

Evaluation of the susceptibility of Portuguese autochthonous flora to the pine wilt nematode (*Bursaphelenchus xylophilus*)



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Introduction

Bursaphelenchus xylophilus is the causal agent of the pine wilt disease and its insect vector is *Monochamus galloprovincialis*. *B. xylophilus* is native to North America, where it causes little damage to trees, however, the most severely affected areas are found in the Far East. *B. xylophilus* was first detected in Portugal, in 1999 (Mota *et al.*, 1999), and it was also the first report of this nematode in a native conifer within the EU. After infection, nematodes move rapidly from the inoculation point and enter woody tissues via resin canals of the xylem and cortex, feeding on their epithelial cells (Ichihara *et al.*, 2000). Once infected, most plants cease resin production and show symptoms of needle chlorosis and usually die in just a few months (Fukuda, 1997). It's widely known that *B. xylophilus* can infect other species of the *Pinaceae* family besides the ones of the *Pinus* genus (Robbins, 1982; Malek, 1984). Nevertheless, it's not known what species of the Portuguese autochthon flora are susceptible to the pine wilt nematode (PWN). In this work we aimed at determining if *Picea abies* and *Cupressus lusitânica* are susceptible species to the PWN. The susceptibility to the PWN was evaluated by means of nematode population, total chlorophyll and phenolic compounds quantification and Scanning Electron Microscopy (SEM).

Materials and Methods

- One year old plants were kept under 8h/26°C light, 16h/24°C darkness cycles with constant 80% of humidity.
- *B. xylophilus*, HF strain (BxHF), was grown on barley seeds with *Botrytis cinerea* at 26°C, in the dark, and extracted using Baermann funnel technique (Fig. 1-4).
- Fifteen seedlings of each plant species were inoculated with 1800 nematodes in a sterile water suspension and symptoms were monitored during 14 days (Fig.5).
- Nematodes were extracted from the stems using Baermann funnel technique and quantified by optical microscopy (Fig.4).
- Chlorophyll extraction and quantification was performed according to Abadía *et al.* (1984).
- Total soluble phenolic compounds were extracted and quantified following Azevedo (2005).
- SEM was used to examine the morphology of *P. abies* and *C. lusitânica*.



Fig. 1 - BxHF growing on PDA.



Fig. 2 - *B. cinerea* growing on PDA.

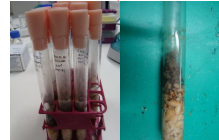


Fig. 3 - BxHF growing on *B. cinerea* (on barley seeds).

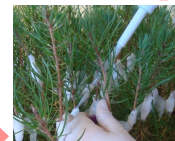
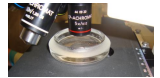


Fig. 5 - Inoculation procedure.



Fig. 4 - Baermann funnel technique.

Nematode quantification



Total chlorophyll and total soluble phenolic compounds quantification



SEM



Results and Discussion

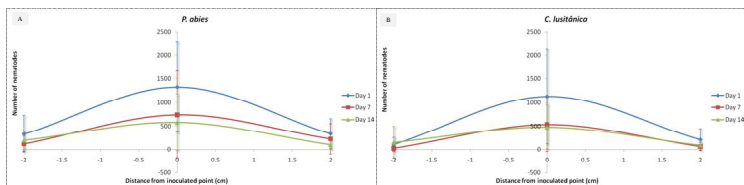


Fig. 6 - Changes in the nematodes population in *P. abies* (A) and *C. lusitânica* (B) seedlings inoculated with BxHF. Each value is the mean of five replicates.

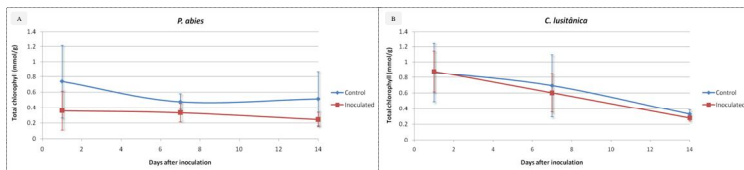


Fig. 7 - Evolution of the amount of total chlorophyll in *P. abies* (A) and *C. lusitânica* (B) seedlings inoculated with BxHF. Each value is the mean of five replicates.

