EVALUATION OF THE SUSCEPTIBILITY OF PORTUGUESE AUTOCHTHONOUS FLORA TO THE PINE WOOD 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* was first detected in Portugal in *Pinus pinaster* trees in 1999 (Mota *et al.*, 1999); this was the first report of the presence of *B. xylophilus* in a native conifer forest 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). Also, phenolic compounds extrusion and plant morphology seem to be associated with the degree of disease susceptibility. 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 wood 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 count, total chlorophyll quantification, phenolic compounds and lignin quantification and Scanning Electron Microscopy (SEM).

Materials and Methods

- One year old plants were kept under 8h/25ºC light, 16h/18ºC darkness cycles with constant 80% humidity.
- *B. xylophilus* HF strain (BxHF – Fig. 1), was grown on barley seeds with *Botrytis cinerea* at 26ºC (Fig. 2), in the dark, and extracted using Baermann funnel technique (Fig. 3).
- Eighty seedlings of each plant species were inoculated with 1000 nematodes in a sterile water suspension (Fig. 4) and symptoms were monitored during 28 days.
- Nematodes were extracted from the stems using Baermann funnel technique and counted under the optical microscope.
- Chlorophyll extraction and quantification was performed according to Abadía *et al.* (1984).
- Total soluble phenolic compounds were extracted and quantified following Azevedo (2005).
- Lignin was extracted and quantified according to Fukushima (2001).
- SEM was used to examine the morphology of *P. abies*, *C. lusitânica* and *P. pinaster* stems.

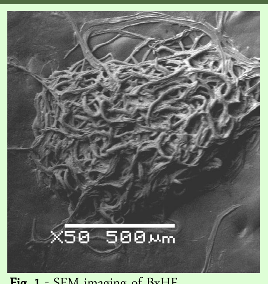

Fig. 1 - SEM imaging of BxHF.


Fig. 2 – BxHF growing on barley seeds.

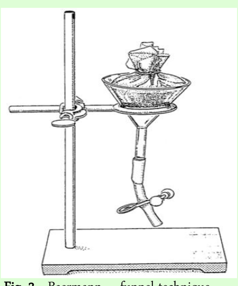
Fig. 3 - Baermann funnel technique.


Fig. 4 - Tree inoculation.

Results and Discussion

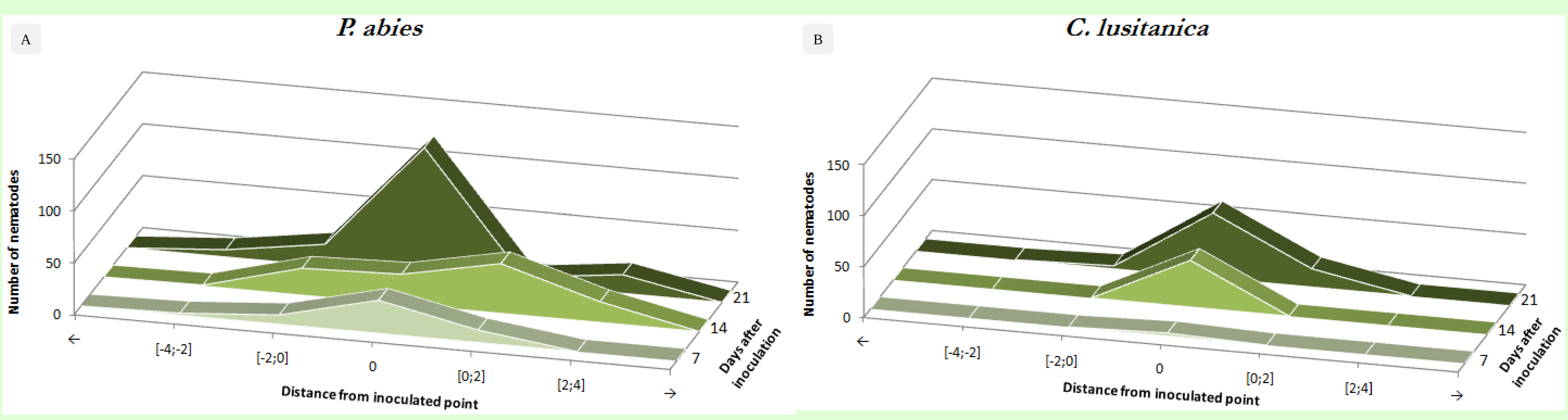

Fig. 5 - Changes in the nematode progression above and below the inoculation point in *P. abies* (A) and *C. lusitânica* (B) seedlings inoculated with BxHF. Each value is the mean of ten replicates.

