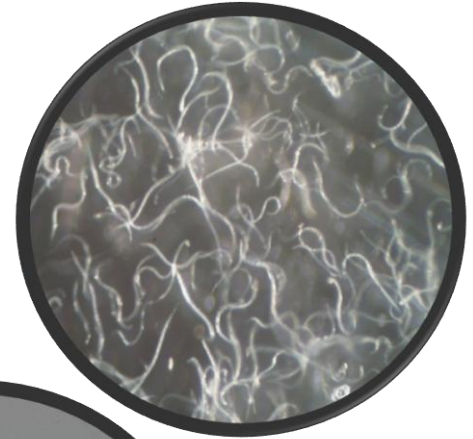


38 µm sieve



1. Nematode extraction from wood samples

Establishing a new isolate of *Bursaphelenchus xylophilus*

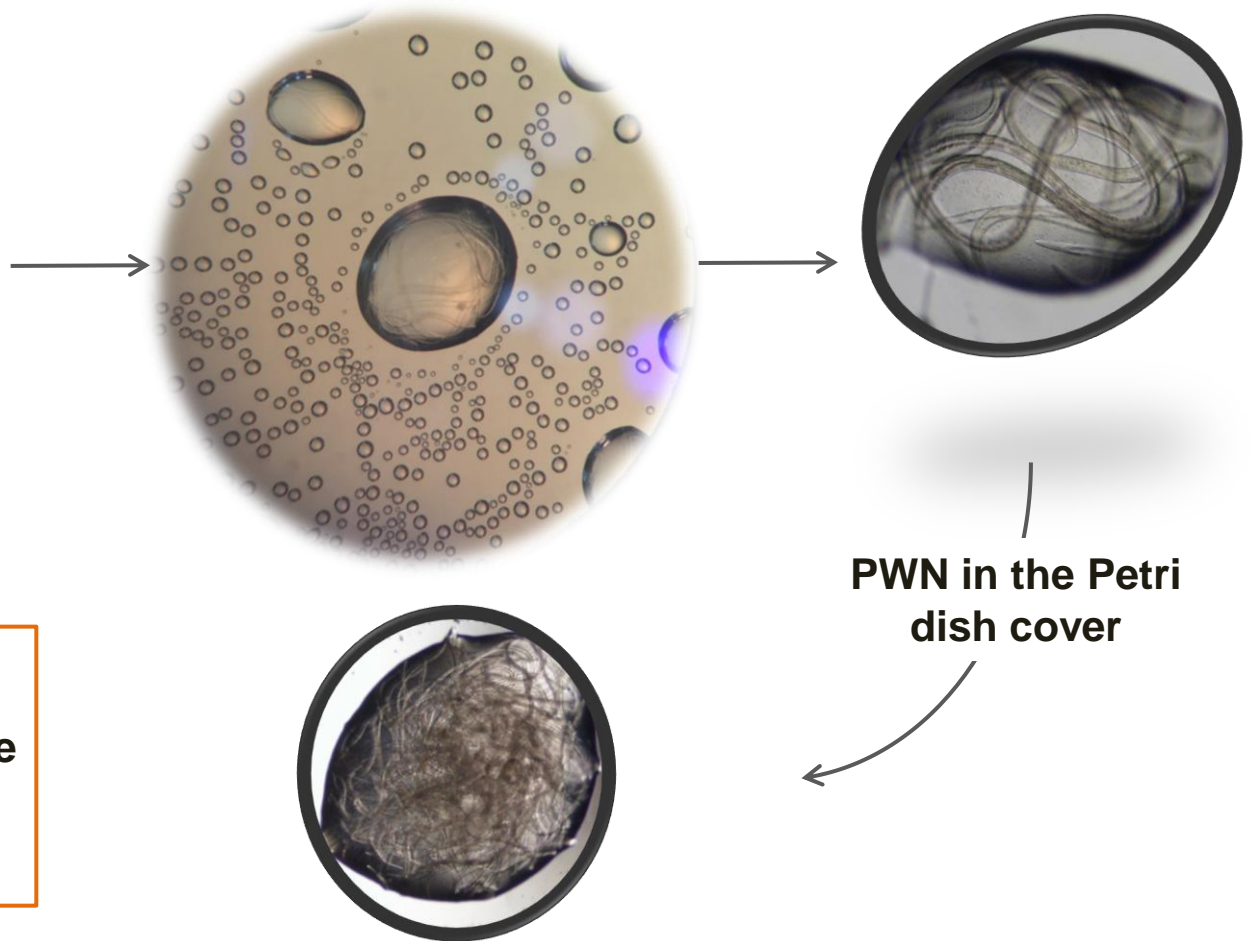


2. Identification (morphology + molecular)

Establishing a new isolate of *Bursaphelenchus xylophilus*



