

Bursaphelenchus xylophilus

The Invisible Enemy of the Coniferous Detection and identification



INSTITUTO DO MAR – CENTRO DO MAR E AMBIENTE (IMAR-CMA)



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FCTUC

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Coimbra, 05.November.2013

Bursaphelenchus species

Valid species (2012) → 117



Europe → 49

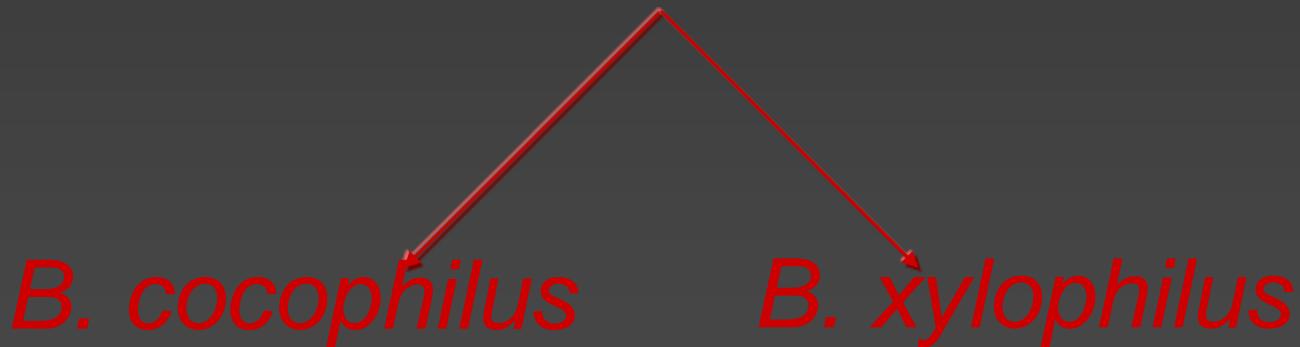
Bursaphelenchus species

Valid species (2012)

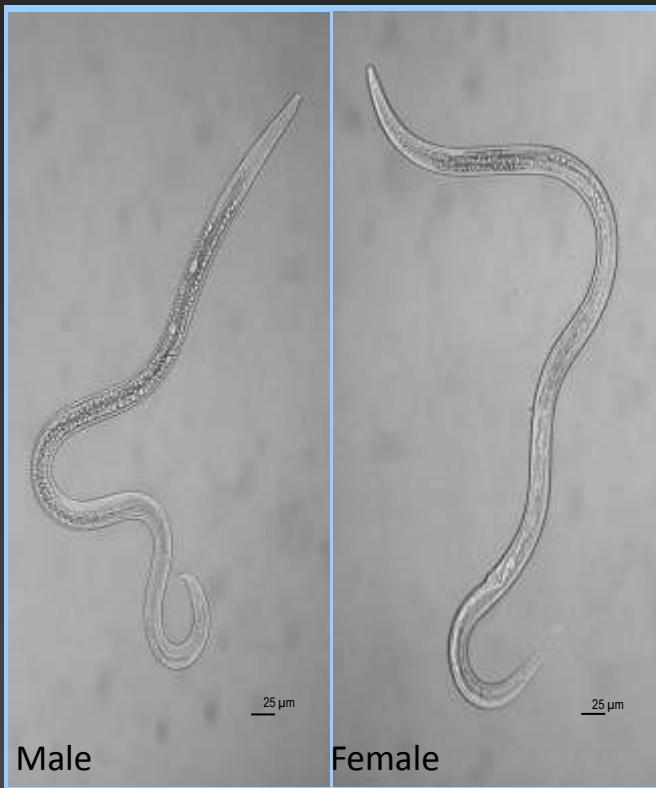


117

Two pathogenic species



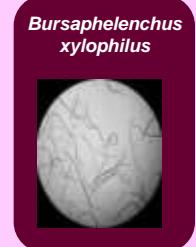
Bursaphelenchus xylophilus



Phylum: Nematoda
Order: Rhabditida
Suborder: Tylenchina
Superfamily: Aphelenchoidea
Family: Aphelenchoididae
Subfamily: Parasitaphelenchinae

Hunt, 2008

Integrated pest management



Sampling and extraction



Crown (Branches)