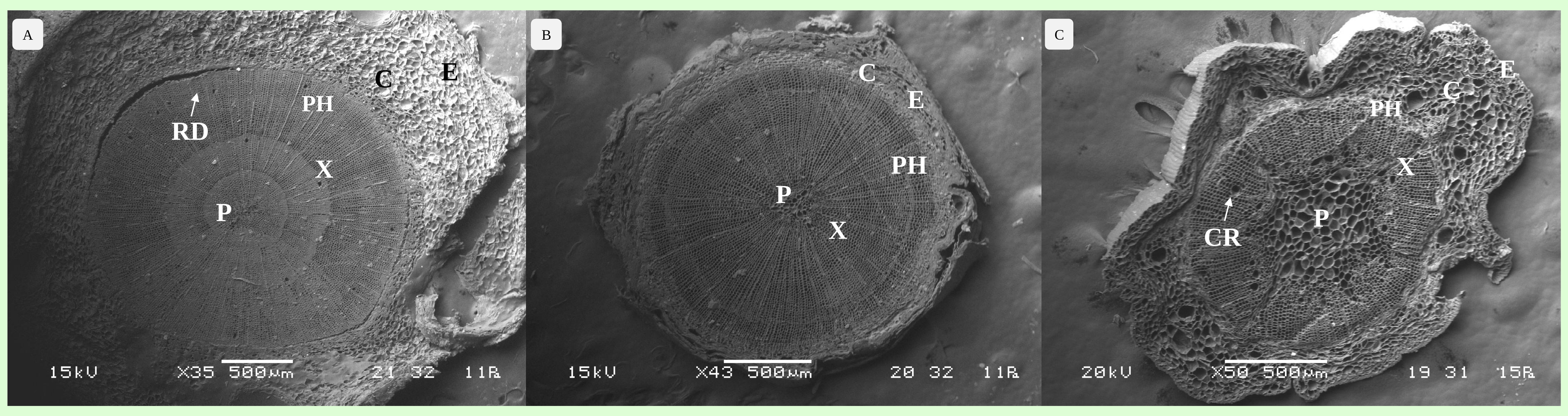

Fig. 9 - SEM imaging of *P. abies* (A), *C. lusitânica* (B) and *P. pinaster* (C) stems showing morphological differences between the three species. E–epidermis; C–cortex; RD–resin duct; PH–phloem; X–xylem; P–pith.

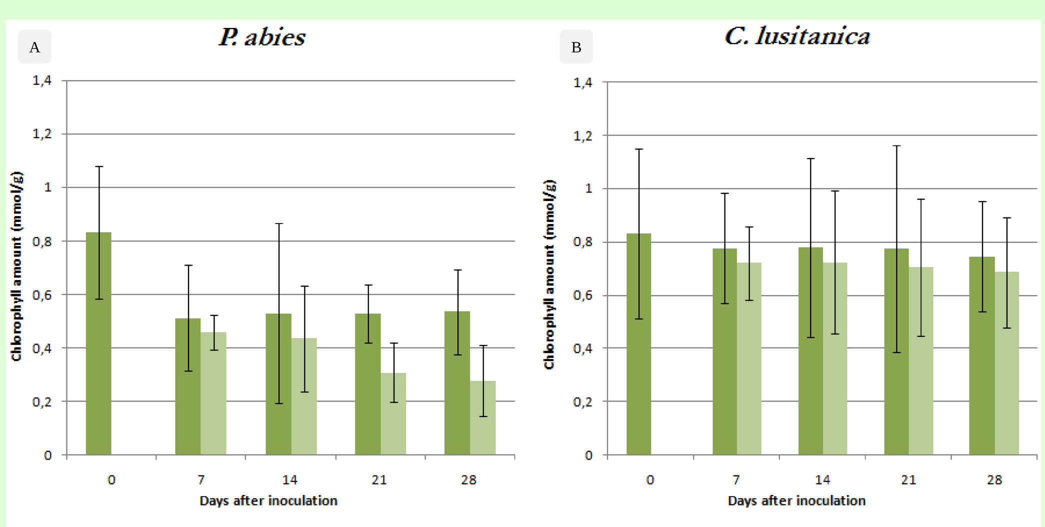

Fig. 6– Changes in total chlorophyll concentration in *P. abies* (A) and *C. lusitânica* (B) seedlings inoculated with BxHF. Each value is the mean of ten replicates. The vertical bars represent the standard deviation.