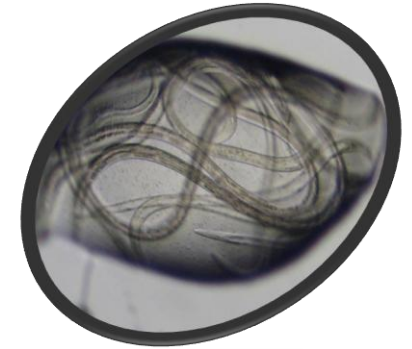
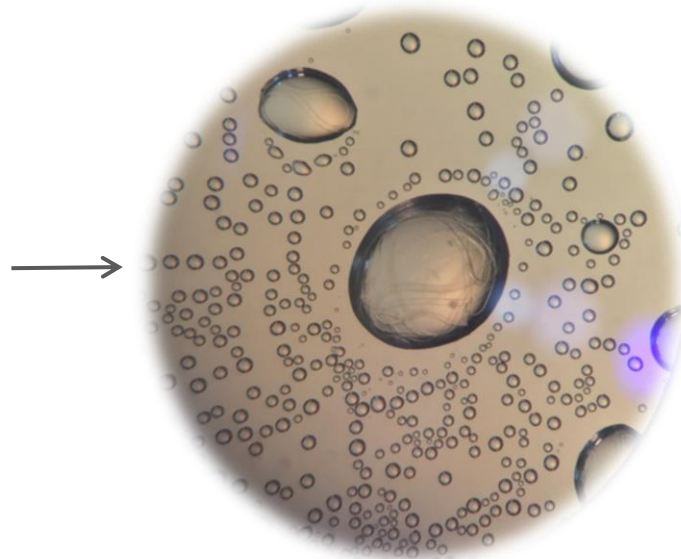
PWN inoculation in 2-weeks old fungal culture after nematodes' disinfection (≈ 50 nem)



PWN in the Petri dish cover

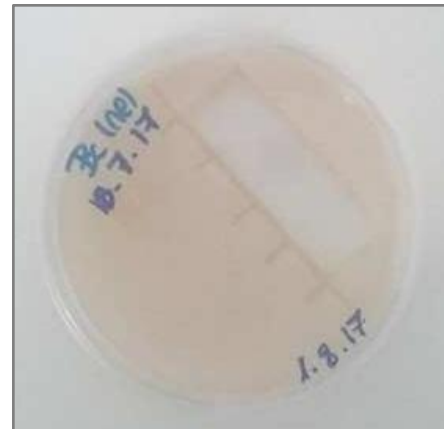
3. Obtaining a new isolate in *Botrytis cinerea* (PDA, 24 1°C)