Trunk 6-8 m

Trunk 1-2 m



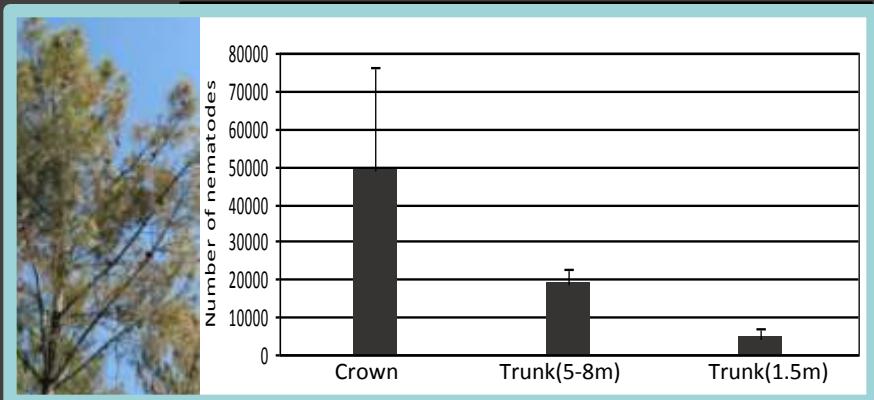
Tray



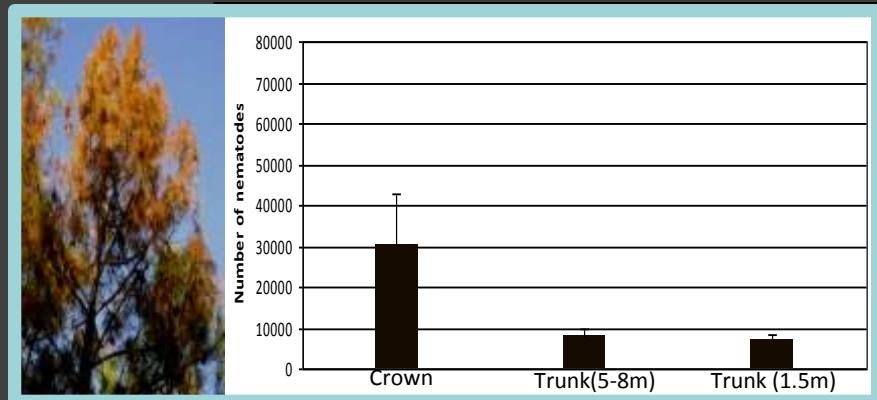
Baermann funnel

Axial distribution in the host tree

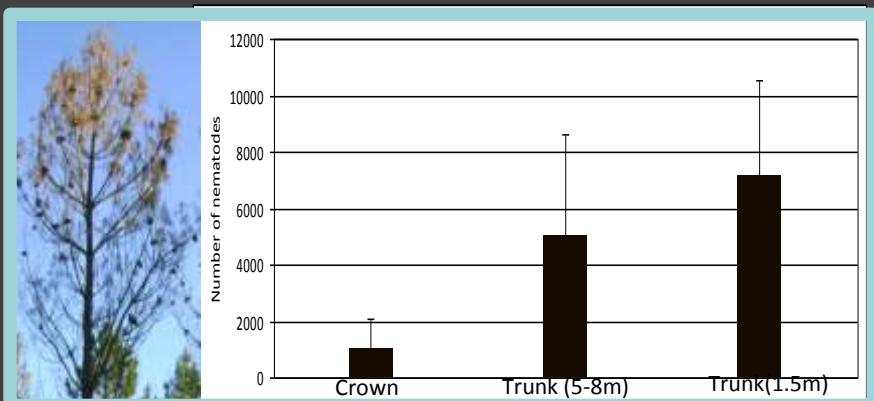
Stage II (10-50% brown leaves)



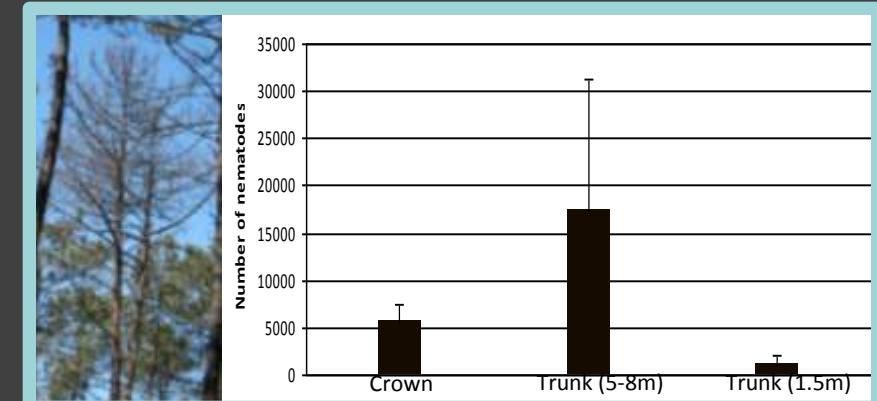
Stage III (50-80% brown leaves)