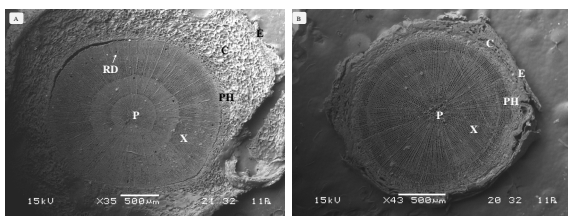


Fig. 9 - SEM imaging of *P. abies* (A) and *C. lusitânica* (B) stems showing morphological differences between the two species. Leg.: E-epidermis; C-cortex; RD-resin duct; PH-phloem; X-xylem; P-pith.

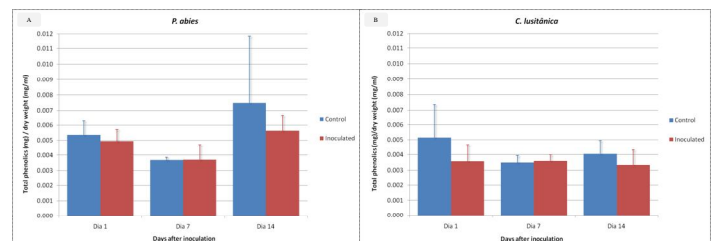


Fig. 8 - Evolution of the amount of total phenolic compounds in *P. abies* (A) and *C. lusitânica* (B) seedlings inoculated with BxHF. Each value is the mean of five replicates.

➤ Neither species presented visible symptoms of disease, either because more time was needed for symptoms appearance, or because these species are resistant to the nematode.

➤ *P. abies* presented a larger amount of nematodes, probably because it's a member of the *Pinaceae* family, and so, has a more identical morphology and physiology to pine species (the preferred host for the PWN) (Fig. 6).

➤ Both in control and infected plants (of both *P. abies* and *C. lusitânica*) the leaf chlorophyll concentration decreased during the experiment, probably due to the mechanic injury inflicted during the inoculation and the action of the nematode population itself, in inoculated plants case (Fig. 8).

➤ The evolution of the amount of total phenolic compounds may indicate that *P. abies* and *C. lusitânica* don't increase the production of phenolics as part of their defense mechanism, or that the relative amount of the different phenolic compounds differs but the total amount of phenolics stays the same (Fig. 8).

➤ *P. abies* has resin ducts that are used by the PWN to move along the plant, feed and reproduce (Ichihara *et al.*, 2000), being, therefore, more similar to the known host *Pinus pinaster* (Fig. 9).

Conclusions and Future Work

The results indicate that the examined species appear to be resistant to the PWN. However, for confirmation of resistance, further studies should be carried out, particularly with a larger number of replicates. Despite the phylogenetic proximity between *P. abies* and *C. lusitânica* and the species of the *Pinus* genus, the preferred host of the PWN, they have not responded in a susceptible way to infection since it wasn't observed significant reduction in total chlorophyll content or an increase in concentration of phenolic compounds. Future work will involve optical microscopy techniques, lignin quantification and the study of genes associated with the defense mechanism of plants to be able to determine with greater certainty the degree of susceptibility of the species studied.

Acknowledgments

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Diapositivo 1

U1 Isto não está muito bem. O nemátode cresce em fungo, não é? Pode é crescer no fungo que cresceu em PDA. Esta placa parece-me da Mariana onde ela punha 1 nemátode mas o que crescia lá eram bactérias! Às vezes a Carla também põe nemátodes em solução nestas placas, mas é para guardar a 4°C.
Utilizador; 07-07-2010

U2 And vertical bars represent standard deviation or standard error?
Utilizador; 07-07-2010

U3 Acho que se costuma dizer inoculation site. Não sei se se pode dizer inoculation point...
Utilizador; 07-07-2010

U4 And vertical bars represent (...)?
Utilizador; 07-07-2010

U5 And vertical bars?
Utilizador; 07-07-2010