Maintaining *Bursaphelenchus xylophilus* isolates



**The entire process:
≈1-1,5 month**

**Cultures can be kept
at low T (≈ 6-7°C) and
the process repeated
each 8 weeks**



Sometimes
nematodes fail to
establish

Timely (!) mass production of *Bursaphelenchus xylophilus*



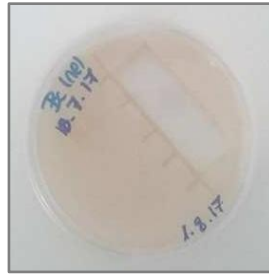
Organic barley autoclaved 2x



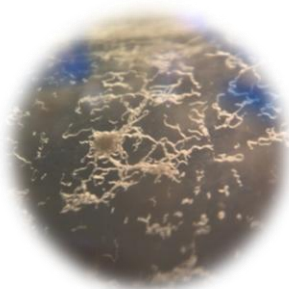
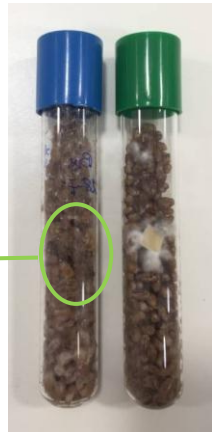
Inoculation with the mycelial mat



2 wk



PWN inoculation



Nematode extraction and counting



Inoculation tests with *Bursaphelenchus xylophilus*



Seedlings and cuttings



Inoculation tests with *Bursaphelenchus xylophilus*



timely and
flawless
inoculum
production
for the
several
ongoing
studies

healthy branches
3x autoclaved



1000 PWN/ mL
25°C, ↑ RH



2-3 wk after



**Keeping
isolates'
virulence**



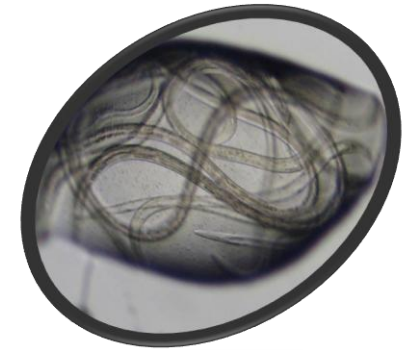
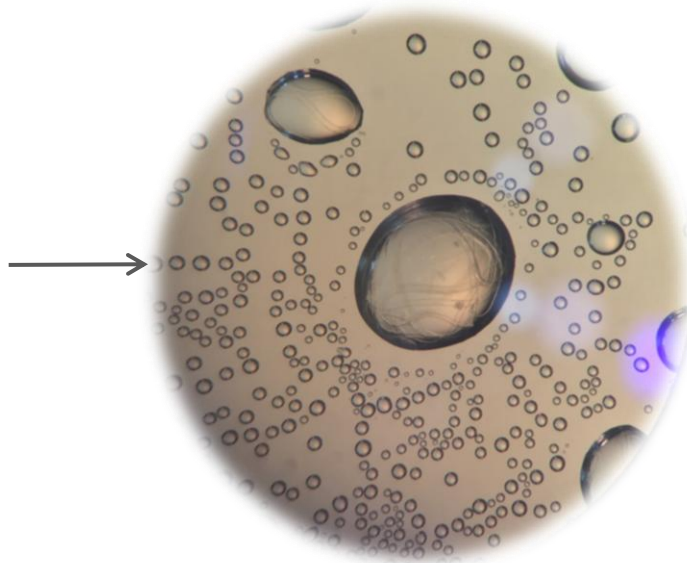
Once a year



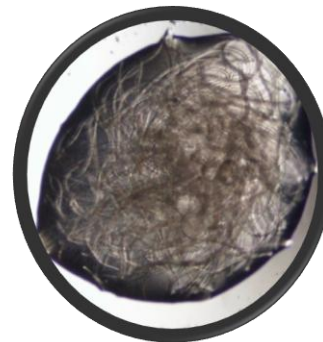
Re-establishing the isolate of *Bursaphelenchus xylophilus*



PWN inoculation in 2-weeks old fungal culture after nematodes' disinfection (≈ 50 nem)



PWN in the Petri dish cover



Isolate in *Botrytis cinerea* (PDA, 24 1°C)



INIAV's Nematology team

Maria L. Inácio

Ana M. Fontes

Helena Vicente

Imaculada Wahnon

Marina Cardoso

Filomena Nóbrega (molecular)

End