Stage IV (80-100% brown leaves)



Stage V (dead tree, without leaves)



PINEWOOD NEMATODE (PWN) IDENTIFICATION



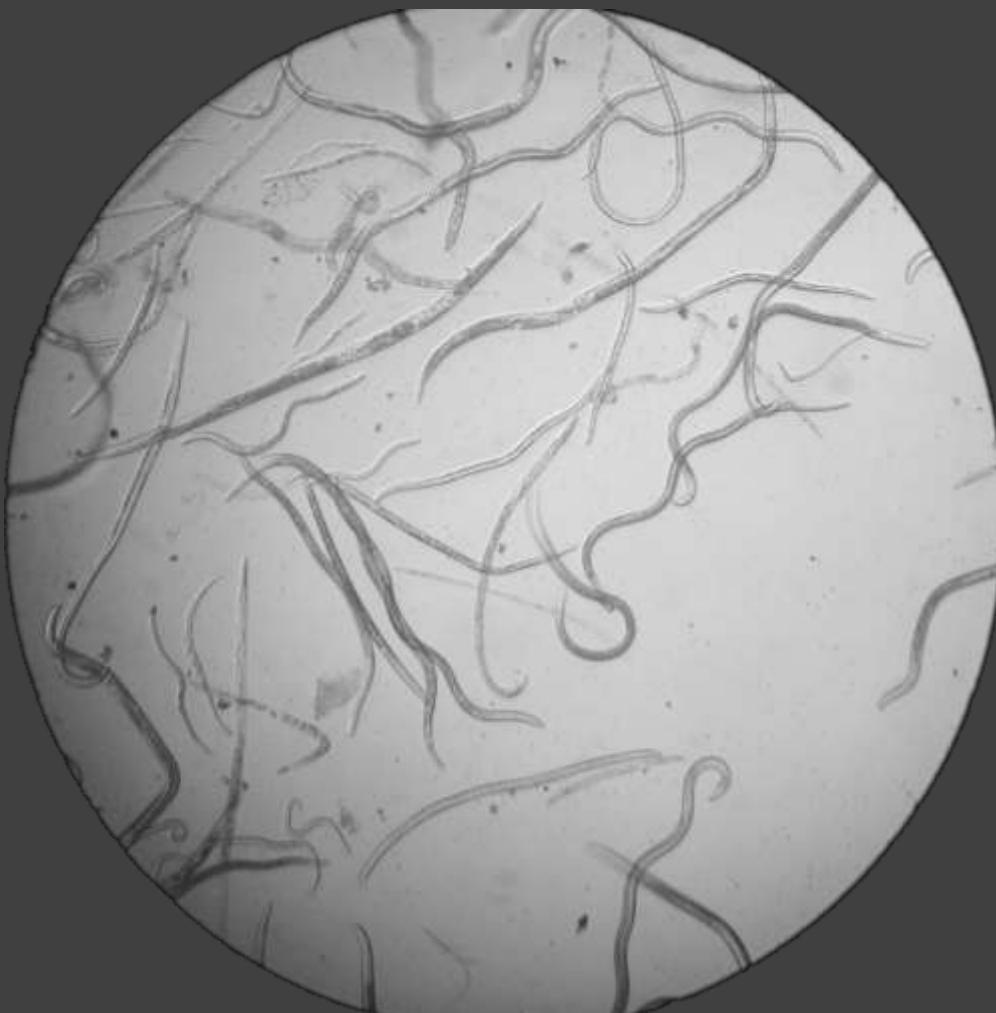
From

Morphology