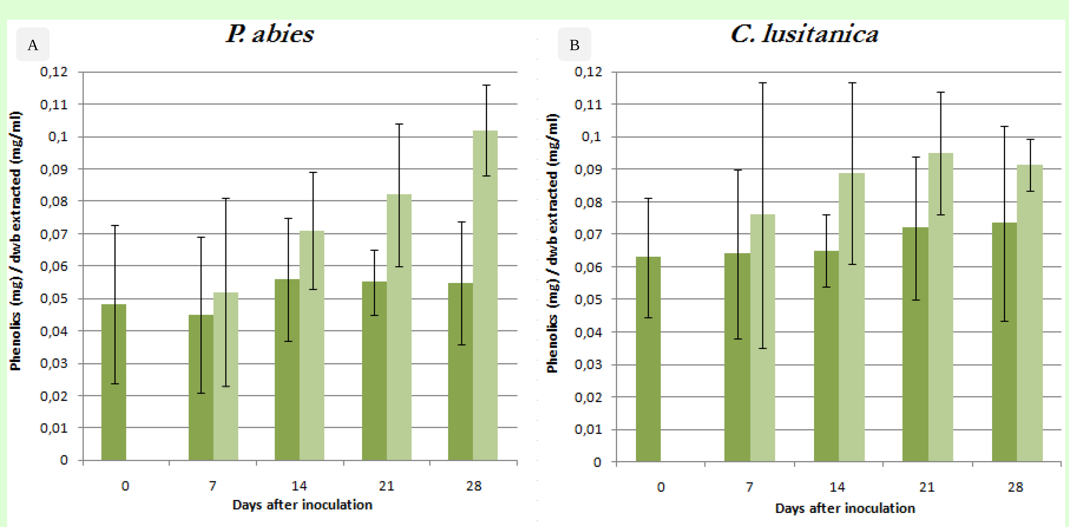

Fig. 7 – Changes in total soluble phenolics concentration in *P. abies* (A) and *C. lusitânica* (B) seedlings inoculated with BxHF. Each value is the mean of ten replicates. The vertical bars represent the standard deviation.

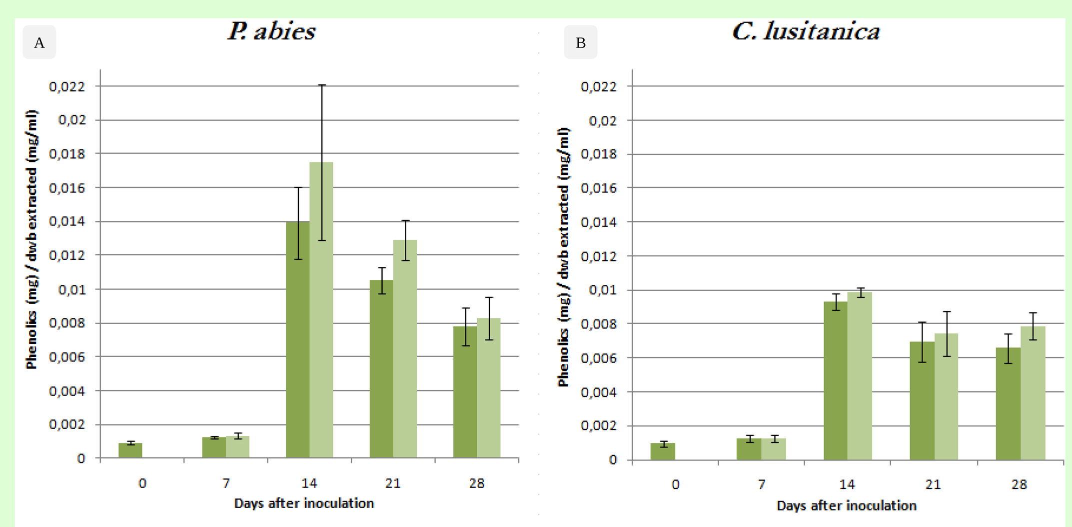

Fig. 8 – Changes in lignin concentration in *P. abies* (A) and *C. lusitânica* (B) seedlings inoculated with BxHF. Each value is the mean of ten replicates. The vertical bars represent the standard deviation.

- Neither species presented visible symptoms of disease, possibly because more time was needed for symptom appearance, or because these species are resistant to the nematode.
- *P. abies* presented a larger number of nematodes, probably because it is a member of the *Pinaceae* family, and so it is best related morphologically and physiologically to pine species (the preferred host for the PWN; Fig. 5).
- This similarity of morphological characteristics between *P. abies* and *P. pinaster* was confirmed by visualizing stems of these species under electron microscopy. *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 *P. pinaster* (Fig. 9).
- Both in control and inoculated plants of either *P. abies* or *C. lusitânica*, the chlorophyll leaf concentration decreased during the experiment. In control seedlings this was probably due to the mechanic injury inflicted during mock inoculation, whereas in infected seedlings this might have been caused by mechanic injury or the action of the nematode population itself (Fig. 6).
- There seems to be an increase in the amount of total phenolics in inoculated plants of either species, probably due to the nematode activity (Fig. 7).
- The lignin content of *P. abies* is significantly increased 14 days after inoculation in both species, which might indicate that they are capable of mounting a defense response against the PWN (Fig. 8).

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 manner to the infection, namely, no external symptoms were observed. Future work will involve optical microscopy techniques and the study of genes associated with known plant defense mechanisms to be able to determine with greater certainty the degree of susceptibility of the species studied to the PWN.

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Acknowledgments

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