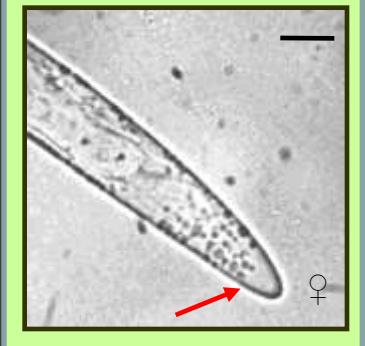
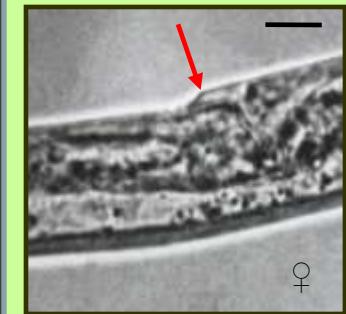
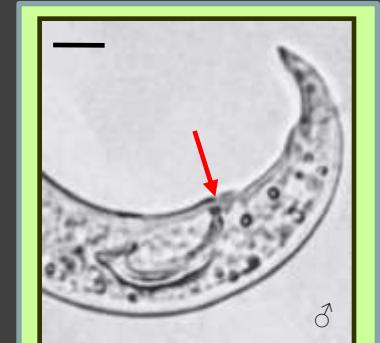
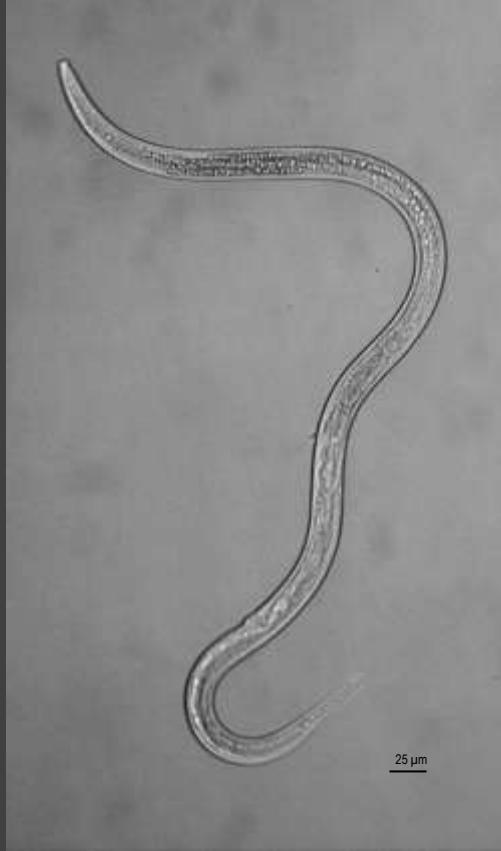
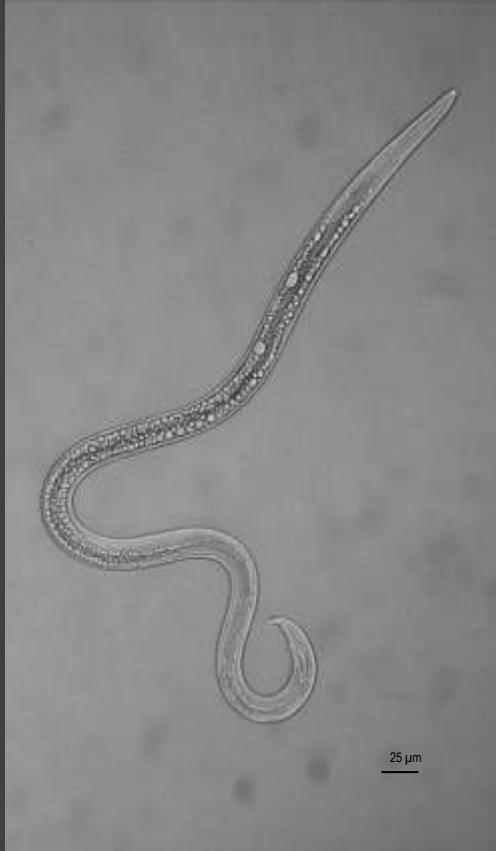


to
Molecular

Morphology



Morphology



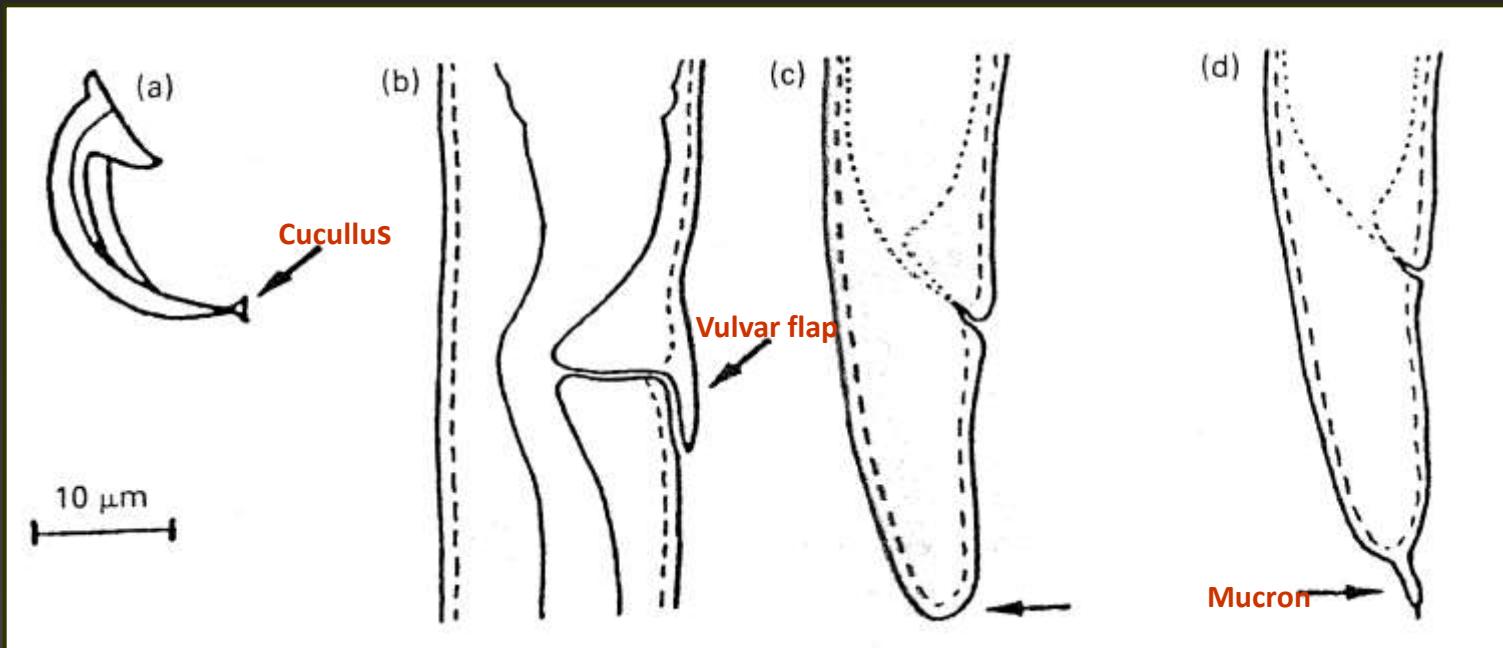
Disc-like projection (cucullus) at the distal end of the spicules.

A vulvar flap.

Female tail with rounded terminus.

10 µm

Morphology

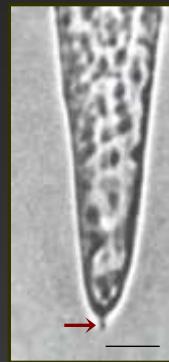
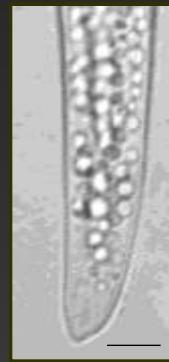
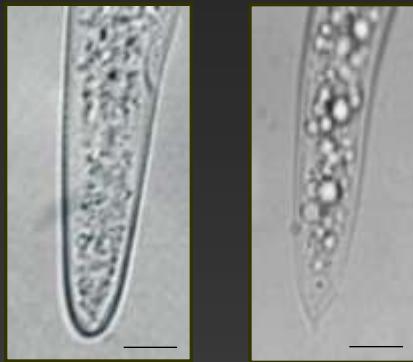


Morphological diagnostic characters

Bursaphelenchus xylophilus and *B. mucronatus*

(Adapted from OEPP, 2001)

Morphology - Female tails variability



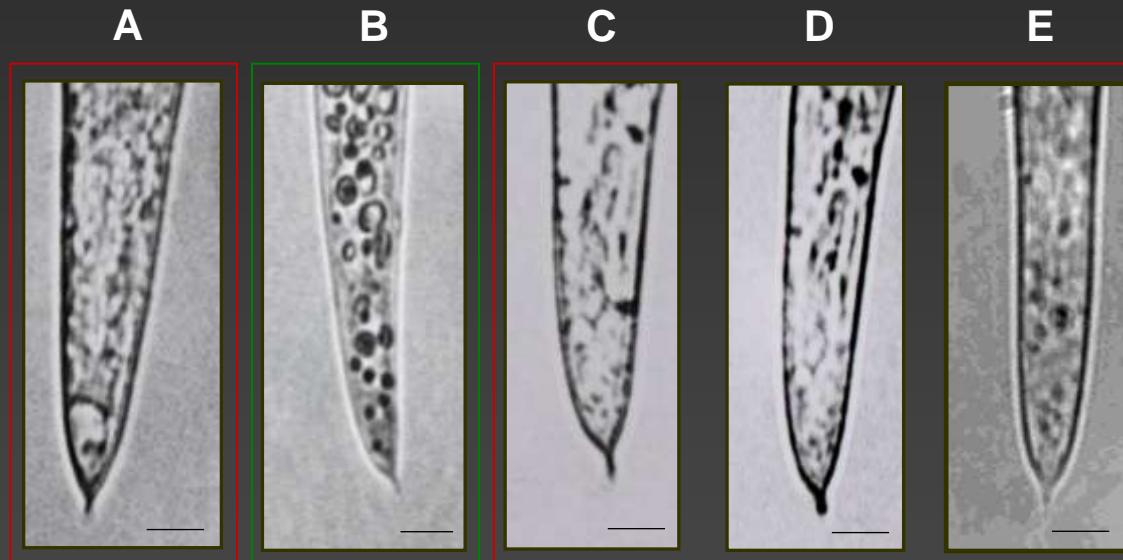
B. mucronatus

B. xylophilus

Bursaphelenchus xylophilus

or

Bursaphelenchus mucronatus ?



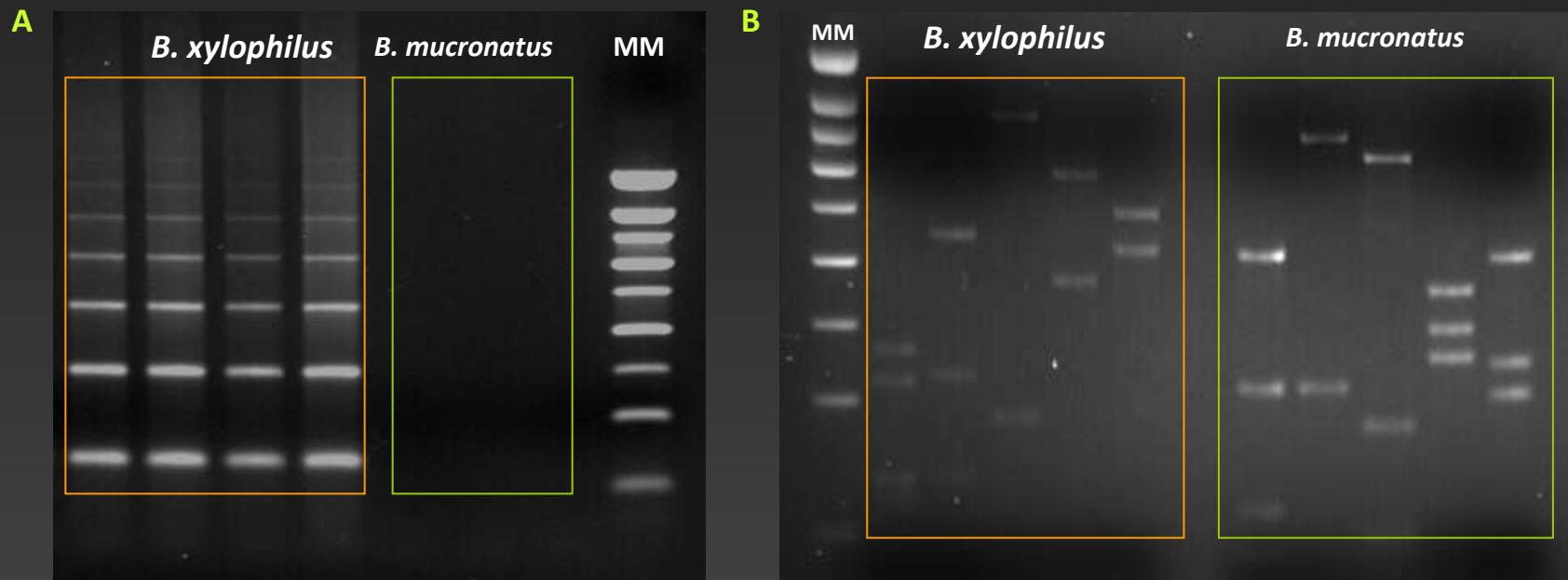
Bars = 10 µm

MORPHOLOGICAL IDENTIFICATION

Disadvantages

- Limited number of useful characters available and developmental stages
- Intraspecific variation
- Be aware of species mixtures (common)
- Time-consuming
- Expertises

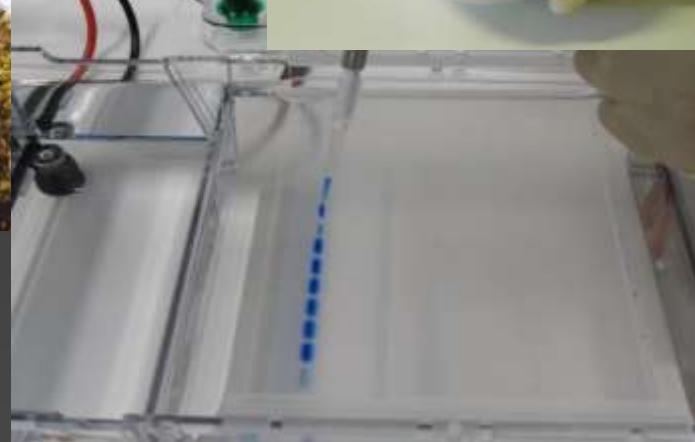
Molecular identification



A – “Single Worm PCR” - using satellite DNA primers set specific for *B. xylophilus*

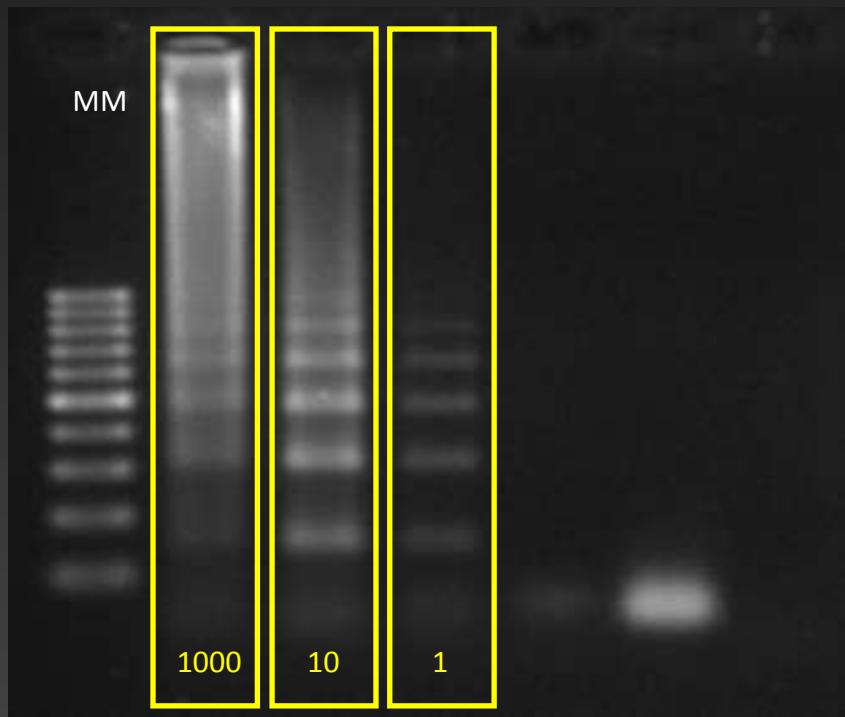
B - ITS-RFLP patterns of *B. xylophilus* and *B. mucronatus* isolates obtained by digestion of amplified rDNA ITS regions with the restriction enzymes *Af*I, *Al*uI, *Hae*III, *Hin*II, and *Msp*I

Molecular identification from wood/insects



Cardoso J.M.S., Fonseca L. and Abrantes I. (2011). Direct molecular detection of the pinewood nematode, *Bursaphelenchus xylophilus*, from pine wood, bark and insect vector *European Journal of Plant Pathology* - DOI: 10.1007/s10658-10011-19915-y.

Molecular identification from wood



Detection of *B. xylophilus* by PCR of the satDNA, using DNA extracted directly from 100 mg of *Pinus pinaster* wood

MM: molecular marker

MOLECULAR IDENTIFICATION CONVENTIONAL PCR

Advantages

- Rapid
- Robust
- Specific primers available for several PWN species
- Multiplex analysis possible

Disadvantages

- Low number of species investigated at molecular level
- Post PCR processing (agarose gel - time consuming, poor precision,

and low sensitivity and resolution,...)

MOLECULAR IDENTIFICATION – NEXT FUTURE

In situ diagnostics

REAL-TIME PCR

Advantages

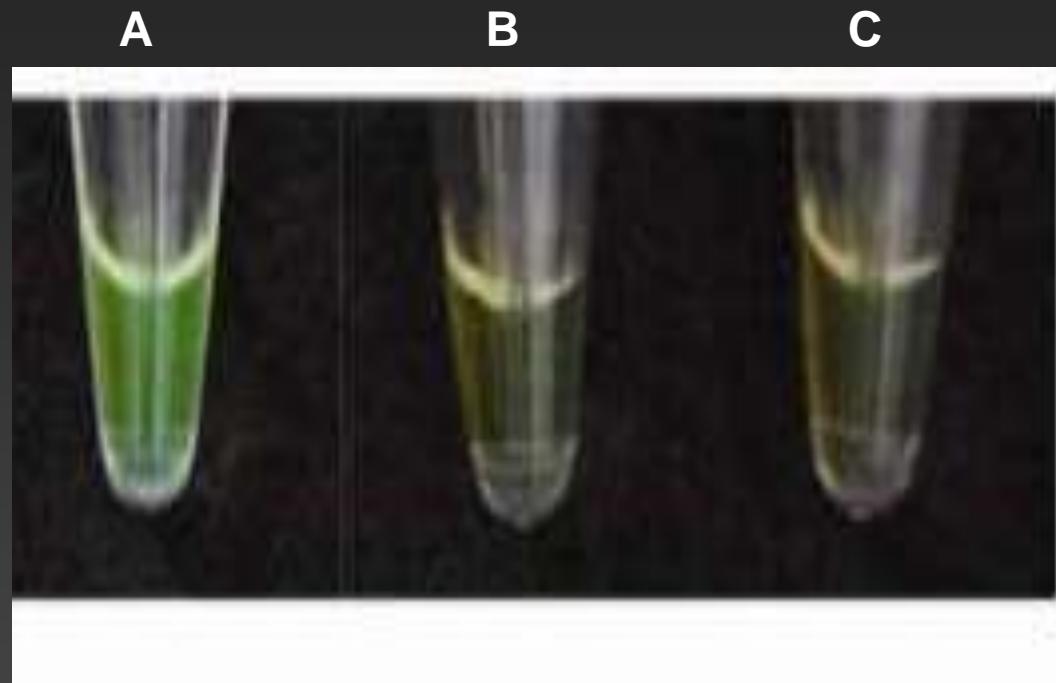
- No-post PCR processing
- Proportionality between fluorescence intensity and amount of DNA synthesized during PCR reaction

MOLECULAR IDENTIFICATION – NEXT FUTURE

In situ diagnostics

LAMP

Loop mediated
isothermal
amplification



Detection of *B. xylophilus* by LAMP of the rDNA ITS specific regions .

A: *B. xylophilus*; B: *B. mucronatus*; C: without DNA (negative control).

Research lines

Detection methods



Genetic studies



Bacteria associated with the pine wilt disease



Pathogenicity of PWN in *Pinus* spp.



Impact of the PWN in the physical and chemical wood properties



Wood/bark treatment



Acknowledgments



Transcriptomic and proteomic approach for understanding the molecular basis of *Bursaphelenchus xylophilus* pathogenicity
PTDC/AGR-CFL/098916/2008

BxTOXIC-Identification of genetic determinants from endophytic bacteria involved in nematode infectivity in PWD
PTDC/AGR-CFL/115373/2009

Thank you!